The 12th International Barley Genetics Symposium

June 26-30, 2016

Minneapolis-St. Paul, Minnesota

United States of America

Program Overview

Sunday June 26	Monday June 27	Tuesday June 28	Wednesday June	Thursday June
	Breakfast	Breakfast	Breakfast	Breakfast
	Welcome	Session 5	Session 8	
	Session 1			Travel to campus
	Session 2	Session 6	Session 9	Presentations in the St. Paul campus fields
	Lunch	Lunch	Lunch	Lunch
	Session 3	Session 7	Session 10	Self-paced field demonstrations
Registration and Poster set-up	Session 4	Flash and Dash	Session 11	Travel to hotel
	Flash and Dash	Poster Session- Even numbers &		
Opening Reception	Poster Session- Odd numbers &	Dinner on your own	Travel to banquet	
	Dinner on your		Banquet	
	Evening Work-shops	Evening Work-shops		
			Travel to hotel	

Key Sessions

Poster Session

Event not held at Commons hotel

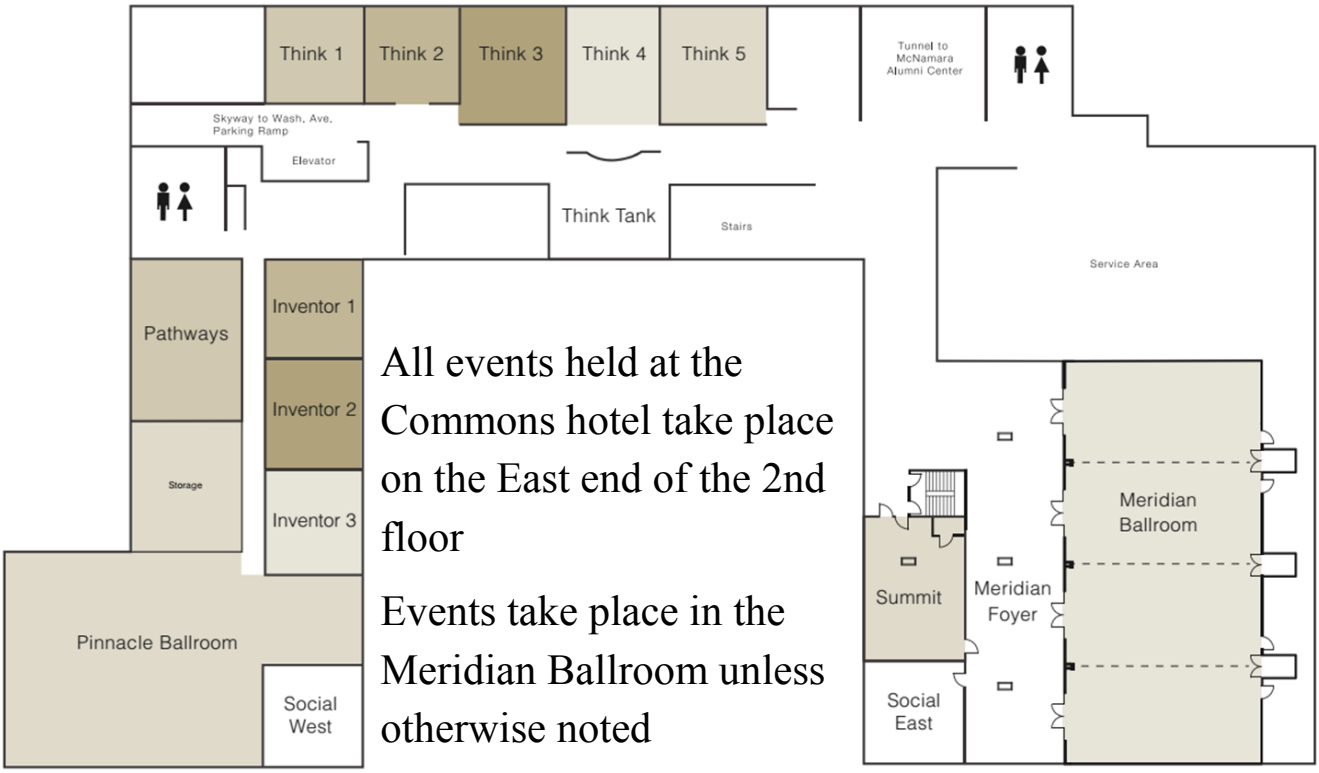
Group travel by bus

Field day at Univ. of Minnesota St. Paul Campus

Presentations are 20 minutes including question time.

Keynote presentations are 30 minutes.

Commons Hotel—2nd Floor



All events held at the Commons hotel take place on the East end of the 2nd floor

Events take place in the Meridian Ballroom unless otherwise noted

IBGS Local Organizing Committee

Kevin Smith, Chair, University of Minnesota
Flavio Capettini, Field Crop Development Centre, Alberta Agriculture and Forestry
Michael P. Davis, National Barley Improvement Committee, American Malting Barley Association
Ruth Dill-Macky, University of Minnesota
Duane Falk, University of Guelph
Patrick Hayes, Oregon State University
Peter L. Morrell, University of Minnesota
Gary J. Muehlbauer, University of Minnesota
Madeline Smith, University of Minnesota
Brian Steffenson, University of Minnesota

IBGS International Committee

Guoping Zhang, China(Chair)
Andreas Graner, Germany
Euclydes Minella, Brazil
Bryan Harvey, Canada
Oga Kovaleva, Russia
Antonio Michele Stanca, Italy
Wayne Powell, United Kingdom
Kazuhiro Sato, Japan
Alan Schulman, Finland
Flavio Capettini, Canada
Chengdao Li, Australia
Kevin Smith, USA

FULL PROGRAM

SUNDAY, June 26th

2:00 PM – 5:00 PM	Registration at the Commons Hotel 2nd Floor Meridian Ballroom Foyer.
2:00 PM – 5:00 PM	Poster Set Up—Think 1, 3, 4, 5 and Inventor 1, 2, 3 (see poster map at registration table)
5:00 PM – 8:00 PM	**Opening Reception at the McNamara Alumni Center** Appetizers and Refreshments, Music, Registration Table

MONDAY, June 27th

7:00 AM - 8:00 AM	Breakfast (Pinnacle)
8:15 AM - 8:45 AM	**Welcome and Opening Remarks** Brian Buhr, Dean College of Food Agricultural and Natural Resource Sciences.
8:45 AM - 10:00 AM	**Session 1: Success Stories in Barley Breeding and Genetics** Presider: **Duane Falk**

KEYNOTE SPEECH

Barley: a research model for the temperate grasses
Robbie Waugh; The James Hutton Institute and University of Dundee, UK

Imidazolinone tolerant barley: an Australian barley breeding success story
David Moody; InterGrain Pty Ltd, Austrailia

From MAS to GS - strong support for modern barley breeding
Viktor Korzun; KWS Lochow GmbH, Germany

10:00 AM - 10:30 AM	Coffee (Foyer)

10:30 AM - 12:05 PM **Session 2: Barley Morphology and Development**
Presider: **Jerry Franckowiak**

KEYNOTE SPEECH

Identification of genes defining culm length in barley
Mats Hansson; Lund University, Sweden

Story of barley domestication
Mohammad Pourkheirandish; The University of Sydney, Austrailia

Global transcriptome profiling of developing leaf and shoot apicies reveals distinct genetic and environmental control of floral transition and inflorescence development in barley
Maria von Korff Shmising; University of Düsseldorf, Max Planck Institute for Plant Breeding Research, Germany

Genome-wide association mapping of root extension in a collection of European winter barley cultivars
Alessandro Tondelli; Genomics Research Centre, Università degli Studi di Milano, Italy

12:05 PM - 1:20 PM Lunch (Meridian Ballroom)

1:20 PM - 2:55 PM **Session 3: Resources I: Genome**
Presider: **Gary Muehlbauer**

KEYNOTE SPEECH

The barley genome
Nils Stein; Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

Genetic diversity in the genome era: a fine-scale map of sequence variation in the barley genome
Martin Mascher; Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

We are the 80%! Transposable elements and genome dynamics in barley
Alan H. Schulman; Luke and University Helsinki, Finland

Genome-wide diversity analysis focused on flowering-related loci sheds light on the history of barley domestication
Artem Pankin; Max Planck Institute for Plant Breeding Research, Germany

2:55 PM - 3:25 PM Coffee (Foyer)

3:25 PM - 5:00 PM **Session 4: Resources II: Germplasm and Populations**
Presider: **Peter Morrell**

KEYNOTE SPEECH

Modelling the genetic architecture of plant development through nested associated mapping
Klaus Pillen; University of Halle, Germany

Barley composite crosses: past and future value in barley breeding and research
Cal Qualset; University of California, Davis, USA

The molecular basis of self-incompatibility in *Hordeum bulbosum*
Katsuyuki Kakeda; Mie University, Graduate School of Bioresources, Japan

A pipeline for rapid development of mapping populations relevant to breeding
Laura Ziems; The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Austrailia

5:00 PM - 5:35 PM	**Flash and Dash and Poster Session** Presider: **Gary Muelbauer**
5:35 PM 6:35 PM	**Poster Session--Odd Posters** (Thinks 1, and 3-5 and Inventor 1-3) Happy Hour with complementary beer and appetizers

Dinner on your own

7:30 PM - 9:30 PM Evening Workshop: Climbar

Alan Schulman: Introduction to ClimBar

Bill Thomas: QTL x E Interactions in Spring Barley

Ernesto Igartua: TBA

Eyal Fridman: High-content yield phenotype under abiotic stresses - integrating armed and remote sensing tools for better phenomics

Patrick Schweizer: Disease assessment in seedlings of ClimBar collection - temperature effects

Søren Rasmussen: Effects of CO_2 and temperature on the quality of barley seeds

TUESDAY June 28th

7:00 AM - 8:00 AM Breakfast (Pinnacle)

8:15 AM - 9:50 AM **Session 5: Abiotic Stresses**
Presider: **Brian Steffenson**

KEYNOTE SPEECH

Genetic characterization of salinity tolerance traits to increase salinity tolerance of crops

Mark Tester; King Abdullah University of Science & Technology, Saudi Arabia

Genetic resources for climate change: wild barley genes for tolerance to waterlogging

Anna Westerbergh; Department of Plant Biology, Uppsala BioCenter, Linnean Centre for Plant Biology in Uppsala, Swedish University of Agricultural Sciences, Sweden

Genetic solution for phosphorus use efficiency (PUE) in barley through candidate genes

Xue Gong; The University of Adelaide, Austrailia

Influence of temperature on recombination in barley

Mikel Arieta; The James Hutton Institute, UK

9:50 AM - 10:20 AM Coffee (Foyer)

10:20 AM - 11:55 AM **Session 6: Biotic Stresses**
Presider: **Ruth Dill-Macky**

KEYNOTE SPEECH

Gene-based approaches to durable disease resistance in Triticeae cereals
Patrick Schweizer; Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

Proteomic comparison of near-isogenic barley germplasm differing in allelic state of major senescence QTL identifies numerous proteins involved in plant pathogen defense
Andreas Fischer; Montana State University, USA

The barley 'nibblerome': defining the set of NB-LRR-type R-genes from a diverse collection of barley
William Jackson; The Sainsbury Laboratory, UK

Modulation of integrated decoy R-genes/transcription factor assembly elicits wheat stem rust resistance responses in barley: rpg4/ Rpg5-mediated Ug99 resistance
Shyam Solanki; North Dakota State University, USA

11:55 PM - 1:10 PM Lunch (Meridian Ballroom)

1:10 PM - 2:55 PM **Session 7: Grand Challenges Discussions**

Breeding with a reference genome – Pathways
Organizers: Pat Hayes, Keving Smith, Lucia Gutierrez, Bill Thomas

Discovery and deployment of durable disease resistance: genetics deployment and management – Pinnacle Ballroom
Organizers: Kelly Turkington, Brian Steffenson, Mohammad Pourkheirandish, Blakely Paynter, Matthew Moscou

Genetic resources and populations available to explore genetic diversity in barley – Meridian Ballroom
Organizers: Peter Morrell, Klaus Pillen, Cal Qualset, Marin Mascher

2:55 PM - 3:25 PM Coffee (Foyer)

3:25 PM - 4:00 PM **Flash and Dash Presentations**
Presider: **Gary Muelbauer**

4:00 PM - 5:30 PM **Poster Session--Even Posters** (Thinks 1, and 3-5 and Inventor 1-3)
Happy Hour with complementary beer and appetizers

Dinner on your own

7:30 PM – 9:00 PM Evening Workshop: Developmental Mutants in Barley: Characterization and Use
Organizer: **Antonio Michele Stanca and Udda Lundqvist**

M. Hansson ,**U.Lundqvist** and **J. Frankowiak**: The history of developmental mutant collections and their use, including the demonstration of the "International Database of Barley Genes and Barley Genetic Stocks.

T. Komatsuda: Origin of cultivated barley through mutation analysis

N. Stein: Barley developmental mutants - accessing underlying genes to support research and breeding

R. Waugh: Semi-sterile mutants and screening population for mutations in target genes

J. Franckowiak: The hooded barley grown in US

L. Schneider: Eceriferum-cqu (cer-cqu) – cluster gene

T. Schnurbusch: Six rowed and irregular spike barley mutants

S. Salvi and **R. Tuberosa**: Mutants of barley roots

G. Muelbhauer: Uniculm and lnt barley mutants

B. Thomas: Mutants for pre-breeding: the vision of the breeder

P. Bregitzer and **U. Lundqvist**: Barley Genetics Newsletter, the existence to-day and its future

P. Bregitzer and **U. Lundqvist**: Barley Genetics Newsletter, the existence to-day and its future.

U. Lundqvist: Coordinators for barley collections – existence to-day and their future

WEDNESDAY June 29th

7:00 AM - 8:00 AM Breakfast (Pinnacle)

8:15 AM - 9:50 AM **Session 8: Breeding Methodologies: Current Approaches and Future Prospects**
Presider: **Pat Hayes**

KEYNOTE SPEECH

Current breeding methodologies and future prospects
Bill Thomas; The James Hutton Institute, UK

Study of barley maturity with new breeding tools
Anne-Marie Bochard; Limagrain Europe

Genome engineering in barley using customizeable endonucleases
Jochen Kumlen; Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany

Marker-based breeding tools inform early generation selection and crossing block decisions
Tyler Tiede; University of Minnesota, USA

9:50 AM - 10:20 AM Coffee (Foyer)

10:20 AM - 11:55 AM — Session 9: Barley End Use: Malting and Brewing

Presider: **Mike Davis**

KEYNOTE SPEECH

The North American malting barley industry
Chris Swersey; Brewers Association, USA

Genome editing to validate genes putatively contributing to grain (1,3:1,4)-β-glucan content
Kelly Houston; The James Hutton Institute, UK

Identification of two key genes controlling chill haze stability of beer in barley
Lingzhen Ye; Institute of Crop Science, Zhejiang University, China

Barley contributions to beer flavor I: impact assessment of variety, location, and genotype x environment
Dustin Herb; Oregon State University, USA

11:55 AM - 1:10 PM — Lunch (Meridian)

1:10 PM - 2:45 PM — Session 10: Barley End Use: Food and Feed

Presider: **Madeline Smith**

KEYNOTE SPEECH

Molecular genetic characterization of key genes for utilization of barley as human food and animal feed
Shin Taketa; Institute of Plant Science and Resources, Group of Genetic Resources and Functions, Okayama University, Japan

Effects of agronomic practices, soil, and climatic zones on the content and molecular structure of dietary fiber constituents in food barley genotypes
Marta Izydorczyk; Canadian Grain Commission, Canada

Regulation of plant development by miRNA-targeted transcription factor families
Marcel Lafos; University of Dundee, UK

The INUDFOOD Report
Brigid Meints; Washington State University, USA

2:45 PM - 3:15 PM — Coffee (Foyer)

3:15 PM - 4:50 PM — Session 11: Plant Breeding and Genetics Education

Presider: **Flavio Capettini**

KEYNOTE SPEECH

Plant Breeder Training Network: collaborative approach to training
Jamie Sherman; Montana State University, USA

Barley breeding in the public interest: perspectives on integrating research, variety development, teaching, and extension in a US land grant university
Patrick Hayes; Oregon State University, USA

Connecting genotype to phenotype in 7-12 classrooms with iTAG barley
Roger Wise; USDA-ARS, Iowa State University, USA

Genetic basis of barley adaptation to the southern cone of South America: using GWAS to analyze phenology in a wide population
Andres Locatelli; Universidad de la República, Uruguay

12th IBGS Celebration Banquet Evening

4:50 PM - 5:00 PM	Announcements
5:45 PM	Board busses outside of Commons Hotel
6:30 PM	Arrive at Padelford RiverBoats
6:30 PM – 9:30 PM	Refreshments, Dinner
	Music by "The Gentleman's Antitemperance League"
9:30 PM	Board busses for Commons Hotel

THURSDAY June 30th

7:00 AM - 8:30 AM	Breakfast (Pinnacle)
8:30 AM	Board Buses
8:30 AM - 9:00 AM	Travel to University of Minnesota St. Paul campus

Barley Genetics Research in the Field University of Minnesota, St. Paul Campus

9:00 AM - 12:00 PM	Field presentations
12:00 PM - 1:00 PM	Lunch
1:00 PM - 3:00 PM	Self-paced demonstrations
3:00 PM	Board Buses
3:00 PM - 3:30 PM	Travel to Commons Hotel

Thank you for visiting us!

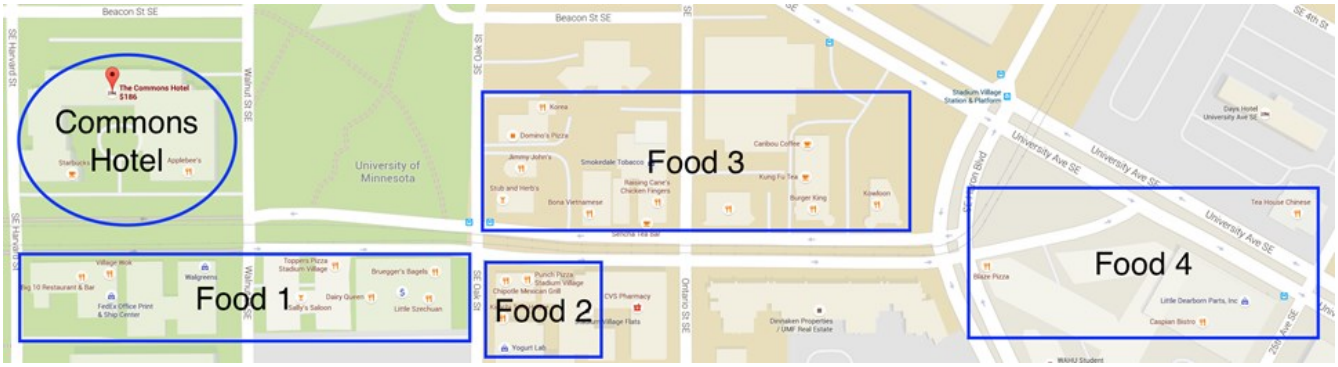
We hope you have a chance to enjoy some of the great things that Minnesota has to offer. Here are some suggestions:

Top 10 Things to do in Minneapolis!

Explore Minneapolis with Nice Ride bike sharing

Great Minneapolis walking tours!

Eating options withing walking distance



Food 1 (1-2 blocks)	Food 2 (2 blocks)	Food 3 (2-3 blocks)	Food 4 (4-5 blocks)
Abduls Afandy	Chipotle Mexican Grill	Bar Luchador	Blaze Pizza
Big 10 Restaurant & Bar	Kimchi Tofu House	Bona Vietnamese	Caspian Bistro
Bruegger's Bagels	Kitty Corner Café	Burger King	Tea House
Bun Mi Sandwiches	Noodles & Company	Caribou Coffee	
Dairy Queen/Orange	Punch Pizza	Domino's Pizza	
Espresso Expose	Yogurt Lab	Hong Kong Noodle	
Haiku Japanese Bistro		Jimmy John's	
Little Szechuan		Korea	
Sally's Saloon		Kowloon	
Village Wok		Kung Fu Tea	
		My Burger	
		Raising Cane's Chicken	
		Sencha Tea Bar	
		Stub and Herb's	

United States Department of Agriculture
National Institute of Food and Agriculture

Thanks to our sponsors!

SUPPORTED BY

BA BREWERS ASSOCIATION

SAMUEL ADAMS

New Glarus Brewing Co. · Drink Indigenous

BAYER

BRIESS MALT & INGREDIENTS Co.
All Natural Since 1876

National Barley Growers Association

SIERRA NEVADA

NEW BELGIUM BREWING

SUMMIT BREWING CO.

Douglas SCIENTIFIC

AB InBev

MONSANTO

BEER INSTITUTE

American Malting Barley Association

1847 R RAHR MALTING CO.

Montana Wheat and Barley

North Dakota Barley Council

syngenta

BioTechnology Institute
University of Minnesota

IDAHO BARLEY COMMISSION

DESCHUTES BREWERY EST 1988

MillerCoors

Malteurop North America

Brewing and Malting Barley Research Institute

WASHINGTON GRAIN COMMISSION

Minnesota Barley

InteGrow Malt LLC

College of Food, Agricultural and Natural Resource Sciences
University of Minnesota

RAPID GENOMICS

Cargill Malt

MCIA MINNESOTA CROP IMPROVEMENT ASSOCIATION

SESSION: 1

KEYNOTE

Barley: a research model for the temperate grasses

Robbie Waugh

The James Hutton Institute and University of Dundee; Robbie.Waugh@hutton.ac.uk

ABSTRACT

As we awaken to the concept that the crop should now also be considered the model, ready access to increasingly powerful research and informational tools complemented by widely available and extensive genetic resources has been driving an resurgence in the use of barley as an experimental system in both applied and fundamental research programs. Crucially, as a major world crop, routes towards testing and translation of discoveries are both immediately relevant and straightforward and can be implemented using a blend of traditional and new breeding technologies. I would argue that this is clearly evidenced in the recent scientific literature. In my presentation I will focus on some of the ways we (and others) have been applying new information and technologies to drive our understanding of how barley has responded to the environmental challenges it has faced in the past as it emerged from its centre of origin and spread throughout the world, and once established, the consequences of intense selection during breeding. I will illustrate and discuss the extensive and worryingly monomorphic tracts present of the current elite barley genome and, time permitting, discuss some of the strategies we are starting to develop to help address this problem.

SESSION: 1

Imidazolinone tolerant barley: an Australian barley breeding success story

David Moody

InterGrain Pty Ltd; dmoody@intergrain.com

Co-authors: M. Materne and C. Pittock

ABSTRACT

In the space of 10 years, imidazolinone tolerant barley in Australia has progressed from the creation of the original imidazolinone tolerant mutant to a national market share exceeding 20%. The popularity of this trait in barley with growers has been (largely) driven by increasing levels of brome grass species (Bromus rigidus and Bromus diandrus) in zero and minimum tillage farming systems, and the ease of control of these weed species using imidazolinone chemistries. Imidazolinone tolerance in barley has also provided growers with a number of additional benefits. Tolerance has allowed barley to be planted into paddocks containing residual imidazolinone herbicides used in the previous crop, with the dry summer and autumn conditions in Australia often resulting in low rates of microbial decomposition. The trait provides growers with the opportunity to achieve higher yields and improve grain quality through earlier sowing into wheat stubbles, due to the ability to remove volunteer wheat in-crop, rather than waiting for the germination and removal of residual wheat by knockdown (non-selective) herbicides. As a result of the combination of advantages the trait provides to barley in Australian farming systems, the relative proportion of imidazolinone tolerant barley to intolerant barley varieties is three-fold higher than the relative proportions of tolerant to intolerant varieties in the other crop species possessing this trait in Australia (canola and wheat).

This paper describes the original development of the trait in barley, the breeding history of the first imidazolinone tolerant barley variety (cv. Scope) and the critical factors that have driven the rapid and successful commercialization of this variety. Further, the paper highlights the future prospects for imidazolinone tolerant variety development and the challenges facing the longevity of this trait in the farming system.

SESSION: 1

From marker-assisted selection (MAS) to genomic selection (GS) - strong support for modern barley breeding

Viktor Korzun

KWS LOCHOW GMBH; viktor.korzun@kws.com

Co-authors: E. Byrne, J. Großer, D. Harrap, J-F. Herbommez, S. Kollers, K. Oldach, M. Schmidt, B. Schinkel, A. Tomerius, N. Wendler, P. Werner, and H. Verstegen

ABSTRACT

Conventional barley breeding is time-consuming and strongly depends on environmental conditions. Therefore, breeders are extremely interested in new technologies that optimize breeding processes. Molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improve selection strategies in breeding. There has been a large and rapid accumulation of genomics tools in the cereal crops during the last decade. These developments have been coupled with the emergence of high throughput technologies, which have allowed advances in molecular marker technology and implementation.

The impact of new molecular tools and technologies available for plant variety development has proven to be essential to optimize and accelerate breeding programs.

Disease resistance and quality are prime targets in practical barley breeding programs. In all cases the breeding process can be accelerated by applying marker-assisted selection (MAS) for developing new material and for improvement of the selection intensity and accuracy. Nevertheless, the recent advance in high density genotyping has opened new possibilities to apply genome-wide markers in Genomic Selection (GS) rather than individual markers as in classical MAS.

Malting quality traits in barley breeding usually has been targeted by using MAS. In this study, the potential to apply genomic selection instead to improve malting quality in two commercial breeding programs of spring and winter barley (Hordeum vulgare L.) was evaluated. Predictive abilities (PA), as derived from cross-validation, for twelve malting quality characters investigated ranged from 0.14 to 0.58 for spring barley and 0.40 to 0.80 for winter barley indicating that Genomic Selection can be successfully applied.

The results of specific applications of molecular markers, potential of genomic selection and practical applications of genomics knowledge in malting barley breeding will be presented and discussed.

SESSION: 2

KEYNOTE

Identification of genes defining culm length in barley

Mats Hansson

Lund University, Department of Biology, Solvegatan 35, 22362 Lund, Sweden;
mats.hansson@biol.lu.se

ABSTRACT

Reduced plant height and culm robustness are quantitative characteristics important for assuring cereal crop yield and quality under adverse weather conditions. A very limited number of short-culm mutant alleles were introduced into commercial crop cultivars during the Green Revolution although more than 1000 different short-culm barley mutants have been isolated and classified in different phenotypic groups according to culm length and additional pleiotropic characters. We are currently employing available short-culm barley mutants in order to identify the genes responsible for regulation of culm length. The mutant lines are from the brachytic, breviaristatum, dense spike, erectoides, semibrachytic, semidwarf, and slender dwarf mutant groups. Our toolbox also includes in-silico gene mapping, a set of near-isogenic lines and various F2-mapping populations. The genes we have identified so far encodes different subunits of heterotrimeric G-proteins as well as brassinosteroid receptors and brassinosteroid biosynthetic enzymes.

References

Braumann, I. and M. Hansson. 2013. “Barley plants with short culm”. Danish Patent Application PA 2013 70363.

Dockter, C., D. Gruszka, I. Braumann, A. Druka, I. Druka, J. Franckowiak, S. P. Gough, A. Janeczko, M. Kurowska, J. Lundqvist, U. Lundqvist, M. Marzec, I. Matyszczak, A. H. Müller, J. Oklestkova, B. Schulz, S. Zakhrabekova and M. Hansson. 2014. Induced variations in brassinosteroid genes define barley height and sturdiness, and expand the “Green Revolution” genetic toolkit. Plant Physiol. 166: 1912-1927.

Zakhrabekova, S., C. Dockter, K. Ahmann, I. Braumann, S. P. Gough, T. Wendt, U. Lundqvist, M. Mascher, N. Stein and M. Hansson. 2015. Genetic linkage facilitates cloning of Ert-m regulating plant architecture in barley and identified a strong candidate of Ant1 involved in anthocyanin biosynthesis. Plant Mol. Biol. 88: 609-626.

SESSION: 2

Story of barley domestication

Mohammad Pourkheirandish

The University of Sydney; mohammad.pourkheirandish@sydney.edu.au

Co-authors: T. Komatsuda

ABSTRACT

Barley is one of the first crops used by ancient farmers during Neolithic agriculture. The most manifest event during its domestication is the appearance of non-brittle rachis. Grain dispersal systems are designed to enable wild to survive in nature, but loss of natural dispersal was essential for agriculture. btr1 and btr2 are non-brittle rachis genes tightly linked and located on the short arm of chromosome 3H. We isolated them by means of positional cloning. Sequence analysis of wild and cultivated barley accessions revealed that non-brittle rachis was generated twice by independent mutations, first in the South (btr1) and then in the North Levant (btr2). The increase of grain number was deliberately selected during early cultivation as another domestication goal. Emergence of six-rowed spike in barley that increases grain number up to three times goes back to the early agriculture. Six-rowed spike gene, vrs1, is located on the long arm of chromosome 2H. The evolutionary study of the gene showed that it was selected four times independently. A notable feature of these three domestication genes is that all of them were created through a dynamic duplication followed by neofunctionalization. Btr1, Btr2 and Vrs1 became dispensable genes unique to Triticeae, and mutation targeted these genes in the domestication process. Wild barley (Hordeum vulgare ssp. spontaneum) always has brittle rachis, two-rowed spikes whereas cultivated barley (ssp. vulgare) non-brittle rachis and two-rowed or six-rowed spikes. “Agriocrithon” barley, exceptionally, have brittle rachis and six-rowed spikes. Here we will present a conclusive answer to the historic question where, when and how agriocrithon was generated.

SESSION: 2

Global transcriptome profiling of developing leaf and shoot apices reveals distinct genetic and environmental control of floral transition and inflorescence development in barley

Maria von Korff Schmising

University of Düsseldorf, Max Planck Institute for Plant Breeding Research;
korff@mpipz.mpg.de

Co-authors: B. Digel

ABSTRACT

The control of shoot development has a strong impact on yield in temperate cereals. However, the genetic and environmental control of developmental processes underlying differences in yield potential are not well understood. We conducted microscopic phenotyping and RNA-sequencing of the developing barley shoot apical meristem (SAM), leaf and stem growth under different photoperiods and temperatures and in introgression lines (ILs) varying at the photoperiod response gene Ppd-H1.

The daylength sensitivity of SAM development revealed two phases, floret primordia initiated under long and short days, whereas successful inflorescence development occurred only under long days. The number of floret primordia initiated until the beginning of stem elongation largely determined the number of final seeds per spike, while Ppd-H1 increased flower fertility during stem elongation. High temperature delayed floral transition and inflorescence development in a Ppd-H1 dependent manner.

The photoperiod- and Ppd-H1-dependent differences in inflorescence development and flower fertility were associated with the induction of barley FLOWERING LOCUS T orthologs: FT1 in leaves and FT2 in MSAs. FT1 expression was coregulated with transcripts involved in nutrient transport, carbohydrate metabolism, and cell cycle regulation, suggesting that FT1 might alter source-sink relationships. Successful inflorescence development correlated with upregulation of FT2 and transcripts related to floral organ development, phytohormones, and cell cycle regulation. This work provides the basis to better understand the genetic control of flowering and yield formation in response to photoperiod and ambient temperature variation in barley.

SESSION: 2

Exploring phenotypic variation for key agronomic and life history traits in a barley legacy collection

Alessandro Tondelli

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Genomics Research Centre, 29017 Fiorenzuola d'Arda, Italy; Università degli Studi di Milano – DiSAA, 20133 Milan, Italy; alessandro.tondelli@entecra.it

Co-authors: Stefano Delbono, Davide Guerra, Chiara Biselli, Nadia Faccini, Marcello Baravelli, Ivana Tagliaferri, M˝ria Megyeri, Pétér Mik½, M˝rta Moln˝r-L˝ng, Hakan Özkan, Joanne Russell, Marco Bink, Fred van Eeuwijk, Michael Alaux, Thomas Letellier, Luigi Cattivelli

ABSTRACT

The WHEALBI (Wheat and barley Legacy for Breeding Improvement) project, supported by EU funding, is taking a multidisciplinary approach to identify, understand and utilise the genetic diversity available in a barley legacy collection of cultivars, landraces and wild relatives. In this frame, a standard phenotypic approach based on "common garden" trials across Europe has been established to test the whole collection (512 accessions) for exploring its adaptative capacity. In the 2014/15 growing season, winter and spring nurseries have been sown in 4 locations (North Italy - CREA, Hungary - ATK, Scotland - JHI and Central Anatolia - Univ. Cukurova) selected to represent different environmental conditions (latitudes from 32Γ to 57Γ north, altitudes from sea level to 1250 m, from continental to arid climatic conditions), following augmented experimental designs and considering a two-rows plot as the experimental unit. The following adaptive traits have been recorded on a plot basis: growth habit, winter survival, flowering time, plant height, (thousand) grain weight, fruiting efficiency. Morphological traits such as awn length and roughness, leaf length and width and peduncle length have been also evaluated in few trials. Such information is providing the phenotypic information required to complement the genomics data generated by exome sequencing the same accessions and to help deciphering the genetic basis of barley adaptation to contrasting environments.

SESSION: 3

KEYNOTE

The barley genome

Nils Stein

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany; stein@ipk-gatersleben.de

Co-authors: Nils Stein, on behalf of The International Barley Sequencing Consortium (IBSC)

ABSTRACT

Barley is one of the most important cereal crop species. It is a close relative to wheat and rye. Its haploid genome size exceeds 5 Gigabases (Gbp), almost twice the size of any fully sequenced organism or crop species. The International Barley Sequencing Consortium (IBSC) started in 2006 a project to establish a map-based high quality reference sequence of barley. After reaching important intermediate results like (i) virtual gene order maps based on sorted chromosome survey sequencing, (ii) a whole genome shotgun draft sequence integrated to a genome-wide physical map and (iii) a draft sequence of the barley genome gene space based on pooled BAC sequencing, IBSC has come close to reach its 10 year milestone of a genome-wide BAC-by-BAC based genome sequence of barley. All seven barley chromosomes were sequenced based on short-reads sequencing-by-synthesis technology along their respective minimum tiling path of the physical map. Sequence data of more than 85,000 BAC clones was assembled and integrated with high density genetic marker information and 3D conformation capture sequencing data (HiC). As a result 4.6 Gbp of the 4.8 Gbp non-redundant sequences could be linearly ordered into seven pseudomolecules representing a first version (v1.0) reference sequence of the barley genome. A nano-channel electrophoresis based optical map of fluorescently labeled high molecular weight DNA was used to independently validate the physical integrity of the scaffolds of the pseudomolecules.

SESSION: 3

Genetic diversity in the genome era: a fine-scale map of sequence variation in the barley genome

Martin Mascher

IPK Gatersleben; mascher@ipk-gatersleben.de

ABSTRACT

Dense genome-wide marker datasets constitute the basis for research into demographic processes and patterns of natural selection among barley cultivars, landraces and wild relatives and make it possible to correlate genotypes with phenotypes in genome-wide association studies. In barley, sequence-based methods such as whole-genome resequencing, exome capture and genotyping-by-sequencing have been implemented to detect and genotype single-nucleotide polymorphisms across the genome. Moreover, nanochannel mapping has made it possible to assess large-scale structural variation between barley genotypes. Integrated analyses of these variation datasets will greatly benefit from the positional information provided by the linearly ordered, highly contiguous genome sequence assembly of cv. 'Morex' generated by the International Barley Genome Sequencing Consortium. In this talk, I will give an overview about the genotypic datasets collected in recent years using different resequencing strategies and discuss the genomic distribution of genetic diversity in context of the map-based reference sequence of barley.

SESSION: 3

Alan Schulman

ABSTRACT

SESSION: 3

Genome-wide diversity analysis focused on flowering-related loci sheds light on the history of barley domestication.

Artem Pankin

Max Planck Institute for Plant Breeding Research, Cologne; pankin@mpipz.mpg.de

Co-authors: A. Pankin, B. Kilian, M. von Korff

ABSTRACT

The central focus of evolutionary studies in crops and their wild progenitors is understanding genetic architecture of domestication syndrome and underlying processes modulating diversity in wild and domesticated forms. Recent advances in barley genomics made it feasible to investigate patterns of genetic diversity in a large-genome cereal and its wild relative on an unprecedented scale. In this study, we set out to investigate the genetic basis of barley domestication with the following questions in mind. Did domestication deplete genetic diversity and to what extent? Do patterns of genetic diversity carry signatures that distinguish demographic history from different modes of selection associated with barley domestication? Which regions of the barley genome were subject to selection during domestication? Did domestication target flowering-related loci?

To this end, we interrogated ~330,000 SNPs in a diverse set of 345 wild and 87 domesticated barley genotypes using custom designed reduced representation sequencing. The SNPs originated from ~ 12,800 loci, enriched for homologs of flowering- and domestication-related candidate genes. Diversity analysis identified unexpectedly high rate of admixture in both wild and domesticated forms, with the latter case predominantly restricted to the landraces. We found evidence of a severe domestication bottleneck, resulting in loss of genetic diversity and maintenance of extended haplotype blocks in strong linkage disequilibrium.

Selection scans using a combination of tests identified multiple targets of selection under domestication. Several tests identified a sweep around genes involved in non-brittle rachis phenotype. Detailed analysis of the sweeps and signatures of selection at individual loci narrowed down the selected regions and suggested prospective targets of domestication, which included the homologs of genes implicated in the regulation of flowering and phenology. The characteristics and map of barley genetic variation will inform future evolutionary and genome-wide association studies and support advancement of barley breeding.

SESSION: 4

KEYNOTE

Modelling the genetic architecture of plant development in barley through nested association mapping

Klaus Pillen

University of Halle; klaus.pillen@landw.uni-halle.de

ABSTRACT

Flowering time and plant development are major determinants of agronomic yield potential and yield stability. We developed the first barley nested association mapping (NAM) population, HEB-25, derived from crossing 25 wild barley donors with one elite barley recipient, and used it to dissect the genetic architecture of plant development. Upon cultivation of 1,420 HEB lines in multi-field trials and applying a genome-wide association study (GWAS), a number of major QTL were identified to control flowering time.

Extending the GWAS study to include developmental traits like shooting time, shoot elongation phase, flowering time, ripening phase, maturity, plant height and thousand grain weight resulted in locating 89 stable QTL controlling those agronomic traits. Several exotic QTL alleles caused drastic effects, potentially useful for breeding. For instance, thousand grain weight was increased by 4.5 g and flowering time was reduced by 9.3 days after substituting elite QTL alleles against exotic QTL alleles at the denso/sdw1 and the Ppd-H1 locus, respectively. We also showed that the exotic allele at the semi-dwarf locus denso/sdw1 is a potential target to increase yield since it uncouples the negative correlation between shoot elongation and ripening phase, resulting in a beneficial effect on thousand grain weight.

Our study demonstrates that nested association mapping of HEB-25 can assist to unravel the genetic regulation of plant development and yield formation in barley. Moreover, we propose that the introgression of wild barley alleles into the elite barley gene pool may assist in fine-tuning key developmental phases to maximize yield in the long run. We conclude that nested association mapping is a valuable genetic tool, allowing to dissect the genetic architecture of important agronomic traits and, simultaneous, to expand the genetic diversity of our modern elite barley gene pool.

SESSION: 4

Barley Composite Crosses: Past and Future Value in Barley Breeding and Research

Cal O. Qualset

University of California, Davis; coqualset@ucdavis.edu

Co-authors: F.R. Gabor, P.L. Morrell

ABSTRACT

Harry V. Harlan, a life-long barley researcher, advanced the concept of creating diversity for barley genetic improvement by creating diverse populations based on multiparent mating schemes in the early 1920s. His resulting populations were designated Composite Crosses (CCs). These populations were advanced for many generations at Davis, CA by Coit A. Suneson and were used by barley breeders who selected and released at least 10 varieties from the CCs. Seven CCs have been advanced for up to 57 generations, making these populations among the oldest active experimental populations in agriculture. Suneson popularized the CCs with his classic paper on evolutionary breeding in 1956 and with his introduction of male-sterile-induced recombination in CC XXI, his 'Paul Bunyan' creation. Since the CCs harbor genetic diversity, natural selection was expected to improve fitness, i.e., grain potential, over time. This has not been realized, but the populations have been shown to be valuable sources for desirable traits, such as scald resistance. R. W. Allard and his students and collaborators used the populations extensively for documenting genetic diversity via isozyme analyses. The positive gene conservation role of the CCs was demonstrated as well as changes in gene frequency as the populations were advanced. Breeders can expect to find useful genes in the CCs now. Future DNA research can investigate population structure and trait associations with the CCs. This report will examine past results and lessoned learned that are relevant to construction of genetically diverse and useful multiparent experimental populations. The future of the Harlan/Suneson CCs will be discussed with respect to generation advancement and secure curation.

SESSION: 4

The molecular basis of self-incompatibility in Hordeum bulbosum

Katsuyuki Kakeda

Mie University, Graduate School of Bioresources, Japan; kakeda@bio.mie-u.ac.jp

Co-authors: K. Kakeda, and R. Asahara

ABSTRACT

Self-incompatibility (SI) in the grasses (Poaceae) is gametophytically controlled by a distinct two-locus genetic system. In the genus Hordeum, two SI species of H. bulbosum and H. brachyantherum are known. The genetic control by the multiallelic loci S and Z, which are located on chromosomes 1(1Hb) and 2(2Hb), respectively, has been confirmed in the SI of H. bulbosum. This wild species is most closely related and cross-compatible to cultivated barley (H. vulgare). Thus, if the SI system could be transferred into barley, it would be of great value in utilizing for F1 hybrid breeding. In our previous studies using diploid H. bulbosum to elucidate molecular mechanisms of the SI system, we have identified a promising candidate of the female S gene, named HPS10 (Hordeum pistil S-specific 10). This gene fulfills the requirements for the female S, namely, complete linkage to the S locus, stigma-specific expression and a high degree of allelic sequence polymorphisms. The gene product was predicted to be a small hydrophilic protein of unknown function. Here we show the results of in vitro pollen bioassay to demonstrate that HPS10 is the female S determinant. We also report our current approaches involving comparative genomics and transcriptome analysis to further search for candidate genes encoding the remaining SI determinants in H. bulbosum.

SESSION: 4

A pipeline for rapid development of mapping populations relevant to breeding

Laura Ziems

The University of Queensland, Queensland Alliance for Agriculture and Food Innovation; l.ziems@uq.edu.au

Co-authors: L.A. Ziems, H. Robinson, C.A.I. Richard, J.D. Franckowiak, G.J. Platz, R. Williams, R.F. Park, D. Singh, L.T. Hickey

ABSTRACT

Generating populations for mapping quantitative trait loci (QTL) in pre-breeding programs is typically a lengthy process, requiring five to seven plant generations to make a cross and develop recombinant inbred lines. The resulting populations often lack relevance to elite breeding material and segregation for extreme plant height and maturity makes it difficult to precisely phenotype complex traits, such as drought adaptation or quantitative disease resistance. Here, we outline a novel pipeline for development of mapping populations that addresses these limitations. We applied this strategy to generate the first multi-parent reference nested-association mapping (NAM) population, suitable for dissecting complex agronomic traits in barley. 'Speed breeding', the rapid generation advance (RGA) technique developed at The University of Queensland, was used to generate 1,375 F4-derived NAM lines within only 18 months. Twenty-one elite breeding lines diverse for a range of abiotic and biotic attributes were selected as founders and nested within three common parents: Commander, Compass and La Trobe, which are cultivars preferred by industry. An incomplete factorial crossing design was adopted, producing a total of 32 families. All plant generations were grown in the speed breeding system, with the exception of the F2 generation, which was grown in the field and subject to selection for plant height and maturity similar to the respective common parent. The population was genotyped using the Diversity Array Technology genotype-by-sequencing platform, generating over 30,000 polymorphic markers. We performed NAM for plant height and maturity, plus investigated the effects of selection during line development. Both known and novel genes for plant development were detected, highlighting the power of this genetic resource to dissect complex traits. Our novel strategy for NAM population development incorporating both RGA and field-based selection has established a powerful pre-breeding platform that permits examination of new alleles within a relevant breeding context.

SESSION: 5

KEYNOTE

Genetic characterization of salinity tolerance traits to increase salinity tolerance of crops

Mark Tester

King Abdullah University of Science & Technology; mark.tester@kaust.edu.sa

ABSTRACT

Forty percent of the world's food is produced under irrigation, and this is directly threatened by over-exploitation and changes in the global environment. In this talk, the focus will be on the use of forward genetic approaches for discovery of genes related to salinity tolerance in barley. Rather than studying salinity tolerance as a trait in itself, we dissect salinity tolerance into a series of components that are hypothesised to contribute to overall salinity tolerance. Two consecutive years of field trials were conducted at the International Center for Biosaline Agriculture, a site with sandy soil and very low precipitation. Drip irrigation systems allowed the control of salinity by supplying plots with low (1 dS/m) and high salinity water (17 dS/m). A barley Nested Association Mapping (NAM) population developed by Klaus Pillen has been used to dissect physiologically and genetically complex traits in response to salt stress. Ten traits related to yield and yield components (e.g. days to flowering, harvest index, 100 seed mass) were recorded and five stress-indices were derived from each of these measurements. We have identified two significant loci located on the long arms of chromosomes 1H and 5H, which are both associated with several traits contributing to salinity tolerance, namely days to flowering, days to maturity, harvest index and yield. The application of this approach provides opportunities to significantly increase abiotic stress tolerance of crops, and thus contribute to increasing agricultural production in many regions.

SESSION: 5

Genetic resources for climate change: wild barley and genes for tolerance to waterlogging

Anna Westerbergh

Department of Plant Biology, Uppsala BioCenter, Linnean Centre for Plant Biology in Uppsala, Swedish University of Agricultural Sciences, Uppsala, Sweden; Anna.Westerbergh@slu.se

Co-authors: M. Sameri, E. Lerceteau-Köhler, and G. Bedada

ABSTRACT

Wild relatives of crops are adapted to a wide range of habitats and stresses. Much of the phenotypic and genetic diversity of the wild populations was lost as a result of human and natural selection during domestication. Hordeum spontaneum, as the ancestor of barley, is therefore a potential source of genes and alleles for improvement of tolerance to stresses in barley. Waterlogging (WL) is one abiotic stress which is projected to increase in agricultural fields in some regions due to global climate change. We have screened H. spontaneum accessions from areas with high precipitation as well as a set of diverse cultivars and have identified WL tolerant genotypes. Based on the large variation found among these accessions we distinguished suitable parents for generating QTL mapping populations. In a doubled-haploid (DH) mapping population derived from a cross between a susceptible cultivar and a tolerant H. spontaneum accession, we identified several QTL with large effect and favorable H. spontaneum alleles for waterlogging tolerance. Some QTL were verified using near-isogenic lines with H. spontaneum segments in a barley background. For comparison, another mapping population based on two cultivars with variation in WL tolerance is evaluated. Transcriptome sequencing (RNA-seq) of highly WL tolerant and susceptible parents, and selected DH lines is carried out. The combined use of screening, mapping and gene expression studies will identify genes, genomic variation and genetic resources important for improvement of WL tolerance in barley. Robust cultivars adapted to changes in climate will provide the farmers with high yield stability, increased global food security and a sustainable agriculture.

SESSION: 5

Genetic solution for phosphorus use efficiency (PUE) in barley through candidate genes

Xue Gong

The University of Adelaide; xue.gong@adelaide.edu.au

Co-authors: Glenn McDonald, Willie Shoobridge, Diane Mather, Rob Wheeler

ABSTRACT

Improving phosphorus (P) use efficiency (PUE) is an important breeding goal for barley, but barley breeding for high PUE varieties has been slow due to a lack of knowledge in molecular mechanism of PUE and understanding in yield responses to P. To tackle these issues, we used two methods: (1) PUE = yield at no-added P relative to yield at added P and (2) P responsiveness = difference in yield between added P and no-added P. PUE indicates a variety's ability to use native soil P and P responsiveness assesses a variety's yield response to P independently of its yield potential. Phenotyping of the two traits using a Commander/Fleet population was conducted in five field environments. Variation of both traits was observed, but a low correlation was found. PUE varied from 31% to 124% and lines showed a higher than average response to added P also displayed the highest yield in the population. Again, unique QTLs for PUE were mapped with grain yield (GY) or P uptake at no added P, but unique QTLs for P responsiveness were co-localized with GY or P uptake at added P. A shared QTL was also identified for PUE and P responsiveness, but the phenotypic effect was contributed by different parental alleles. These results demonstrated improving PUE, P responsiveness and P uptake is improving GY. Thus, our work provided experimental evidence to cultivate barley varieties that can not only use native soil P efficiently when P is limited, but also response to P when P is available. Assisted with information of whole genome shotgun sequences of Commander and Fleet, candidate genes underlying the QTLs for PUE and P responsiveness are revealed. Molecular markers derived from candidate genes would decrease the time for farmers to realize on-farm benefits from improved varieties.

SESSION: 5

Influence of temperature on recombination in barley

Mikel Arrieta

James Hutton Institute; mikel.arrieta@hutton.ac.uk

Co-authors: I. Colas, R. Waugh, and L. Ramsay

ABSTRACT

Breeding work relies fundamentally on recombination but the control of this process is not fully understood especially in crop plants. In barley the distribution of meiotic crossover events is highly skewed meaning that, substantial proportions of the chromosomes are inherited together as large linkage blocks, preventing the generation of novel gene combinations and useful variation that could be exploited in breeding programmes. An ability to modify the pattern of recombination in these species could therefore have profound impact on the breeding of the crops.

Historical and more recent work has shown that abiotic stress such as high temperature can affect the distribution of chiasmata and thus could provide the means to manipulate recombination within a breeding programme.

As part of a recently funded Marie Curie ITN entitled Control of Meiotic Recombination: Arabidopsis to Crops (COMREC) co-ordinated by Prof FCH Franklin (University of Birmingham) the effect of temperature on recombination in barley is being undertaken at the James Hutton Institute, (Dundee, Scotland).

The initial experimentation has concentrated on cytogenetics of affected meiocytes and the genetic mapping of progeny from F1 plants that have been subjected to a sustained period of heat stress. This work has confirmed the reported effect of temperature on the distribution of crossovers and recombination in barley (Higgins et al 2012, Phillips et al 2015); showing increases in recombination in peri-centromeric regions in the genetic maps derived from F2 progeny of heat stressed F1 plants compared to those derived from progeny from control F1plantsf.

Research is now focused on performing experiments using SSD trays as a higher throughput working-platform, that allow shorter heat shock treatments to be applied, allowing the dissection of the critical effect on crossovers and recombination.

Results of these and other experiments will be presented.

SESSION: 6

KEYNOTE

Gene-based approaches to durable disease resistance in *Triticeae* cereals

Patrick Schweizer

IPK Gatersleben; schweiz@ipk-gatersleben.de

ABSTRACT

Broad-spectrum, quantiative pathogen resistance is of high importance to plant breeders due to its durability. However, it is usually controlled by multiple quantitative trait loci and therefore, challenging to handle in breeding practice. Knowing about the underlying genes would allow its more targeted utilization by allele introgressions. With the available omics tools and data of barley and one of its major fungal pathogens, the powdery mildew fungus *Blumeria graminis* f.sp. *hordei*, at hand we are now enabled to functionally address genes for defense and attack on both sides of this plant-pathogen interaction at a genome-wide scale. To identify genes that mediate race-nonspecific resistance of barley to *B. graminis* we combined a functional-genomics approach based on genomewide transcript profiling and transient-induced gene silencing (TIGS, 1400 genes) with a genetic approach consisting of association- and Meta-QTL mapping plus analysis of copy-number variation. This guided us to a shortlist of approximately 50 candidates with converging evidence for an important role in race-nonspecific resistance of barley. We have started marker-assisted introgression of potentially valuable alleles of some of these candidate genes in barley, followed by assessment of multiple pathogen resistance. On the pathogen side, host-induced gene silencing (HIGS) can inform us about effectors and other molecular weapons that are critical for successful host invasion.

SESSION: 6

Proteomic Comparison of Flag Leaves from Barley Germplasm Varying at a Major Senescence QTL Identifies Numerous Proteins with Functions in Pathogen Defense

Andreas Fischer

Montana State University; fischer@montana.edu

Co-authors: Katelyn E. Mason, Jonathan K. Hilmer, Walid S. Maaty, Benjamin D. Reeves, Paul A. Grieco, Brian Bothner, and Andreas M. Fischer (presenting author)

ABSTRACT

Recent research has established the importance of leaf and whole-plant senescence for barley yield and quality, pointing to the interdependence of senescence timing, nutrient use efficiency, grain nutrient content and yield. It has been suggested that senescing tissues are particularly vulnerable to plant pathogens, further complicating breeding towards an ideal combination of senescence traits. In this context, we have performed a proteomic comparison of flag leaves of late-senescing barley variety 'Karl' and a near-isogenic early-senescing line, '10_11', differing in the allelic state of a chromosome six locus encompassing the HvNAM-1 gene. Protein samples at 14 and 21 days past anthesis were analyzed using both two-dimensional gel-based and label-free quantitative mass spectrometry-based ('shotgun') proteomic techniques. This approach identified >9,000 barley proteins, and one-third of them were quantified. Analysis focused on proteins that were significantly ($p \leq 0.05$; difference ≥ 1.5-fold) upregulated in early-senescing line '10_11' as compared to 'Karl', as these may be functionally important for senescence. Proteins in this group included several membrane and intracellular receptors, glucanases, enzymes with possible roles in cuticle modification, classical pathogenesis-related proteins, membrane transporters and proteins involved in DNA repair. From the global analysis, a common theme of plant pathogen defense arose. Additionally, several proteases and elements of the ubiquitin-proteasome system were upregulated in line '10_11'; these proteins may be involved in nitrogen remobilization and in the regulation of both senescence and plant defense reactions. Together, our data shed new light, at the protein level, on the importance of plant defense reactions during senescence, on senescence regulation, and possibly on crosstalk between senescence regulation and plant-pathogen interactions.

SESSION: 6

The barley 'nibblerome': defining the set of NB-LRR-type R-genes from a diverse collection of barley

William Jackson

The Sainsbury Laboratory, Norwich, UK; william.jackson@tsl.ac.uk

Co-authors: Inmaculada Hernández-Pinzón, Matthew Moscou

ABSTRACT

Plant pathogens are a major impediment to sustainable food production, particularly in light of an increasing human population. One of the major forms of protection against plant pathogens comes from the plant itself – in the form of resistance genes (R genes) – which defend the plant in a pathogen specific manner, typified by the hypersensitive response (HR). The majority of R genes in plants encode NB-LRRs, which are cytosolic proteins with a nucleotide-binding (NB) domain and several leucine-rich repeat (LRR) domains. NB-LRR encoding genes exist in complex loci, meaning that correct assembly and annotation from next-generation sequencing data is typically an extremely difficult task. Using the Morex whole genome shotgun reference assembly as a starting point, we found that approximately 420 NB-LRRs are present in the annotated Morex genome. The ~420 NB-LRRs are unequally distributed across the genome, with particular enrichment on chromosome arms: 1HS, 2HS, 6HL, and 7HS. Here we describe a substantial extension of the number of annotated NB-LRRs, which was achieved using leaf transcript-based approaches utilising a collection of 38 elite, landrace, and wild barley accessions to assess allelic diversity. We also describe the physical anchoring of many of these R gene candidates to chromosomes and POPSEQ ordered contig assemblies. High expression was observed for many NB-LRR encoding R genes, including Mla and Rpg5. In parallel, we have developed an exome capture method (RenSeq) specifically targeting NB-LRRs from barley. The annotated NB-LRRome will be a critical resource in the rapid identification of disease resistance genes.

SESSION: 6

Modulation of integrated decoy R-genes/transcription factor assembly elicits wheat stem rust resistance responses in barley: rpg4/Rpg5-mediated Ug99 resistance

Shyam Solanki

Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA; shyam.solanki@ndsu.edu

Co-authors: Shyam Solanki, Jonathan Richards, Gazala Ameen and Robert Brueggeman

ABSTRACT

The rpg4/Rpg5 locus, harboring three tightly linked genes, Rpg5, HvRga1, and HvAdf3 from barley line Q21861, have been genetically and functionally characterized and shown to provide resistance against many races of the wheat stem rust pathogen Puccinia graminis f. sp. tritici (Pgt) including race TTKSK (Ug99). Predicted RPG5 and HvRGA1 proteins are NLRs and RPG5 has an additional C-terminal kinase domain. Initial qPCR of the three genes and microscopy data suggests that rpg4/Rpg5-mediated resistance engage in an early pathogen recognition event manifested as HvAdf3 down regulation and later invoke a strong ETI response 48 hours post inoculation (HPI). A yeast-two-hybrid prey library developed from RNA of barley line Q21861 48 HPI was screened utilizing the RPG5 kinase domain as bait. The, putative barley one-zinc-finger transcription factor, HvVOZ1, was identified. This is the first ever report of interaction between an R-protein kinase domain and putative transcription factor. To validate the role of HvVOZ1 we are using VIGS to determine if the silencing of HvVOZ1 compromises Pgt resistance. RPG5, HvRGA1, and HvVOZ1 proteins with fluorescent tail are being developed to determine protein localization and interactions in-planta. We hypothesize that the RPG5-PK domain acts as an integrated decoy that HvVOZ1 binds to possibly negatively regulate defense activation or binds after defense activation as part of the signaling complex. As is hypothesized in the integrated decoy systems, the second NLR protein HvRGA1 may be guarding this VOZ1-RPG5 interaction or guarding the RPG5-STPK domain from Pgt rpg4/Rpg5-Avr effector manipulation and detection triggers defense responses. To identify other genes involved in the resistance pathway we are characterizing two independent Q21861 fast neutron induced mutants FN617 and FN618, susceptible to Pgt race TTKSK using Genotype-by-Sequencing of RIL populations and RNA-seq. The characterization of rpg4/Rpg5-mediated resistance will establish this pathosystem as a model to understand cereal host-pathogen interactions.

SESSION: 8

KEYNOTE

Current breeding methodologies and future prospects

William Thomas

The James Hutton Institute; Bill.Thomas@hutton.ac.uk

Co-authors: , Luke Ramsay, Joanne Russell, Isabelle Colas, Robbie Waugh, Hazel Bull, Kelly Houston, Mark Looseley

ABSTRACT

Key issues in barley breeding are cycle time and recombination. For example, the current spring barley breeding cycle within a European company is approximately 3 years as Concerto was first recommended in the UK in 2009, followed by a selection from a Concerto cross (Odyssey) in 2012, and then a selection from an Odyssey cross (Octavia) in 2015. Working with other breeders' germplasm extends the cycle to 5 to 6 years as Quench, a parent of Concerto but bred by a different company, was first recommended in 2007. This rapid cycling is generally being achieved without the use of doubled haploids as it is more likely to lengthen a commercial breeder's cycle in the spring crop rather than shorten it. Doubled haploids are used more routinely in the winter crop where the breeding cycle within a company is approximately 5 years with Saffron, recommended in 2005, being a parent of KWS Cassia (2010), which in turn was a parent of KWS Infinity (2015). We will consider how markers have impacted barley breeding programmes to facilitate such rapid breeding cycles and how the cycle could be further shortened. Genomic selection (GS) could be an option to shorten the cycle by removing phenotyping between crossing cycles. GS has therefore been the subject of much research over the past 5 years but there is little information about how successful it really is when omitting a phenotyping step. We believe that the real barrier to major improvement in the barley crop is the lack of recombination in centromeric regions and that the unlocking of novel centromeric haplotypes could lead to major future improvements.

SESSION: 8

Study of barley maturity with new breeding tools

Anne-Marie BOCHARD

Limagrain Europe; anne-marie.bochard@limagrain.com

Co-authors: Doriane Hamernig, Stéphanie Chauvet, Sophie Capon, Jérémy Derory

ABSTRACT

Barley breeding is challenging, due to the diversity of morphology, phenology and end use of this species: six-row v two-row, spring v winter, malting v feed barley types. In a competitive environment, breeders are always on the look-out for new approaches to improve their breeding programs. They have to combine high-throughput phenotyping data, environment characterization, understanding of gene architecture and prediction of genomic values.
In the field, new traits, such as reflectance or canopy temperature, are measured on a broad scale thanks to drone-based technologies and environments can be described and clustered with abiotic data (temperature, rainfall…). In the lab, recent advances in genome sequencing make gene identification much easier while for complex traits, Genome Wide Association Study (GWAS) and Genomic Selection (GS) have become standard breeding methods.

To illustrate the implementation of new tools in breeding, a study of barley grain maturity (flowering to ripening stages) is presented. In a context of climate variability, breeders have to select varieties adapted to specific environments and a better understanding of these environments and the required maturity are key to this adaptation.

While flowering time is intensely studied - the role of photoperiod response loci PPD-H1 and PPD-H2, and vernalization response loci VRN-H1 and VRN-H2 in flowering time have been recently reviewed by Bentley et al (2013) – maturity is less well-understood. Maturity is a generic term covering grain filling period, physiological maturity, and ripening. Each stage duration influences grain composition, yield, yield stability and adaptation to environmental stresses. These stages can be described with the Zadoks scale (Zadoks et al, 1974) but their duration cannot be easily evaluated on a breeding scale, with thousands of plots.
Visible and near-infrared canopy reflectances (Araus et al, 2001), measured with drone-based technologies, were used to calculate Normalized Difference Vegetation Index (NDVI). These measurements, taken during grain filling and ripening periods, are associated with specific plant characteristics (chlorophyll content, pigments…) and allowed to detect the starting point and duration of senescence, key elements to describe maturity.

Using the method described by Bouffier et al (2015), breeding environments were clustered according to their environmental scenarios i.e. occurrence of environmental limiting factors during key developmental phases.

Once genotypes have been characterized for maturity traits and environments clustered by similarities, GS (Meuwissen et al. 2001) and GWAS (Kang et al, 2010) were performed using medium density SNP array to detect loci involved in maturity and to predict breeding values.
Methods and results are presented and the main advantages and pitfalls of these new tools are discussed.

Araus, J.L., J. Casadesus, and J. Bort. (2001). Recent tools for the screening of physiological traits determining yield. p. 59–77. In M.P. Reynolds et al. (ed.) Application of physiology in wheat breeding. CIMMYT, Mexico, DF.

Bentley AR, Jensen EF, Mackay IJ, Honicka H, Fladung M, Hori K, Yano M, Mullet JE, Armstead IP, Hayes C, Thorogood D, Lovatt A, Morris R, Pullen N, Mutasa-Go_ttgens E, Cockram J (2013) Flowering time. In: Cole C (ed) Genomics and breeding for climate-resilient crops, vol 2. Springer, Berlin, pp 1–67

Bouffier, B., Derory, J., Murigneux, A., Reynolds, M., & Le Gouis, J. (2015). Clustering of Environmental Parameters Discriminates Drought and Heat Stress Bread Wheat Trials. Agronomy Journal, 107(4), 1489-1503.

Kang, H. M., Sul, J. H., Service, S. K., Zaitlen, N. A., Kong, S. Y., Freimer, N. B., ... & Eskin, E. (2010). Variance component model to account for sample structure in genome-wide association studies. Nature genetics, 42(4), 348-354.

Meuwissen TH, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829

Zadoks, J.C., T.T. Chang, and C.F. Konzak. (1974). A decimal code for the growth stages of cereals. Weed Res. 14(6):415–421. doi:10.1111/j.1365-3180.1974.tb01084.x

SESSION: 8

Genome engineering in barley using customizable endonucleases

Jochen Kumlehn

IPK Gatersleben; kumlehn@ipk-gatersleben.de

Co-authors: J. Kumlehn, S. Hiekel, N. Budhagatapalli, H. Trautwein, K. Zelkowska, M. Gurushidze, and G. Hensel

ABSTRACT

Genome engineering is a breakthrough technology that facilitates site-directed modifications of genomic DNA in planta. It offers versatile novel possibilities for both the experimental elucidation of gene functions and the improvement of crop performance. Aiming to establish site-directed mutagenesis in Triticeae cereals, target gene-specific transcription activator-like effector nucleases (TALENs) were designed and barley mutant plants generated via transfer of a pair of TALEN-coding expression units into haploid cells from which transgenic doubled haploid (DH) plants can be produced. Owing to the haploid founder cells and whole genome duplication taking place in the course of the regeneration process, some of the resultant primary mutants proved instantly homozygous, as was indicated by non-segregating progeny. In a second approach, we produced two types of plant lines each carrying only one of the two required TALEN-units. TALEN cleavage activity was then induced in hybrid progeny derived from crossings of complementary pairs of these lines. By this principle, many independent and heritable mutations can be produced even if only a few TALEN-transgenic lines are available. Whereas primary mutants are typically chimeric, mutant dissolution and genetic fixation was readily achieved by generating doubled haploids. To allow for optimization of nuclease construct design and routine prevalidation of target-specific constructs before embarking on the laborious stable barley transformations, a transient expression test was established which indicates cleavage activity via frame shift reconstitution of a reporter gene. In addition, we exemplified homology-dependent genome editing in barley cells using a customized DNA-repair template, which allows to precisely predefine not only the genomic target locus but also the resultant DNA sequence. More recently, we have also used RNA-guided endonucleases (RGENs), which derive from the bacterial CRISPR/CAS immune system, to generate site-directed mutations in barley plants.

SESSION: 8

MARKER-BASED BREEDING TOOLS INFORM EARLY GENERATION SELEECTION AND CROSSING BLOCK DECISIONS

Tyler Tiede

University of Minnesota; tied0068@umn.edu

Co-authors: A.H. Sallam, A.J. Lorenz, and K.P. Smith

ABSTRACT

Genomic selection (GS) is becoming a routinely used tool in plant breeding. The University of Minnesota barley breeding program has been using and evaluating GS since 2009. We have found that predictions, based on genome-wide markers, can be accurate enough to warrant replacing phenotypic selection at some stages of the breeding program, particularly for traits that are difficult or expensive to measure. For example, we have found that genomic selection for Fusarium head blight resistance and grain yield is equivalent to phenotypic selection, but substantially less expensive and can be implemented earlier in the breeding cycle. In addition, prediction accuracy can be improved through proper optimization and maintenance of the training dataset. In addition to using GS to select lines for advancement and crossing, we have recently evaluated the use of genome-wide markers to select parent combinations to maximize genetic variance (__G^2) in bi-parental breeding populations. Previously, metrics such as the phenotypic, genetic, and kinship-based (estimated from genomewide markers) distances between parents have been tested for their ability to predict __G^2. In general there is little to no correlation between these metrics and __G^2. One explanation for this is the inability of such methods to explicitly model the segregation of associated genetic loci (i.e. QTL). This concern has been addressed by three recently proposed methods that, at varying levels, predict __G^2 by explicitly modeling the segregation of QTL. We evaluated the accuracies of all six __G^2 prediction models using field-based estimates of __G^2 from 40 bi-parental barley breeding populations and found that methods that explicitly model the segregation of QTL were superior. Specifically, a method (PopVar) that uses the genomic estimated breeding values of simulated bi-parental populations most accurately predicted __G^2.

SESSION: 9

KEYNOTE

Chris Swersey

Brewers Association; chris@brewersassociation.org

ABSTRACT

SESSION: 9

Genome editing to validate genes putatively contributing to grain (1,3;1,4)-_-glucan content.

Kelly Houston

The James Hutton Institute; kelly.houston@hutton.ac.uk

Co-authors: Abdellah Barakate a, Matt Tucker b, Miriam Schreiber c, Edwin R. Lampugnani d, Monika Doblin d, Rachel A Burton b, Geoffrey B. Fincher b, Robbie Waugh a

ABSTRACT

a The James Hutton Institute, Dundee, Scotland, UK.
b ARC Centre of Excellence in Plant Cell Walls, School of Agriculture, Food and Wine, University of Adelaide, South Australia 5064.
c University of Dundee at The James Hutton Institute, Dundee, Scotland, UK.
d ARC Centre of Excellence in Plant Cell Walls, Plant Cell Biology Research Centre, School of Botany, The University of Melbourne, Parkville VIC 3010.

The cell wall polysaccharide (1,3;1,4)-_-glucan in cereal grain has been shown to have human health benefits such as reducing the risk of coronary heart disease1. However, high levels of (1,3;1,4)-_-glucan are an undesirable component of grain destined for the brewing and malting industries because it clogs filters and causes haze in beer. While members of the cellulose synthase-like CslF gene family have already been implicated in the synthesis of (1,3;1,4)-_-glucan, such as HvCslF6 and HvCslH2,3 , we are interested in validating candidates which contribute to (1,3;1,4)-_-glucan content in the mature grain identified in a GWAS study of this trait4.
The genome editing technique CRISPR-CAS9 has become increasingly popular in the last couple of years. We generated knockout lines using CRISPR-CAS9 for HvCslF6, and several candidates from a recent GWAS on mature grain (1,3;1,4)-_-glucan content4 . We will characterise these knockout lines for various components of their cell wall biology, including (1,3;1,4)-_-glucan and starch content, in a range of tissues and developmental stages. I will describe our overall strategy and the progress we have made towards clarifying the contribution of these genes towards (1,3;1,4)-_-glucan content in the barley grain.

1. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2005/ucm108543.htm

2. Burton RA et al., (2006). Science 311: 1940-2.

3. Doblin et al., (2009). PNAS 106: 5996–6001.

4. Houston et al., (2014). BMC Genomics 15:907.

SESSION: 9

Identification of two key genes controlling chill haze stability of beer in barley

Lingzhen Ye

Institute of Crop Science, Zhejiang University, China; mylz1987@163.com

Co-authors: Yuqing Huang, Fei Dai, Huajiang Ning, Chengdao Li, Meixue Zhou, Guoping Zhang

ABSTRACT

In bright beer, haze formation is a serious quality problem, degrading beer quality and reducing its shelf life. The quality of barley (Hordeum vulgare L.) malt, as the main raw material for beer brewing, largely affects the colloidal stability of beer. In this study, the genetic mechanism of the factors affecting beer haze stability in barley was studied. Quantitative trait loci (QTL) analysis of alcohol chill haze (ACH) in beer was carried out using a Franklin/Yerong double haploid (DH) population. One QTL, named as qACH, was detected for ACH, and it was located on the position of about 108cM in chromosome 4H and can explain about 20% of the phenotypic variation. Two key haze active proteins, BATI-CMb and BATI-CMd were identified by proteomics analysis. Bioinformatics analysis showed that BATI-CMb and BATI-CMd had the same position as qACH in the chromosome. It may be deduced that BATI-CMb and BATI-CMd are candidate genes for qACH, controlling colloidal stability of beer. Polymorphism comparison between Yerong and Franklin in the nucleotide and amino acid sequence of BATI-CMb and BATI-CMd detected the corresponding gene specific markers, which could be used in marker-assisted selection for malt barley breeding.

SESSION: 9

Barley contributions to beer flavor I: Impact assessment of variety, location, and genotype x environment interaction on beer flavor

Dustin Herb

Oregon State University; Dustin.Herb@oregonstate.edu

Co-authors: R.C. Graebner, S.P. Fisk, T.P. Nielsen, D. Carey, T. Shellhammer, P.M. Hayes

ABSTRACT

Barley grain contains significant amounts of positive flavor compounds; however the impact of variety and environment on flavor is not well understood. In this study we evaluated the beer flavor potential of three barley varieties (Full Pint; AC Metcalfe; Klages) grown in replication in three environments (Corvallis, OR, USA; St. Paul, MN, USA; Saskatoon, SK, CA). The resulting grain was micromalted by Rahr Malting. Sierra Nevada Brewing Co. and New Glarus Brewing Co. both conducted congress wort sensory assessments. The controls were AC Metcalfe high and low color malts. Gas chromatography and mass spectrometry (CG-MS) at Sierra Nevada revealed that the principle volatile compounds present across samples were dimethylsulfide (759164.8 m/z), 3-methyl butanal (72583.2 m/z), 2-methyl butanal (144753.2 m/z), hexanol (129991.8 m/z), phenylacetaldehyde (393667.1 m/z), ocimene quintoxide (757448.1 m/z), and methyl mercaptan (835.5 m/z) and contributed flavors such as fruity, floral, cooked corn, nutty, potato chips, biscuit, malty, and cereal. There were off-odors as well. Concentrations of dimethylsulfide and trans-2-hexanal were highest in Full Pint, while 2- and 3-methyl butanal and methyl mercaptan was highest in AC Metcalfe, and hexanal in Klages. Principle component analysis (PCA) indicated that variety and environment account for 44% and 21% respectively of the variation in mass-spec abundance analyte concentrations across samples. In a blind sensory assessment, panelists distinguished unique flavors and preferences across the difference varieties and environments, suggesting that barley variety and production environment play a significant role in beer flavor.

SESSION: 10

KEYNOTE

Molecular Genetic Characterization of Key Genes for Utilization of Barley as Human Food and Animal Feed

Shin Taketa

Institute of Plant Science and Resources, Group of Genetic Resources and Functions, Okayama University, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan.; staketa@rib.okayama-u.ac.jp

Co-authors: T. Tonooka2, E. Himi1, and T. Kato3

ABSTRACT

Molecular identification and characterization of key genes that would be suited for barley consumption as human health food are our current major research aims. We briefly summarize the achievements of our Japanese barley genetics and breeding group. Emphases are placed on genes for seed characteristics, including (1) hulled or naked caryopsis (Nud), (2) phenol reaction, syn., polyphenol oxidases (PPOs), (3) (1,3;1,4)-beta-D-glucan synthesis (HvCslF6) and (4) proanthocyanidin less (ant mutants). These genes appear to elevate the values of food barley, in terms of better edibility and functional component properties. Promising morphological genes that may enhance animal preference of whole crop barley will also be briefly presented, with a stress on genes that shorten and soften the stiff and harmful awns (lks2).

SESSION: 10

Effects of agronomic practices and soil and climatic zones on the content and molecular structure of dietary fibre constituents in food barley genotypes

Marta Izydorczyk

Canadian Grain Commission; marta.izydorczyk@grainscanada.gc.ca

Co-authors: T. McMillan, S. Bazin, J. O'Donovan

ABSTRACT

The recently developed food barley genotypes are excellent sources of soluble and insoluble dietary fibres, primarily arabinoxylans and (1_3)(1_4)-_-D-glucans . Barley _-glucans are important against two of the most prevalent diseases in industrialized countries, i.e., cardiovascular disease and diabetes. The health benefits of arabinoxylans are less known, but studies showed their positive effects on cecal fermentation, production of short-chain fatty acids, and reduction of serum cholesterol. The health benefits of barley _-glucans are dose-dependent. In addition, the physiological benefits of _-glucans are associated also with their viscosity building properties linked to the high molecular weight of these polymers. While it is known that food barley genotypes contain relatively high amount of _-glucans, little is known about the effects of environment and agronomic practices on the content and molecular properties of fibre constituents. The objectives of this project were to determine how certain agronomic practices (nitrogen fertilization and seeding rates) and environmental factors affect the level and properties of dietary fibre constituents in two food barley varieties, CDC Rattan and CDC Hilose. Field experiments were conducted for three years at five locations in western Canada. The effects of nitrogen rate (60 and 120 kg/ha), and seeding rate (200, 300 and 400 seeds/m2) were determined. Generally, the increasing nitrogen rates significantly increased the content of protein, but decreased the content of starch in the grain. Increasing rates of nitrogen fertilization had small but statistically significant effect on the content of arabinoxylans and insoluble dietary fibre, but no effect on the content of _-glucans and soluble dietary. The increasing seeding rates significantly decreased the content of _-glucans and soluble fibre, but increased the content of arabinoxylans and insoluble dietary fibre. The results indicated significant effects of genotypes, environments, and seeding rates on the composition, molecular structure and weight of barley dietary fibre constituents.

SESSION: 10

Analysis of miRNA-regulated transcription factors in barley

Marcel Lafos

University of Dundee / Plant Sciences Department; Marcel.Lafos@hutton.ac.uk

Co-authors: S. McKim

ABSTRACT

Plant breeders face the challenge of delivering larger crop yields within the context of climate change. A major route to sustainable increases in productivity is by modifying plant architecture to suit specific environmental conditions. To support this effort, we must understand how developmental pathways and gene expression networks interact to determine important agronomic traits such as seed quality and number. Gene expression networks are driven through the activity of multiple transcription factor (TF) families while the actual network read-out is balanced through a combination of antagonism between different transcription factors, environmental and feedback regulation, miRNA-targeting, hormonal crosstalk and higher-order regulatory mechanisms such as chromatin remodelling and epigenetics.
In cereal crops such as barley, developmental phenotypes important to breeding include plant height and grain density, which are determined by internode elongation in the stem and inflorescence, respectively. A miRNA-regulated APETALA2-like (AP2-like) transcription factor, HvAP2, has been shown to regulate internode length in barley (Houston K. and McKim S. et al., 2013). We are using the miRNA-resistant HvAP2 barley semi-dwarf mutant, Zeo1.b, as a tool to analyse the networks controlling internode elongation by identifying targets of HvAP2 regulation and defining interactions between HvAP2 and hormonal signalling pathways. In other plant systems, AP2-like TFs participate in an antagonistic network with another set of miRNA-regulated transcription factors, the SQUAMOSA PROMOTER BINDING-LIKE (SPL) proteins, to control phase-specific traits and developmental timing. We are using a variety of approaches, including TF-ChIP-seq and the expression of miRNA-resistant transcripts, to identify and manipulate components of the barley AP2-SPL network and to investigate their role in internode growth and other agronomic traits.

SESSION: 10

The INUDFOOD Report

Brigid Meints

Washington State University; brigid.meints@wsu.edu

Co-authors: P. Batlle, L. Cistué, S. Fisk, L. Helgerson, K. Murphy, I. Romagosa, W. Thomas, and P. M. Hayes

ABSTRACT

The INUDFOOD (International hull-less food barley) trial represents the start of an international collaboration directed at the rapid development of diverse food barley germplasm resources. It arose from our goal to increase agronomic performance and provide growers and consumers with a range of grain colors, flavors, textures, and processing attributes. The end-goal of this trial is to release varieties that are best adapted to the regions where the trial is being grown. We produced doubled haploids (using anther culture) from crosses of OSU food germplasm with selected German winter barleys that have excellent agronomic performance and high levels of winter hardiness. After several years of phenotypic selection for agronomic and quality traits, selected lines were advanced to the INUDFOOD trial. There are 30 lines in this trial, 13 selected by OSU, 14 selected by Dr. Cistué and colleagues and three hulled check varieties. The experimental germplasm is all doubled haploid and hull-less and includes waxy and non-waxy starch types with moderately high grain _-glucan content. This project focuses on breeding exclusively hull-less barley for food end-uses due to the additional processing steps that become necessary in the presence of an adhering hull. The INUDFOOD trial was planted in fall 2013 at four locations: Corvallis, OR, USA; Pullman, WA, USA; Lleida, Spain; and Dundee, Scotland. In the fall of 2014, it was planted at the same four locations as well as at Mount Vernon, WA, USA. Agronomic and quality traits and resistance to biotic and abiotic stresses were measured at each location based on regional relevance. Preliminary results look promising; a number of the hull-less entries rival or out-yield the checks and have excellent agronomic and food quality ratings.

SESSION: 11

KEYNOTE

Plant Breeder Training Network: Collaborative approach to training

Jamie Sherman

Montana State University; jsherman@montana.edu

Co-authors: Jamie Sherman, Mary Brakke, Deana Namuth-Covert, Mao Thao, Frances Lawrenze, Gwen Nugent, Ashu Guru and Amy Kohmetscher

ABSTRACT

A national priority for the US is to strengthen capacities for cultivar improvement not only for ongoing food, feed and fiber sufficiency, but also to meet new demands for fuels and challenges from climate change. A supply of well-prepared plant breeders and support personnel is a primary capacity need. A collaborative model of plant breeder training, called the Plant Breeder Training Network (PBTN), was created and assessed, to address issues imposed by the changing landscape of plant breeding in the US. The creation of the PBTN was a primary goal of the Triticeae Coordinated Agricultural Project (TCAP), funded by USDA-NIFA. Through the PBTN more than 100 graduate students were mentored by TCAP PIs. Surveys of TCAP and non-TCAP students indicate that TCAP students had greater levels of independent participation in a number of plant breeding skills, including both technical and personal/professional skills. TCAP students also reported greater confidence in a number of knowledge and skill areas. TCAP students also developed a larger network in part through the online environment, increasing their scope of national collaboration. The PBTN provided online learning materials and courses in which hundreds have participated, including students and professionals from outside the project. By tracking student behavior in the online environment, data were archived, including time spent in a single session, number of sessions spent in the environment, materials accessed and quiz grades. These data will be used to inform online course best learning practices, which can help students gain the most from their online learning experiences and also guide instructional design that best supports self-paced learners. Here we will share strengths and challenges of the PBTN in hopes of improving plant breeder training globally.

SESSION: 11

Barley Breeding in the Public Interest: Perspectives on Integrating Research, Variety Development, Learning Experiences, and Extension in a U.S. Land Grant University

Patrick Hayes

Oregon State University ; patrick.m.hayes@oregonstate.edu

Co-authors: P.M. Hayes, all current and past members of the OSU Barley Project, and our worldwide network of collaborators

ABSTRACT

Barley is one of last major crops in the U.S. where the public sector is still actively engaged in cultivar development. A number of factors have contributed, historically, to this unique status. An additional set of factors could lead, in the near term, to a shift towards greater private sector delivery of varieties and public sector delivery of knowledge and training. A retrospective analysis of the Oregon State University (OSU) Barley Project is useful as an example of the outcomes that can be achieved when a public program receives sufficient support to engage in the Land Grant mission of (i) contributing to the fundamental body of knowledge, (ii) stimulating economic development through variety release, and (iii) assisting in forming an educated citizenry. Keys to the success of the program have been a balance of public (e.g. USDA Collaborative Agricultural Project grants) and industry funds (e.g. American Malting Barley Association); an opportunity to explore challenges and opportunities with no obvious immediate practical application (e.g. facultative growth habit and barley contributions to beer flavor); the integration of research and learning (e.g. graduate and undergraduate student research; the Oregon Wolfe Barleys); and an extensive international network based on unrestricted collaboration and exchange. Considerations for future public sector barley breeding include: shrinking public investment in plant breeding research with applied outcomes; expansions (e.g. the craft sector) and consolidations (e.g. the multinational sector) in the malting and brewing industries; an increased emphasis on innovative undergraduate and graduate teaching and experiential learning (with concomitant increases in time commitments); and the mantra that public sector variety development can generate sufficient revenue to be self-sustaining.

SESSION: 11

Connecting Genotype to Phenotype in 7-12 Classrooms with iTAG Barley

Roger Wise

USDA-ARS / Iowa State University; rpwise@iastate.edu

Co-authors: L. McGhee, N. Hayes, R. Schuck, J. Gonzalez, L. Maffin, T. Hubbard, E. Whigham, and G. Fuerst

ABSTRACT

iTAG "Inheritance of Traits and Genes" is a NSF-sponsored Research Experience for Teachers (RET) to promote understanding of the relationship between genotype and phenotype, which is the core foundation for modern genomics projects. Using the diverse Oregon Wolfe Barley population as the model, we have created a self-sustaining, inquiry-based curriculum comprising lab and classroom activities for high school students to learn concepts in plant development, phenotypic diversity, genetics, and/or genomics. Three key systems are presented; homeotic mutations, domestication, and epistasis. Through inquiry based learning, students become better-informed citizen scientists. iTAG Barley is aligned to the National Science Standards, and thus, can be adapted to any state standards. Teachers and workshop participants conduct pre- and post content-based survey of iTAG concepts. Results of the assessment are incorporated into publications and presented at NSTA and ASPB conferences.

The curriculum for iTAG Barley is available as teacher and student versions [PDF or digital textbook (iTAG for iPAD; https://itunes.apple.com/us/book/itag-barley-9-12-curriculum/id959451733?mt=11)], and includes NSF-funded thermal cyclers, microcentrifuges, gel boxes, transilluminators, pipetteman, and reagents. It has been implemented in >35 high-school classrooms from 2009-2014, impacting >800 students, half of which were underrepresented from urban to rural communities. The project is continuing to expand its reach with the first iTAG Barley workshop hosted by Iowa State University July 28th-31st, 2015. Workshop organizers and participants collectively will use iTAG Barley in 53 classes during the 2015-16 school year, impacting an additional 1,400 high school students. Additional workshops for summer 2016 are already being planned at Iowa State University in Ames, IA and Tuskegee University in Tuskegee, AL.

Supported by the National Science Foundation - Plant Genome grants 09-22746 and 13-39348.

SESSION: 11

Genetic basis of barley adaptation to the southern cone of South America: using GWAS to analize phenology in a wide population

Andres Locatelli

Departamento de Produccion Vegetal, Est. Exp. “Dr. Mario A. Cassinoni”, Facultad de Agronomia, Universidad de la Republica; aloca@fagro.edu.uy

Co-authors: L. Gutierrez, L. Viega, A. Castro

ABSTRACT

Phenology is a determinant factor for the adaptation of any crop, particularly in temperate conditions short growing season. Uruguayan conditions during barley growing season are defined by a relatively mild winter and a sharp increase in temperature during spring, which results in a high risk of heat stress during the grain filling period. In order to understand adaptation and to study novel gene combinations for yield potential increase, knowledge about the genetic factors affecting phenology is essential. In this research we study a wide population in order to confirm the previous work performed in adapted germplasm and to look for novel alleles. We used 297 spring barley genotypes from diverse origins. The population was genotyped with 1096 SNPs from BOPA1. It was phenotyped in four field experiments in Paysandu (Uruguay) during two years and with contrasting planting dates. A total of twelve phenotypes were measured, (lenght of GS00-GS20, GS00-GS30, GS20-GS30, GS30-GS49, GS00-Z49, GS49-GS90 and photoperiod response in all of them). A comprehensive GWAS was performed and QTLs were detected for all traits, with a total of 28 QTL regions associated to phenology traits. Most of the QTL involved more than one linked SNP, more than one trait and more than one environment. The long arms of 1H and 4H and the short arms of 2H, 3H and 5H were the regions of the most important QTLs. Four new QTL regions associated to phenology under our conditions were detected on chromosomes 1H, 6H (2) and 7H. Diversity analysis and LD analysis of detected QTLs suggest that phenology and most precisely photoperiod response may be a key determinant of the population structure. Our results are a contribution to expand the knowledge about genes with potential for breeding use in the region.

12th IBGS Poster Presentations

Think 5 – Posters 1-24

Poster Number	Name	Title
1	Tilahun Abebe	Analysis of the barley transcriptome during drought stress at the reproductive stage
2	Amina Abed	How close is close enough? Use of genomic selection across different but related breeding programs
3	Anil Adhikari	Screening Ethiopian and Eritrean Barley Accessions for Resistance to the Net Form of Net Blotch
4	Olga Afanasenko	Mapping seedling resistance to different isolates of Pyrenophora teres f. teres in barley cultivar Harbin
5	Alireza Akhavan	Genetic and Pathogenic Population Structure of the Net Blotch Pathogen of Barley and the Effectiveness of Currently-Used Sources of Resistance in Western Canada
6	Umme Aktari Nadira	Identification of the germplasm with low phosphorus stress tolerance and studies on the tolerant mechanisms in Tibetan wild barley
7	Gazala Ameen	rcs5 is a wall associated kinase gene that putatively functions as a dominant susceptibility factor in the Barley- Cochliobolus sativus interaction
8	Ahmad Alqudah	The genetic architecture of barley plant stature
9	Reda Amezrou	Genome wide association studies of barley net blotch resistance in Morocco
10	Tefera Tolera Angessa	Barley landraces – the underutilized genetic resources.
11	Noemir Antoniazzi	Genetic evolution of Barley Brewing in Agroindustrial Agraria Cooperative
12	Matthew Aubert	Molecular and Genetic Characterisation of Early Aleurone Development in Barley
13	Theresa Asabea Ayirebi	Genomic Selection for agronomic important traits in two-rowed winter barley
14	Ana Badea	Development of High Resolution Melting Assay for Quick and Accurate Molecular Screening for the Presence of Functional *Rpg*5 Alleles in Barley
15	Ana Badea	Survey of Tocols and Oil Levels in Whole Grain, Pearling Fractions and Brewer's Spent Grains of Malting and Hulless Food Barley Genotypes
16	Franz-Werner Badeck	Effects of sowing date and climatic conditions on two and six-row barley yield and quality
17	Agim Ballvora	Genetic and molecular analysis of epistatic interactions in flowering time pathways identified in a spring barley MAGIC population
18	Sebastian Beier	Exploring the barley genome with BARLEX
19	DAMIEN BEILLOUIN	Development and evaluation of a genotypic crop model for winter barley

Think 5 – Posters 1-24; Think 4 – Posters 25-60

Poster Number	Name	Title
20	DAMIEN BEILLOUIN	Which malting barley genotype characteristics are adapted to low input system: an experimental approach
21	Sébastien Bélanger	Genotyping-by-Sequencing on Pooled Samples (Pool-GBS) and its Use in Measuring Segregation Bias during the Course of Androgenesis in Barley
22	Andrea Bellucci	Genomic Prediction in Spring Barley
23	HAJER BEN GHANEM	Epidemiological Evolution of Barley foliar diseases in Tunisia
24	Therese Bengtsson	Genetic diversity and population structure in Nordic spring barley
25	FATIHA BENTATA	Analysis of Diversity genetic of Moroccan Net blotch populations of barley using AFLP markers
26	Timm Bernhard	CMS-based breeding of winter barley hybrids for bioenergy use
27	Natalie Betts	Transcriptomic analysis of individual barley grain tissues at maturity and during very early germination
28	Hongwu Bian	Efficient Targeted Genome Editing on Vitamin E-Biosynthesis Related Gene in Barley by a CRISPR-Cas9 System
29	Olena Bilynska	Use of chemically modified starch as a solidifying agent of medium for barley haploid production in anther culture in vitro
30	Phil Bregitzer	Transposon Tagging Resources in Hordeum vulgare
31	Maree Brennan	Grain skinning in malting barley: understanding the environmental and genetic influences
32	Davide Bulgarelli	Barley as a model to study host-microbiota interactions in crop plants
33	Hazel Bull	Identification and Characterisation of the Barley Row-Type Gene, VRS3
34	Andy Burkhardt	Screening nested association mapping population for root-lesion nematode, Pratylenchus neglectus, resistance/susceptibility
35	Fangbin Cao	Identification of specific genes for Al tolerance in Tibetan wild and cultivated barleys
36	Ludovic Capo-chichi	Association of SNP markers and chlorophyll fluorescence parameters with low temperature tolerance in spring barley
37	Steven Carlsen	Characterization of spot form net blotch of barley using host-pathogen genetics
38	Maria Cristina Casao	Spike architecture and downstream genes in VRS3 mutants
39	Austin Case	Mapping of Stem Rust Resistance Genes Rpg2 and Rpg3 in Barley
40	Ana M. Casas	A new Vrs1 allele identified in 2-row Spanish landraces
41	Ariel Castro	Genetic control of phenology in INIA Ceibo x Norteña Carumbé under temperate conditions in South America
42	Ariel Castro	Phenology and breeding origin are the main determinants of genetic diversity structure in South American barley
43	Antony Chapman	Required for Mla resistance 3, a new player in barley powdery mildew resistance?
44	M. Ilhan Çağırgan	Outcrossing Percentage of Genetic Male-steriles Derived from “Baronesse” in a Lowland Mediterranean Environment

Think 4 – Posters 25-60; Think 3 – Posters 61-84

Poster Number	Name	Title
45	Cecilie Christensen	CAD gene knock-out with CRISPR/Cas9 in barley to improve bioethanol production
46	Helen Collins	Changes in the biochemical composition, morphology and transcript profiles of genes, during starch and cell wall synthesis and degradation from anthesis to germination of barley grain
47	Suong Cu	Genetic variation for malting quality responses to different protein levels in barley grain
48	Fei Dai	A draft genome of hulless barley and its domestication of cold adaptation and tolerance
49	Carla de la Fuente Cantó	Recombinant Chromosome Substitution Lines (RCSLs) as a source of genetic variation for drought stress tolerance in barley
50	Vera Draba	Allele mining of wild barley resistance genes using a nested association mapping (NAM) approach
51	Peter Dracatos	Putting rust to sleep in Australia: Breeding for durable resistance to rust diseases in barley
52	Lisa Eichel	Fine mapping and towards cloning of resistance gene rym7 against Barley mild mosaic virus.
53	Ammar Elakhdar	Analysis of Molecular Diversity and Population Structure in Barley (*Hordeum vulgare L.*) inferred with SSRs
54	Mouldi EL FELAH	Barley in Tunisia : a long-way breeding story
55	J. Mitch Elmore	¬¬¬¬Identification of host targets of Blumeria candidate secreted effector proteins.
56	Duane Falk	Re-domesticating Barley
57	Weiyao Fan	Mapping loci associated with seed phytic acid in barley (Hordeum vulgare L.).
58	Scott Fisk	The OSU Malt Lab: Bridging the Gap between Barley and Beer on a Research Scale
59	Enrico Francia	Regulatory mechanisms of barley frost tolerance, the continuing conundrum of CBF genes
60	Jerome Franckowiak	A Chromosome Walk Using Bowman Backcross-derived Barley Lines
61	Eyal Fridman	Analysis of GxE interactions in barley HEB-25 population and acceleration to gene isolation by the ComSeq approach
62	Megan Getz	QTL mapping of head and seed morphology in a nested association mapping panel
63	Michael Gines	Candidate qRT-PCR Reference Genes For Barley that Demonstrate Better Stability Than Traditional Housekeeping Genes
64	Shawn Goggins	Environmental associations for cold tolerance in barley
65	baojian guo	Comparative proteomic analysis of two different application cultivars in barley (Hordeum vulgare L.
66	Ganggang Guo	Allelic variation and geographic distribution of vernalization genes HvVRN1 and HvVRN2 in Chinese barley germplasm
67	Sanjiv Gupta	Availability and utilization of resistance genes for foliar diseases in commercial barleys
68	Sanjaya Gyawali	Genome wide association study (GWAS) of resistance to barley spot blotch in South Asia
69	Allison Haaning	Genome-wide association study of tillering traits in field-grown barley

Think 3 – Posters 61-84; Think 1 – Posters 85-108

Poster Number	Name	Title
70	Ning Han	miR393-mediated repression of HvTIR1/AFB2 regulates root growth during seedling development and under Aluminum treatment in barley
71	Patrick Hayes	Has the time come for barley to go naked?
72	Patrick Hayes	The Oregon Promise population: the search for barley contributions to beer flavor leads to unexpected opportunities.
73	Xiaoyan He	A Novel β-Expansin Gene HvEXPB7, Cloned in the Root Hairs of Tibetan Wild Barley, Improves Root Hair Growth under Drought Stress
74	Joshua Hegarty	Mapping and Deployment of Tolerance to Cereal Yellow Dwarf Virus In Two-Rowed Spring Malting Barley
75	Laura Helgerson	The Oregon State University doubled haploid lab: status update and the development of facultative 2-row malting barley germplasm
76	Dustin Herb	Genome-wide association mapping of low temperature tolerance (LTT) in barley to improve crop efficiency under climate change
77	Paul Herzig	CHARACTERIZATION OF AGRONOMIC TRAITS AND LOCATING EXOTIC GENES, WHICH CONTROL AGRONOMIC TRAITS UNDER CONTRASTING NITROGEN SUPPLY IN THE WILD BARLEY NESTED ASSOCIATION MAPPING POPULATION HEB-25
78	Hiroshi Hisano	Identification of the genomic region responding to amenability of Agrobacterium-mediated transformation in barley
79	Traci Hoogland	Genetics of forage quality traits in a two-row nested association mapping population
80	Parastoo Hoseinzadeh	TOWARD FINE MAPPING OF A POWDERY MILDEW RESISTANCE GENE IN A HORDEUM VULGARE/ BULBOSUM INTROGRESSION LINE
81	Gongshe Hu	Effects of m351 barley (Hordeum vulgare L.) mutation on grain quality and its applications
82	Matthew Hunt	miRNA discovery in barley (Hordeum vulgare L.) and the powdery mildew pathogen, Blumeria graminis
83	Yadong Huang	Two barley quantitative trait loci associated with Fusarium head blight resistance exhibit differential resistance mechanisms to Fusarium graminearum infection
84	Ernesto Igartua	Natural variation in FLOWERING LOCUS T, HvFT1
85	Ernesto Igartua	What is PpdH2 doing in winter varieties?
86	Ernesto Igartua	Yield effects of flowering time genes in Mediterranean conditions
87	Marta Izydorczyk	Quality response of new malting barley varieties to increasing nitrogen fertilization rates in western Canada
88	Zahra Jabeen	NHX-type Na+/H+ anti-porter gene expression under different salt levels and allelic diversity of HvNHX in wild and cultivated barleys
89	Ahmed Jahoor	Genomic Prediction in Spring Barley.
90	Abderrazek Jilal	Single Spike Selection (SSS): a different strategy for an efficient barley breeding
91	Zhu Jinghuan	Genetic evidence of local adaption and long distance migration in Blumeria graminis f. sp. hordei populations from China

Think 1 – Posters 85-108; Inventor 1 – Posters 109-132

Poster Number	Name	Title
92	Nejdet Kandemir	Improving the drought tolerance of some barley cultivars through marker assisted selection
93	Andy Flavell	The barley chromatin epigenome
94	Ajit Kharub	Barley research in India: Challenges and opportunities
95	ANIL KUMAR KHIPPAL	Conservation agricultural practices to improve quality and productivity of malt barley
96	Helmut Knüpffer	Analysis of legacy evaluation data of a large barley germplasm collection
97	Takao Komatsuda	Gene regulation and editing of the barley cleistogamy 1
98	Thomas Kono	The role of deleterious substitutions in crop genomes
99	Ravi Koppolu	Barley multiflorus2.b (mul2.b) regulates rachilla determinacy
100	DINESH KUMAR	Genotypic and location effect on grain protein content of barley under sub-tropical climates
101	LOKENDRA KUMAR	Genetic Evaluation of Barly Germplasm for Lodging Tolerance under Sub-Tropical Climates of India
102	Vishnu Kumar	Malt barley research in India and future prospects
103	BERHANE LAKEW	Barley Research and Development in Ethiopia
104	Alessandro Tondelli Laura Rossini	Genome-wide association mapping of root extension in a collection of European winter barley cultivars
105	W.G. Legge	AAC Connect two-row malting barley combines desirable agronomics, quality and disease resistance including lower DON accumulation
106	Linda Legzdina	Comparison of Spring Barley (*Hordeum vulgare* L.)Population Genetic Diversity and Performance under Organic and Conventional Farming Systems
107	Li Lei	The evolution of species range limits in wild barley and its effects on deleterious mutations
108	YUEQIANG LENG	The gene conferring susceptibility to spot blotch caused by Cochliobolus sativus is located at the Mla locus in barley cultivar Bowman
109	Jens Léon	Identification of genetic and phenotypic traits in the root system of drought tolerant spring barley (Hordeum vulgare)
110	Maria Imaculada Pontes Moreira Lima	Evaluation of barley genotypes to fusarium head blight (FHB) under favorable environment for the disease
111	Maria Imaculada Pontes Moreira Lima	Occurrence and damage of head blast in barley in Brazil
112	Ruiming Lin	Virulence diversity in the populations of barley spot blotch pathogen Bipolaris sorokiniana in China

Inventor 1 – Posters 109-132; Inventor 2 – Posters 133-156

Poster Number	Name	Title
113	Chaochih Liu	Genomic Structural Variations in Two Genomic Regions of Wild Barley
114	Andres Locatelli	Using QTL analysis of waterlogging tolerance in barley to measure its impact on plant growth and leaf chlorosis
115	Mark Looseley	Introgression of quantitative resistance to Rhynchosporium commune into elite winter barley through marker assisted backcrossing.
116	Haiye Luan	Physiological and proteomics mechanism of barley response to waterlogging
117	Ramamurthy Mahalingam	Shotgun proteomic analysis of the seed proteomes of two-row and six-row barley
118	Rekha Malik	Genetic relatedness studied at molecular level for huskless barley (Hordeum vulgare)
119	Bram Marynissen	Combining drought tolerance and malt quality
120	Matthew Martin	Barley Rph1-15 Near Iso-genic lines in susceptible cultivar 'Bowman'
121	Oadi Matny	The origin of stem rust resistance in Hordeum
122	Andreas Maurer	GENOMIC DISSECTION OF PLANT DEVELOPMENT AND ITS IMPACT ON YIELD COMPONENTS IN BARLEY THROUGH NESTED ASSOCIATION MAPPING
123	Sarah McKim	Growing Up – Developmental Genetics of Internode Elongation
124	Brigid Meints	Food Barley Breeding for Flavor, Nutrition, and Color
125	Sara Giulia Milner	BRIDGE: Biodiversity informatics for harnessing barley genetic diversity hosted at the genebank of IPK Gatersleben
126	Euclydes Minella	Improvements in acid soil tolerance of Brazilian barley will require new allelic combinations
127	David Moody	Australian Barley Breeding: The Public to Private Transition
128	Imrul Mosaddek Ahmed	Physiological mechanism, stress-specific proteins for the tolerance to combined stress of drought and salinity in Tibetan wild barley
129	Matthew Moscou	Dual specificity at the Mla locus confers resistance to barley powdery mildew and wheat stripe rust
130	Gary Muehlbauer	Do the same sets of genes regulate tiller and leaf development?
131	C. Walter Newman	Barley is Better for Food and Health
132	Jeffrey Neyhart	Investigating GxE in a Two-Row Barley Genomic Selection Pilot Study
133	Joseph Nyachiro	Agronomic and quality variation in lines derived from a 'Vivar' barley mutant population
134	Joseph Nyachiro	The development of Quick Cooking Barley using hulless barley
135	Ron Okagaki	Convergence and divergence of axillary meristem and leaf developmental pathways in barley
136	Alex Ollhoff	Building a Bridge to Barley Germplasm Resources with the National Small Grains Collection NAM Population
137	Frank Ordon	A new QTL identified for drought stress tolerance and leaf senescence in juvenile barley

Inventor 2 – Posters 133-156; Inventor 3 – Posters 157-180

Poster Number	Name	Title
138	Frank Ordon	Genome wide association studies for resistance to Pyrenophora teres f. teres and Coch-liobolus sativus in barley (Hordeum vulgare)
139	Frank Ordon	Locating QTL for resistance to net blotch (Pyrenophora teres f. teres) in a wild barley nested association mapping population
140	Frank Ordon	Towards genomics based isolation of resistance genes against soil-borne barley yellow mosaic virus disease (BaYMV, BaYMV-2, BaMMV)
141	Mehmet Tufan Oz	Generation of transgenic barley expressing Blumeria effector candidate (BEC)1019 to unravel host signaling pathways
142	Zhifen Pan	Wide variations of major grain components, pasting properties and in vitro starch digestibility of Tibetan hull-less barley and its effects on the textural characteristics and predicted functional values of processed cookies
143	Blakely Paynter	Influence of the Alt1 gene on the agronomic performance of barley on acidic soils in Western Australia
144	Carlos Perez-Cantalapiedra	Fine-mapping of a powdery mildew QTL by exome sequencing
145	Dragan Perovic	Assessment of genomic resources and Next-Generation-Sequencing technology for resistance breeding in barley
146	Luke Linz	Cost-effective and accurate SNP genotype analysis of barley samples using the Array Tape® Platform and KASP™ genotyping chemistry
147	Ana Poets	Recombination patterns among spring barley populations
148	Kenton Porker	Opportunities to improve barley yield potential and adaptation by optimising development pattern
149	Juncang Qi	Effects of Different Drought Intensity on the Morphology of Barley Seedling Roots
150	Hai long QIAO	Salt Tolerance Identification and Evaluation of Barley Varieties
151	Dandan Qin	Characterization and Fine mapping of a Novel Barley Stage Green-Revertible Albino Gene (HvSGRA) by Bulked Segregant Analysis based on SSR assay and Specific Length Amplified Fragment Sequencing
152	Do Mornhinweg	New pest threat to barley in the U.S.
153	Mohammad Pourkheirandish	Fine Mapping of Rph12, a Gene Conferring Resistance to Leaf Rust in barley
154	Luke Ramsay	Association genetic analysis of nitrogen use efficiency in northern european winter barley
155	Abdur Rashid	Extraction of high-quality RNA from germinating barley (Hordeum vulgare L.) seeds containing high levels of starch.
156	Volodymyr Radchuk	Molecular Mechanisms of Assimilate Transfer in the Developing Barley Grains
157	Sajid Rehman	Identification of novel sources of resistance to powdery mildew in barley by Focused Identification of Germplasm Strategy (FIGS) approach
158	Jonathan Richards	High-Resolution Mapping Reveals Candidate Genes at Major and Minor Effect Loci Conferring Susceptibility to Net Form Net Blotch on Barley Chromosome 6H

Inventor 3 – Posters 157-180; Pinnacle Ballroom – Posters 181-230

Poster Number	Name	Title
159	Fulvia Rizza	Diversity in frost tolerance and adaptive traits in barley germplasm of different origin
160	Fulvia Rizza	Effects of elevated CO2 on growth, yield and grain composition of four barley varieties
161	Ghizzoni Roberta	Fusarium langsethiae as an emerging toxins producer in malting barley
162	Silvio Salvi	Describing and Cloning Root Mutants In Barley
163	Joanne Russell	A genome wide analysis of key genes controlling diastatic power activity in winter and spring barley.
164	stephanie saade	Identifying the components of salinity tolerance using a Nested Association Mapping barley population
165	Shun Sakuma	Identification of deficiens (Vrs1.t) responsible for rudimental lateral spikelets and enlarged central grains
166	Saida Salah-Mlaouhi	Abiotic Stress of *Barley* with Rise Temperature conjugated to Soil Salinity in an Irrigated Area by Treated Waste Water in Tunisia
167	Ahmad Sallam	Genome-wide Association Mapping of Heading date in the Wild Barley Diversity Collection
168	Kazuhiro Sato	Isolation of seed dormancy QTL Qsd1 in barley
169	Karl Schmid	Improving genomic prediction in barley breeding with variable selection methods
170	Alan Schulman	ClimBar: An Integrated Approach to Evaluate and Utilize Genetic Diversity
171	Alan Schulman	Retrotransposons and Their Role in Genome Structure and Dynamics in Barley
172	Thorsten Schurbusch	Domesticated barley (Hordeum vulgare L.) originated from an Intermedium type of wild barley
173	AJEET SINGH SHEKHAWAT	Genetic Analysis of Barley (Hordeum vulgare L.) Genotypes Under Normal and Limited Moisture Conditions
174	Roshan Sharma Poudel	You need two to Tango: Identification of candidate effectors/avirulence genes that interact with the wheat stem rust resistance locus rpg4/Rpg5
175	Lizette Schneider	Wax biosynthesis expands beyond Arabidopsis to the diketone synthase (DKS) polyketide pathway.
176	Jaswinder Singh	A Malting quality QTL on chromosome 4H harbors a key gene which influences β-glucan activity in barley
177	Jogendra Singh	Association and Multivariate Analysis of Yield and its Components in Hulless Barley (Hordeum vulgare L)
178	Brian Steffenson	Low Temperature Tolerance of Cultivated and Wild Barley Germplasm in Autumn-Sown Field Trials in Minnesota.
179	Brian Steffenson	On the Possible Origin of Stem Rust Resistance Genes in Barley
180	Eric Stockinger	Implication of CBF2A–CBF4B genomic region copy numbers in affecting expression of other FR-H2 CBFs
181	Dongfa Sun	Genetic divergence, population structure of wild barley and origin of cultivated barley
182	Dongfa Sun	Inheritance, molecular identification and utilization of a novel dwarfing germplasm “Huaai 11” in barley

Pinnacle Ballroom – Posters 181-230

Poster Number	Name	Title
183	Saemundur Sveinsson	
184	Daniel Sweeney	Non-destructive whole grain high-throughput phenotyping for malt quality traits in malting barley
185	Dimitri SZABO	On the interest of Research Resource Planning1 tool in data handling for barley breeding
186	Karima Taibi	Virulence of Moroccan Pyrenophora teres f. teres revealed by international differential barley genotypes
187	cong tan	BarleyVarDB: an integrated database of barley genomic variants
188	Zenith Tandukar	Towards positional cloning and functional analysis of glossy mutants in barley
189	William Thomas	Improving the processability of malting barley
190	William Thomas	Improving winter barley malting quality
191	Filipa Tome	Regulation of inflorescence development in response to photoperiod in barley
192	Alessandro Tondelli	A major QTL on chromosome 7HS controls seed germination under salt stress in the 'Nure' x 'Tremois' barley mapping population
193	Claudia Toniazzo	MALTING QUALITY IMPROVEMENT AND MOLECULAR MARKERS TO ASSESS GENETIC DIVERSITY IN BARLEY
194	James Tucker	Phenotypic Assessment of a Set of Two-row Barley Genotypes with Differential FHB Resistance and DON Accumulation
195	Thomas Turkington	An overview of scald and net blotch resistance screening at Lacombe and Edmonton, Alberta, Canada
196	Yerlan Turuspekov	Genetic and phenotypic variation of spring barley accessions from Kazakhstan and USA
197	Wilma van Esse	Genetic dissection of tiller development in barley
198	THIRULOGACHANDAR VENKATASUBBU	Dosage-dependent expression of duplicated genes regulated barley leaf and spikelet development and brought two-rowed barley into existence
199	Liliana Vasilescu	A comparison study of agronomical and physiological traits in a set of varieties released in Romania and Italy during the last 40 years
200	Ramesh Verma	Addressing the global challenges for barley improvement through CGIAR Research Program on Dryland Cereals
201	Ramesh Verma	Novel sources of resistance to stripe rust in ICARDA barley germplasm
202	Sadaram Verma	Genetic improvement for grain yield and quality traits in barley (Hordeum vulgare L.)
203	Marcus Vinje	Comparative expression analysis of hordein and beta-amylase in developing barley grains
204	Andrea Visioni	Optimizing test locations and identifying promising genotypes in ICARDA barley breeding program
205	Asuka Takahashi	Relationship between *Hordoindolines* and barley pearling characteristics
206	Agatha Alexandra Walla	Phenotypical Dissection of a High Tillering Mutant in Barley
207	Hugh Wallwork	Detection of minor gene resistance to net form net blotch under controlled environment conditions

Pinnacle Ballroom – Posters 181-230

Poster Number	Name	Title
208	Junmei Wang	A high density map reveals better markers for the new semi-dwarf gene on chromosome 7H in barley
209	Rui Wang	Molecular mapping of a single dominant gene for resistance to a new pathotype of Cochliobolus sativus in barley
210	Yajun Wang	Map-based cloning of *Rphq11*: a QTL conferring partial resistance to adapted and non-adapted rust species in barley
211	WHEALBI Consortium	WHEALBI: Applying state of the art genomic tools, methods and approaches to characterise barley and wheat genetic resources from across their geographical range as sources of genes and alleles for use in crop improvement
212	Mathias Wiegmann	CROSSTALK BETWEEN FLOWERING TIME AND ABIOTIC STRESS TOLERANCE IN HALLE EXOTIC BARLEY-YIELD
213	Ronja Wonneberger	Identification of molecular mechanisms in the Drechslera teres – barley pathosystem and analysis of genetic diversity and population structure of a Norwegian D. teres population
214	Dezhi Wu	Cadmium uptake is mediated by a manganese transporter, HvNramp5 in barley
215	Ping Yang	Evolution of recessive resistance genes against the bymovirus disease of barley
216	Lu Yin	Fine Mapping Coincident QTL for Multiple Traits Linked with Gpc1 on Chromosome 6H of Barley
217	Helmy Youssef	Six rowed spike 2 (Vrs2) – a central regulator of spike development in barley
218	Behzad Talle	Manipulating Male Fertility in Barley (Hordeum vulgare L.)
219	Jennifer Zantinge	A customised single nucleotide polymorphism (SNPs) panel for molecular marker assisted selection within an applied spring barley breeding program.
220	wen zang	Genetic and histological studies of durable loose smut resistance mediated by Un8 in barley
221	Jianbin Zeng	Integration of Transcriptome, Proteome and Metabolome Analysis Reveals the Mechanisms of Low-Potassium Tolerance in Barley
222	Alessandro Tondelli	Exploring phenotypic variation for key agronomic and life history traits in a barley legacy collection
223	Roger Wise	Inter-chromosomal transfer of immune regulation during barley-powdery mildew interactions
224	Takashi Yanigasawa	Japanese Barley Cultivars with high β-glucan Content
225	Qisen Zhang	Identifications of significant genes controlling malting qualities in barley genome
226	xinzhong zhang	QTL mapping of glume-opening angle in barley (Hordeum vulgare L.)
227	Meixue Zhou	Breeding barley for waterlogging tolerance: key mechanisms and ready-to-use molecular markers
228	Min Zhu	Evaluating predictive values of various physiological indices for salinity Stress tolerance in barley and wheat
229	Min Zhu	Evaluation of physiological characteristics conferring waterlogging tolerance in barley accessions
230	Monika Zwirek	Mechanisms Underlying Row-Type Determination in Cultivated Barley

POSTER: 1

Analysis of the barley transcriptome during drought stress at the reproductive stage

Tilahun Abebe

University of Northern Iowa; Tilahun.Abebe@uni.edu

Co-authors: T.B. Mersha and A. Poleksic

ABSTRACT

Drought at the reproductive stage can influence yield by limiting the availability of nutrients translocated from photosynthetic and storage organs. In barley, the spike organs (lemma, palea, and awn) play a major role in grain development. Previously, we investigated changes in the transcriptome of the lemma, the palea, the awn, and the developing kernel in response to drought stress in Morex barley using the Barley1 Genome Array. Although the Barley1 Genome Array has expanded our understanding of transcriptome dynamics in response to environmental perturbations, it does not contain all the estimated 30,000 _ 32,000 barley genes. We have expanded our investigation of changes in the transcriptome of the lemma, the palea, and the awn of barley during stress using next-generation sequencing (NGS). We also included the flag leaf in this study to compare gene expression differences between the spike organs and the leaf during stress. Barley plants were exposed to moderate drought stress at the early grain-filling stage by withholding water for four days. Transcript abundance was compared using 50 bp-long single reads generated by Illumina Genome Analyzer II. We found that the flag leaf and the awn express more genes than the lemma and the palea. We will discuss gene expression differences between the four organs during drought stress and how the NGS data compares with our previous study using the Barley1 Genome Array.

SECTION:
Abiotic stresses

POSTER: 2

How close is close enough? Use of genomic selection across different but related breeding programs

Amina Abed

Laval university, Canada; amina.abed.1@ulaval.ca

Co-authors: M. Jean, S. Marchand, M. Lacroix and F. Belzile

ABSTRACT

The genetic complexity of some economically important traits in barley (*Hordeum vulgare* L.), such as resistance to *Fusarium* head blight (FHB), is very challenging because of the poor quality of the limited phenotypic information available to breeders early in the selection process. To overcome this challenge, genomic selection (GS) represents an attractive alternative. Genomic selection is based on a statistical model describing the relationship between phenotype and genotype. This model is derived from the detailed characterization of a training population (TP) composed of lines meant to capture the gene pool of a breeding program. This model can then be used to predict the phenotypes of new selection candidates (SCs) for which only the genotype is known. The response of genomic prediction depends on various factors. The composition of the TP in relation to the SCs is an important factor in maintaining a high degree of predictive accuracy. In this study, we have evaluated the genomic prediction accuracy for FHB tolerance when using a TP and SCs from two different, but interrelated breeding programs in Eastern Canada. A collection of 260 advanced lines from the UL (Laval University) program were used to train the GS model, while a collection of 50 lines from a private program (C_r_la Inc.) were used as SCs. Dense genotypic information was obtained using a genotyping by sequencing (GBS) approach and two different pipelines were used to call about 25,000 polymorphic SNPs. We first investigated the genetic relationship between the TP and the SCs by examining both population structure and genetic relatedness. The populations did not show any stratification and the lines seemed closely related. The genotypic data and genomic selection model were used to predict the phenotype of the SC lines and we report on the accuracy of these predictions.

SECTION:
Success stories in barley breeding and genetics
Biotic stresses
Breeding Methodologies: current approaches and future
Plant Breeding and Genetics Education

POSTER: 3

Screening Ethiopian and Eritrean Barley Accessions for Resistance to the Net Form of Net Blotch

Anil Adhikari

University of Minnesota; ruthdm@umn.edu

Co-authors: R. Dill-Macky and M. J. Smith

ABSTRACT

The net form of net blotch (NFNB), caused by Pyrenophora teres f. sp. teres (Ptt), is one of the most common and economically important foliar diseases of barley. NFNB frequently reduces yield and grain quality in the northern great plains of the United States. Eight isolates of Ptt, collected from North Dakota (ND) and Minnesota (MN) were tested for virulence on a differential set of 30 barley lines. Based on these results three ND isolates and one MN isolate were selected, based on their varying virulence profiles, for further studies. These four isolates were used to screen a collection of 268 spring barley accessions originating from Ethiopia and Eritrea (EEBC) in separate experiments. The EEBC accessions were grown in the greenhouse (18-22oC, 18 h light) in a randomized complete block design with three replicates. Two week-old seedlings were inoculated to runoff with a conidial suspension (20,000-25,000 spores/ml), incubated in a dew chamber at 100% relative humidity for 24 hours before being returned to the greenhouse bench. The inoculated plants were assessed seven days after inoculation using the Tekauz scale where 1-3 is resistant (R), 4-5 is moderately resistant (MR), 5-7 is moderately susceptible (MS) and 8-10 is susceptible (S). Preliminary analysis of the data indicated that 0.4-5.3% of the lines were R, 12.0-47.7% were MR, 44.7-73.7% were MS and 2.2-13.9% were S to the four isolates tested. One accession (EEBC 123) was resistant to all four Ptt isolates in this study. Association mapping study was conducted in TASSEL 4.0 using a mixed linear model with an additive kinship matrix (K model). Significant hits were observed on chromosome six, which was consistent with previously reported studies for NFNB resistance.

SECTION:
Biotic stresses

POSTER: 4

Mapping seedling resistance to different isolates of *Pyrenophora teres f. teres* in barley cultivar Harbin

Olga Afanasenko

All-Russian Reserch Institute for Plant Protection; olga.s.afan@gmail.com

Co-authors: D. Mather, A. Anisimova, N. Mironenko, G. Platz

ABSTRACT

One of the most widely distributed and harmful diseases of barley world-wide is net form of net blotch caused by *Pyrenophora teres f. teres* (Ptt). In order to standardise characterisation of *P. teres f. teres* populations globally, an international set of 9 differentials was developed (Afanasenko et al., 2009). Cultivar Harbin from this set demonstrated good differential ability and has been used in local sets of differentials in other countries.

The objective of this research was to map QTL associated with seedling resistance to Ptt in cv. Harbin using the doubled haploid (DH) population Harbin x Harrington. Harrington is also included in the international set of barley differentials as a susceptible check.

Three single conidial isolates of Ptt of different origins and virulence spectra were used for inoculation of detached first seedling leaves of parents and DH lines. All isolates used were avirulent to cv. Harbin and virulent to Harrington. The infection response was recorded 5 days after inoculation, using the 10-point scale of Tekauz (1985).

DNA samples of Harbin, Harrington and each of the 92 doubled haploid lines were assayed for SNP markers using DArTseq[a] technology (Diversity Arrays Technology Pty Ltd, University of Canberra, Bruce, Australia). The major QTL Rpt2-3HHarbin, (LRS 86.3, 72.4, and 44.3 for different Ptt isolates) controlling resistance to the three Ptt isolates mapped to chromosome 3HS at interval 37,46 _ 44,24 cM, and a QTL of small effect was identified on chromosome 6HS, which was contributed by the susceptible parent Harrington. In addition, the isolate from Belarus revealed a very small effect on chromosome 1H (LRS 15.4), which was contributed by Harbin.

SECTION:
Resources I: Genome

POSTER: 5

Genetic and Pathogenic Population Structure of the Net Blotch Pathogen of Barley and the Effectiveness of Currently-Used Sources of Resistance in Western Canada

Alireza Akhavan

University of Alberta; akhavan@ualberta.ca

Co-authors: T.K. Turkington, H. Askarian, A. Tekauz, K. Xi, W.G. Legge, J.R. Tucker, and S.E. Strelkov

ABSTRACT

Genetic and pathogenic diversity was assessed in western Canadian populations of *Pyrenophora teres f. teres* (Ptt) and *P. teres f. maculata* (Ptm). In total, 110 distinct alleles were identified in 13 microsatellite loci, 19 of which were shared between the two forms, while 75 were specific to Ptt and 16 were specific to Ptm. Among the Ptt isolates, 94 alleles were detected with an average of 7.2 alleles per locus, while for Ptm isolates, 35 alleles were detected with an average of 2.7 alleles per locus. Significant genetic differentiation (PhiPT = 0.230, P = 0.001) was revealed among all populations, with 77% of the variation occurring within populations and 23% between populations. Subsequently, 39 Ptt and 27 Ptm isolates were assessed for pathogenic variation on a set of barley differentials. Cluster analysis revealed 16 and 13 pathotype groupings, respectively, among the Ptt and Ptm isolates. The differentials CI 5791 and CI 9820 were resistant to all isolates of Ptt except one, while the differential CI 9214 was resistant to all isolates of Ptm except two. Separate growth cabinet seedling experiments also were conducted to evaluate the reaction of net form net blotch (NFNB) and spot form net blotch (SFNB) resistant barley genotypes against representative pathotypes of Ptt and Ptm. The NFNB resistance in the cultivars ÔVivarÕ and ÔCDC HelgasonÕ was overcome by two and three of the Ptt pathotypes, respectively. Resistance to SFNB in ÔCDC MeredithÕ was overcome by all pathotypes tested. The results suggest that western Canadian populations of Ptt and Ptm are genetically and pathogenically diverse, and that some isolates are capable of overcoming the resistance in certain barley cultivars.

SECTION:
Biotic stresses

POSTER: 6

Identification of the germplasm with low phosphorus stress tolerance and studies on the tolerant mechanisms in Tibetan wild barley

Umme Aktari Nadira

Institute of crop science, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, P.R. China; nadira@zju.edu.cn

Co-authors: I.M. Ahmed, G.P. Zhang

ABSTRACT

The experiments were carried out to evaluate the genotypic difference in low phosphorus (LP, 10 μM Pi) stress tolerance using 104 Tibetan wild and 20 cultivated barley genotypes. The results showed that plant growth and P accumulation were significantly reduced in the plants exposed to LP stress relative to normal P (500 μM Pi) condition, while some genotypes of wild and cultivated barley increased root length and dry weight under LP stress. There were significant differences among the 104 Tibetan wild barley genotypes in the growth parameters examined under LP stress, suggesting a wide genetic diversity among the tested genotypes. Significantly genotypic differences were also observed in the relative values of the examined physiological parameters and mineral nutrients in responses to LP stress. Three Tibetan wild barley genotypes, XZ77, XZ99 and XZ167, and two cultivated barley genotypes, ZD9 and B1034 were identified as high tolerance or moderate tolerance to LP. In addition, these genotypes showed considerable differences in the change of biomass accumulation, root/shoot dry weight (DW) ratio, root morphology, organic acid secretion, carbohydrate metabolism, ATPase (Adenosine triphosphatase) activity, P concentration and accumulation under LP in comparison with the control (normal P level). The results prove the potential of Tibetan wild barley in developing barley cultivars with high tolerance to LP stress and understanding the mechanisms of LP stress tolerance in plants.

SECTION:
Abiotic stresses

POSTER: 7

Rcs5 is a wall associated kinase gene that putatively functions as a dominant susceptibility factor in the Barley- Cochliobolus sativus interaction

Gazala Ameen

Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA; gazala.ameen@ndsu.edu

Co-authors: T. Drader, L. Sager, B. Steffenson, A. Kleinhofs and R.S. Brueggeman

ABSTRACT

Spot blotch, caused by the ascomycete fungus, Cochliobolus sativus is a globally important foliar disease of barley. Resistance to spot blotch identified from the line NDB112, designated Rcs5 has remained effective for more than 40 years in the Upper Midwest. Previous studies determined that Rcs5 is located on the short arm of chromosome 7H and high-resolution genetic mapping delimited Rcs5 to an ~0.32cM region between the flanking markers ctg1584 and ctg7. Utilizing the barley genome sequence of cultivar (cv) Morex, four wall-associated kinase (WAK) genes were identified in the Rcs5 region designated HvWAK2-5. To validate the candidate WAK genes as Rcs5 virus induced gene silencing (VIGS) was utilized to post-transcriptionally silence the WAK genes. Two replications of the initial VIGS experiments using two barley stripe mosaic virus (BSMV)-VIGS constructs specific to HvWAK2 (BSMV-WAK2) and a second construct targeting HvWAKs 3, 4 and 5 (BSMV-WAK345) were conducted. This preliminary VIGS data showed that silencing HvWAK2 produced no response in the cv Morex (resistant; Rcs5+) or cvs Steptoe or Harrington (susceptible; rcs5-). However, the BSMV-HvWAK345 silencing construct resulted in shifts from susceptibility towards resistance in Steptoe and Harrington and no response in Morex. Further analysis of Steptoe x Morex F2 individuals showed a 1 (resistant):3 (susceptible) segregation ratio suggesting that rcs5 is recessive in nature. A 635-bp insertion in the Steptoe HvWAK3 gene introduces an additional 206 aa encoding a putative Ca2+ binding domain, which translates as part of a predicted functional WAK protein, correlates with all susceptible genotypes. Further allele analysis and BSMV-VIGS constructs specific to each WAK gene are being tested. We hypothesize that HvWAK3 encodes the RCS5 dominant susceptibility protein targeted by C. sativus to induce programmed cell death in an inverse-gene-for-gene manner suggesting that rcs5 is actually a dominant susceptibility gene that facilitates disease in the susceptible lines.

SECTION:
Biotic stresses

POSTER: 8

The genetic architecture of barley plant stature

Ahmad Alqudah

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK); alqudah@ipk-gatersleben.de

Co-authors: R. Koppolu; G. M. Wolde; A. Graner; T. Schnurbusch

ABSTRACT

Plant stature in temperate cereals is predominantly controlled by tillering and plant height as complex agronomic traits, representing important determinants of grain yield. This study was designed to reveal the genetic basis of tillering at five developmental stages and plant height at harvest in 218 worldwide spring barley (*Hordeum vulgare* L.) accessions under greenhouse conditions. The accessions were structured based on row-type classes [two- versus six-rowed] and photoperiod response [(photoperiod-sensitive (Ppd-H1) versus reduced photoperiod sensitivity (ppd-H1)]. Phenotypic analyses of both factors revealed profound between group effects on tiller development. To further verify the row-type effect on plant height and tiller number, Six-rowed spike 1 (vrs1) mutants and their two-rowed progenitors. Data analysis showed that, wild-type (Vrs1) plants were significantly taller and had more tillers than mutants suggesting a negative pleiotropic effect of row-type locus on both traits. Our genome-wide association scans further revealed highly significant associations, thereby establishing a link between the genetic control of row-type, heading time, tillering and plant height. We further show that associations for tillering and plant height are co-localized with chromosomal segments harboring known plant stature-related phytohormone and sugar-related genes. This work demonstrates the feasibility of the GWAS approach for identifying putative candidate genes for improving plant architecture.

SECTION:

POSTER: 9

Genome wide association studies of barley net blotch resistance in Morocco

Reda Amezrou

ICARDA; a.reda@cgiar.org

Co-authors: R.P.S Verma, L. Belqadi, S. Chao, M. Arbaoui, S. Rehman and S. Gyawali

ABSTRACT

Net blotch of barley caused by *Pyrenophora teres f. teres* (net form) and *Pyrenophora teres f. maculata* (spot form) are important foliar diseases of barley crop worldwide. In Morocco, the yield losses can reach up to 35% on susceptible cultivars under severe disease incidence. The resistance to Moroccan isolates is poorly understood and very few sources of resistance are available. The objectives of this study are to identify the genomic regions conditioning resistance to net form of net blotch (NFNB) and spot form of net blotch (SFNB), in 336 barley genotypes originating from ICARDA germplasm, as well as to identify markers tightly linked with these loci, to help in better integration of resistance in improved germplasm. An Association Mapping panel (AM-15) was genotyped using 9K iSELECT SNP markers. The panel was phenotyped in three hotspot locations in Morocco (Marchouch, Jemma Shaim, and Sidi El-Aidi research stations) for seedling and adult stage resistance in α-lattice design experiment. Association analysis using mixed linear model (structure and kinship as covariate) were employed to identify SNP markers associated with net blotch resistance. A total of 92 marker-trait associations were detected. Seven and six QTLs associated with resistance to NFNB resistance at seedling and adult stage respectively were identified on all seven barley chromosomes. A total of seven QTLs associated with seedling resistance to SFNB were identified on chromosomes 3H, 5H and 7H while four QTLs located on 3H and 7H were associated with adult stage resistance. Out of the 24 identified QTLs, five correspond to previously mapped net blotch resistance QTLs, QRpt6, QRpts4 and Rpt7. The 19 novel loci identified in the current study may represent a broad spectrum of net blotch resistance and will be useful in marker-assisted barley improvement.

SECTION:
Biotic stresses
Plant Breeding and Genetics Education

POSTER: 10

Barley landraces - the underutilized genetic resources

Tefera Tolera Angessa

Murdoch University; T.Angessa@Murdoch.edu.au

Co-authors: C. Grime, R. Boyd, S. Broughton, and C. Li

ABSTRACT

In contrast to commercial cultivars, considerable genetic diversity has been widely acknowledged in barley landraces. The narrow genetic base in modern commercial barley varieties cultivated in Australia and North America is partly attributed to the dominance of 37 barley landraces as founding progenitors. In spite of their considerable genetic variation, barley landraces are an underutilized genetic resource in commercial variety development. This is in contrast to the evaluation and exploitation of landraces for resistance to biotic and tolerance to abiotic stress factors. Over 10,000 barley entries from international origins were studied for adaptation to Australian environments, tolerance to abiotic stress, or resistance to common Australian barley foliar diseases. Over 20 years of studies utilising barley landraces, commercial varieties, doubled haploid populations and advanced breeding lines has identified barley genotypes, particularly landraces, with a range of useful traits. They include tolerance to abiotic stress factors such as boron toxicity e.g. a two rowed Ethiopian landrace and a six-rowed Algerian landrace, heat stress tolerance e.g. a landrace from Pakistan, water-logging tolerance e.g. a landrace from China, frost tolerance e.g. a landrace from Tibet, aluminium toxicity, salinity, alkalinity; and resistance to biotic stress factors such as powdery mildew, scald, net type net blotch, and spot type net blotch. An additional striking observation is the level of variation in phenology traits that was observed in a wider barley gene pool which was evaluated for more than 11 years. This report summarises genotypes that have been identified as tolerant to abiotic stress factors and resistant to biotic stress factors common in Australia, and the level of phenological variation in both spring and winter barleys. Understandably, linkage drag and the pressure on breeding programs to release improved varieties in a short period of time contribute to the underutilization of landraces in breeding programs. However, recent developments in molecular genetics may help to overcome the problem of linkage drag and enhance the use of landraces in developing commercial varieties.

SECTION:
Resources II: Germplasm and Populations

POSTER: 11

Genetic evolution of Barley Brewing in Agroindustrial Agraria Cooperative

Noemir Antoniazzi

FAPA - Fundação Agraria de Pesquisa Agropecuaria; noemir@agraria.com.br

ABSTRACT

The cultivation of barley for brewing purposes in Brazil began in 1930 and at Agraria Agroindustrial Cooperative in 1973. The research with brewing barley at the Agraria Cooperative in partnership with brewing companies and public research (Embrapa), in order to obtain cultivars adapted to the AgrariaÕs region of operation, started in 1976. The evolution of barley productivity grew over the years and with the cycles of the cultivars. In 1983 when the cycle of the Antarctica 1 cultivar ended, the average yield was 1671 kg ha-1. In 1984 the Antarctica 5 cultivar was introduced, also coming from the Antarctica Paulista Company breeding program. The cycle of this cultivar was until 1992 and promoted an increase of 42.3% in productivity compared to Antarctica 1 cultivar. In 1993 a new cycle began, with the BR 2 cultivar, developed by Embrapa Wheat. This cultivar was maintained in cultivation until 2002 and registered the same level of productivity, however, with greater yield stability. The last cycle where a cultivar dominated the area of cultivation in the Agraria Cooperative, started in 2003 with the BRS 195, also belonging to Embrapa. This cultivar caused a revolution in barley crops in the Agraria's region, reaching an yield average of the 4676 kg ha-1 2008 harvest during its seven years of cultivation, provided an increase of 59.2% in productivity. Starting with 2010 harvest, no barley cultivar has managed to reach 50% of the crop area of the Agraria Cooperative, providing a better blend of cultivars in the field, improving the performance of the malt industry.

SECTION:
Plant Breeding and Genetics Education

POSTER: 12

Molecular and Genetic Characterisation of Early Aleurone Development in Barley

Matthew Aubert

University of Adelaide; matthew.aubert@adelaide.edu.au

Co-authors: R.A. Burton, and M.R. Tucker

ABSTRACT

Cereal grains are important for human health and nutrition and can be utilised for a number of processes in the brewing, fibre and food industries. Within the cereal grain, an endosperm tissue layer known as the aleurone contains high levels of dietary fibre and antioxidants and is known to be crucial for grain germination. In Hordeum vulgare (barley), the aleurone releases enzymes during germination that stimulate the release of starchy energy reserves, and this is utilised in the production of malt for brewing. Despite the economic importance of aleurone development, few details are available in regards to molecular cues controlling the differentiation of the aleurone from the inner starchy endosperm cells. Therefore this project aims to investigate and characterise the molecular and genetic basis for aleurone development in barley, and determine how intercellular communication controls the differentiation of individual endosperm cell layers. To achieve this we have investigated phenotypic differences in aleurone development across a number of barley varieties using UV autofluorescence microscopy. Two barley cultivar panels, including one from the University of Adelaide barley breeding program and a second spring 2-row European panel, are being assessed. Each panel has been genotyped, thus providing genetic marker information for use in genome wide association studies (GWAS) and quantitative trait loci (QTL) mapping. Preliminary results reveal correlations between various phenotypic traits such as the number of aleurone cell layers and aleurone thickness, and have revealed cultivars that significantly deviate from these trends. The successful completion of the project objectives will provide an enhanced fundamental understanding of endosperm differentiation during cereal grain development and contribute mechanistic information that can be utilised for crop improvement.

SECTION:
Barley morphology and development

POSTER: 13

Genomic Selection for agronomic important traits in two-rowed winter barley

Theresa Asabea Ayirebi

University of Copenhagen; taa@plen.ku.dk

Co-authors: A.M. Torp, J.D.Jensen, L. Janss, and S.K. Rasmussen

ABSTRACT

Genomic selection, a process where all marker effects covering the entire genome are simultaneously estimated to calculate genomic estimated breeding values (GEBVs) of individuals for selection is reported as a promising tool for accelerating genetic gains in polygenic traits. Its incorporation in plant breeding is more of a reality now than it was some few years ago through the advancement in statistical analysis as well as technologies for genomic studies. Previous simulation studies as well as studies of empirical data have shown a great potential of this tool in barley breeding. This study aims at developing genomic prediction models for seed quality traits in winter barley and identifying the genes controlling these traits. Data on protein content, seed specific weight and seed fractions (of 2.8mm, 2.5mm and 2.2mm diameter) were used. The data used was collected on 184 elite winter barley lines originating from a commercial breeding program. These lines had been grown at three different locations in Denmark and genotyped using the barley 9K iSelect SNP chip.
Here we report findings from the comparison of different statistical models for their prediction abilities in the quality traits mentioned above. Results from association study performed to identify the genomic regions associated with these polygenically controlled traits would also be reported.
incredible tool for accelerating genetic gains. Its incorporation in plant breeding is more of a reality now than it was some few years ago through the advancement in statistical analysis as well as technologies for genomic studies. Previous simulation studies as well as studies of empirical data have shown a great potential of genomic selection in barley breeding. Here we report results from the comparison of different statistical models for their prediction abilities in agronomic traits such as protein content, seed specific weight and seed fraction weight of elite winter barley lines originating from a commercial breeding program. Furthermore, we also report on an association study performed to identify the genomic regions associated with these three polygenically controlled traits.

SECTION:
Plant Breeding and Genetics Education

POSTER: 14

Development of High Resolution Melting Assay for Quick and Accurate Molecular Screening for the Presence of Functional Rpg5 Alleles in Barley

Ana Badea

Agriculture and Agri-Food Canada; ana.badea@agr.gc.ca

Co-authors: B. Steffenson, W.G. Legge, J. Wang and R. Brueggeman

ABSTRACT

The highly virulent stem rust *Puccinia graminis f. sp. tritici* race TTKSK has emerged as a potentially serious threat to global wheat and barley production. Previous studies reported that susceptible barley genotypes such as Golden Promise (GP) contain a single cytosine insertion within the first exon resulting in a frame shift and a predicted truncated RPG5 protein. The molecular markers (Rpg5-LRK) that amplify across the leucine rich repeat (LRR) region to protein kinase domain in the resistant cultivars and from the LRR to protein phosphatase domain in the susceptible cultivars are used for routine screening for the presence of the functional Rpg5 gene. However, GP-like susceptible alleles will also amplify in this assay. Thus, the accessions producing this amplicon still need to be further analyzed for the presence of the GP allele that gives false positive results. This is performed by sequencing across the region, which is laborious and time consuming. Thus, a high-throughput method to identify the cytosine insertion is needed. High Resolution Melting (HRM) analysis is a relatively new, post-PCR analysis method that is gaining popularity for genotyping. To apply this methodology, primers for HRM assay were designed and validated by screening 26 barley accessions. These accessions were previously phenotyped for reaction to the TTKSK and QCCJ races. They were also screened with the Rpg5-LRK molecular markers and sequenced for the presence of the cytosine insertion. The HRM assay consistently and accurately distributed the 26 accessions into two independent clusters, with and without cytosine insertion, that perfectly match the sequencing results. Our findings suggest that the designed HRM assay is a robust genotyping method that can be used for high-throughput screening of germplasm for the presence of functional Rpg5 alleles and thus alleviating the need for direct sequencing of the amplicons.

SECTION:
Abiotic stresses

POSTER: 15

Survey of Tocols and Oil Levels in Whole Grain, Pearling Fractions and Brewer's Spent Grains of Malting and Hulless Food Barley Genotypes

Ana Badea

Agriculture and Agri-Food Canada; ana.badea@agr.gc.ca

Co-authors: K. Swallow, S.P. Johnston, M.S. Izydorczyk, and W.G. Legge

ABSTRACT

Vitamin E is a complex of eight structurally related compounds, four tocopherols and four tocotrienols, cumulatively referred to as tocols. Structural differences among the tocols are small, but have significant impacts on their metabolism and specific health benefits. The α-tocopherol has been found to reduce the risk of ischemic heart disease and cataracts, and also enhance immune system functionality while the tocotrienols have neuro-protective, anti-carcinogenic and cholesterogenesis-inhibiting proprieties not exhibited by tocopherols. Barley grain is unique because, unlike other cereals and oilseeds, it contains all eight tocol vitamers. Barley oil has long been recognized as a potential functional oil due to its health benefits owed in part to its high levels of tocols.

In the current study, the tocols composition and content as well as oil content were investigated from whole grain, pearling fractions and brewer's spent grains of 26 different malting and hulless food barley genotypes grown at two sites in Manitoba, Canada in 2014. In general, genotypic variation in the content and ratio of eight tocols was observed with the tocotrienols having a higher representation than tocopherol, regardless of sample type. The total tocols content in the whole grain was slightly higher in the hulless food barley than in the malting barley genotypes (67.3µg/g vs 60.2µg/g). Moreover, it was higher in the brewer's spent grain (109.3 µg/g) and pearling fractions than in the whole grain with the 5-10% pearling fraction recording the highest values (396.2µg/g). A significant increase was observed for oil content in the brewer's spent grain samples and the 5-10% pearling fraction versus whole grain samples (7.2 and 10.9% vs 2.1%). Our results suggest that the brewerÕs spent grain and pearling fractions of hulless food barley could potentially be used as novel functional food ingredients or extracted to yield oils enriched in health-promoting tocols.

SECTION:
Barley end use: food/feed
Barley end use: malting and brewing

POSTER: 16

Effects of sowing date and climatic conditions on two and six-row barley yield and quality

Franz-Werner Badeck

CREA-GPG; franz-werner.badeck@entecra.it

Co-authors: A. Gallo, G. Giuberti, F. Rizza, A. Gianinetti, A.M. Stanca, M. Baronchelli, A. Petrini, F. Masoero

ABSTRACT

Four barley varieties currently under cultivation in Italy, Aldebaran and Tea, usually sown for production of animal feed and of facultative and spring growth habit, and Scarlett and Tunika, commonly used for malt production, both of spring growth habit, were grown in a three year field trial. The varieties were selected in Austria, Germany and Italy with largely diverse pedigrees. The aim was to evaluate the potential for the use of winter barley for animal feeding profitting from higher yields in winter cultivation and avoiding potential summer high temperature and drought stresses.
The trials located at Tolentino (Macerata, Marche, Italy) were done with autumn as well as spring sowing. The climate conditions were characterised by humid growing seasons without relevant freezing stress and occasional high temperature episodes. The agronomic performance as well as quality traits were determined.
Results:
1) Overall, quality traits of barley grain were sufficiently good as compared to the barley tabular data reported by the Cornell Net Carbohydrate and Protein System (CNCPS) dynamic nutrition system independently of the vocation of the varieties for feed and malting.
2) Apart from the widely observed negative correlation between yield and grain protein content, there was no indication of major trade offs between yield and quality traits. Quality appears to respond in a largely independent manner to environmental drivers than does yield.
3) Especially, quality traits did not differ between autumn and spring sown conditions.
4) Thus, barley can be used as a qualitatively adequate component of animal feed, produced with autumn sowing and thereby profiting from the higher yields in autumn sowing, consuming water when precipitation is seasonally abundant and avoiding the dry and hot summer season.
5) Genetic variability in phenological traits, quality and yield can be exploited for selection of varieties to serve in specifically targeted environments.

SECTION:
Barley end use: food/feed

POSTER: 17

Genetic and molecular analysis of epistatic interactions in flowering time pathways identified in a spring barley MAGIC population

Agim Ballvora

Co-authors: N. Pesaran Afsharyan, W. Sanneman, J. Léon

ABSTRACT

Barley is the fourth most important cereal after rice, maize and wheat that provides food and feed globally. As the world food demand is increasing every year, it is necessary to investigate how to increase the yield for important crops such as cereals. As a model plant for cereals, barley provides the opportunity to study complex traits such as yield and flowering time. Flowering time is one of the major traits that effect yield. It is defined as the transition from vegetative to reproductive stage in plants and has been studied intensively in the recent years. Several genes have been identified in the pathways leading to flowering particularly in the model plants. Recent studies in barley have demonstrated the importance of epistatic interactions in the domestication-related traits like heading date, plant height and yield. The first spring barley MAGIC population from an eight-way cross of seven German landraces and one modern variety was established which is an important tool for studying epistatic interactions. A wide variation in flowering time was detected using MAGIC DH lines in two consecutive years which was not observed in originating parents. Several interacting loci have been identified. Among others, QTL interactions were found between chromosomal regions on 5H and 7H harboring flowering time genes Vrn-H1 and Vrn-H3. For a deeper understanding of molecular basis of epistatic interactions of flowering time genes, we aim to analyze the temporal and organ specific regulation of gene expression.

SECTION:
Barley morphology and development

POSTER: 18

Exploring the barley genome with BARLEX

Sebastian Beier

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben; beiers@ipk-gatersleben.de

Co-authors: C. Colmsee, A. Himmelbach, T. Schmutzer, N. Stein, U. Scholz, The International Barley Genome Sequencing Consortium, M. Mascher

ABSTRACT

The progress in sequencing and mapping technology has enabled the construction of advanced genomics resources in species with large and complex genomes. Developing draft genome assemblies are often fragmented and represented by a multi-layered framework of disparate resources. Understanding the relationship between these inhomogeneous data sets often benefits from intuitive visualization to uncover links. Towards this aim, large volumes of data from multiple sources have to be integrated into a complex infrastructure. Existing genome browsers are not well suited for this task as they expect all genomic features to be anchored to a finished linearly ordered reference sequence. We developed the genome explorer BARLEX as a central repository for all available genomic resources of barley (Hordeum vulgare L.). BARLEX is built around the 4.9 Gb long genome-wide physical map of barley consisting of more than half a million BAC clones and links it to dense genetic, optical, conformation-capture maps and an annotated whole-genome shotgun assembly. A web-based interface presents data in tabular and graphical format and makes all information and published sequence data associated with shotgun assemblies, physical contigs and annotated genes accessible with a few mouse-clicks. A novel graph-based visualization strategy was implemented to show overlaps between adjacent bacterial artificial chromosomes (BACs) based on sequence data and provides an intuitive view of all available resources in a given genomic region. BARLEX is designed as an expandable system to accommodate the steady accumulation of sequence data and provides insight into the construction of the non-redundant reference sequence of barley from fragmented BAC assemblies. The cereal research community can now take full advantage of the integrated sequence and mapping resources of the barley draft genome sequence, which is publicly accessible at http://barlex.barleysequence.org.

SECTION:
Resources I: Genome

POSTER: 19

Development and evaluation of a genotypic crop model for winter barley

Damien Beillouin

INRA (french national institute for agricultural research);
damien.beillouin@versailles.inra.fr

Co-authors: M Leclere, R. Trepos, A. Gauffreteau, M-H. Jeuffroy

ABSTRACT

The progression of areas under low input management is an European priority. Good adaptation between varietal characteristics (i.e. resistance to abiotic stresses), crop management and the environment are necessary for targeting good economic performances of low input systems. Dynamic crop models are useful tools to define both crop ideotypes/cultivar advice and management ideotypes. This study aims at developing and evaluating a dynamic barley crop (*Hordeum vulgare* L.) model including genotypic parameters. Based on an existing dynamic wheat model, parameters were adapted to barley species using three sources of information: a literature review, specific experimentations and a sensitivity analysis of the existing crop model. The predictive quality of the newly developed model was then evaluated with an independent experimentation laid out for 2 successive years with 4 genotypes and 4 Nitrogen levels. Out of the 60 parameters of the sub-model N accumulation, plant growth and yield, 38 were considered as non-depending on the species because of describing general physiologic process. Twenty-two parameters were adapted to barley s. The maximum yield, maximum thousand kernel weight, maximum number of grains per m^2 and the reduction of grain per m^2 caused by Nitrogen deficiency were considered as genotypic parameters. The relative mean square errors of prediction of the model were 18% and 10% respectively for the yield and the grain protein content. The relative mean square error of prediction of the nitrogen nutrition index at flowering and the total nitrogen grain uptake were below 15%. Impacts of the different Nitrogen management on the yield and grain protein content were well predicted. However, model improvement seemed necessary to fully take into account genotypic variability. The model will be used to identify barley ideotypes adapted to low input system.

SECTION:
Abiotic stresses
Breeding Methodologies: current approaches and future
Plant Breeding and Genetics Education

POSTER: 20

Which malting barley genotype characteristics are adapted to low input system: an experimental approach

Damien Beillouin

INRA (french national institute for agricultural research);
damien.beillouin@versailles.inra.fr

Co-authors: JF Herbommez, C. Perrot, A. Gauffreteau, M-H. Jeuffroy

ABSTRACT

The progression of areas under low input management is an European commission priority. Good adaptation between varietal characteristics (i.e. resistance to biotic and abiotic stresses), crop management and the environment are necessary for targeting good economic performances of low input systems. This study aims at identifying barley crop characteristics favorable to low input crop management. A split-plot experiment with crop management (conventional and low-input) as main factors and genotypes (20 genotypes chosen for their genetic diversity) as sub-plots were conducted in seasons 2013-2014 and 2014-2015 in 7 sites across France. Yield and its component were measured on the three replicates (min. $8m^2$). Grains were considered suitable for the malting industry if the grain protein content was between 9 and 11% and more than 90% of grains have a size over 2.5 mm. A hierarchical clustering was performed on genotypes based on the proportion of Site_year where they meet or not the malting criterions. In low input management, only 33% of the Genotype_environment combinations respected the malting specifications compared to 58% for the conventional system. However, in low input system, genotypes exhibited highly variable performances: some genotypes fulfilled the malting criterions in 66% of the Site_years whereas others never reached the malting specifications. A significant correlation (0.63) between the calibrated yield and the malting potential of genotypes was found. Groups of genotypes with a high proportion of plump grains showed a significant higher thousand-kernel weight and maximum genotypic kernel-weight and a significant lower maximum number of grains per m^2. Groups with a high rate of grain protein content over 9% did not exhibit significant different genotypic characteristics. The grain protein content was more largely impacted by the characteristics of the crop management. Develop low input barley production will thus led to define simultaneously coherent crop and management ideotypes.

SECTION:
Abiotic stresses
Plant Breeding and Genetics Education

POSTER: 21

Genotyping-by-Sequencing on Pooled Samples (Pool-GBS) and its Use in Measuring Segregation Bias during the Course of Androgenesis in Barley

Sébastien Bélanger

Université Laval; sebastien.belanger.1@me.com

Co-authors: P. Esteves, I. Clermont, M. Jean, F. Belzile

ABSTRACT

Estimation of allelic frequencies at numerous loci is often required in breeding, but genotyping many individuals at many loci can be expensive. In this work, we have developed a genotyping-by-sequencing (GBS) approach for estimating allelic frequencies on pooled samples (Pool-GBS) and used it to examine segregation distortion in two populations of doubled haploid (DH) barley lines. In a first phase, we genotyped each line of one population of DH lines individually and exploited these data to explore a strategy enabling us to call SNPs on pooled reads. We measured both the number of SNPs called and the variance of the estimated allelic frequencies at various depths of coverage on a subset of reads containing 5 to 25M reads. We show that allelic frequencies could be cost-effectively and accurately estimated at a depth of 50 reads/SNP using 15M reads. This Pool-GBS approach yielded 1,984 segregating SNPs whose allelic frequency estimates were both highly reproducible (CV=10.4%) and correlated (r = 0.9167) with the ÒtrueÓ frequency derived from analysing the individual DH lines. In the segregating population used in this first phase, five genomic regions exhibited a significant segregation distortion. In a second phase, we used this Pool-GBS approach to investigate segregation bias throughout the course of androgenesis, from microspores to a population of regenerated plants. No strong bias was detected among the microspores resulting from the meiotic divisions, whereas significant biases could be shown to arise during the formation of embryos and the regeneration of whole plants. In conclusion, this methodology provides an attractive approach to easily and efficiently estimate allelic frequencies in research materials unsuitable for individual analysis. In addition, it sheds light on the process of androgenesis in barley, revealing that segregation biases occur only during the in vitro phase of this process.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 22

Genomic Prediction in Spring Barley

Andrea Bellucci

Aarhus University, Tjele, Denmark; andrea.bellucci@mbg.au.dk

Co-authors: V. Edriss, J. D. Jensen, J. Orabi, A. Jahoor, J. Jensen

ABSTRACT

In plant breeding schemes, genomic prediction (GP) has been demonstrated to be an efficient strategy to reduce time and costs to obtain elite genotypes. GP is based on the creation of a training set constituted of lines both genome-wide genotyped and tested at different locations. These are subsequently used to obtain a model for the outcome prediction on new breeding material based solely on the genotypic information, without the need of a direct measure of the traits of interest. GP efficiency has been proved in animal breeding as well as in several crops like maize, wheat and ryegrass. In the future, genotypic platform with growing amount of markers screened at reduced costs will further increase GP efficiency. Aim of the present study is to test GP for barley and evaluate the possibility to include such strategy in future breeding programs. To this end, 3 sets of 350 barley breeding lines were tested for 2 years at three locations at Nordic Seed (Galten, Denmark). All the lines were genotyped with the iSelect Illumina 9k chip and approx. 4500 SNP markers were employed in the analysis. GP was carried on using the GBLUP model accounting fixed effects as well as random effects as GxE interactions. Several traits regarding agronomical performances and quality parameters were included in the analysis. Prediction accuracies as correlation between corrected phenotypes and genomic estimated breeding values between sets will be presented. Additionally, strategies to improve GP will be discussed.

SECTION:

POSTER: 23

Epidemiological Evolution of Barley foliar diseases in Tunisia

Hajer Ben Ghanem

INRA Tunisia; Hajeur_bg@yahoo.com

Co-authors: H. Ben Ghanem & M. Elfelah

ABSTRACT

In Tunisia, barley is the second most cultivated cereal straw (half a million hectares annually) after durum wheat and won in these areas last 3 years for him to be equivalent (over 600,000 hectares) . It is subject to a number of foliar diseases whose incidence is increasing due to the epidemiological impact of climate change particularly in barley areas, which would limit more production levels and product quality. Whatever productive and having a satisfactory level of adaptation, barley varieties currently grown in Tunisia, sin somewhere in their susceptibility to mildew, to net blotch and to scald. For these reasons, besides the selection for the stability of grain yield levels (tolerance to lack of water) and good technological quality, particular attention was paid to the selection of barley varieties with a resistance level / satisfactory tolerance to major diseases of barley in Tunisia.
This study focused on the epidemiological evolution of powdery mildew, net blotch and scald in barley in semiarid upper northwest of Tunisia, Kef during the period 2001-2011. The method was developed by Sharma and Duveiller (2003), using the scoring scale double (00-99). The first level D1 indicated the vertical level of the disease on plants and D2 indicated the contested part of the leaf area. The disease severity was calculated according to the formula: Disease severity (%) = [(D1 / 9) x (D2 / 9)] x 100. The results obtained showed that powdery mildew was unlike other diseases, much more stable in terms of vulnerability to drastic changes in the environment, because the causal fungus (*Blumeria graminis*) is required, via the now common mutations and the sexual cycle in its air populations, with the identification of a gene resistance by identifying markers via OWB-related Projects developing populations: "The Oregon Wolfe Barley population . Note also, the increasing severity of net blotch of barley in the Maghreb zone, which lived around 100 million people and 90 million livestock heads in front of a water scarcity and unprecedente rangeland degradation (2). Indeed, the trend line net blotch (Dreschleria teres) is rising with a slope of 30%, indicating the epidemiological evolution of the causal fungus. On scald (Rhychosporium secalis), there was a resistance level germplasm rated satisfactory, with a balance of two forms of the fungus and the fact that it can be controlled by means other than the genetic resistance.

SECTION:
Biotic stresses

POSTER: 24

Genetic diversity and population structure in Nordic spring barley

Therese Bengtsson

Swedish University of Agricultural Sciences, Department of Plant Breeding;
therese.bengtsson@slu.se

Co-authors: T. Bengtsson, A. Jahoor, J. Orabi, and PPP Barley Consortium.

ABSTRACT

This study is a part of the Public Private Partnership (PPP) on pre-breeding initiated by the Nordic Council of Ministers. The main goal for the Barley PPP project is to lay the foundation for effective cereal breeding for disease resistance and harvest stability in changing climatic conditions that meet current and future challenges in the Nordic region. The partners within the first phase (2012-2014) of the barley PPP project were Boreal Plant Breeding (Finland), Graminor Breeding AS (Norway), Agricultural University of Iceland (Iceland), Lantmännen Lantbruk, (Sweden), Nordic Seed and Sejet Planteforaedling I/S (Denmark) and Swedish University of Agricultural Sciences (Sweden).
179 barley lines (30 from each participating breeding company) chosen to represent the available genetic variation in current elite Nordic barley germplasm were genotyped with Illumina iSelect 9K SNP barley chip and with 48 microsatellite markers spanning over the seven chromosomes. A Mantel test showed a strong correlation between the SSR-based Modified Roger's genetic distances and the SNP-based simple matching coefficient, with a Pearson's value of $r^2 = 0.864$. However, the average polymorphic information content (PIC) value and the genetic diversity (H') was higher for the SSR markers than for the SNP markers.
The structure analysis revealed four and three groups based on the SSR and SNP markers, respectively and was further confirmed by principle coordinate analysis (PcoA). The results show that the collection has a clear level of structure, largely depending on the row-type, but also on geographical location. The genome-wide association study (GWAS) analysis identified markers linked to several traits, such as earliness and nematode and powdery mildew resistance.

SECTION:
Resources II: Germplasm and Populations

POSTER: 25

Analysis of Diversity genetic of Moroccan Net blotch populations of barley using AFLP markers

Fatiha Bentata

INRA MOROCCO; bentataiav@yahoo.fr

Co-authors: M.Labhilili, A. El Aissami, S. Bary, CH. Yeslem and J. Ibijbijen.

ABSTRACT

Net blotch caused by *P. teres f. teres* is the most harmful foliar disease in barley generating significant economic losses in Morocco. Populations of P. teres f. teres were collected from different regions of Morocco; Thirty five P. teres f. teres isolates single conidial were isolated and were subject to molecular study using AFLP technique. Out of fourteen primers combinations tested, four primers combinations were selected to disclose the polymorphism between the different P. teres f. teres isolates. The molecular characterization of these isolates showed high degree of polymorphism reaching 95% and identifying 25 specific genotypes. The genetic variability of the different isolates of P. teres f. teres within and between Moroccan regions was highlighted disclosing no linkage between the isolates and their geographical origins. This result might be due to informal material flow between regions.
Key words: Barley, Net blotch, Pyrenophora teres f. teres, AFLP, genetic diversity

SECTION:
Biotic stresses

POSTER: 26

CMS-based breeding of winter barley hybrids for bioenergy use

Timm Bernhard

Department of Plant Breeding, IFZ Research Centre for Biosystems, Land Use and Nutrition, Justus Liebig University, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany; timm.bernhard@agrar.uni-giessen.de

Co-authors: W. Friedt, R. Snowdon, B. Wittkop

ABSTRACT

Winter barley (*Hordeum vulgare* L.) is the third most important crop in Germany, mainly used for animal fodder. On the other hand winter barley can also be an interesting supplement to maize in catch crop production systems to enhance the bioenergy production per area. Thanks to its very early maturation, it can be sown as a winter catch crop and harvested prior to sowing maize or sorghum as a second bioenergy crop. Besides the increase of annual dry matter (DM) yields per area, this can reduce ecological problems like soil erosion or leaching of nutrients during winter. Presently, the majority of registered barley varieties are true breeding inbred lines. However, breeding efforts are today tending towards the incorporation of hybrid varieties, which potentially combine higher yields with a better yield stability and stress tolerance. Although a few investigations have demonstrated the grain yield advantage of barley hybrids, little work has been done focusing on the heterotic character of total DM yield of whole plant silage.
For an effective and cost-efficient seed production of hybrid barley a cytoplasmic male sterility (CMS) system is needed to ensure high hybridity. The common CMS system is known to be temperature-dependent and photo-sensitive, which currently presents a major problem for production of homogeneous hybrid seeds. Besides these environmental factors, the genetic background of potential female lines also affects the reversion of sterility, causing seed set in selfed ‘sterile’ mother lines. In first experiments, dependence of both the CMS mother lines and their maintainers on the genetic background could be observed.
The objectives of this study are the investigation of barley test hybrids for their use as whole plant silage, to discover how temperature influences the sterility/fertility of CMS lines, and to investigate how the genetic background influences undesired fertility restoration in sterile CMS-lines.

SECTION:
Plant Breeding and Genetics Education

POSTER: 27

Transcriptomic analysis of individual barley grain tissues at maturity and during very early germination

Natalie Betts

University of Adelaide, South Australia; natalie.betts@adelaide.edu.au

Co-authors: N.S. Betts, O. Berkowitz, N. Hall, V. Srivastava, H.M. Collins, N.J. Shirley, R.A. Burton, B. Skadhauge, J. Whelan, V. Bulone and G.B. Fincher

ABSTRACT

A mature barley grain contains nutrients and resources to support germination and growth of the young seedling until the plant becomes self-sustaining through photosynthesis. Mature grain also stores RNA and proteins to enable an immediate response to the water uptake that signals the beginning of germination. However, analyses of individual tissues at maturity and during very early germination have been hindered due to the difficulties in isolating tissues from dry, brittle grain. Previous work has focussed on tissue combinations and aleurone layers or protoplasts from embryo-less grain or grain that has been germinating for at least 24h.

Here, we describe a novel technique to wet the barley grain that does not initiate germination, but does allow dissection and subsequent extraction of RNA and proteins. The grain was separated into individual tissues: embryo, scutellum, aleurone, palea/lemma, pericarp/testa, starchy endosperm and the crushed cell layer. The clean aleurone sheet was divided into thirds along the distal_proximal axis. Microscopy of grain tissue sections revealed minimal cross-contamination between tissue types.

High-quality RNA was obtained from the living tissues: embryo, scutellum and aleurone. RNA of lower quality could also be extracted from the remaining non-living tissues except for the crushed cell layer, from which no RNA was recoverable. This technique for grain dissection allows, for the first time, tissue-specific identification of gene and protein expression profiles, e.g. for enzymes and their inhibitors.

RNAseq was performed on samples from grain of the elite Australian malting variety 'Navigator' at maturity and after 2h, 6h and 24h of germination. A searchable database of transcript levels is being prepared and will be made publicly available. Proteomics analysis will support the transcriptomics data. Initial results, with an emphasis on genes important for malting and cell wall remodelling, will be presented and discussed.

SECTION:
Resources I: Genome

POSTER: 28

Efficient Targeted Genome Editing on Vitamin E-Biosynthesis Related Gene in Barley by a CRISPR-Cas9 System

Hongwu Bian

Institute of Genetics, Key Laboratory for Cell and Gene Engineering of Zhejiang Province, College of Life Sciences, Zhejiang University, Hangzhou, China; Tel: 86-571-88206536; hwbian@zju.edu.cn

Co-authors: C. Liu, M. Wang, F. Guo, N. Han, J. Wang, M. Zhu

ABSTRACT

CRISPR-Cas9 system is being utilized as a powerful RNA-guided genome targeting modification technology in crop improvement. Up to now, it is no reports about the application of CRIPSR-Cas9 system as a genome editing tool in barley (*Hordeum vulgare*). Vitamin E is important for human health especially _-tocopherol which has the highest Vitamin E activity and is easy to be absorbed by human. Suppression of homogentisate geranylgeranyl transferase (HGGT) might contribute to the α-tocopherol accumulation in the Vitamin E-biosynthesis pathway. Here, we chose HGGT gene as a targeted gene to apply CRIPSR-Cas9 system in barley. Three guide RNAs (gRNAs) with a 20-nt seed region were designed to pair with HGGT gene site which are followed by the protospacer-adjacent motif (PAM). Protoplast transient expression system was used to estimate the efficiency of genome editing in barley. We compared the expression levels of Cas9 protein of various CRIPSR-Cas9 system vectors. By analyzing the RNA-guided genome-editing events, the mutation efficiency at HGGT site was estimated to be extremely different among the three gRNAs. Further sequence analysis of the PCR products showed that single gRNA system caused mainly point mutation (T-C or A-G replacement) and insertion/deletion (indel) mutations (1-8 bp lengths) at 20 bp targeted sites. Our results demonstrated that CRISPR-Cas9 system can be applied for gene targeting and genome editing in barley, and analysis of the mutation efficiency at protoplast level can provide reliable experimental selection of effective gRNAs for further stable transformation.

SECTION:

POSTER: 29

Use of chemically modified starch as a solidifying agent of medium for barley haploid production in anther culture in vitro

Olena Bilynska

Yuriev Plant Production Institute of National Academy of Agricultural Sciences of Ukraine; bilynska_genetics@yuriev.com.ua

Co-authors: P. G. Dulnev

ABSTRACT

Morphogenetic structure induction and plant regeneration in anther culture in vitro, which is known to be an effective technique for haploid and doubled haploid production, depends on different factors, including solidifying agent of medium. The aim of the study was to determine gelling properties and the effect of new chemically modified starch D-5aM on spring barley (*Hordeum vulgare* L.) haploid production via androgenesis in vitro. Six barley varieties with different in vitro androgenic ability and 22 F1 hybrid cross combinations from a program for waxy-barley breeding were used as initial material. Both control inductive medium NMSmod.2 and regenerative medium were solidified with 0.8 % agar "Difco". Concentration of D-5aM, which was added to the inductive medium instead of agar, was 12.0 %. Investigations have shown that mean green plant regeneration frequency in varieties was increased from 10.8 % on agar solidified medium to 27.7 % on starch solidified medium, having reached maximum 71.7 in Modern. This variety is a carrier of dominant gene Lks1 (awnless), and it was selected as donor both of this trait and of high androgenetic ability. Mean green plant yield was increased from 6.7 to 16.6 % with maximum at 31.5 % in hybrids obtained via cross of new Ukrainian barley varieties with lines which were carriers of waxy-gene and possessing low androgenetic ability. Within a program for waxy-barley breeding, 1,264 plants were regenerated. Chemically modified starch D-5aM used as a gelling agent of the medium for barley anther culture in vitro promoted induction of embryoids -structures with a high regeneration capacity. This perspective starch preparation is recommended for application in plant biotechnology as less costly agar substitution.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 30

Transposon Tagging Resources in *Hordeum vulgare*

Phil Bregitzer

USDA-ARS; phil.bregitzer@ars.usda.gov

Co-authors: J. Singh, P. G. Lemaux, R. H. Brown, S. Singh, and H. Q. Tan

ABSTRACT

Barley transposon tagging, using a two-component system based on the biology of the maize Activator-Dissociation (Ac-Ds) transposable element, was initiated by this group in the late 1990s with the objective of providing a forward and reverse genetics resource to the barley genetics community. In this system, near-minimal terminal sequences derived from Ac (252 and 317 bp of 5' and 3' terminal sequences, respectively), border an expression cassette bearing the selectable marker bar. This recombinant Ds transposon (Ds-bar) was introduced into barley (cv. Golden Promise) and mobilized via crosses to Golden Promise plants expressing Ac transposase. Approximately 140 lines carrying single, independent Ds-bar insertions have been characterized and released as germplasm stocks, and are available to researchers worldwide via the USDA-ARS National Plant Germplasm System. Several insertions were in genes. For instance, one line contains an insertion in the putative barley miRNA172 gene, and displays a striking abnormal phenotype in spike architecture and development. In other lines, reactivation of Ds elements that were linked to important malting quality QTLs lead to identification of Ds insertions in genes such as β-GAL1 and β-amylase-like that are potentially associated with malting quality. The transposon insertions in the majority of these lines have been mapped and the sequences into which they have inserted determined. Aspects of Ds-bar transposition in barley that are critical to using this tool, such as transposition frequencies and the n ature of transposition sites, have been determined and will be discussed.

SECTION:
Resources I: Genome
Resources II: Germplasm and Populations

POSTER: 31

Grain skinning in malting barley: understanding the environmental and genetic influences

Maree Brennan

Crop and Soil Systems, SRUC, Edinburgh, United Kingdom; Maree.Brennan@sruc.ac.uk

Co-authors: W.T.B. Thomas, P.E. Hedley, J. Morris, T. Shepherd, L. Ramsay, C.F.E. Topp, S.P. Hoad

ABSTRACT

Grain skinning, a defect in malting barley, is the whole or partial detachment of the husk from the underlying caryopsis during harvest or post-harvest handling. Grain skinning decreases malting efficiencies due to uneven germination, a key step in the malting process. The whole malting supply chain is adversely affected by grain skinning, from breeders and growers through to maltsters, brewers and distillers. Assessment of skinning severity in more than 200 spring barley cultivars from the Association Genetics of UK Elite Barley (AGOUEB) collection that have been genotyped with 7000 SNPs, among different growing environments, indicates that there is genotypic variation in susceptibility to the defect. An association genetics approach has been used to identify quantitative trait loci (QTL) that may be useful for screening against skinning in future breeding programmes. Additionally, environmental conditions that increase the severity of grain skinning have been identified and used to investigate the physiological and genetic influences on skinning severity among cultivars. Different environmental conditions alter the composition of a lipid “cementing layer” on the caryopsis surface, influencing the quality of husk adhesion and therefore skinning severity. Measurement of cementing layer thickness suggests that the composition of the cementing material has more influence on husk adhesion than the amount. Differential expression of genes in contrasting controlled environments has been examined in seven spring barley varieties, and candidate genes identified that may have a role in skinning severity, some of which are potentially involved in lipid biosynthetic pathways. This project is building our knowledge of how genotype and environmental factors together influence the severity of grain skinning, so that risk of this condition may be minimised in future varieties.

SECTION:
Barley end use: malting and brewing

POSTER: 32

Barley as a model to study host-microbiota interactions in crop plants

Davide Bulgarelli

Plant Sciences, School of Life Sciences, University of Dundee at the James Hutton Institute. Invergowrie DD2 5DA, Scotland, United Kingdom; d.bulgarelli@dundee.ac.uk

Co-authors: R. A. Terrazas, S. Robertson, K. Balbirnie and D. Bulgarelli

ABSTRACT

Plants host distinct microbial communities in the vicinity of and in association with their roots. These communities, designated the plant microbiota, engage in symbiotic relationships with their hosts that range from parasitism to mutualism. Therefore, the microbiota emerges as an extended plant phenotype capable of influencing crop yield. However, the host genetic determinants shaping the plant microbiota remain largely unknown.

My group uses barley as a model to gain fundamentally novel insights into the genetic network linking a plant genome and its associated microbiota. Using next-generation sequencing approaches, we previously characterised ~45 Gbp of the (meta)genome of the microbiota associated with wild and domesticated barley genotypes. Remarkably, this work revealed a significant effect of the plant genotype on the diversity of the microbiota, possibly representing a footprint of barley domestication.

To identify the host genetic determinants of the barley microbiota, we extended our initial investigation to a broader panel of ecologically referenced accessions. We analysed the microbiota of more than 150 plant samples grown in two agricultural soils and we are defining the recruitment units of the microbiota interfering with plant growth. This discovery will be key in guiding future genetic investigations of barley-microbiota interactions. In a parallel line of research, we demonstrated that root hair development correlates with microbiota diversification, suggesting that root system architecture contributes, at least in part, to microbiota recruitment.

Collectively, our work is paving the way for the “breeding for the microbiota”, where functions of the rhizosphere microbiota could be deployed to enhance crop yield by targeting specific genes of the plants.

SECTION:
Resources II: Germplasm and Populations
Biotic stresses

POSTER: 33

Identification and Characterisation of the Barley Row-Type Gene, VRS3

Hazel Bull

The James Hutton Institute; Hazel.bull@hutton.ac.uk

Co-authors: A.J.Flavell; R.Waugh; W.T.B. Thomas

ABSTRACT

Barley row-type describes the number of grains present at a node on the barley spike. Two forms exist amongst cultivated barley: two-rowed with only the central of three spikelets fertile producing a single grain at a node and six-rowed with all three spikelets fertile, producing three grain at a node. Twelve regions of the barley genome have been associated with the row-type character with specific genes identified at three loci, VRS1, VRS4 and INT-C. Advancements in the understanding of the genetic control underpinning barley row-type enables the identification of potential mechanisms for improving yield and yield architecture within the cereals.

This study used genetic linkage mapping in segregating F2 populations to refine the genetic location of the row-type locus, VRS3, to 16 candidate genes on barley chromosome 1H. Sequencing candidate loci in 32 vrs3 induced mutant alleles identified VRS3.

VRS3 was further characterised as a potential means of improving grain uniformity within cultivated six-rowed barley, through phenotypic assessment of grain size in varying allele combinations of VRS3, VRS1 and INT-C. The addition of six-rowed alleles at these loci was found to improve balance between central and lateral grain parameters, resulting in a more uniform grain sample.

Analysis of gene expression found Vrs3 to be constitutively expressed across a diverse panel of barley tissues. Moreover, detailed study within the developing inflorescence suggests a role for Vrs3 in the regulation of the row-type genes VRS1 and INT-C.

SECTION:
Barley morphology and development

POSTER: 34

Screening nested association mapping population for root-lesion nematode, Pratylenchus neglectus, resistance/susceptibility

Andy Burkhardt

Montana State University; andrew.burkhardt@montana.edu

Co-authors: S. Briar, L. Elmore, M. Getz, T. Hoogland, J. Sherman

ABSTRACT

Root-lesion nematode (RLN) has become an increasing pathogen among Montana cereal production (Johnson, 2007). Continuous cropping of susceptible wheat and barley can lead to economically damaging populations. Using carrot disc culture defined by Moody et al. (1973) and greenhouse screening protocols optimized by Keil et al. (2009) we screened lines from the Barley World Core for *P. neglectus* resistance in a growth chamber using a randomized complete block design with 4 replications. Barley lines were inoculated 10 days after planting with 400 P. neglectus juveniles of varying growth stages. Nine weeks after inoculations, nematodes were extracted g from soil and roots using Baermann funnel and misting chamber techniques, respectively. Extracted nematodes were counted on a counting slide. A Pf/Pi (final to initial population) ratio was calculated as the quotient of the initial number of nematodes inoculated and the final number of nematodes extracted after the screening. These ratios were then analyzed for significant differences. The Barley world Core lines screened are parents in a Barley Two Row Nested Association Mapping Panel (NAM), consisting of 100 families and about 7500 lines. A goal of this study is to genetically dissect for root-lesion nematode resistance by mapping in the 2 row NAM. A long term goal is to utilize newly identified genes to improve barley for nematode resistance.

SECTION:
Biotic stresses

POSTER: 35

Identification of specific genes for Al tolerance in Tibetan wild and cultivated barleys

Fangbin Cao

Department of Agronomy, College of Agriculture and Biotechnology, Zijingang Campus, Zhejiang University, Hangzhou, China; fangbincao@163.com

Co-authors: H. Dai, F. Wu

ABSTRACT

To better understand the mechanisms involved in Al toxicity and tolerance in plants, microarray technology was used to evaluate changes in gene expression in roots of two contrasting Tibetan wild barley genotypes XZ16 and XZ61 and cv. Dayton under 200 μM Al stress. With the use of Affymetrix Genechip, a comparison of RNA expression profiles was made between control and Al-treated barley seedlings. A total of 15 genes were upregulated in XZ16 but downregulated in XZ61, or 682 upregulated in XZ16 but unchanged in XZ61, 553 genes unchanged in XZ16 but unchanged in XZ61. These genes were identified as Al-responsive in XZ16. We found that some of the genes were associated with the function of signal factors including abscisic acid, Ca2+, salicylate, gibberellins, jasmonic acid and ethylene. These signal factors improved the expression of a series of identified genes which could be categorized mainly in detoxificants, transporters, lipid metabolism, defense, transcription and carbohydrate metabolism, etc. Furthermore, these genes in XZ16 attributed the Al tolerance mechanisms consist of ROS scavenge, ion homeostasis, production of ATP, synthesis of acetyl-CoA, protein conformation and stabilization, DNA replication, glycolysis, TCA cycle, cell wall biosynthesis and elongation remodeling, etc. Thus, not only citrate secretion, the ABC transports, some types of ATPases and several genes relevant to tricarboxylic acid cycle were newly found to be associated with the Al resistance in XZ16.

SECTION:
Abiotic stresses

POSTER: 36

Association of SNP markers and chlorophyll fluorescence parameters with low temperature tolerance in spring barley

Ludovic Capo-chichi

Alberta Innovates-Technology Futures; Ludovic.Capo-chichi@albertainnovates.ca

Co-authors: J. Nyachiro, P. Juskiw, A. D. Beattie, J. J. Slaski

ABSTRACT

Low soil and air temperatures in the spring occur due to Arctic air masses bringing down sub-zero temperatures to the Canadian Prairies. Each year, millions of dollars can be lost due to these frigid temperatures causing seedling mortality, reduced vigor, and yield reduction. The use of chlorophyll fluorescence to measure photosynthetic efficiency in plants under stress is now widespread. The objectives of this study were to 1) evaluate the effects of low temperatures on emergence and seedling performance among spring barley germplasm, 2) introgress cold hardiness genes from winter-hardy barley into spring barley, and 3) conduct an association mapping study to identify markers linked to alleles for improved cold tolerance. Untreated field-grown seeds of 95 genotypes were sown in pots containing field soil. Two replicates of 25 seeds for each genotype were seeded 1.5 cm deep in pots. Seedling emergence was assessed at 5 °C, 10 °C and 15 °C. Cold treatments consisted of cold acclimation and freeze shocking of the seedlings at the three-leaf stage. Prior to cold treatments, the chlorophyll fluorescence values FV/FM varied from 0.739 to 0.766. Values measured 24 hours after cold acclimation varied from 0.125 to 0.752. Under freezing-shock treatment, the values varied from 0 to 0.517, leading to irreversible damage for non-hardy genotypes. The survival rates are higher under cold acclimation than under freeze shock treatment. Frost injury was visually rated as well as being inferred from the measurement of the chlorophyll fluorescence parameter ($FV/FM = (FM - F_0)/FM$). Cold acclimation was highly correlated with survival rates and showed wider variation among genotypes for cold tolerance than the freezing shock did. A total of 7864 SNPs were used for the genome wide association analysis (GWAS). The GWAS analyses identified 39 positive SNP associations with the chlorophyll fluorescence of which 19 were under cold acclimation and 20 were under freezing shock treatment.

SECTION:
Resources I: Genome
Abiotic stresses
Breeding Methodologies: current approaches and future

POSTER: 37

Characterization of spot form net blotch of barley using host-pathogen genetics

Steven Carlsen

North Dakota State University; steven.carlsen@ndsu.edu

Co-authors: J. M. Richards, S.S. Xu, J.D. Faris, R. S. Brueggeman, T. L. Friesen

ABSTRACT

Spot form net blotch (SFNB) caused by the necrotrophic fungal pathogen P. teres f. maculata is a major foliar pathogen of barley. Currently little is known about the P. teres f. maculata-barley interaction. To further understand SFNB, pathogen and host genetics approaches will be used to dissect the P. teres f. maculata-barley interaction. The fungal genetics of a *P. teres f. maculata* mapping population, FGOB10Ptm-1 × SG1 will be utilized to explain the virulence/avirulence present in the pathogen. A mapping population of 118 progeny generated from the FGOB10Ptm-1 × SG1 cross was phenotyped using 13 barley genotypes demonstrating a differential reaction to the two parental isolates. Genotyping was performed using a restriction associated DNA (RAD) GBS approach optimized for the Ion Torrent PGM. A total of 983 SNP markers were distributed across 16 linkage groups with a total map size of 1650 cM. QTL analysis identified more than 25 genomic regions associated with P. teres f. maculata virulence. On the host side, populations between popular Upper Midwest malting varieties (Tradition, Pinnacle, AC Metcalfe) and resistant lines (PI 67381, PI 84314) are being used to genetically characterize resistance/lack of susceptibility. Host populations will be genotyped using a PCR based genotype-by-sequencing (GBS) method, optimized for the Ion Torrent PGM. Population specific panels will be designed utilizing the 9K SNP data from the T-CAP T3 database to ensure even distribution of markers across all barley chromosomes. The development of robust barley genetic maps and the generation of quality phenotypic data will allow for the genetic characterization of SFNB resistance and/or susceptibility. Host progeny containing all resistance and/or lack of susceptibility QTL will then be utilized in backcrossing strategies to develop elite prebreeding lines with improved SFNB resistance.

SECTION:
Biotic stresses

POSTER: 38

Spike architecture and downstream genes in VRS3 mutants

Maria Cristina Casao

The James Hutton Institute; Cristina.Casao@hutton.ac.uk

Co-authors: M. Zwirek, S. McKim, H. Bull, M. Lafos, V. Patil, R Waugh

ABSTRACT

Productivity is one of the major concerns of grain crops and is the most important economic driver of barley improvement. However, yield and yield stability are complex traits controlled by multiple genes and influenced by agronomic and environmental conditions. One of the most important yield components is spike architecture, which can develop either two- or six rows of grains. This morphological and developmental switch is generally accepted to result from recessive mutations in VRS genes. One of the VRS gene of particular interest, VRS3, promotes a partial or incomplete six- row, called “intermedium” and a double-awned phenotype.

To understand how the VRS3 phenotype is elaborated, we are identifying the downstream genes involved in producing the striking intermedium spike phenotype. We are conducting RNA-seq expression analysis at two different developmental stages, in the two-rowed wild type barley (cv Bowman) and the intermedium Bowman-derived introgression line containing the vrs3 mutation. In this experiment, apices at the same developmental stages are being harvested for morphological characterization using SEM and in-situ hybridization. By revealing global changes in gene expression driven by VRS3 function, we expect to provide novel tools that allow breeders to tailor varieties with higher productivity adapted to different environments.

SECTION:
Barley morphology and development

POSTER: 39

Mapping of Stem Rust Resistance Genes Rpg2 and Rpg3 in Barley

Austin Case

University of Minnesota; case0197@umn.edu

Co-authors: B.J. Steffenson, G. Macharia

ABSTRACT

The barley stem rust resistance genes Rpg2 and Rpg3 confer resistance to a variety of *Puccinia graminis f. sp. tritici* (Pgt) and *Puccinia graminis f. sp. secalis* (Pgs) races. The source of Rpg2 is Hietpas-5 (CIho 7124), a selection made from Oderbrucker in 1921 in Wisconsin. Oderbrucker is a landrace from Germany. Rpg3 is derived from GAW 79-3 (PI 382313) a landrace from Ethiopia. While these genes are known to confer intermediate to high levels of resistance to isolates of Pgt and Pgs their chromosomal location is unknown. To investigate the resistance conferred by these genes and map their location in the barley genome we crossed Hietpas-5 and GAW 79-3 to Hiproly (PI 60693), a highly susceptible to stem rust. Two mapping populations were created by single seed descent to create F5:7 RIL. These populations were then genotyped with Genotype by Sequencing and a map representing all seven chromosomes of barley was constructed. Plants were evaluated in the field for reaction to Pgt race QCCJB, MCCFC and HKHJC in St. Paul, Minnesota and for reaction to African stem rust in Njoro, Kenya. Data were then used in QTL mapping to detect significant marker trait associations. Significant QTL on chromosome 2H were detected in all locations for in the Rpg2 mapping population. Suggesting, Rpg2 is located on the long arm of chromosome 2H. In the Rpg3 mapping population, significant hits were detected on the short arm of chromosome 5H and 4H. The likely location of Rpg3 is the short arm of 5H based on race specificity; however, it is possible that the resistance carried by GAW 79-3 is multigenic.

SECTION:
Resources I: Genome
Biotic stresses

POSTER: 40

A new Vrs1 allele identified in 2-row Spanish landraces

Ana M. Casas

CSIC; acasas@eead.csic.es

Co-authors: B. Contreras-Moreira, S. Sakuma, M.P. Gracia, M. Moralejo, J.L. Molina-Cano, T. Komatsuda, E. Igartua

ABSTRACT

Vrs1, the gene determining the type of spike in barley has been extensively studied. The wild dominant allele encodes a homeodomain-leucine zipper transcription factor whose activity results in a two-rowed spike, whereas the recessive allele produces a six-row phenotype. Phylogenetic analysis of barley cultivars identified two alleles in two-rowed types and at least four different alleles in six-rowed barleys. Previous results genotyping with MWG699, a marker closely linked to Vrs1, suggested different geographic origins for six-row alleles, among them the vrs1.a2 allele originated in the Western Mediterranean. Using this same marker, we showed that a large proportion of Spanish and Moroccan landraces (both 2- and 6-rowed) as well as Moroccan Hordeum spontaneum lines, all shared the same haplotype. We then analyzed the sequence of the Vrs1 gene in those lines. All (11) six-rowed barleys sequenced carried the vrs1.a2 allele, but we found different Vrs1 alleles among 2-rowed types: the material from Morocco, both wild (3) and cultivated (2), carried the Vrs1.b2 allele, which was absent from the Spanish 2-rowed landraces studied. The most common allele among these was Vrs1.b3, in 46 lines out of 53 evaluated. The other seven lines presented a new Vrs1 allele, Vrs1.b5. This new allele contains a 'T' insertion in exon 2, originally proposed as the causal mutation giving rise to the 6-row vrs1.a2 allele, but has an additional upstream deletion that results in the change of 15 amino acids and a potentially functional protein. These results add a new hypothesis to the origin of the 6-rowed vrs1.a2 allele, which could result from a mutation either at the Vrs1.b2 allele (classical hypothesis) or at the newly found Vrs1.b5 allele identified in Spanish landraces.

SECTION:
Resources I: Genome

POSTER: 41

Genetic control of phenology in INIA Ceibo x Norteña Carumbé under temperate conditions in South America

Ariel Castro

Departamento de Producción Vegetal, Est. Exp. "Dr. Mario A. Cassinoni", Facultad de Agronomía, Universidad de la República; vontruch@fagro.edu.uy

Co-authors: P. Grignola, S. Bartaburu, A. Locatelli, J. Mosqueira, and L. Viega

ABSTRACT

Understanding the genetic control of phenology, in particular the control of pre-anthesis phases, is key for the development of novel genotypes with tailored phenology. In South American conditions (and particularly in Uruguay) an increase in the length of the critical period (GS32-GS49) should not result in delayed grain filling in order to profit from a higher in yield potential. In order to advance in the knowledge of the issue we studied a RIL population derived from the cross of two adapted varieties with contrasting phenology: INIA Ceibo (late variety with photoperiod sensitivity) and Norteña Carumbé (early and insensitive). The population was genotyped with 134 SNPs and 9 SSRs. The population was phenotyped in five field experiments (two years, contrasting planting dates) and a total of ten phenotypes were measured, (lenght of GS00-GS22, GS22-GS30, GS30-GS49, GS00-GSZ49, GS49-GS90 and photoperiod response in all of them). Two QTLs, on chromosomes 2H and 3H with coincident location with eps2S and denso, explained most of the phenotypic variation for anthesis date: denso affected the lenght of GS22-GS30 and eps2S affected the lenght of GS30-GS49. Photoperiod sensitivity for anthesis time was explained by differences in the magnitude of these effects on contrasting planting dates and effects on 2H (coincident with Ppd-H1).

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 42

Phenology and breeding origin are the main determinants of genetic diversity structure in South American barley

Ariel Castro

Departamento de Producción Vegetal, Est. Exp. "Dr. Mario A. Cassinoni", Facultad de Agronomía, Universidad de la República; vontruch@fagro.edu.uy

Co-authors: S. Brandariz, L. Gutierrez, A. Locatelli, and P. Speranza

ABSTRACT

Barley (*Hordeum vulgare* L.) is an important crop in the southern cone of South America where it is mainly used for malting. Understanding the structure of the genetic diversity available for breeding programs is essential for the decision-making process. This includes understanding the genetic basis of traits of interest and the relationships among germplasm sources that are being used. The aim of this study was to analyze the genetic diversity of the germplasm used in a malting barley breeding in South America by studying commercial varieties, advanced lines from breeding programs and their common ancestors in order to understand the historical evolution of the diversity through breeding and to identify the main factors driving that evolution. To address this objective we used several approaches combining ancestry, phenotypic and genetic data together with QTL analysis information for the same population. Analyses of phenotypic diversity showed the effect of phenological traits on yield and grain size traits but they also showed a lack of correlation between agronomic and malting traits, which can theoretically allow for cumulative breeding progress for both groups of traits. Coancestry clustered genotypes according to their origin but these groupings were not highly correlated with marker-based, QTL-based or phenology-based clusterings. The highest correlation between marker-based and QTL-based clustering was recovered when using phenology-related QTL. Phenological traits were significantly different among marker-based clusters reflecting the effect of phenology on the structure of the population. The use of several and independent approaches to study diversity complemented each other and provides a more complete picture of it.

SECTION:
Resources I: Genome
Resources II: Germplasm and Populations
Breeding Methodologies: current approaches and future

POSTER: 43

Required for Mla resistance 3, a new player in barley powdery mildew resistance?

Antony Chapman

Iowa State University; antonyc@iastate.edu

Co-authors: P. Surana, M.R. Hunt, G. Fuerst and R.P. Wise

ABSTRACT

Barley alleles of Mildew resistance locus a (Mla) encode c. 30 variants of CC-NB-LRR proteins. These proteins recognise corresponding avirulence (AVR) effectors secreted by the obligate fungal pathogen, *Blumeria graminis f. sp. hordei* (Bgh), triggering defense. Key factors in this resistance pathway include Required for Mla12 Resistance 1 (Rar1), Suppressor of G-two allele of skp1 (SGT1), Heat-shock protein 90 (HSP90) and several WRKY and MYB transcription factors (TFs).

Fast-neutron mutagenesis of barley CI 16151 (Mla6) generated the mutant m11526, which displayed susceptibility to the avirulent isolate Bgh 5874 (AVRa6). Complementation tests revealed that the susceptibility was not due to mutations in Rar1 or Mla6. Sequence-based analysis also ruled out other known members of the Mla resistance pathway, notably SGT1, HSP90 and all WRKY/MYB TFs. This suggests that the mutation has occurred in a novel gene, which we designated as Required for Mla resistance 3 (Rar3). Several strategies are being used to isolate Rar3, including transcript based cloning, exome capture, and genome resequencing.

Crosses of m11526 with lines carrying other Mla alleles (Mla1, Mla7, Mla10, Mla12, Mla13 & Mla15) were used to indicate which alleles do and, most intriguingly, do not require Rar3 to function. This differential requirement of Rar3 by Mla alleles is reminiscent of Rar1 dependence. These data suggest that RAR3 functions in the Mla pathway at, or upstream of, RAR1. Lastly, Barley1 GeneChip differential transcriptome analysis revealed that while Rar1 mutants affect expression of a dozen genes, Rar3 influences over one-thousand.

Supported by NSF-PGRP grants 09-22746 and 13-39348.

SECTION:
Biotic stresses

POSTER: 44

Outcrossing Percentage of Genetic Male-steriles Derived from “Baronesse” in a Lowland Mediterranean Environment

M. Ilhan Çağırgan

Akdeniz University, Antalya, Turkey; cagirgan@akdeniz.edu.tr

Co-authors: A. Adak, N. Başkaya Demir, H. Topuz

ABSTRACT

Baronesse is a two–row feed barley that is every breeder’s dream about excellent yield potential and very wide adaptation. It was bred in Germany and has been marketed in Pacific Northwest, USA. The high number of spontaneous genetic male sterile (ms) mutants isolated from farmers’ fields in Pullman, WA, in 1997 were previously characterized, and revealed that they were completely sterile under isolation and at least two of them, Bns-40ms and Bns-54ms, were non-allelic to each other. Consequently, we used them extensively to pyramid drought tolerance-related traits by developing two-, double-, and 8-way crosses manually, resulting better performing pure lines and sub-populations from ”Baronesse”. To develop recurrent selection strategies facilitated by male sterility in Antalya, Turkey, we have studied recently random outcrossing characteristics of the male-sterile spikes segregating in 19 sub-populations. The genetic material was grown in drill plots of 6 row and 7 m long and planted on 30 November 2012 applying regular agronomic practices for barley. The range for days to heading was 12 days among the sub-populations; the late ones were winter types. Fifty ms spikes at harvest, where available, were sampled and outcrossing percentage was calculated dividing number of seed set by number of spikelet. Outcrossing ranged between 1.74-9.50%, much higher than the dryland upland values obtained previously in Kθzθlkaya, Burdur. The highest value was obtained in a spring X winter type cross (7037). The range for percentage of ms-spikes at least with one-seed set was 26-78%. The crosses sharing a winter and/or early heading component have shown a tendency to higher outcrossing percentage by providing, probably, turgidity to lengthen the period to receipt of the pollens by stigma and viable pollen shedding. In conclusion, there were clear differences in outcrossing and agronomic characteristics among the populations generated by their genetic make-up, which may be exploited successfully for practical breeding purposes to develop populations and pure lines better than “Baronesse”.

SECTION:

POSTER: 45

CAD gene knock-out with CRISPR/Cas9 in barley to improve bioethanol production

Cecilie Christensen

University of Copenhagen, Faculty of Science, Institute of plant and environmental sciences; cslc@plen.ku.dk

Co-authors: C. Gao, Y. Wang, B. J¿rgensen & S. K. Rasmussen

ABSTRACT

The aim of this project is to increase bioethanol production from barley straws by reducing and/or alter the composition of lignin. This will be done by knocking-out the genes coding for cinnamyl alcohol dehydrogenase (CAD), controlling the last step in the lignin biosynthesis with the new CRISPR/Cas9 genome editing method.
Barley straws are an abundant source for alternative energy. They contain cellulose that can be converted into bioethanol and are often considered a waste product. However, cellulose is tightly bound by lignin which is recalcitrance to saccharification. Down-regulating of Cinnamyl Alcohol Dehydrogenase (CAD) encoding the last step in the biosynthesis of mono-lignols have shown to benefit the saccharification process and thereby increase bioethanol production. Here we will use the new CRISPR/Cas9 genome editing method to knock-out the CAD2 gene in barley. Active sgRNA target site for CAD2 verified in barley protoplast have been used for CRISPR/Cas9 transformation of barley plants.

SECTION:
Plant Breeding and Genetics Education

POSTER: 46

Changes in the biochemical composition, morphology and transcript profiles of genes, during starch and cell wall synthesis and degradation from anthesis to germination of barley grain

Helen Collins

University of Adelaide; helen.collins@adelaide.edu.au

Co-authors: H Lou, L Wilkinson, SF Khor, NJ Shirley, S Cu, M. Marchetti, B Skadhauge, RA Burton, GB Fincher, NS Betts

ABSTRACT

The endosperm of barley begins development as a large, multinucleate cell called a syncytium. Between 4 and 6 days after pollination (DAP), this central vacuole undergoes cellularisation until the endosperm is completely compartmentalized into cells (Wilson et al., 2006). During the next 3 weeks, the central endosperm undergoes differentiation, depositing starch and protein into cells and building cell walls, mainly of (1,3;1,4)- β -glucans and arabinoxylans. The aleurone appears around the exterior of the endosperm and can easily be distinguished by 10 DAP. At approximately 28 DAP, the grain begins to mature and lose water (Wilson et al., 2012).

Mature dry barley grain consists of approximately 65% starch, 15% cell walls and 10% protein (MacGregor and Fincher, 1993). Upon hydration, hormones are released from the embryo and migrate to the scutellum and aleurone, causing a number of lytic enzymes to be released or produced de novo (O'Brien et al., 2011). These enzymes migrate into the starchy endosperm, hydrolysing the stored reserves that are in turn transported back to support the growing embryo.

During both the development and germination of barley, transcription occurs of a large number of genes encoding enzymes in the starch and cell wall biosynthetic and hydrolytic pathways. In this study, we have tracked the transcript profiles of key genes in these pathways during grain development and germination using quantitative-PCR (Q-PCR) and compared these profiles to compositional and morphological changes within the grain using biochemical and immunohistochemical techniques.

SECTION:
Barley morphology and development

POSTER: 47

Genetic variation for malting quality responses to different protein levels in barley grain

Suong Cu

The University of Adelaide, School of Agriculture, Food and Wine;
suong.cu@adelaide.edu.au

Co-authors: S. Stewart, K. Porker, J. Lahnstein, S. Degner, R. A. Burton, M. Marchetti, B. Skadhauge, O. Olsen, G. B. Fincher and J. Eglinton

ABSTRACT

Protein content of barley grain is influenced by many factors, including agronomic management, climate and cultivar. The interactions between grain protein content and functional quality characteristics are well understood and form the basis for malting barley selection and trade. Improvements in the quality of new varieties and emerging interest in differences in processing behaviour provide an opportunity to examine genetic variation in grain protein levels and their relationships with malt quality. In this study, four Australian barley varieties were grown under various nitrogen regimes to produce grains with a range of protein levels. The changes in physical and biochemical characteristics of the grain were investigated. The most prominent effects were found in hordein contents, (1,3;1,4)-β-glucan content and fine structure. The responses differed between varieties. No significant effects were observed in grain hardness, arabinoxylan content, amylose/amylopectin ratio or in the hordein D fraction. Correlations with malting quality parameters including α-amylase, β-amylase, apparent attenuation limit, diastatic power, malt protein, soluble protein, Kolbach index, free amino acid nitrogen, hot water extract, wort (1,3;1,4)- β-glucan and viscosity were investigated.

SECTION:
Barley end use: malting and brewing

POSTER: 48

A draft genome of hulless barley and its domestication of cold adaptation and tolerance

Fei Dai

Department of Agronomy, Zhejiang University, Hangzhou, China; fdai@zju.edu.cn

Co-authors: X.L. Wang, Z.H. Chen, and G.P. Zhang

ABSTRACT

Cultivated barley (*Hordeum vulgare* L.) is one of the earliest domesticated crops in the world. Low temperature is one of the major abiotic stresses limiting crop yield and distribution. A better understanding of low temperature tolerance mechanisms is imperative for developing the crop cultivars with high tolerance. The Tibetan hulless (naked) barley, also called "Qingke" in Chinese, is a main food crop in the Tibetan Plateau, characterized by its high altitude. In this study we investigated the genome of the Tibetan hulless barley and its domestication in cold adaptation and tolerance. The results showed that the hulless barley had existed at the early domestication stage of, barley, in view of its difference from modern cultivated barley at genomic level and closely genetic similarity with wild barley. Meanwhile, Tibetan hulless barley has developed the adaptation to low temperature and high altitude. In addition, it harbors a myriad of mutations favorable for its adaptation to cold environment. Furthermore, we performed a RNA-sequencing experiment using two barley genotypes differing in freezing tolerance (Nure, tolerant and Tremois, sensitive), to determine the transcriptome profiling and genotypic difference under freezing shock treatment. Some key genes associated with Ca2+ signaling, PtdOH signaling, CBFs pathway, ABA pathway, jasmonate pathway and amylohydrolysis pathway were identified. Accordingly, we constructed the pathway network, which may provide a platform for better understanding the functions of genes involved in low temperature tolerance in barley, as well as the domestication of the relevant genes. Based on a draft genome sequence of Tibetan hulless barley and the large amounts of RNA sequencing data, we made the discussions on the domestication of cold adaptation genes or pathways in cultivated barley.

SECTION:
Abiotic stresses

POSTER: 49

Recombinant Chromosome Substitution Lines (RCSLs) as a source of genetic variation for drought stress tolerance in barley

Carla de la Fuente Cantó
The James Hutton Institute, Invergowrie, DD2 5DA, Dundee, UK;

Co-authors: T.S. George, J. Russell, C.A. Hackett and R. Waugh

ABSTRACT

Recombinant Chromosome Substitution Lines (RCSLs) derived from a cross between an elite barley cultivar 'Harrington' used as the recurrent parent, and Hordeum vulgare subsp. spontaneum accession from a dry region in the Fertile Crescent, as the donor parent, are being used to identify chromosome regions associated with drought tolerance (Matus et al., 2003). Twenty-nine RCSLs representing the whole genome of the wild barley accession through overlapping and flanking chromosomal introgressed regions have been selected for phenotypic evaluations under controlled and field conditions. In addition to this, the genotypic architecture of the lines has been determined for 1845 polymorphic SNP markers from the 9K iSelect SNP chip for barley.

The field trials were carried out under rainout shelters to evaluate the effect of water stress in the RCSLs performance during 2013 and 2014 growing seasons. Morphological, developmental and agronomic traits showed a consistent and wide variation over both years. A mixed model analysis was designed to define the regions of the exotic genome that might be contributing to the phenotype observed as well as the groups of lines with contrasting response to drought. We have been able to locate major QTLs for traits related to the drought tolerance phenotype and we have found evidence indicating that introgression of the wild progenitor parent contributes to enhanced agronomic performance of the elite cultivar under these conditions.

With this information, root phenotyping experiments under controlled conditions were set up using extreme lines. We found genotypic variation in the development of the root system at the seedling stage suggesting that root traits may play an important role in how the RCSLs perform in water-limited conditions in the field. Work is underway to define the chromosome regions associated with the root system architectural traits segregating in the 29 RCSLs and its effect on the aboveground performance under drought.

Matus, I., Corey, A., Filichkin, T. et al. (2003). Development and characterization of recombinant chromosome substitution lines (RCSLs) using Hordeum vulgare subsp. spontaneum as a source of donor alleles in a Hordeum vulgare subsp. vulgare background. Genome, 46, 1010-1023.

SECTION:
Abiotic stresses

POSTER: 50

Allele mining of wild barley resistance genes using a nested association mapping (NAM) approach

Vera Draba

Martin-Luther University Halle-Wittenberg (MLU), Institute of Agricultural and Nutritional Sciences, Chair of Plant Breeding, Betty-Heimann-Str. 3, D-06120 Halle (Saale), Germany; vera.draba@landw.uni-halle.de

Co-authors: D. Kopahnke, B. Büttner, A. Maurer, F. Schnaithmann, F. Ordon, G. Schweizer, and K. Pillen

ABSTRACT

Wild barley is an important resource for new resistance genes against different barley diseases. One possibility to tap into the wealth of this genetic diversity is the recently implemented nested association mapping (NAM) approach (Yu et al. 2008, Genetics 178, 539-551; McMullen et al. 2009, Science 325, 737-740).

Therefore in our group the barley NAM population HEB-25 has been developed during the last years, consisting of 1,420 lines, originating from crosses of the spring barley cultivar Barke with 25 accessions of wild barley (*Hordeum vulgare ssp. spontaneum* and *H. v. ssp. agriocrithon*). The complete HEB-25 population has been genotyped with the barley Infinium iSelect 9k SNP chip (Illumina), characterizing the allelic constitution of 7,864 SNPs in total.

In cooperation with the Julius Kühn-Institute, Quedlinburg and the Bavarian State Research Center, Freising-Weihenstephan, seedlings and young plants of HEB-25 were phenotypically characterized in regard to resistance against three important barley diseases, leaf rust (*Puccinia hordei*), scald (*Rhynchosporium commune*) and net blotch (*Pyrenophora teres*). Additionally, adult plant resistance against leaf rust in field was examined in three consecutive years at the Kühnfeld experimental station of the University of Halle.

Subsequently, the obtained data were subjected to genome wide association studies (GWAS) in order to locate genes, which confer pathogen resistance across the NAM population or within individual families of HEB-25. We detected several beneficial QTL across almost all barley chromosomes controlling the traits of our interest and in some cases the exotic donor allele conferred disease resistance.

Finally, we identified some HEB-lines with combined resistances against at least two of the three barley leaf diseases under examination.

SECTION:
Biotic stresses

POSTER: 51

Putting rust to sleep in Australia: Breeding for durable resistance to rust diseases in barley

Peter Dracatos

University of Sydney; peter.dracatos@sydney.edu.au

Co-authors: D. Singh, and R.F Park

ABSTRACT

In Australia, barley (*Hordeum vulgare* L.) is the second most important cereal crop, with an estimated annual production of seven million tonnes. With a reputation for consistent, contaminant free high yielding barley varieties, Australia currently has the highest malting selection rate of all barley exporting countries. However, barley is susceptible to foliar diseases, including the Puccinia rust pathogens. Leaf rust, caused by Puccinia hordei, is one of the most destructive foliar diseases of barley, and has caused significant yield losses in many regions where barley is grown. Yield reductions of up to 32% have been reported in certain susceptible barley cultivars in both Australia and North America. Due to potentially adverse environmental effects of fungicides, the most preferable and cost-effective means of controlling barley leaf rust is through the development and deployment of durable host resistance. In cereals, two major types of resistance have been described for rust pathogens, seedling resistance and adult plant resistance (APR). Seedling resistance genes are effective at all stages of crop development and are often characterized by a hypersensitive response. Numerous genes conferring seedling resistance to *P. hordei* (Rph) have been identified (Rph1-Rph19, Rph21-Rph22); however, virulence matching most of these genes has been detected. In some regions, including South Australia, the presence of the alternate host *Ornithogalum umbellatum* ('Star of Bethlehem') can permit sexual recombination and increase the likelihood of new virulent pathotypes developing. Unlike major gene (all stage) resistance, single APR genes confer minor levels of resistance and their expression is dependent on growth stage, environment, inoculum load and pathogen virulence. APR genes also tend to be additive, meaning that combinations of two or more can lead to very high levels of resistance. This paper will outline the recent progress made at PBI-Cobbitty Australia in the genetic analysis, marker assisted selection and positional cloning of new sources of Adult Plant Resistance to leaf rust in barley.

SECTION:
Success stories in barley breeding and genetics
Resources II: Germplasm and Populations
Biotic stresses

POSTER: 52

Fine mapping and towards cloning of resistance gene rym7 against Barley mild mosaic virus.

Lisa Eichel

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK); eichel@ipk-gatersleben.de

Co-authors: A. Habekuss, P. Yang, E. Laubach, F. Ordon, N. Stein

ABSTRACT

Barley (*Hordeum vulgare* L.) is one of the four most important cereal crop species. Barley production is affected by a numerous diseases that cause high economic damages. One of these, the Barley yellow mosaic virus disease, is caused by different strains of the Barley Yellow Mosaic Virus (BaYMV) and the Barley Mild Mosaic Virus (BaMMV) complex. The virus belongs to the genus of Bymovirus of the family Potyviridaee. First described in Germany in 1978 it is now widespread across Europe. The soil born plasmodiophorid Polymyxa graminis Led., an obligate intercellular parasite of cereal roots, serves as a vector for virus transmission. It survives in the soil as highly persistent resting spores. Under appropriate conditions in late winter or early spring mobile zoospores are released which infect the host`s root system and transfer the virus. Infected plants show a pale green leave phenotype with a typical mosaic pattern on the youngest leaves. At maturity the whole plant habitus is severely stunted. Infections in susceptible barley genotypes can lead to over 50% yield loss. Breeding for resistance is the only measure to control the disease and a number of recessive resistance loci have been identified in the barley genome. Two of the underlying genes, rym4/5 and rym11, both conferring complete resistance, have been cloned recently. Our contribution to the current project COBRA is to isolate the gene rym7, that is located on chromosome 1H close to the centromeric region and confers partial resistance to BaMMV, but not to BaYMV and BaYMV-2, under greenhouse and field conditions. The differential response of the gene is of special interest since the isolation of the gene may provide important insights at the strain specific regulation of virus and host factors in this host-pathogen system.

SECTION:
Biotic stresses
Plant Breeding and Genetics Education

POSTER: 53

Barley in Tunisia : a long-way breeding story

Mouldi El Felah

National Agronomic Research Institute of Tunisia (INRAT); elfelah.mouldi@gmail.com

Co-authors: H. Ben Ghanem

ABSTRACT

Barley (*Hordeum vulgare* L.) is one of the oldest crops in the world and played a key role in agriculture and science development. In the world, barley is used mainly for feed (55-60%), for malt (30-40%), for food (2-3%) and for seed (5%). Average world barley production is about 140 million tons (2003-2007). For Maghreb countries, barley production in the late decade (2000-2009) was about 3.172 million tons, adjusted by average importation of 1.384 million tons, with annual total need of 45.556 million quintals. For Tunisia, annual total barley were estimated to 0.9 million ton, with 0.4 million tons produced in the country.

Tunisian barley improvement program have been reviewed for the late 98 years (1914-2008). Local barleys characterization revealed intensive information on secondary evolution of Hordeum tribe within regional distribution across the country depending on uses, farming systems and eco-geographic adaptation. Barley improvement started early in 1893. During this period, more than 40 barley varieties were cultivated in the country. Rihane registered in 1987, has been widely cultivated in the country and in the region and participate actively in barley production records.

SECTION:
Success stories in barley breeding and genetics

POSTER: 54

Analysis of Molecular Diversity and Population Structure in Barley (*Hordeum vulgare* L.) inferred with SSRs

Ammar Elakhdar

Kyushu University, Japan; a.elakhdar@kyudai.jp

Co-authors: M. Abd EL-Sattar, K. Amer and T. Kumamaru

ABSTRACT

Global environmental change and increasing human population emphasize the urgent need for higher yielding and better adapted crop plants. One strategy to achieve this aim is to exploit the wealth of so called landraces of crop species, representing diverse traditional domesticated populations of locally adapted genotypes. In this study, we investigated a comprehensive set of 153 barley germplasm collected from several sources spanning 18 years. The whole collection was genotyped using 69 SSR markers to assess the genetic diversity and population structure. Out of the 69 locus analyzed, 64 turned out to be polymorphic, detected 261 alleles with an average of 4.08 alleles per locus. The highest PIC value was estimated for GMS021/ 1H and scssr00103/ 6H (0.82) with a mean of 0.49 over the 64 polymorphic loci. The markers Bmac0154, GMS021, GBM1214, Bmag0125, Bmac0030, Bmac0096, scssr09398 and scssr07970, demonstrated a high level of allelic diversity and were found most informative (GD > 0.75) on the set of genotypes analyzed, makes them ideal markers for differentiating between barley genotypes. The analysis of molecular variance AMOVA revealed highly significant $P \leqslant 0.001$ genetic variance within and among subpopulation. The results of population STRUCTURE and Neighbor-joining dendrogram suggesting that the accessions most likely separate into three subpopulations based on the genetic distances among the genotypes, the results may be explained by more diverse set of genotypes with respect according to origin and growth habit, our results also suggest that landraces are a source of valuable germplasm for sustainable agriculture in the context of future climate change.

SECTION:
Resources II: Germplasm and Populations
Abiotic stresses

POSTER: 55

Identification of host targets of Blumeria candidate secreted effector proteins.

J. Mitch Elmore

USDA-ARS / Iowa State University; jmelmore@iastate.edu

Co-authors: W. Xu, P. Surana, A. Chapman, G. Fuerst, D. Nettleton, and R.P. Wise

ABSTRACT

Powdery mildew disease caused by the obligate biotrophic fungus *Blumeria graminis f. sp. hordei* (Bgh) is a serious threat to barley production worldwide. The interaction between Bgh and barley represents a model pathosystem to dissect the molecular basis of plant disease resistance and susceptibility. Recent genome sequencing of Bgh has revealed the presence of ca. 540 candidate secreted effector proteins (CSEPs) that are predicted to be employed by the pathogen to manipulate host cell physiology during infection. To date, the vast majority (99%) of identified CSEPs remain to be functionally characterized. Transcriptome sequencing of barley lines containing the Mla6 resistance gene and isogenic immune system mutants (mla6, rar3, bln1, and mla6/bln1) was used to profile host and pathogen gene expression during infection with Bgh isolate 5874 (AVRa6). Two major waves of CSEP differential expression were detected over time: the first wave of ca. 100 CSEPs coincided with fungal penetration and the second wave of yet another set of 40 CSEPs during establishment of mature haustoria. The data also indicate that Bgh is able to sense compromised resistance functions in the immune system mutants and modify expression of its effector repertoire accordingly. CSEPs expressed at different stages of infection and within different plant genotypes are predicted to target distinct host pathways. To understand the roles of these CSEPs during infection, we have undertaken a yeast two-hybrid screen using a sequencing-based approach to identify and rank potentially interacting proteins. CSEPs with unique expression patterns are used as baits to screen a full length-enriched and normalized cDNA library derived from infected barley tissue. Initial screening has uncovered potential protein-protein interactions between CSEPs and barley proteins. The results from this study will be used to characterize the host proteins and pathways that are targeted by Blumeria in order to establish a successful infection.

SECTION:
Resources I: Genome
Biotic stresses

POSTER: 56

Re-domesticating Barley

Duane Falk

University of Guelph; dfalk@uoguelph.ca

ABSTRACT

Wild relatives of many domesticated crop species have been used mainly as a source of specific genes for increased disease resistance. It is often difficult to efficiently exploit these gene pools because they lack a number of essential traits necessary for cultivation and management using modern agricultural technology. Many of these traits were developed in our modern crops as part of the process of domestication. These traits are usually associated with seed dispersal, seed size, seed coats, and seed dormancy that are essential to survival in the wild, but undesirable in domesticated crops. By incorporating these 'domestic' traits into the wild species, they may be managed using standard farming methods. It would then be easier to select desirable gene combinations within these diverse and variable populations, and more effectively introgress these selected traits into modern, elite germplasm and cultivars. Cultivated barley (*Hordeum vulgare*) and its wild progenitor, *H. spontaneum*, can be used as a model to demonstrate this concept and illustrate the increased efficiency and effectiveness of this approach to 'taming' wild relative germplasm. Applying recurrent selection, via an efficient genetic male sterile system within re-domesticated H. spontaneum, may lead to greater genetic diversity for nutritional quality, agronomic traits, processing quality, and even yield, than currently exists within cultivated barley. Re-domesticating barley on a much broader scale than the original domestication event(s); could help address the current issue of lack of useful diversity in the existing very narrow elite barley gene pool. A diversity of H. spontaneum backgrounds adapted to widely different ecological niches can be used as starting materials to increase the potential adaptation and adaptability, and range of end uses of the resulting materials.

SECTION:
Resources II: Germplasm and Populations

POSTER: 57

Mapping loci associated with seed phytic acid in barley (*Hordeum vulgare* L.).

Weiyao Fan

University of Copenhagen; weiyao@plen.ku.dk

Co-authors: A. M. Torp, L. Legzdina and S. K. Rasmussen

ABSTRACT

Barley (*Hordeum vulgare* L.) is a major grain cereal worldwide. In barley, phytic acid (PA or myo-inositol hexakisphosphate) is the primarily storage of phosphate. While minerals in seeds become unavailable to human and monogastric animals by binding with PA. Developing cultivars with low phytic acid (lpa) mutations has become important in barley breeding.
Two different types of low PA barley mutants from cv. 'Alexis' were identified in previous work. A-type grains (A2A, A8A) contained very high levels of free phosphate, low levels of PA and trace amounts of other phosphate-containing compounds. B-type grains (A5B) increased free phosphate and moderately decreased PA without additional P-compounds. In this work, we are trying to map lpa loci in populations derived from these lpa mutants and to identify molecular markers closely associated with PA in barley. We started from B-type mutant A5B. The A5B mapping population was composed of 550 crossing individuals of F4 progenies from mutant A5B and cv. 'Jumara' (normal phytic acid variety). 185 simple sequence repeat (SSR) primers in 7 chromosomes are available for testing. 100 SSR primers for the whole-genome have been tested and 45 showed polymorphism between two parents. Based on the free phosphorus content in seeds, we have found 2 polymorphic makers in 4H between the DNA pools for now. We are working on the A-type population as well now and trying to find other potential linked loci and markers. Then we will try to fine mapping the linked loci we found.

SECTION:
Plant Breeding and Genetics Education

POSTER: 58

The OSU Malt Lab: Bridging the Gap between Barley and Beer on a Research Scale

Scott Fisk

Oregon State University; scott.fisk2@oregonstate.edu

Co-authors: T. Filichkin, P.M. Hayes, L. Helgerson, D. Herb

ABSTRACT

Up to this point there has been a gap in the malting industry between micro-malting and industrial production. The OSU Malt Lab will assist in closing this gap. Our pilot malting unit was built as a collaborative effort of the OSU Barley Project, the OSU Fermentation Science Program, and the OSU School of Mechanical, Industrial, and Manufacturing Engineering. The unit is designed as an all-in-one system as each step of the malting process takes place within a single vessel with the capacity to malt 100-300 pounds of grain. This is a sufficient amount for making research scale beers using typical pilot brewing facilities. Brewing may be performed in the OSU Fermentation Science Pilot Brewing facility or by brewer cooperators. Beers made in these pilot plants will be more representative of actual beer production. This will allow brewers to assess brewhouse performance and flavor characteristics earlier in the variety development process. The feedback from brewers and/or sensory panels will also allow the breeding program to more effectively breed and select for positive flavor attributes. Development of malt analysis capacity is currently underway. Malting and malt analysis will be integrated into the research and training programs in Barley Breeding and Fermentation Science at OSU.

SECTION:
Barley end use: malting and brewing

POSTER: 59

Regulatory mechanisms of barley frost tolerance, the continuing conundrum of CBF genes

Enrico Francia

Dept. Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, Pad. Besta, Reggio Emilia, I 42122, Italy; enrico.francia@unimore.it

Co-authors: C. Morcia, M. Pasquariello, V. Mazzamurro, J. Milc, L. Mareri, E. Endrighi, F. Rizza, V. Terzi, A.M. Stanca, N. Pecchioni

ABSTRACT

Low freezing temperature is one of the primary limiting factors that affect barley yield potential. Freezing tolerance depends on a period of cold acclimation that involves a highly integrated physiological response and in which the action of AP2 CBF transcription factors plays a major role in reconfiguring the plant transcriptome. Quantitative genetic studies demonstrated that a large part of the observed phenotypic variation in frost tolerance is explained by two major loci (QTL), FR-H1 and FR-H2 on chromosome 5H. A cluster of more than 13 CBF genes maps coincident with FR-H2 suggesting them as candidates. Recently, ca. 1.5 Mb of sequence that harbour the entire HvCBF cluster in the barley reference cultivar 'Morex' has been physically mapped, sequenced and assembled. This sequence represents a useful genomic tool for structural and evolutionary comparisons of FR-H2 in germplasm accessions. Variation in copy number (CNV) at FR-H2 using qPCR was performed in a barley diversity panel in order to identify resistance-associated copy number variants at specific subclusters of HvCBF genes, unravel whether the effect of FR-H2 is linked to CNV at a single gene or to a 'HvCBF-number game', and verify its dependency on the allelic state at VRN-H1/FR-H1. Copy number relative quantity results evealed a CNV involvement only for some of the investigated genes. Through an accurate phenotyping that combined Fv/Fm and field survival, relationship of phenotypic values to CNV effects were investigated revealing that the more resistant genotypes carry a higher copy number of certain CBFs in combination with a lower CNV at others. Further structural validation is being undertaken constructing a BAC library of the frost tolerant cultivar Nure. Roughly 50% of the trait could be explained after the VRN-H1 allelic effect is fixed in the model.

SECTION:
Resources I: Genome

POSTER: 60

A Chromosome Walk Using Bowman Backcross-derived Barley Lines

Jerome Franckowiak

University of Minnesota; jfranck@umn.edu

Co-authors: K. Beaubien, E. Schiefelbein, G. Velasquez Rodrigiez, and K.P. Smith

ABSTRACT

To facilitate study of genetic diversity in barley, *Hordeum vulgare*, over 800 phenotypic variants and induced mutants were backcrossed into the two-rowed spring cultivar Bowman (PI 483237), which served as a common genetic background. The resulting Bowman-backcross derived (BW) lines were selected to retain specific phenotypic traits from their donor parents. Druka et al. (2011) identified molecular markers associated with the DNA segments from donor parents retained in each of the BW lines. For many morphological variants, the relative positions of controlling genes in the seven barley chromosomes can be visualized by planting the BW lines in a specific order based on the estimated position of the genes. For this 'chromosome walk' demonstrated at the 12th International Barley Genetics Symposium, approximately 250 BW lines in were planted in one meter rows on the University of Minnesota, St Paul Campus. Seed stocks for this planting were provided by the Department of Plant Sciences, North Dakota State University at Fargo.

SECTION:
Barley morphology and development

POSTER: 61

Analysis of GxE interactions in barley HEB-25 population and acceleration to gene isolation by the ComSeq approach

Eyal Fridman

ARO, Volcani Center; fridmane@agri.gov.il

Co-authors: Silberman, R., Bonfil, D., Koren, M., Klaus, P., Shental, N.

ABSTRACT

Enhancing crop biodiversity is a key strategy to cope with challenges posed on agricultural production by climate change, under prediction for increased incidences of abiotic stresses. Crop wild relatives contain a wealth of gene alleles useful for modern agriculture that are absent from the cultivated gene pool, including rich repertoire of alleles regulating adaptation to biotic and abiotic stresses. The barley multi-parent mapping population (HEB-25) is the first population designed for nested association QTL mapping (NAM) with wild relatives as donors. It is composed of 25 'families' of app. 60 BC1S4 lines derived from crosses of the Hordeum vulgare cv. Barke to 25 wild barley (H. spontaneum) donors, in total the population composed of 1420 lines. This design takes advantage of both historic and recent recombination events to achieve high mapping resolution, high statistical power and allelic richness that capture the potential of the H. spontaneum gene pool.

A high-content phenotypic pipeline is implicated to allow semi-controlled field trials that capture whole-plant phenotype of the population under well and poor watered regimes. The genotype by environment interaction component, i.e. wild alleles with conditional effects on yield-associated trait, is integrated within the original mixed linear statistical model (including population structure and family relatedness). Residual heterozygosity allows testing possible heterozygous advantage (overdominance), which has implication for hybrid breeding. The genomic scan identified DRY1.1 (HsDry1.1), one major QTL on chromosome 1 that show drought-conditioned and overdominant effect on grain yield. No pleiotropic effects on biomass or heading indicate that there may be are other ways than timing flowering to achieve drought resilience. A GBS-based recombinant screening using the ComSeq approach, i.e. recombinant identification by ComSeq, is implicated to achieve single gene resolution of this genome scan.

SECTION:
Barley morphology and development

POSTER: 62

QTL mapping of head and seed morphology in a nested association mapping panel

Megan Getz

Montana State University; megan.getz@msu.montana.edu

Co-authors: L. Elmore, T. Hoogland, A. Burhardt, D. Hole, R. Horsley, and J. Sherman

ABSTRACT

Barley (Hordeum vulgare) is used for malt, animal feed and human food. Released varieties containing specific qualities are targeted to these uses. However, high yield and large uniform seed are consistently valued. Breeders require genetic diversity to make improvements, and sometimes use un-adapted lines in crosses to provide new alleles. A more difficult prospect, because of linkage drag of poor alleles, is using un-adapted lines to improve quality or yield. In an attempt to access new alleles from un-adapted lines, a two-row Nested association mapping (NAM) population was created from the core barley germplasm collection. The goal of this study is to identify QTLs associated with head and seed morphology, and uniformity, which are important traits for both yield and quality. Others have found major genes that control the six-row versus two-row head type affecting yield, plumpness, and seed uniformity. Here, the NAM population is being used to genetically dissect genes specific to two-rowed lines that impact head and seed morphology. To create the population, lines from the world core were genotyped and 100 that were genetically diverse were selected to be crossed with Conlon, a high yielding, plump, spring variety. Approximately 100 lines from backcrossed progeny were selected in hopes of exposing new alleles while eliminating some un-adapted traits. This seed was then grown in Bozeman, MT where phenotypic and phenological data were collected. Five heads from each of the lines were collected for 30 families that were segregating for head morphology. Digital image analysis is being used to measure length and width of heads as well as kernel count, density, and size. The phenotypic data along with the genotypic maps, will be used to discover QTLs which affect head morphology and eventually yield, plumpness, protein content, and kernel uniformity.

SECTION:
Abiotic stresses
Breeding Methodologies: current approaches and future

POSTER: 63

Candidate qRT-PCR reference genes for barley that demonstrate better stability than traditional housekeeping genes

Michael Gines

USDA; Michael.C.Gines@outlook.com

Co-authors: T. Baldwin, A. Rashid, K. Klos, P. Bregitzer, E. Jellen, J. Maughan

ABSTRACT

Gene transcript expression analysis is a useful tool for correlating gene activity with plant phenotype. For these studies, an appropriate reference gene is necessary to quantify the expression of target genes. Classic housekeeping genes have often been used for this purpose, but may not be consistently expressed across tissues or genotypes, and empirical data for such genes in barley is lacking. Publically available bioinformatic resources allow prediction of gene expression stability for candidate reference genes through in silico analysis. Using the EST profile viewer data from Unigene, we estimated the expression stability of 655 barley genes. The twenty most-stable genes as predicted by in silico analysis were evaluated in the barley cultivar 'Conlon' by qRT-PCR across seven tissues. The five genes with the most stable expression were identified as a zinc finger A20 & AN1 domain-containing stress-associated protein, a 3-ketoacyl-CoA thiolase like protein, superoxide dismutase [Cu-Zn], elongation factor EF-2, and malate dehydrogenase. The expression stability of these genes was further evaluated across the same set of tissues in 'Golden Promise' and 'Harrington'. These results may be broadly applicable to barley, as these cultivars are genetically diverse and commonly used.

SECTION:
Barley morphology and development

POSTER: 64

Environmental associations for cold tolerance in barley

Shawn Goggins

University of Minnesota, Plant Biological Sciences Program; sgoggins@iastate.edu

Co-authors: A. M. Poets, F. Katagiri, P. L. Morrell.

ABSTRACT

Barley is produced from the equator to inside the Arctic Circle. The spread of barley from the initial region of cultivation has occurred in the last 8,000 to 3,000 years. During that timeframe, barley has undergone adaptation to a wide range of environmental conditions, resulting in production at both much higher altitude and latitudes than in the home range. Landraces accessions potentially capture much of the adaptive genetic variation associated with this production across the prehistoric geographic range of the species that is ~4 times the range of the wild progenitor East to West and ~7 times the range North to South. Using ~6,500 single nucleotide polymorphisms (SNPs), we perform environmental association analyses, making use of both differences in allele frequency across the range and explicit association with environmental variables. We emphasize the identification of variants with potential to contribute to cold tolerance, as this could improve overwintering in winter barley production environments.

SECTION:
Success stories in barley breeding and genetics

POSTER: 65

Comparative proteomic analysis of two different application cultivars in barley (Hordeum vulgare L.)

Baojian Guo

Yangzhou University; rgxu@yzu.edu.cn

Co-authors: H. Luan, S. Lin, C. Lv, X. Zhang, R. Xu

ABSTRACT

The grain protein contents of barley seeds were significantly difference between feed and malting barley cultivars. However, the molecular mechanism of grain protein content formation is still an area to be understanding. To better understand the underlying molecular basis of grain protein content formation of barley, differential proteomic analysis between Yangsimai 3 (high grain protein content) and Naso Nijo (low grain protein content) cultivar was performed by using a combination of 2DE and MALDI-TOF-MS. In total, 502 reproducibly protein spots in grain proteome of barley were detected with silver staining in a pH range of 4-7 and 6-11, among which 41 (8.17%) detected protein spots were differentially expressed between Yangsimai 3 and Naso Nijo, 34 protein spots corresponding to 23 different proteins were identified, which were grouped into eight categories. Secondly, Enolase (spot 274) and ADP-glucose pyrophosphorylase (spot 271) were specific expressed in barley Yangsimai 3, which could be contribute to the grain protein content formation. In addition, B hordein as a major storage protein was high expressed in Yangsimai 3, which could be contribute to the difference of grain protein content in barley varieties. Most noticeably, the malting quality is characterized by an accumulation of serpin protein, alpha-amylase/trypsin inhibitor CMb and Alpha-amylase inhibitor BDAI-1. These results indicated that barley cultivars between Yangsimai 3 and Naso Nijo displayed significant difference on protein levels, which may be responsible for the observed difference of grain protein content.

SECTION:
Resources II: Germplasm and Populations

POSTER: 66

Allelic variation and geographic distribution of vernalization genes HvVRN1 and HvVRN2 in Chinese barley germplasm

Ganggang Guo

Institute of Crop Science, Chinese Academy of Agricultural Sciences;
guoganggang@caas.cn

Co-authors: D. Dondup, J. Zhang, Z. Sang, L. Zhang, D. Xu, X. Yuan, N. Tashi

ABSTRACT

Knowledge of the allelic variation and epistatic interaction of vernalization genes is crucial to understanding seasonal growth habit and adaptability of genetic resources in cultivated and wild barley. In this research, 1,398 Chinese barley accessions were characterized with molecular markers for vernalization genes HvVRN1 (V1) and HvVRN2 (V2). Different combinations of vernalization haplotypes were present at different frequencies in wild barley, landraces and modern cultivars. All of the known HvVRN1 haplotypes except HvVRN1-7 (V1-7) were present in the Chinese barley collection chosen for this study. Haplotype V1-8 occurred at the highest frequency in spring V1 alleles, and V1-1, V1-4 and V1-8 had broad geographic distributions in China. Haplotypes V1-2 and V1-3 are restricted to modern cultivars as they were introduced in foreign germplasm. A total of 18 HvVRN1/HvVRN2 (V1/V2) multi-locus haplotypes were identified. Most of the V1/V2 haplotypes were present in materials from the Yellow-Huai River Valley and Middle-Lower Yangtze Valley ecological zones, birthplace of Chinese farming. The majority combinations in landraces were V1-8/V2 and v1/V2, but v1/v2 made up the highest proportion of modern cultivars. Haplotypes V1-5/v2, V1-10/V2 and V1-9/V2 exhibited strong regional adaptability. Our results confirmed the presence or absence of conserved regulatory elements and the length of the first intron of HvVRN1 plays a determinant role in the vernalization requirement of barley. This study provides useful information for breeding and utilization of Chinese barley germplasm.

SECTION:
Resources I: Genome

Barley end use: food/feed

POSTER: 67

Availability and utilization of resistance genes for foliar diseases in commercial barleys

Sanjiv Gupta

Murdoch University and Department of Agriculture and Food Western Australia;
S.Gupta@murdoch.edu.au

Co-authors: J. Bradley, S. Westcott, H. Luo, E. Walker and C. D. Li1

ABSTRACT

The disease resistance profiles of barley varieties change with the adoption and emergence of new pathotypes of foliar leaf diseases in Australia. It constitutes a significant limitation to sustainable barley production. The lack of resistance to diseases like net blotches, scald, leaf rust and powdery mildew have significant impact on yield and grain quality mainly through reduced grain size. Reduced grain yields and low malting quality reduce returns to growers and affect domestic and overseas exports for its marketability. It is important that production of high quality barleys remain reliable. A marker assisted approach has been deployed where characterised resistance genes are sought to improve resistance profiles of the commercially important barley varieties. As many as nine resistance genes (1H, 5H APR for powdery mildew; 6H and 7H for spot form of net blotch; 3H, 4H, 6H for net form of net blotch; 6H and 7H for barley scald), characterised from different sources of germplasm lines, are currently being transferred via backcrossing into many barley varieties using tightly linked markers for respective genes. The various backcrossed material is at different generations of development and are being progressed in a marker-assisted fashion. The choice of barley varieties is primarily based upon its commercial value, adoption and susceptibility to a range of foliar diseases. This work has progressed under the National Barley Foliar Pathogen Variety Improvement Project with an aim to develop suite of resistant materials for its use and adoption. Once resistance genes are fully integrated, it is assumed that the commercial value, adoption and success of the barley varieties will be significantly enhanced with the change of their resistance profiles. The shift in resistance profiles will influence the decisions of growers and advisers of their varietal choices who need to manage leaf diseases with the deployment of barley varieties in Australia.

SECTION:
Resources II: Germplasm and Populations

POSTER: 68

Genome wide association study (GWAS) of resistance to barley spot blotch in South Asia

Sanjaya Gyawali

ICARDA; S.Gyawali@cgiar.org

Co-authors: S.S. Vaish, P.K Singh, A.S. Kharub, S.R. Vishwakarma, R. Selvakumar, S. Chao, S. Rahman, and R.P.S. Verma

ABSTRACT

Spot blotch, caused by Cochliobolus sativus (anamorph: Bipolaris Sorokiniana) is one of the important foliar diseases of barley in South Asia. However, limited information on genetic resistance of spot blotch resistance in barley in South Asia is available. Thus identification of new sources of resistance to spot blotch and genomic regions conditioning resistance, was initiated using an Association Mapping panel (AM-15) of 336 barley genotypes of ICARDA. The AM-15 was genotyped using 9K iSELECT SNP markers in USDA ARS Fargo and phenotyping for spot blotch resistance done at two host spot locations (Varanasi and Faizabad) in India during 2014-2015. The disease severity was recorded using double digit score (% incidence on flag and next below leaves) in four readings after flowering stages. Area under the disease progress curve (AUDPC) was used in the GWAS study. Association analyses using both generalized linear model (GLM) and mixed linear model (MLM) identified same SNP markers associated with spot blotch resistance. Population structure and kinship was used as covariate to control false positives in association study. New sources of spot blotch resistance were observed that were effective in South Asia. The AUDPC ranged from 282 (AM-310, resistant) to 1245 (NBYT13-12 and SBYT2-13_3219 highly susceptible). Three moderately resistant genotypes identified were (IG:155493, Esperance Orge 289, and CI5289) with AUDPC less than 300 and the highest AUDPC up to 1245. Significant spot blotch resistant QTL were mapped in 4H (11_10113; 18.61 cM; 12_20143, 67.91 cM and 11_10751, 82.99 cM), 5H (SCRI_RS_236744), and 6H (SCRI_RS_156616) while one marker with unknown position (SCRI_RS_7517). The R2 of the significant markers ranged from 3.31-4.8%. The QTLs identified in this study will be useful in the marker-assisted selection of barley genotypes for spot blotch resistance in South Asia.

SECTION:
Biotic stresses

POSTER: 69

Genome-wide association study of tillering traits in field-grown barley

Allison Haaning

University of Minnesota; haani001@umn.edu

Co-authors: K.P. Smith and G.J. Muehlbauer

ABSTRACT

The majority of yield in barley comes from inflorescences that develop on lateral stems called tillers. Despite the fundamental relationship between tiller production, or tillering, and yield, the underlying genetics are not well understood. In this study, the global genetic diversity of barley represented in the USDA-ARS National Small Grains Core Collection (NSGCC) was exploited to maximize detection of quantitative trait loci (QTL) associated with tillering. A subset of spring two-row and six-row lines was selected by including the most genetically diverse lines from the NSGCC. Lines were genotyped using the Infinium 9K iSelect platform and GBS and grown in the field in Saint Paul, Minnesota in 2014 and 2015. Data for heading date, weekly tiller numbers per plant, and numerous other traits were collected throughout development. As expected, two-row lines produced more tillers than six-row lines on average, but, despite this, one-quarter of all six-row lines produced more tillers than the two-row average. The week tillering stopped and average tiller number were both weakly, positively correlated with heading date. Genome-wide association mapping was used to detect QTL associated with different tillering traits. In 2014 three QTL were detected: one on chromosome 2H overlapping the Photoperiod-H1 (Ppd-H1) locus, which controls photoperiod sensitivity, and two others on 5H and 7H. Four QTL were detected in 2015: one on 3H overlapping a circadian gene, Lux1; the same one on 5H as 2014; and two others on 3H. For heading date, the most significant QTL detected in 2014 overlapped Ppd-H1 and the most significant QTL detected in 2015 overlapped Lux1, indicating that detection of different tillering QTL in 2014 and 2015 was influenced by genes controlling heading date. Drastically different tillering capacity between 2014 and 2015 coupled with low heritability estimates for tillering traits also indicates that tillering is strongly influenced by environment.

SECTION:
Resources II: Germplasm and Populations

POSTER: 70

miR393-mediated repression of HvTIR1/AFB2 regulates root growth during seedling development and under Aluminum treatment in barley

Ning Han

Zhejiang University; ninghan@zju.edu.cn

Co-authors: B. Bai, H. Bian, J. Wang, M. Zhu

ABSTRACT

miR393, which is a conserved microRNA family across the angiosperms, post-transcriptionally regulates mRNAs for the F-box auxin receptors TIR1/AFBs. miR393/target regulatory module have been demonstrated to play crucial roles in plant development and responses to biotic and abiotic stresses in Arabidopsis and rice. In this study, we investigated biological function of the miR393 in barley. Using a modified form of 5'-RACE (rapid amplification of cDNA ends) assay, combined with degradome data analysis, we identified two TIR1/AFB homologs MLOC_56088 and MLOC_9864 as the targets of HvmiR393. Transgenic plants overexpressing miR393 displayed a pleiotropic phenotype, including longer primary root length, increased number of adventitious roots and enhanced resistance to exogenous auxin. Meanwhile, transgenic plants expressing an artificial target mimic directed against miR393 (MIM393), with decreased level of miR393, showed opposite phenotype to that of 35S:MIR393. We also found that exposure of barley roots to Al inhibited the accumulation of HvmiR393, and 35:MIR393 plants exhibited enhanced resistance to Al treatment. Our data suggests a mechanism underlying how developmental and environmental cues affect root growth through miRNA-mediated auxin signalling regulation.

SECTION:
Barley morphology and development

POSTER: 71

Has the time come for barley to go naked?

Patrick Hayes

Oregon State University; patrick.m.hayes@oregonstate.edu

Co-authors: S. Fisk, T. Filichkin, L. Helgerson, D. Herb, and B. Meints

ABSTRACT

Is it time for barley to leave the hull behind? The adhering lemma and palea are signature features of barley grain that have persisted for the past 10,000 years of barley production largely due to the filtration function of the hulls in brewing. Advances in brewing technology have provided other filtration options, perhaps rendering the hull obsolete. Absence of hulls leads to higher malt extract and a lower concentration of tannins, which contribute off-flavors and astringency to finished beer. So what's not to like about naked malts? If not for beer, then for what the hull? Composed primarily of insoluble fiber, the hull has minimal value in human nutrition. In fact, removal of the hull through pearling can compromise the whole-grain status while increasing processing cost of the resulting foods. The hull has little to no feed value for non-ruminant animals and there are many alternative sources of cellulose for ruminant diets. Finally, the hull accounts for ~ 15% of the total grain yield per unit area meaning the yield data for hulled barley is inflated, or at least disingenuous. Why not be honest and state actual grain yields? Prior to taking the naked plunge, several factors need be taken into consideration, including pleiotropic effects of the nud gene, linkage drag, and the catch-up that will be needed to realize the agronomic potential of naked barley germplasm. These challenges can all be addressed with the current breeding and genetics toolkit.

SECTION:

POSTER: 72

The Oregon Promise population: the search for barley contributions to beer flavor leads to unexpected opportunities

Patrick Hayes

Oregon State University; patrick.m.hayes@oregonstate.edu

Co-authors: D. Carey, L. Cistue, B. Echávarri, T. Filichkin, S. Fisk, L. Helgerson, C. Henson, D. Herb, H. Hisano, R. Jennings, P. Kramer, C. Martens, B. Meints, M. Moscou, K. Sato, R. Thiel, W. Thomas

ABSTRACT

The Oregon Promise doubled haploid mapping population consists of 200 doubled haploid lines derived from the cross of Golden Promise x Full Pint. Golden Promise, which entered commercial production in the 1960's, remains a favorite of craft brewers due to contributions to beer flavor and a workhorse for molecular biologists due to its efficiency for transformation, although it represents a different genetic grouping compared to current European malting varieties. Full Pint (a Triumph (Trumpf) derivative) was originally released as the germplasm "BCD47" in 2011 due to its role as a donor of stripe rust resistance QTL alleles. Subsequently, it was identified as making unique and favorable contributions to beer flavor and released as "Full Pint" in 2014. It entered commercial production in 2015 and is featured in identity preserved specialty malts and a range of craft beer brands. Golden Promise and Full Pint are both semi-dwarves but at different loci (ari-e and sdw1 respectively). The doubled haploid population has been used for a range of phenotyping and genotyping projects. Phenotyping data include: malting quality, nano-brew sensory assessments, stripe rust resistance, leaf rust resistance, mildew resistance, plant height, spike length and kernel number, and grain yield. Genotype data include: Eureka Genomics SNPs, RNA-Seq-based SNPs, and GBS. The population is serving as a platform for mapping QTLs for the aforementioned phenotypes and map-based cloning of selected candidate genes. Selected Oregon Promise DH lines with the Golden Promise "transformability haplotype" will be tested as candidates for genome editing using CRISPR/Cas9. And last but not least, the population will optimistically yield one or more varieties that combine agronomic performance and unique contributions to beer flavor. Collaborators are invited to join us in realizing the Oregon Promise.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 73

A novel β-expansin gene HvEXPB7, cloned in the root hairs of Tibetan wild barley, improves root hair growth under drought stress

Xiaoyan He

Department of Agronomy, College of Agriculture and Biotechnology, Zijingang Campus, Zhejiang University, Hangzhou 310058, China; hexiaoyan@zju.edu.cn

Co-authors: G.P. Zhang, and F.B. Wu

ABSTRACT

Drought is a major limiting factor for crop production. One of the cost-effective solutions for sustainable crop production in water-limiting areas is crop genetic improvement for higher drought tolerance. Root hair is an important organ in uptake water and nutrients, and plays an important role in drought tolerance. Tibetan wild barley is rich in genetic diversity and provides elite genes for crop improvement in such abiotic stress tolerance as drought. Here, root hair phenotype of two contrasting Tibetan wild barley genotypes (drought-tolerant XZ5 and drought-sensitive XZ54) and drought-tolerant cv. Tadmor were compared in response to polyethylene glycol-induced drought stress. Drought induced pronounced development of root hair was only observed in XZ5. Comparative transcriptome profile in root hairs of the three genotypes in response to drought stress revealed 36 drought tolerance-associated genes in XZ5, including 16 genes specifically highly expressed in XZ5 but not Tadmor under drought. The full length cDNA of a novel _-expansin gene (HvEXPB7), being the unique root hair development related gene in the identified genes, was cloned. The sequence comparison indicated that HvEXPB7 carried both DPBB_1 and Pollon_allerg_1 domains. Comparative analysis of CDS and promoter sequences of HvEXPB7 among the three barley genotypes revealed that this gene reserves the highest genetic polymorphism and root hair specific cis-elements in XZ5. Tissue expression analysis revealed that HvEXPB7 is predominantly expressed in roots, subcellular localization verified that HvEXPB7 is located on the plasma membrane. Barley stripe mosaic virus induced gene silencing (BSMV-VIGS) of HvEXPB7 led to severely suppressed root hairs both under control and drought conditions, and significantly reduced K uptake in XZ5. Our findings highlight and confer the significance of HvEXPB7 in root hair growth under drought stress in XZ5, and provide a novel insight into the genetic basis for drought tolerance in Tibetan wild barley.

SECTION:

POSTER: 74

Mapping and deployment of tolerance to cereal yellow dwarf virus in two-rowed spring malting barley

Joshua Hegarty

University of California, Davis; jmhegarty@ucdavis.edu

Co-authors: I.A del Blanco, L. Gallagher, B. Falk and J. Dubcovsky

ABSTRACT

Cereal yellow dwarf virus (CYDV-RPV) causes a serious viral disease affecting small grain crops around the world. In the US, it is frequently present in California where it has been a major impediment to the release of an adapted two row malting barley variety due to significant yield reductions. To identify genetic loci associated with tolerance to CYDV, a segregating population of 184 recombinant inbred lines (RIL) from a cross of the California adapted malting barley line Butta 12 and the CYDV tolerant Madre Selva was used to construct a genetic map including 180 polymorphic markers mapping to 163 unique loci. Tolerance to CYDV was evaluated in replicated experiments where the population was challenged by aphid mediated inoculation with the isolate CYDV-RPV. Quantitative trait loci (QTL) analysis revealed the presence of two major QTL for CYDV tolerance from Madre Selva on chromosomes 2H (Qcyd.MaBu-1) and 7H (Qcyd.MaBu-2), and 4 QTL of less effect from Butta 12 on chromosomes 3H, 4H, and 2H. Additional populations were developed to refine the genetic position of the two major effect loci and new markers have been designed for more effective marker assisted selection of these. Furthermore, two of the lines from this mapping population, MP103 and MP179, combine all major QTL for CYDV tolerance with very good agronomic performance and malting quality (similar to Butta 12). These lines are currently in state wide yield and quality trials as candidates for variety release.

SECTION:
Barley morphology and development

POSTER: 75

The Oregon State University doubled haploid lab: status update and the development of facultative 2-row malting barley germplasm

Laura Helgerson

Oregon State University; laura.helgerson@oregonstate.edu

Co-authors: T. Filichkin, J. Daker, S. Fisk and P. Hayes

ABSTRACT

Breeding barley is a long-term process that requires multiple cycles of self-pollination to achieve complete homozygosity. Doubled haploid (DH) production leads to complete homozygosity in a single generation, thus bypassing the complications of field, greenhouse, or off-season generation advance. The Oregon State University Barley Project started a DH production facility in 2011. From 2013 – 2016, we provided DH lines on a fee-for-service basis to external users. The lab is now focused on collaborative research and breeding efforts. Our DH production system is based on anther culture. There is a high frequency of spontaneous chromosome doubling. Start to finish requires 9-12 months for winter and spring genotypes, respectively. Genotype responsiveness to anther culture remains a key determinant of overall efficiency. We are using the DH lab to develop a community resource for facultative malting 2-row germplasm with superior low temperature tolerance. This project, supported by the American Malting Barley Association with a special supplement from Sierra Nevada Brewing, is directed at producing DH lines from F1s contributed by participating programs. The resulting germplasm will be shared and tested by cooperating programs.

SECTION:
Resources II: Germplasm and Populations
Biotic stresses

POSTER: 76

Genome-wide association mapping of low temperature tolerance (LTT) in barley to improve crop efficiency under climate change

Dustin Herb

Oregon State University; Dustin.Herb@oregonstate.edu

Co-authors: A. Cuesta-Marcos, S. Fisk, P.M. Hayes

ABSTRACT

Low temperature tolerance can be an indirect path to greater water use efficiency (WUE) in environments with winter precipitation. In such environments, the grain yields of fall-sown barley are typically 20% greater than those of spring-sown barley. The challenge is achieving sufficient LTT, aka winter survival. We assembled a very large GWAS panel (n = 941) which was evaluated at 14 locations around the world in 2013/2014 and 2014/2015. The panel includes 300 lines from the TCAP Facultative-Winter 6 row set derived from elite lines at Oregon State University and University of Minnesota Breeding programs, and originally developed to map QTLs for nitrogen use efficiency (NUE) and water use efficiency. Additionally, the panel contains winter/facultative accessions submitted by breeders from around the world and collections assembled by the AGOUEB and ExBarDiv Projects led by the James Hutton Institute and University of Dundee, UK. Genotyping was done with the Illumina 9K chip in several separate runs based on accession collections. After combining all the information available and filtering for data quality we currently have 6683 SNPs in 941 lines. Differential winter survival was observed in four of the test environments: ID, MN, OH (USA) and Alberta, Canada. One environment experienced complete winter kill (NE, USA), while there was 100% survival in the remaining 9 environments which generated useful data on a range of biotic stress resistance, morphology, and phenology phenotypes. The GWAS validated the effects of know LTT-related genes (FR-H1, FR-H2, FR-H3, PPD-H2) and identified significant QTLs on all other chromosomes. Interestingly, VRN-H2 was not a significant determinant of LTT. Characterization of these novel QTLs in terms of the physiological bases and candidate genes is underway.

SECTION:
Success stories in barley breeding and genetics

POSTER: 77

Characterization of agronomic traits and locating exotic genes which control agronomic traits under contrasting nitrogen supply in the wild barley nested association mapping population HEB-25

Paul Herzig

Martin-Luther-University Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, Plant Breeding; paul.herzig@landw.uni-halle.de

Co-authors: A. Maurer, V. Draba, K. Pillen

ABSTRACT

Plant response to nitrogen (N) deficiency is a crucial component of yield and is of special importance in future agriculture to increase nitrogen use efficiency (NUE) to avoid nitrogen pollution of water and soil. Moreover, to maintain sustainable barley production there is an increased demand of new nitrogen stress tolerant cultivars. However, the reservoir of novel useful alleles in the elite barley gene pool is low. This loss of genetic diversity in barley during domestication is counteracted by utilizing nested association mapping (NAM). Therefore, to create HEB-25, the first barley NAM population, 25 highly divergent wild barley accessions were crossed with the elite barley cultivar Barke. This population consists of 1,420 highly diverse BC1S3 lines.

In the present study we utilized HEB-25 to investigate five agronomic traits (e.g. flowering time, plant height), six yield component traits (e.g. number of ears per square meter, thousand grain weight) and plot yield under contrasting N supply with a 60 kg N/ha difference during the seasons 2014 and 2015. We found variation between N treatments and among HEB lines for most traits. Furthermore we carried out genome-wide association studies (GWAS) to detect favorable exotic alleles, which regulate agronomic traits under nitrogen stress.

These exotic alleles can be of particular interest for breeders to develop new cultivars with an increased nitrogen efficiency. Subsequent analyses will be conducted to determine the content of grain nutrients. These measurements include the macro nutrients N, P, K, Mg, Ca and S, as well as the micro nutrients Cu, Mn, Zn, B, Fe, Al and Mo.

SECTION:
Resources II: Germplasm and Populations
Abiotic stresses

POSTER: 78

Identification of the genomic region responding to amenability of Agrobacterium-mediated transformation in barley

Hiroshi Hisano

IPSR, Okayama University; hiroshi.hisano@rib.okayama-u.ac.jp

Co-authors: Y. Motoi and K. Sato

ABSTRACT

An efficiency of genetic transformation in crop plant is dependent on the cultivars or genotypes. In a case of barley, cv. Golden Promise is one of few reliable cultivars for Agrobacterium-mediated transformation. However the genetic factors of transformation amenability of barley are still unknown. Here we used the F_2 generation of immature embryo derived from a cross between cvs. Haruna Nijo and Golden Promise as explant to identify the genomic region responding to successful transformation. Haruna Nijo is a recalcitrant cultivar for genetic transformation. Three thousand and thirteen of F2 immature embryos were co-cultivated with A. tumefaciens strain AGL1 carrying a binary vector, pIG121-Hm, and finally sixty independent PCR-positive transgenic barley plants resistant to hygromycin were obtained. By a platform of 384-SNP GoldenGate Assay (Illumina), the genotypes of all transgenic barley plants were determined by genome wide 124 markers. As the results of Chi-square test for each markers, the significant segregation distortion with expected Mendelian proportions of 1:2:1 ($df=2$, $p<0.01$) was observed on the regions of 2H and 3H chromosomes. In addition, the minor but significant segregation distortion with assumed dominant proportions of 3:1 or 1:3 ($df=1$, $p<0.05$), was observed with multiple regions on 2H, 4H, 5H and 6H. According to allele frequency of these markers, the distortions of marker segregation in 2H, 3H, 4H, and 6H was caused by the allele of Golden Promise and in 5H by Haruna Nijo allele. It is speculated that these regions are necessary or favorable genomic regions for Agrobacterium-mediated barley transformation.

SECTION:
Abiotic stresses

POSTER: 79

Genetics of forage quality traits in a two-row nested association mapping population

Traci Hoogland

Montana State University; tracihoogland@gmail.com

Co-authors: C. Chalupsky, P. Merta, M. Getz, A.Burkhardt, L. Elmore and J. Sherman

ABSTRACT

Forages are where plant breeding meets animal nutrition. On average, a 1% increase in forage digestibility (IVDMD) translates to a 3.2% increase in average daily weight gain of beef cattle, which not only means that less forage can feed more animals, but also more efficient use of water and energy. Despite its importance, much remains unclear about the genetics controlling forage nutritional quality in barley. Breeders are dependent on genetic diversity to improve crops and are in search of new alleles. One source of potentially beneficial alleles could be un-adapted lines held in the National Small Grains Collection (NSGC). As part of the Triticeae CAP a number of these lines were genotyped. Genetically diverse lines were then chosen as parents for a nested association mapping (NAM) population that was created by crossing unadapted parents to an adapted 2-row parent, Conlon. An additional cross was made back to Conlon to lessen the effect of negative genes. Ninety-nine families of between 50 and 100 lines were developed via single-seed decent. The BC1F6 generation was planted in single row plots in Bozeman, MT in 2015 and evaluated for morphological and agronomic traits. Preliminary observations indicate that forty of the families (2300 total lines) segregate for plant biomass. To further narrow the population, the parents of these families were screened in a preliminary greenhouse trial to determine their economically important forage quality traits: including ADF, NDF, CP and IVDDM. Based on the forage quality results of the parents, families will be selected to be part of a 2016 forage field trial, which will result in QTL mapping of forage quality. The long-term goal of this study is the genetic dissection of forage quality traits and the eventual utilization of QTLs in forage quality improvement.

SECTION:
Breeding Methodologies: current approaches and future
Plant Breeding and Genetics Education

POSTER: 80

Toward fine mapping of a powdery mildew resistant gene in a Hordeum vulgare bulbosum introgression line

Parastoo Hoseinzadeh

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466 Stadt Seeland, Germany; hoseinzadeh@ipk-gatersleben.de

Co-authors: M. Mascher, B. Ruge-Wehling, P. Schweizer, N. Stein

ABSTRACT

Powdery mildew (PM) caused by Blumeria graminis f.sp. hordei (Bgh) is one of the main foliar diseases on barley (Hordeum vulgare L.) leading to severe economic losses worldwide. For disease resistance, wild barley relatives have been considered as valuable genetic diversity resources in barley breeding. However, most sources of PM resistance have been overcome by the emergence of new virulent races within the pathogen. Therefore, new durable and broadly acting sources of resistance are necessary for a long-term success of disease resistance in barley. The secondary gene pool of barley (Hordeum bulbosum) is a potential source of resistance genes for barley improvement. Despite of some crossing barriers, the hybridization and recombination between two Hordeum genomes allow the introgression of H. bulbosum chromosome segments into barley carrying novel resistance genes.

The aim of the project is to positionally clone a H. bulbosum-derived resistance gene introgressed to cultivated barley. Novel Single Nucleotide Polymorphism (SNP) markers will be developed by genotyping-by-sequencing for Hordeum vulgare/bulbosum introgressions lines conferring resistance to Bgh. Resistant recombinants with small introgression size and a high marker density will be used for map-based cloning of genes underlying Bgh resistance. Based on the physical/genetic map, gene-flanking markers will be employed to identify a barley BAC contig corresponding to the orthologous region of the H. bulbosum genome. RNA sequencing approach on Bgh-infected leaf segments of the near isogenic lines will be exploited to identify candidate gene(s) within the target genetic interval.

SECTION:
Resources II: Germplasm and Populations
Barley end use: food/feed

POSTER: 81

Effects of m351 barley (Hordeum vulgare L.) mutation on grain quality and its applications

Gongshe Hu

USDA-ARS; Gongshe.Hu@ars.usda.gov

ABSTRACT

The chemical induced barley mutation of m351 was identified for its low level of mixed-linkage (1-3,1-4) beta-D-glucan (MLG) in grains. The MLG decrease in m351 was associated with increased levels of fructans and crude fiber, but maintained the same plant characteristics under field conditions. The mutation was mapped on chromosome 7H. Molecular cloning of the CslF6 gene from the m351 line revealed the presence of a point mutation, causing a substitution of alanine for threonine at position 849 in the amino acid sequence of the corresponding protein. The resultant protein retains some functionality and affects other components in the m351 grain. The metabolic changes associated with MLG reduction in m351 is the first case reported of a partially functional CslF6 gene in cereal grains. The mutant consistently showed low beta-glucan in malting and the mutant allele could be used to control low beta-glucan in the malting process. Fermentation experiments indicated that m351 also produced better feed by-product with higher protein and lower fiber in bioethanol production. The characterization of m351 contributes to better understanding of the functional effects of the CslF6 gene and the grain end-use quality improvement.

SECTION:
Biotic stresses

POSTER: 82

miRNA discovery in barley (Hordeum vulgare L.) and the powdery mildew pathogen, Blumeria graminis

Matthew Hunt

Iowa State University; mhunt@iastate.edu

Co-authors: P. Surana, G. Fuerst and R.P. Wise

ABSTRACT

The interaction between barley (Hordeum vulgare L.) and the obligate biotroph Blumeria graminis f. sp. hordei (Bgh) potentially involves hundreds of secreted effector and host proteins. These effector proteins allow the fungus to suppress the host's innate immunity and to uptake nutrients from the plant. Barley has evolved an effective defense against Bgh through resistance genes (R genes) including Mildew resistance locus a (Mla), that recognize Bgh effector proteins and trigger immunity. Our group has recently shown that regulation of resistance through the Mla pathway is dependent on two barley miRNAs. miR9863 targets distinct Mla alleles to attenuate NLR-triggered disease resistance, whereas Mla itself controls miRNA398 and Cu/Zn-SOD to regulate Bgh-responsive cell death.

To identify additional small RNAs involved in Mla triggered resistance, we carried out Illumina small RNA sequencing of the CI 16151 (Mla6) progenitor and four fast-neutron-derived resistance signaling mutants. Three biological replications of a split-plot based design were inoculated with Bgh 5874 (AVRa6), harvested at 0, 16, 20, 24, 32, and 48 hours, and small RNA (sRNA) libraries were prepared from the 90 barley-Bgh treatments for Illumina Hi-Seq 2500 sequencing. Several hundred conserved and novel putative sRNAs in barley and Bgh were identified from the resulting 2.1 billion reads (average 24.2 million reads/treatment) and differential expression analysis was carried out to look for significant differences among wild-type progenitor and mutant derivatives. Within and trans-kingdom target predictions were initiated for both barley and Blumeria, facilitating a genome-wide knowledge base of the sRNA players in Mla specified resistance.

SECTION:
Barley end use: malting and brewing
Plant Breeding and Genetics Education

POSTER: 83

Two barley quantitative trait loci associated with Fusarium head blight resistance exhibit differential resistance mechanisms to Fusarium graminearum infection

Yadong Huang

University of Minnesota; huan0277@umn.edu

Co-authors: L. Li, K.P. Smith, G.J. Muehlbauer

ABSTRACT

Fusarium graminearum infects barley spikes and causes Fusarium head blight (FHB), a major disease problem worldwide. Resistance to FHB is partial and controlled by quantitative trait loci (QTL) of which two are located on barley chromosomes 2H bin8 (2Hb8) and 6H bin7 (6Hb7). To understand the molecular mechanisms of FHB resistance, transcriptomes of near-isogenic lines (NILs) carrying Chevron-derived resistant alleles for the two QTL and recurrent parents (M69 and Lacey) were investigated with RNA sequencing after F. graminearum or mock inoculation. A total of 2,083 FHB-responsive transcripts were detected and provide a gene expression atlas for the barley-F. graminearum interaction. Comparative analysis of the 2Hb8 resistant (R) NIL and M69 revealed that the 2Hb8 R NIL exhibited an elevated defense response in the absence of fungal infection and responded quicker than M69 upon fungal infection. The 6Hb7 R NIL displayed a more rapid induction of a set of defense genes than Lacey during the early stage of fungal infection. Overlap of differentially accumulated genes were identified between the two R NILs, suggesting that certain defense mechanisms may represent basal resistance to F. graminearum and/or general biotic stress response and were expressed by both resistant genotypes. Long noncoding RNAs (lncRNAs) have emerged as potential key regulators of transcription. A total of 12,366 lncRNAs were identified of which 604 were FHB responsive. The current transcriptomic analysis revealed differential mechanisms conferred by two QTL in response to F. graminearum infection and identified genes and lncRNAs that were associated with FHB resistance.

SECTION:
Biotic stresses

POSTER: 84

Natural variation in FLOWERING LOCUS T, HvFT1

Ernesto Igartua

CSIC; igartua@eead.csic.es

Co-authors: B. Contreras-Moreira, J. Loscos, M. P. Gracia, A.M. Casas

ABSTRACT

The barley ortholog of FLOWERING LOCUS T, HvFT1, also called VrnH3, is the main integrator of the photoperiod and vernalization signals leading to the transition from the vegetative to the reproductive stage. Results gathered by us and other groups for the last years have repeatedly identified variation in this gene related with flowering time QTL in mapping populations and also in genome wide association studies. Differences in the promoter, SNPs in the first intron and also copy number variation have all being associated with phenotypic and expression differences, resulting in earlier or later heading. The first reports found that mutations in the HvFT1 first intron differentiated plants with dominant and recessive alleles, with large phenotypic effect on time to flowering. The catalog of polymorphisms at this gene with potential phenotypic effect has been enlarged with copy number variation and sequence variation at the promoter. There is variation in the number of copies of the HvFT1 gene, apparently related to growth habit. A large set of winter genotypes, with a functional VrnH2 allele, has one copy of VrnH3, whereas variable number (1-5), was found in also a large set of spring or facultative barleys (without VrnH2). The dominant VrnH3 allele, which overrides the vernalization requirement of winter VrnH1 and VrnH2 alleles, is found only in Nordic barleys and carries a particular structure of the gene, with one promoter and variable number of transcribed regions. Using two indels from the promoter region and allele-specific markers for two SNPs in the first intron, we were able to classify four VrnH3 haplotypes, which showed differences in heading time among Spanish landraces. We will present results from several mapping populations and association analyses to contribute to describe the different polymorphisms that should be taken into consideration when analyzing this gene and its phenotypic effects.

SECTION:
Biotic stresses

POSTER: 85

What is PpdH2 doing in winter varieties?

Ernesto Igartua

CSIC; igartua@eead.csic.es

Co-authors: I. Karsai, T. Kiss, M.P. Gracia, A.M. Casas

ABSTRACT

Temperatures during barley growing season have been on the rise in the Mediterranean basin over the last 40 years. Under these circumstances, winter cereal farmers are exposed to a difficult choice of cultivars for autumn sowing, from spring cultivars in warm areas to strictly winter cultivars. The choice must take into account frost probability for the region. The most common process found in winter cereals to achieve frost tolerance is vernalization, although high frost tolerance can also be attained in cultivars almost devoid of vernalization requirement. We have carried out an experiment with winter barley subjected to insufficient vernalization to test the influence of allelic diversity at genes HvFT1 (VrnH3) and HvFT3 (PpdH2) on development.

The experiment used selected plants of the population Esterel x SBCC016, grown in growth chambers. Three F4 lines of each of the four haplotypes determined by HvFT1 and HvFT3, selected with markers, were tested under three different day lengths, 8, 12 and 16h light. The plants were deliberately chosen to be strictly winter types). They were partially vernalized (45 days) before being transferred to the day length chambers, to test the performance of the haplotypes under conditions close to the natural target of the study. The plants showed a large range of duration of development and other phenotypic traits in response to day length, but also between the genetic haplotypes tested. The relationship of the phenotypic development with the expression levels of genes HvFT1, HvFT3, VrnH1 and VrnH2 will be presented and discussed, together with possible agronomic implications. Flowering time is closely related to HvFT1 expression, which seems controlled by a balance between its positive regulator VrnH1 and its well-known repressor VrnH2, but also by an epistatic interaction between VrnH2 and HvFT3, with further implications on tillering.

SECTION:
Barley morphology and development

POSTER: 86

Yield effects of flowering time genes in Mediterranean conditions

Ernesto Igartua

CSIC; igartua@eead.csic.es

Co-authors: M.P. Gracia, A. Monteagudo, F.J. Ciudad, A.M. Casas

ABSTRACT

Evidences of QTL studies and biogeography of landraces in the Western Mediterranean suggest a large role of vernalization and photoperiod genes on agronomic performance of barley in the region. Two hypotheses have been put to test, a possible agronomic advantage of the dominant allele of VrnH2 over the recessive (vrnH2), and a better adaptation of a haplotype combining a reduced vernalization requirement (VrnH2/VrnH1-4) and an active PpdH2 over a typical winter type (VrnH2/vrnH1/ppdH2).
Sets of lines were selected from two biparental populations with the aid of molecular markers, according to their haplotypes of flowering time genes. Four sets of 25 lines were made in SBCC097/Plaisant, considering their haplotypes at VrnH1 and PpdH2. Eight sets of 8-16 lines were prepared in SBCC145/Beatrix, as per their haplotypes at VrnH2, VrnH3 and PpdH1 (also all the lines had the Beatrix, spring allele at VrnH1). Equal amounts of seeds of these 12 sets of lines were bulked, and sown in field trials for up to three years and two sowing dates per year, normal (November) and late (early February). There were significant differences in grain yield among the bulks. There was a significant yield advantage of the VrnH2 over vrnH2 bulks in SBCC145/Beatrix (16%) and of the PPdH2 bulks in SBCC097/Plaisant (6%). Although the differences were significant across sowings, they were even higher in the autumn one. Small differences in heading date could not account for the grain yield differences found. The relationship of these results with winter temperatures in the experimental locations will be studied and reported. These differences could be due to either a pleiotropic effect of the genes targeted by this study, or to other so far undetected linked genes with large agronomic effect. These hypotheses will be tested further over the next years.

SECTION:
Resources II: Germplasm and Populations

POSTER: 87

Quality response of new malting barley varieties to increasing nitrogen fertilization rates in western Canada

Marta Izydorczyk

Canadian Grain Commission; marta.izydorczyk@grainscanada.gc.ca

Co-authors: T. McMillan, D. Shaluk, S. Parsons, J. O'Donovan

ABSTRACT

Barley grown in western Canada is known for its adequate content of protein, resulting in high levels and activity of various hydrolytic enzymes required for proper modification of cell walls and proteins during malting and for rapid degradation of starch to fermentable carbohydrates during mashing. While application of nitrogen fertilization can increase barley yield, it can also impair malting quality by increasing protein to undesirably high levels. Barley with excessive grain protein content is difficult to modify and results in lower extract levels. The objective of this study was to determine the effects of increasing nitrogen fertilization rates on the malting behavior of recently registered in Canada malting barley varieties, AAC Synergy, CDC Kindersley, Voyageur, and Cerveza, compared to the most commonly grown variety, AC Metcalfe. Field experiments were conducted for three years at six locations in western Canada with four rates of nitrogen fertilization (0, 25, 50 and 100 kg/ha). While increasing rates of nitrogen fertilisation generally resulted in increased grain protein levels and had a negative effect on the cell wall modification and the amount of malt extract, not all varieties responded similarly. AAC Synergy and CDC Kindersley showed lower levels of protein at higher nitrogen fertilization rates. AAC Synergy and CDC Kindersley produced malt with higher friability values and wort with lower levels of _-glucan than the other three varieties. Although malt friability decreased as nitrogen rates increased, the decrease in friability was not as dramatic for AAC Synergy as for the other varieties. AAC Synergy and CDC Kindersley produced wort with relatively low levels of _-glucan that were relatively independent of the increasing nitrogen fertilization rates. These results suggest that barley varieties may differ with respect to their ability to resist the negative effects of increasing nitrogen fertilization rates on certain malting quality parameters.

SECTION:
Resources II: Germplasm and Populations

POSTER: 88

NHX-type Na+/H+ anti-porter gene expression under different salt levels and allelic diversity of HvNHX in wild and cultivated barleys

Zahra Jabeen

Department of Agronomy, College of Agriculture and Biotechnology, Zijingang Campus, Zhejiang University, Hangzhou 310058, PR China,Department of Biosciences, COMSATS Institute of Information Technology, 44000 Islamabad, Pakistan; kubra_119@yahoo.com

Co-authors: N. Hussain, J. B. Zeng, Y. Han, S. Cai, F. Irshad and G. Zhang

ABSTRACT

Salinity tolerance is a multi-faceted trait attributed to various mechanisms. Wild barley cultivars of Tibet, commonly known as Tibetan barley is highly specialized to grow under harsh environmental conditions of Tibet and is well-known for its rich germplasm with high tolerance to abiotic stresses. The present study focused on determining the profile of expression of HvNHX isoforms gene in wild and cultivated barley under salt stress. In leaves and roots, expression of HvNHX1 and HvNHX3 in XZ16 and CM72 was up-regulated at all time as compared with sensitive one. The HvNHX2 and HvNHX4 isoforms were also induced by salt stress, although not to the same extent as HvNHX1 and HvNHX3. Gene expression analysis revealed that the HvNHX1 and HvNHX3 are the candidate genes which could have the function of regulators of ions by sequestration of Na+ in the vacuole. HvNHX1 and HvNHX3 showed a wide range of sequence variation in amplicon, identified via single nucleotide polymorphisms (SNPs). Evaluation of the sequencing data of 38 barley genotypes including Tibetan wild and cultivated varities showed polymorphisms including SNPs, small insertion and deletion (INDELs) sites in the targeted genes HvNHX1 and HvNHX3. Comprehensive analysis of the results revealed that Tibetan wild barley have distinctive alleles of HvNHX1 and HvNHX3 which confer tolerance to salinity. It was also observed that less Na+ was accumulated in the root of XZ16 as compared to the other genotypes visualized by CoroNa-Green, a sodium-specific fluorophore. XZ16 is the tolerant genotypes, showing least reduction of root and shoot dry weight under moderate (150 mM) and severe (300 mM) NaCl stress. Evaluation of genetic variation and identification of salt tolerance mechanism in wild barley could be promoting approaches to unravel the novel alleles involved in salinity tolerance.

SECTION:
Barley end use: malting and brewing

POSTER: 89

Genomic prediction in spring barley

Ahmed Jahoor

Nordic Seed A/S; ahja@nordicseed.com

Co-authors: A. Bellucci, V. Edriss, J. D. Jensen, J. Orabi, J. Jensen

ABSTRACT

In plant breeding schemes, genomic prediction (GP) has been demonstrated to be an efficient strategy to reduce time and costs to obtain elite genotypes. GP is based on the creation of a training set constituted of lines both genome-wide genotyped and tested at different locations. These are subsequently used to obtain a model for the outcome prediction on new breeding material based solely on the genotypic information, without the need of a direct measure of the traits of interest. GP efficiency has been proved in animal breeding as well as in several crops like maize, wheat and ryegrass. In the future, genotypic platform with growing amount of markers screened at reduced costs will further increase GP efficiency. Aim of the present study is to test GP for barley and evaluate the possibility to include such strategy in future breeding programs. To this end, 3 sets of 350 barley breeding lines were tested for 2 years at three locations at Nordic Seed (Galten, Denmark). All the lines were genotyped with the iSelect Illumina 9k chip and approx. 4500 SNP markers were employed in the analysis. GP was carried on using the GBLUP model accounting fixed effects as well as random effects as GxE interactions. Several traits regarding agronomical performances and quality parameters were included in the analysis. Prediction accuracies as correlation between corrected phenotypes and genomic estimated breeding values between sets will be presented. Additionally, strategies to improve GP will be discussed.

SECTION:
Abiotic stresses

POSTER: 90

Single Spike Selection (SSS): a different strategy for an efficient barley breeding

Abderrazek Jilal

INRA-Morocco; abderrazek_2001@yahoo.fr

Co-authors: M.E.H. Maatougui, A. Bari and H. Ouabbou

ABSTRACT

Barley is the best nutraceutical, multiuse and widely adapted cereals in the world. Classical barley breeding strategies for desired characteristics in potential new cultivars are time, money and efforts consuming. Single spike selection method from parental crossing to material fixing has many advantages in conducting, monitoring, screening and selecting the segregating material resulting in a pure foundation seed of a new cultivar. Interplants competition associated with seed vigor at every breeding generation represents the basic of the selection pressure. Due to the ability of large scale screening in a small flat or slope area, this strategy can also be used under collaborative breeding for biotic & abiotic stresses with minimum of effort and maximum benefit for the welfare of farmers.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 91

Genetic evidence of local adaption and long distance migration in Blumeria graminis f. sp. hordei populations from China

Zhu Jinghuan

The National Barley Improvement Centre, Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China; jinghuanz@163.com

Co-authors: Y. Zhou, Y. Shang, W. Hua, J. Wang, Q. Jia, J. Yang

ABSTRACT

Barley powdery mildew_caused by the obligate biotrophic fungus Blumeria graminis f. sp. hordei is one of the most devastating diseases in the winter barley-growing regions of China. The genetic diversity of this fungus was assessed by analyzing 75 B. graminis f. sp. hordei isolates belonging to 12 pathotypes using seven primer combinations of amplified fragment length polymorphism (AFLP) markers. The six sampling locations were 300 km to 2,200 km apart. The Highest gene diversity indices were observed within Baoshan (H = 0.21) in Yunnan province and Putian (H = 0.21) in Fujian province. Higher gene flow values were found between Baoshan and other populations (Nm = 2.010 to 5.765) except Yancheng in Jiangsu province and Jiaxing in Zhejiang province and between Putian and other populations (Nm = 2.110 to 5.423) except Jiaxing though a long geographical distance among some locations. A closer correlation between the genetic groups and origins of the B. graminis f. sp. hordei isolates and a significant population differentiation ($P < 0.005$ or $P < 0.001$) between each B. graminis f. sp. hordei population pair were detected. These results suggested that in spite of local adaptation of B. graminis f. sp. hordei populations to hosts a frequent long distance gene flow existed among these populations in China as shown in the previous study based on virulence assessment, and the centers of genetic diversity and primary inoculum origins of Bgh were probably located in those barley-growing areas in southwestern Yunnan province and southeastern Fujian province.

SECTION:
Plant Breeding and Genetics Education

POSTER: 92

Improving the drought tolerance of some barley cultivars through marker assisted selection

Nejdet Kandemir

Gaziosmanpasa University; nkandemir53@gmail.com

Co-authors: I. Saygili and B. Aslan

ABSTRACT

We are transferring three QTL regions that confer drought tolerance from cv. Tadmor into two malting (cvs. Aydanhanθm and Sladoran) and two feed (cvs. Bolayır and Baronesse) barley cultivars. These QTL regions are: a) on chromosome 6(6H) in a 30 cM interval flanked by Bmag0496 and Scssr00103 markers, b) on chromosome 1(7H) in a 38 cM interval flanked by Gbm1464 and Gbms141 markers, and c) on chromosome 2(2H) in a 34 cM interval flanked by Scssr03381 and Bmag125 markers. The QTL regions are being transferred by a crossing, followed by four backcrossing and a selfing generations. Controlled growth conditions and embryo culture are employed to hasten the breeding procedure. During backcrossing and selfing generations, QTL regions are monitored through linked DNA markers. About 10 polymorphic SSR or INDEL markers are used to monitor the QTL regions in each cross. In addition, at least 50 polymorphic markers distributed throughout the genome are used to screen against donor genetic background to allow fast recovery of recurrent parent backgrounds. BC4F2 lines are being developed and will be studied under rainfed conditions in two locations of Tokat Province. Changes in drought tolerance characteristics of recurrent parents will be determined based on seed yield and drought related traits such as carbon isotope discrimination, relative water content, amount of chlorophyll, chlorophyll fluorescent parameters, and leaf and canopy temperatures. Effects of transferred QTL regions on feed and malting quality features of recurrent barley cultivars will also be investigated. Developed lines could be used for fine mapping, as well as for sequence based cloning, of drought tolerance QTL.
[This study has been supported by The Scientific and Technological Research Council of Turkey TUBITAK (Project No: TOVAG 113O934). This poster will be presented at the 12th International Barley Genetics Symposium, Minneapolis, June, 26-30, 2016.]

SECTION:

POSTER: 93

The barley chromatin epigenome

Andy Flavell

University of Dundee at JHI; a.j.flavell@dundee.ac.uk

Co-authors: K. Baker, T. Dhillon, I. Colas, N. Cook, I. Milne, L. Cardle, M. Mascher and M. Bayer

ABSTRACT

Combinations of histones carrying different covalent modifications are a major component of epigenetic variation. We have mapped nine modified histones in the barley seedling epigenome by ChIP-seq and used peak sharing to define ten chromatin states representing local epigenetic environments in the barley genome. Five states map mainly to genes and five to intergenic regions. Two genic states involving H3K36me3 are preferentially associated with constitutive gene expression, while an H3K27me3-containing genic state is associated with differentially-expressed genes. The ten states display striking distribution patterns that divide barley chromosomes into three distinct global environments. First, telomere-proximal regions contain high densities of H3K27me3 covering both genes and intergenic DNA, together with very low levels of the repressive H3K27me1 modification. Flanking these are gene-rich interior regions that are rich in active chromatin states, greatly decreased levels of H3K27me3 and increasing amounts of H3K27me1 and H3K9me2. Lastly, H3K27me3-depleted pericentromeric regions contain gene islands with active chromatin states separated by extensive retrotransposon-rich regions that are associated with abundant H3K27me1 and H3K9me2 modifications. We propose an epigenomic framework for barley whereby intergenic H3K27me3 specifies facultative heterochromatin in the telomere-proximal regions and H3K27me1 is diagnostic for constitutive heterochromatin elsewhere in the barley genome. These data have been made available to the user community as a web-based searchable browser at http://ics.hutton.ac.uk/jbrowse/barley-chip/

SECTION:
Resources I: Genome

POSTER: 94

Barley research in India: challenges and opportunities

Ajit Kharub

Inadian Institute of Wheat and Barley Research, Karnal-132001 INDIA;
askharub@gmail.com

Co-authors: D. Kumar, V. Kumar, R. Malik, R.P.S. Verma and I. Sharma

ABSTRACT

Barley in India is grown in marginal, problematic and resource poor soils as a rainfed crop except some malt barley under contract farming. India's annual production of barley is around 1.6-1.8 m tons and area under cultivation stabilized around 0.65-0.70 m ha with per hectare yield of around 2.4 qt. In addition to direct livestock/ human consumption, barley is utilized by the beer industry, food and feed processing industry. Annual demand of malt is estimated to be around 4.0-4.5lakh tones and increasing due to more consumption of beer among youngsters. Majority of the barley produced is consumed within the country, only small quantity of feed barley is exported to Middle East. The barley research in India has been progressed with development of improved varieties (90 after AICBIP initiation) for different purposes as feed, malt, fodder and food under varied agroclimatic conditions (plains, hills, irrigated, rainfed, salt stress, biotic and abiotic stress) and efforts are on to develop varieties for changing climatic conditions. A substantial progress in enhancing yield by reducing the losses from biotic stress, increasing the seed size, lodging resistance and tolerance to salinity stress has been made by adopting the appropriate breeding approaches. Molecular studies targeted the problems of disease incidences (rust, leaf blight), insect pest infestations (aphid) and malting quality (beta glucan). ICAR-Indian institute of wheat and barley research also promote public private partnership in malt barley production for better grain and malt quality. The future challenges in India are lodging, improved varieties for food purpose, improvement in malt characteristics mainly husk, beta glucan and diastatic power, biotic and abiotic stress, climate resilient varieties, quality seed availability, assured market and price, area expansion, capacity building and linkages with national and international organisations, industries, extension workers and farmers.

SECTION:
Barley morphology and development

POSTER: 95

Conservation agricultural practices to improve quality and productivity of malt barley

Anil Kumar Khippal

ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana, India; anilkhippal@gmail.com

Co-authors: D. Kumar, J. Singh, R. P. S. Verma, J. Singh, R. Sheokand, A. Kumar, L. Kumar, R. Malik and I. Sharma

ABSTRACT

Climate change is manifesting itself in many ways across the Indian subcontinent and among the indicators; regional variations as well as reduced number of rainy days, untimely rains and sudden increase in temperature at crop maturity can be noticed. Marginal and sub-marginal farmers, having small land holdings for cultivation dominate agriculture in many Asian countries. Small changes in temperature, rainfall and terminal heat stress could have significant effect on quality and productivity of different crops. Malt barley being an industrial crop plays an important role in economy of the country and the barley farmers, so needs special attention to increase malt quality and productivity. So there is a great need for climate smart technologies. Considering these points, experiments were formulated to maximize the productivity and quality of malt barley with farmers' participatory research mode during rabi 2013-14 and 2014-15 with nine treatments and three replications in randomized block design at Karnal, India.
Zero till sown barley with rice residue retention @ 6 t ha-1 after direct seeded (3597 kg ha-1) and un-puddled transplanted rice (3544 kg ha-1) resulted in significantly higher grain yield of malt barley. Lowest grain yield (3157 kg ha-1) was recorded when barley was sown after transplanted puddled rice. Returns over variable cost (_ 23835 ha-1) and B:C ratio (1.74) were maximum when malt barley was sown with zero till technique with rice residue retention @ 6 t ha-1 after reduced till direct seeded rice. Dry weight of weeds was reduced significantly with residue retention @ 4 t ha-1 and 6 t ha-1. Significant differences were found for all the seed quality parameters except germination% due to rice residue retention. Hectolitre weight, bold grain percent and 1000 grain weight was highest when barley was sown in direct seeded rice field with rice residue @ 6 t ha-1.

SECTION:
Success stories in barley breeding and genetics

POSTER: 96

Analysis of legacy evaluation data of a large barley germplasm collection

Helmut Knüpffer

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK); knupffer@ipk-gatersleben.de

Co-authors: M. Oppermann and S. Weise

ABSTRACT

The germplasm collection of barley (Hordeum vulgare L.) of the present Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, comprises more than 15,000 accessions of spring and winter barley. Since 1946, this material has been sown in the field for seed multiplication, and a number of traits were observed and recorded during each multiplication. Till the establishment of the cold chambers for storing the seed in 1975, the samples were sown for rejuvenation every 2-3 years. Since that, the frequency of multiplication was drastically reduced, due to the longer storageability. Thus, several thousand accessions that existed in the genebank collection before 1975, were cultivated in three or more different years, and thus observation data (scores) exist with repetitions. These data will be statistically analysed, and results will be presented. Accessions with interesting combinations of traits will be shown.

SECTION:
Barley end use: malting and brewing

POSTER: 97

Gene regulation and editing of the barley cleistogamy 1

Takao Komatsuda

National Institute of Agrobiological Sciences; takao@affrc.go.jp

Co-authors: N. Anwar, M. Ohta, T. Yazawa, A. Tagiri, M. Sakuma, T. Nussbaumer, M. Pourkheirandish

ABSTRACT

Floret gaping in cereals is promoted by the swelling of the lodicule. Cleistogamy, a closed flowering, occurs in a limited germplasm of barley. Cleistogamy in barley is under the control of cleistogamy 1 (cly1), a gene which encodes a member of the APETALA2 transcription factor family. A sequence polymorphism at a miR172 target site within cly1 is associated with lodicule development and hence with the cleistogamy phenotype. Our working hypothesis is that miR172 activity reduces the abundance of AP2 protein in the lodicule primordium. An transcriptome analysis of sRNA sequence reveals that the immature barley spike includes an abundant population of miR172 molecules. RNA In situ hybridization showed that miR172 and cly1 mRNA co-localize in the lodicule primordium indicating their interaction. The expression profile of the identified miR172s was supported by qPCR, where among the three known barley miR172 genes, only the product of miR172a is abundant in the immature spike. We demonstrate some direct evidence to show that the interaction between cly1 mRNA and miR172a is an important component of the mechanism underlying lodicule development. Experiments are in progress to determine whether cly1 down-regulation is achieved by either miR172 cleavage of cly1 mRNA or by the repression of its translation. The implication is that AP2 translation is de-repressed in cly1 as suggested by a Western-blotting using polyclonal antibodies. Modification of cly1 by genome editing approach will be presented.

SECTION:
Resources II: Germplasm and Populations

POSTER: 98

The role of deleterious substitutions in crop genomes

Thomas Kono

University of Minnesota Department of Agronomy and Plant Genetics;
konox006@umn.edu

Co-authors: F. Fu, M. Mohammadi, P. J. Hoffman, C. Liu, R. M. Stupar, P. Tiffin, K. P. Smith, J. C. Fay, P. L. Morrell

ABSTRACT

Populations experience a continual input of new mutations with fitness effects rang-ing lethal to adaptive. While the distribution of fitness effects (DFE) of new mutations is not directly observable, many mutations likely have either no effect on organismal fitness or are deleterious Historically, it has been hypothesized that populations carry many mildly dele-terious variants as segregating variation, which may decrease the mean absolute fitness of the population. Recent advances in sequencing technology and sequence conservation-based metrics for predicting the functional effect of a variant permit examination of the per-sistence of deleterious variants in populations. The issue of segregating deleterious varia-tion is particularly important for crop improvement, because the demographic history of domestication and breeding allows deleterious variants to persist and reach moderate fre-quency, potentially reducing crop productivity. In this study, we use exome resequencing of thirteen cultivated barley lines and genome resequencing of seven cultivated soybean lines to investigate the prevalence and genomic distribution of deleterious variation in the pro-tein-coding regions of crop genomes.

SECTION:
Barley morphology and development

POSTER: 99

Barley multiflorus2.b (mul2.b) regulates rachilla determinacy

Ravi Koppolu

IPK; koppolu@ipk-gatersleben.de

Co-authors: R. Koppolu, H. Youssef and T. Schnurbusch

ABSTRACT

Spike inflorescence is a characteristic feature of tribe Triticeae which includes economically relevant crops like barley (Hordeum vulgare L.), wheat (Triticum spp.), and rye (Secale cereale L.). Within the spike, spikelets (grain bearing structures) are borne on a rachis (spike inflorescence axis) extension called "rachilla". In wheat, the rachilla is indeterminate and produces up to 10 florets per spikelet. Whereas in barley the rachilla is determinate and restricts the number of florets to one per spikelet. However, in a barley mutant called multiflorus2.b (mul2.b), the rachilla becomes indeterminate and produces two florets per spikelet. The rachilla indeterminacy is restricted to lateral spikelets, whereas the central spikelets have determinate rachilla. With the aim of identifying the gene underlying mul2.b locus, we have developed two F2 mapping populations, Morex _ MC(mul2.b) and MC(mul2.b) _ Montcalm. From our phenotypic analysis, we identified that mul2.b shows a semi-dominant inheritance pattern. By conducting exome capture experiment using wild type and mutant bulks (25 and 27 individuals respectively) from the Morex _ MC(mul2.b) population we could localize the mul2.b genetic interval to the centromeric region on chromosome 2H. Currently we are in the process of narrowing down the mul2.b genetic interval by screening large F2 mapping populations from Morex _ MC(mul2.b). Identification of mul2.b gene may provide important insights into the differences in floret developmental programs in barley, wheat, and other Triticeae species. mul2.b also offers potential to improve barley yields since there is a chance to increase the yield/spike by four times over wild type.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 100

Genotypic and location effect on grain protein content of barley under sub-tropical climates

Dinesh Kumar

ICAR-Indian Institute of Wheat & Barley Research, Karnal -132 001, India; dkbiochem69@gmail.com

Co-authors: R.P.S. Verma, S. Narwal, J. Singh, R. Malik, V. Kumar, A. Khippal, L. Kumar, A.S. Kharub and I. Sharma

ABSTRACT

Barley (Hordeum vulgare L.) is a very ancient grain and is considered as a sacred grain the Indian culture. However, over the years the area and production of barley decreased in India mainly because of availability of higher yielding dwarf wheat genotypes and development of irrigation facilities. The area of barley is more or less stabilized from past one decade, possibly because of increasing use of barley by the malt and food industry The malt made from barley is mainly used for brewing and some portion goes for making health foods. For brewing the protein content should be low (preferably between 9.0-11.0%), but for food malt, it has always been desirable to have higher protein quantity and quality. In case of sub-tropical climates of India the total barley crop duration is 130-140 days with effective grain filling period of 30-40 days. The restricted grain filling period influences the grain composition and getting higher protein content along with bold grains in barley grown under sub-tropical climates of India is a big challenge. The present investigation was undertaken during the year 2012-13 at seven locations to find out the genotypic differences in the grain protein content; influence of growing locations on protein content; to identify genotypes with higher protein component having higher bold grain percentage and evaluation of malt quality of selected high protein genotype/s. There was significant effect of genotype and growing location on the protein content of grain. Four genotypes (BCU 2241, BCU 5070, BK 306 and BK 316) were unique in the sense that besides higher protein content (≥ 14.0% dwb), these had higher bold grain percentage (> 85%) also. The value of lysine content in BK 306 (3.36 mg/100 g) was comparable to check DWRUB 52 (3.49 mg/100 g protein). The malt quality of BK 316 was at par to best control. The genotypes identified are now being used for improvement of protein content in hulless barley improvement programme and are useful resources for food malt improvement activities.

SECTION:
Barley morphology and development

POSTER: 101

Genetic evaluation of barly germplasm for lodging tolerance under sub-tropical climates of India

Lokendra Kumar

ICAR-Indian Institute of Wheat & Barley Research, Karnal, India – 132 001;
dr.lokendrakumar@yahoo.com

Co-authors: RPS Verma, J. Singh, D. Kumar, A.S. Kharub, A. Khippal, R. Malik, A. Kumar Sharma, and I. Sharma

ABSTRACT

Barley (Hordeum vulgare L.) is one of the most ancient crops in India and world, however in last few decades the area of barley has come down. In India, the crop is getting revived in last two decades and its area is more or less stabilized mainly because of increasing demand by malt and food industry. Barley crop is bestowed with many desirable traits like salt tolerance and drought tolerance and owing of these attributes it is most suitable crop of rain fed areas and marginal lands, but to get desirable malt quality attributes in the grain, malt barley is being grown under high input conditions in the country. However this crop is very prone to lodging problem and this drawback restricts its cultivation in high input conditions. Since past few years, due to prevailing adverse climatic conditions (heavy rainfall, winds and hailstorms) especially during crop maturity, a huge loss is occurring in the yield and quality of barley. With the establishments of new malt and breweries units in India, the demand of quality malt is increasing year by year and problem of lodging is resulting in shortfall in supply of quality grain to the industry. To overcome this problem and meet out the ever growing demand of quality malt from shrinking land holdings the development of lodging resistance varieties is the need of hour. In order to develop lodging resistant genotypes of barley a set of 349 exotic germplasm was screened at Karnal, India during 2014-15 under natural conditions. The crop maturity period (during February/March) was marred with frequent rains, strong winds and hailstorm. A total of 32 genotypes were identified to have lodging tolerance along with several desirable agronomic and physiological traits. These genotypes have great potential for high input malt barley improvement programme in India.

SECTION:
Barley end use: malting and brewing

POSTER: 102

Malt barley research in India and future prospects

Vishnu Kumar

ICAR-IIWBR, INDIA; vishnupbg@gmail.com

Co-authors: R.P.S. Verma, A.S. Kharub, D. Kumar, and I. Sharma

ABSTRACT

Malt barley improvement in India was initiated with several introductions viz. Peatland, Pedigree, Manchuria, Odessa, Clipper, Golden Promise, Midas etc. but these varieties could not popularize due to late maturity, low 1000 grain weight and grain yield (in Indian climate) and mainly due to lack of industrial support/premier prices. Malt barley improvement programme was again re-looked in early nineties and two introductions namely ALFA 93 and BCU73 were released for commercial cultivation in India. Presently, with concerted efforts and research interventions several good malt barley varieties have been released for timely (DWRUB52, DWRB92, DWRB101 etc.) and late sown (DWRB73, DWRUB64 and DWRB91) conditions. These malt barley varieties are having 78-80% malt extract, 100-105oL diastatic power, 63-65 kg/hl hectolitre wt., 10.5-11.5 % protein content. The average grain yield for these two row malt barley varieties have been depicted as 50 q/ha for timely sown and nearly 40 q/ha under late sown conditions with high to moderate resistance for stripe and brown rust and leaf blights. In northern plains of India, barley crop flowers in 85-95 days and harvests in nearly 130-135 days. Therefore, crop grain filling period is very less (35 days) and even the ripening stage coincides with high temperature as terminal heat, which reduces the activities of ADP-glucose pyrophosphorylase (AGPase) and congenial for more accumulation of sulphur poor C hordeins. However, the varieties are performing well for grain yield and different quality parameters viz. hectolitre wt., malt extract, filtration rate, diastatic power etc. The future breeding needs further attention for higher malt extract, diastatic power, better friability, filtration rate and low beta-glucan, husk and protein contents with optimum value of free amino nitrogen. The special emphases are also required for epiheterodendrin (EPH), lipoxygenase (LOX) activity and integration of molecular tools for malt barley genetic enhancement.

SECTION:
Resources II: Germplasm and Populations

POSTER: 103

Barley research and development in Ethiopia

Berhane Lakew

Ethiopian Institue of Agricultural Research (EIAR), Holetta Agricultural Research Center, KWS; berhanekaz@yahoo.com

Co-authors: W. Fikadu, B. I. G. Haussmann, R.von BrBroock

ABSTRACT

Barley (Hordeum vulgare L.) is one of the major cereal crops grown in Ethiopia and its production is an old heritage with a large number of landraces and traditional practices. The landraces have an international recognition for their useful traits to modern agriculture through provision of genes for resistance to diseases, high nutritional quality and abiotic stresses. The Ethiopian Institute of Agricultural Research (EIAR) with the support and collaboration of international organizations carry out barley research and development activities for the last fifty years. over the years, more than 50 food and malt barley varieties have been released by the national and regional research programs through local germplasm selections and introductions. Recently, a research collaboration between Ethiopia, and Germany through GIZ- Capacity Development (CD) Seed project KWS and the German Breeders Association has been started. The collaborative project focuses to enhance the efficiency of barley and wheat breeding through a combination of conventional and modern tools such as double haploids and marker assisted selection techniques; increase quality production of early generation seeds of released varieties and capacity development. In line with this, farmers based seed multiplication (FBSM) and participatory variety selection trial were conducted with full participation of farmers. To this effect, a total of 293 farmers (250 women) were participated in both activities. The FBSM activity enhanced quality seed production and more than 15.0 tons seed produced and utilized to improve productivity in the community. The participatory breeding approach also enhanced the active participation of farmers in variety selection suitable to their growing environments and farming systems in the context of climate change. The EIAR, GIZ-CD Seed and KWS partnership will continue in germplasm exchange, provision of research facilities and assisting in capacity building of the national research staff through short and long term trainings.

SECTION:
Success stories in barley breeding and genetics
Barley end use: malting and brewing

POSTER: 104

Genome-wide association mapping of root extension in a collection of European winter barley cultivars

Alessandro Tondelli, Laura Rossini

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Genomics Research Centre, 29017 Fiorenzuola d'Arda, Italy; Università degli Studi di Milano – DiSAA, 20133 Milan, Italy; alessandro.tondelli@entecra.it

Co-authors: G. Bretani, W. Xue, G. Verderio, S. Delbono, L. Cattivelli

ABSTRACT

Root extension in cereals is an extremely plastic trait exhibiting high variation in relation to the genetic background and to environmental conditions. The study of root system is particularly important in the Mediterranean area, where genetic improvement of drought tolerance on winter barley is a relevant breeding target. Here we aimed at exploring the natural genetic variation in root extension in a collection of European winter barley cultivars (67 two-rowed and 75 six-rowed, released between 1921 and 2006). For each genotype, three plants were grown in cylindrical pots (rhizotrons) with diameter of 10 cm and 50 cm height, filled with siliceous sand. Plants were collected at the 4 leaf stage (Zadocks stage 14), when roots were separated from shoots and scanned. The obtained images were analyzed by using the winRHIZO software to calculate the total root extension, as the sum of lengths of primary and secondary roots. The whole experiment was replicated three times, showing repeatability of 0.53. The same collection was previously genotyped for >7000 iSelect SNP markers, providing a powerful tool for association mapping of root traits. Genotype-phenotype association with the R-GAPIT package identified a significant genomic region on chromosome 5H-bin7, that has been scrutinized for candidate genes and alleles with a putative role in the trait under study.

SECTION:
Success stories in barley breeding and genetics

POSTER: 105

AAC Connect two-row malting barley combines desirable agronomics, quality and disease resistance including lower DON accumulation

W.G. Legge

Agriculture and Agri-Food Canada; bill.legge@agr.gc.ca

Co-authors: J.R. Tucker, A. Tekauz, R.A. Martin, T.M. Choo, B.A. Blackwell, and M.E. Savard

ABSTRACT

Fusarium head blight (FHB), caused by Fusarium graminearum Schwabe, continues to be one of the most damaging diseases of barley in western Canada due mainly to effects of the mycotoxin deoxynivalenol (DON) on grain quality. Combining lower DON content with good agronomic traits including high yield, resistance to other diseases and malting quality in a commercially acceptable package has been difficult. AAC Connect (tested as TR12225 and registered on April 1, 2016) is a new two-row malting barley variety developed by Agriculture and Agri-Food Canada's Brandon Research and Development Centre, Brandon, Manitoba, from the cross TR04282/BM9831D-229, where TR04282 = Harbin/TR253//TR253 with Harbin being an exotic, two-row source of FHB resistance from China. AAC Connect was 11% higher yielding than AC Metcalfe and 5% higher yielding than CDC Copeland over 2 years of cooperative testing in western Canada. It performed particularly well in the eastern Black and Brown Soil Zones, where it out-yielded AC Metcalfe by 14%. It has shorter, stronger straw and heavier, plumper kernels than the checks combined with similar days to heading and maturity. AAC Connect has a good disease resistance package, including resistance to spot-form net blotch, surface-borne smuts and stem rust; intermediate resistance to net-form net blotch; and moderate resistance to spot blotch and FHB with lower DON content. It had 24% lower DON content than AC Metcalfe over 12 site-years in Manitoba FHB nurseries. It has a promising malting quality profile with higher malt extract and friability than the checks, lower wort viscosity, beta glucan content within the lower range of the checks, diastatic power and alpha amylase approaching AC Metcalfe, and generally similar to the checks for the remaining malting traits. AAC Connect has a desirable combination of agronomic, malting quality, and disease resistance traits including lower DON accumulation.

SECTION:
Barley morphology and development

POSTER: 106

Comparison of spring barley (Hordeum vulgare L.) population genetic diversity and performance under organic and conventional farming systems

Linda Legzdina

State Priekuli Plant Breeding Institute; Linda.Legzdina@priekuliselekcija.lv

Co-authors: D.E. Ruņģis, I. Mežaka, I. Ločmele, D. Piliksere, N. Venta

ABSTRACT

Increasing genetic diversity in crops can ensure stability, adaptability, reduce disease severity, and improve competitive ability with weeds and nutrient uptake efficiency. Solution for increasing diversity within a variety of self-pollinating species is growing of populations which include higher levels of diversity if compared to pure lines and mixtures and are able to develop itself in time by adapting to the respective environment and providing buffering capacity. After hybridization population can be multiplied in the environment of interest with natural selection applied resulting in increase of frequency of better adapted plants. Our research aims at comparison of three population types: bi-parental populations (two parental genotypes), complex populations (more than two parents combined in one cross) and composite cross populations (CCPs, bulked dialell crosses among group of 10 parents). This presentation will include the preliminary results on examination of genetic diversity by nine SSR markers and testing under organically and conventionally managed field trials of four bi-parental populations, six complex populations and three CCPs. The study on genetic diversity found that in bi-parental populations average number of alleles ranged from 2.5 to 4.9, in one complex population on average 3.4 alleles per loci were detected, whereas in CCPs it was from 5.3 to 5.9. Thus CCPs showed larger intra-population variation than other types of studied populations. Two season results show that significantly higher average grain yield was obtained for one CCP and one complex population surpassing the average of a group of five control varieties (3.74 t/ha) by 24 and 14%, respectively. To compare the average yield of the three population types, there was a tendency for higher yield for CCPs and lower yield for bi-parental populations (3.92 t/ha versus 3.68 t/ha), however, under organic location in one of the two seasons complex populations provided the best result.

SECTION:
Biotic stresses
Barley end use: malting and brewing

POSTER: 107

The evolution of species range limits in wild barley and its effects on deleterious mutations

Li Lei

University of Minnesota, Department of Agronomy and Plant Genetics; llei@umn.edu

Co-authors: T. J. Y. Kono, P. J. Hoffman, C. Liu, F. R. Gaibor, S. Wyant, K. P. Smith, J. C. Fay, and P. L. Morrell

ABSTRACT

Though barley is cultivated from the equator to the Arctic Circle, the progenitor species, Hordeum vulgare ssp. spontaneum, occupies a relatively narrow latitudinal range. A number of models have been put forward to explain the limits of geographic range within species. Population genetic models invoke a number of constraints, including recent arrival of the species in the current habitat, metapopulation dynamics at the range periphery, source-sink dynamics, maladaptive gene flow and genetic constraints on adaptation. Currently it is unknown which of these factors contribute most directly to species range limits in wild barley or how the cultivated species has escaped these constraints. In several of these models, limited effective population size (Ne) in peripheral populations may play a role in adaptive constraints. Limited Ne also means that selection is less effective at removing deleterious mutations. To determine how species range limits interact with the proportion of variants that are deleterious we will explore how wild barley species range limits evolve and how they impact deleterious variants. To address this question we performed exome resequencing for 75 wild barley accessions from distinct subpopulations. Our comparative analysis seeks to determine if peripheral populations have lower nucleotide diversity, smaller population size and more deleterious mutations than populations at the center of the range.

SECTION:
Resources II: Germplasm and Populations
Breeding Methodologies: current approaches and future

POSTER: 108

The gene conferring susceptibility to spot blotch caused by Cochliobolus sativus is located at the Mla locus in barley cultivar Bowman

Yueqiang Leng

North Dakota State University; yueqiang.leng@ndsu.edu

Co-authors: M. Zhao, R. Wang, B. J. Steffenson, R. Bruggeman, and S. Zhong

ABSTRACT

Spot blotch caused by Cochliobolus sativus is an important disease in many barley production areas, but the molecular mechanisms underlying susceptibility to the disease are not well understood. In this study, we identified and mapped a dominant gene conferring susceptibility to the pathotype 2 isolate ND90Pr of C. sativus in barley cultivar Bowman. Using a doubled haploid population derived from the cross between Calicuchima-sib and Bowman-BC, which are resistant and susceptible to isolate ND90Pr, respectively, we showed that a single gene (designated as Scs1) control the susceptibility contributed by Bowman-BC. Scs1 was mapped to the same location as Rcs6 on the short arm of chromosome 1H, which confers resistance to isolate ND90Pr and contributed by Calicuchima-sib. Using a genome-wide putative linear gene index of barley (Genome Zipper), we constructed a genetic map with 12 cleaved amplified polymorphism (CAPS) markers spanning a 59.1 cM genomic region with the Scs1 locus. Further fine mapping with 944 F2 individuals derived from a cross between Bowman and ND 5883 indicated that Scs1 is a dominant gene and located at an interval of 0.766 cM genomic region. This genomic region covers a physical distance of 250 kb homologous to the Mla locus cloned from the barley cv. Morex. With sequences retrieved from the genome sequence of Bowman for the Scs1 region, we identified 17 markers co-segregating with Scs1. Sequence analysis of these markers showed that ten of them were disease resistance (R) gene homologs containing either full or partial domains of coiled-coil (CC)-nucleotide-binding site (NBS)-leucine-rich repeat (LRR), and 5 out of the 10 R gene homologs were highly homologous to the powdery mildew resistance genes at the Mla locus with identity ranging from 83% to 94%. Further characterization of the candidate gene for Scs1 is in progress.

SECTION:
Resources II: Germplasm and Populations

POSTER: 109

Identification of genetic and phenotypic traits in the root system of drought tolerant spring barley (Hordeum vulgare)

Jens Léon

Co-authors: J. Palczak, T. Wojciechowski, J. Lynch, B. Kilian, F. Ordon, A. Ballvora

ABSTRACT

Drought stress is a major limiting factor of crop productivity and yield quality. The root system of a plant contributes substantially to a healthy development of the whole organism by supplying it with water and nutrients by anchoring it in the soil. Plants that make root faster and deeper and show a high root number and branching density are probably more drought stress tolerant than others due to a better soil exploration. Furthermore, narrow xylem vessels lead to higher hydraulic resistance and reduced water transport within the plant and thus to efficient use of exploited water. The goal of this study is to uncover the allelic variability for root morphology traits, dynamic of root development and the reaction to drought stress. The investigated population consists of 192 spring barley genotypes (Hordeum vulgare) including wild forms and cultivars from over forty countries. Experiments were performed under field conditions and under drought stress (rain out shelter) in three consecutive years. The “Shovelomics” technology was used to analyze root architectural traits like the number of nodal roots, their growth angles, branching densities and the number of seminal roots. In addition, number of shoots per plant and their biomass was determined. In total 10 morphological traits were scored. To get inside into anatomy of roots laser tomography analysis of root cross sections is performed. The images provide information about total root area, area of cortex and stele, number of xylem vessels and total xylem areas. Genotyping based on 9k iSelect Chip and GBS revealed 6.272 polymorphic SNP markers with information about their position in the genome left after marker cleaning process.

Data on correlations between phenotyped root morphological and anatomical traits and the treatments will be shown. Furthermore, marker-trait associations identified using genome-wide association analysis will be presented and discussed.

SECTION:
Plant Breeding and Genetics Education

POSTER: 110

Evaluation of barley genotypes to fusarium head blight (FHB) under favorable environment for the disease

Maria Imaculada Pontes Moreira Lima

Embrapa Wheat; maria-imaculada.lima@embrapa.br

Co-authors: E. Minella, M.Z. Miranda, C.A. Mallmann, and D. Schmitz

ABSTRACT

Fusarium head blight (FHB) caused by Gibberella zeae (Fusarium graminearum) is a major barley disease in Southern Brazil because the associated mycotoxins. The objectives of this work were to evaluate grain infection by F. graminearum and the production of the mycotoxin deoxynivalenol (DON) in FHB favorable environment.

An experiment was seeded at Embrapa Wheat, Passo Fundo, RS, Brazil in 2013, in the "FHB Nursery". The trial was established in a randomized block design with six genotypes and three replications in of seven five meter long plots. At heading, wheat grains with perithecia of G. zeae were spread on the soil surface followed by plant watering. Fifty spikes were harvested, threshed and a 100 g sample of grain was classified in 2.8, 2.5, 2.2 and < 2.2 mm sieves. DON quantification was made in 2.8 + 2.5 mm grains samples.

One hundred seeds of each class, after asepsis, were plated on PDA medium and incubated in a 12 h photoperiod, 24 Γ C ± 2 Γ C temperature for six days. The incidence of F. graminearum was very low and was statistically different only for PFC 2008067 in the grain class < 2.2 (7.7%).There were no statistical differences among genotypes for classes 2.5 and < 2.2. The infection of F. graminearum in line PFC 2008067 (10.7%) was different and inferior to PFC 2007103 (24.0%) and PFC 2008012 (24, 7%) for class 2.5. BRS Cauê (26.7%) differed statistically from PFC 2008067 (7.7%) in the for the < 2.2 mm class. The lowest levels of DON in _g/kg were quantified in BRS Cauê (263) and PFC 2008067 (253) and the largest in PFC 2008012 (480). DON was not detected in genotypes PFC 2008058. The results confirm that even in low F. graminearum grain infection, can produce DON at low detected levels.

SECTION:
Abiotic stresses

POSTER: 111

Occurrence and damage of head blast in barley in Brazil

Maria Imaculada Pontes Moreira Lima

Embrapa Wheat; maria-imaculada.lima@embrapa.br

Co-authors: E. Minella

ABSTRACT

The fungus Pyricularia grisea (Magnaporthe grisea) was first reported infecting barley in Brazil in 2000 on leaves and in 2003 on spikes. The characteristic symptoms in spikes, the main organ affected, are premature discoloration above the point of infection by the pathogen, which occurs in the rachis. The grains formed above the infection point are usually smaller, due to the interruption of the translocation of water and nutrients. In this study we measured the damage of the blast in barley spikes, cultivar BR 2, under natural infection in the field of Embrapa Wheat, Passo Fundo, RS, in 2002.

Twenty-two spikes with symptoms of blast and 22 spikes without symptoms were marked. At maturity they were harvested separately and threshed by hand. The weight of a thousand kernels (TWK) and the kernel plumpness (KP) were determined

The TKW of the affected spikes weighed 13.7 g less than those of healthy spikes, with a reduction of around 30%. The KP of healthy ears was 35.1% higher for grains Class 1(> 2,5 mm) and 22.6 and 12.5, lower for classes 2 and 3, (<2.5 > 2.2 and < 2.2 mm) respectively, than the diseased spikes. The results show that the blast in barley spikes can significantly reduce grain size and weight suggesting that, in case of widespread epidemics, the disease may treat barley yield and production in Brazil.

SECTION:
Biotic stresses

POSTER: 112

Virulence diversity in the populations of barley spot blotch pathogen Bipolaris sorokiniana in China

Ruiming Lin

Institute of Plant Protection, Chinese Academy of Agricultural Sciences; linruim@hotmail.com

Co-authors: H. Guo, F. Wang, J. Feng, S. Xu

ABSTRACT

Spot blotch is widely found where barley is grown. It is caused by Bipolaris sorokiniana (Sacc.) Shoemaker, whose teleomorph is Cochliobolus sativus. Spot blotch usually initiates significant yield losses in regions with a warm, humid climate condition. It is the most destructive disease on barley in the northeast China, including Heilongjiang province and eastern part of Inner Mongolia. Yield reduction is as high as 20%, even up to 40% in some fields. The most effective control method is to utilize durable resistance cultivars. However, there is no document of virulence variation in C. sativus populations in these areas. Through selecting barley germplasm with more than fifty strains collected across northeast China, a set of differential cultivars was set up, including 12 cultivars such as Ganpi 2, Zaoshu 3, Mengpimai 3,Kenpimai 7, Kenpimai 9,Kenpimai 11, Secale cereale Lrye, Morex, Tradition, ND 5883, Bowman and ND B112. Every cultivar is with different genetic background and the last three ones are used as differentials in the United States. When only using three American differentials, fifty strains were grouped into three virulence types: no virulence (1 strain), low virulence (36 strains) and intermediate virulence (13 strains). While tested with the other 9 differential cultivars plus one highly susceptible MIRCO, 14 strains are low virulent, 31 intermediate virulent and 5 high virulent. However, when using the twelve differentials listed to analyze virulence diversity, we found the number of low virulence, intermediate virulence and high virulence strains is 26, 23 and 1 respectively. It was supposed that results of virulence diversity of pathogen populations with the 12 differentials are more reasonable and will be used to facilitate the development of barley resistance cultivars to spot blotch. Besides, our results also indicated a higher virulence diversity level in the populations of C. sativus in the northeast China.

SECTION:
Biotic stresses

POSTER: 113

Genomic structural variations in two genomic regions of wild barley

Chaochih Liu

University of Minnesota; liux1299@umn.edu

Co-authors: L. Lei, P.L. Morrell

ABSTRACT

A number of large structural variants in both wild and cultivated barley have been detected by cytological studies. Detected variants have included both chromosomal inversions and translocations. However, cytological studies are laborious and generally do not permit a broad survey of accession from across the geographic range of a species. Chromosomal inversions are noted for their role in maintaining locally adaptive variants, but at the cost of creating partial reproductive isolation. The most recently reported putative structural variants in wild barley occur on chromosomes 2H and 5H. The putative variants were detected on the basis of SNP frequency divergence and elevated linkage disequilibrium. These chromosomal regions also have the strongest signals genome-wide, for association with environmental variables, notably rainfall and temperature regime. We make use of tests of interchromosomal measures of linkage disequilibrium to attempt to distinguish inversions from chromosomal translocation. Using whole exome resequencing data from wild barley, we seek to further lineate the regions of barley chromosomes impacted by putative structural variants. Along with investigating putative variants, we will also be able to identify population genetic approaches that provide the best utility for identification of structural variations.

SECTION:
Resources I: Genome
Biotic stresses

POSTER: 114

Using QTL analysis of waterlogging tolerance in barley to measure its impact on plant growth and leaf chlorosis

Andres Locatelli

Departamento de Producción Vegetal, Universidad de la República; aloca@fagro.edu.uy

Co-authors: G. Quero, L. Viega and A. Castro

ABSTRACT

Waterlogging can be a key limiting factor for crop yield, particularly in the case of barley. Annual rainfall in Uruguay have an average between 1000 and 1200 mm, with high variation between years. In addition to that, a significant fraction of the soils in the production areas has limited water storage capacity and problematic drainage, making the ocurrence of hipoxia during short periods a common situation. In this research we used bi-parental QTL analysis to study the genetic basis of tolerance to waterlogging in barley, analyzing 84 RIL derived from the cross between two adapted varieties with contrasting tolerance leves: cv INIA Ceibo (tolerant) and cv. Norte•a Carumbé (less tolerant). The population was genotyped with 134 SNPs and 9 SSRs. It was phenotyped in two growth chamber experiments and one greenhouse experiment. In these experiments waterlogging conditions were imposed during 15 days, starting at GS13 (chamber experiments) and GS30 (greenhouse experiment). All experiments include a control treatment. In the growth chamber we measured leaf chlorosis (LC) during flooding and root biomass (R), above ground biomass (AG), R/AG ratio and root volume and density at the end of the flooding interval. In the greenhouse experiment we measured LC during flooding. A total of nine QTL were detected for LC on chromosomes 3H (1), 5H (1), 6H (1) and 7H (3). For reduction of plant weight and volume we detected six QTL on chromosomes 3H (1), 5H (1), 6H (1) and 7H (3). At least two QTL (on chromosomes 6H and 7H) for both traits have coincident location. Our preliminary results support the idea of a genetic basis for waterlogging tolerance, a particular complex trait. At the moment we are analyzing the effects of a period of hipoxia on yield components in order to elucidate the consequences of it on crop productivity.

SECTION:
Resources I: Genome

POSTER: 115

Introgression of quantitative resistance to Rhynchosporium commune into elite winter barley through marker assisted backcrossing.

Mark Looseley

The James Hutton Institute; mark.looseley@hutton.ac.uk

Co-authors: A.C. Newton, P. Werner, D. Harrap, D. Guy

ABSTRACT

Rhynchosporium commune is a highly destructive fungal pathogen of barley. Varietal resistance to R. commune has traditionally focussed on the incorporation of single major resistance genes, which, whilst giving large initial gains, are frequently defeated by changing pathogen populations. Advances in marker technology has provided the tools necessary for breeders to design varietal resistance that is based on multiple quantitative resistance effects, each of relatively small effects; contributing to a more durable form of resistance. The effectiveness of this approach for producing broad-based varietal resistance was studied. Multiple candidate resistance QTL were identified from two mapping populations, and introgressed into an elite winter variety (KWS Cassia) using marker assisted backcrossing to produce a set of Near-isogenic lines (NILs) incorporating single or multiple resistance QTL. NILs were characterised phenotypically using disease nursery trials, quantitative PCR, single-spore isolate tests and confocal microscopy using GFP expressing R. commune isolates.

Clear resistant phenotypes were identified from disease nursery trials for a number of candidate QTL whilst alternative contrasting race-specific effects on fungal growth morphology were also identified. High density genotyping of selected NILs provided improved map resolution for a number of candidate QTL. NILs carrying multiple QTL introgressions demonstrated a clear increase in resistance scores compared to single QTL NILs, demonstrating the effectiveness of combing multiple quantitative resistances as a method for designing varietal resistance to R. commune.

SECTION:
Abiotic stresses
Breeding Methodologies: current approaches and future

POSTER: 116

Physiological and proteomics mechanism of barley response to waterlogging

Haiye Luan

Yangzhou University; rgxu@yzu.edu.cn

Co-authors: B. Guo, C. Lv, X. Zhang, R. Xu

ABSTRACT

Waterlogging is one of the major harmful abiotic stresses to crops in low-lying areas and causes great yield losses. Two lines TF57 and TF58 with similar agronomic characters but different waterlogging-tolerance were selected from DH population derived from TX9425_Franklin to investigate physiological and proteomics mechanism of of barley response to waterlogging. Under waterlogging, the sensitive line TF57 showed obvious symptoms including stunting, yellowing leaves and dwarfing. The chlorophyll content (SPAD value) and autioxidant enzyme activities in leaves significantly reduced in TF57 but the malondialdehyde contents significantly increased comparing to normal growing conditions. In addition, a few adventitious roots appeared. On the contrary, autioxidant enzyme activities of leaf and root were significantly increased in TF58, a large number of adventitious roots and developed aerenchyma was observed in TF58 under waterlogging stress. Comparative proteomic analysis in leaf, adventitious roots, root nodal and seminal root were performed and 326 detected protein spots were differentially expressed between TF58 and TF57. Among which one hundred differentially expressed protein spots were identified by using MALDI-TOF-MS, which are involved in energy, metabolism, stress, protein modification, RNA, Light reaction, Calvin cycle, cytoskeleton, redox and development. The ATP synthase CF1 beta subunit, oxygen-evolving enhancer protein, adenosine diphosphate glucose pyrophosphatase were up-regulated in the leaf of TF58. Pyruvate decarboxylase, glutamine synthetase, aminocyclopropane carboxylate oxidase, glutathione s-transferase, NADP-dependent malic enzyme, caffeic acid o-methyltransferase, guanine nucleotide-binding protein subunit beta were differentially expressed in root and root nodal. Taken together, TF58 and TF57 lines displayed significant difference in physiological response under waterlogging stress, as well as expression differences between TF58 and TF57 at protein abundances and multiple functions of differentially expressed proteins may contribute to waterlogging tolerance of barley.

SECTION:
Biotic stresses

POSTER: 117

Shotgun proteomic analysis of the seed proteomes of two-row and six-row barley

Ramamurthy Mahalingam

USDA-ARS, Cereal Crops Research Unit; mali.mahalingam@ars.usda.gov

ABSTRACT

Proteins play a vital role in determining the quality of barley in malting and brewing end-uses. A comprehensive inventory of all the barley seed proteins would be a useful resource for identifying novel protein markers associated with barley malting quality. Many of the proteomics studies in barley have been conducted using the 2D-PAGE followed by MALDI-TOF or MS/MS analysis. In this study we explored the use of shotgun proteomics to analyze the differences in the seed proteomes of the two-rowed barley cultivar 'Conrad' and six-rowed barley cultivar 'Lacey'. Dry seeds were used for isolating the total proteins in acetone. Precipitated proteins were reduced, alkylated and digested with trypsin. Typsin digested peptides were desalted by solid phase extraction using the C18 cartridges. Digested peptides were analyzed using HPLC coupled to tandem mass spectrometry on a hybrid linear ion trap mass spectrometer equipped with a nanoelectrospray ion source. MS spectra of tryptic digests were collected over an m/z range of 380-2400. Raw MS/MS data files from Xcalibur software were submitted to Proteome Discoverer software with the Mascot search engine for peptide/ protein identification. Scaffold software was used to validate MS/MS based peptide and protein identification. Three biological replicates of the two seed samples were analyzed. This study led to the identification of nearly 1000 barley seed proteins, the largest, when compared with the published seed proteome studies in soybeans and quinoa using a similar strategy. A detailed quantitative and qualitative analysis of the similarities and differences in the seed proteomes of the two-row and six-row barley will be presented.

SECTION:
Abiotic stresses

POSTER: 118

Genetic relatedness studied at molecular level for huskless barley (Hordeum vulgare)

Rekha Malik

ICAR-Indian Institute of Wheat and Barley Research; rekhasmalik1@gmail.com

Co-authors: J. Singh, S. Verma, R. Kumar, D. Kumar, V. Goyal, L. Kumar, A.S. Kharub, R.P.S. Verma, and I.Sharma

ABSTRACT

Barley (Hordeum vulgare L.) is fourth major crop in the world that has been under cultivation since ancient times. It is classified into hulled and hulless barley according to the grain type. In India, hulless barley is cultivated in the higher Himalayas during the summer season and in some tribal regions during the main winter season. Hulless barley has many positive characteristics for feed, food and malt uses. Recent trends in India also indicate the popularization of the naked barley with higher beta-glucan content becoming popular for breakfast food. The genetic improvement of naked barley is a big challenge as not much information is available about the genetic variability of hulless barley for designing hybridization program. Keeping in view, forty genotypes of hulless barley including 5 Indian released varieties, 2 advanced lines and 33 exotic lines were screened with a set of 35 SSR markers for genetic diversity studies during ICAR-ICARDA collaborative research programme for dryland cereals. Molecular weights for microsatellite products, in base pairs, were estimated and the summary statistics including the number of alleles per locus and polymorphism information content (PIC) values were determined. Total 60 alleles were scored with number of alleles ranging from 1 to 3 with an average of 1.66 alleles per locus. The band fragment size varied from 100 bp to 800 bp and PIC values varied between from 0.0 to 0.58. DARwin software package was used to group these genotypes for diversity analysis that clustered these genotypes in five groups depending on their genetic variability. This study identifies genetically diverse genotypes which may be used for breeding purposes as hulless barley improvement is being paid more emphasis these days in India with potential for feed, food and industrial uses.

SECTION:
Resources I: Genome

POSTER: 119

Combining drought tolerance and malt quality

Bram Marynissen

UGent; bram.marynissen@ugent.be

Co-authors: G. Haesaert

ABSTRACT

Spring barley is an important small grain cereal in semi dry areas (e.g. Ethiopia, Egypt, India,..). A significant percentage of Spring barley is grown for malt production although malting quality is frequently not sufficient. Besides a low malting quality local varieties are very often characterized by poor disease and drought tolerance and a consequently low yield level.

My PhD study will develop a new generation of varieties adapted to semi-arid growing conditions. These varieties will combine adaptation to local growing conditions with good malting quality. Therefore a diverse collection of approximately 50 genotypes of Spring barley will be phenotyped (e.g. yield, biomass, tillering, number of spikes, thousand kernel weight, grains per spike, grain plumpness, time to anthesis and duration of grain filling period) and genotyped (SNP's). Experiments in greenhouses as well as field experiments under different growing conditions will be carried out. Based on these information breeding values will be calculated and prediction models will be constructed using the Progeno software. Crossing results will be validated by using dihaploids. These doubled haploids will be produced by using the androgenesis in vitro method.

SECTION:
Barley end use: food/feed

POSTER: 120

Barley Rph1-15 Near Iso-genic lines in susceptible cultivar ‘Bowman’

Matthew Martin

University of Minnesota, PLPA DEPT; mjmartin@umn.edu

Co-authors: O. Chicaiza, J. Caffarel, A. Druka, R. Waugh, J. D. Franckowiak, B. J. Steffenson

ABSTRACT

Leaf rust of barley, caused by the fungal pathogen Puccinia hordei, occurs worldwide wherever the crop is grown. Yield losses ranging from 10 to 63% can occur on susceptible cultivars depending on the onset and severity of epidemics. In the last 70 years, 23 major effect resistance genes have been identified in various accessions of cultivated barley (Hordeum vulgare ssp. vulgare), wild barley (Hordeum vulgare ssp. spontaneum), and bulbous barley grass (Hordeum bulbosum). To efficiently characterize virulence diversity in populations of P. hordei, the individual Rph genes should be divided into near iso-genic lines (NILs) via backcrossing into a uniform susceptible background of barley. A set of NILs for Rph1-15 were developed by back-crossing 12 differential lines and wild species into the susceptible cultivar ‘Bowman’. From 1 to 8 backcrosses were completed for 95 alleles of Rph1-15. The final selection of lines for the differential host series of NILs will be made based on both the similarity of infection types between the donor parent and the NIL and also single nucleotide polymorphism (SNP) data indicating the size of the introgression around the Rph genes. This set of NILs should be useful for differentiating races of P. hordei and for clarifying resistance gene specificity.

SECTION:
Abiotic stresses
Plant Breeding and Genetics Education

POSTER: 121

The origin of stem rust resistance in Hordeum

Oadi Matny

University of Minnesota/Plant Pathology Department; onmatny@umn.edu

Co-authors: A. Hulse, B. Steffenson

ABSTRACT

Puccinia graminis f. sp. tritici, known as pathogen caused stem rust disease on barley, and it is a serious problem on barley growing areas of the world. The best way to control this disease is by find a new source of resistance gene and uses it in barley breeding program. Rpg1 gene is one of the most resistance genes to stem rust, and it is use in barley breeding programs to get a long term resistance to P. graminis f. sp. tritici pathogen in barley cultivars. Rpg1 gene has been found in the landrace in Switzerland, there is a few race of stem rust as known Rpg1- virulent like TTKST and QCCJB. The object of this study is to know the origin region source of the Rpg1 in wild barley by characterized the reaction type of HKHJC race of stem rust on the wild barley germplasm collection from WBDC , ICARDA (Syria), N.I. Vavilov Institute of Plant Industry/St. Petersburg (Russia) , Institute for Cereal Crops Improvement, Tel Aviv University/Tel Aviv (Israel) , The Institute of Evolution and University of Haifa/Haifa (Israel). Five hundred twenty nine accessions of wild type were exam for stem rust HKHJC race which is diagnostic to detect Rpg1. From the stem rust phenotyping 24 (4.5%) barley accessions were showed resistance reaction to HKHJC race, 18 (75%) of barley accession was originally from Medal East. These results keep the door opened toward possibility of the original region of Rpg1 source or maybe there is another gene control resistance reaction to HKHJC race. These results want to be confirmed by molecular assay, so far we still working in this project.

SECTION:
Biotic stresses

POSTER: 122

Genomic dissection of plant development and its impact on yield components in barley through nested association mapping

Andreas Maurer

University of Halle, Germany; andreas.maurer@landw.uni-halle.de

Co-authors: V. Draba, K. Pillen

ABSTRACT

Time of flowering is a key agronomic trait that has a major impact on crop yield. Therefore, understanding the genetic and environmental factors that trigger a plant to flower has been of major interest during the last decades and many questions could be answered. However, there is a growing interest in dissecting the genetic architecture of flowering time in more detail to fine-tune plant development in regard to maximizing yield. Thus, we utilized the barley nested association mapping population HEB-25 to map quantitative trait loci (QTL) regulating life history traits, plant height and the yield component thousand grain weight through genome wide association studies (GWAS).
After conducting GWAS numerous QTL could be defined. By comparing their impact across traits we formulated five general developmental trends: 1) Flowering time QTL affect multiple developmental traits simultaneously; 2) Genetic effects on flowering time are more pronounced than on shooting time; 3) Duration of the ripening phase compensates for early/late flowering; 4) Duration of ripening influences thousand grain weight; 5) Developmental QTL also affect plant height. Apart from that several new QTL that partially act phase-specific could be defined. In regard to thousand grain weight we found a clear gain with increasing duration of the ripening phase. However, a long duration of shoot elongation has also to be considered when maximizing yield, which is hampered by the negative correlation of both traits. We showed that the wild allele at the semi-dwarf locus denso is a promising target to increase yield since it uncouples this negative correlation.
The genomic dissection of key developmental stages based on HEB-25 will further assist to unravel plant developmental pathways. Moreover, the targeted introgression of useful wild alleles into the elite breeding pool in barley may enable fine-tuning of developmental phases to maximize yield.

SECTION:
Resources II: Germplasm and Populations

POSTER: 123

Growing up – Developmental genetics of internode elongation

Sarah McKim

University of Dundee; sarah.mckim@hutton.ac.uk

Co-authors: V. Patil, M. Lafos, and R Waugh

ABSTRACT

Domestication of cereals from their wild relatives involved often drastic changes in their architecture - the arrangement, form and function of their body parts, as the earliest farmers cultivated trait variants associated with increased productivity. Genomic resources developed for barley have vastly improved its molecular tractability, helping researchers identify transcription factors which regulate architecture. However, to realise the full potential of molecular breeding in barley, we should learn how these factors are regulated, how they interact and their downstream targets. We address this knowledge gap by applying a combination of fine-scale phenotyping, genetic analyses, functional transgenics and genome-wide analyses, to define the molecular networks underlying barley architectural traits. We are particulaly interested in stem growth. Here, I will show that microRNA172-downregulation of the HvAPETALA (HvAP2) transcription factor promotes internode elongation by controlling both internode meristem activity and elongation of specific cell populations. I will also present how we use precise transcriptomic sampling to reveal spatiotemporal switches in gene expression along the developing internode, including possible targets of HvAP2 essential for internode meristem maintenance. Taken together, our research delivers a developmental genetic understanding of cereal internode elongation at a greater depth than ever before.

SECTION:
Barley morphology and development

POSTER: 124

Food barley breeding for flavor, nutrition, and color

Brigid Meints

Washington State University; brigid.meints@wsu.edu

Co-authors: P. M. Hayes, S. Fisk, L. Helgerson, S. Lyon, S. S. Jones

ABSTRACT

Colored barley has been grown as human food for millennia in many parts of the world, but feed and malting barley with a white seed-coat have dominated the commodity market. Blue, purple, and black seed coats in barley are found predominately in landraces from areas of the world where barley remains a staple crop; these regions include the Himalayas, Ethiopia, and the Andes. These lines are not well adapted to the Pacific Northwest and so it is necessary to introgress the color traits into adapted material with good quality. Breeding and selecting barley varieties with unique color traits can bring recognition and distinction to grains grown in this region and the quality characteristics will make them desirable to bakers, chefs, consumers, and companies looking to create distinct products with grain. Through this work we are seeking to create nutritionally superior barley lines with excellent baking and culinary qualities that are immediately recognizable by their color. It has been shown that purple and blue lines contain anthocyanins and black lines contain melanin, contributing to the antioxidant properties of the grain. Because the genes regulating the blue, black, and purple color traits are distinct and the colors exist in separate layers (blue in the aleurone, black and purple in the pericarp) it is possible to stack these traits for increased phenolic content. We requested and received 47 accessions of colored barley from the National Small Grains Germplasm Collection in Aberdeen, ID to use as parents in a crossing block. Successful F1 seed from these crosses is currently being increased to begin selection. Additionally, colored barley breeding lines that are currently in the F3, F4, and F7 stages were planted at multiple locations in 2015. These lines are currently being evaluated for agronomic quality, nutritional value, and flavor.

SECTION:
Barley end use: food/feed

POSTER: 125

BRIDGE: Biodiversity informatics for harnessing barley genetic diversity hosted at the genebank of IPK Gatersleben

Sara Giulia Milner

IPK-Gatersleben; milner@ipk-gatersleben.de

Co-authors: A. Himmelbach, M. Oppermann, S. Weise, A. Graner, H. Knüpffer, U. Scholz, A. Börner, M. Mascher, N. Stein

ABSTRACT

Genomics and biodiversity informatics are rising as fundamental tools to harness genetic resources harbored in germplasm collections, which represent essential albeit mostly untapped reservoirs of genetic diversity for crop research and improvement. The BRIDGE project aims to molecularly characterize more than 20,000 accessions of domesticated (Hordeum vulgare L. subsp. vulgare) and wild (Hordeum vulgare L. subsp. spontaneum (K. Koch) Thell.) barley hosted at the German ex situ genebank at IPK Gatersleben, by means of a genotyping-by-sequencing (GBS) approach. GBS data will be analyzed in context of barley genomic framework to study population structure and genetic diversity patterns and also to mine allelic variation at breeding-relevant traits. A novel warehouse infrastructure will provide a systematic valorization to the upcoming genomics data and a link to the passport and phenotypic data accumulated by the IPK genebank conservation menagement. Here, we describe our bioinformatics pipeline for read mapping, variant detection and genotyping. First results of the GBS analysis conducted on more than 6,000 barley accessions will be presented.

SECTION:
Resources I: Genome
Resources II: Germplasm and Populations

POSTER: 126

Improvements in acid soil tolerance of Brazilian barley will require new allelic combinations

Euclydes Minella

Embrapa Wheat; Euclydes.Minella@embrapa.br

Co-authors: J.R. Ferreira, J.F. Pereira, P.R. Ryan, E. Delhaize, and C.A. Delatorre

ABSTRACT

More than 50% of the agricultural soils in Brazil have toxic levels of aluminum (Al3+). The Al3+ can limit root growth negatively interfering with water and nutrient uptake. Many plant species have evolved mechanisms to cope with the Al3+ stress and, among them, is the efflux of organic acids by the root tip. In Brazilian barley, a single major gene appears to control the Al3+ tolerance and, for this reason, the opportunity to introgress additional Al3+ tolerance genes derived from natural germplasm is limited. Here, we investigated the Al3+ tolerance in a collection of 51 Brazilian barley genotypes comparing the trait to seven foreign cultivars, six wild barley and one transgenic line overexpressing the wheat TaALMT1 gene. Moreover, we evaluated the polymorphism of two molecular markers linked to the HvAACT1 gene. Both TaALMT1 and HvAACT1 encode transporters responsible for the efflux of malate and citrate by the root apices that are thought to confer tolerance by chelating Al3+. In hydroponics, only six genotypes showed greater root growth than Antarctica 01, the Brazilian genotype used as a tolerant control. However, in the short-term soil experiment, that number was reduced to the two conventional cultivars Dayton and Murasakimochi. The transgenic line showed the greatest root growth in soil and performed better than any of the conventional genotypes. In hydroponics, the genotype Golden Promise, recognized as Al3+ sensitive, was similar to Antarctica 01 suggesting a low level of tolerance among the Brazilian materials. In the short-term soil experiment, a marker based on a 21-bp indel was associated with the root growth while only the two conventional cultivars showing the greatest performance had the 1 kb insertion in the promoter region of the HvAACT1 gene. This insertion is correlated with greater gene expression and citrate efflux and is probably the reason why these two cultivars had the greatest tolerance. In order to further improve the Al3+ tolerance of Brazilian barley, we suggest the introgression of the HvAACT1 allele containing the 1 kb insertion in the promoter as well as the TaALMT1 transgene.

SECTION:
Abiotic stresses

POSTER: 127

Australian barley breeding: The public to private transition

David Moody

InterGrain Pty Ltd; dmoody@intergrain.com

ABSTRACT

Australian barley breeding is rapidly moving from being predominately publically funded to a fully privately funded activity. This paper describes the industry structural environment that enables a successful transition between these models, principally strong legislative support and a royalty collection system with sufficient efficiency to sustain plant breeding activities. In the Australian case, the establishment of an End Point Royalty (EPR) system has been possible due to the majority of barley deliveries being received by a limited number of bulk handling and storage companies, and a major percentage of the barley crop being marketed by a small number of grain traders who assist with EPR collection. Critically, the EPR system is well supported by the majority of growers who provide correct variety declaration upon delivery, especially for the higher value grain segregations. The efficiency of the EPR system is lower in those regions in Australia with higher domestic feed barley consumption (principally dairy and beef feedlots); lower rates of EPR collection in these areas can be attributed to different attitudes of both the growers and end-users to the value of specific varieties.

There are additional issues that need to be addressed for a transition to a successful private breeding model. The business structures of the breeding entities must be sufficiently robust to allow survival in the face of fluctuating market shares and, in the case of EPR based businesses, volatile production environments. Continued access to outputs of pre-breeding activities and technology development is required, and business relationships need to be developed to ensure an ongoing supply and exchange of germplasm. Solutions to these issues in the Australian context will be discussed by drawing on examples from the transition from public to privately funded wheat breeding, which is closer to reaching the stage of being considered a mature industry sector.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 128

Physiological mechanism, stress-specific proteins for the tolerance to combined stress of drought and salinity in Tibetan wild barley

Imrul Mosaddek Ahmed

Post Doctoral Researcher, Department of Agronomy, College of Agriculture and Biotechnology, Zijingang Campus, Zhejiang University, Hangzhou 310058, China and Senior Scientific Officer, Plant Physiology division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur-1701, Bangladeah; imrulahmed@zju.edu.cn or imrulbau@gmail.com

Co-authors: U.A. Nadira, G.P. Zhang, F.B. Wu

ABSTRACT

Drought and salinity are the major abiotic stresses that limit agricultural production and dramatically threaten the food supply worldwide. Compared to the other cereals, barley (Hordeum vulgare L), exhibiting high drought and salinity tolerance, but its growth and production are also greatly affected by drought and salinity. Development of barley for improved drought and salinity resistance requires the knowledge of physiological mechanisms and genetic control of the contributing traits. Tibetan annual wild barley is rich in genetic variation. Here, we demonstrate the physiological and molecular differences between Tibetan wild (XZ5, drought-tolerant; XZ16, salinity/aluminum-tolerant) and cv. CM72 (salinity tolerant) in response to individual and combined stresses (D+S) of drought (4% soil moisture, D) and salinity (S). Tibetan wild barley XZ5/XZ16 is more tolerant to combined stress of drought and salinity than cv CM72. The stress tolerance mechanism of wild barley is attributed to lower Na+/K+ ratio, enhanced water use efficiency (WUE), increased capacity of antioxidative enzymes to scavenge reactive oxygen species (ROS), increased activities of the carbohydrate and secondary metabolism related enzymes showed a greater increase in XZ5/XZ16 over cv CM72 during vegetative stage. Furthermore, comparative proteomic analysis identified 34 differentially expressed proteins (DEPs) related to tolerance to drought and salinity alone or a combination. Differentially regulated proteins predominantly had functions in photosynthesis, but also in detoxification, energy metabolism, and protein biosynthesis. Importantly, identification of stress responsive proteins and higher expression of their related genes i.e. SAM3, BSA1, PDX11 and TUFA in Tibetan wild barley represents the stress adaption mechanisms. The roles of the corresponding genes were further characterized by employing virus-induced gene silencing (VIGS). Our findings add knowledge that wild barley is a treasure trove of useful genes and offer rich sources of genetic variation which can be exploited in future efforts for breeding barley for multiple stress tolerance.

SECTION:
Abiotic stresses

POSTER: 129

Dual specificity at the Mla locus confers resistance to barley powdery mildew and wheat stripe rust

Matthew Moscou

The Sainsbury Laboratory; matthew.moscou@tsl.ac.uk

Co-authors: I.Hernández-Pinzón, Matthew Gardiner, J. Ferguson, A. Dawson, J. Bettgenhaeuser, P. Green, P. Emmrich, A. Hubbard, R. Bayles, B.B.H. Wulff, J.D.G. Jones, E.R. Ward

ABSTRACT

In 1894, Eriksson established the concept of host species specificity of stripe rust (Puccinia striiformis) on monocot hosts by observing a clear preference for the host species from which it was isolated. Fourty-five years later, Straib screened a large collection of stripe rust isolates from Europe, west and central Asia, and South America with a range of wheat accessions, and importantly, the barley cultivars Fong Tien and Heils Franken. Straib observed a striking contrast, Fong Tien exhibited near universal susceptibility to stripe rust irrespective of the host it was isolated, whereas Heils Franken was completely resistant to all forms of stripe rust isolated on wheat. We initiated an investigation to define the genetic architecture of the host species specificity of wheat stripe rust (P. striiformis f. sp. tritici). Mapping resistance from 16 accessions of barley identified a major locus conditioning resistance to wheat stripe rust mapped to the barley powdery mildew resistance locus, Mla. Fine-mapping resistance established that these two resistance specificities are in complete coupling. To define the gene content in the region, we developed a BAC library for CI 16153, a near-isogenic line containing Mla7 in the cultivar Manchuria. Sequencing of the minimal tiling path for the Mla7 locus using PacBio sequencing has shown that substantial structural variation exists at the Mla locus. In parallel to map-based cloning, we adopted an allele mining approach by sequencing the leaf transcriptomes for 38 diverse barley accessions. Cloning and functional analysis will establish the relationship of multiple pathogen recognition specificity at the Mla locus and its role in maintaining the host species specificity of stripe rust.

SECTION:
Biotic stresses

POSTER: 130

Do the same sets of genes regulate tiller and leaf development?

Gary Muehlbauer

University of Minnesota; muehl003@umn.edu

Co-authors: R. Okagaki and S. Heinen

ABSTRACT

Shoot architecture in barley is defined by tiller number and vigor, and leaf number and morphology. Thus, these structures play a major role in biomass and grain yield and understanding the genetic control of tiller and leaf development may result in crop improvement. Tiller development is divided into three stages: (1) initiation of the axillary meristem; (2) development of an axillary bud with leaves; and (3) outgrowth. Leaves are composed of the proximal sheath and distal blade separated by the ligule which is an epidermally-derived fringe of tissue. My laboratory is studying a collection of low tillering mutants including: uniculm2 (cul2) and uniculme4 (cul4). Cul4 mutants exhibit a liguleless phenotype and are low tillering. Cul4 encodes a homolog for the Arabidopsis BLADE-ON-PETIOLE gene and is expressed in the ligule and axillary meristem, indicating that axillary meristem and ligule development are under the genetic control of similar genes (Takavol et al., 2015). Cul2 mutants exhibit wildtype leaf development and do not tiller due to the lack of axillary bud development. To identify suppressors of the cul2 mutant phenotype, we mutagenized homozygous cul2 seeds and identified two mutant alleles that produced tillers. Allelism tests demonstrated that the cul2 suppressors were allelic and also allelic to the Eligulum-a (Eli-a) gene. Eli-a mutants exhibit a dwarf, low tillering, and liguleless phenotype. RNA-seq analysis and sequencing multiple alleles identified and demonstrated that Eli-a encodes an unknown protein. RNA in situ hybridization of wildtype barley showed that Eli-a was expressed in the leaf margins, near the leaf vasculature and in the ligule but not in the axillary meristems. Our results show that Eli-a regulates the development of ligule and tiller development, and reinforces a model whereby ligule and axillary meristem development are controlled by some of the same sets of genes.

SECTION:
Barley morphology and development

POSTER: 131

Barley is better for food and health

C. Walter Newman

Newman Associates; cwn@3rivers.dbs

Co-authors: R. K. Newman

ABSTRACT

Ancient peoples valued barley as a healthy sustaining food for many millennia as recorded in numerous archeological studies. Barley was a serious contender to wheat and other grains for food until the advent of improved milling techniques. This led to a greater use of wheat to produce high quality flour for breads, a major source of calories for the human population. Modern consumers are now seeking foods to improve their health due to greatly increased incidences of obesity, diabetes, heart disease and colonic abnormalities. Discoveries in medical and nutritional sciences have opened a food market for barley due to the recognition of the positive effects of beta-glucans (BG) on human health. With the knowledge of health benefits derived from BG, numerous studies were undertaken with barley that revealed a genetic linkage to increased BG. The waxy gene has possibly the greatest effect on increasing BG concentrations in barley. Although, it has not been thoroughly tested, it has been reported that in the presence of the waxy gene in barley, there is not only an increased level of BG, but there is a distinct difference in the molecular structure of the BG molecule. The hull-less gene also increases BG levels in barley kernels since the hull contains very little if any of this fiber. Studies have shown that barley has a very low glycemic index, thus diets containing sufficient barley are effective in reducing and controlling body mass. The composition of the barley kernel is an ideal choice for healthy diets. Interest in utilizing barley in food products is currently on the upswing as evidenced by recent research publications and development of enticing recipes.

SECTION:
Barley end use: food/feed

POSTER: 132

Applying Genotyping-by-Sequencing (GBS) and Population Sequencing (POPSEQ) to Predict Genetic Variance in Two-Row Barley Breeding

Jeffrey Neyhart

University of Minnesota; neyha001@umn.edu

Co-authors: K.P. Smith

ABSTRACT

The ability to accurately predict the genetic variance (V_G) that may result from a cross would be invaluable to breeders. Several methods to predict V_G have been proposed recently that incorporate genomic prediction and virtual bi-parental populations. Some of these methods require marker genetic map positions, information not readily available for newer genomics technologies such as genotyping-by-sequencing (GBS). Here we demonstrate the use of population sequencing (POPSEQ) in barley (*Hordeum vulgare* L.) to assign genetic positions of *de novo* GBS markers for predicting V_G. A 183-line training set was established and evaluated in six environments for days to heading, plant height, and grain yield. Forty bi-parental populations were created from parents within this set, and using the R package PopVar, we simulated *post hoc* 34 of these populations. This package uses marker effects estimated by RR-BLUP to predict the mean (μ), V_G, and superior progeny mean (μ_{sp}) of each virtual population. We used the same marker effects and observed GBS genotypes for 1,020 F_3 progeny across the 34 populations to obtain genomic estimated breeding values (GEBVs), from which population predictions of μ, V_G, and μ_{sp} were made. Correlations between the PopVar and GEBV predictions of V_G were moderate (0.19 – 0.39), but mirrored the cross-validation accuracy of each trait (0.43 – 0.89). Correlations for μ_{sp} were high (0.85 – 0.93) and significant ($\alpha = 0.05$). To determine the optimal resource allocation for validating predictions, we conducted simulations using varying trait architecture, marker density, and size of breeding populations. Simulation results will be included in the poster. This pilot experiment demonstrates the utility of POPSEQ information to order and position genomic data for use in breeding predictions. Additionally, we outline procedures to be used in validation experiments with intentionally selected crosses and larger population sizes.

SECTION:
Resources I: Genome
Resources II: Germplasm and Populations
Breeding Methodologies: current approaches and future

POSTER: 133

Agronomic and quality variation in lines derived from a ‘Vivar’ barley mutant population

Joseph Nyachiro

Alberta Agriculture and Forestry, Field Crop Development Centre, 5030 - 50 Street, Lacombe, Alberta T4L 1W8, Canada.; joseph.nyachiro@gov.ab.ca

Co-authors: P. Juskiw and J. Vidmar

ABSTRACT

Induced mutation is a method of developing new genetic variability in breeding programs. The desirable mutants generated may be developed directly into new cultivars, or used in crossing programs to develop new cultivars. A mutant population developed from the cultivar ‘Vivar’ was used in a TILLinG (Targeting Induced Local Lesions in Genomes) by sequencing study. TILLinG by sequencing is a reverse genetic technique that uses traditional mutagenesis coupled with high-throughput sequencing for discovery of mutations in loci of interest. One objective of this study was to determine the phenotypic variability in the mutant population, select germplasm with desirable agronomic and quality traits, and advance using traditional forward genetic techniques. In 2014, a sub-set of 16 lines derived from the mutant population and four check cultivars ‘Chigwell’, ‘Kasota’, ‘Sundre’ and Vivar were tested in a preliminary two-replicate field trial at the Field Crop Development Centre (FCDC) in Lacombe. Plots were arranged in a completely randomized block design. Variation in grain yield was observed, ranging from 3,088 (in an early low lysine line) to 8,713 kg ha-1, compared to Vivar at 8,569 kg-ha-1. Test weight varied from 61 to 65 kg–hL-1, plumpness 48 to 72%, days to anthesis 50 to 57 d, maturity 96 to 97 d, and plant height 68 to 85 cm. Grain quality was measured on a subset of 8 mutant lines using previously developed near-infrared spectroscopy calibrations. Protein ranged from 11.1 to 18.6%, protein digestibility 52 to 82%, starch 53.0 to 61.2%, digestible energy content 2,939 to 3,567 kcal/kg, lysine 0.38 to 0.69%, total fiber 14.3 to 20.4%, and soluble fiber 3.44 to 5.24%. Overall, variations of desirable agronomic and quality traits that may benefit FCDC’s breeding program were found in lines derived from the Vivar mutant population.

SECTION:
Resources II: Germplasm and Populations

POSTER: 134

The development of Quick Cooking Barley using hulless barley

Joseph Nyachiro

1. Alberta Agriculture and Forestry, Field Crop Development Centre, 5030 - 50 Street, Lacombe, Alberta T4L 1W8, Canada.; joseph.nyachiro@gov.ab.ca

Co-authors: M. Nakonechny, H. Qi, J. Yao and F. Temelli

ABSTRACT

There is increasing interest in incorporating whole grain barley into North American diets due to the health benefits associated with dietary fiber. Barley is rich in β-glucan, which is a soluble fiber that has been shown to lower cholesterol, a risk factor for heart disease. Compared to other crops, barley also has a low glycemic index, making it useful for maintaining blood glucose levels in diabetics. In 2006, the United States Federal Drug Administration approved the use of a barley health claim provided the food product contains at least 0.75 g of β -glucan per serving. In 2012, Health Canada followed suit with their own health claim, requiring the food product to contain at least 1 g of β -glucan per serving. In response to these claims, Progressive Foods Inc. initiated the development and production of a Quick Cooking Barley barley food product that allows consumers a convenient way to include barley into their everyday diets. This novel, patented process buffs, pre-cooks and dries hulless barley so that it can be cooked quicker while maintaining the characteristics and nutritional quality of the whole grain. This process reduces cooking time from 45 minutes to 15 minutes for 35% pearled barley, and from 60 minutes to 18 minutes for 5% pearled barley. Further research has indicated that Quick Cooking Barley has an improved β -glucan solubility under physiological conditions over regular pearled barley (increasing from 44.3% to 57.8%), although the viscosity varied depending on how the barley grain was treated prior to cooking. The final product also had a higher water-binding capacity due to partially gelatinized starch, which will contribute to a higher satiety when consumed. Quick Cooking Barley can be used in numerous recipes as an alternative to rice, pasta or potatoes and gives consumers a quick and tasty way to increase their dietary fiber intake.

SECTION:
Barley end use: food/ feed

POSTER: 135

Convergence and divergence of axillary meristem and leaf developmental pathways in barley

Ron Okagaki

University of Minnesota; okaga002@umn.edu

Co-authors: S. Heinen, and G.J. Muehlbauer

ABSTRACT

The genetic regulation of leaf and axillary meristem development has recently been shown to share sets of common genes. To further investigate the relationship between leaf and axillary meristem development, we are using two barley mutants uniculme4 (cul4), and eligulum-a (eli-a). In addition to a low-tillering phenotype, the cul4 mutant exhibits a liguleless phenotype. The eli-a also exhibits a liguleless phenotype and also inhibits axillary meristem development and tiller number. CUL4 encodes a homolog to the Arabidopsis BLADE-ON-PETIOLE gene (Takavol et al., 2015), and the ELI-A gene encodes an unknown protein. RNA in situ hybridizations of CUL4 shows signal in the ligule and axillary meristem, and ELI-A shows signal in the ligule, leaf margins and in pre-sclerenchyma cells near the vascular bundles. Double mutant analysis identified a strong interaction between cul4 and eli-a in leaves but not axillary meristems. Cul4 mutants lack ligules and have normal auricles, while both ligules and auricles are much reduced in eli-a mutants. Cul4; eli-a double mutants have a more extreme leaf phenotype than either single mutant; ligules and auricles were not detected, indicating an additive interaction during leaf development. Noteworthy, axillary meristem development and tillering is reduced in both cul4 and eli-a mutants, but tillering is not further reduced in the double mutant, indicating a potential epistatic interaction. Our results argue that although CUL4 and ELI-A function in both axillary meristem and leaf development, their roles in genetic pathways controlling axillary meristem and leaf development are unlikely to be the same.

SECTION:
Barley morphology and development

POSTER: 136

Building a bridge to barley germplasm resources with the National Small Grains Collection NAM population

Alex Ollhoff

University of Minnesota; ollho007@umn.edu

Co-authors: K.P. Smith

ABSTRACT

New genetic diversity must be incorporated into barley breeding programs if we are to efficiently meet market demands and mitigate the environmental impacts of agriculture. Germplasm collections are the best source of novel genetic diversity for plant breeding, but their accessibility is limited by our ability to phenotype many accessions of unadapted germplasm. To meet this challenge we, under the auspices of the Triticeae Coordinated Agriculture Project, created a nested association mapping (NAM) population bridging the genetic diversity in the National Small Grains Collection (NSGC) to barley breeding programs. The population was created by crossing 91 diverse parents from the NSGC Core Collection to the cultivar Rasmusson and creating ~80 recombinant inbred lines per cross. The population and founder parents have been characterized for plant height, bacterial leaf streak disease severity, and heading date. This population is also being characterized for SNPs using genotyping by sequencing. This poster will include an analysis of phenotypic variation across the population. Improved plant genetic resources and analysis strategies will allow plant breeders to make more efficient progress toward a wider range of breeding objectives.

SECTION:
Resources II: Germplasm and Populations

POSTER: 137

A new QTL identified for drought stress tolerance and leaf senescence in juvenile barley

Frank Ordon

Julius Kuehn-Institute (JKI), Institute for Resistance Research and Stress Tolerance; frank.ordon@jki.bund.de

Co-authors: G. Wehner, C. Balko, K. Humbeck

ABSTRACT

Premature leaf senescence induced by drought stress is a main factor for yield losses in barley (Hordeum vulgare L.). Research in drought stress tolerance has become more important as due to climate change the number of drought periods will increase and tolerance to drought stress has become an important goal in barley breeding. Therefore, the aim of this study was to identify genomic regions involved in drought tolerance and leaf senescence in early developmental stages of barley. For this purpose phenotyping, genotyping and expression analyses were conducted on 156 genotypes and based on these data genome wide association studies (GWAS) were performed. After a four weeks stress period (BBCH 33) six physiological parameters for drought stress and leaf senescence were determined in the control and stress variant in greenhouse pot experiments. Leaf colour and biomass yield the main traits for leaf senescence and drought stress were significantly correlated (r=0.36) under drought stress conditions and significant phenotypic variation was observed. Analysis of variance revealed significant genotype and treatment effects. Based on these phenotypic data and 3,212 polymorphic SNPs with a minor allele frequency >5 % derived from the Illumina 9k iSelect SNP Chip, 38 quantitative trait loci (QTL) were detected under stress conditions. Major QTL for drought stress and leaf senescence were located on chromosome 2H and 5H. Expression analyses of a set of 14 genes involved in drought stress and early leaf senescence on these 156 genotypes resulted in the identification of 13 eQTL of which one is located in the same region of chromosome 5H as the QTL for biomass yield and leaf colour under drought stress. Respective markers may be used in future barley breeding programmes for improving tolerance to drought stress and leaf senescence.

SECTION:
Abiotic stresses

POSTER: 138

Genome wide association studies for resistance to Pyrenophora teres f. teres and Cochliobolus sativus in barley (Hordeum vulgare)

Frank Ordon

Julius Kuehn-Institute (JKI), Institute for Resistance Research and Stress Tolerance; frank.ordon@jki.bund.de

Co-authors: F. Novakazi, A. Anisimova, O. Afanasenko, D. Kopahnke

ABSTRACT

Pyrenophora teres f. teres (PTT) and Cochliobolus sativus (CS) are the causal agents of the net type of net blotch and spot blotch in barley, respectively. Both fungal pathogens are widely spread and cause high yield losses. The most cost effective and environment-friendly way to prevent and control infection is growing resistant cultivars. In order to identify sources of resistance, in a first step more than 10,000 barley accessions including landraces and commercial cultivars were screened for resistance to PTT and CS under greenhouse and field conditions, 449 barley accessions derived from the centres of barley diversity, and expressing different levels of resistance to respective pathogens were selected.
Next, greenhouse experiments were conducted with these 449 accessions with two PTT and CS isolates, respectively. Three week old plantlets were inoculated with a spore suspension of 5000 spores/ mL and assessed 14 dpi. Furthermore, PTT field trials were conducted in so-called “summer hill trials” in Germany with an artificial inoculation with naturally infected barley straw. CS field trials were conducted in Belarus with a natural infection. First results of the greenhouse experiments showed 177 genotypes (about 39%) to be resistant against PTT isolate No 13 from Saint Petersburg. In parallel respective genotypes were analysed by the 9k iSelect ship. Based on phenotypic data and genotypic data genome wide association studies were conducted in order to identify quantitative trait loci (QTL) associated with resistance against net blotch and spot blotch. First results of these studies are presented.

SECTION:
Biotic stresses

POSTER: 139

Locating QTL for resistance to net blotch (Pyrenophora teres f. teres) in a wild barley nested association mapping population

Frank Ordon

Julius Kuehn-Institute (JKI), Institute for Resistance Research and Stress Tolerance; frank.ordon@jki.bund.de

Co-authors: T. Vatter, D. Kopahnke, K. Pillen

ABSTRACT

Net blotch, caused by the fungus Pyrenophora teres f. teres, is an important foliar disease of barley causing high yield losses. The identification of QTLs and underlying genes conferring resistance to this fungus is the basis for targeted and sustainable breeding approaches aiming to improve net blotch resistance in modern barley cultivars and to broaden the genetic base of resistance. Therefore, a SNP-based nested association mapping (NAM) approach was used to map QTL for resistance derived from H. spontaneum. To achieve this, the barley nested association population (HEB-25) comprising 1420 BC1S3 lines in 25 families originating from a cross of 25 wild barley accessions (H. spontaneum) with the cultivar Barke was screened for resistance in two-year field trials using a summer-hill-design. Best linear unbiased estimates (BLUES) for area under disease progress curve and average ordinate were calculated. Using these and 5704 informative SNPs obtained from the 9k iSelect barley chip nested association mapping was conducted. Results indicate a high variability in net blotch resistance between and within families of the NAM-population. In summary, SNPs highly associated to net blotch resistance were detected on chromosomes 2H, 4H and 6H. In a next step, additional markers will be generated by exome capture in order to achieve more detailed information on genes underlying respective QTL for Pyrenophora resistance.

SECTION:
Biotic stresses

POSTER: 140

Towards genomics based isolation of resistance genes against soil-borne barley yellow mosaic virus disease (BaYMV, BaYMV-2, BaMMV)

Frank Ordon

Julius Kuehn-Institute (JKI), Institute for Resistance Research and Stress Tolerance; frank.ordon@jki.bund.de

Co-authors: S. Färber, K. Perner, D. Perovic, A. Habekuß, N. Stein, A. Graner

ABSTRACT

Soil-borne Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV) are the causal agents of barley yellow mosaic virus disease of winter barley in Europe and East Asia. Due to the transmission by the soil-borne plasmodiophorid Polymyxa graminis, chemical measures to prevent high yield losses are neither effective nor ecological sound. Thus, breeding for resistance is of prime importance. Up to now, nine loci conferring resistance to the different strains of BaMMV and BaYMV are known. In order to get detailed information on the structure and function of resistance genes effective against this disease, map based cloning was conducted to isolate the resistance gene rym13 located in the telomeric region of chromosome 4HL and a resistance gene located in the centromeric region of chromosome 5H conferring resistance against BaYMV and BaYMV-2.
Marker saturation of the target intervals was conducted using all available sequence information in barley, synteny to rice, sorghum, Brachypodium and sequence information of barley included in the genome zipper. Phenotyping of respective segmental RILs derived from high resolution mapping populations was conducted by mechanical inoculation (BaMMV) and in field trials (BaMMV/BaYMV/BaYMV-2) followed by DAS-ELISA. The resistance gene located on chromosome 5H was mapped in an interval of 0.22% recombination. Candidate genes were identified using exome capture sequencing of the parental lines resulting in 249 Morex contigs containing 256 genes. Two candidate genes were discovered of which one is co-segregating with the resistance locus. For rym13 in addition genotyping-by-sequencing (GBS) of 132 RILs and deep sequencing of two differentiating segmental RILs was performed, which leads to the identification of candidate genes which are presently re-mapped in the high resolution mapping population.

SECTION:
Biotic stresses

POSTER: 141

Generation of transgenic barley expressing Blumeria effector candidate (BEC)1019 to unravel host signaling pathways

Mehmet Tufan Oz

Co-authors: W. Xu, R.P. Wise, F. Altpeter

ABSTRACT

Mildews, caused by obligate fungal pathogens, are major threats to cereal grain production worldwide. Effector proteins secreted by these pathogens modulate, inhibit, or accelerate host processes to co-opt the cellular environment for nutrient acquisition and colonization. As such, effectors serve as optimal probes to decode host signaling pathways. We focus on the interaction of barley with the powdery mildew fungus, Blumeria graminis f. sp. hordei (Bgh), to address fundamental questions in host resistance and susceptibility. Among more than 500 predicted effectors, Blumeria effector candidate (BEC)1019 is a single-copy gene encoding a putative, secreted metalloprotease. BEC1019 is evolutionarily conserved among at least 91 other diverse fungi, including obligate plant pathogens, necrotrophic animal pathogens, and free-living non-pathogens. It was recently demonstrated that BEC1019 suppresses hypersensitive reaction and enhances virulence in barley. Here we report overexpression of BEC1019 in barley cv. Golden Promise via Agrobacterium-mediated genetic transformation. Transgenic plants were regenerated following selection with hygromycin. Expression levels of BEC1019 gene variants with or without a signal peptide (SP) and an affinity purification tag (HPB tag), and production of transgenic seed progenies are reported. Transgenic plants will be crossed with resistance signaling mutants for biochemical (e.g. pull-down assay) and genetic assays to determine cellular localization and confirm target interactions.

SECTION:
Biotic stresses
Resources II: Germplasm and Populations

POSTER: 142

Wide variations of major grain components, pasting properties and in vitro starch digestibility of Tibetan hull-less barley and its effects on the textural characteristics and predicted functional values of processed cookies

Zhifen Pan

Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China; panzf@cib.ac.cn

Co-authors: X.-Q. Deng, Q. Li, T. Nyima, Y.-W. Tang, X.-Q. Zeng, G.-B. Deng, H. Long, and M.-Q. Yu

ABSTRACT

As the staple food crop in Tibet for thousands of years, hull-less barley is becoming worldwide desirable food crop due to many healthy functions. However, there are few systematical studies on the difference of barley foods derived from different cultivars. In this study, 23 hull-less barley were selected to investigate major grain components, pasting properties and in vitro starch digestibility of wholegrain flour and the textural characteristics and predicted functional values of corresponding cookies. The results were as following: (1) Wide variations among wholegrain flours were detected. The amylose, β-glucan and protein contents ranged from 0% to 26.27%, 4% to 9.49% and 9.46% to 18.66%, respectively. The peak time, initial pasting and peak temperature varied from 2.47 to 5.33 min, 50.5 to 59.1℃, 66.8 to 92.5℃, respectively. Most of pasting viscosity parameters also varied greatly. In term of starch digestibility, the time to reach the starch digestion plateau ranged from 60 to 120 min and the ultimately digested starch ranged from 49% to 61%, much lower than that of white bread. (2) Waxy wholegrain flour had significantly higher protein and β-glucan content, faster hydrolysis rate during early stage, but lower maximum starch digested levels, HI and GI compared to non-waxy types. (3) Cookies showed similar starch digestibility compared with raw flours except for significantly higher hydrolysis rate during early stage. Like the raw materials, waxy cookies also showed faster hydrolysis during early stage, lower maximum digested levels, HI and GI compared to non-waxy types. In addition, waxy cookies had higher hardness, crispness and chewiness. (4) Correlation analysis suggested amylose, β-glucan and protein have strong effects on pasting properties of wholegrain flour and textural properties and functional value of cookies. This study could provide information for producing hull-less barley foods with high nutritional and healthy value.

SECTION:
Barley end use: food/feed

POSTER: 143

Influence of the Alt1 gene on the agronomic performance of barley on acidic soils in Western Australia

Blakely Paynter

Department of Agriculture and Food, Western Australia;
blakely.paynter@agric.wa.gov.au

ABSTRACT

Soil acidity is a major production constraint in the south-west of Western Australia affecting more than 14 million ha of wheatbelt soils. More than 70% of surface soils (0-10cm) and almost 50% of subsurface (10-30cm) soils are below the target pH(Ca) of 5.5 and 4.8, respectively. As soil pH drops aluminium (Al) is released into the soil solution. In most WA wheatbelt soils Al reaches toxic levels when subsurface pH(Ca) falls below 4.8. As roots are unable to effectively grow through acidic subsurface soil the plants are restricted in their access to stored subsoil water for grain filling, especially barley given its sensitivity to Al toxicity. The release of barley varieties with improved Al tolerance has the potential to lift the grain yield of barley by 10-40% on those soils and reduce in paddock variability.

The aim of this study was two-fold: (1) compare the performance of barley carrying the Alt1 gene barley against standard barley and wheat cultivars; and (2) determine if the management (nitrogen and seed rate) required to optimise productivity differs between cultivars with and without the Alt1 gene.

In the first study, barley carrying the Alt1 gene was able to match wheat for grain yield on two acidic, sandy textured sites in two contrasting seasons. In the second study, two Al tolerant BC4 barley lines out-yielded their parental line by 34% across six trials because they grew more tillers and produced heavier kernels. They were also able to extract more water from the soil (measured to 1 m). There was no evidence, however, that barley carrying the Alt gene would respond differently to management inputs on acidic soils, they were just higher yielding (especially in lower rainfall seasons).

SECTION:
Abiotic stresses

POSTER: 144

Fine-mapping of a powdery mildew QTL by exome sequencing

Carlos Perez-Cantalapiedra

CSIC; cpcantalapiedra@eead.csic.es

Co-authors: B. Contreras-Moreira, C. Silvar, D. Perovic, F. Ordon, M.P. Gracia, E. Igartua, A.M. Casas

ABSTRACT

Powdery mildew in barley is caused by the biotrophic fungus Blumeria graminis f. sp. hordei. Colonization of leaf surfaces results in severe yield losses in temperate latitudes worldwide. Up to date, most mildew resistance loci (Ml genes) have not been cloned, with the exception of mlo and Mla genes. Cloning efforts to identify them relied on cumbersome procedures, due to the lack of genomic resources and the large and highly repetitive nature of barley genome. In the last decade, new resources have been made available which accelerate barley research and breeding. In this work, we take advantage of those resources to fine map a powdery mildew resistance QTL on 7HL, through the development of a high-resolution mapping population followed by exome sequencing of recombinant lines with contrasting resistance phenotypes. Exome capture data allowed delimiting the physical position of the QTL to a single physical contig. The analysis of available genomic references led to the characterization of the gene composition in the region, revealing a cluster of NBS-LRRs taking up most of the QTL. We followed a non-standard pipeline to assemble reads from mixed mappings, causing heterozygous variants in NBS-LRR loci. Overall, the results suggest that NBS-LRR genes, absent from the reference and the susceptible genotypes, could be functional and responsible for the powdery mildew resistance. The procedure followed is an example of the use of next generation sequencing tools to tackle the challenges of gene cloning when the template is absent from the reference.

SECTION:
Biotic stresses

POSTER: 145

Assessment of genomic resources and Next-Generation-Sequencing technology for resistance breeding in barley

Dragan Perovic

1Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, 06484Quedlinburg, Germany; dragan.perovic@jki.bund.de

Co-authors: C. Silvar, M. M. Martis, T. Nussbaumer, J. Koenig, D. Kopahnke, A. Habekuss, E. Schliephake, B. Kilian, J. Plieske, N. Haag, R. Rauser, J. Keilwagen, V. Korzun, B. Steffenson, K. F.X. Mayer, F. Ordon

ABSTRACT

The aim of this work was to assess the usability of modern technologies and barley genomic resources in deciphering novel sources of resistance identified in landraces and Hordeum bulbosum and their use in barley breeding.
Firstly, the doubled haploid (DH) population derived from the cross between the landrace MBR1012 and the cultivar Scarlett that was previously used for the mapping of resistance to leaf rust was genotyped on the 9K iSelect chip. A map of 2863 markers covering 1468,34 cM was used for the genetic dissection of the resistance to three monoconidial isolates and QTL detection of field resistances to net blotch and for construction of a consensus map that holds a total of 6,405 markers.
The second aim was to exploit the BYDV tolerance of DH-lines derived from the backcross (Emir × H.bulbosum) × Emir. The analysis included localization of the introgressed fragment, mapping of PCR and 9K iSelect markers, exome capture screening and RNASeq profiling after virus infection. Analysis allowed narrowing down the introgression carrying the BYDV-tolerance to about 3 cM and detection of 12 cis down regulated genes.
The third aim was to estimate the convergence of barley genomic resources, GenomeZipper (GZ) and POPSEQ, at the genome-wide level and to assess their usefulness in breeding by analysing seven known loci. Comparison of barley GenomeZipper and POPSEQ maps to a consensus map yielded an accuracy of 97.8% and 99.3%, respectively. The fine scale comparison involved seven genetic regions on five chromosomes harbouring major genes and quantitative trait loci (QTL) for disease resistance. In total, 179 GenomeZipper loci were analyzed and 69 polymorphic markers were developed. 89.1% of these were allocated within the targeted loci. Forty-four markers allowed the identification of fingerprinted contigs. The results revealed the presence of novel resistance genes and provided the tools for their efficient deployment in barley breeding.

SECTION:
Resources II: Germplasm and Populations

POSTER: 146

Cost-effective and accurate SNP genotype analysis of barley samples using the Array Tape® Platform and KASPΣ genotyping chemistry

Luke Linz

Douglas Scientific; luke.linz@douglasscientific.com

Co-authors: L.B. Linz, R.L. Howard, L. Hinton and J.E. Holme

ABSTRACT

As our collective knowledge of genetic sequences and their associated traits continues to increase, the importance and usefulness of Single Nucleotide Polymorphisms (SNPs) has become increasingly apparent. SNPs are valuable tools for many aspects of plant and animal genetic research, ranging from marker assisted selection to trait associations and disease prediction, and are particularly important for agricultural crop development. With the increase in the use of SNPs comes the need for economical and reliable methods of SNP genotyping to allow plant breeding programs to take full advantage of the available genetic information. The Array Tape Platform in conjunction with KASPΣ genotyping chemistry helps barley breeders address this need. Array Tape enables miniaturized reaction volumes and is supported by two automated systems, Nexar and IntelliQube, which allow laboratories to process more samples in less time, while increasing quality and reducing human error. This presentation discusses how Array Tape, KASP chemistry, and its supporting automation can be used for high throughput SNP analysis of barley samples. We will demonstrate the features and benefits of this technology, highlighting the advantages of a streamlined workflow and reduced reaction volumes, while maintaining the accurate and reliable SNP genotyping results required for barley breeding programs.

SECTION:

POSTER: 147

Recombination patterns among spring barley populations

Ana Poets

University of Minnesota; agonzale@msi.umn.edu

Co-authors: A. Ollhoff, G. Brown-Guedira, and K.P. Smith

ABSTRACT

The number, location of recombination events and their frequency vary among individuals. The most commonly used methods to identify the genes underlying traits assume that recombination frequencies are consistent across families. Additionally, these methods often suffer from the presence of too little (e.g. in QTL mapping) or too much recombination (e.g. in association mapping). Nested association mapping (NAM) populations help resolve the issue by combining the power of detection of a QTL mapping population with the resolution gain from more recombination in an association mapping population. We used a six-row spring barley NAM population of 7,700 RILs representing 91 families to characterize recombination in spring barley. A combination of exome capture and genotyping-by-sequencing technology were used to collect dense genome-wide single-nucleotide polymorphism data. These data were used to develop a dense consensus genetic map and examine variation in fine scale recombination patterns among barley families.

SECTION:
Resources I: Genome
Resources II: Germplasm and Populations

POSTER: 148

Opportunities to improve barley yield potential and adaptation by optimising development pattern

Kenton Porker

University Of Adelaide, School of Agriculture Food and Wine, Barley Breeding Program; kenton.porker@adelaide.edu.au

Co-authors: J. Eglinton, S. Coventry, N. Fettell

ABSTRACT

Recognising crop phenology is controlled by complex genotype by environment interactions, an approach combining fundamental physiological research and genomics is required to manipulate phenology for further yield improvement. This paper discusses the possibilities for improving yield potential using field trials investigating variation in crop phenology and yield in elite genotypes adapted to Australian environments. Improved yield potential is associated with increased grain number and grain weight. The pre-anthesis developmental stage is critical in determining grain number. During the critical period from awn primordium (when grain number potential is set) to anthesis, a proportion of reproductive structures fail to survive. This suggests GxE interaction between potential and actual grain number. Using a novel multivariate approach the development of a pheno-meteorological model facilitated the dissection of the environmental cues that modulate crop development. The findings revealed even in adapted cultivars there is large variation for the duration of spike growth phases relative to other phenological stages. Using an adapted cultivar Compass as a benchmark it is shown that grain weight can be increased independently of grain number through subtle adjustments in its phenological pattern, particularly by spending a greater proportion of its lifecycle in the spike growth phase. This indicates the possibility to improve final grain number and yield potential by manipulating the genes associated with sensitivity to environmental cues during the pre-anthesis period. A matrix of phenology gene data will now be assembled to expand the model and validate their relative function in controlling crop development. It will be discussed how focused research on the physiological basis of yield can complement breeding.

SECTION:
Abiotic stresses

POSTER: 149

Effects of different drought intensity on the morphology of barley seedling roots

Juncang Qi

Shihezi University; shzqjc@qq.com

Co-authors: H. Hui, L. Lin, L. Liao, C. Wang, H. Cheng

ABSTRACT

The objective of this experiment was to study the effects of different drought intensity on the growth of barley seedling roots. Barley seedlings were grown in pots, in which the soil water content was maintained at 85% of field capacity(FC), 55% of FC, 45% of FC and 35% of FC by irrigating with distilled water daily, respectively. Some conventional morphological and absorption capacity indexes in four different drought resistant barley cultivars were investigated. The results showed that the longest seminal root, the number of tips, tissue water content of the same cultivars under different drought stress gradients are all decreased, the number of forks, root/shoot ratio showed a rising trend in general, and the rhizosheath, root hair density raised at first and then decreased; there is a certain complementary effect among root activity, total absorption area and total active absorption area. However, cultivars with different drought resistance also differ in adaption to water stress, the strong drought resistance cultivars can hold larger roots, have a greater root and soil interaction and a higher absorption capacity than the drought sensitive cultivars. In conclusion, different cultivars have already evolved its own way to adapt to different drought intensity. Thus, the root morphological traits can be used as the screening indicator in barley drought resistance.

SECTION:
Abiotic stresses

POSTER: 150

Salt Tolerance Identification and Evaluation of Barley Varieties

Hai long Qiao

Institute of Agricultural Sciences in the Coastal Area in Jiangsu, Yancheng, Jiangsu 224002; hlqiao80@163.com

Co-authors: H. Chen

ABSTRACT

One-fifth of irrigated agriculture is adversely affected by soil salinity. Hence, developing salt-tolerant crops is essential for sustaining food production. Barley is one of the salt tolerant crop cultivated in world wide. The effect of salt stress on the germination, growth, yield and quality of different barley cultivars were studied. The result showed that with increasing of salt stress concentration, salt injury index of seed, content of soluble sugar and proline in barley shoot were increased, with the increasing of salt stress concentration, but the rate of K+/Na+ was reduced. There are some differences between different barley cultivars. Through the saline soil (soil salt concentration is 4 δ) and desalination soil test, found that the spike number, grain number per spike of barley under saline soil conditions ware significantly lower than desalination soil, but the1000 grain weight of barley under saline soil conditions was higher than desalination soil. Soil salinity not only caused the barley yield decline, but also reduced the seedlings chlorophyll synthesis and the accumulation of barley grain nitrogen, the average nitrogen content of barley grain under desalination soil was 0.15% lower than saline soil.

SECTION:
Abiotic stresses

POSTER: 151

Characterization and fine mapping of a novel barley stage green-revertible albino gene (HvSGRA) by bulked segregant analysis based on SSR assay and specific length amplified fragment sequencing

Dandan Qin

Institute of food crops, Hubei Academy of Agricultural Science, Hubei Wuhan, 430064 China; hnqdd@163.com

Co-authors: J. Dong, F. Xu, G. Guo, S. Ge, Q. Xu, Y. Xu, M. Li

ABSTRACT

Leaf color variations are common in plants. Herein we describe a natural mutant of barley cultivar Edamai No.6, whs18, whose leaf color showed stable and inheritable stage-green-revertible-albino under the field condition. Leaf color of whs18 was green at seedling stage, while the seventh or eighth leaf began to show etiolation, and albino leaves emerged after a short period. The newly emerged leaves of whs18 began to show stripe white before jointing stage, and normal green leaves emerged gradually. The duration of whs18 with abnormal leaf color lasted for about three months, which had some negative impacts on yield-related-traits. Further investigations showed that the leaf color variation of whs18 was associated with changes in chlorophyII content and chloroplast development. Genetic analysis revealed that the trait was controlled by a single recessive nuclear gene, and was designed as HvSGRA in this study. Based on the F2 population derived from Edamai No.9706 and whs18, we initially mapped the HvSGRA gene on the chromosome 2HS using SSR and BSA. GBMS247 on chromosome 2HS showed co-segregation with HvSGRA gene, and the genetic distance between the other marker GBM1187 and HvSGRA gene was 1.2 cM. Using BSA with SLAF-seq also identified this region as candidate region. Finally, the HvSGRA interval was narrowed to 0.4 cM between morex_contig_160447 and morex_contig_92239, which were anchored to two adjacent FP contigs, contig_34437 and contig_46434, respectively. Furthermore, six putative genes with high confidence in this interval were identified by POPSEQ. Further analysis showed that the substitution from C to A in the third exon of fructokinase-1-like gene generated a premature stop codon in whs18, which may lead to loss function of this gene. The current study lays foundation for hierarchical map-based cloning of the HvSGRA gene and utilizing the gene/trait as visualized maker in molecular breeding of barley in the future.

SECTION:
Resources II: Germplasm and Populations

POSTER: 152

New pest threat to barley in the U.S.

Do Mornhinweg

USDA-ARS Wheat, Peanut, and other Cereal Crops Research Unit, Stillwater, OK; domornhinweg@ars.usda.gov

Co-authors: D.W. Mornhinweg, G.J. Puterka, M.J . Brown, R.W. Hammon, M. Franklin and T. Grasswitz

ABSTRACT

Sipha maydis (Passerini), hedgehog grain aphid (HGA), has historically attacked a large variety of wild grasses and cereal crops in Europe, the Middle East, Near East, Central Asia, Pakistan, and India. HGA has dual damage potential. Its feeding causes significant plant damage to small grains due to injection of phytotoxic saliva but it is also a vector of the most serious disease of wheat and barley world-wide, barley yellow dwarf virus, BYDV. S. maydis was reported as a newly introduced pest invading grasses and cereals in S. Africa in 1991 and Argentina in 2002. By 2004 the aphid was reported to cause significant damage to wheat in Argentina resulting in yield reduction. Surveys in Argentina from 2004 - 2006 found HGA mainly on cultivated barley and wheat. HGA was first reported in the United States in 2007 where it was found infesting wild rice in California. It has since been reported on wheat in Georgia in 2012, on an oat cover crop within the Albuquerque, NM city limits in 2014, and in 2015 found to be established on grasses in Grand Junction, Colorado. Its potential to significantly damage barley and wheat has made it important to determine its distribution and hosts range in the small grains growing regions of the US. The objectives of our study was to determine how wide-spread S. maydis has become and its range of wild and cultivated hosts in the Rocky Mountain and Great Plains states. Results of the surveys to date will be presented.

SECTION:

POSTER: 153

Fine Mapping of Rph12, a Gene Conferring Resistance to Leaf Rust in barley

Mohammad Pourkheirandish

University of Sydney; mohammad.pourkheirandish@sydney.edu.au

Co-authors: M. Pourkheirandish, D. Singh, R. F. Park

ABSTRACT

Cultivated barley can be affected by four different rust diseases: leaf rust, crown rust, stripe rust and stem rust, of which the most devastating is leaf rust (causal agent, Puccinia hordei). Genetic resistance is one of the most important strategies to protect barley from devastating rust pathogens. The molecular determinants underlying resistance to leaf rust in barley are not well understood. The locus Rph12 is one of the few Rph loci that are complex, involving multiple resistance alleles with diverse isolate-specific recognition. We mapped Rph12 to the long arm of chromosome 5H using two doubled haploid populations derived from crosses Baudin (Rph12) X WI3407 (rph12) and Baudin X RAH1995 (rph12) with 92 lines for each; and one Recombinant Inbred Line population derived from Baudin X CI9214 (rph12) with 89 lines. Mapped markers were derived from more than 22,000 DArT-seq marker loci. In all three populations tested, the Rph12 phenotype completely linked with a cluster of markers derived from long arm of 5H (130-134 cM in 'Bowman' consensus genetic maps). The physical window of Rph12 was narrowed down to less than 900Mb. Flanking markers of the corresponding marker cluster carried 29 predicted barley genes using barley genome sequence. A high-resolution map will be constructed using more than 2000 F2 gametes with the aim of cloning of the first leaf rust resistance gene in barley.

SECTION:

POSTER: 154

Association genetic analysis of nitrogen use efficiency in Northern European winter barley

Luke Ramsay

The James Hutton Institute; Luke.Ramsay@hutton.ac.uk

Co-authors: G. Barral-Baron, D. Harrap, A. Booth, J. Russell, R. Waugh, P.Werner and W. Thomas

ABSTRACT

In order to investigate the genetic control of nitrogen use efficiency an association genetics study was carried out using over 160 elite Northern European winter barley cultivars selected to represent over 50 years of European barley breeding. These lines were grown in five site/season trial combinations with known available soil mineral nitrogen content with each trial compromising of three fertiliser regimes (no nitrogen applied, an intermediate and a feed nitrogen application) in a split plot design with partial replication.
Plots were kept free from disease with a standard fungicide regime, agronomic traits were scored on the trials, grab samples were taken from each plot prior to harvest and split into grain and straw with the latter oven dried and analysed for nitrogen content. Harvested grain samples were analysed for nitrogen content by near-infra-red transmittance. These data coupled with yield data allowed the calculation of measures of nitrogen uptake efficiency and utilisation efficiency (both total and grain). Analysis of the data revealed that there was considerable genetic and genotype x environment variation for all the traits measured. Association analysis using eigenanalysis to account for population sub-structure found significant QTL associated with all traits measured including nitrogen uptake efficiency and utilisation efficiency. Most of these NUE QTL showed interactions with the environment and many were co-located with yield QTL. The position of these QTL suggested a number of candidate genes involved in nitrogen metabolism and transport. The potential involvement of some of these genes in nitrogen use efficiency in barley and the more general implications of the interactions found, will be discussed.

SECTION:
Abiotic stresses

POSTER: 155

Extraction of high-quality RNA from germinating barley (Hordeum vulgare L.) seeds containing high levels of starch.

Abdur Rashid

USDA-ARS, Small Grains and Potato Germplasm Research Unit, 1691 S. 2700 W., Aberdeen, Idaho 83210 ; abdur.rashid@ars.usda.gov

Co-authors: M. Gines, T. Baldwin, K. Esvelt Klos and P. Bregitzer

ABSTRACT

Comparative evaluation of gene expression levels can lead to improved understanding of the gene networks underlying traits of economic importance. Extraction of high-quality RNA from germinating barley seeds that contain high levels of starch is of vital importance for analysing the expression of candidate genes of malting quality traits through techniques such as quantitative real-time polymerase chain reaction (qRT-PCR). Most of the existing RNA extraction protocols published in the literature for seed tissues fail to yield high quality RNA in barley because elevated levels of starch contaminate RNA by co-precipitation. This causes sample solidification and hinders RNA dissolution in nuclease-free water, reducing both yield and quality. Here we describe an extraction protocol for high quality RNA from germinating barley seeds based on Li and Trick (2005), but with substantial modifications. RNA extracted with this protocol is of excellent quality and could be used for expression analysis of genes in germinating seeds of cereal crops, including barley, by qRT-PCR.

SECTION:
Resources I: Genome

POSTER: 156

Molecular Mechanisms of Assimilate Transfer in the Developing Barley Grains

Volodymyr Radchuk

Insitute of Plant Genetics and Crop Plant Research (IBK), Gatersleben, Germany; radchukv@ipk-gatersleben.de

Co-authors: D. Weier, H. Rolletschek, L. Borisjuk

ABSTRACT

Barley is grown for its seeds and as such it is one of the major cereal crops grown in temperate climates. Therefore, understanding of barley grain development and seed filling is important for improving grain yield. In the developing grain, endosperm and embryo are symplastically isolated from the maternal tissues. Using high-field NMR and 13C-labelled sucrose, we have shown non-invasively that nucellar projection together with endosperm transfer cells are main tissues for assimilate allocation from maternal tissues towards endosperm. The nucellar projection cells form a complex tissue with the progressive cell turnover. Continuous gradient is observed from the dividing cells in the meristematic zone to the dying cells facing the endosperm transfer cells. This turnover is likely important for assimilate transfer via the nucellar projection.

As seen from electron microscopy, symplastic assimilate transfer occurs in small meristematic cells of the nucellar projection. In differentiated cells, apoplastic pathway for assimilate transfer becomes predominant. These cells develop thick cell walls with numerous cell wall invaginations. Numerous transporters are expressed including sucrose exporters SWEETs. Especially SWEET11a, SWEET11b and SWEET15 are shown to play important role in sugar allocation within the nucellar projection and sugar export to apoplastic cavity at maternal-filial margins. Sucrose transporter 1 is highly expressed in endosperm transfer cells and may be involved in assimilate import to endosperm. Alternatively, sucrose can be cleaved by cell wall invertase and produced hexoses can be transported into endosperm by hexose-transporting SWEET4 those transcripts are also highly abundant in the endosperm transfer cells. Assimilate transfer from the nucellar projection involves programmed cell death (PCD). The Jekyll gene is expressed exclusively in the nucellar tissues of the developing caryopsis. Down-regulation of Jekyll transcription leads to drastic reduction of PCD in the nucellar tissues due to interruption of transport route for assimilates. Severely impaired ability to accumulate storage products in the endosperm leads to the formation of small-sized seeds at maturity. A subfamily of vacuolar processing enzyme VPE2a-VPE2d encodes a cysteine proteinase with caspase-1-like activity that is exclusively expressed in the nucellus and nucellar projection. Simultaneous repression of VPE2a, VPE2b and VPE2d transcription in the nucellar tissues impairs PCD and has also an impact on endosperm development. The lower starch accumulation in the VPE2-repressed grains is probably due to disrupted assimilate transfer route from the nucellar projection to endosperm. Therefore, it is proposed that VPE2a-VPE2d represents a further mechanism for maternal control of grain filling. Interplay and role of different molecular mechanisms in assimilate transfer in the developing barley grains is discussed.

Barley is grown for its seeds and as such it is one of the major cereal crops grown in temperate climates. Therefore, understanding of barley grain development and seed filling is important for improving grain yield. In the developing grain, endosperm and embryo are symplastically isolated from the maternal tissues. Using high-field NMR and 13C-labelled sucrose, we have shown non-invasively that nucellar projection together with endosperm transfer cells are main tissues for assimilate allocation from maternal tissues towards endosperm. The nucellar projection cells form a complex tissue with the progressive cell turnover. Continuous gradient is observed from the dividing cells in the meristematic zone to the dying cells facing the endosperm transfer cells. This turnover is likely important for assimilate transfer via the nucellar projection.

As seen from electron microscopy, symplastic assimilate transfer occurs in small meristematic cells of the nucellar projection. In differentiated cells, apoplastic pathway for assimilate transfer becomes

predominant. These cells develop thick cell walls with numerous cell wall invaginations. Numerous transporters are expressed including sucrose exporters SWEETs. Especially SWEET11a, SWEET11b and SWEET15 are shown to play important role in sugar allocation within the nucellar projection and sugar export to apoplastic cavity at maternal-filial margins. Sucrose transporter 1 is highly expressed in endosperm transfer cells and may be involved in assimilate import to endosperm. Alternatively, sucrose can be cleaved by cell wall invertase and produced hexoses can be transported into endosperm by hexose-transporting SWEET4 those transcripts are also highly abundant in the endosperm transfer cells. Assimilate transfer from the nucellar projection involves programmed cell death (PCD). The Jekyll gene is expressed exclusively in the nucellar tissues of the developing caryopsis. Down-regulation of Jekyll transcription leads to drastic reduction of PCD in the nucellar tissues due to interruption of transport route for assimilates. Severely impaired ability to accumulate storage products in the endosperm leads to the formation of small-sized seeds at maturity. A subfamily of vacuolar processing enzyme VPE2a-VPE2d encodes a cysteine proteinase with caspase-1-like activity that is exclusively expressed in the nucellus and nucellar projection. Simultaneous repression of VPE2a, VPE2b and VPE2d transcription in the nucellar tissues impairs PCD and has also an impact on endosperm development. The lower starch accumulation in the VPE2-repressed grains is probably due to disrupted assimilate transfer route from the nucellar projection to endosperm. Therefore, it is proposed that VPE2a-VPE2d represents a further mechanism for maternal control of grain filling. Interplay and role of different molecular mechanisms in assimilate transfer in the developing barley grains is discussed.

SECTION:

POSTER: 157

Identification of novel sources of resistance to powdery mildew in barley by Focused Identification of Germplasm Strategy (FIGS) approach

Sajid Rehman

International Center for Agriculture Research in the Dry Areas (ICARDA); s.rehman@cgiar.org

Co-authors: A. Bari, B. El-Yousfi, A. Amri, A. Tsivelikas, S. Gyawali and R. P. S. Verma

ABSTRACT

Barley powdery mildew (PM) is a recurrent problematic disease of economic significance worldwide. Genetic resistance (mlo-type) has been used as an effective tool to curb its effect on barley, as it confers durable race non-specific resistance since its introgression. It has been observed recently that the mlo resistance is temperature dependent and at higher temperatures, in particular in rain-fed dry areas, it has lost its effectiveness. The identification and deployment of new sources of resistance/ genes from ex situ collections/gene banks, can help to limit yield losses due to new virulent races. However given the rarity of these genes, such novel genetic variation is hard to find from a fixed subset and/or currently available core collections. To circumvent this, we have used the FIGS and predictive analytical models to develop subsets. Two subsets of germplasm developed along with a random sub set (as a check) to compare the likelihood of the PM-resistance sources, were screened with a mixture of field isolates of PM from Morocco. In first FIGS subset 63 out of 212 accessions and in the second FIGS subset, 35 out of 140 barley accessions were resistant. Whereas, only 2 out of 25 accessions were found resistant in the random subset, indicating that the proportion of resistant accessions is more in FIGS subsets than the random set. Furthermore, resistance to PM was found to be confined in accessions originating from Ethiopia, Iran, Turkey, Ukraine, Greece, Egypt, and Afghanistan. The molecular markers are being used to characterize the resistance sources for their diversity. The FIGS has helped in the identification of new sources of resistance which may be incorporated in the elite barley germplasm at global scale.

SECTION:
Biotic stresses
Breeding Methodologies: current approaches and future

POSTER: 158

High-resolution mapping reveals candidate genes at major and minor effect loci conferring susceptibility to net form net blotch on barley chromosome 6H

Jonathan Richards

North Dakota State University; jonathan.richards@ndsu.edu

Co-authors: T.L. Friesen, and R.S. Brueggeman

ABSTRACT

Net form net blotch (NFNB), caused by the necrotrophic specialist fungal pathogen Pyrenophora teres f. teres (Ptt), is an economically important foliar disease of barley. Utilizing diverse barley lines and pathogen isolates, NFNB resistance/susceptibility often maps to the centromeric region of barley chromosome 6H. Barley cultivars (cv) Rika and Kombar harbor dominant susceptibility at the 6H locus to Ptt isolates 6A and 15A, respectively. Utilizing 2,976 recombinant gametes from a Rika/Kombar population and SNP markers in the region, the major effect susceptibility to Pyrenophora teres (Spt1) locus was delimited to ~0.29 cM. Within this region, an LRR-receptor like gene, Spt1.cg, was identified. Spt1.cg allele analysis determined that it contains a conserved C-terminal LRR, diverged N-terminus, and predicted transmembrane domain. The allele analysis using diverse barley lines shows remarkable correlation with the presence of Rika or Kombar alleles and susceptibility to Ptt isolates 6A and 15A, respectively. Additionally, Montana isolates TA5 and TD10 are virulent on cv Morex and the susceptibility or resistance was mapped to the 6H locus in the Steptoe x Morex DH population. The cv Morex contains a third Spt1.cg allele found in several other barley lines that corresponds to susceptibility to these Montana isolates, suggesting that susceptibility to several isolates of Ptt may be mediated through interactions with different alleles of a diverse dominant susceptibility gene. Phenotypic analysis of the critical recombinants identified two additional minor effect QTL, Spt2 and Spt3 in the region. Spt3 was delimited to a ~ 0.06 cM region corresponding to a physical region of ~105 kb spanning two BAC clones. Sequencing of the physical region revealed a single phosphoenolpyruvate carboxykinase (PEPCK) gene potentially representing a new class of genes implicated in host disease susceptibility. Validation of these candidate genes will be attempted through BSMV-VIGS, mutant analysis, and development of stable transformants.

SECTION:
Biotic stresses

POSTER: 159

Diversity in frost tolerance and adaptive traits in barley germplasm of different origin

Fulvia Rizza

CREA-GPG; fulvia.rizza@entecra.it

Co-authors: Karsai, I., Morcia, C., Badeck, F-W, Terzi, V., Pagani, D., Kiss, T. and Stanca, A.M.

ABSTRACT

Changing climatic conditions with warming winters and shifts in the frequencies of drought, intense rainfall and cold spell episodes together with the concomitant shifts in the geographical distribution of potentially arable areas increase the challenges for the selection of new varieties. In this context, we aim to contribute to a better understanding of the determinants of barley frost tolerance (FT) and improvement of markers applicable in MAS. To this end, a diversity panel of 121 barley genotypes including 62 six-row and 59 two-row types with different growth habit and origin was analysed. The haplotypes of vernalisation and photoperiod genes were determined with PCR and correlation analyses with the results of 12 frost resistance tests performed.

The relevance of allelic combinations of VRN-H1/VRN-H2 for FT demonstrated with earlier studies (Rizza et al. 2011, Crop Science) could be confirmed with these experiments with a larger set of genotypes.

The predictive power of polymorphisms in VRN-H1 intron 1 region in FT modulation was shown to be significantly higher than that of the VRN-H1 promotor polymorphism.

Facultative genotypes had similar or higher (under sub-optimal hardening conditions) FT of facultative than winter genotypes.

A significant role of genes regulating long-day and short-day photoperiodic responses in FT modulation was found.

The most parsimonious model (assessed with the Akaike Information Criterion) for prediction of FT based on the polymorphisms in VRN-H1 intron 1 region, VRN-H2 and PPD-H2 explained 72% of the variation in FT.

SECTION:
Abiotic stresses

POSTER: 160

Effects of elevated CO2 on growth, yield and grain composition of four barley varieties

Fulvia Rizza

CREA-GPG; fulvia.rizza@entecra.it

Co-authors: F.W. Badeck, S. Afshari Behbahanizadeh, F. Reggiani, A. Moschella, E. Ceotto, A.M. Stanca

ABSTRACT

Two double-rowed (Cometa, Pariglia) and two six-rowed (Alimini, Ponente) barley varieties were grown under free air carbon dioxide enrichment (FACE) in the fields of the institute for plant genomics at Fiorenzuola d'Arda, Italy. The target concentration for CO2 in the FACE plots was 570 ppm as compared to the ambient CO2 concentration of about 400 ppm.
Plant phenology was not affected by the elevated CO2 treatment. FACE significantly increased aboveground biomass production and stem density, while grain yield showed a non-significant increasing trend, only. The yield of Alimini did not respond to FACE.
Grain nitrogen concentration decreased in FACE significantly by 8% on average, while straw nitrogen concentration remained unchanged. The responses of leaf chlorophyll content and a derived nitrogen balance index to FACE varied in the course of plant development.
Substantial between varietal variation was found for the response ratios (FACE/ambient) of yield, grain nitrogen content and stem density suggesting high genetic variability in the response of barley varieties to elevated CO2.

SECTION:
Abiotic stresses

POSTER: 161

Fusarium langsethiae as an emerging toxins producer in malting barley

Ghizzoni Roberta

Genomics Research Centre (CREA-GPG), Council for Agricultural Research and Economics , Via San Protaso 302, I-29017 Fiorenzuola d'Arda (PC), Italy; roberta.ghizzoni@entecra.it

Co-authors: V. Terzi , C. Morcia, G. Tumino, F. Badeck, V. M.T. Lattanzio, M. Pascale, E. Lysøe, A. M. Stanca

ABSTRACT

Mycotoxin contamination of barley grains is an outstanding problem for the beer-malt production chain, both from a safety and industrial point of view. Monitoring activities can effectively contribute to control mycotoxins through early detection and quantification of fungal infections on plants and grains and taxonomical characterization of the invading fungal species , therefore allowing an early identification of emerging mycotoxigenic species. A four years survey of malting barley grown in several Italian environments has evidenciated the emerging occurrence of T-2 and HT-2 toxins, two of the most toxic members of type-A trichothecenes. Molecular diagnostics has identified Fusarium langsethiae as the main fungal species responsible for this contamination. Variability in the susceptibility to this fungal contamination has been observed among different barley cultivars. The fate of T-2 and HT-2 - and of their glycosilated forms - has been followed throughout the malt production chain, starting from naturally contaminated grains. Key steps for the reduction of toxin level have been identified during malting process. Finally, a draft sequence of F. langsethiae genome has been produced laying the basis for a better understanding of its biology.

SECTION:
Barley end use: malting and brewing

POSTER: 162

Describing and Cloning Root Mutants In Barley

Silvio Salvi

DipSA - University of Bologna, Bologna, Italy; silvio.salvi@unibo.it

Co-authors: R. Bovina, S.G. Milner, M. Mascher, N. Stein and R. Tuberosa

ABSTRACT

Despite the importance of mutants in plant biology investigations and plant breeding, only a limited number of root mutants have been described in crop species. Recently, the interest in mutant collections has increased due to the availability of new genomics-assisted mapping and cloning approaches (eg. mapping by sequencing) enabling the exploitation of mutant collections in a forward-genetics fashion. In this context, we performed a screening of a barley mutant population (cv. Morex background) formerly produced for TILLING purposes (Talamè et al. 2008, Plant Biotech J. 6:477-485), to identify and clone root morphology mutants. By means of a paper-roll method, 3,071 M5 mutant families were phenotyped at the seedling stage and several tens of putative root mutants were identified. Mutants were grouped in three categories: root growth rate/length (short and long, 77%), root morphology (coiling, geotropic, etc., 15%) and root hairs (hairless, shorthairs, etc., 8%). Four mutants were utilized in experimental crosses and their Mendelian inheritance confirmed. A SNP array-based bulk-segregant analysis applied on the experimental crosses allowed us to quickly map the mutations at cM-range interval. NGS-based approaches are being tested both on phenotypic bulks and single mutant lines in order to streamline the process of linking a mutant with the underlying gene, thus making our population a useful resource for functional genomics in cereals.

SECTION:

POSTER: 163

A genome wide analysis of key genes controlling diastatic power activity in winter and spring barley.

Joanne Russell

James Hutton Institute; joanne.russell@hutton.ac.uk

Co-authors: M. Looseley, L. Ramsay, P. Hedley, A. Booth, H Bull, M. Bayer, S. Hoad, R. Waugh and W.T.B. Thomas.

ABSTRACT

Diastatic power (DP) is a measure of starch conversion in the malt and is therefore a key attribute in the purchase of malting barley for grain distilling. Whilst there is clear evidence of breeding progress in the UK for some aspects of barley malting quality, such as increased hot water extract and reduced grain nitrogen content, there has been little progress in others such as DP. Nevertheless, there is still considerable variation in DP among winter and spring barley cultivars and the challenge is therefore to identify the genes controlling this very complex trait.

Our approach makes use of pre-existing data and resources to identify subsets of germplasm that contrast for diastase activity. Pools of high and low DP in spring and winter germplasm have been identified and we have used divergent SNP frequencies within and between pools to identify genomic regions controlling the character. This strategy is relatively coarse; therefore a more detailed strategy to identify potential candidate genes is required. This is being achieved by analysing the pools of lines for variation in their expressed gene sequences using exome capture of over 26,000 genes (Mascher et al., 2013). Potentially, we will be able to directly identify causal polymorphisms leading to the differences in diastase activity. These potential will then be used to generate allele-specific KASP markers, which will be validated in independent germplasm sets from field-grown breeder's lines.

The validated markers will then be released to the breeders, testing authorities, and maltsters for immediate use in their programmes so that improved varieties will come through the selection process.

SECTION:
Barley end use: malting and brewing

POSTER: 164

Identifying the components of salinity tolerance using a Nested Association Mapping barley population

Stephanie Saade

King Abdullah University of Science and Technology (KAUST);
stephanie.saade@kaust.edu.sa

Co-authors: A. Maurer, M. Shahid, H. Oakey, C. Brien, B. Berger, S. M. Schmöckel, S. Negrão, K. Pillen, M. Tester

ABSTRACT

Producing sufficient food for nine billion people by 2050 will be constrained by soil salinity, especially in irrigated systems. To improve crop yield, greater understanding of the genetic control of traits contributing to salinity tolerance is needed. Here, we exploit natural variation in exotic germplasm by taking a genome-wide association approach to a new nested association mapping population of barley called HEB-25. HEB-25 is investigated under both controlled and field conditions. Growth of seedlings is measured in control and saline conditions using non-destructive, high-throughput imaging facilities available at The Plant Accelerator® (Adelaide, Australia). In addition, the effect of salinity on harvest index, yield, and other important agronomic traits is evaluated in the field at the International Center for Biosaline Agriculture (ICBA, Dubai, UAE), where plants are irrigated with fresh and saline water. An association study is conducted combining the phenotypic data collected from the two-year field trial and the genotypic information available from a 9k SNP chip. We dissect the genetic architecture of flowering time under high salinity. In addition, we identify a locus on chromosome 2H where, under saline conditions, lines homozygous for the wild allele yielded 30% more grain than did lines homozygous for the Barke allele. Introgressing this wild allele into elite cultivars could markedly improve yield under saline conditions.

SECTION:
Abiotic stresses

POSTER: 165

Identification of deficiens (Vrs1.t) responsible for rudimental lateral spikelets and enlarged central grains

Shun Sakuma

Kihara Institute for Biological Research, Yokohama City University;
ssakuma@yokohama-cu.ac.jp

Co-authors: K. Hori, J. Wu, A. Tagiri, U. Lundqvist, T. Komatsuda

ABSTRACT

Barley spikes are divided broadly into two categories by lateral spikelet fertility such as two-rowed (sterile) and six-rowed (fertile). Ethiopian two-rowed landrace var. deficiens is characterized by two-rowed with the rudimental lateral spikelets and larger central spikelet compared with normal two-rowed type. Deficiens barleys are now the major in UK winter barley growing indicating that deficiens has higher yield potential from the spike architecture. Old reference shows that deficiens is one of the Vrs1 allele (called as Vrs1.t), however, its molecular genetics is not yet understood. Here, we wanted to clone the gene controlling deficiens phenotype and explore contribution to the yielding potential. First, we developed five bi-parental F2 mapping population and investigated phenotypic segregation. All they segregated as a monogenic trait (fitting to 1: 2: 1 = normal: intermedium: deficiens) indicating single gene controls deficiens. Fine mapping revealed that Vrs1.t locates on the ~420 kb in Morex genome of the long arm of chromosome 2H. This region includes only one candidate gene and showed three amino acid changes between deficiens type and normal two-rowed cultivars. We then characterized deficiens mutant and found a single amino acid change among three changes suggesting this amino acid change is responsible for deficiens phenotype. The deficiens mutant showed 11% and 16% increased grain length and width than that of wildtype. No changes of spikelet number and spike length were found. Expression analysis showed deficiens gene expressed only in developing immature spike. These results suggest that deficiens type contributes to increase grain size and has a potential of improving yield in two-rowed barley.

SECTION:
Barley morphology and development

POSTER: 166

Abiotic stress of barley with rise temperature conjugated to soil salinity in an irrigated area by treated waste water in Tunisia

Saida Salah-Mlaouhi

National Institute for Agricultural Research of Tunisia (INRAT);
saidamlouhi@gmail.com

Co-authors: A. Boujelben and M. Elloumi

ABSTRACT

Climate change and soil salinity pose a serious problem for growth and sustainable development. They also threaten progress made in reducing poverty. Rural communities and small farmers will probably be the most affected. According to previous studies, temperature rises by 1 and 2°C conjugate to soil salinity could have a negative impact on crop yields in Mediterranean region including Tunisia. Such impacts could affect economic activities, ecosystems and tourism. Further to face drought and reduce conventional water pressure, we use unconventional resources such wastewater treated which constitutes an exploitation form of very important potential waste water in Tunisia. So, we tried to predict the long-term effects of soil salinity, climate change and increasing doses of waste water treated irrigation on barley yields in Tunisia, given its importance for development of livestock sector.
We used a biophysical approach based on simulation with an agronomic model "Cropsyst" specialized to simulate yield crop. This model was used to simulate barley yields irrigated with treated wastewater according climatic changes for 30 years from 2011 to 2040.
Main results show that yields will be adversely affected by soil salinity and more with temperature rises. Considering only soil salinity effect, during the first decade (2011/2020), barley yields averages simulated are goshawk of 2.5 and 2.6 tons per hectare. In the second decade (2021/2030), these averages will decrease of 0.2 tons per hectare, about 7% compared to the first decade.
During the third decade (2031/2040), reductions will be around 27% compared to the first decade and 17% compared to the 2nd decade.
With temperature rise of 1 and 2°C, barley yields will accuse declines about 31%. Therefore, we adopted four irrigation techniques in which we increase of 20%, 40%, 60% and 100% the doses initially applied by farmers. So, water irrigation supplementation could increase yields up to 58%.

SECTION:
Abiotic stresses

POSTER: 167

Genome-wide association mapping of heading date in the Wild Barley Diversity Collection

Ahmad Sallam

University of Minnesota; sall0029@umn.edu

Co-author: B.J. Steffenson

ABSTRACT

The Wild Barley Diversity Collection (WBDC) is a valuable resource for increasing the genetic diversity of agronomic traits in cultivated barley. It is composed of 318 accessions from diverse ecogeographic regions across the habitat range of Hordeum vulgare ssp. spontaneum. This collection was phenotypically evaluated for heading date under vernalized and unvernalized conditions in greenhouse environments across two years. To identify heading date QTL, the WBDC was genotyped using GBS genotypig platform, generating about 23,000 markers. To position loci conferring heading date, we performed genome-wide association mapping using 1) mixed models that accounts for kinship and population structure and 2) a G model that corrects for background chromosomal marker effects to increase the power of QTL detection.

SECTION:
Resources II: Germplasm and Populations

POSTER: 168

Isolation of seed dormancy QTL Qsd1 in barley

Kazuhiro Sato

Okayama University; kazsato@rib.okayama-u.ac.jp

Co-authors: K. Takeda, T. Matsumoto, and T. Komatsuda

ABSTRACT

Seed dormancy in wild barley is an adaptation trait to prevent germination and escape from summer heat in arid environments. Cloning dormancy genes in barley may contribute to understand the domestication process, and it will facilitate optimizing the trait in cultivated barley for pre-harvest sprouting and malting. Genetic factors controlling seed dormancy have been reported as quantitative trait loci (QTLs). Of these QTLs, Qsd1 at the centrometic region of 5HL has been most frequently identified and showed the largest effect across mapping populations including Haruna Nijo (HN) (H. vulgare ssp. vulgare) x wild barley H602 (H. vulgare ssp. spontaneum). A recombinant chromosome substitution line with segments of Qsd1 from H602 in a HN genetic background was developed and Qsd1 mapped with the derived 910 BC3F2 plants and 4,792 BC3F3 plants. Two gene candidates were estimated both directing 3' ends toward the 345bp sequences between genes. The alignment of H602 and HN genomic sequences showed SNPs and limited the mutation region within 9,467bp by the crossing-overs within two genes. Mapping of Haruna Nijo full length cDNAs on genomic sequences indicated only one non-synonymous substitution on a gene. The results of QTL analysis among eight mapping populations, on which Qsd1 was detected, agreed to have this substitution with the same dormancy effect of QTL allele. The association analysis of cultivars with known seed dormancy also supported the substitution as a cause of reduced seed dormancy in cultivated barley. RNAi knock down and complementation transgenic experiments also proved that the identified gene is responsible for seed dormancy in barley.

SECTION:
Abiotic stresses

POSTER: 169

Improving genomic prediction in barley breeding with variable selection methods

Karl Schmid

Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany; karl.schmid@uni-hohenheim.de

Co-author: P. Thorwarth

ABSTRACT

Genomic selection is becoming an important component of crop breeding programs. A key step is the selection of a model for predicting the genomic estimated breeding value. Different statistical models are evaluated using a cross-validation approach to find the model which maximizes the prediction ability given the phenotypic and marker information of a training population. Although prediction abilities in barley and other crops can be high, knowledge about the influence of different parameters such as the genetic architecture, the population size and the optimal number of markers on the prediction ability is still limited. One approach to improve prediction abilities is to remove markers with little effect on prediction ability by giving them a penalty or by removing them from the marker set with a feature selection algorithm. We implemented a simulated annealing approach with an early stopping criterion to select subsets of genetic markers that maximize the prediction ability in a Genomic Best Linear Unbiased Prediction model (GBLUP). This optimized GBLUP model (SA_GBLUP) was compared to classical variable selection models, such as the Least Absolute Shrinkage and Selection Operator (LASSO) and the Elastic Net (EN) using simulations and empirical data. We simulated 32 data sets that differed in sample size (100, 250, 500, 750), heritabilities (0.25 and 0.75), QTL numbers (10 and 50) and linkage disequilibrium (0.05 and 0.2), with a fixed number of markers per chromosome (1,000). To get a robust estimate of the performance of the different genomic prediction models, we repeated each of the 32 simulations 10 times and validated the analysis for each of the 320 data sets 48 times, resulting in 15.360 tested data sets. SA_GBLUP increased prediction ability in small populations (<250) and retained more markers linked to QTLs in the model than LASSO or EN especially with low genome-wide LD and low heritabilities. In large training populations of 750 individuals, the performance of the different models was similar. We currently validate these methods in several commercial German winter barley breeding programs and will present the results and make recommendations for the application of variable selection in barley breeding.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 170

ClimBar: An integrated approach to evaluate and utilize genetic diversity

Alan Schulman

Luke & Univ. Helsinki; alan.schulman@helsinki.fi

Co-authors: S. K. Rasmussen, M. Mosehlion, E. Fridman, A. Tondelli, L. Cattivelli, T. Dalmay, L. Rossini, E. Igartua, A. Casas, S. Beguería, P. Schweizer, R. Waugh, B. Thomas, and J. Russell

ABSTRACT

European agriculture anticipates an unprecedented combination of stress factors, production threats and quality needs due to climate change. Various regions of Europe will be affected differently. Barley & wheat domestication, and landrace formation in Europe, were under very different climates than those emerging now. Alleles needed for sustainable, resilient, quality yields in a changed climate are likely not combined in current haplotypes of elite barley cultivars. These alleles are likely found in diverse landraces and wild relatives in the Mediterranean basin and Fertile Crescent -- areas that prefigure expected climate change. New precision, high-throughput phenotyping tools are essential to find trait-allele associations needed for future-climate breeding. Combining genetics, genomics, modelling, molecular biology, morphology, and physiology, ClimBar takes an interdisciplinary approach to develop a strategy for breeding an increased resilience to climate change in barley. ClimBar, a project under the framework of FACCE ERA-NET Plus Joint Programming Initiative on Climate Smart Agriculture, will identify genome regions, genes, and alleles conferring the traits needed to breed resilient barley varieties adapted to the climatic conditions predicted for 2070 in different European environments. Adapted, resilient germplasm created using ClimBar data, tools and models will provide food-chain security, economic stability and environmental sustainability.

SECTION:
Abiotic stresses

POSTER: 171

Retrotransposons and their role in genome structure and dynamics in barley

Alan Schulman

Luke & Univ. Helsinki; alan.schulman@helsinki.fi

Co-authors: M. Jääskeläinen, W. Chang, J. Tanskanen

ABSTRACT

Retrotransposons are ubiquitous and abundant in eukaryotic genomes. They are responsible for much of the variation in monoploid genome size over evolutionary time and have direct or indirect effects on genes by both insertional mutagenesis and epigenetic mechanisms. Annotation of the newly completed barley genome indicates that over 74% is comprised of long terminal repeat (LTR) retrotransposons. Retrotransposon copies may be passed to the next generation by one of two ways: replication of existing copies as part of the chromosome; synthesis and propagation of new copies by the combined activities of RNA polymerase II and reverse transcriptase by a “copy and paste” life cycle virtually identical to that of retroviruses, excepting (so far as has been directly demonstrated) the ability to readily move from organism to organism infectively. The retrotransposon life cycle within the cell involves both nuclear and cytoplasmic phases. Integrated copies are transcribed within the nucleus, but translation and virus-like particle assembly takes place in the cytoplasm. Following replication, the cytoplasmic, packaged cDNA copies need to be brought back into the nucleus for integration of new copies to occur. We are interested in analyzing the intracellular trafficking of retrotransposon components, using the BARE retrotransposon family of barley as the model system, which comprises c. 10% of the genome. The goal is to develop an understanding of the mechanisms controlling the propagation of retrotransposons and consequences for the genome and plant.

SECTION:
Resources I: Genome

POSTER: 172

Domesticated barley (Hordeum vulgare L.) originated from an Intermedium type of wild barley

Thorsten Schurbusch

IPK_Gatersleben; thor@ipk-gatersleben.de

Co-authors: H. M Youssef; M. Mascher; M. A. Ayoub; N. Stein; B. Kilian; T. Schnurbusch

ABSTRACT

Barley (Hordeum vulgare L.) was domesticated from its wild two-rowed ancestor H. vulgare ssp. spontaneum (C. Koch.) Thell. To clarify the phylogenetic relationship between two- and six-rowed domesticated barley, and to better understand the role of the intermedium row-type during barley domestication history, phenotypic analyses and re-sequencing of three known row-type genes (six-rowed-spike 1 (Vrs1), Vrs4 and Intermedium –c, Int-c) was performed in a worldwide collection of Intermedium-barleys (302 accessions), wild barley ssp. spontaneum (100), ssp. agriocrithon (10) and H. bulbosum (3) accessions. Measuring lateral floret length and width revealed the presence of two distinct row-type groups within wild barley; one row-type form with enlarged but infertile lateral florets, designated here as ssp. spontaneum-Intermedium, and one group with significantly smaller lateral florets, similar to domesticated two-rowed barleys, designated here as ssp. spontaneum-Distichon. 93.7% of the Intermedium-barley collection, all ssp. spontaneum-Intermedium and H. bulbosum carried the two-rowed allele Vrs1.b in combination with the six-rowed allele Int-c.a. Further network analyses revealed that ssp. spontaneum-Intermedium, phylogenetically closer to H. bulbosum, is the direct ancestor of ssp. spontaneum-Distichon and all domesticated barleys. All ssp. spontaneum-Distichon accessions, however, possessed only two-rowed alleles at both Vrs1 and Int-c; whereas ssp. agriocrithon carried six-rowed specific alleles at all tested loci. Vrs1 transcript levels in wild and Intermedium-barleys predominantly correlated negatively with lateral spikelet fertility as expected, indicating that Intermedium-barleys are a rich genetic resource for increasing lateral spikelet fertility in breeding programs. A revised model of barley evolution and domestication is discussed.

SECTION:
Barley morphology and development

POSTER: 173

Genetic Analysis of Barley (Hordeum vulgare L.) Genotypes Under Normal and Limited Moisture Conditions

Ajeet Singh Shekhawat

Rajasthan Agricultural Research Institute, Sknau, Jobner, Rajasthan, India;
shekhawatdrajeet@gmail.com

Co-authors: J. Shrimali

ABSTRACT

The present investigation was carried out to estimate genetic variability, character association and path coefficient among 30 genotypes of barley for thirteen morphological characters. These genotypes were planted in Randomized Block Design with three replications during rabi 2012-13 at Research farm, College of Agriculture, SKRAU, Bikaner under normal and limited moisture conditions. Analysis of variance indicated presence of considerable variability for all the thirteen characters. High genotypic coefficient of variance and phenotypic coefficient of variance were recorded for plant height, number of effective tillers per plant, spike length, biological yield per plant, relative water content and seed yield per plant under both environments. High estimates of heritability along with high genetic advance as percent of mean were observed for plant height, number of effective tillers per plant, spike length, biological yield per plant, relative water content and seed yield per plant under normal and limited moisture condition. Therefore, these characters can aid in selection programme.

The result from character association indicated that seed yield per plant had significant and positive correlation with plant height, biological yield per plant, test weight, number of spikelet's per spike and spike length in normal and limited moisture condition at both levels. Path coefficient revealed that biological yield per plant and harvest index in both the environments. On the basis of mean performance of seed yield per plant, the genotypes RD-2787, RD-2660, RD-2794, RD-2550 and RD-2668 for normal condition and RD-2660, RD-2787, RD-2816 and RD-2508 for limited moisture condition were found superior. These genotypes may further be utilized in breeding programme for improving grain yield in barley

Result obtained from the present study based on variability parameters, correlation and path coefficient analysis revealed that selection programme based on number of biological yield per plant might prove effective in enhancing productivity level in barley.

SECTION:
Barley morphology and development

POSTER: 174

You need two to Tango: Identification of candidate effectors/avirulence genes that interact with the wheat stem rust resistance locus rpg4/Rpg5

Roshan Sharma Poudel

North Dakota State University; roshan.sharmapoudel@ndsu.edu

Co-authors: J. Richards, S. Solanki, and R. Brueggeman

ABSTRACT

Stem rust, a potential economic disease of wheat and barley is caused by the pathogen Puccinia graminis f. sp. tritici (Pgt). The majority of the prevalent Pgt races in the US have been effectively controlled by the dominant stem rust resistance gene Rpg1 in barley for nearly 70 years. However, the emergence of the race QCCJ and the highly virulent African race TTKSK that are virulent on Rpg1 deems stem rust as a threat to barley production. The cloning of the only effective QCCJ/TTKSK resistance locus in barley, rpg4/Rpg5, and functional characterization of the proteins underlying this resistance which include the two NBS-LRR resistance like Rpg5 and HvRga1 and the actin depolymerization factor HvAdf3, are hampered by the lack of the rpg4/Rpg5 avirulence effector from the pathogen. The molecular and functional study of rpg4/Rpg5-mediated resistance suggests that the mechanism of resistance follows the integrated decoy model. However, the Pgt effectors/avirulence proteins that manipulate the putative protein kinase integrated decoy domain of Rpg5 need to be identified to validate this hypothesis. This research aimed to identify the avirulence protein/s initially utilizing a panel of 37 rust isolates collected in ND since 1970 with differential reactions on rpg4/Rpg5 and Rpg1. Restriction site Associated DNA-Genotyping by Sequencing (RAD-GBS) was performed on these isolates to generate robust genotypic data containing 4919 informative SNPs. The genotyping information from RAD-GBS was then used to select 24 diverse (9 avrRpg4/Rpg5-/AvrRpg1+, 8 Avrrpg4/Rpg5+/avrpg1- and 7 avrRpg4/Rpg5-/avrRpg1-) isolates that are being used to conduct RNAseq on stem rust susceptible barley variety Harrington 5 days post inoculation with the 24 diverse Pgt isolates. The RNAseq data will be used to identify Puccinia graminis SNPs within genes expressed during the infection process that will be added to previous RAD-GBS generated SNPs to conduct association mapping (AM).

SECTION:
Biotic stresses

POSTER: 175

Wax biosynthesis expands beyond Arabidopsis to the diketone synthase (DKS) polyketide pathway.

Lizette Schneider

Copenhagen University, Denmark; lizette.schneider@biol.lu.se

Co-authors: N. M. Adamski, C. E. Christensen, D. B. Stuart, S. Vautrin, M. Hansson, C. Uauy and P. von Wettstein-Knowles

ABSTRACT

Aliphatic compounds on plant surfaces, called epicuticular waxes, are the first line of defense against pathogens and pests, contribute to reducing water loss and determine other important phenotypes. Aliphatics can form crystals affecting light refraction resulting in a color change allowing identification of mutants in their synthesis or transport. The present study discloses three such Eceriferum genes in barley; Cer-c, Cer-q and Cer-u known to be tightly linked and functioning in a biochemical pathway forming dominating amounts of _-diketone and hydroxy-_-diketones plus some esterified alkan-2-ols. These aliphatics are present in many Triticeae as well as dicotyledons such as Eucalyptus and Dianthus. Recently developed genomic resources and mapping populations in barley defined these genes to a small region on chromosome arm 2HS. Exploiting Cer-C and -U potential functions pinpointed five candidates of which three were missing in apparent cer-cqu triple mutants. Sequencing more than 50 independent mutants for each gene confirmed their identification. Cer-C determines a chalcone synthase-like polyketide synthase, designated diketone synthase (DKS), Cer-U a lipase/carboxyl transferase and Cer-U a P450 enzyme. All were highly expressed in pertinent leaf sheath tissue of wild type. A physical map revealed the order Cer-c, Cer-u, Cer-q with the flanking genes 101 kb apart, confirming they are a gene cluster, Cer- cqu. Homology based modeling suggests that many of the mutant alleles affect overall protein structure or specific active site residues. The rich diversity of identified mutations will facilitate future studies of the three key enzymes involved in synthesis of plant apoplast waxes.

SECTION:

POSTER: 176

A malting quality QTL on chromosome 4H harbors a key gene which influences β-glucan activity in barley

Jaswinder Singh

McGill University; jaswinder.singh@mcgill.ca

Co-author: S. Singh

ABSTRACT

Malting quality represents a comprehensive effect of a number of interacting traits, inheritance of which is complex and controlled by quantitative trait loci (QTL). QTL2, one of the important QTLs affecting various malting quality traits like malt extract, beta-glucan, diastatic power and α,-β- amylases has been located on chromosome 4H. Dissecting QTL2 to characterise important genes affecting these traits was the focus of the current study. We employed a synteny based approaches to hunt for the malting related genes harbouring QTL2 of barley. Our barley-rice synteny study of QTL2 region detected 24 candidate genes; one of them (MSB12) shows differential gene expression, among commonly used malting and feed barley varieties. We hypothesize that this gene is a major gene that influences malt extract or level of β-glucan, and thus can control the malting quality. Biochemical analyses including protein-carbohydrate binding assays and ELISA confirm the relationship of MSB12 and β-glucan. This will lead to develop new molecular breeding tools for barley malting quality and a better understanding of malting quality traits, that display quantitative variation.

SECTION:
Resources I: Genome
Breeding Methodologies: current approaches and future
Barley end use: malting and brewing

POSTER: 177

Association and multivariate analysis of yield and its components in hulless barley (Hordeum vulgare L)

Jogendra Singh

ICAR-Indian Institute of Wheat & Barley Research, Karnal, India - 132 001;
jogendrasail@yahoo.co.in

Co-authors: RPS Verma, D. Kumar, L. Kumar, A. Khippal, R. Malik, V. Kumar, S. Ahlawat, K.P Singh, A. S. Kharub and I. Sharma

ABSTRACT

Hulless or naked barley is an important cereal crop in the high altitude areas of Himalayas in India. Now-a-days, it is becoming popular as human food due to presence of higher beta glucan content, since healthy effects of the soluble-fibre rich barley products have been officially approved and consumers' current interest in nutrition might boost the status of barley as human food in India. Therefore, there seems to be great scope for hulless barley cultivation in Indian plains also. Although, very few number of hulless barley varieties have been evolved for commercial cultivation in the hill as well as plain areas of India, hitherto, lack of availability of high yielding varieties of naked barley is restricting its commercial cultivation. In this direction, 40 germplsm lines received from ICARDA have been evaluated for adaptability, high yield and resistance to diseases and insect-pests in different agro-climatic conditions during 2014-15. Plant height and tillers/plant revealed positive direct effect and significant positive correlation with seed yield/plant. However, days to maturity and spike length were observed to have indirect effects on seed yield. The cluster analysis classified the 40 genotypes into 7 clusters. Cluster VI had maximum genotypes (10) whereas cluster IV showed single genotype. In clusters I, II and V, indigenous as well as exotic genotypes were distributed while clusters VI and VII consisted of only exotic genotypes. The maximum intra-cluster distance was revealed in cluster V while the highest inter-cluster distance was observed between cluster IV and VII, indicating maximum genetic diversity between these groups. The genotypes selected from these clusters may be used in hybridization for achieving the high heterotic progeny. Cluster I showed high mean value for plant height, spike length and 1000-grain weight while cluster IV exhibited high mean value for tillers/plant and yield/plot. Similarly, cluster V revealed high mean value for days to heading and maturity. This indicates that genotypes of clusters I, IV and V could be used for improvement of above mentioned traits.

SECTION:
Resources II: Germplasm and Populations

POSTER: 178

Low temperature tolerance of cultivated and wild barley germplasm in autumn-sown field trials in Minnesota.

Brian Steffenson

University of Minnesota; bsteffen@umn.edu

Co-authors: T. Szinyei, I. Loskutov and O. Kovaleva

ABSTRACT

Autumn-sown barley has many advantages over traditional spring-sown barley such as higher yield potential, higher nitrogen/water use efficiency, and various ecological services. Considering the increased demand for local malting barley by the burgeoning craft brewing industry and the need for a more diversified cropping system, there is great potential for establishing an autumn-sown barley cropping system in the northern United States—providing sufficient low temperature tolerance (LTT) can be bred into adapted cultivars. To identify additional sources of LTT for barley breeding, we evaluated two diverse panels of germplasm: 1) 2,214 accessions of landraces, cultivars, and breeding lines from the N. I. Vavilov Institute of Plant Industry (VIR) in St. Petersburg, Russia and 2) seven accessions of wild barley (H. vulgare subsp. spontaneum) from the Wild Barley Diversity Collection (WBDC) that survived several previous Minnesota winters to varying degrees. Germplasm was sown in October of 2014 and assessed for survival in April of 2015. Although major gains have been made for LTT in the Minnesota barley breeding program, the severe winter of 2014-15 killed off >99% of the breeding lines. Yet in a parallel experimental trial of VIR germplasm, 268 (12.1%) accessions survived the winter. Most of the surviving VIR accessions were from Russia, but at least one originated from 36 other countries. All seven of wild barley accessions died out completely in the winter of 2014-15, even though they exhibited moderate levels of LTT in previous trials. The VIR germplasm represents an important new and diverse genepool from which to breed barley for enhanced LTT.

SECTION:
Abiotic stresses
Biotic stresses

POSTER: 179

On the possible origin of stem rust resistance genes in barley

Brian Steffenson

University of Minnesota; bsteffen@umn.edu

Co-authors: A. Hulse, O. Matny, A. Sallam, and A. Case

ABSTRACT

Stem rust, caused by Puccinia graminis f. sp. tritici, is a serious disease of barley in a number of production areas around the world. To date, six major genes have been described including Rpg1, Rpg2, Rpg3, rpg4, Rpg5, and rpg6. Rpg1 has been used extensively in barley breeding programs across the northern Great Plains of North America and has protected the crop from serious losses for over 70 years. However, races from North America (QCCJB) and Africa (TTKSK) with virulence for Rpg1 are known. The only other genes widely used in commercial breeding are rpg4 and Rpg5, which are very closely linked on chromosome 5H. These two genes act in concert with other linked genes to confer resistance to the Rpg1-virulent races of QCCJB and TTKSK. To assess whether the three commercially used resistance genes might have originated in the wild progenitor of barley (Hordeum vulgare subsp. spontaneum), we evaluated different germplasm panels of wild barley. The Wild Barley Diversity Collection (WBDC), consisting of 318 ecogeographically diverse accessions from across the range of the subspecies, was evaluated for reaction to the North American P. g. f. sp. tritici race HKHJC at the seedling stage because it is virulent for the rpg4/Rpg5 complex, allowing the detection of Rpg1 and possibly other resistance genes at the seedling stage. From this test, only two accessions (WBDC094 and WBDC238, both from Jordan) exhibited consistently low (resistant) infection types suggestive of the presence of Rpg1. However, molecular assays for Rpg1 were negative, indicating the two accessions either carry a diverged version of Rpg1 or a different gene conferring HKHJC resistance. Recent data suggest a possible origin of Rpg1 in six-rowed landraces from eastern Switzerland (Steffenson et al. 2015, Alpine Botany). As part of a large effort to identify resistance to the widely virulent African race of TTKSK in Hordeum, we evaluated the WBDC plus 593 other wild barley accessions at the seedling stage. Only 13 (1.4%) accessions exhibited consistently low infection types to race TTKSK. Molecular assays revealed that 11 of these 13 accessions carried Rpg5. Nine of the Rpg5-carrying accessions were from Central Asia, while the remaining two were from Israel. Thus, the origin of the important rpg4/Rpg5 resistance gene complex is wild barley.

SECTION:
Biotic stresses

POSTER: 180

Implication of CBF2A–CBF4B genomic region copy numbers in affecting expression of other FR-H2 CBFs

Eric Stockinger

Department of Horticulture and Crop Science, The Ohio State University/Ohio Agricultural Research and Development Center, Wooster, OH 44691, USA; stockinger.4@osu.edu

Co-authors: T. Dhillon

ABSTRACT

The C-Repeat Binding Factors (CBFs) are DNA-binding transcriptional activators functioning at an early step in the cold acclimation response of Arabidopsis thaliana. In barley, a CBF gene cluster resides at FROST RESISTANCE-H2, one of two loci having major effects on winter-hardiness. FR-H2 was revealed in a winter × spring population derived from 'Nure' × 'Trèmois'. Expression analyses of N×T recombinants indicate a subset of genes, CBF2 and CBF4, are expressed to higher levels in recombinants possessing the 'Nure' FR-H2 allele. Sequencing the respective alleles reveals 'Nure' harbors 2–3 copies of CBF2A and CBF4B as a consequence of tandem iteration of the genomic region encompassing these genes, whereas 'Trèmois' harbors single copies, supporting association between gene copy number and transcript levels. More recent studies indicate transcripts other FR-H2 CBF are also increased in CBF2A overexpressing plants, suggesting CBF2A–CBF4B genomic region copy numbers also affects expression of other CBFs. Here we explore further the relationship between gene copy number and transcript levels using 'Admire', a winter barley line accumulating multiple FR-H2 CBF transcripts to relatively high levels, and a group of lines related by descent to 'Admire'. DNA blot hybridization indicated the CBF2A–CBF4B region is present in 7–8 copies in 'Admire', and that it is highly variable in copy number across the lines related to 'Admire'. In lines having greater CBF2A–CBF4B copy numbers, CBF12, CBF14, and CBF16 transcript levels were higher relative to lines having fewer copy numbers, although these other FR-H2 CBFs did not show copy number differences. Chromatin immunoprecipitation indicated CBF2 was at the CBF12 and CBF16 promoters at normal growth temperatures. These data support a scenario in which CBF2A–CBF4B genomic region copy numbers affect expression of other FR–H2 CBFs through a mechanism in which CBF2 protein activates their expression.

SECTION:
Resources II: Germplasm and Populations

POSTER: 181

Genetic divergence, population structure of wild barley and origin of cultivated barley

Dongfa Sun

Huazhong Agricultural University, China ; sundongfa1@mail.hzau.edu.cn

Co-authors: X. Ren,Y. Wang, S. Yan and G. Sun

ABSTRACT

Genetic divergence of the chromosomal regions with domesticated and non-domesticated genes in barley was examined to reveal effect of natural selection on shaping diversity. It was found that diversity ratios ((diversity of wild type – diversity of cultivated type)/diversity of wild type*100%) of the domesticated regions on 5H, 1H and 7H were higher than those of non-domesticated regions. Averaged diversity among six chromosomes was 33.73% different in domesticated region, and 27.56% different in the non-domesticated region, between wild and cultivated barley. This indicates that natural selection played important role on shaping genetic diversity. Our results showed that the Tibetan wild barley distinctly diverged from Southwest Asian (Near East) wild barley, and that Central Asian wild barley was related to Southwest Asian wild barley. Chinese domesticated barley shared the same haplotypes with Tibetan wild barley. Our results confirmed that Tibet is one of the origin and domestication centers for cultivated barley, and in turn supported a polyphyletic origin of domesticated barley. Comparison of haplotype composition among geographic regions revealed gene flow between Eastern and Western barley populations, suggesting that the Silk Road might have played a crucial role in the spread of genes. The six-rowed spike 1 (vrs1) locus revealed that the nucleotide diversity, number of haplotypes, haplotype diversity and per-site nucleotide diversity in two-rowed barley were higher than those in six-rowed barley. Wild barley had a higher grain protein content (GPC) (10.44%) than cultivated barley in average. Two unique haplotypes (Hap2 and Hap7) caused by a base mutations (at position 544) in the coding region of the NAM-1 gene might have a significant impact on the GPC. SNPs and haplotypes of NAM-1 associated with GPC in barley provide a useful method for screening GPC in barley germplasm. The Tibetan wild accessions with lower GPC could be useful for malt barley breeding.

SECTION:
Resources II: Germplasm and Populations

POSTER: 182

Inheritance, molecular identification and utilization of a novel dwarfing germplasm "Huaai 11" in barley

Dongfa Sun

Huazhong Agricultural University, China ; sundongfa1@mail.hzau.edu.cn

Co-authors: X. Ren, L. Liu, G. Sun and C. Li

ABSTRACT

More than 30 dwarfing or semi-dwarfing genes have been reported, but few have been exploited in barley breeding. The newly discovered dwarfing germplasm "Huaai 11" carries desirable agronomic traits such as shortened stature and early maturity. Genetic factors or QTL controlling agronomic, quality, physiological traits in the Huaai 11 were investigated in order to efficiently use the novel dwarf germplasm.

Our result indicated that dwarfism of Huaai 11 is controlled by a single recessive gene, btwd1, which is nonallelic with genes br, uzu, sdw1 and denso, and mapped onto chromosome 7HL, closely linked to Bmac031 and Bmac167 with genetic distances of 2.2 cM.

A total of 20 QTL underlying seven plant height components traits were mapped onto 2H, 3H, 5H, 6H and 7H. The large effects QTL controlling these plant height components traits were found near the btwd1 on 7H. The QTL on 7H accounted for 27.19% to 59.73% of phenotypic variation on these traits. Seventeen QTL for ten other agronomic and one quality traits were mapped onto five chromosomes. Six QTL, Qhd2-12, Qmsl2-15 and Qmsl7-7, Qgwp7-7, Qgws7-7and Qtgw7-10 were detected in all years. The QTL on 7H has effect on grain weight per plant, grain protein content, and main spike length, accounting for 35.11%, 45.74% and 54.88% phenotypic variation, respectively.

A total of 38 QTLs on 1H, 2H, 3H, 4H, 6H and 7H underlying 7 physiological and 3 morphological traits at the pre-filling stage were detected, and explained 6.53% - 31.29% phenotypic variation. The QTLs flanked by marker Bmag829 and GBM1218 on chromosome 2H were associated with net photosynthetic rate, stomatal conductance, flag leaf area, flag leaf length, flag leaf width, relative chlorophyll content and leaf nitrogen concentration. Two QTL cluster regions associated with physiological and morphological traits, one each on the 2H and 7H, were observed.

SECTION:
Plant Breeding and Genetics Education

POSTER: 183

[No title]

Saemundur Sveinsson

Agricultural University of Iceland ; saemundur@lbhi.is

Co-authors: M. Göransson, H.S. Hilmarsson, A. Helgadottir, J. Hermannsson and J.H. Hallsson

ABSTRACT

Agriculture in Iceland is shaped by a wet, cool and short growing season, which makes cereal production challenging. Furthermore, autumns are characterized by rainy and stormy weather. Winters are characterized by repeated freeze-thaw cycles making cultivation of winter barley impossible. Despite these unfavorable weather conditions, cultivation of spring barley in Iceland has been quite successful in certain regions of the country. Barley fields have grown 10 fold from about 500 ha in 1995 to about 5,000 ha in 2015, with yields averaging about 3.7 tons per ha (8.3 US tons per acre). The expanded barley cultivation can be attributed to many factors, such as increased farmer experience and higher temperatures in recent decades. Another important factor is the use of better adapted barley cultivars, which have been bred for the short growing season. Several early six-rowed cultivars bred for Northern-Scandinavia have been adopted by Icelandic farmers. Unfortunately, many of these tend to be vulnerable to the strong winds in early autumn and they frequently suffer lodging and straw breaking. The Agricultural University of Iceland (AUI) has been running a barley breeding program since 1994, focusing on developing early maturing varieties with a strong straw. Several cultivars have been released, which have been widely adopted by Icelandic farmers. Alongside the breeding work, AUI runs research projects to understand the genetics behind the earliness trait in barley. Our experience demonstrates the importance of local breeding and research efforts for crop improvement, especially for places with a small market like Iceland.

SECTION:

Plant Breeding and Genetics Education

POSTER: 184

Non-destructive whole grain high-throughput phenotyping for malt quality traits in malting barley

Daniel Sweeney

Cornell University ; dws275@cornell.edu

Co-authors: M.E. Sorrells

ABSTRACT

Malt quality traits are becoming increasingly important in malting barley varieties as the craft brewing industry and the demand for high quality malt grow. Phenotyping for these traits is expensive, slow, and requires a substantial amount of seed, making early generation testing for malt quality highly impractical in barley breeding programs. The Single Seed Analyzer (SSA) at Cornell University can non-destructively measure protein, β-glucan, and seed weight on a large number of individual seeds and detect variation between and within genotypes using near infrared spectroscopy. We are characterizing seed protein, β-glucan, and seed weight for eight two-row spring malting barley varieties grown in multiple locations across two years in New York State using the SSA. GxE interactions for each trait and predictions across environments are being modeled. The SSA results will be correlated with malt quality phenotypic data from the USDA Cereal Quality Lab at the University of Wisconsin, Madison. Successful implementation of the SSA for malting barley quality analysis will provide a high-throughput phenotyping tool to screen important segregating malt traits in early stages of the breeding cycle.

SECTION:

Barley end use: malting and brewing

POSTER: 185

On the interest of Research Resource Planning1 tool in data handling for barley breeding

Dimitri Szabo

Doriane SAS ; dimitri.szabo@supagre

Co-authors: T. Duminil and F. Royer

ABSTRACT

Modern breeding techniques such as genome-scale marker assisted selection and high-throughput phenotyping are huge generators of great potential data. Combined with the now easily accessible sensor and environmental data, it makes data handling a real challenge in order to make this information a source of improvement in breeding.
Yet, breeding organizations are lacking tools to give them a true opportunity to analyse and get concrete indicators out of genotypic, phenotypic and processing data altogether, with restricted time and resources. When existing, such tools are often not accessible for small breeding programs. Moreover, most of these data handling and analysis solutions are combinations of various software or solutions which imply a certain complexity and can cause difficulties for global, multidimensional analysis.
In the particular case of barley with its diverse uses, including complex end chain processes for brewery, integrated approaches can be particularly beneficial for breeding organizations. Indeed, possible combination of metadata and data from every step of the breeding process in a unique solution for all campaigns would help malting barley commercial breeders fit the changing beer quality standards accentuated by craft beers development. Besides, integrated approaches in data management and analysis will also support innovative long term processes such as hybrid barley breeding, requiring long monitoring notably due to male sterilization using CMS, and further pedigree studies implying specific and complex analysis algorithms (e.g. evaluating parents combining ability).
This presentation discusses the possibilities offered by Research Resource Planning (RRP) to be used as data handling tool for barley breeders, and the benefits provided in terms of integrated analysis and decision making, according to various barley breeding program using RRP, focusing on brewing and hybridization specific cases.

SECTION:

Breeding Methodologies: current approaches and future

POSTER: 186

Virulence of Moroccan *Pyrenophora teres f. teres* revealed by international differential barley genotypes

Karima Taibi

University of Rabat ; ktaibi1@gmail.com

Co-authors: F. Bentata, S. Rehman, M. Labhilili, A. El Aissami, R.P.S. Verma and S. Gyawali

ABSTRACT

Pyrenophora teres f. teres (Ptt), causing net blotch in barley, is an important and frequently isolated leaf pathogen across the globe. The virulence spectrum of Ptt from North Africa including Morocco is poorly understood. Sixteen barley genotypes were challenged, at seedling stage, with 15 Ptt isolates that were collected from different agroecological zones of Morocco. The experiment was conducted in a factorial arrangement of treatments in a randomized complete block design with three replicates. The ANOVA revealed highly significant ($P < 0.001$) effects of genotype (G), isolate (I) and GxI interaction explaining 23.2, 62.5, and 13.9% of the variation, respectively. Therefore, the current study revealed highly diverse virulence pattern of Moroccan isolates. Furthermore, the results indicated that minor virulence of Ptt isolates dominated over virulence interaction. In addition, Taffa (6-rowed) and Aglou (2-rowed), had the highest level of resistance to Ptt, while Coast and Rabat071 were the most susceptible genotypes. Pt2, Pt7, Pt8, and Pt4 were being the most virulent isolates, while Pt10 and Pt11 were the least virulent isolates. The emergence of the new Ptt pathotypes, which were highly virulent to durable resistance in Rabat071 posed a risk of breaking down the currently deployed resistance to net blotch in Morocco. A careful evaluation and selection of Ptt isolates based on minor virulence pattern to barley genotypes is essential for successful barley breeding program for resistance to net blotch in Morocco.

SECTION:

Resources II: Germplasm and Populations

Biotic stresses

POSTER: 187

BarleyVarDB: an integrated database of barley genomic variants

Cong Tan

Murdoch university ; Cong.Tan@murdoch.edu.au

Co-authors: B. Chapman, Q.S. Zhang, G.F. Zhou, X.Q. Zhang, R. Barrero, M. Bellgard and C.D. Li

ABSTRACT

Barley variation database (BarleyVarDB, http://brl.ccgapps.com.au/Barley/) provides comprehensive information of barley genomic variants, which were discovered by whole genome sequencing (30~50X) of 21 barley accessions including wild barleys from Israel and Tibet and cultivated barley from Australia, Europe and Japan. The genomic variants stored in BarleyVarDB mainly include single nucleotide polymorphisms (SNPs) and short insertions and deletions (InDels, 1-30 bps), which were identified using standard analysis workflow with strict filtering and evaluated to be of high accuracy. PCR primer pairs for each InDel variant were designed and assessed using the electronic polymerase chain reaction (e-PCR). Meanwhile, 100 InDel markers were randomly selected to validate the accuracy through PCR and gel electrophoresis. Variants of SNPs and InDels were allocated into corresponding genomic regions based on predicted gene models. Genetic effect (changing of coding product) of each variant was also recommended. BarleyVarDB can be retrieved with identifier of each SNPs and InDels, InDel marker identifier, physical genomic region, gene identifiers as well as genetic effects. In addition, BarleyVarDB can provide an overview of all genomic variants and molecular markers along each chromosome using Genome Browser and sequence alignment between query sequence and assembled WGS sequences of each accession using BLAST. BarleyVarDB will enhance the efficiency for functional genomics research and marker-assisted breeding in barley.

SECTION:
Resources I: Genome

POSTER: 188

Towards positional cloning and functional analysis of glossy mutants in barley

Zenith Tandukar

University of Minnesota ; tandu002@umn.edu

Co-authors: G.J. Muehlbauer

ABSTRACT

Epicuticular waxes provide a primary water resistant barrier and protection against different environmental stresses like pests, radiation and disease pathogens. Association mapping in an advanced backcross NAM population segregating for waxy phenotypes mapped a QTL on a ~5 cM region on chromosome 3HL, and another QTL to a ~3 cM region on chromosome 1HS. The QTL on chromosome 3HL and 1HS are coincident with classical barley mutants glossy sheath 2 (gsh2) and Eceriferum-yy (Cer-yy), respectively. The gsh2 mutants are characterized by an allelic series of recessive mutations exhibiting a lack of epicuticular waxes in the leaf, sheath and spike, while the Cer-yy mutants are characterized by an allelic series of dominant mutations showing a lack of wax in the spike. The objectives of this project were to characterize the phenotypes of both mutants and to isolate the genes responsible for the waxy phenotype. Scanning electron microscopy confirmed the dramatic reduction of wax on the mutants compared to wild type. A near-isogenic line (NIL) of a gsh2 mutant allele in the cv. Bowman genetic background was used to create F2 mapping populations with four elite barley cultivars. Fine mapping gsh2 with a set of 20 polymorphic SNPs in the MassARRAY system and KASP assays identified markers within 3 cM of the gene, corroborating mapping results of the AB-NAM population. Additional fine mapping combined with examination of the barley genome and comparative genomics analysis with brachypodium and rice will enable the discovery of the genes responsible for these traits. Drought response of the NIL pairs carrying mutant alleles for gsh2 and Cer-yy in the Bowman genetic background and Bowman will be discussed.

SECTION:
Resources II: Germplasm and Populations
Abiotic stresses

POSTER: 189

Improving the processability of malting barley

William Thomas

The James Hutton Institute ; Bill.Thomas@hutton.ac.uk

Co-authors: L. Ramsay, J. Russell, P.E. Hedley, J. Morris, H. Bull, K. Houston, and I. Slaiding

ABSTRACT

Plant breeding has improved the malt extract and thus the amount of beer that can be produced per tonne of malt but maltsters and brewers still encounter problem batches that do not process properly. Processability is therefore second after malt extract on the Institute of Brewing and Distilling's 'wish list' of desirable characters. Problems are much more apparent in samples that are less than ideal, as even poor malting quality varieties give adequate levels of malt extract and process efficiently when grown under optimum conditions. This target will become increasingly important as climate change is likely to result in harsher and more variable environments for malting barley production.

We have grown samples of UK spring and winter malting barley under malting and feed nitrogen management and analysed samples for nitrogen, beta-glucan, and total pentosan contents, as well as grain weight and dimensions. The samples were micro-malted and analysed for malt extract, wort viscosity, and wort beta-glucan content. Campden BRi developed the Vmax test to estimate the filterability of processed malt samples and this was applied as a final analysis in our malt phenotyping. All of the lines have been genotyped with 7000 SNPs and the results of association analysis of the phenotypes will be combined with the results of gene expression analyses on a subset of the lines to identify key genomic regions affecting malt processability.

SECTION:
Barley end use: malting and brewing

POSTER: 190

Improving winter barley malting quality

William Thomas

The James Hutton Institute ; Bill.Thomas@hutton.ac.uk

Co-authors: The IMPROMALT Consortium

ABSTRACT

Results from the AGOUEB project highlighted a significant reduction in the malt extract potential of UK winter barley malting cultivars when compared to the spring and that this difference equated to a reduction in spirig yield produced per tonne of malt of approximately 15l. All UK winter malting barley cultivars trace back to Maris Otter, which effectively is derived from Proctor but in a winter background. The introduction of Triumph provided a notable increase in malt extract potential compared to Proctor and further improvements have been made subsequently yet genotyping shows that a number of key Triumph derived regions found in UK spring malting cultivars are absent in the UK winter malting cultivars.

The IMPROMALT Consortium was formed to provide a partnership between academic, breeding, and end-user organisations to further refine these key segments of the barley genome and conduct a targeted backcross introgression programme that would transfer the spring segments from two spring barley lines into five different winter barley genotypes, two of which were 6 row. Refinement of the interval is being achieved by augmenting the AGOUEB dataset with analysis of UK spring and winter barley lines that have been added to the recommended list since 2006. Data accumulated so far has resulted in an approximate halving of the target segments, facilitating more precise introgression.

SECTION:

Barley end use: malting and brewing

POSTER: 191

Regulation of inflorescence development in response to photoperiod in barley

Filipa Tome

Cluster of Excellence on Plant Sciences (CEPLAS) Institute for Plant Genetics Heinrich Heine University Düsseldorf c/o Dept. Plant Developmental Biology Max Planck Institute for Plant Breeding Research ; tome@mpipz.mpg.de

Co-authors: B. Digel and M. von Korff

ABSTRACT

Variation in flowering time has a strong impact on yield in the long-day crop plant barley. Under long day conditions, PHOTOPERIOD 1 (Ppd-H1) acts as a major positive regulator of flowering by inducing FT1, the barley homolog of FLOWERING LOCUS 1 in Arabidopsis.
By analyzing the effects of day length and Ppd-H1 on barley development, we identified two major phases of shoot development based on differences in the day-length sensitivity of the shoot apical meristem: floral transition occurred under long and short days, whereas fertile florets and seeds only developed under long days. These differences in floret fertility were associated with natural variation at Ppd-H1 and expression of its downstream targets HvFT1 in the leaves and HvFT2 in the main shoot apex (MSA). RNA-sequencing of a developmental series of developing leaves and MSAs revealed that the expression of HvFT1 correlated with transcripts involved in nutrient transport and carbohydrate metabolism, suggesting that HvFT1 may alter source-sink relationships. Successful inflorescence development under long days correlated with upregulation of HvFT2 and transcripts related to floral organ development, phytohormones, and cell cycle regulation.

SECTION:

Barley morphology and development

POSTER: 192

A major QTL on chromosome 7HS controls seed germination under salt stress in the 'Nure' x 'Tremois' barley mapping population

Alessandro Tondelli

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Genomics Research Centre, 29017 Fiorenzuola d'Arda, Italy ; alessandro.tondelli@entecra.it

Co-authors: W. Xue, J. Yan, J. Cheng and L. Cattivelli

ABSTRACT

Seed germination is an important and vulnerable stage in the life cycle of crops and determines the following seedling establishment and plant growth. Comparing with the information currently available about salt tolerance physiology and bio-chemistry in vegetative plants, the mechanisms of salt tolerance in seeds is relatively poorly known. Barley is normally considered as a "halophyte" when compared with other crops. Here, a new experimental approach was tested on the 'Nure' x 'Tremois' doubled-haploid mapping population in order to determine dynamic curves describing the ability of seeds to germinate under different concentrations of salt solution. QTL analysis identified a major QTL on chromosome 7HS with LOD values up to 15.5 and explaining 46% of the observed phenotypic variation. Moreover, correlations between grain weight, grain protein and beta-glucan content and salt tolerance have been examined. This new major QTL could provide a robust evidence for better understanding the response of seed germination under salt stress conditions.

SECTION:
Abiotic stresses

POSTER: 193

Malting quality improvement and molecular markers to assess genetic diversity in barley

Claudia Toniazzo

Embrapa Trigo ; claudiatoniazzo@hotmail.com

Co-authors: S. P. Brammer, E. Minella, P. Wiethölter and A. Caverzan

ABSTRACT

Barley breeding for malting quality aims to pyramid in agronomic superior genotypes genes associated with quality. Molecular markers can be an effective tool in breeding programs by accessing polymorphism at the DNA. In this study we estimated the genetic distance among 12 elite genotypes used by the Embrapa barley breeding program in Brazil. Seed samples of the genotypes were collected in a yield trial seeded in 2012 in three and four locations in the Rio Grande do Sul and Paran˜ states, respectively. Micromalting and quality analysis were performed by VLB in Germany and ABINBEV in USA. Kolbach Index, β-glucans, α-amylase and diastatic power were the quality parameters measured. The molecular analysis was performed using 16 specific microsatellite markers in agarose gel electrophoresis. All quality parameters of all genotypes were highly influenced by the environment in the different locations. BRS Brau, BRS Korbel had the highest overall value for malt quality whereas Victor Graeff/RS was the site with the best environmental conditions for the expression of the quality variables. Some of the cultivars/lines tested had a superior malt quality profile than Scarlett, an international high quality standard. In the microsatellite analysis and linking genotypic data with phenotypic markers, the genotypes that excelled in malt quality appeared in the same groups of the ones established by genetic diversity. However, the microsatellite genetic distances produced more groups than the quality analysis. Therefore, the use of molecular markers showed greater specificity of the data and could be used in malting barley breeding programs.

SECTION:
Barley end use: malting and brewing

POSTER: 194

Phenotypic assessment of a set of two-row barley genotypes with differential FHB resistance and DON accumulation

James Tucker

Agriculture and Agri-Food Canada/ University of Manitoba ; james.tucker@agr.gc.ca

Co-authors: A. Badea, W.G.D. Fernando and W.G. Legge

ABSTRACT

Fusarium head blight (FHB) primarily incited by *Fusarium graminearum* Schwabe, is a ubiquitous disease of barley (*Hordeum vulgare L.*) on the Canadian prairies. Infected grains are deemed unsuitable for use in animal feed or malting and brewing industries primarily due to contamination of mycotoxins, such as deoxynivalenol (DON). Extremely low acceptance thresholds have been set by industry, which has posed a challenge for breeders. The relationship between initial fungal infection and DON production is complex for barley, where visual symptoms often do not correlate well with DON content in matured grains. Disease nurseries require epidemic levels of infection to differentiate between genotypes, which may not be possible in all years due to annual weather conditions. While breeding for low DON accumulation in barley has been difficult, several cultivars have been developed that exhibit low DON content. As global markets becoming increasingly stringent, breeders are required to develop cultivars with exceedingly low DON content. As traditional breeding methods may be approaching practical limits, new methodologies will need to be investigated to further lower DON content in barley cultivars. Genomic selection could offer benefits through placing less reliance on expensive chemistry assays, reducing the number of breeding lines that need to be screened, and allow for selection in years when nurseries are sub-optimal. A substantially large (n=400) and diverse set of two-row barley genotypes were phenotyped in three environments in Manitoba (Brandon, Carberry and Carman) over two growing seasons (2014-15). Data collected for these genotypes will be used to develop models of genomic association which will be presented here. If successful, genomic selection will be pursued for enhancing FHB resistance in the two-row-malting barley breeding program at Agriculture and Agri-Food Canada's Brandon Research and Development Centre.

SECTION:

Biotic stresses

POSTER: 195

An overview of scald and net blotch resistance screening at Lacombe and Edmonton, Alberta, Canada

Thomas Turkington

Lacombe Research Centre, Agriculture and Agri-Food Canada ;
kelly.turkington@agr.gc.ca

Co-authors: K. Xi, F. Capettini, P. Juskiw, W.G. Legge, J. Nyachiro, A. Badea and A.D. Beattie

ABSTRACT

Leaf diseases including scald and net blotch are among the most important biotic factors impacting barley yield, grain filling, and end use quality in Alberta, Saskatchewan and Manitoba. Annual yield losses due to leaf diseases can range from 5-15%, but for individual fields losses may be as high as 20-40%. Incorporation of leaf disease resistance into agronomically adapted barley varieties is an important component of barley breeding programs in Western Canada. Currently, leaf disease screening nurseries are a joint effort among federal, provincial and university breeding and plant pathology programs. Each year 6,000-8,000 hill plots are tested for scald reaction at the Lacombe Research and Development Centre (LRDC), and 2,000-3,000 hill plots are also screened by Alberta Agriculture and Forestry (AAF) at the University of Alberta, in Edmonton, AB. At LRDC, field nurseries for net-form net blotch and spot-form net blotch are also established with approximately 1,000-1,500 hill plots in each. Hill plots are typically comprised of germplasm, breeding lines, checks, a selection of currently registered barley varieties, and candidate barley lines in the Prairie Recommending Committee for Oat and Barley (PRCOB) Western Cooperative Tests. Approximately three to four weeks after seeding, the Lacombe and Edmonton scald screening sites are each inoculated once with infested-straw from the previous year and twice with a conidiospore suspension of several isolates. For the net blotch nurseries, spreader rows of susceptible lines are used and all hill plots are inoculated approximately four to five weeks after seeding with autoclaved winter wheat grain infested with several isolates of the net- or spot-form followed by a second set of inoculations one to two weeks later. In addition, net- or spot-form net blotch infested straw is spread over the respective hill plot nurseries about four to five weeks after seeding. At both locations, irrigation is used to promote disease development. Disease assessments are done on individual hill plots using a 0 to 9 scale, where 0 is no disease and 9 represents a hill plot with greater than 50% of the lower, middle and upper leaves diseased. At Lacombe, the initial ratings are typically done in early to mid-July for scald, mid- to late-July for both forms of net blotch, and again three to four weeks later. At Edmonton the scald rating is typically done towards the end of July. Scores from both sites are tabulated and recommendations regarding disease resistance levels are sent to cooperating breeders for use in assessing lines to advance, crosses to make, and as part of other studies (e.g. the development of molecular markers).

SECTION:
Biotic stresses

POSTER: 196

Genetic and phenotypic variation of spring barley accessions from Kazakhstan and USA

Yerlan Turuspekov

Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan ; yerlant@yahoo.com

Co-authors: B. Sariev, V. Chudinov, A. Abugalieva, L. Tokhetova, G. Sereda, V. Tsygankov, A. Ortaev, A. Yespanov, A. Rsaliev, T. Blake, S. Chao and S. Abugalieva.

ABSTRACT

In this study we assessed genetic and phenotypic variation of spring barley accessions from Kazakhstan and the USA. The collection consisted from 92 samples from Kazakhstan and 574 accessions from the USA and was studied in seven regions of Kazakhstan during 2009-2011. The analyses allowed the identification of a large number of accessions from the USA with high agronomic performances in several regions of Kazakhstan. In addition, the collection tested for key grain quality traits, including protein content. Ninety two accessions from Kazakhstan were genotyped by using 9K SNP Illumina array. The genetic variation was studied using selected polymorphic SNPs for Kazakh samples and already available 3K SNP data for the US barley accessions. Genetic and phenotypic data used for GWAS mapping of QTL associated with agronomic traits, grain quality parameters and resistance to stem rust and spot blotch diseases.

SECTION:
Resources II: Germplasm and Populations

POSTER: 197

Genetic dissection of tiller development in barley

Wilma van Esse

Max Planck Institute for plant breeding reseachr ; vanesse@mpipz.mpg.de

Co-authors: G.W. van Esse, A. Walla, C.B. Liller, M. Koornneef and M. von Korff

ABSTRACT

Cereal crop yield is determined by different yield components such as the number of spike bearing branches (tillers), the number of seeds per spike and seed weight. Optimizing yield by changing the number of branches is an effective strategy in several crops. However, this approach is restricted by negative correlations between the yield components. In barley, for example, an increase in seed number per spike can result in a reduction in tiller number. This negative correlation has often been attributed to a resource limitation occurring at the end of the development. Recent evidence indicates that the same genes or regulatory modules affect both shoot and inflorescence development. This suggests that a resource limitation or reallocation of assimilates is not the only determinant of the trade-off between shoot and inflorescence branching.
Recently, we performed a detailed phenotypic analysis on row-type mutants to study their pleiotropic effects on tillering. Two main groups of row-type mutants were observed, one group with an increased number of seeds per spike and a tillering phenotype at early development (vrs3, int-c) or only at maturity (vrs1, vrs4), and a second with a reduction in both seeds per spike and tiller number (int-l, als, int-b). Altered tiller number occurring at early developmental stages suggest that these loci act in both inflorescence and shoot branching. In contrast, reduction of tiller number occurring only at the end of the development (vrs1,vrs4) is likely due to resource competition. To gain insight into the genetic pathways that affect both shoot and inflorescence branching we performed RNA-sequencing on apex tissue of row-type/tillering mutants at three developmental stages. This transcriptional profiling provides a starting point to unravel how genes affecting shoot and inflorescence branching are regulated and in turn control downstream targets.

SECTION:
Barley morphology and development

POSTER: 198

Dosage-dependent expression of duplicated genes regulated barley leaf and spikelet development and brought two-rowed barley into existence

Thirulogachandar Venkatasubbu

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) ; venkatasubbu@ipk-gatersleben.de

Co-authors: G. Govind, T. Rutten, G. Hensel, J. Kumlehn, L. Eschen-Lippold, J. Lee, T. Schnurbusch, N. Sreenivasulu and M. Kuhlmann

ABSTRACT

Spike architecture (two-rowed vs. six-rowed) is one of the major domestication traits in barley (*Hordeum vulgare* ssp. *vulgare*), which also influences grain yield. The homeobox transcription factor HvHOX1 (VRS1), unique to the tribe Triticeae, expressed in barley spikes and affects the fertility of lateral spikelets causing the two-rowed spike observed in its wild–type progenitor (*Hordeum vulgare* ssp. *spontaneum*). Mutation, deletion or reduction in transcript level of this gene can restore the fertility of lateral spikelets and transform two-rowed barley into six-rowed barley. It was proposed that the HvHox1 was only expressed in lateral spikelet meristems and had evolved from a duplication event of HvHox2, which was expressed in all organs of the plant. Here, we show that HvHox1 expression is present in both central and lateral spikelet meristems but with different dosage levels. We also found that HvHox1 and HvHox2 display similar spatiotemporal but different dosage-dependent expression during spikelet and leaf development. Both proteins have similar dimerization and DNA binding properties, but exhibit differences in their transactivation property. Expression of these genes in barley leaves is the prime evidence of the narrow leaf versus broad leaf phenotypes observed in two- and six-rowed barley types, respectively. Overexpression of HvHox2 under HvHox1 promoter (partially) recovers the fertility of lateral spikelets in transgenic two-rowed barley, suggesting that HvHOX2 is a positive regulator of spikelet development. From the higher leaf nitrogen (%) in six-rowed barley, we explain how the (increased fertile) spikelets in six-rowed spikes are supported by the broad leaf phenotype. Transcriptome analysis during early leaf development in mutant (six-rowed) and wild type (two-rowed) barley showed that HvHOX1 suppresses cell proliferation in leaf cells. We exemplify the mechanistic function of these proteins in barley leaf and spikelet development, thereby providing intriguing evidence how two-rowed barley was brought into existence and influenced the domestication and adoption of six-rowed barley.

SECTION:
Barley morphology and development

POSTER: 199

A comparison study of agronomical and physiological traits in a set of varieties released in Romania and Italy during the last 40 years

Liliana Vasilescu

National Agricultural Research and Development Institute Fundulea, Calarasi (Romania) ; liliana@ricic.ro

Co-authors: F. Rizza, F-W. Badeck, R. Alberici, A.M. Stanca, S. Delbono, E. Alionte, E. Petcu, M. David and V. Terzi

ABSTRACT

A set of 50 cultivars was analysed in a field experiment in two sites, Fiorenzuola d'Arda (PC), Northern Italy and Fundulea, Southern Romania. Eighteen varieties, released in the winter barley breeding program of Romania from 1968 to 2013 were selected to represent the history of barley breeding activity at NARDI Fundulea. Thirty varieties, most of them with winter growth habit, were derived from the breeding work at the CREA of Fiorenzuola since 1979. Two other cultivars from central Europe were included as controls with high winter survival capacity. The first 2014-2015 season trials were done with small plots in three replications. Plant morpho-physiological traits were studied to monitor development with measurements of canopy reflectance spectra (e.g. normalized difference vegetation index, NDVI), phenological surveys, digital images of the plots taken for analyses of cover fraction, surveys of diseases, plant height, chlorophyll and flavonoid content, and seeds quality. The correlation with the main yield components was analysed. High variability was found not only between old and modern cultivars but also within modern cultivars of the same geographical origin. These materials represent a source of genes useful for crosses in the respective breeding activities. Further investigation is planned for the 2015-2016 season to extend the study to GxE effects, of special relevance to identify complex physiological traits determinant for plant development, adaptation and resistance to abiotic stress for application in a modern physiological breeding.

SECTION:

Barley morphology and development

POSTER: 200

Addressing the global challenges for barley improvement through CGIAR Research Program on Dryland Cereals

Ramesh Verma

ICARDA ; r.verma@cgiar.org

Co-authors: S. Gyawali, A. Amri, Z. Bishaw, A. Niane, A. Al-Abdallat, S. Udupa, A. Visioni, S. Rehman and M. Baum

ABSTRACT

The International Center for Agricultural Research in Dry Areas (ICARDA) has a global mandate for barley improvement, specifically for dry areas across the globe. ICARDA has reorganized the spring and winter barley research under the CGIAR Research Program on Dryland Cereals to address the requirements of North and East Africa, Central, West and South Asia regions. The program targets germplasm improvement for optimal and stressed environments to address the feed, forage, food and malt uses in spring barley from its new base at Rabat, Morocco and winter barley program from Ankara, Turkey. The partnership with focal country in each region is the new strategy, which utilizes the complementing capabilities with national programs. Each year, >15,000 advanced lines are evaluated for various agronomic, biotic and abiotic stress tolerances, and quality parameters at ICARDA. The focal countries help in evaluation of the elite germplasm for target region(s). In 2014-2015 cropping season, 339 sets of international trials & nurseries were distributed to more than 60 collaborators in 35 countries. The process has helped the partner countries to release more than 250 barley varieties across globe during 1977-2014, as direct introduction. More than 14 varieties have been released in last three years only. Improvement for malting barley and nutritional qualities (Zn, Fe, and β-Glucan) are new initiatives. Since the demand for industrial uses of barley is on sharp rise in East Africa and South Asia, contract farming and seed production through private-public partnership have big potentials to raise the benefits to the small holder farmers. Another aspects for such partnership includes application of advanced molecular research and technologies including the doubled haploid. Genomics are also supported under CRP Dryland Cereals. Post-harvest and value addition aspects still need the collaboration with advanced research institutes and industry to make research development real.

SECTION:
Success stories in barley breeding and genetics

POSTER: 201

Novel sources of resistance to stripe rust in ICARDA barley germplasm

Ramesh Verma

ICARDA ; r.verma@cgiar.org

Co-authors: R. Selvakumar, P.S. Shekhawat, O. P. Gangwar, S.C. Bhardwaj, S. Rehman and S. Gyawali

ABSTRACT

Stripe rust of barley (*Puccinia striiformis* Westend. f. sp. *Hordei*) occurs worldwide and host resistance is the most economically and environmentally appropriate option as the use of fungicides is not very common in low input crops like barley. Breakdown of host resistance by new emerging pathotypes necessitates the identification of new sources of resistance and the understanding of genetic mechanisms or diversity for developing and deploying resistant barley cultivars. A total of 336 diverse barley genotypes consisting of released cultivars, advanced lines, differentials and local landraces from ICARDA, were screened for seedling and adult plant resistance (APR) to stripe rust in India. Seedling resistance test was done against each of the five prevalent races {Q (5S0), 24 (0S0-1), 57 (0S0), M (1S0), and G (4S0)} at ICAR-IIWBR, Shimla. The field evaluation for resistance was done at ARS, Durgapura (Rajasthan) in three years (2013 to 2015), and at IIW&BR Karnal (Haryana) in 2014 under artificial epiphytotics using the mixture of these five races. Twelve barley genotypes (ARAMIR/COSSACK, Astrix, C8806, C9430, CLE 202, Gold, Gull, Isaria, Lechtaler, Piroline, Stirling, and Trumpf) were resistant to all five races at seedling stage as well as under field screening. Another 42 genotypes were found with slow rusting ability (< 1000 AUDPC values from a range of 520-3600) under heavy inoculum load, restricting the final disease score up to 40MS/S, while the susceptible ones scored up to 100S. It is indicated that resistant genotypes possess novel genes for absolute as well as durable resistance, though more research is warranted for gene postulation in the identified sources. The GWAS is in progress to identify genomic regions conferring resistance in these genotypes.

SECTION:
Resources II: Germplasm and Populations
Biotic stresses

POSTER: 202

Genetic improvement for grain yield and quality traits in barley (*Hordeum vulgare* L.)

Sadaram Verma

CCS HAU Hisar ; verma.sadaram@gmail.com

Co-authors: Nil

ABSTRACT

Development of high yielding cultivars is the primary objective of most barley breeding programmes. Breeders' success in this regard may be measured by comparing the modern cultivars with the older ones. Substantial yield increases have been obtained in the state (about three fold) and at the national level (about 54%) during the last four decades. It has been achieved through alteration of biomass partitioning to the grains or improving the harvest index in the newly developed varieties namely - BH885, BH902, BH946 and BH959. These varieties have better yield potential, more resistance to diseases, better adaptability and excellent malting attributes than the old varieties. For genetic improvement in barley for yield, selection priorities have been given to various characters according to intended use of the grain. Traditionally, high grain yield had always received top priority. Yield has been affected by many characters other than plant height such as resistance to lodging, shattering and prevalent diseases. At the same time emphasis has been placed on selection for market factors such as test weight, kernel plumpness and kernel colour. These varieties have high yield, strong and short straw, plump kernels, medium maturity, high test weight, no shattering, lax ear, thin husk, smooth awns, short rachilla hairs, resistance to prevalent diseases, good kernel colour and good malt quality. The traditionally involved approaches of breeding relying on generating and screening large number of crosses and progeny lines based on attributes of economic interest in a sample environment have yielded results. At the same time the role of physiological and molecular understanding in improving the efficiency of breeding programme have been examined and correlation of these traits with yield and malting quality traits were established.

SECTION:
Success stories in barley breeding and genetics

POSTER: 203

Comparative expression analysis of hordein and beta-amylase in developing barley grains

Marcus Vinje

USDA-ARS Cereal Crops Research Unit ; marcus.vinje@ars.usda.gov

ABSTRACT

Hordeins are the major seed storage proteins (SSP) in the barley grain. They account for the majority of all proteins in the mature grain. Hordeins accumulate and are stored during grain development. Their primary function is to act as nitrogen, carbon, and sulfur reserves. Beta-amylase is a starch degrading enzyme that is important to fermentable sugar production during malting. Beta-amylase is different from the other enzymes involved in fermentable sugar production in that it is expressed and stored in the developing grain in a similar pattern as the hordeins. Previous studies have shown an increased amount of hordein and beta-amylase in the mature grain when barley is exposed to nitrogen, drought, and heat. Beta-amylase has been long speculated to be a SSP or have a dual function as an enzyme and a SSP. High beta-amylase activity is a desirable trait as it is positively and significantly correlated to diastatic power. However, high levels of beta-amylase are correlated to high levels of hordeins and total protein. In order for barley to meet malting quality standards protein levels must be within a defined range. Increased beta-amylase levels can potentially lead to barley not meeting malting quality due to high total protein levels. The expression patterns of hordein and beta-amylase were determined in developing barley caryopsis from 5 days after anthesis (DAA) to 35 DAA to assess whether there is an exploitable difference between hordein and beta-amylase accumulation.

SECTION:
Barley morphology and development

POSTER: 204

Optimizing test locations and identifying promising genotypes in ICARDA barley breeding program

Andrea Visioni

ICARDA, Biodiversity and Integrated ; a.visioni@cgiar.org

Co-authors: M. Singh, S. Rehman, S. Gyawali and R.P.S. Verma

ABSTRACT

Barley (*Hordeum vulgare* L.) is an important cereal crop with versatile use as animal feed, forage and human consumption in the low rainfall situations around the world. Recently the industrial use in malting and brewing is also becoming popular in the developing countries with the possibilities of additional income from premium price for better grain quality. ICARDA has taken a note of this and initiated the high input program targeted towards malting and high value feed barleys. There is need for developing high yielding barley genotypes suitable for the mandate regions to meet growing demand. In order to optimize the test locations and identify the better genotypes this study undertook the evaluation of a total of 544 genotypes with seven checks at three locations (Terbol in Lebanon; Marchouch and Allal Tazi in Morocco) with diverse agro-ecological conditions. Genotypes were evaluated in eight trials sets (six of hulled and two of hull-less genotypes). The mixed models were fitted to evaluate variance components, heritability, genotype x environment interactions and predicted means for identifying the high yielding genotypes. Specific adaptation of genotypes to the locations was assessed in terms of the genotypes performance overall the locations and adding the specific environment effect as GxE interaction, denoted as GGE and presented as GGE-bi-plot. Amongst the three locations, Terbol showed maximum discrimination of genotypes and genotypes specifically adapted to each location followed by Marchouch. Significance of response of different sets of genotypes to location varied with trials and location. GxE interaction across locations was significant for days to heading, days to maturity, plant height, spike length and grain yield. Several hulled genotypes with higher grain yield and early flowering time were identified in the three environments for further evaluation in other barley growing environments in Asia and Africa.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 205

Relationship between Hordoindolines and barley pearling characteristics

Asuka Takahashi

NARO, Western Region Agricultural Research Center, Fukuyama 721-8514;
atakhash@affrc.go.jp

Co-authors: A. Takahashi, T. M. Ikeda, T. Yoshioka, T. Yanagisawa

ABSTRACT

Pearled barley grains are used in miso, distilled spirits, and mixed with boiled rice, in Japan. Barley is recently gaining interest because it is rich in dietary fiber, _-glucan. The barley pearling characteristics: grain hardness, pearling time, broken grain rate after pearling, glassy grain rate, are important factors for the pearling quality. Barley seed proteins, hordoindolines, are homologues of wheat puroindolines, which are associated with grain hardness. Barley hordoindoline genes are known to comprise Hina and Hinb, and Hinb consists of two Hinb genes, Hinb-1 and Hinb-2. In our previous study, we showed that the Hinb-2b frame shift (null) mutation increased grain hardness (Theor Appl Genet 120:519–526, 2010). Grain hardness, pearling time, broken grain rate, and glassy grain rate were measured using near-isogenic lines (NILs) derived from a cross between a line with the Hinb-2a (Shikoku hadaka 109) and a line with the Hinb-2b (Shikoku hadaka 84). In the mutant lines, we found significantly higher grain hardness, longer pearling time, lower broken grain rate and higher glassy grain rate. Grain hardness index (HI) of Shikoku hadaka 84 with the Hinb-2b and Shikoku hadaka 84 with the Hinb-2a was 72.2 and 52.6, respectively. The glassy grain rate increased from 69.1% to 82.9% in the mutant lines. The pearling time of the mutant lines was also increased from 600 sec to 707 sec, but the broken grain rate of the mutant lines decreased from 30.5% to14.7%. It suggests that the Hinb-2b null mutation resulting an increase in grain hardness also increased in pearling time but decreased in broken grain rate. These results are the first clear evidence that hordoindoline gene plays a major role in the barley pearling quality.

SECTION:

POSTER: 206

Phenotypical dissection of a high tillering mutant in barley

Agatha Alexandra Walla

Max Planck Institute for Plant Breeding ; walla@mpipz.mpg.de

Co-authors: W.G. van Esse, M. Koorneef and M. von Korff

ABSTRACT

Shoot architecture is a key trait that contributes to the final yield in temperate cereal crops. In grass species like barley (*Hordeum vulgare* L.), tillering or the formation of lateral branches at non-elongated internodes is determining the final shoot architecture. Tillers are formed in the leaf axils of lower leaves where axillary meristems are initiated and develop into axillary buds which can grow out into tillers. Each developed tiller is capable of the production of a seed bearing inflorescence, hence contributes to the final yield. Cultivated barley is characterized by low synchronous tillering as an adaptation to agricultural practices, while wild barley (*H. vulgare* spp. *spontaneum*) shows high and asynchronous tillering. The objective of the present study was to identify genetic components regulating tiller number variation in barley.
We performed a detailed phenotypic analysis of a high tillering mutant in barley under field and control conditions. In both environments, the mutant line revealed pleiotropic effects including an altered spike, leaf number and leaf morphology phenotype. Interestingly, the selected mutant showed an altered plastochron which might be causative for the high tillering phenotype. In addition, we carried out RNA-sequencing and genetic mapping experiments to locate the causative mutation on the barley chromosome and to identify genetic pathways affected by the mutation.

SECTION:

Barley morphology and development

POSTER: 207

Detection of minor gene resistance to net form net blotch under controlled environment conditions

Hugh Wallwork

South Australian Research and Development Institute ; hugh.wallwork@sa.gov.au

Co-authors: M. Butt and E. Capio

ABSTRACT

A technique for rapidly growing barley plants to flag leaf emergence involving extended day lengths has been used for pathotyping a wide range of isolates of net form net blotch (NFNB) from around South Australia on adult plants of 24 locally grown varieties. The technique provides results that are repeatable and which can be achieved within 7 weeks of sowing the seed. The tests reliably detect minor gene or adult plant resistance to NFNB, being the form of resistance most common in Australian varieties and breeding programs. Almost all isolates evaluated have shown different pathotype profiles as would be expected from a pathogen that undergoes sexual reproduction each year. Pathotype profiles reflect recent changes in virulence on varieties as observed in variety trials and crops across South Australia. The test results support observational evidence for durable resistance in the varieties Clipper, SloopSA and Schooner.
Pathotype profiles gained from these tests have been used to select the best strains for evaluating breeding lines and varieties in disease screening nurseries as well as in controlled environment conditions. New barley doubled haploid populations have been developed to investigate the genetic basis of durable resistance using specific isolates from this work. Phenotyping of these populations under controlled conditions and in field nurseries is commencing in 2016.

SECTION:
Biotic stresses

POSTER: 208

A high density map reveals better markers for the new semi-dwarf gene on chromosome 7H in barley

Junmei Wang

Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China ; wangjunmei@mail.zaas.ac.cn

Co-authors: J. Yang, Q. Zhang, J. Zhu, Q. Jia, W. Hua, Y. Shang, C. Li and M. Zhou

ABSTRACT

Reducing plant height has played an important role in improving crop yields. The success of a breeding program relies on the source of dwarfing genes. For a dwarfing or semi-dwarfing gene to be successfully used in a breeding program, the gene should have minimal negative effects on yield and perform consistently in different environments. In a population of 182 doubled-haploid (DH) lines, derived from the cross between a Japanese malting barley and a Chinese feed barley, a QTL for plant height was identified on 7H by using SSR markers and DArT markers. This QTL showed no significant effects on other agronomic traits and yield components and consistently expressed in the six environments. To find a more closely linked marker for this semi-dwarf gene, DH population was re-genotyped with DArTSeq, which ended up with more than 20,000 markers. Several DArT and SNP markers showed closer linkage to the gene. These markers will be further used to fine map the gene using F2 populations which segregate only in this QTL and near isogenic lines.

SECTION:
Resources II: Germplasm and Populations

POSTER: 209

Molecular mapping of a single dominant gene for resistance to a new pathotype of *Cochliobolus sativus* in barley

Rui Wang

North Dakota State University ; shaobin.zhong@ndsu.edu

Co-authors: R.S. Brueggeman, Y. Leng and S. Zhong

ABSTRACT

Spot blotch, caused by *Cochliobolus sativus*, is an economically important disease on barley in Upper Midwest region of the USA and Prairie Provinces of Canada. A new pathotype (pathotype 3 isolate ND4008) of the causal pathogen was recently identified in this region, which can overcome the spot blotch resistance widely used in six-row barley cultivars. To identify and characterize genetic loci conferring resistance to this new pathotype, genetic analysis of spot blotch resistance was conducted using a cross between a highly resistant line (PI 235186) and a highly susceptible accession (PI 356741). Seven F1 plants from the PI 235186/PI 356741 cross were resistant to isolate ND4008 and 212 F2 individual plants derived from one of the F1 plants segregated into a 3 (resistant) to 1 (susceptible) ratio (λ^2 =0.23, p=0.63), suggesting a single dominant gene (Rcsp3-1) controls the resistance in PI 235186. The result was confirmed by genetic analysis of the F2:3 families derived from individual F2 plants of the cross. Rcsp3-1 was mapped to the short arm of chromosome 6H based on bulk segregant analysis using 194 SSR markers and genotyping-by-sequencing using 20 SNP markers in the F2 population. To saturate the genetic region containing Rcsp3-1, six co-dominant and two dominant markers were developed based on the genome sequences of barley cv. Morex in the Ensembl Plants database. The resistance gene was finally localized to a ~6.5 centimorgan (cM) region between markers 2548555 and 368749, which spans 2.8 Mbp in the physical map. Rcsp3-1 is localized at the same region with the QTL Rcs-qtl-6H-P3 identified based on genome-wide association analysis of 1480 barley accessions. The identified resistance gene and the associated molecular markers will be useful for developing barley varieties with spot blotch resistance to this new pathotype of *C. sativus* in breeding programs.

SECTION:
Plant Breeding and Genetics Education

POSTER: 210

Map-based cloning of Rphq11: a QTL conferring partial resistance to adapted and non-adapted rust species in barley

Yajun Wang

Wageningen University and Research Center ; yajun1.wang@wur.nl

Co-authors: F.K.S. Yeo and R.E. Niks

ABSTRACT

Partial resistance of barley to *Puccinia hordei* and non-host resistance to non-adapted rust fungi inherits polygenically. The two types of resistance seem to share some genes and have a similar prehaustorial mechanism of resistance, but partial resistance is less strong than non-host resistance of barley. Partial resistance to adapted rusts fungi seems, therefore, like a weak form of non-host resistance to non-adapted rust fungi. QTL gene Rphq11 against *P. hordei* isolate 1.2.1 was mapped in seedlings stage of the mapping populations L94 x 116-5 and in Steptoe x Morex, and located at the middle of chromosome 2HL. The resistance allele was contributed by 116-5 and Steptoe. Rphq11 explains 34% of the phenotypic variance in latency period of seedlings. A chromosomal fragment containing Rphq11 had been introgressed from Steptoe into SusPtrit (susceptible to at least 11 non-adapted rust fungi, and highly susceptible to *P. hordei*), resulting in a near-isogenic line, SusPtrit-Rphq11. Compared to SusPtrit, SusPtrit-Rphq11 caused longer latency period of *P. hordei*, and also showed lower infection frequency when inoculated with six non-adapted rust species. Using a high-resolution NIL-F2 population consisting of 3953 individuals, we mapped Rphq11 to a 0.15 cM interval between WBE129 and WBE307, and it co-segregated with WBE144. A BAC contig from the SusPtrit BAC libary that covers the whole genomic region from WBE129 to WBE307 was constructed. BAC sequencing showed the BAC contig to be about 250 kb, on which five genes were predicted. Further search for recombinants indicated that the five candidate genes were reduced to only one. The functional study is in progress.

SECTION:

Biotic stresses

POSTER: 211

WHEALBI: Applying state of the art genomic tools, methods and approaches to characterise barley and wheat genetic resources from across their geographical range as sources of genes and alleles for use in crop improvement

WHEALBI Consortium ; Joanne.Russell@hutton.ac.uk

Co-authors: WHEALBI Consortium

ABSTRACT

Temperate small grain cereals are grown on half of the cultivated area of Europe and are a key element of the European agricultural economy. Indeed, Europe is the number one producer of wheat and barley globally. With demands set to rise by 40% in the next 35 years, it is necessary to increase crop yields while reducing the environmental impact. To meet these challenges, the WHEALBI (Wheat and barley Legacy for Breeding Improvement) project, supported by EU funding, is taking a multidisciplinary approach to identify, understand and utilise the genetic diversity available in wheat and barley landraces and wild relatives. We have chosen to focus on a carefully selected set of 500 accessions from extensive ex situ collections which cover the geographical and agro-ecological adaptive range of barley cultivation. Comprehensive molecular variant analysis by exome sequencing (exome capture arrays) and evaluation of key agronomic and life history traits (common gardens and precision phenotyping) is providing unique datasets to decipher the genetic basis of adaptation to environmental conditions. We anticipate that such key information will help develop pre-breeding material and breeding tools to improve environmental stress tolerance of future barley varieties. Our intention is to make the genotypic and phenotypic data, along with the biological materials, available to the entire research community to facilitate the informed use of biological diversity in academically and commercially led research and development.

SECTION:
Resources II: Germplasm and Populations

POSTER: 212

Crosstalk between flowering time and abiotic stress tolerance in halle exotic barley-yield

Mathias Wiegmann

Martin-Luther-University Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, Chair of Plant Breeding, Betty-Heimann-Str. 3, 06120 Halle (Saale), Germany ; mathias.wiegmann@landw.uni-halle.de

Co-authors: A. Maurer and K. Pillen

ABSTRACT

One of the major challenges that mankind faces is the ability to feed the ever-growing population. The plant breeding sector can provide one possible solution through breeding improved varieties. The optimal time point of flower induction is critical for developing well adapted varieties because this developmental stage determines to a high extent the final yield.
In our study, 48 selected lines of the Halle Exotic Barley-25 (HEB-25) population (Maurer et al. 2015) are used to investigate the crosstalk between flowering time (FTi) and yield performance as well as abiotic stress tolerance. HEB-25 is a spring barley nested association mapping (NAM) population and was created to exploit the high genetic diversity of wild barley. These 48 lines are selected first for their agronomic performance and second for the allelic segregation at four major flowering time loci Ppd-H1, Vrn-H1, Vrn-H3 and Denso/Sdw1. This subset of lines is referred to as HEB-YIELD (HEB-YLD).
This population is planted at four locations around the world (Scotland, Germany, Jordan and Dubai) to investigate the effect of different environments and abiotic stresses on flowering time, stress tolerance and yield. These four locations differ from each other in regard to environmental conditions, including day length. Since this latter factor is a key regulator of flowering time in plants, we will study the effect of day length on important traits, such as yield and stress tolerance.
Two trait categories, life history traits (e.g. shooting and awn emergence time) and grain yield traits (e.g. thousand grain weight and grain protein content), will be recorded.
The strategic aim of this study is to improve the understanding of pleiotropic effects of flowering time regulators (e.g. Ppd-H1) and how they account for yield (and its components) under stress.

SECTION:
Abiotic stresses

POSTER: 213

Identification of molecular mechanisms in the *Drechslera teres* _ barley pathosystem and analysis of genetic diversity and population structure of a Norwegian *D. teres* population

Ronja Wonneberger

Norwegian University of Life Sciences (NMBU) ; ronja.wonneberger@nmbu.no

Co-authors: A. Ficke, A. Vivian-Smith, T. Friesen and M. Lillemo

ABSTRACT

The necrotrophic fungus *Drechslera teres* causes net blotch disease in barley by secreting necrotrophic effectors (NEs) which, in the presence of corresponding host susceptibility factors (SF), act as virulence factors in order to enable host colonization. At present the resistance within most Norwegian cultivars is insufficient.

This study aims at detecting QTL associated with resistance and susceptibility in the Nordic barley breeding material and at discovering new NE _ SF interactions. This knowledge together with an understanding of the genetic background of the Norwegian net blotch population will be utilized to speed up resistance breeding.

Resistance of a segregating mapping population of a cross between the closely related Norwegian varieties Arve and Lavrans to three Norwegian *D. teres* isolates was assessed at seedling stage in the greenhouse and in adult plants in the field. QTL mapping revealed four major QTL on chromosomes 4H, 5H, 6H and 7H. The 5H and 6H QTL accounted for up to 47% and 14.1% of the genetic variance, respectively, and were found both in seedlings and adult plants with the latter QTL being an isolate-specific association. The high correlation of seedling and adult resistance (R^2=0.49) suggests that components of adult plant resistance can be predicted already at the seedling stage.

Selected isolates and their culture filtrates will be screened on selected barley lines to characterize novel NE - SF interactions and to map the corresponding sensitivity loci. Effector protein candidates will be purified and further analysed to verify their effect on disease development.

Additionally, 365 Norwegian *D. teres* isolates and a selection of globally collected isolates are currently being ddRAD genotyped in order to obtain SNP markers to study the genetic diversity and population structure of the current Norwegian fungal population. This data will also allow us to perform Genome Wide Association Studies (GWAS) to identify potential novel NE genes.

SECTION:
Biotic stresses

POSTER: 214

Cadmium uptake is mediated by a manganese transporter, HvNramp5 in barley

Dezhi Wu

Zhejiang University, China; Institute of Plant Science and Resources, Okayama University, Chuo 2-20-1, Kurashiki 710-0046, Japan ; wudezhi@zju.edu.cn

Co-authors: N. Yamaji, M. Yamane, M. Kashino, K. Sato and J.F. Ma

ABSTRACT

It is known that uptake of Cd in rice is mainly mediated by a Mn transporter, OsNramp5 (Sasaki et al., 2012). Here, we isolated and characterized a homolog of OsNramp5 in barley, HvNramp5. HvNramp5 shows 84% identity with OsNramp5. Expression of HvNramp5 in yeast showed transport activity for both Mn and Cd. HvNramp5 was mainly expressed in the roots and its expression was not affected by Cd and deficiency of Zn, Cu and Mn, but slightly up-regulated by Fe deficiency. Furthermore, different from rice, HvNramp5 showed similar expression level in the root tip and basal root region. Analysis of laser microdissection samples showed that the expression of HvNramp5 was higher in the outer root cell layers than in the inner cell layers. This is consistent with immunostaining result. Transient expression of HvNramp5-GFP in onion revealed its localization at the plasma membrane. Knockdown of HvNramp5 resulted in a significant reduction in root growth under low Mn concentrations, decreased Mn and Cd concentrations in both the roots and shoots. Furthermore, the Cd concentration in the roots and shoots were decreased with increasing Mn concentrations in the external solution in the wild-type barley, but not in the RNAi lines. Taken together, these results indicate that HvNramp5 is a transporter for Mn uptake and that Cd uptake is mediated by this transporter in barley.

SECTION:
Abiotic stresses

POSTER: 215

Evolution of recessive resistance genes against the bymovirus disease of barley

Ping Yang

Institute of Plant Biology, University of ZOrich ; ping.yang@botinst.uzh.ch

Co-authors: A. HabekuΛ, B.J. Hofinger, K. Kanyuka, B. Kilian, A. Graner, F. Ordon and N. Stein

ABSTRACT

Co-evolution between plants and pathogens is an antagonistic perpetual process driving resistance gene evolution in species. Little is known about the evolution of sequence diversity in recessive virus resistance genes. Recently we cloned by map-based approaches two barley host factor genes HvPDIL5-1 and HvEIF4E, the causal genes of recessive resistance loci rym1/11 and rym4/5, respectively, that confer broad-spectrum and complete resistance to the agriculturally important Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV). We further analyzed natural variation in a broad collection of wild (*Hordeum spontaneum*) and domesticated barleys (*Hordeum vulgare*) by re-sequencing the full-length coding sequence of the two host factor genes. Two types of gene evolution conferred by sequence variation in domesticated barley, but not in wild barley were observed. Whereas resistance conferring alleles of HvEIF4E exclusively contained non-synonymous amino acid substitutions (including in-frame sequence deletions and insertions), loss-of-function alleles were predominantly responsible for the HvPDIL5-1 conferred bymovirus resistance. Moreover, a strong correlation between the geographic origin and the frequency of barley accessions carrying resistance conferring alleles was evident for each of the two host factor genes, indicating strong adaptive selection for bymovirus resistance in cultivated barley from East Asia.

SECTION:
Resources II: Germplasm and Populations
Biotic stresses

POSTER: 216

Fine mapping coincident QTL for multiple traits linked with gpc1 on chromosome 6H of barley

Lu Yin

University of Minnesota ; yinxx134@umn.edu

Co-authors: K.P. Smith, E.L. Schiefelbein, G.V. Rodrigiez, K.A. Beaubien and A.H. Sallam

ABSTRACT

Barley with bright kernels (low kernel discoloration or KD level), low mycotoxin deoxynivalenol accumulation, resistance to Fusarium head blight (FHB), net blotch and bacterial leaf streak, relatively low grain protein (GP) content (115 - 135 g/kg), and good agronomic traits (high yield, low lodging, low stem breakage, desirable heading and senescencing dates) are desirable for the malting and brewing industries. Quantitative trait loci (QTL) studies and other genetic evidence have associated these traits with a region of chromosome 6H near bin 6 of barley. In addition, this coincident QTL 6H region is orthologous to Gpc-B1 influencing protein concentration in wheat. Unfortunately, some of these traits are in unfavorable associations posing a challenge for breeding. For example, the cultivar Chevron has good FHB and KD resistances, but high (undesirable) GP and poor agronomic traits; while the cultivar Karl has low GP but discolored kernels. Whether the genetic associations among these traits are due to linkage or pleiotropy remains unknown. We developed a mapping population comprised of recombinant near isogenic lines carrying a segment of Chevron spanning this 6H region in the background of the commercial cultivar Lacey. The objectives of this project are to fine map the 6H region for the above traits, and recover, if possible, recombinants with favorable associations. Genotype-by-sequencing will be implemented to give dense marker coverage in the target region. Each of the 11 traits indicated above was evaluated in at least two field or greenhouse trials in 2015. After analysis of pehnotypic and genotypic data, QTL mapping was performed through QTLcartographer.

SECTION:
Barley end use: malting and brewing
Plant Breeding and Genetics Education

POSTER: 217

Six rowed spike 2 (Vrs2) - a central regulator of spike development in barley

Helmy Youssef

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) ; youssef@ipk-gatersleben.de

Co-authors: K. Eggert, R. Koppolu, A. Fazeli, T. Rutten, G. Govind, U. Lundqvist , N. Sreenivasulu and T. Schnurbusch

ABSTRACT

Studies investigating the genetic basis of different row-types including their lateral spikelet fertility have a long history in barley. Naturally occurring row-type loci underlying the six-rowed-spike (vrs) phenotypes were identified for vrs1 (Komatsuda et al. 2007), vrs5 (syn. Intermedium-c; Ramsay et al. 2011) and labile (lab), while vrs2, vrs3 and vrs4 (Koppolu et al. 2013) were only found after mutagenesis. Thus, the molecular identification of row-type loci represents promising means to better understand the genetic regulation of spike fertility in barley. In this study, detailed phenotypic analyses of vrs2 mutant spikes in comparison to two-rowed cultivar (Bowman) using SEM analysis showed that, vrs2 mutants possess a distinct spike phenotype with additional spikelet meristems at the base (7-9 spikelets/rachis node), enlarged lateral spikelets - few of them producing awns - and irregular lateral spikelets setting seeds along the spike. This gradual increase of lateral spikelet fertility from the top to the base of the spike may imply putative nutritional, transcriptional or hormonal gradients along the spike. To elucidate the underlying genetic determinant for this interesting phenotype we followed a map-based cloning approach to identify the vrs2 gene. Here we found a novel gene belonging to a class of transcriptional regulators implicated to play a role during grass inflorescence development. Phylogenetic analysis of this protein family showed that it exclusively occurs in land plants with a grass-specific branch containing vrs2 gene. Vrs2 transcript levels in different plant organs of wild type (Bowman) showed that it is expressed in some organs such as tiller buds and spike meristems but not in leaves. Transcriptional, hormonal and nutritional measurements revealed that specific transcripts, plant hormones and metabolites can be related to the seen developmental gradient along the spike. In this study we will show that vrs2 has a central regulatory role during spike development of barley

SECTION:
Success stories in barley breeding and genetics

POSTER: 218

Manipulating Male Fertility in Barley (Hordeum vulgare L.)

Behzad Talle

School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, UK; behzad.talle@nottingham.ac.uk

Co-authors: Fernandez Gomez J. and Wilson Z.A.

ABSTRACT

Plant reproductive organs are major contributors to providing sources of energy for humans. A successful pollen dispersal that synchronises with ovule maturity is pivotal for fertilisation and seed formation. Study of Arabidopsis flower has divided anther and pollen development into 14 stages, during which stamens are formed from the floral meristem, mature pollen develops inside the anther lobes and are finally dispersed from the anthers through dehiscence. The final step of anther development, anther dehiscence, is important to ensure that viable pollen is able to reach the stigma. However the failure of anthers to release viable pollen is potentially a useful tool for hybrid seed production in agronomically important crops, such as barley. Three principal transcription factors have been identified to be involved in regulating secondary wall thickening in Arabidopsis anthers; these are MYB26 and two NAC domain transcription factors (NST1 and NST2). It has been shown in Arabidopsis that NST1 and NST2 redundantly regulate formation of secondary wall thickening in the anther via MYB26. In this study the putative orthologues of NST1 and NST2 in barley (Hordeum vulgare L.) were identified. The identified sequences were used to make silencing constructs (SRDX and RNAi) and generate barley HvNST1 and HvNST2 silencing lines. The repressor lines of HvNST1SRDX and HvNST2SRDX showed reductions in fertility of more than 90%; this was accompanied by reduced spike size and high levels of sterile flowers in the spike. These lines also had reduced plant height and weaker stems compared to wild type. The expression analysis of the target genes in the silencing lines showed a positive link between the three main transcription factors' expression and fertility. These lines are being used to further study the relationship between the main genes involved in formation of secondary wall thickening in the anthers and also the targets of NST1 and NST2 in barley anthers. Further exploitation of these transcription factors can pave the way for providing a reliable system of hybrid seed production in economically important cereals such as barley.

SECTION:

POSTER: 219

A customised single nucleotide polymorphism (SNPs) panel for molecular marker assisted selection within an applied spring barley breeding program.

Jennifer Zantinge

Alberta Agriculture and Forestry ; jennifer.zantinge@gov.ab.ca

Co-authors: S. Xue, M. Holtz, P. Juskiw, J. Nyachiro, M. Aljarrah, J. Helm, Y. Kabeta, K. Xi, L. Oatway, K. Kumar and F. Capettini

ABSTRACT

The Field Crop Development Centre (FCDC), based in Lacombe, Alberta (Canada) conducts breeding programs for feed, forage and food barley (2-row, 6-row and hulless), 2-row malting barley, spring and winter triticale, and wheat. These breeding programs are focused on developing improved cultivars for production in western Canada. New varieties must be high yielding, lodging resistant, adapted to unique regional growing conditions, resistant to major diseases and meet the quality demands of the malt, feed or food industry. At present, cereal lines are primarily selected using phenotypic data (field testing, wet chemistry and NIRS), although a limited amount of marker assisted selection has been applied. If genotyping data is to be used routinely as a selection method within our current breeding program, our genotyping assays must be done quickly, economically and accurately. Recent advances in sequencing, bioinformatics, and the accumulation of knowledge on genetic diversity, genes and QTLs, has made genotyping methods more cost effective and accurate. As a new approach we identified about 100 SNP markers that are customised for trait selection within FCDC spring barley breeding lines. We have focused on SNPs linked to disease resistance (scald, net blotch, Fusarium head blight, spot blotch and stripe rust), malt quality enzymes, dormancy, nitrogen use efficiency and in vitro fiber digestibility. With this panel, we plan to speed up and reduce the cost of genotyping across breeding programs, by simultaneously genotyping multiple genes/QTLs on one array.

SECTION:
Breeding Methodologies: current approaches and future

POSTER: 220

Genetic and histological studies of durable loose smut resistance mediated by Un8 in barley

Wen Zang

University of Saskatchewan ; wen.zang@usask.ca

Co-authors: P.E. Eckstein, M. Colin, X.M. Zhang, D. Voth, A. Himmelbach, S. Beier, N. Stein, G.J. Scoles and A.D. Beattie

ABSTRACT

Loose smut of barley, caused by *Ustilago nuda* (Jens.) Rostr. (*U. nuda*), is a common disease in North America. Durable loose smut resistance under the control of the Un8 gene has been effective for more than half a century. Here, we used a population of 4,625 lines segregating for Un8 to delimit the Un8 gene to a 0.108 cM interval on chromosome 1HL and identified the minimal tiling path for the Un8 locus using two flanking markers. One candidate gene located close to an Un8 co-segregating marker showed high sequence identity to a disease resistance gene containing two protein kinase domains.
Sequence analysis of the candidate gene from 24 cultivated and 4 wild barley accessions representing diverse geographic regions showed it contained one intron with the translation start site located in the second exon. Between resistant and susceptible accessions, no sequence differences could be found in the first exon, only one SNP was present within the intron and 16 SNPs were identified in the second exon. Alignment of the deduced amino acid sequences indicated that all resistant accessions shared the same protein sequence and 13 amino acid variations unique to the deduced protein sequence in resistant accessions differentiated it from the deduced protein sequences in susceptible accessions. After analyzing ~2 kb of the upstream promoter region in resistant and susceptible accessions, several SNPs in potential cis-regulatory elements unique to resistant accessions were identified.
Based on histological and PCR results, the resistance mediated by the Un8 gene in barley appears to be expressed early after germination in the seedling stage.

SECTION:

Biotic stresses

POSTER: 221

Integration of transcriptome, proteome and metabolome analysis reveals the mechanisms of low-potassium tolerance in barley

Jianbin Zeng

Zhejiang University, China ; zengjb@zju.edu.cn

Co-authors: G. Zhang

ABSTRACT

Understanding of physiological and molecular mechanisms of low-potassium (K) tolerance in crops is imperative for developing the cultivars with high-K use efficiency. In this study, RNA-Seq based transcriptome profiling, TMT based proteomics and GC-MS based metabolomics were performed on the two wild barley genotypes (XZ153, low-K tolerant and XZ141, low-K sensitive), and a low-K tolerance cultivar (Ludaomai), to determine the genotypic difference in response to low-K stress. Totally, 692 differentially expressed genes were identified under two K levels, including transcription factors, transporters and kinases, oxidative stress and hormone signaling related genes. Meanwhile, a total of 129 proteins related to low-K tolerance were identified, which were mainly involved in defense, transcription, signal transduction, etc. In addition, 61 key metabolites were identified and their concentrations were significantly affected by low-K stress. In general, amino acids accumulation was enhanced in both roots and leaves, while TCA cycle was inhibited. Additionally, the concentration of the negatively charged amino acids and organic acid were decreased in leaves and roots, but more positively charged amino acids were increased, possibly in order to maintain the balance of charges in the cytoplasm. The results showed that wild barley accession XZ153 has a higher capability of K absorption and use efficiency than other two genotypes under low-K. A rapid response to low-K stress in XZ153 is attributed to its more K uptake and accumulation in plants. Meanwhile, it was also found that the ethylene response pathway and phenylpropanoid pathways may account for the genotypic difference in low-K tolerance, and accumulation of glucose and other sugars, thus ensuring the energy supply, could be also a physiological basis for better low-K tolerance of barley. The current results confirmed the possibility of Tibetan wild barley in providing low-K tolerant genetic resource and developing the cultivars with low-K tolerance.

SECTION:
Abiotic stresses

POSTER: 222

Exploring phenotypic variation for key agronomic and life history traits in a barley legacy collection

Alessandro Tondelli

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Genomics Research Centre, 29017 Fiorenzuola d'Arda, Italy; alessandro.tondelli@crea.gov.it

Co-authors: S. Delbono, D. Guerra, C. Biselli, N. Faccini, M. Baravelli, I. Tagliaferri, M. Megyeri, P. Mikó, M. Molnár-Láng, H. Özkan, J. Russell, M. Bink, F. van Eeuwijk, M. Alaux, T. Letellier, L. Cattivelli

ABSTRACT

The WHEALBI (Wheat and barley Legacy for Breeding Improvement) project, supported by EU funding, is taking a multidisciplinary approach to identify, understand and utilise the genetic diversity available in a barley legacy collection of cultivars, landraces and wild relatives. In this frame, a standard phenotypic approach based on "common garden" trials across Europe has been established to test the whole collection (512 accessions) for exploring its adaptative capacity. In the 2014/15 growing season, winter and spring nurseries have been sown in 4 locations (North Italy - CREA, Hungary - ATK, Scotland - JHI and Central Anatolia - Univ. Cukurova) selected to represent different environmental conditions (latitudes from 32Γ to 57Γ north, altitudes from sea level to 1250 m, from continental to arid climatic conditions), following augmented experimental designs and considering a two-rows plot as the experimental unit. The following adaptive traits have been recorded on a plot basis: growth habit, winter survival, flowering time, plant height, (thousand) grain weight, fruiting efficiency. Morphological traits such as awn length and roughness, leaf length and width and peduncle length have been also evaluated in few trials. Such information is providing the phenotypic information required to complement the genomics data generated by exome sequencing the same accessions and to help deciphering the genetic basis of barley adaptation to contrasting environments.

SECTION:
Resources II: Germplasm and Population

POSTER: 223

Inter-chromosomal transfer of immune regulation during barley-powdery mildew interactions

Roger Wise

USDA-ARS / Iowa State University; rpwise@iastate.edu

Co-authors: P. Surana, D. Mistry, R. Xu, A. Chapman, G. Fuerst, J. Dickerson and D. Nettleton

ABSTRACT

Powdery mildew fungi infect over 650 monocot and 9,000 eudicot plants, yet, Triticeae grain crops are among the most important that suffer yield loss. Blumeria graminis f. sp. hordei (Bgh) is the causal agent of barley powdery mildew and a model system to investigate the interactions of obligate biotrophic pathogens with their hosts. Reaction to Bgh is mediated by several mildew resistance (Ml) loci, which trigger barley-Blumeria interactions in a gene-for-gene manner. Expression quantitative trait locus (eQTL) mapping associates quantitative gene expression with polymorphisms in segregating populations, and as such, has been used to identify key regulators for traits. Genome-wide transcriptome analysis of the Q21861 X SM9010 doubled haploid population identified major trans eQTL hotspot clusters on chromosomes 2HL and 1HS. Embedded within these clusters are the resistance loci, MlLa and Mla1, which associate with 961 and 3,296 genes, respectively, corresponding to Bgh penetration and haustorial growth. Moreover, of the 961 genes regulated by MlLa during penetration, control of 299 of these was transferred to Mla1 during haustorial growth. Time-course transcript analysis discerned two opposing scenarios: 1) down-regulation in compatible interactions after Bgh penetration and initiation of haustoria, as compared to incompatible interactions -- this pattern was designated “PacMan”, or 2) sustained up-regulation in compatible interactions, as compared to incompatible interactions -- designated “i (inverse) PacMan”. The first scenario represents host defense genes that are suppressed by pathogen effectors, whereas the second scenario may represent a situation where the pathogen is inducing defense, but then co-opts these genes for its own purposes. Interestingly, ~30% of genes associated with interchromosomal transfer of immune regulation were classified as PacMan or iPacMan. We postulate that the functions of genes regulated by alternate chromosomal positions are repurposed as part of a conserved immune complex to respond to different pathogen attack scenarios.

SECTION:
Biotic Stresses

POSTER: 224

Japanese Barley Cultivars with high β-glucan Content

Takashi Yanigasawa

Institute of Crop Science, NARO Institute of Crop Science, NARO
2-1-2 Kannondai, Tsukuba, Ibaraki 305-8518, Japan; tyanagi@affrc.go.jp

ABSTRACT

Barley is used for many foods such as pearled barley. Improvement of the grain composition and quality is an important objective in barley breeding in Japan. _-glucan, a major component of polysaccharides in the cell walls of the barley endosperm, is a very important source of dietary fiber, which has effects of lowering blood cholesterol levels. Barley usually contains 2–3% _-glucan in grains. I introduce here some recently released barley cultivars with high _-glucan content. The recently released cultivar 'Beau Fiber' with a lys5h mutation has an approximately 10% _-glucan content per weight in both whole grain and pearled grain. Bread mixed with 'Beau Fiber' flour is a value-added food, and Chiffon cakes made with 'Beau Fiber' flour are available commercially. Recently, granola (cereal consisting of rolled barley with dried fruits and nuts) made with grains of 'Beau Fiber' has also gone on sale. Waxy barley cultivars are conventionally used for specialized foods, such as 'dango' and 'mochi'. Waxy barley results in a sticky texture, which is a desired feature for boiled pearled barley in Japan. Boiled pearled barley made from waxy cultivars usually has higher eating quality than that of non-waxy cultivars. The waxy phenotype is associated with high _-glucan content (ca. 5–6% in grains). Waxy cultivar 'Daishimochi', which has purple grains, is cultivated in western Japan. 'Kirarimochi' is both amylose-free (waxy) and proanthocyanidin (PA)-free (ant 28.494) cultivars. PA was shown to be responsible for this browning reaction, and PA-free (ant) mutants have been used to reduce browning and show no discoloration after boiling and incubation. 'Waxy Fiber' has very high _-glucan content (ca. 11% in grains) with both lys5h mutation and waxy character. Barley cereal (barley puff) made with 'Waxy Fiber' are available.

SECTION:

POSTER: 225

Identifications of significant genes controlling malting qualities in barley genome

Qisen Zhang

Australian Export Grains Innovation Center ; qisen.zhang@aegic.org.au

Co-authors: C. Li

ABSTRACT

Molecular marker technologies have been used for studying malting quality traits, including malting extracts, diastatic power, grain protein concentrations, free amino acid contents, grain plumpness, test weight, viscosity, α-amylase and β-glucan. Many QTL for these traits have been identified and used for improvement of barley varieties in barley breeding. Sequencing of barley genomes has provided great resources for investigation of malting quality traits. Genomic information for several different barley such as wild and domesticated barley has become available. We have developed a tool to localize malting quality QTL to physical map of barley genome. We have mapped over 10100 molecular markers with DNA sequences to the barley physical map. This tool can be used to establish the size of a chromosome region in base pairs, obtain DNA sequences and examine candidate genes flanked by molecular markers. New molecular markers which specifically target the potential genes can be designed and used for fine mapping. We have used this tool to investigate genes responsible for diastatic power, viscosity, grain protein concentrations and free amino acid contents in malting barley. This tool can also be used for identification of genes controlling other economically important traits such as yields and disease resistance.

SECTION:

Barley end use: malting and brewing

POSTER: 226

QTL mapping of glume-opening angle in barley (*Hordeum vulgare L.)*

Xinzhong Zhang

Yangzhou University ; rgxu@yzu.edu.cn

Co-authors: G. Lan, C. Lv, B. Guo, S. Lin, J. Ma and R. Xu

ABSTRACT

Cleistogamous and chasmogamous are two opposite phenotypes for barley during flowering. The glume-opening angle is an important factor affecting production of hybrid barley seeds. In present study, 247 DH lines derived from angnongpi7/Yang0187 were used to identify and validate QTLs associated with barley glume-opening angle in different environments using SSR markers. Three QTLs associated with glume-opening angle were mapped on chromosome 2H and 7H using a 584.1 cM genetic linkage maps constructed by 100 SSR markers. The major QTL qGOA-2H-1 was on chromosome 2H with flanking marker ebmac0415 and GBM1498, explaining 65.8% of the phenotypic variance. Eliminating DH lines with the major QTL by functional marker, the two minor QTLs were validated at all three locations across two seasons. QTLs identified in this study preliminarily revealed the genetic mechanism of glume-opening angle. A functional marker of major QTL made it possible to use marker assisted selection in barley breeding programs. Comparing the two minor QTLs to rice chromosomes showed that the minor genes may be transcription factor genes in MADS-box family.

SECTION:

Resources I: Genome

POSTER: 227

Breeding barley for waterlogging tolerance: key mechanisms and ready-to-use molecular markers

Meixue Zhou

University of Tasmania ; mzhou@utas.edu.au

Co-authors: X.C. Zhang, S. Shabala, L. Shabala, C.D. Li, X. Huang, F.R. Zeng, G.F. Zhou and S. Broughton

ABSTRACT

Barley waterlogging tolerance is a complex trait which is composed by various physiological components. Over the last several years, we have conducted a series of studies on physiological mechanisms contributing to waterlogging tolerance in barley and associated the QTL mapping of overall waterlogging tolerance with QTL mapping of some physiological traits. The ability of root cells to maintain MP and take up nutrients, plant tolerance to soil elemental toxins and secondary metabolites produced under anaerobic conditions, and the formation of aerenchyma in roots under waterlogging conditions are all important to plant's overall waterlogging tolerance. Among all the mechanisms, the fast formation of aerenchyma under waterlogging stress has been found to be the major tolerance mechanism. This has been confirmed through QTL mapping of aerenchyma formation in three different DH populations. A single major QTL, which is located in a similar position, was found in all three populations. This QTL was co-located at the same position as the QTL for waterlogging tolerance. Further studies showed that allelic differences exist with the allele from a wild barley accession being more effective in forming aerenchyma, which leads to better waterlogging tolerance. Several molecular markers were found to co-segregate with the gene from the wild barley for aerenchyma formation. These markers will be used in a pre-breeding program to create barley germplasm with waterlogging tolerance. Preliminary results also revealed that the formation of aerenchyma was also controlled by different mechanisms in Yerong and the wild barley.

SECTION:
Abiotic stresses

POSTER: 228

Evaluating predictive values of various physiological indices for salinity stress tolerance in barley and wheat

Min Zhu

University of Tasmania ; min.zhu@utas.edu.au

Co-authors: M. Zhou, L. Shabala and S. Shabala

ABSTRACT

Soil salinity tolerance is a complex trait - both physiologically and genetically - and the issue of which mechanism or trait has bigger contribution towards the overall plant performance is still hotly debated in the literature. In this work, a large number of barley (*Hordeum vulgare* L. and *Hordeum spontaneum* L.) and wheat (*Triticum aestivum* and *Triticum turgidum*) cultivars were screened using a broad range of physiological characteristics. In general, 46 barley genotypes and 49 wheat cultivars were grown under glasshouse condition and exposed to moderate salinity stress for 5 weeks (200 mM NaCl and 150 mM NaCl respectively). In barley, the overall salinity tolerance correlated positively with stomata density, leaf K+ concentration and the relative contribution of inorganic ions towards osmotic adjustment in the shoot, occurring in moderate salinity stress. The results indicate the importance of increasing stomata density as an adaptive tool to optimise efficiency of CO^2 assimilation under moderate saline condition, as well as benefits of the predominant use of inorganic osmolytes for osmotic adjustment in barley. While in wheat, bread wheat and durum wheat showed different tolerance mechanisms. Most of the bread wheats showed better Na+ exclusion that was associated with higher relative yield. Leaf K+/Na+ ratio and leaf and xylem K+ contents were the major factors determining salinity stress tolerance in wheat. Other important traits included high xylem K+ content, high stomatal conductance and low osmolality. These results indicate that Na+ sequestration ability is much stronger in durum compared with bread wheat, most likely as a compensation for its lesser efficiency to exclude Na+ from transport to the shoot. Taken together, all the aforementioned physiological traits can be recommended as efficient screening methods for salinity-tolerant genotypes in both barley and wheat.

SECTION:
Abiotic stresses

POSTER: 229

Evaluation of physiological characteristics conferring waterlogging tolerance in barley accessions

Min Zhu

University of Tasmania ; min.zhu@utas.edu.au

Co-authors: W.G. Legge, M. Zhou and A. Badea

ABSTRACT

Waterlogging is becoming one of the challenging issues for modern agriculture globally, due to the aggravation of climate change. However, waterlogging tolerance in crops is a rather complex trait, both physiologically and genetically. In case of barley, waterlogging stress causes chlorophyll, protein, and RNA degradation, and also reduces the concentration of nutrients like nitrogen, phosphorus, metal ions, and minerals in the shoot. Hence, this calls for an urgent need to understand physiological mechanisms underlying waterlogging tolerance, so as to breed waterlogging-tolerant barley cultivars. In this work, a large number of barley (*Hordeum vulgare* L.) cultivars and accessions from various origins were screened using a broad range of physiological characteristics in both greenhouse and field conditions, aiming to evaluate each of the physiological traits and their contributions towards the overall plant performance under waterlogging stress. Our results to date showed that leaf chlorophyll content decreased dramatically 7 days after waterlogging treatment in the field and the results from greenhouse work suggest a significant positive correlation between healthy leaf rating and fresh weight, indicating the importance of maintaining chlorophyll content as an adaptive tool to optimize efficiency of photosynthesis and carbon fixation. In conclusion, chlorophyll content can be used as an efficient screening method for developing waterlogging-tolerant barley genotypes.

SECTION:
Abiotic stresses

POSTER: 230

Mechanisms underlying row-type determination in cultivated barley

Monika Zwirek

The James Hutton Institute ; monika.zwirek@hutton.ac.uk

Co-authors: M. Lafos, V. Patil, N. Bonar, S.M. McKim and R. Waugh

ABSTRACT

Row-type determines the number of kernels produced at each node on the barley rachis. Each node carries three single-flowered spikelets - one central and two laterals. Based upon the fertility of the lateral spikelets, barley is classified into two- and six-row varieties. In the two-rowed spikes, only the central spikelet is fertile and produces grain, while in the six-rowed types all three spikelets yield grains. Given the potential for increasing yield, there has been a long-term interest in identifying and understanding the causal genetic mechanism(s) underlying this developmental switch. We now know the identity and phenotypes of five SIX-ROWED SPIKE (VRS) genes involved in determining row-type in cultivated barley. However, we lack any mechanistic understanding of the basis for the two- to six-row conversion and, in particular, whether and how the VRS genes interact directly or indirectly to control this vital agronomic trait.

Our research addresses this knowledge gap through a combination of genetic and phenotypic analyses and by constructing protein interaction and gene expression networks using different vrs mutants. Here, we will present a detailed ontogenetic analysis of spikelet fertility for known vrs mutants (isogenic lines within the Bowman background) using scanning electron microscopy which has allowed us to identify critical stages for lateral row fertility. We will also show how double vrs combinations interact in controlling and shaping row number and additional agronomic traits in barley. Finally, using in situ hybridisation and qPCR, we will show the spatial and temporal expression pattern of known VRS genes in mutants and wild-type inflorescences during development.

SECTION:
Barley morphology and development

Barley in Tunisia: a long-way breeding story

M. El Felah

Field Crop Lab.[1]INRAT, Rue Hédi Karray, 2049, Ariana, Tunisia
PH: (+216) 71 230-024, E-mail: elfelah.mouldi@gmail.com

OBJECTIVES

To reveal markers that help in the study of secondary evolution of *Triticeae* tribe within Tunisian Barley Landraces.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest crops in the world and played a key role in agriculture and science development. In the world, barley is used mainly for feed (55-60%), malt (30-40%), food (2-3%) and seed (5%). In the world, average barley production is estimated to 140 million tons (2003-2007). For Tunisia, annual total need was 0.9 million ton, with 0.4 millions quintals produced in the country. Average Maghreb barley production in the late decade (2000-2009) was 3.172 million tons, adjusted by average importation of 1.384 million tons, with annual total need of 45.556 million quintals (El Felah et al, 2015).

The barley varieties (Martin, Ceres) are carefully cultivated only for the production of malt. The marginalization of barley cultivation continued until the 70s, despite the country's independence in 1956. After the bitter experience of collectivism in the 60s and the economic recovery of the 70s, Tunisia wanted self-sufficient in agricultural products, mainly milk and derivatives, red and white meats and other products. For this it was necessary to increase the size of sheep and cattle and therefore significantly increase feed. So we started to give importance to barley which, despite annual average plantings half a million hectares, yield levels and production remained very low. Local barley, introduced improved and included in the catalog do not have a real impact on the quantitative and qualitative improvement of the national barley production (El Felah, 2011; Gharbi et El Felah; 2013).

INRAT barley improvement program review for the late 98 years was done to show achievements and how to improve our research activities to face new challenges (Scharen and al, **1986**). Local barley landraces analysis revealed regional distribution across the country depending on uses, farming systems and eco-geographic adaptation within littoral and continental areas. Barley improvement started early in the late century. 108.787 barley lines from INRAT (32%), ICARDA (43%), CIMMYT (22%) and ACSAD (3%) were evaluated during the period (1914–2008). During this period, 17 barley varieties were registered and widely cultivated in the country. Rihane registered in 1987, has been widely cultivated in the country and in the region and participate actively in barley production records.

MATERIALS AND METHODS

This study involved evaluation of 148 barley germplasm samples collected in 75 island and mainland sites in central and southern Tunisia (El Felah, 1998). It aims the evaluation of polymorphism of storage proteins in barley. This is to the revelation of genetic markers that help in the study of the evolution of this species. This work was performed in electrophoresis cereal lab in Bari Germplasm Institute of Italy. The method used was that of Shewry et al,

1978 and Cooke et al (1983), which we have made some changes related to extracts samples in the stacking gels (Kralova et al, 1991).

RESULTS AND DISCUSSION

The tested 148 samples gave 56 profiles with a polymorphism rate of 38%. Hordeins B and C being the most polymorphic, have generated 44 B and 40 C diagrams. Similarly, we noted the presence of 2 to 9 band B and 2 to 7 band C. The relatively high polymorphism found in the central coastal and central island (83% and 73%), shows the importance of the gene flow of germplasm coming from different regions and skills of farmers acceptance of new introductions barley crops for possible crop adaptation. This was confirmed by the allele frequencies of storage proteins in grains. Indeed, the allele frequencies hordeins B showed two polymorphic classes of hordeins central class (insular, coastal and inland) characterized by alleles frequencies from 41 to 49% and a second own ranks of continental coastline of southern Tunisia with frequencies from 36 to 38%. For the other polymorphe hordeins class C, different polls island, coastal and inland were more or less similar and ranged from 32 to 37% (Table 1). Typically, Gabès oasis farmers usually cultivate Sfira forms as barley dual uses and produce forage for direct pasture or after cutting. Observed similarity in alleles frequencies of the polymorphe hordeins classes B and C in southern coastal areas (HB = 38%; HC = 38%) and in southern continental areas (HB = 34%; HC = 36%) showed the flow of migration of indigenous genes continental urn to littoral urn and vice versa. In another hand, a second collecting mission of sixty accessions, collected from the central Island of Kerkena (sites of El Attaya, Mellita, and El Khmara), were evaluated for hordein patterns using SDS-PAGE. Results showed a high polymorphism for B and C hordein bands, with the identification of eighteen distinct barley chemotypes (figure 1). Notable changes in B and C banding patterns, including band shortening, discoloration or total disappearance of a specific storage protein (El Felah and al, 2004). Since B-hordein is indicative of good grain quality, the modification or disappearance of B-hordein would therefore indicate a reduced grain quality in the barley populations at Kerkena during the elapsed period (El Felah and al, 2006).

This lead us to conclude that dual purpose barley varieties are not actually concerned by total yield proteins of hordeins B and C and that the indigenous peoples of other regions (central island, central coast, central and southern continental island) practiced unconsciously mass selection to choose barley ecotypes rich in protein content and prefered for food. Moreover, these areas are popular and known for barley uses for human consumption (Bsisa, Zammit, Hail, Dardoura, Dchich, Bazine, Barkoukech, Glia, Kisra ...). Hordeins B may play a key role as markers for quality and identification of food barley. We did observe that endosperm color frequencies evolution depend a lot on barley landrace site and on the gender issue, (Bettaieb-Ben Kaab and al, 2005; El Felah and Medimagh, 2006). In Tunisia, the local grown barley is the product of all this panoply of gene routes between the native gene flow in the Middle East and through urn of Eastern Mediterranean. Local forms of barley are grown generally in small traditional farming systems where farmers practiced subsistence traditional farming with local empirical knowledge. The local barley map in Tunisia indicates that anthropological and sociological factors within cultural practices have significantly influenced the empirical establishment of barley local genetic resources over time. Moreover, the selected barleys ‘’Djebali’’ in northern Tunisia encountered mainly in Mogods and Kroumirie mountains were in fact, barley ecotypes from the central and continental barley landrace ‘’Frigui’’ brought by ships owners transhumating for summer grazing. These forms of long spikes, bearded, semi-compact likely helped diversify the local Tunisian barley forms with ‘’Tarichti’’ and formed a landrace of open area between the North and South of the country where other forms were

received from cyrenaic mediterraneen areas and early, within Punic and Roman introductions throughout the islands and the central mediterraneen littoral areas. The island forms ‘’Jerbi’’ in Djerba, ‘’Beldi’’ in Kerkennah and ‘’Sfira’’ in the oasis of Gabes significantly helped to generate other forms much more tolerant to drought, heat and salt and used mainly for human consumption as local Souihli, Ardhaoui and Arbi barleys, (El Felah and Medimagh, 2005).The elucidation of specific key candidate gene essential for amino acid regulation and prolamine metabolism help for future breeding strategies to quality and phenotype response to climate change.

Table 1. Areas, Regions (landraces) Collection Sites of barley accessions Tunisian south of the Tunisian Dorsal

Zone	**Landrace / Region**	**Collecting Sites**	**Ecotype**	**EPP/Ec**	**EPP/Eco (%)**	**HA RDI (HA/ TH) (%)**	**HB RDI HB/ TH (%)**	**HC RDI HC/ TH (%)**	**HD RDI HD/ TH (%)**	**Total Hord. ZDI (%)**
Littoral Central	Sahel	Téboulba Mahdia	Souihli	5/6	83	8 (12)	32 (47)	23 (33)	5 (8)	68 (8,5)
Littoral Sud	Oasis	Gabès	Sfira	7/15	47	12 (15)	30 (38)	30 (38)	7 (9)	79 (9,8)
Continental Central	Le Kairouanais	Route Sfax-Kairouan km 68	Frigui Tarichiti Sahli Beldi	7/19	36	8 (11)	37 (49)	24 (32)	6 (8)	75 (8,5)
Continental Sud	Médenine	El Fedja	Ardhaoui	5/11	45	11 (20)	20 (36)	19 (34)	5 (10)	55 (6,8)
Insulaire Central	Île de Kerkennah	Mellita Ouled Kacem Ouled Bou Ali Attaya	Beldi	11/15	73	16 SMP=4 (13)	51 SMP=13 (43)	40 SMP=10 (33)	12 SMP=3 (11)	119 (14,9)
Insulaire Sud	Ile de Djerba	Jorf 1 km 2 Jorf 2 km 8 Houmet Essouk 1 El Khmara Houmet Béni Dighet Djerba 1	Jerbi	33/54	61	52 SMP=9 (13)	167 SMP=28 (41)	151 SMP=25 (37)	34 SMP=6 (9)	404 (50,5)
Total	5	14	8	68/120	56	107 ZMP=7	337 ZMP=24	287 ZMP =20,5	69 ZMP=5	800 ZMP=57

Abbreviations: **EPP**: Electrophoretic Polymorphism Pattern; **Eco**: Ecotype; **HA**: Hordeins A; **HB**: Hordeins B; **HC**: Hordeins C; **HD**: Hordeins D; **RDI**: Regional Diversity Index; **TH**: Total Hordeins; **ZDI**: Zonal Diversity Index; **ZMP**: Zonal Mean pattern; **SMP**: Site Mean Pattern.

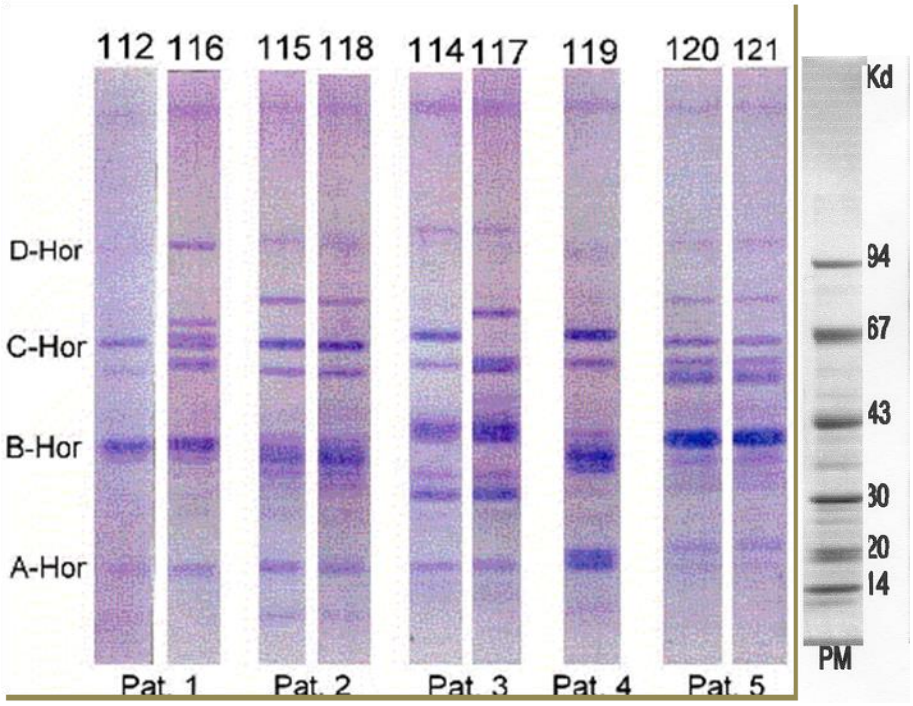


Figure1: Polymorphism Analysis of storage proteins of Tunisian local barleys by SDS-PAGE and characterization of profiles under extinction or gradual drift. The example of accessions of Kerkena Island (sites of El Attaya, Mellita, and El Khmara) (Accessions 112, 114, 115, 116, 117, 118, 119, 120 and 121) that segregate by 5 patterns (Pr.1 to Pr.5).
PROTEIN Markers (PM): phosphorylase (94 kd), bovine serum albumin (67 kD), Ovalbumin (43 kd), Anydrase Carbonic (30 kd), Trypsin soybean I (20 kd) and α-lactoglobulin (14 kd).

REFERENCES

Bettaieb-Ben Kaab, M. El Felah, M.H. Ghorbal and M. Laurière, 2005. Relationships between some hordein components and quality properties in two tunisian barley varieties as influenced by nitrogen fertilization. CZECH ACADEMY OF AGRICULTURAL SCIENCES, Czech Journal of GENETICS AND PLANT BREEDING, 41, 2005 (1): 11-16. Volume 41-ISNN 1212-1975.

Cooke R.J. and Cliff E.M., 1983. Barley cultivar characterization by electrophoresis I. A method for the polyacrylamide gel electrophoresis of hordein proteins. J. Nat. Inst.Agric., Bot. 16: 1-16.

EL FELAH M. 1998. Evaluation de 423 écotypes d'orge collectés en Tunisie (Centre et Sud). Utilisation en amélioration variétale pour la sélection d'idéotypes adaptés à différentes régions bioclimatiques. Thèse de Doctorat de Biologie, Faculté des Sciences de Tunis, Université Tunis II, 4 Juillet 1998, 301 p.

M. El Felah, L. Bettaieb Ben Kaab & N. Chalbi, 2004. Tunisian insular barley landraces : A significant hordein drift. In : 9th IBGS Proceedings Edited by Ing. Jaruslav Spunar, CSc., Jarmila Janicova, Agricultural Research Institute Kromeriz, Ltd. Brno, Czech Republic; 20-26 June, 2004. 1079 p., 39-42.

EL FELAH M. and MEDIMAGH S., 2005. Food Barley in Tunisia. In: «Food Barley : Imporatance, uses and local Knowledge»; Editors : Stefania Grando & Helena Gomez Macpherson; 2005- ICARDA, (ISBN : 92-9127-173-0), 156 p. : 29-35.

El Felah M. & Bettaieb-Ben Kaab L. 2006. Barley in situ-conservation: a significant risk. IPBS, August 20-25, 2006, Mexico City. In: Book of Poster Abstracts, "International Plant Breeding Symposium, 2006", Honoring John Dudley, Editor: Sophie Higman, p. 37.

El Felah M., 2011. L'orge en Tunisie : Historique, état actuel et perspectives. Ann. de l'INRAT, Volume 84, 2011; 07–34.

Gharbi, M. S. et El Felah, M.- 2013. Les céréales en Tunisie: plus d'un siècle de recherche variétale. Annales de l'INRAT, Numéro Spécial – Centenaire de l'INRAT (1913-2013); Volume 86: 45-68.

M. EL FELAH, M. S. GHARBI, H. BEN GHANEM et M. ELLOUMI 2015. Les céréalesen Tunisie entre mythe et réalité. Annales de l'INRAT, 2ème Numéro Spécial Centenaire, Volume 88, 2015 ; 1-17.

Kralova M. et al, 1991. Vybrané metody chemiké analÝzy půd a rostlin. ČSAV, studie 12.90, Academia Praha, 152s.

Scharen A.L., Schaller M., El Felah M. and Srivastava J. (International Review Team), 1986. Meeting the Challenge – Cereal Improvement in Tunisia; INRAT/ICARDA: A Cooperative Project to Improve Cereal Production in Tunisia. This Joint Report has been published by ICARDA (Aleppo – Syria) in English version (23 p.) and French version [Répondre au défi. Amélioration des céréales en Tunisie], (33 p.).

Shewry P.R., Pratt H.M., Finch R.A. and Miflin B.J., 1978. Genetic analysis of hordeins polypeptides from single seeds of barley. Herdity 40: 463-466.

SECTION: Success stories in barley breeding and genetics

Analysis of Molecular Diversity and Population Structure in Barley (*Hordeum vulgare L.*) inferred with SSRs

A. Elakhdar [1, 2], M. Abd EL-Sattar[2], K. Amer[3] and T. Kumamaru[1]*
Faculty of Agriculture, Kyushu University, Japan[1].
Faculty of Agriculture, Alexandria University, Egypt[2].
Field Crop Research Institute, Agricultural Research Center, Egypt[3].
* Corresponding Author: PH: 81-92-642-3057, e-mail: kumamaru@agr.kyushu-u.ac.jp

OBJECTIVES

We studied genetic diversity and population structure in the spring barley using microsatellite (SSR) markers, which provided the basis for targeted research activities.

INTRODUCTION

Barley (*Hordeum vulgare L.*) is one of the oldest cultivated crops in the world. Today, it constitutes the fourth most cultivated cereal in the world after maize, rice, and wheat (FAO 2015, http://www.fao.org/home/en/). In this crop, as in others, genetic diversity has declined following domestication and intense selection in modern plant breeding programs: it is highest in wild barley, *H. vulgare subsp. spontaneum*, intermediate in landraces or parents of mapping populations, and lowest in elite cultivars, over the last 100 years, modern plant breeding has led to the development of elite cultivars that have often been based on a single genotype (pure lines, hybrids or clones) with improved yield potential, quality, and pest resistance traits (Fischbeck, 2003). Today, most barley landraces have disappeared from practical farming. Many of them are still maintained in ex-situ repositories. Globally, landraces represent the largest part of barley germplasm conserved in gene banks (Anonymous, 2008).

In order to breed and improve barley cultivars for future needs, it is very important to search for new sources of useful variation and to understand the relationship among cultivated and wild populations, focusing on the centers of barley domestication and diversification. Breeding barley strategies in Egypt, included screening of local and exotic materials, crossing blocks, and yield test trials (exotic annually providing from ICARDA, CIMMYT and ACSAD) and INRA; Europe, to support breeding objectives (Noaman, 2008).

Currently, Genome-wide association analysis (GWAS) and association mapping project is in progress on 472 germpalsm, with the aim of identifying molecular markers for traits important for low input under the Egyptian conditions. As a first step in this project, we report a preliminary characterization of the population structures in part of our panel.

MATERIALS AND METHODS

Plant materials- From 472 Egyptian spring barley germplasm adapted to a wide range of climates in Egypt, a set of 153 cultivars and promising lines were used in this study to represent the Egyptian barley diversity.

Pedigree- The pedigree of the lines was obtained from several sources (ICARDA, CIMMYT, ACSAD and INRA) and archived at Agricultural Research Center (ARC) in Sakha; N31.3°, E30.9°, Egypt. The populations were collected since 1998 and growing annually as parental sources in the same geographical areas; a.elakhdar@kyudai.jp for more details.

DNA extraction- Five seeds of each line were germinated on growth chamber in Institute of plant genetic resources at Kyushu University, Japan; young leaves (3 cm^2) of each genotype were collected to extract DNA according the CTAB methods protocol suggested by (Doyle and Dolye, 1990).

Genotyping using SSR markers- Sixty-nine SSRs markers were selected based on their mapping position in the barley genome, covering all seven chromosomes (Table 1).

Genetic diversity and population differentiation- The number of alleles, gene diversity, heterozygosity and polymorphism information content (PIC) values, were determined for all loci across the total population using PowerMarker 3.25 (Liu and Muse 2005). Genetic variation within and among populations was estimated by AMOVA using GeneAlEx6.5 (Peakall and Smouse 2012). The population structure of the 153 genotypes considered was inferred using STRUCTURE 2.3.4 (Pritchard et al., 2000) based on 69 SSRs markers. This approach uses a Bayesian clustering analysis to assign individuals to clusters (K). STRUCTURE simulations were performed with the number of presumed clusters from K=1 to K=10 and five runs per K value. For each run, the initial burn-in period was set to 100,000 Markov Chain Monte Carlo (MCMC) iterations. The most probable number of clusters was determined by plotting the estimated likelihood values [LnP (D)] as a function of K. Furthermore, delta (K) values were also calculated as proposed by (Evanno et al., 2005). Neighbor-Joining tree were performed using MEGA6 (Tamura et al., 2013).

RESULTS AND DISCUSSION

To the best of our knowledge, the present study is the first that directly compares the population diversity of Egyptian barley landraces, even at a field (data not shown) level, with a representative pool of Egyptian barley germpalsm. In particular, using SSRs, the landrace collections spanned 18 years from several test trials experiments (IBON, IBCB, SCB, ON-FARM, PNBYT, F5-SAL, F5-DR and local varieties). This collection comprised of 153 genotypes (36 and 117 naked and hulled barley, respectively) included 11 commercial varieties were analysed (Bellucci et al., 2013).

Out of the 69 SSRs analysed, 64 turned out to be polymorphic and 5 monomorphic. The 64 polymorphic SSRs resulted in 261 alleles averaging 4.08 alleles per locus (Table 1). The highest number of alleles (10) in polymorphic SSRs was scored for Bmac0030/ 4H. The highest PIC value was estimated for GMS021/ 1H and scssr00103/ 6H (0.82), with a mean of 0.49 over the 64 polymorphic loci. The markers Bmac0154, GMS021, GBM1214, Bmag0125, Bmac0030, Bmac0096, scssr09398 and scssr07970,demonstrated a high level of allelic diversity and were found most informative (GD > 0.75) on the set of genotypes analyzed, the results agree with (Elakhdar et al., 2016b).

The highest value of Δk was observed with three clusters (Fig.1) suggesting that the accessions most likely separate into three subpopulations, which is in contrast to the three populations found in the set 416 genotypes (Bellucci et al., 2013) and ten populations in the set of 1485 spring barley landrace (Pasam et al., 2014). Neighbor-joining dendrogram results shown three main clusters (Fig. 2), and agree with Structure analysis revealed several major clusters with low bootstrap support and generally good separation of accessions in terminal nodes often consistent with the known origin. This may be explained by more diverse set of genotypes with respect according to origin and growth habit (Pasam et al., 2014; Elakhdar et al., 2016a; Elakhdar et al., 2016b). Table 2 gives the results of the AMOVA analysis. Considering the genetic diversity between and within the populations, highly significant ($P \leq 0.001$) genetic variance was observed between and within subpopulations recorded a total 49 % and 51 % of total variance, respectively, with main genetic variation occurred within subpopulations (Elakhdar et al., 2016b). In Conclusions; the 153 genotypes great genetic diversity and good sample to represent the total core collection size of 472 genotypes. Increased marker coverage and precise phenotypic data for our collection will help to identify candidate genes also for agronomic and adaptation-related traits using GWAS (Comadran, 2012) .

Table 1. Diversity statistics for 143barley accessions inferred with 69 SSR markers.

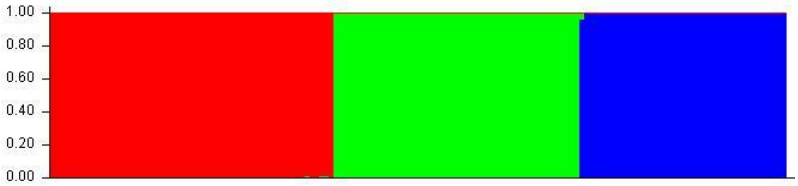
Marker*	Ch	NA	GD	He	PIC	Marker	Ch	NA	GD	He	PIC
EBmac0501	1	2	0.33	0.00	0.28	Bmac0030	4	10	0.81	0.91	0.78
Bmag770	1	8	0.70	0.23	0.65	HVMLOH1A	4	3	0.66	0.00	0.59
Bmac0154	1	7	0.75	0.02	0.71	EBmac0701	4	5	0.72	0.00	0.67
Bmac0213	1	5	0.59	0.03	0.50	scssr18005	4	4	0.65	0.00	0.59
Bmag382	1	3	0.26	0.00	0.24	Bmac0181	4	3	0.59	0.89	0.50
GMS021	1	7	0.82	0.08	0.80	GBM1221	4	2	0.47	0.00	0.36
GBM1278	1	3	0.51	0.58	0.43	GBM1323	4	2	0.50	0.00	0.37
GBM1434	1	3	0.48	0.09	0.42	GBM1482	4	2	0.50	0.00	0.37
GBM1451	1	3	0.63	0.00	0.56	GBM1501	4	1	0.00	0.00	0.00
GBM1461	1	3	0.66	0.00	0.59	GBM5210	4	2	0.38	0.00	0.31
GBM1480	1	5	0.67	0.00	0.62	Bmac0096	5	5	0.76	0.00	0.73
EBmac0695	1	4	0.74	0.00	0.69	Bmac0113	5	2	0.03	0.00	0.03
scssr10477	1	4	0.47	0.02	0.44	GMS001	5	4	0.71	0.00	0.66
Bmag0125	2	6	0.78	0.00	0.75	scssr02306	5	3	0.55	0.00	0.48
EBmac0415	2	4	0.67	0.00	0.60	scssr10148	5	4	0.73	0.00	0.68
Bmag749	2	5	0.58	0.03	0.50	GBM1164	5	3	0.62	0.00	0.55
scssr08447	2	3	0.58	0.00	0.51	GBM1176	5	2	0.50	0.00	0.37
Bmac0093	2	4	0.55	0.00	0.45	GBM1227	5	2	0.49	0.00	0.37
GBM1208	2	5	0.58	0.00	0.53	GBM1483	5	2	0.36	0.00	0.29
GBM1214	2	5	0.76	0.34	0.72	EBmac0602	6	6	0.69	0.00	0.65
GBM1218	2	2	0.49	0.21	0.37	Bmag0009	6	2	0.33	0.00	0.28
GBM1459	2	4	0.69	0.00	0.64	Bmac0316	6	7	0.69	0.01	0.64
scssr03381	2	3	0.66	0.00	0.59	GMS006	6	2	0.36	0.00	0.29
scssr07759	2	3	0.52	0.01	0.41	scssr09398	6	9	0.82	0.57	0.79
HvLTPPB	3	4	0.70	0.00	0.64	scssr00103	6	4	0.73	0.35	0.68
Bmac0209	3	6	0.78	0.00	0.74	Bmag0011	7	3	0.63	0.00	0.56
EBmac0871	3	5	0.77	0.00	0.73	EBmac0603	7	5	0.52	0.01	0.44
GMS116	3	6	0.74	0.06	0.70	Bmag0120	7	5	0.75	0.01	0.70
scssr10559	3	3	0.66	0.00	0.59	HVCMA	7	4	0.67	0.00	0.60
GBM1078	3	2	0.02	0.02	0.02	HvID	7	5	0.70	0.01	0.65
GBM1280	3	2	0.50	0.00	0.37	scssr07970	7	6	0.78	0.80	0.75
GBM1405	3	1	0.00	0.00	0.00	HVTUB	2/4	3	0.23	0.00	0.21
GBM1413	3	1	0.00	0.00	0.00	HvLOX	5/6	4	0.71	0.00	0.66
scssr20569	3	2	0.48	0.03	0.36	HvWaxy4	6/7	1	0.00	0.00	0.00
scssr25538	3	1	0.00	0.00	0.00	Mean		4	0.55	0.08	0.49

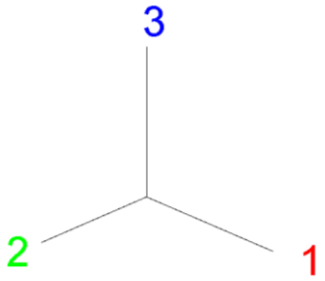
- *source: Grain Genes data base; http://wheat.pw.usda.gov/GG2/index.shtml
- Ch; chromosome, NA; No. allele, GD; gene diversity, He; heterozygosity, PIC; Polymorphism Information Content.

Table 2. Analysis of the molecular variance using SSR markers, performed at different levels.

Source	df	SS	MS	Est. Var.	%
Among subpopulations	2	2584.2	1292.1	12.5	49%
Among Individuals	150	3626.2	24.1	11.2	44%
Within Individuals	153	263.0	1.7	1.7	7%
Total	305	6473.5		25.481	100%

(a)





(b)

Fig.1. Population structure of 153 landraces using 69 SSR inferred by STRUCTURE for K=10. Genotypes were ordered according to their membership coefficient (Q) values to one group, Group I (red) = 53, Group II (green) = 41 and Group III (blue) = 36 barley accessions.

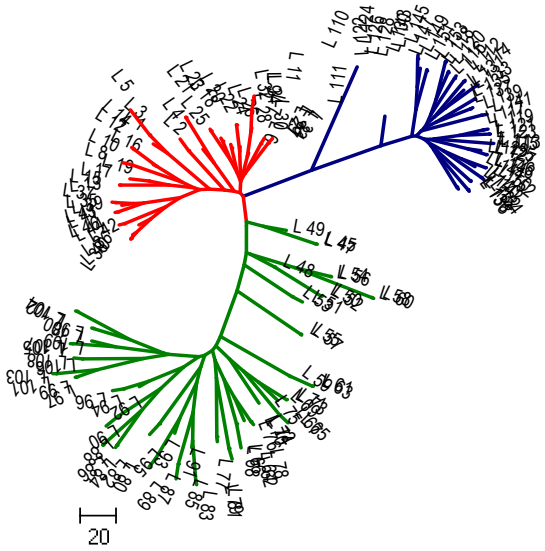


Fig. 2. Dendogram obtained through cluster analysis of the barley landraces on the basis of allele frequencies across mapped SSR markers.

REFERENCES

Anonymous. 2008. Global strategy for the ex-situ conservation and use of barley germplasm. pp. 1–65.

Bellucci, E., Bitocchi, E., Rau, D., Nanni1, L., Ferradini1, N., Giardini1, N., Rodriguez, M., Attene, G., and Papa R. 2013. Population structure of barley landrace populations and gene-flow with modern varieties. PLoS One 8(12): e83891.

Comadran, J., Kilian, B., Russell, J., Ramsay, L., and Stein, N. 2012. Natural variation in a homolog of Antirrhinum CENTRORADIALIS contributed to spring growth habit and environmental adaptation in cultivated barley. Nat Genet 44: 1388–1392.

Doyle, J., and Doyle, JL. 1990. Isolation of plant DNA from fresh tissue. Focus, v.12, p.13-15.

Elakhdar, A., Abdelsattar, M., Amer, K., Assma, R., and Kumamaru, T. 2016b. Population structure and marker-trait association of salt tolerance in barley (*Hordeum vulgare L.*). Comptes rendus Biologies, Under Pressed.

Elakhdar, A., Abdelsattar, M., Ame, K., and Kumamar, T. 2016a. Genetic diversity analyzed by microsatellite markers among barley (*Hordeum vulgare L.*) genotypes with different adaptations to saline conditions. Australian J. Crops Sci., accepted.

Evanno, G., Regnaut, and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14(8): 2611-2620.

Fischbeck, G. 2003. Diversification through breeding. In: von Bothmer R, van Hintum Th, Knùpffer H, Sato K, editors. Diversity in barley (*Hordeum vulgare)*. Amsterdam: Elsevier Science B.V. 29–52.

Jonathan K. Pritchard, M. S. a. P. D. (2000). Inference of population structure using multilocus Genotype Data. Genetics (155945–959.).

Liu, K., and Muse, SV. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21(9): 2128-2129.

Noaman, M. 2008. Barley development in Egypt: Proceedings of the 10th International Barley Genetics Symposium. 5-10 April 2008, Alexandria, Egypt, 3- 15.

Pasam, R Sharma, R., Walther, A., Özkan, H., Graner, A., and Kilian, A. 2014. Genetic diversity and population structure in a legacy collection of spring barley landraces adapted to a wide range of climates. PLoS One 9(12): e116164.

Peakall, R., and Smouse, PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. Bioinformatics 28(19): 2537-2539.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0.Mol Biol Evol.; 30(12): 2725–2729 .

Barley Breeding in the Public Interest: Perspectives on Integrating Research, Variety Development, Learning Experiences, and Extension in a U.S. Land Grant University

P.M. Hayes[1], all current and past members of the OSU Barley Project, and our worldwide network of collaborators

[1]Department of Crop and Soil Science; Oregon State University; Corvallis, OR 97333
Phone: 541-737-5878 email: Patrick.m.hayes@oregonstate.edu

OBJECTIVES:

Share with the 12th International Barley Genetics Symposium participants the impact that student training has had on plant breeding with the Oregon State University Barley Project.

INTRODUCTION:

Barley is one of last major crops in the U.S. where the public sector is still actively engaged in cultivar development. A number of factors have contributed, historically, to this unique status. An additional set of factors could lead, in the near term, to a shift towards greater private sector delivery of varieties and public sector delivery of knowledge and training. A retrospective analysis of the Oregon State University (OSU) Barley Project is useful as an example of the outcomes that can be achieved when a public program receives sufficient support to engage in the Land Grant mission of (i) contributing to the fundamental body of knowledge, (ii) stimulating economic development through variety release, and (iii) assisting in forming an educated citizenry. Keys to the success of the program have been a balance of public (e.g. USDA Collaborative Agricultural Project grants) and industry funds (e.g. American Malting Barley Association); an opportunity to explore challenges and opportunities with no obvious immediate practical application (e.g. facultative growth habit and barley contributions to beer flavor); the integration of research and learning (e.g. graduate and undergraduate student research; the Oregon Wolfe Barleys); and an extensive international network based on unrestricted collaboration and exchange. Considerations for future public sector barley breeding include: shrinking public investment in plant breeding research with applied outcomes; expansions (e.g. the craft sector) and consolidations (e.g. the multinational sector) in the malting and brewing industries; an increased emphasis on innovative undergraduate and graduate teaching and experiential learning (with concomitant increases in time commitments); and the mantra that public sector variety development can generate sufficient revenue to be self-sustaining.

MATERIALS AND METHODS:

Students were recruited, funded, supported, and engaged in all aspects of the OSU Barley Project. They assumed primary responsibility for the preparation of their theses and the resulting manuscripts.

RESULTS AND DISCUSSION

Since 1986, 27 students have completed graduate degrees (21 MS, 6 PhD) at OSU with barley breeding and genetics as the focus. There are currently two graduate students in the program, both pursuing PhD's. Cumulatively, these students have contributed - directly and indirectly - to the release of 10 varieties, 3 registered germplasms, and 10 mapping populations; 144 journal articles, 13 germplasm registrations, and 13 book chapters. Student research projects focused on traits and breeding methods integrated with overall breeding objectives. Traits include malting quality, food quality, agronomic performance, low temperature tolerance, growth habit, durable disease resistance, and input use efficiency. Breeding methods included phenotypic, marker-assisted, and genomic selection. Breeding objectives include facultative/winter 6-row and 2-row malting, food, forage, and feed varieties. Engaging students in plant breeding is, on paper, more expensive than accomplishing the same tasks with full-time staff. However, the integration of student research and breeding objectives has led to serendipitous and synergistic outcomes that would not have been predicted and that would not have been possible to justify *a priori* on a cost-recovery basis. Student contributions, ideas and outcomes are deeply appreciated. Many thanks to Nusret Zencirci M.S. 1989 ; Somvong Tragoonrung M.S. 1989; Chris Schön M.S. 1990 ; Sue Kolar M.S. 1990 ; Chen Fuqiang Ph.D. 1991; Judy Holmer M.S. 1992; Adeline Oziel M.S. 1993; Odianosen Iyamabo Ph.D. 1994; Aihong Pan M.S. 1994; Abdoulaye Traore M.S. 1994; Doris Prehn M.S. 1994; Ergun Ozdemir M.S.1994; Theerayut Toojinda Ph.D. 1998; Luis Marques-Cedillo Ph.D. 2000; Ariel Castro Ph.D.2002; Ivan Matus Ph.D. 2002; Jarislav vonZitzewitz M.S., Ph.D. 2004, 2010; Carlos Rossi M.S. 2005; Kelley Richardson Ph.D. 2006; Juan Rey ; M.S. 2008; Kale Haggard M.S. 2008; Yada Chutimanitsakun Ph.D. 2012; Scott Fisk M.S. 2011; Natalie Graham M.S. 2011; Brigid Meints M.S. 2014; Ryan Graebner M.S. 2014 ; Araby Belcher Ph.D. 2015; Dustin Herb Ph.D. continuing; Javier Hernandez Ph.D. continuing.

Improving the Drought Tolerance of Some Barley Cultivars through Marker Assisted Selection

N. Kandemir*, I. Saygili and B. Aslan
Gaziosmanpasa University, Faculty of Agriculture, Department of Crop Science, Tokat, Turkey.
* Corresponding Author: PH: (533) 369 8277, Email: nkandemir53@gmail.com

OBJECTIVES

To transfer three drought tolerance QTL from cv. Tadmor into four barley cultivars via marker assisted backcross breeding.

INTRODUCTION

Drought is a major yield limiting factor for crops worldwide. Drought tolerance is associated with many traits such as chlorophyll content, carbon isotope discrimination, osmotic potential, osmotic adjustment, relative water content, flowering time, root structure (Mir et al., 2012) and stoma conductivity (Cattivelli et al., 2007). Therefore, it involves many genes with relatively minor effects compounded by differences in plant phenology. Transfer of multiple loci responsible for drought resistance through classical plant breeding strategies is difficult, and a marker assisted approach can facilitate easier monitoring of genes during breeding generations.

Certain genomic regions in barley have been reported to carry drought tolerance loci in Tadmor x Er/Apm cross. A QTL was found on chromosome 6 around WG286 and Dhn4 markers (Teulat et al., 1998). This QTL was also associated with relative water content, osmotic potential, osmotic adjustment and 1000-seed weight. Another QTL detected on barley chromosome 2 around EBmac684 marker was associated with carbon isotope discrimination, a trait related to water use efficiency and yield stability of crops (Teulat et al., 2002). A third QTL for drought tolerance was mapped on barley chromosome 1(7H) around Acl3 marker. This QTL was reported to explain 5.8 and 12.3% of the variation for relative water content and osmotic potential, respectively (Teulat et al., 1998). This genomic region was also associated with plant height, harvest index, carbon isotope discrimination (Teulat et al., 2002). Favorable alleles for all three QTL regions were conferred by cv. Tadmor. Because of their associations with yield related traits, these three QTL regions are good candidates for marker assisted breeding of drought tolerance.

Barley has good genetic maps that consist of many RFLP, SSR and InDel markers (Ramsay et al., 2000; Wenzl et al., 2006; Varshney et al., 2007; Zhou et al., 2015). These are codominant markers that can be transferred between different maps. SSR markers have the advantage of carrying higher number of alleles and, hence, polymorphism rate in addition to ease of use as PCR marker. However, close size of different alleles and stutter bands make their analysis complicated (Arif et al., 2010). InDels are relatively newer type of markers that also have ease of use as PCR markers. Though their low number of alleles may seem to be a disadvantage, clear differences of allele sizes eliminate problems related to their genotyping.

Despite many QTL mapping studies about drought tolerance in barley (Baum et al., 2003; Teulat et al., 1998; Teulat et al., 2003; Mir et al., 2012; Guo et al., 2008; Chloupek et al., 2006), breeding efforts that employ the knowledge gained through these studies are rare. The

gap between the number of QTL studies and breeding programs that use those QTL (Xu and Crouch, 2008) are also evident in drought tolerance of barley. Determined by many sub-traits, drought tolerance is especially suitable for marker assisted breeding.

MATERIALS AND METHODS

Three QTL regions for drought tolerance are being transferred from cv. Tadmor into two malt (cvs. Aydanhanım and Sladoran) and two feed (cvs. Bolayır and Baronesse) barley cultivars through marker assisted backcross breeding. Tadmor is a cultivar developed from a Syrian landrace and has been extensively used in QTL studies for drought tolerance.

Crosses and backrosses are being made in greenhouses under controlled conditions. Embryo culture has been employed to hasten the time to complete a generation. In addition to foreground selection using markers linked to QTL regions, we employ background selections with about 50 markers scattered throughout the genome. Our target is to complete the breeding in BC_4F_2 generation with a satisfactory level of recurrent parent background recovery.

SSR and InDel markers are being used for background and foreground selections. These are mostly single copy markers. Markers have been selected based on their locations in barley maps. First, polymorphism screenings have been made between donor parent Tadmor and four recurrent parents. Some of the markers have been mapped in an F_2 population of 90 plants from Tadmor x Baronesse F_2 population which has the highest number of polymorphic loci for all three QTL regions. Markers were mapped using MapDisto Software (Lorieux, 2012). Because of uncertainties about the exact location of QTL regions (Zeng, 1994), transfers are being made as about 30 cM sized genomic pieces. These regions will be fragmented later to allow higher resolution mapping of respective QTL regions.

PCR products were run on 3% MetaPhor™ agarose gels with 1% TBE buffer. DNA was visualized via ethidium bromide added to the gels, using a gel image system (Vilber Lourmat CN-08). Bands were analyzed using BioCapt v.11.02 software. Marker polymorphism rates were determined using PIC values, which were calculated according to the following formula: $PIC = 1-\sum P^2_i$, where P_i is the frequency of i^{th} allele (Anderson et al. 1993).

RESULTS AND DISCUSSION

Polymorphic markers between cv. Tadmor, donor parent, and cvs. Aydanhanım, Bolayır, Baronesse and Sladoran, recurrent parents, were shown in Figure 1. Chromosome 1 and 2 QTL regions are monitored by about ten markers and chromosome 6 QTL region is monitored by about five markers. In addition, about 50 markers are used to select against donor parent to allow fast recovery of recurrent parent genomic background.

Both SSR and InDel markers were used for polymorphism screening. We compared polymorphism results of 40 InDel and 66 SSR markers on a panel of nine barley cultivars including cvs. Durusu, Tokak 157/37, Arta and Yıldız in addition to donor parent (Tadmor) and four recurrent cultivars (Aydanhanım, Bolayır, Baronesse and Sladoran) in our backcross program. An average of 2.409 alleles were determined for each SSR marker. Average number of alleles for InDel markers were 1.875, which was clearly less than that of SSRs. However, average polymorphic information content of SSR and InDel markers were similar (0.354 and 0.313, respectively). This finding may imply that some of the alleles found in SSR markers

are rare alleles and they may not be very useful to distinguish most cultivars. Alleles of InDel markers seem to have a more equal distribution among barley cultivars. Considering clear differences in allele sizes, relatively new InDel markers offer a good potential for marker assisted selection as well as genetic diversity studies. Clear size differences could also facilitate use of more convenient and cheap gel systems rather than difficult and expensive ones like polyacrylamide and special agarose gels.

Markers had been selected based on their map positions in barley consensus 2007 SSR map (Varshney et al., 2007). Since some of the markers were not mapped on the same mapping population and distances were calculated only in consensus maps, we wanted to map them in the same mapping population. Ninety F_2 plants from Tadmor x Baronesse cross, which was the cross with the highest number of polymorphic markers, were used. Results were given in Figure 2. Map distances between markers are close to or slightly higher than what was reported by Consensus 2007 SSR map (Varshney et al., 2007).

BC_3F_1 lines have been obtained so far by transferring about 30 cM wide genomic pieces from Tadmor into recurrent parents using a marker assisted backcross program. A gel picture of backcross lines using an InDel marker (InDel6063) was given in Figure 3. Through use of markers for background selection against genomic background of donor parent, BC_4F_2 lines with a minimum amount of genomic regions from donor parent could be developed. BC_4F_3 seeds will be evaluated under field conditions for drought tolerance in two locations.

Changes in drought tolerance characteristics of recurrent parents will be determined based on seed yield and drought related traits such as carbon isotope discrimination, relative water content, amount of chlorophyll, chlorophyll fluorescent parameters, and leaf and canopy temperatures. Effects of transferred QTL regions on feed and malting quality features of recurrent barley cultivars will also be investigated. Developed lines could be used for fine mapping as well as for sequence based cloning of drought tolerance QTL.

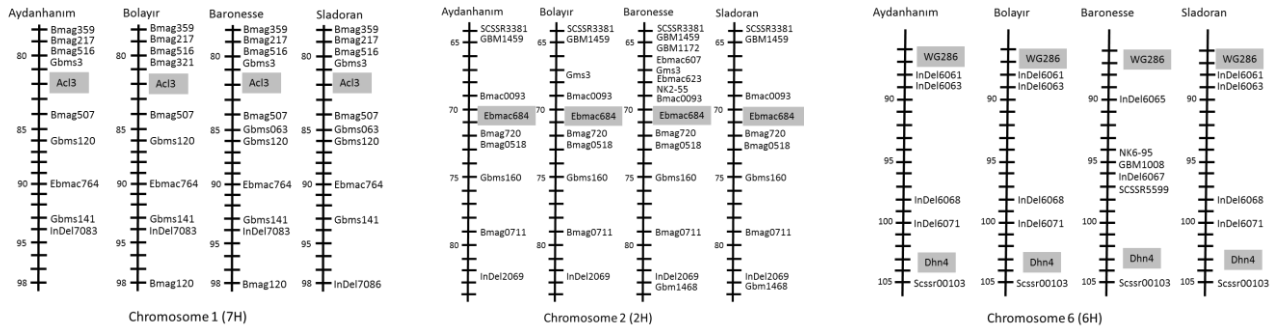


Figure 1. Polymorphic markers for three drought tolerance QTL regions in four backcrossing program between Tadmor and four recurrent parents. Shaded boxes show approximate location of linked markers in original QTL mapping. Distances between markers are based on barley Consensus 2007, SSR map (Varshney et al., 2007).

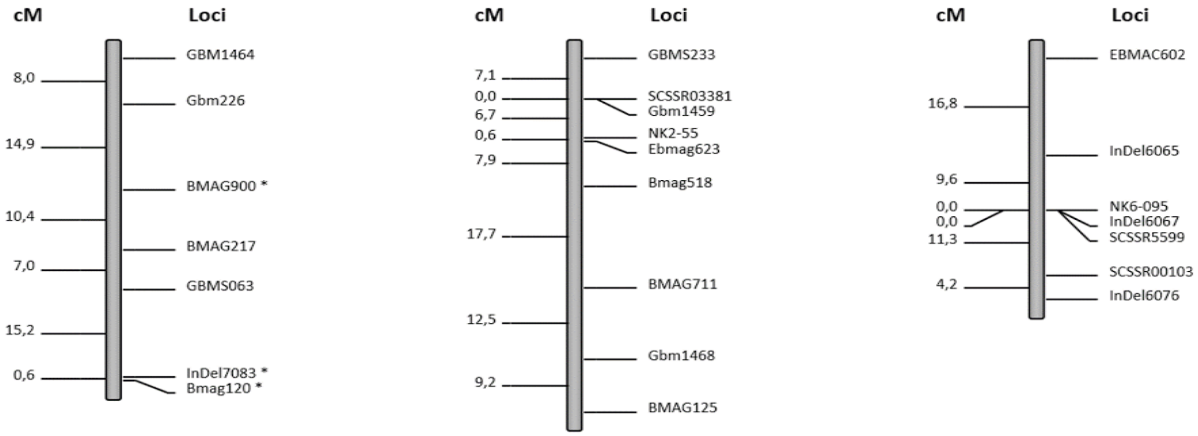


Figure 2. Mapping of polymorphic markers on QTL regions in an F_2 population of 90 plants from Tadmor x Baronesse cross. NK markers have been developed by us.

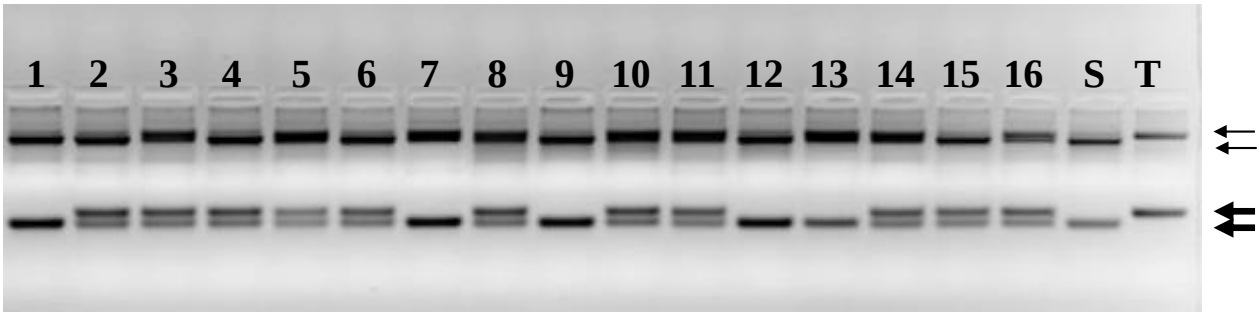


Figure 3. Genotyping of BC_1F_1 lines from Tadmor x Sladoran cross using InDel6063 markers in a 3% MetaPhor agarose gel. S, cv. Sladoran; T, cv. Tadmor. Large arrows show alleles of InDel6063. Bands marked by smaller arrows belong to a second loading on the same gel.

ACKNOWLEDGEMENT

This study has been supported by The Scientific and Technological Research Council of Turkey TUBITAK (Project No: TOVAG 113O934).

REFERENCES

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D. and Sorrells, M.E. 1993. Optimizing parental selection for genetic linkage maps. Genome 36:181-186.

Arif I.A., Khan H.A., Shobrak M., Al Homaidan A.A., Al Sadoon M., Al Farhan A.H., Bahkali A.H. 2010. Interpretation of electrophoretograms of seven microsatellite loci to determine the genetic diversity of the Arabian Oryx. Genetics and Molecular Research 9: 259–265.

Baum, M., Grando, S. and Backes, G. 2003. QTLS for agronomic traits in the mediterranean environment identified in recombinant inbred lines of the cross 'Arta' × *H. spontaneum* 41–1. Theoretical Applied Genetics 107:1215–1225.

Cattivelli, L., Rizza, F., Badeck, F.W., Mazzucotelli, E., Mastrangelo, A.M., Francia, E., Mare, C., Tondelli, A. and Stanca, A.M. 2008. Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crop Research 105:1-14.

Chloupek, O., Forster, B.P. and Thomas, W.T. 2006. The effect of semi-dwarf genes on root system size in field-grown barley. Theoretical Applied Genetics 112:779–786.

Guo, P., Baum, M., Varshney, R., Graner, A., Grando, S. and Ceccarelli, S. 2008. QTLs for chlorophyll and chlorophyll fluorescence parameters in barley under post-flowering drought, Euphytica 163:203–214.

Lorieux, M .2012. MapDisto: fast and efficient computation of genetic linkage maps. Molecular Breeding 30:1231–1235.

Mir, R.R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R. and Varshney, R.K. 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crop. Theoretical Applied Genetics 125:625-645.

Ramsay, L., Macaulay, M., Ivanissevich, S.D., Maclean, K., Cardle, L., Fuller J., Edwards, K.J., et al. 2000. A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005.

Teulat B., This D., Khairallah M., Borries C., Ragot C., Sourdille P. 1998. Several QTLs involved in osmotic-adjustment trait variation in barley (Hordeum vulgare L.) Theoretical Applied Genetics 96:688–98.

Teulat B., Merah O., Sirault X., Borries C., Waugh R., This D. 2002. QTLs for grain carbon-isotope discrimination in field-grown barley. Theoretical Applied Genetics 106:118–126.

Teulat, B., Zoumarou-Wallis, N., Rotter, B., Ben-Salem, M., Bahri, H. and This, D. 2003. QTL for relative water content in field-grown barley and their stability across Mediterranean environments. Theoretical Applied Genetics 108:181–188.

Varshney, R.K., Marcel, T.C., Ramsay, L., Russell, J., Roder, M.S., Stein, N., Waugh, R., Langridge, P., Niks, R.E. and Graner, A. 2007. A high density barley microsatellite consensus map with 775 SSR loci. Theoretical Applied Genetics 114:1091-1103.

Wenzl, P., Li, H., Carling, J., Zhou, M., Raman, H., Paul, E., Hearnden, P., Maier, C., Xia, L., Caig, V., Ovesna, J., Cakir, M., et al., 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206.

Xu, Y. and Crouch, JH. 2008. Marker-assisted selection in plant breeding: from publications to practice. Crop Science 48:391–407.

Zhou, G., Zhang, Q., Tan, C., Zhang, X.Q. and Li, C. 2015. Development of genome-wide InDel markers and their integration with SSR, DArT and SNP markers in single barley map. BMC Genomics 16:804.

From marker-assisted selection (MAS) to genomic selection (GS) - strong support for modern barley breeding

V. Korzun[1]*, E. Byrne[2], J. Großer[1], D. Harrap[2], J-F. Herbommez[3], S. Kollers[1], K. Oldach[1], M. Schmidt[1], B. Schinkel[1], A. Tomerius[1], N. Wendler[1], P. Werner[2], and H. Verstegen[1]

[1]KWS LOCHOW GMBH, Ferdinand-von-Lochow-Straße 5, 29303 Bergen, Germany
[2]KWS UK Ltd., 56 Church Street, Thriplow, Hertfordshire SG8 7RE, UK
[3]KWS MOMONT RECHERCHE SARL, 7 rue de Martinval, 59246 Mons en Pevele, France
*Corresponding author: VK: viktor.korzun@kws.com

Key words: barley, molecular breeding, marker-assisted selection (MAS), genome-wide association (GWAS), Genomic Selection (GS)

Conventional barley breeding is time-consuming and strongly depends on environmental conditions. Therefore, breeders are extremely interested in new technologies that optimize breeding processes. Molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improve selection strategies in barley breeding. There has been a large and rapid accumulation of genomic tools in barley during the last decade. These developments have been coupled with the emergence of high throughput technologies, which have allowed advances in molecular marker technology and implementation. The impact of new molecular tools and technologies available has proven to be essential to optimize and accelerate barley breeding programs.

Yield, disease resistance and malting quality are prime targets in practical barley breeding programs. In all cases the breeding process can be accelerated by applying marker-assisted selection (MAS), marker-assisted backcrossing (MABC) and/ or genomic selection (GS) for developing new material and for improvement of the selection intensity and accuracy.

Example 1: MAS for resistance genes barley BaYMV/BaMMV

The soilborne barley yellow mosaic virus (BaYMV)/barley mild mosaic virus (BaMMV) complex was first detected in Germany in 1978 and since then, the viruses have spread over large parts of Europe and became one of the most important diseases of winter barley (Ordon et al. 2009). Resistant sources are quite frequent in the primary gene pool of barley and several monogenic, predominantly recessive resistance genes (*rym 1-18*) have been detected and mapped. In Central Europe, *rym5* is most widespread and was introgressed in elite populations by molecular markers. MAS became the method of choice for introgression because (i) the resistance to BaYMV cannot be screened on a single plant level in the greenhouse, (ii) the resistance is recessive, (iii) pyramiding of several genes is necessary (Werner et al. 2007) due to newly arisen strains. The *rym4/rym5* locus has been cloned by map-based cloning and turned out to be the translation initiation factor 4E (*Hv-eIF4E* (Stein et al. 2005). By resequencing 1,000 entries of the Gatersleben Genebank, several new alleles of this resistance gene have been detected (Ordon et al. 2009), illustrating the power of allele mining once the gene sequence is available. Identification of the respective gene has opened a fully new prospective now through the recently discovered CRISPR/Cas9 technology.

Genome-wide association scans (GWAS) have become a common approach to dissect the factors controlling complex traits in barley (Pasam et al. 2012; Rode et al. 2012; Ziems et al. 2014). However, this method frequently leads to the detection of false-positive marker-trait associations (MTAs) and strongly depends on additional validation steps before large scale introgressions of detected marker-traits into elite germplasm are justified. One efficient method to validate marker-trait associations is to test for respective MTA in independent bi-parental populations segregating for the relevant alleles at the associated locus, as recently

well demonstrated in winter barley by Lüders et al. (2016). Marker-trait associations that have been verified can be directly implemented in barley breeding for the selection of parental lines and marker-assisted selection.

Malting quality traits in barley breeding traditionally have been targeted by using marker-assisted selection. Nevertheless, the recent advance in high density genotyping has opened new possibilities to apply genome-wide markers in Genomic Selection (GS) rather than individual markers as in classical MAS.

Example 2: Genomic Selection of malting quality traits

Genomic selection has been applied to various plant species, mostly for yield or yield related traits including grain yield, thousand kernel weight and resistances against diseases. Despite the breeding material being highly related and a long history of breeding for quality traits, there is still considerable variation in those traits. This opens the opportunity to further improve those traits through the application of GS.

In the frame of the InnoGRAIN-MALT project, the potential to apply GS to twelve malting quality traits in breeding programs of spring and winter barley (*Hordeum vulgare* L.) was assessed. Phenotypic means were calculated over three and four years with three to five locations per year. Heritabilities for malting traits ranged from 0.50 to 0.98. Predictive abilities (PA), as derived from cross-validation, ranged from 0.14 to 0.58 for spring barley and 0.40 to 0.80 for winter barley (Tab. 1). Furthermore, it could be shown that (i) due to the genetic structure spring- and winter-barley need to be analysed separately; (ii) a training set of around 100 individuals can give sufficient PA and correlations to unobserved data (e.g. when phenotypes of one year were removed before analysis) in a breeding program with highly related material and (iii) an increased training set size leads to higher PA with a reduced standard deviation, showing the potential to improve the accuracy by increasing the training set size (Schmidt at al. 2016).

The results from this study clearly indicate the power of GS to improve selection efficiency in malting barley breeding programs. Deployment of this approach in barley greatly helps to reduce cost intensive phenotyping for quality traits, increases selection intensity and can shorten breeding cycle length. A comparable result is expected for application of GS in barley for grain yield.

Fast development of genomics resources and tools in barley during the last decade has strongly sped up the process of gene identification and validation, enormously increased the use of molecular tools in practical breeding and thus supports a greater selection gain in barley.

Tab. 1: Predictive abilities (PA) derived from a 5 by 5 cross validation with their standard deviation (sd) and sizes of the training sets (#TS) for the twelve analysed malting quality traits in spring- and winter barley. The traits are alpha- and beta-amylase (AMA, AMB), extract (EXT), free amino nitrogen (FAN_L), final attenuation (Fin_At), friability (FRI), beta-glucan content (GLU), Kolbach index (KOL), malting loss (LOSS), soluble nitrogen (NIT), protein content (PRT) and viscosity (VIS).

	Spring barley			Winter barley		
Trait	PA (mean)	PA (sd)	#TS	PA (mean)	PA (sd)	#TS
AMA	0.444	0.151	99	0.564	0.300	102
AMB	0.142	0.218	118	0.606	0.150	102
EXT	0.558	0.072	424	0.625	0.175	102
FAN_L	0.497	0.218	65	0.572	0.165	102
Fin_At	0.495	0.120	424	0.732	0.113	102
FRI	0.552	0.077	424	0.788	0.113	102
GLU	0.479	0.096	423	0.798	0.173	102
KOL	0.487	0.106	424	0.556	0.178	102
LOSS	0.419	0.101	322	0.652	0.165	102
NIT	0.583	0.074	424	0.551	0.150	102
PRT	0.521	0.064	424	0.399	0.158	102
VIS	0.449	0.111	424	0.744	0.116	102
mean	0.469	0.117		0.632	0.163	

ACKNOWLEDGMENTS

InnoGRAIN-MALT project was supported by Grant (FKZ 0315960) from the Bundesministerium für Bildung und Forschung (BMBF) within the framework of the PLANT 2030 program.

REFERENCES

Lüders, T., Ahlemeyer J., Förster J., Weyen J., Roßa E., Korzun V., Lex J., Friedt W., Ordon F. 2016. Verification of marker–trait associations in biparental winter barley (*Hordeum vulgare* L.) DH populations. Mol Breeding 36:14 DOI 10.1007/s11032-016-0438-2.

Ordon, F., Habekuss, A., Kastirr, U., Rabenstein, F., and Kühne, T. 2009. Virus resistance in cereals: Sources of resistance, genetics and breeding. J. Phytopathol. 157:535-545.

Pasam RK., Sharma R., Malosetti M., van Eeuwijk FA., Haseneyer G., Kilian B., Graner A. 2012. Genome-wide association studies for agronomical traits in a worldwide spring barley collection. BMC Plant Biol 12:16.

Rode J., Ahlemeyer J., Friedt W., Ordon F. 2012. Identification of marker-trait associations in the German winter barley breeding gene pool (*Hordeum vulgare* L.). Mol Breed 30:831-843

Schmidt, M., Kollers S., Maasberg-Prelle A., Großer J., Schinkel B., Tomerius A., Graner A., Korzun V. 2016. Prediction of malting quality traits in barley based on genome-wide marker data to assess the potential of genomic selection, Theor Appl Genet 129, pp 203-213.

Stein, N., Perovic, D., Kumlehn, J., Pellio, B., Stracke, S., Streng, S., Ordon, F., and Graner, A. 2005. The eukaryotic translation initiation factor 4E confers multiallelic recessive Bymovirus resistance in *Hordeum vulgare* (L.). Plant J. 42:912-922.

Werner, K., Friedt, W., and Ordon, F. 2005. Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). Mol. Breed. 16:45-55.

Ziems LA., Hickey LT., Hunt CH., Mace ES., Platz GJ., Franckowiak JD., Jordan DR. 2014. Association mapping of resistance to *Puccinia hordei* in Australian barley breeding germplasm. Theor Appl Genet 127:1199-1212.

Analysis of miRNA-regulated transcription factors in barley

M. Lafos[1] and S. McKim[1]*
[1]Dep. of Plant Sciences, University of Dundee, Dundee, UK
*Corresponding author: SM: +44 1382 568916, Email: s.mckim@dundee.ac.uk

OBJECTIVES

To identify barley genes encoding SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE (SPL) transcription factors, and establish their phylogenetic relationship to *SPL* genes in other plant species. To investigate the developmental roles of HvSPLs by expression profiling, miRNA-targeting analyses and transgenic manipulation.

INTRODUCTION

SPL genes, first discovered in snapdragon (Klein et al., 1996) and then described in *Arabidopsis* (Cardon et al., 1999), maize (Hultquist and Dorweiler, 2008) and rice (Yang et al., 2008), encode plant-specific transcription factors (TFs) involved in essential developmental programs such as reproductive timing, patterning and growth (reviewed in Schmid and Huijser, 2011). In particular, multiple studies in crop plants found clear links between SPL activity and branching, both vegetative (tillering) and reproductive (inflorescence architecture), as well as flowering time, seed size and plant stature (Wang L, et al., 2015; Chuck GS, et al., 2014; Chuck G, et al., 2010; Silva et al., 2014; Liang WH, et al. 2014; Luo L, et al., 2012; Jiao Y, et al., 2010), suggesting that SPLs are major regulators of agronomic traits. However, little is known about SPL function in the temperate cereals such as barley and wheat.

SPLs are part of a complex and conserved miRNA-TF network

Analyses of *SPL* gene families also revealed that multiple members encode transcripts containing complementary binding sequences to *microRNA156* (*miR156*). Evidence in *Arabidopsis*, maize and rice suggests that *miR156*-binding leads to *SPL* transcript cleavage and/or translational inhibition, a layer of post-transcriptional control which plays a key role in modulating many SPL-specific functions, including SPL control of developmental timing (Wu and Poethig, 2006; Schwab et al., 2005).

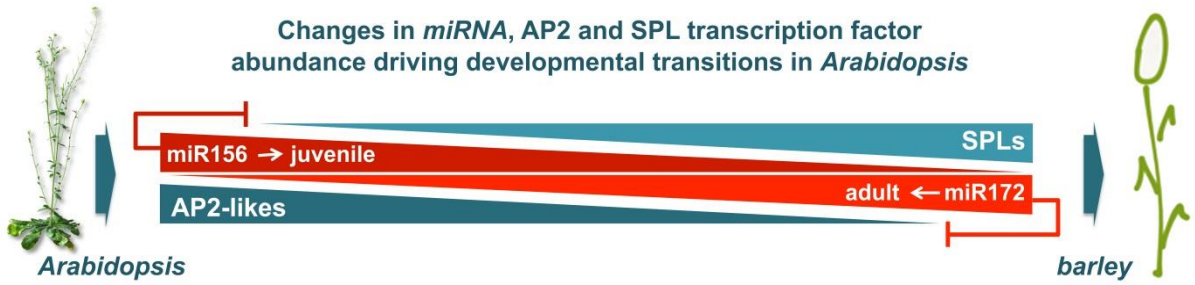


Figure 1: Antagonism between *AP2-SPL* network components.

In fact, we now know that *miR156* regulation of SPL activity works within a deeply conserved network defined by antagonistic interactions between *miR156* and another micro RNA, *miR172*, which targets APETALA2 (AP2)-like TFs. Abundant early in the lifecycle, *miR156* promotes juvenile characteristics such as leaf and tiller production by inhibiting SPL function. Over time, levels of *miR156* decline and the associated increase in SPL activity leads to promotion of adult features and flowering. In parallel, *miR172* abundance increases to advance differentiation of adult features and flowering through the suppression of AP2-like activity (**Fig.1**).

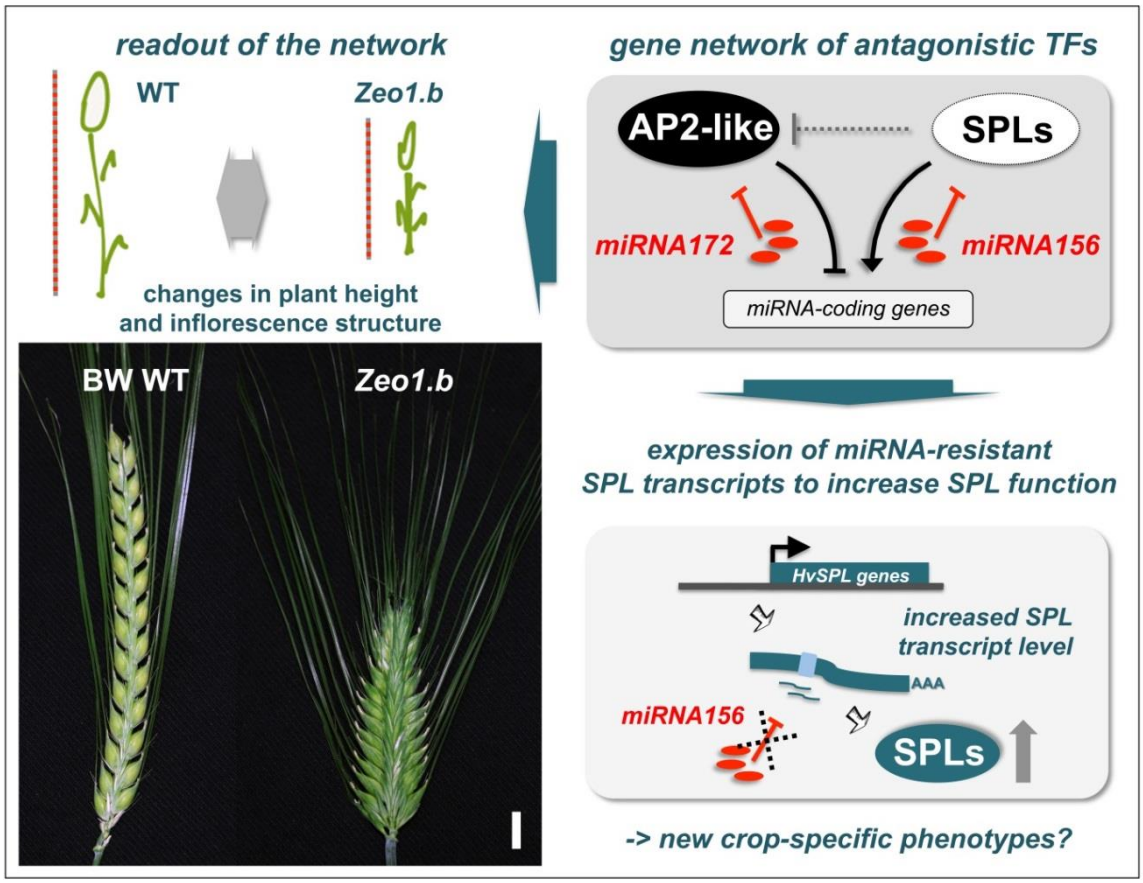


Figure 2: The phenotype of *Zeo1.b* represents an example of the readout of the *AP2-SPL* network in barley, unbalanced by increased *AP2* expression. Scale bar (bottom, left) indicates 1 cm.

The dense spike and late-heading phenotype of the barley *Zeo1.b* mutant, which expresses an *AP2* transcript resistant to *miR172*-mediated cleavage, represents one example of phenotypic change caused by unbalancing the *AP2-SPL* network (Houston K, McKim SM, et al., 2013). Inspired by this mutant, we decided to further unbalance the network by increasing SPL activity (**Fig.2**). Here, we analyse barley *SPL* genes to explore their function during barley development and their potential influence on important agronomic traits limiting yield, especially inflorescence architecture, plant height, grain number and quality. Therefore we are generating transgenic barley lines

expressing miRNA-resistant *SPL* transcripts. To further resolve this network, downstream targets of the involved TFs will be identified by TF-ChIP-seq analysis.

MATERIALS AND METHODS

Identification and sequence analysis of barley *SPL* genes

Arabidopsis and rice *SPL* gene family sequences are well described and easily accessible at the corresponding web resources, 'Rice Genome Annotation Project' (http://rice.plantbiology.msu.edu/annotation_community_families.shtml) and 'TAIR' (http://www.arabidopsis.org/browse/genefamily/sbp_box_genefamily.jsp); (Cardon G, et al., 1999; Yang Z, et al., 2008). By searching the publicly available barley genome resources, 'EnsemblPlants' (http://plants.ensembl.org/index.html) and 'IPK barley BLAST server' (http://webblast.ipk-gatersleben.de/barley) by BLAST, we identified several barley genes encoding proteins with the conserved SBP box signature matching the consensus sequence: 'CQVEGCKADLASAKEYHRRHKVCEAHSKAPRVVVAGQEQRFCQQCSRFHA LSEFDQAKRSCRRRLAGHNRRRRKPQP'.

Sequence and phylogenetic analyses

Sequence analyses were done with the NCBI 'standalone BAST_V2.2.31' tool and custom-made Python-scripts using Python_V3.1.1. The genomic map positions of *OsSPL*genes were received from the genome browser of the 'Rice Genome Annotation Project'. Map positions were determined through the given map positions of the corresponding morex_contig-fragments derived from the 'IPK barley BLAST server' (http://webblast.ipk-gatersleben.de/barley). The relative genomic position of the *OsSPL* and *HvSPL*-genes were plotted on the genomic rice and barley maps by using the R-package 'ggbio_V1.18.5' (Yin T, et al., 2012), R-Studio_V0.99.893 and R_V3.2.4. Phylogenetic analyses were performed with the software program MEGA6.06. Phylogenetic trees were generated by using the 'Maximum Likelihood Phylogeny Test' with '2000 Bootstrap Replications'.

HvSPL gene expression analyses

In-house RNA-seq resources developed by R. Waugh, P. Hedley and colleagues from the James Hutton Institute, were mined for information about *HvSPL* gene expression. These resources contained gene expression information from eight different barley tissues collected from cv. Morex. (Consortium, The International Barley Genome Sequencing, 2012).

RESULTS AND DISCUSSION

Identification of barley SPLs

SPLs are defined by the SBP box, a unique DNA-binding domain containing two zinc-binding pockets, where the second zinc finger overlaps with a nuclear localisation signal (Yamasaki et al., 2004; Birkenbihl R. et al., 2005). *Arabidopsis* and rice (*Oryza sativa*) are known to have 17 and 19 *SPL* genes, respectively. We identified 18 genes

in the barley genome encoding proteins harbouring a typical SBP box with two zinc fingers of the type 3xCys1xHis and 2xCys1xHis1xCys. These genes, hereafter referred to as *HvSPL*s, were named based on their homology to rice *OsSPL* genes, determined by the best BLAST hit. Six of the 18 *HvSPL* genes encode transcripts containing a *miR156*-binding site, 'gtgctctctctcttctgtca'.

Genomic location and phylogeny

Since SPL functions are best understood in model species such as rice and *Arabidopsis*, we focused on these species to identify orthologous *SPL* genes in barley. To examine homology amongst *HvSPL* and *OsSPL* genes, we compared chromosomal location, genomic synteny as well as determined their phylogenetic relationship (**Fig.3**). Comparison of the chromosomal position of rice and barley *SPLs* reveals conserved clusters within chromosomes as well as genomic rearrangements; however, these rearrangements are in agreement with the overall syntenic relationship among rice and barley chromosomes (Mayer et al., 2011), as depicted in **Fig.3** for HvChr2, OsChr4 and OsChr7. Further detailed analyses of the genomic context as well as functional analyses to support orthologous relationships are ongoing.

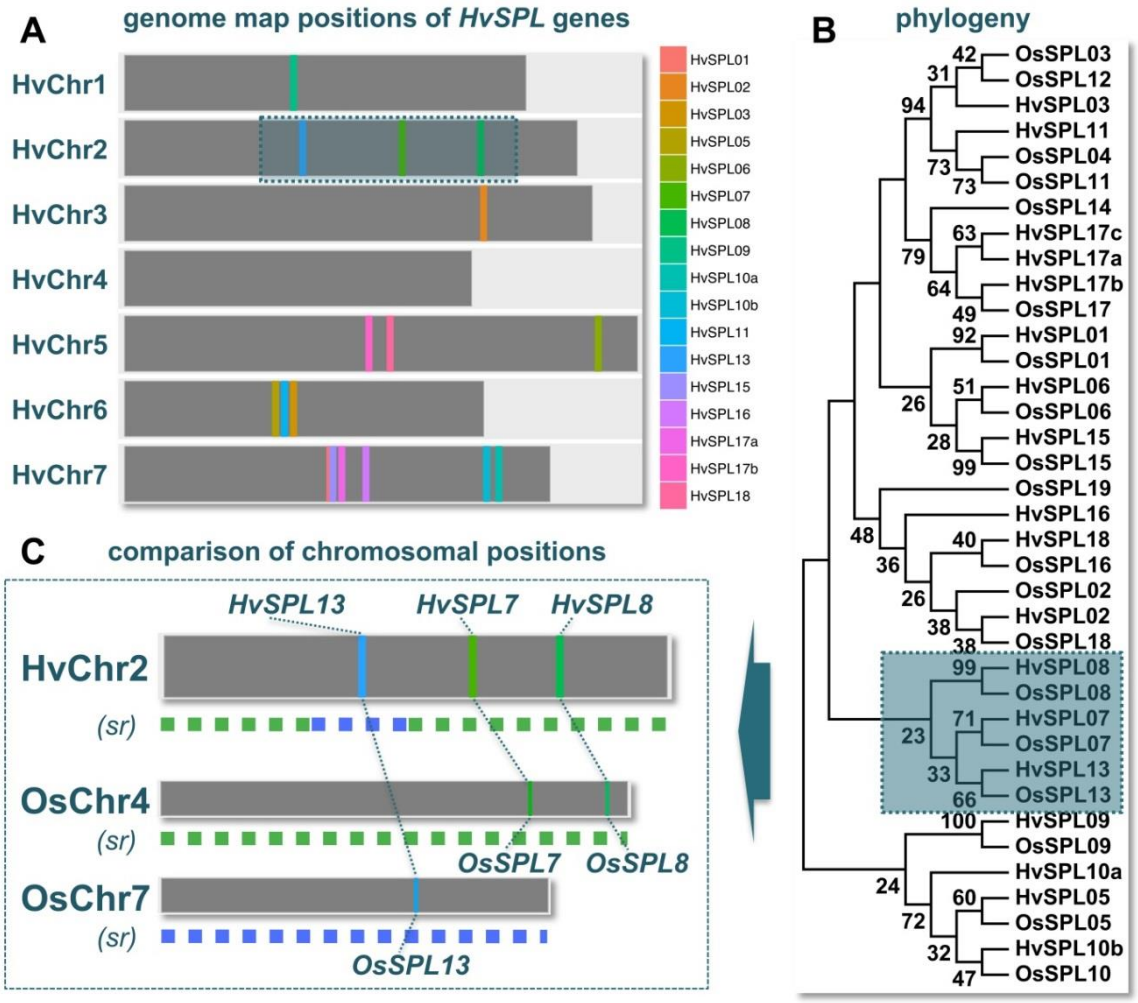


Figure 3: Map position and phylogenetic analysis of *HvSPL* genes. The genomic map positions in cM of *OsSPL* and *HvSPL* genes were plotted to the chromosomes with the R-package 'ggbio'. Maximum likelihood phylogeny trees were generated by MEGA6 with 2000 Bootstraps. The high bootstrap values (bottom, right) indicate a high homology of the putative orthologous gene pairs *SPL08*; *SPL07* and *SPL13* among rice and barley. The comparison of the chromosomal positions (bottom, left) shows that *SPL07* and *SPL08* cluster together in both species, *SPL13* is located in a syntenic region (sr; Mayer et al., 2011).

Functional analysis in barley

Detailed comparison of developmental expression patterns will also help to identify potentially shared function across species. To characterise individual *HvSPL* genes, we are determining their developmental expression pattern and their regulation by *miR156*. As a first step, we accessed expression data resources from the James Hutton Institute (courtesy of R. Waugh; **Fig.4**). This data set reveals expression data for all 18 *HvSPL* genes. Most *HvSPLs* are mainly lowly expressed and rather inflorescence than leaf-specific expressed. We also detected expression in other tissues but rarely as highly expressed as in the inflorescence (data not shown). This pattern of *HvSPL* gene expression later in development and specifically in the developing inflorescence is similar to the previously described developmental expression patterns of *OsSPL* genes (Xie K. et al., 2006). We anticipate that HvSPLs will have developmental roles within the inflorescence based on phenotypes of *OsSPL* mutants which show major differences in inflorescence architecture. Since the available expression data is limited, we decided to analyse the expression of *HvSPL* genes in more detail. Hence, we collected selected tissues at specific developmental stages to determine the spatio-temporal expression of *HvSPLs* during barley development.

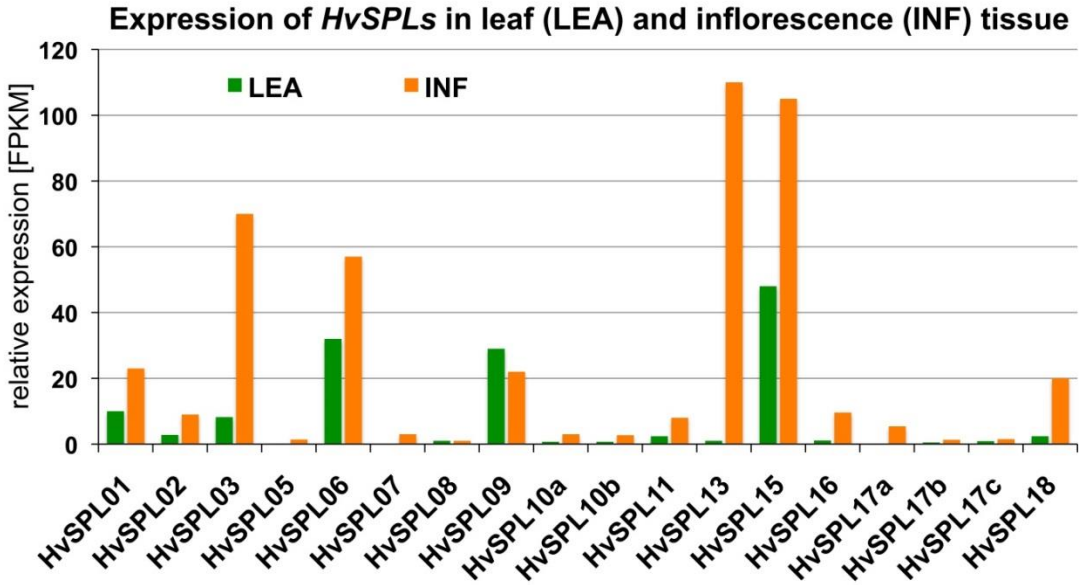


Figure 4: *HvSPL* gene expression analyses. The analysis is based on a barley RNA-seq data set provided by the James Hutton Institute, described by The International Barley Genome Sequencing Consortium (2012)**.**

Conclusions

Since our barley sequence analyses confirmed that certain barley *SPL* transcripts harbour *miR156* target sites, we are investigating whether these binding sites are associated with transcript cleavage and defining where and when this takes place during barley development. Long-term approaches based on transgenic barley lines expressing *miR156*-resistant *HvSPL* transcripts will enable detailed functional analyses of the *HvSPL* genes and may reveal new barley-specific SPL functions. Identification of direct TF-target genes by TF-ChIP-seq will add a complete new layer of complexity to the *AP2-SPL* gene network by revealing through which pathways AP2 and SPL function is realized. Meanwhile, the combination of detailed gene expression studies and analyses of the syntenic relationship of putative orthologous gene pairs among species like rice, maize and barley will contribute to our understanding of candidate genes influencing agronomic traits in barley.

REFERENCES

Birkenbihl, Rainer P, Guido Jach, Heinz Saedler, and Peter Huijser. 2005. "Functional Dissection of the Plant-Specific SBP-Domain: Overlap of the DNA-Binding and Nuclear Localization Domains." *Journal of Molecular Biology* 352 (3): 585–96.

Cardon, G, S Höhmann, J Klein, K Nettesheim, H Saedler, and P Huijser. 1999. "Molecular Characterisation of the Arabidopsis SBP-Box Genes.." *Gene* 237 (1): 91–104.

Chuck, G, C Whipple, D Jackson, and S Hake. 2010. "The Maize SBP-Box Transcription Factor Encoded by Tasselsheath4 Regulates Bract Development and the Establishment of Meristem Boundaries." *Development (Cambridge, England)* 137 (9): 1585–85.

Chuck, George S, Patrick J Brown, Robert Meeley, and Sarah Hake. 2014. "Maize SBP-Boxtranscription Factors Unbranched2and Unbranched3affect Yield Traits by Regulating the Rate of Lateral Primordia Initiation." *Proceedings of the National Academy of Sciences* 111 (52): 18775–80.

Consortium, The International Barley Genome Sequencing. 2012. "Nature11543." *Nature* 491 (7426). Nature Publishing Group: 711–16.

Houston, Kelly, Sarah M McKim, Jordi Comadran, Nicola Bonar, Ilze Druka, Nicola Uzrek, Elisa Cirillo, et al. 2013. "Variation in the Interaction Between Alleles of HvAPETALA2 and microRNA172 Determines the Density of Grains on the Barley Inflorescence.." *Proceedings of the National Academy of Sciences* 110 (41): 16675–80.

Huijser, P, and M Schmid. 2011. "The Control of Developmental Phase Transitions in Plants." *Development (Cambridge, England)* 138 (19): 4117–29.

Hultquist, Judd F, and Jane E Dorweiler. 2008. "Feminized Tassels of Maize Mop1 and Ts1 Mutants Exhibit Altered Levels of miR156 and Specific SBP-Box Genes." *Planta* 229 (1): 99–113.

Jiao, Yongqing, Yonghong Wang, Dawei Xue, Jing Wang, Meixian Yan, Guifu Liu, Guojun Dong, et al. 2010. "Regulation of OsSPL14 by OsmiR156 Defines Ideal Plant Architecture in Rice." *Nature Publishing Group* 42 (6): 541–44.

Klein, J, H Saedler, and P Huijser. 1996. "A New Family of DNA Binding Proteins Includes Putative Transcriptional Regulators of the Antirrhinum Majus Floral Meristem Identity Gene SQUAMOSA.." *Molecular & General Genetics : MGG* 250 (1): 7–16.

Liang, Wei-hong, Fei Shang, Qun-ting Lin, Chen Lou, and Jing Zhang. 2014. "Tillering and Panicle Branching Genes in Rice." *Gene* 537 (1). Elsevier B.V.: 1–5..

Luo, L, W Li, K Miura, M Ashikari, and J Kyozuka. 2012. "Control of Tiller Growth of Rice by OsSPL14 and Strigolactones, Which Work in Two Independent Pathways." *Plant and Cell Physiology* 53 (10): 1793–1801.

Mayer, Klaus F X, Mihaela Martis, Pete E Hedley, Hana Šimková, Hui Liu, Jenny A Morris, Burkhard Steuernagel, et al. 2012. "Unlocking the Barley Genome by Chromosomal and Comparative Genomics." *The Plant Cell Online* 23 (4): 1249–63.

Schwab, Rebecca, Javier F Palatnik, Markus Riester, Carla Schommer, Markus Schmid, and Detlef Weigel. 2005. "Specific Effects of MicroRNAs on the Plant Transcriptome." *Developmental Cell* 8 (4): 517–27.

Silva, Geraldo Felipe Ferreira e, Eder Marques Silva, Mariana da Silva Azevedo, Mike Anderson Corazon Guivin, Daniel Alves Ramiro, Cassia Regina Figueiredo, Helaine Carrer, Lázaro Eustáquio Pereira Peres, and Fabio Tebaldi Silveira Nogueira. 2014. "microRNA156-Targeted SPL/SBP Box Transcription Factors Regulate Tomato Ovary and Fruit Development." *The Plant Journal : for Cell and Molecular Biology* 78 (4): 604–18.

Wang, Lei, Shengyuan Sun, Jiye Jin, Debao Fu, Xuefei Yang, Xiaoyu Weng, Caiguo Xu, Xianghua Li, Jinghua Xiao, and Qifa Zhang. 2015. "Coordinated Regulation of Vegetative and Reproductive Branching in Rice.." *Proceedings of the National Academy of Sciences* 112 (50): 15504–9.

Wu, Gang, and R Scott Poethig. 2006. "Temporal Regulation of Shoot Development in Arabidopsis Thaliana by miR156 and Its Target SPL3.." *Development (Cambridge, England)* 133 (18): 3539–47.

Xie, Kabin, Congqing Wu, and Lizhong Xiong. 2006. "Genomic Organization, Differential Expression, and Interaction of SQUAMOSA Promoter-Binding-Like Transcription Factors and microRNA156 in Rice.." *Plant Physiology* 142 (1): 280–93.

Yamasaki, Kazuhiko, Takanori Kigawa, Makoto Inoue, Masaru Tateno, Tomoko Yamasaki, Takashi Yabuki, Masaaki Aoki, et al. 2004. "A Novel Zinc-Binding Motif Revealed by Solution Structures of DNA-Binding Domains of Arabidopsis SBP-Family Transcription Factors." *Journal of Molecular Biology* 337 (1): 49–63.

Yang, Zefeng, Xuefeng Wang, Shiliang Gu, Zhiqiu Hu, Hua Xu, and Chenwu Xu. 2008. "Comparative Study of SBP-Box Gene Family in Arabidopsis and Rice." *Gene* 407 (1-2): 1–11. doi:10.1016/j.gene.2007.02.034.

Yin, Tengfei, Dianne Cook, and Michael Lawrence. 2012. "ggbio: an R package for extending the grammar of graphics for genomic dataGb-2012-13-8-R77." *Genome Biology* 13 (8). BioMed Central Ltd: R77.

Barley Research and Development in Ethiopia

Berhane Lakew[1]*, Wondimu Fikadu[1], Bettina Haussmann [2], Reinhard von Broock[3]
[1]Ethiopian Institute of Agricultural Research (EIAR), Holetta Agricultural Research Center
[2] KWS, Grimsehistr.31 P.O.Box 1463, 3755 Einbeck, Germany
[3] KWS, Ferdinand-von-Lochow-Strabe5, 29303 Bergen, Germany
* Corresponding Author: berhanekaz@yahoo.com

INTRODUCTION

Barley (***Hordeum vulgare*** L.) is one of the major cereal crops grown in Ethiopia and its production is an old heritage with a large number of landraces and traditional practices. The landraces have an international recognition for their useful traits to modern agriculture through provision of genes for resistance to diseases, high nutritional quality and abiotic stresses. Barley is the fifth important cereal crop in Ethiopia after *teff*, maize, sorghum and wheat. (CSA, 2014). It is produced on about 1 million hectares of land from which 1.90 million tons of grain is produced annually (Table 1). The average national yield of barley is about 1.96 tons per hectare ha, which is low compared to the world average of 3.1 t/ha . Both food and malt barley are grown side by side sharing similar agro-ecologies. The share of malting barley in the national production is low (about 10- 15 %) as compared to food barley. The estimated annual production of malt barley ranges from 225 thousand tons to 267 thousand tons.

Table 2 National Estimates of Area, Production and Yield of Major Cereal Crops in 2014 crop season

Crops	No. of small holder farmers	Area ('000ha)	Production ('000t)	Yield (t/ha)
Cereals	13,419,762	9,848.75	21,583.52	
Teff	6,613,090	3,016.52	4.418.64	1.46
Maize	8,809,221	1,994.81	6,491.54	3.25
Sorghum	4,788,499	1,677.49	3,828.87	2.28
Wheat	4,746,231	1,605.65	3,925.1	2.44
Barley	4,461,616	1,019.48	1,908.26	1.96

Source: CSA, 2014

In the barley-based farming systems of the highlands of Ethiopia, smallholder farmers have very few alternative crops. One source of income could be growing malting barley, which has dependable local buyers in the country (Bayeh and Berhane, 2011) and possibly export market.

RESEARCH ACHIEVEMENTS

The Ethiopian Institute of Agricultural Research (EIAR) with the support and collaboration of international organizations carry out barley research and development activities for the last fifty years. The research objective was to ensure food and nutritional security through availability of sustainable improved food barley technologies and contribute to import substitution of barley malt and grain through continuous generation and supply of improved technologies. Over the years, more than 50 food and malt barley varieties have been released with appropriate packages by the national and regional research programs through local germplasm selections, hybridization and selections and introductions.

Food barley

A considerable genetic progress was attained for grain yield and important agronomic traits on food barley varieties generated from 1975 to 2006 (Wondimu et al., 2014). The yield gain has risen at an average rate of 42.96 kg ha^{-1} (1.19%) $year^{-1}$of release (Fig. 1). Similarly, harvest index was also improved with 0.004 $yr^{-1}$and the progress occurred at annual rate of 1.58% increase for the last three decades. However, plant height was reduced with an average annual genetic gain of - 0.39 cm (-0.33%) $year^{-1}$.

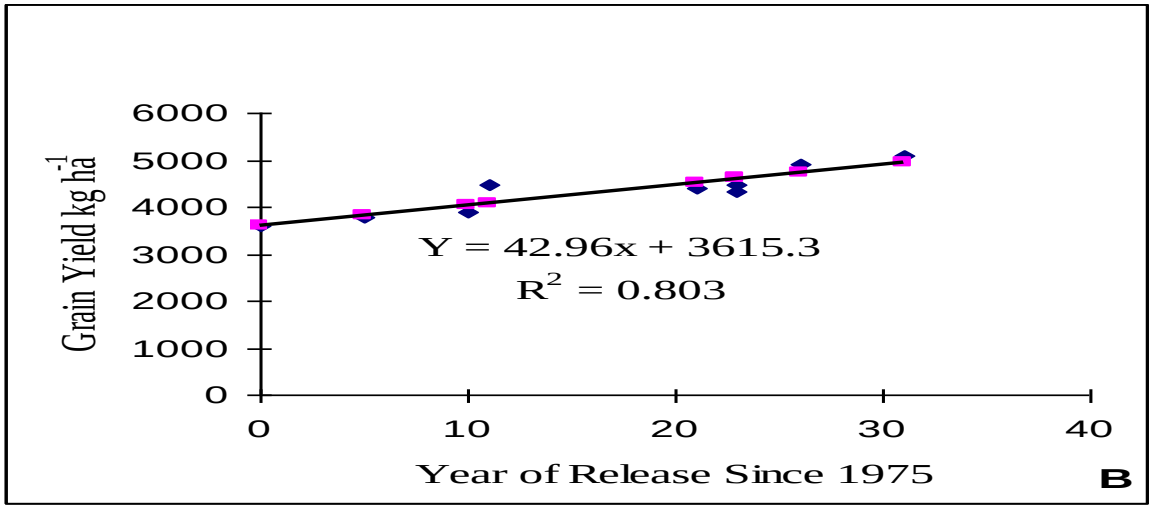


Figure 1. Plot of grain yield gain of food barley varieties against years of release of varieties

Malt barley

The malt barley research program have so far released and registered 16 malt barley varieties. Of which, 11 varieties are under production at different scales in the potential malt barley growing environments. The varieties and associated production packages are contributing to increased income source of small holder farmers in the highlands and enhance the local malt production supply to the malt factory. Grain yield potential of malting barley has risen at an average annual rate of 28.95 kg ha^{-1} (0.88%) $year^{-1}$ since 1979 (Fig. 2). Moreover, kernel plumpness was significantly improved as kernel size ≥ 2.5 mm showed significant improvement (0.27%) $year^{-1}$ and non standard seed size, i.e. ≤ 2.2 mm was substantially to -0.21% $year^{-1.}$ (Wondimu et al., 2013).

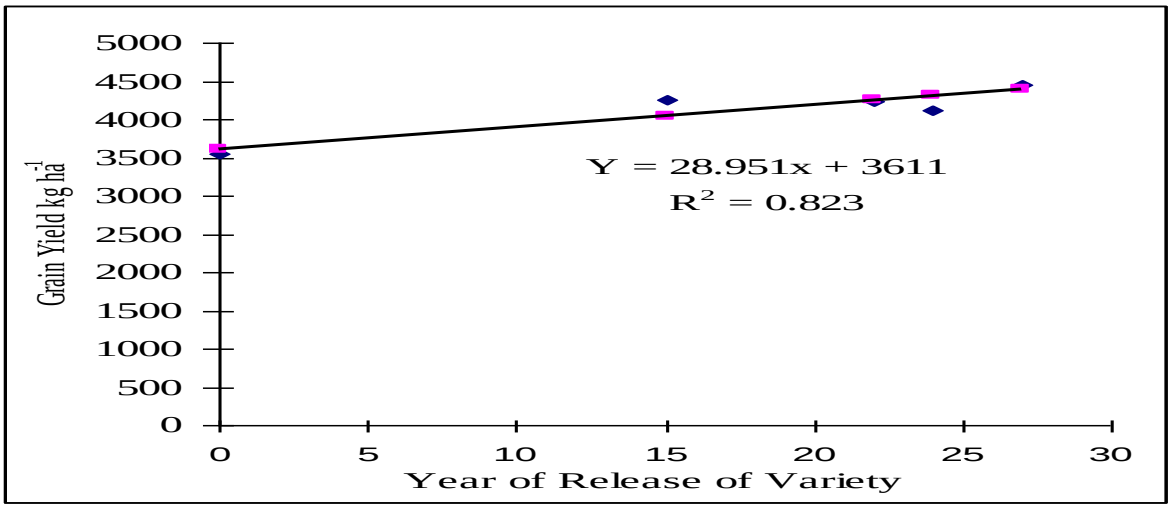


Figure 2. Plot of grain yield gain of malt barley varieties against years of release of varieties

Partnership to Strengthen Research and Development in Barley and Wheat

Recently, a research collaboration between Ethiopia, and Germany through GIZ-Capacity Development (CD) seed project, KWS and the German Breeders Association has been started. The collaborative project focuses to enhance the efficiency of barley and wheat breeding through a combination of conventional and modern tools such as double haploids and marker assisted and genomic selection tools; increase quality production of early generation seeds of released varieties and capacity development. In line with this, community based seed multiplication (CBSM) and participatory variety selection trials are being conducted with full participation of farmers since 2013.

Community based seed multiplication(CBSM)

Improved seed is the basic component to improve crop productivity and increase production. However, the availability of this component is not sufficient to meet local farmers' requirement. This is mainly due to the limited capacity of the existing national and regional seed enterprises of the country and absence of private seed producers mainly for self pollinated crops. To this effect, a multi-disciplinary integrated community based seed production was established by the Holetta agricultural research center at Robe gebeya and Telech kebele in wolmera woreda to address the need of the framers within their farming system on major crops including barley, wheat, highland maize, highland pulses, potato and linseed. The aim is to strengthen the informal seed system and enhance seed production, seed quality and marketing at local community level.

At welmera wereda, Rob-gebeya kebele, 253 volunteer farmers (246 female and 6 male) selected and trained on seed production and management aspects. At planting, 2.32 t of pre basic food barley variety (HB 1307) provided. Based on the training given, almost all farmers used row planting. The CBSM activity enhanced quality seed production and more than 15.0 tons seed produced and utilized to improve

productivity in the community. Production and productivity is not possible without a sufficient seed in the system. Therefore, promoting community based seed multiplication program can be one of the best options to alleviate the existing seed shortage in the country. The activity continued with the engagement of more farmers and support from the CD-seed project.

Participatory variety evaluation of elite food and malt barley varieties

A participatory variety evaluation on 10 elite food and malt barley varieties including a local variety was conducted at wolmera woreda - Robe Gebeya and Telecho kebele on three farmers' fields to obtain farmers view and preferences on the varieties for further promotion and adoption. The varieties were evaluated by farmers at flowering and maturity stages. Some agronomic data and farmers overall assessment score is shown in Table 2.

Table 2. Summary of participatory variety selection at Telecho kebele, 2013 cropping season

SN	Genotype	Type	DMA	TKW	HLW	GY (kg/ha)	FSS (1-3) Scale *
1	Cross 41/98	Food	138.33	48.67	68.3	5453.2	2
2	EH 1493	Food	138.33	54.93	68.97	4491.0	2
3	HB 1307	Food	141	49.73	65.33	4942.0	3
4	Baleme (farmers variety)	Food	139.67	56.27	65.57	4054.2	3
5	IBON 174/03	Malt	128.33	52.13	64.57	4092.8	3
6	Bekoji-1	Malt	141	49.2	71	3818.7	3
7	EH 1847	Malt	141	47.47	68.6	4263.2	2
8	Shege	Food	141	49.33	63.67	4893.7	3
9	BLLUP/Petunia	Food	128.33	43.6	57.77	2599.7	1
10	Kedo Guraghe (farmers variety)	Food	138.67	55.07	63.93	4701.8	2
Mean			137.57	50.64	65.77	4331.0	2
CV (%)			0.04	6.75	3.05	17.7	11.99
LSD(5%)			2.46	5.87	3.44	1315.1	0.5

* Farmers Selection Score (FSS) depending on the traits of interest 1= poor and 3= very good

Famer's selection criteria for genotypes of interest

- Spike size/length (farmers associate the size of spike and row orientation with yield potential)
- High Tillering capacity
- Early vigor
- Lodging resistance (stiff straw)
- Quality for local food *(injera*)and beverage *(tela*) preparation
- High biomass- Straw yield for animal feed

- Market value (white color and grain size)

Based on the farmers preferences, two malt barley varieties (IBON174/03 and Bekoji-1) and one food barley (HB 1307) variety are being promoted and popularized in the project area through CBSM and scaling up activities. As a result high demand for improved seeds is created and market linkage established.

Double Haploid Production (DH)

Application of double haploid techniques on barley was initiated in 2014 through the CD-seed project with technical back stopping from KWS- Einbech, Germany. The aim is to enhance the efficiency of the barley breeding program and accelerate the variety development process. The activity is progressing on protocol optimization and green DH plant production at the Holetta national biotechnology laboratory. The focus is on malt barley to develop disease resistant and high yielding malt barley varieties with acceptable malt quality standards.

Future plan

The Ethiopian Institute of Agricultural Research will continue and strengthen its collaboration with the GIZ-CD Seed project and KWS in the following areas:

- Enhance exchange of trait-specific germplasms of barley and wheat to widen the genetic base of Ethiopian breeding materials
- Accelerate barley and wheat breeding via application of doubled haploid (DH) technique
- Strengthen integration of biotechnological tools (e.g. marker-assisted selection (MAS) , Genomic selections (GS) for diagnostic disease resistance and quality traits) in wheat and barley breeding programs
- Implement screening of barley landraces for acid soil tolerance through molecular markers approach and farmer-participatory barley breeding program
- Strengthen community based seed multiplication for quality seed production and enhance access of improved seeds of barley and wheat to farmers
- Capacity development

References

Bayeh Mulatu and Berhane Lakew. 2011. Barley Research and Development in Ethiopia:- an overview. In. Mulatu B. Grando, S. (eds.). 2011 *Barley research and development in Ethiopia*. Proceedings of the 2nd National Barley Research and Development Review Workshop, 28-30 November 2006, Holetta, Ethiopia PO.Box 5466, Aleppo , Syria. Pp xiv+391.

Central Statistical Agency (CSA). 2015. Report on Area and Production of Major Crops. Agricultural sample Survey 2014 / 2105. Vol. I.

Wondimu Fekadu, Amsalu Ayana and Habtamu Zelekle. 2013. Improvement in Grain Yield and

Malting Quality of Barley (*Hordeum vulgare* L.) in Ethiopia. *Ethiop. J. Appl. Sci. Technol.* 4(2):
37-62 (*2013*).

Wondimu Fekadu, Berhane Lakew and Zewdie Wondatir. 2014. Advance in improving morpho-agronomic and grain quality traits of barley (Hordeum vulgare L.) in Central Highland of Ethiopia. Advanced Science Journals of Agricultural Science**.** Vol 1, No 1.

Genetic diversity in the genome era: a fine-scale map of sequence variation in the barley genome

Martin Mascher

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Corrensstraße 3, 06466 Seeland, Germany
PH: +49 (0) 39482 5243, Email: mascher@ipk-gatersleben.de

Abstract

Dense genome-wide marker datasets constitute the basis for research into demographic processes and patterns of natural selection among barley cultivars, landraces and wild relatives and make it possible to correlate genotypes with phenotypes in genome-wide association studies. In barley, sequence-based methods such as whole-genome resequencing, exome capture and genotyping-by-sequencing have been implemented to detect and genotype single-nucleotide polymorphisms across the genome. Integrated analyses of these variation datasets will greatly benefit from the positional information provided by the linearly ordered, highly contiguous genome sequence assembly of cv. 'Morex' generated by the International Barley Genome Sequencing Consortium. In this talk, I will give an overview about the genotypic datasets collected in recent years using different resequencing strategies and discuss the genomic distribution of genetic diversity in context of the map-based reference sequence of barley.

Genome sequencing for assessing intra-specific diversity

The construction of high-quality reference genome sequence is often followed by the construction of haplotype maps (HapMaps) for assessing genome-scale intra-specific diversity. Prominent examples in crop plants include the HapMaps of maize (Gore et al., 2009), rice (Huang et al., 2010), sorghum (Morris et al., 2013), and wheat (Jordan et al., 2015) are underway and have already demonstrated the impact on population genomics and attempts of resolving the genetic basis of key quantitative agronomic traits. Whereas HapMap projects for small genome species can be performed by whole genome sequencing (WGS), genotyping density and population size need to be well balanced in large genome species, where complexity reduction is necessary prior to sequencing to obtain sufficient sequencing depth for reliable variant calling. Once the data have been collected, HapMaps can provide insights into nucleotide diversity, recombination rate, decay of linkage disequilibrium and repeat content, underpin genome-wide association studies for important traits (Huang and Han, 2013) and identify targets of domestication and crop improvement (Hufford et al., 2012)

Exome sequencing

Exome sequencing uses oligonucleotide probes to selectively enrich sequencing libraries for fragments from specific regions of the genome. An exome capture liquid array targeting ~ 60 Mb of mRNA-coding exons is available for high-coverage

resequencing of the barley genespace (Mascher et al., 2013). A consortium led by researchers of IPK Gatersleben, the James Hutton Institute and the University of Minnesota has recently sequenced the exomes of a representative collection of geo-references of landraces and wild accessions of barley and explored the patterns of diversity in this panel (Figure 1). Their data also indicate that geo-climatic variables associated with temperature and dryness were at least partly responsible for driving the adaptive response as the barley crop dispersed across temperate regions of the world (Russell et al., 2016).

Genotyping-by-sequencing for characterizing germplasm collections

Genotyping-by-sequencing (GBS) employs digestion with restriction enzyme for complexity reduction (Elshire et al., 2011). Its highly streamlined wet-lab protocol make GBS the method of choice for the cost-efficient sequenced-based characterization of very large germplasm collections encompassing thousands of individuals (Romay et al., 2013). Researchers at IPK Gatersleben are currently using GBS to collect at ~ 50,000 SNPs variants for all ~22,000 accessions in the barley collection of the German Federal *ex situ* genebank. First results on ~7,000 winter barley accessions indicate that this data can provide a deeper understanding of barley's breeding history and inform genebank management decisions.

The map-based reference sequence of barley

The limited resolution has long hampered the assessment of sequence diversity in the non-recombining peri-centromeric regions of the barley genome (Kunzel et al., 2000). The International Barley Genome Sequencing Consortium has recently constructed a high-quality reference assembly of the barley genome by sequencing a minimum tiling path of overlapping bacterial artificial chromosomes (BACs). Chromatin interaction frequencies obtained by chromosome conformation capture sequencing (Lieberman-Aiden et al., 2009) were used to derive a linear order of nearly all BAC-based sequence scaffolds. For the first time, an accurate and comprehensive sequence map of large peri-centromeric regions is now available. Re-analyzing the available resequencing datasets in the context of this sequence resource indicate that the high-quality barley genome will empower map-based cloning of genes residing in these regions and population genetic analyses to understand that the origin of large stretches of reduced sequence diversity in domesticated barley in particular on chromosomes 1H and 4H.

Conclusions and outlook

Genome-wide re-sequencing datasets of diversity diversity are key to study the demographic history of barley, identify regions of the genome selected during domestication and subsequent dispersal and crop improvement, and to link genotype and phenotype by genome-wide association mapping. Powerful resequencing strategies together with a high-quality reference sequence will lead to many exciting results in barley genomics.

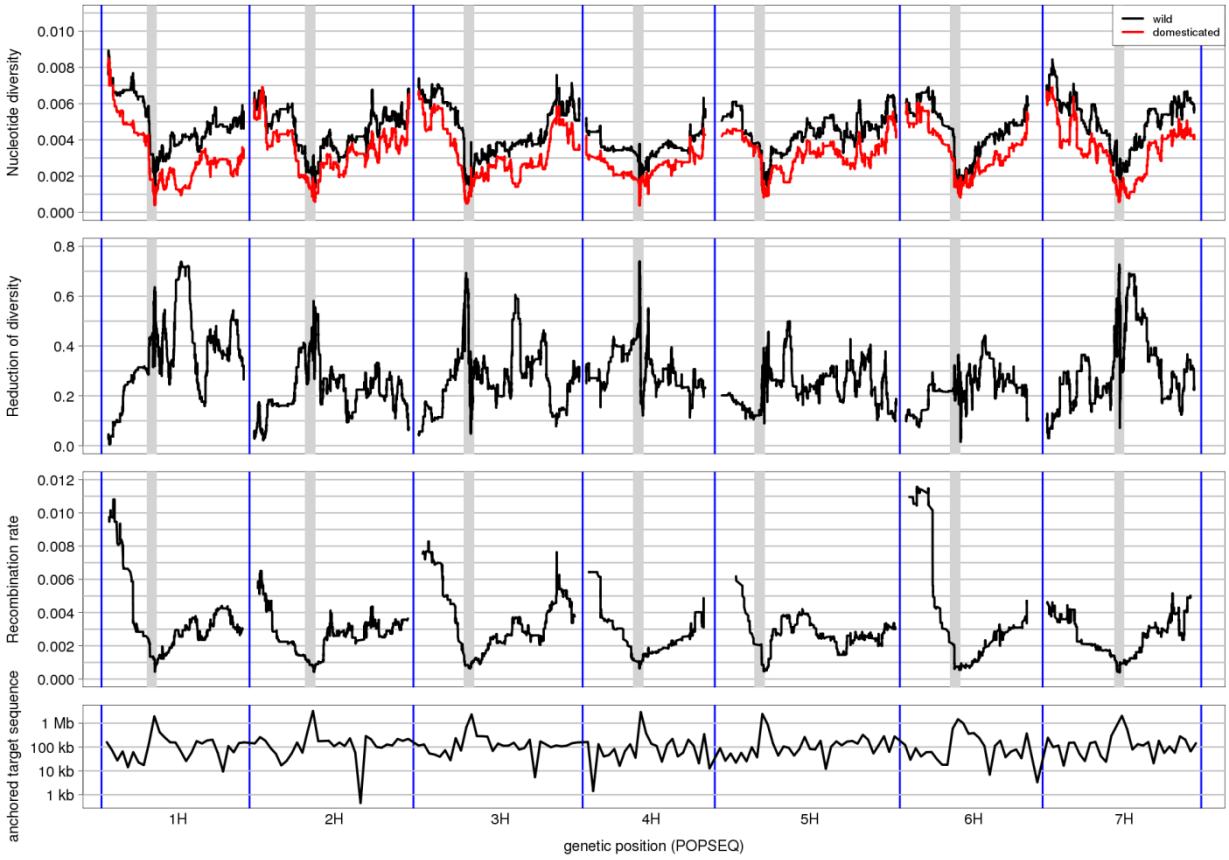


Figure 1: Exome diversity in genome. (A) Nucleotide diversity in 91 wild (black) and 137 domesticated barley accessions (red) as revealed by more than 1.6 million single nucleotide polymorphisms. Diversity is reduced towards the genetic centromeres (marked in gray). **(B)** Reduction in diversity in domesticated barley relative to the wild accessions. Several region of reduced diversity coincide with known domestication genes (e.g. *btr1/2* on chromosome 3H or *nud* on chromosome 7H. **(C)** Population-scaled recombination rate is correlated with nucleotide diversity. **(D)** Distribution of exome capture target regions along the genome.

References

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One **6:** e19379

Gore MA, Chia JM, Elshire RJ, Sun Q, Ersoz ES, Hurwitz BL, Peiffer JA, McMullen MD, Grills GS, Ross-Ibarra J, Ware DH, Buckler ES (2009) A first-generation haplotype map of maize. Science **326:** 1115-1117

Huang X, Han B (2013) Natural Variations and Genome-Wide Association Studies in Crop Plants. Annu Rev Plant Biol

Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y, Li C, Zhu C, Lu T, Zhang Z, Li M, Fan D, Guo Y, Wang A, Wang L, Deng L, Li W, Lu Y, Weng Q, Liu K, Huang T, Zhou T, Jing Y, Li W, Lin Z, Buckler ES, Qian Q, Zhang QF, Li J, Han B (2010)

Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet **42:** 961-967

Hufford MB, Xu X, van Heerwaarden J, Pyhajarvi T, Chia JM, Cartwright RA, Elshire RJ, Glaubitz JC, Guill KE, Kaeppler SM, Lai J, Morrell PL, Shannon LM, Song C, Springer NM, Swanson-Wagner RA, Tiffin P, Wang J, Zhang G, Doebley J, McMullen MD, Ware D, Buckler ES, Yang S, Ross-Ibarra J (2012) Comparative population genomics of maize domestication and improvement. Nat Genet **44:** 808-811

Jordan KW, Wang S, Lun Y, Gardiner LJ, MacLachlan R, Hucl P, Wiebe K, Wong D, Forrest KL, Consortium I, Sharpe AG, Sidebottom CH, Hall N, Toomajian C, Close T, Dubcovsky J, Akhunova A, Talbert L, Bansal UK, Bariana HS, Hayden MJ, Pozniak C, Jeddeloh JA, Hall A, Akhunov E (2015) A haplotype map of allohexaploid wheat reveals distinct patterns of selection on homoeologous genomes. Genome Biol **16:** 48

Kunzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics **154:** 397-412

Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sandstrom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science **326:** 289-293

Mascher M, Richmond TA, Gerhardt DJ, Himmelbach A, Clissold L, Sampath D, Ayling S, Steuernagel B, Pfeifer M, D'Ascenzo M, Akhunov ED, Hedley PE, Gonzales AM, Morrell PL, Kilian B, Blattner FR, Scholz U, Mayer KF, Flavell AJ, Muehlbauer GJ, Waugh R, Jeddeloh JA, Stein N (2013) Barley whole exome capture: a tool for genomic research in the genus Hordeum and beyond. Plant J **76:** 494-505

Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, Riera-Lizarazu O, Brown PJ, Acharya CB, Mitchell SE, Harriman J, Glaubitz JC, Buckler ES, Kresovich S (2013) Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proc Natl Acad Sci U S A **110:** 453-458

Romay MC, Millard MJ, Glaubitz JC, Peiffer JA, Swarts KL, Casstevens TM, Elshire RJ, Acharya CB, Mitchell SE, Flint-Garcia SA, McMullen MD, Holland JB, Buckler ES, Gardner CA (2013) Comprehensive genotyping of the USA national maize inbred seed bank. Genome Biol **14:** R55

Russell J, Mascher M, Dawson IK, Kyriakidis S, Calixto C, Freund F, Bayer M, Milne I, Marshall-Griffiths T, Heinen S, Hofstad A, Sharma R, Himmelbach A, Knauft M, van Zonneveld M, Brown JWS, Schmid KJ, Kilian B, Muehlbauer G, Stein N, Waugh R (2016) Adaptation of barley to different environments revealed in the exomes of a range- wide collection of landraces and wild relatives. submitted

Proteomic Comparison of Flag Leaves from Barley Germplasm Varying at a Major Senescence QTL Identifies Numerous Proteins with Functions in Pathogen Defense

Katelyn E. Mason[1,2], Jonathan K. Hilmer[1,3], Walid S. Maaty[1,4], Benjamin D. Reeves[1], Paul A. Grieco[1], Brian Bothner[1,3], Andreas M. Fischer[5]*
Chemistry and Biochemistry Department[1], Proteomics, Metabolomics and Mass Spectrometry Facility[3], Department of Plant Sciences and Plant Pathology[5], Montana State University, Bozeman, MT 59717. Forensic Science Center, Lawrence Livermore National Laboratory, 7000 East Ave., Livermore, CA 94550 (current address)[2]; Department of Nucleic Acid and Protein Structure, Agricultural Genetic Engineering Research Institute, Agriculture Research Center, 9 Gamaa Street, Giza, Egypt (current address)[4]
*Corresponding author: PH: (406) 994-5908, E-mail: fischer@montana.edu

OBJECTIVES

To characterize the proteome of barley leaf senescence, especially as controlled by a chromosome six grain protein locus encompassing the *HvNAM-1* gene.

INTRODUCTION

We have previously characterized barley germplasm varying in senescence timing, namely the late-senescing variety 'Karl' and a near-isogenic early-senescing line ('10_11'), using biochemical, molecular and gene chip-based methods to compare flag leaf senescence and grain protein accumulation (Heidlebaugh et al., 2008; Jukanti and Fischer, 2008; Jukanti et al., 2008). This germplasm varies in the allelic state of a chromosome six locus encompassing the *HvNAM-1* transcription factor gene (Distelfeld et al., 2008; Distelfeld et al., 2014). The role of NAC transcription factors in senescence regulation is well-established (Uauy et al., 2006; Distelfeld et al., 2014), but *NAC* genes are also involved in plant adaptation to biotic and abiotic stresses (Al-Daoud and Cameron, 2011; Chen et al., 2014; McGrann et al., 2015), suggesting possible roles in crosstalk between plant development and stress responses. *HvNAM-1* expression is strongly induced in flag leaves after anthesis (Distelfeld et al., 2014). Differences between 'Karl' and '10_11' flag leaves identified in previous as well as the present studies from our laboratory therefore depend on the accelerated-senescence phenotype, as controlled by *Hv*NAM-1. Our previous transcriptomic analysis (Jukanti et al., 2008) found that numerous '*Senescence-Associated Genes*' (*SAGs*), but also a series of genes coding for pathogenesis-related proteins are upregulated in line '10_11' as compared to 'Karl' flag leaves.

To establish how differences between 'Karl' and '10_11' found at the mRNA levels impact protein abundance, proteomic data are necessary. Plant productivity parameters for both model and crop species can be defined by integrating plant genome and transcriptome annotations with proteomic and metabolomic studies (Fukushima and Kusano, 2014). For the present study, separation and quantification of 'Karl' and '10_11' flag leaf proteins was achieved by two-dimensional difference in-gel electrophoresis (2D-DIGE) and label-free quantitative mass spectrometry based proteomics ('shotgun proteomics'), leading to an extensive protein-level comparison of early- *vs.* late-senescing barley germplasm.

MATERIALS AND METHODS

Plant material - Barley seeds were planted in a small randomized block design containing four blocks each of variety 'Karl' and line '10_11' in a glasshouse of the Montana State University Plant Growth Center. A 22 °C / 18 °C day : night temperature regime was maintained in the glasshouse, and days were extended to a 16 h photoperiod when required, using Son-Agro 430-W high-pressure sodium lamps (Philips, Somerset, NJ, USA). Plants in potting soil were fertilized with 250 mL Peter's Professional General Purpose fertilizer (4 g L^{-1}, Scotts-Sierra Horticultural Products Company, Marysville, OH) per 4-liter pot with three plants every alternate week until anthesis, starting at two weeks past germination. When plants reached anthesis, each shoot used for further experimentation was labeled with its exact anthesis date. Flag leaves (leaf blades only) harvested at 14 and 21 dpa were flash-frozen in liquid nitrogen and stored at -80 °C until protein extraction. For each harvest time point, four to six 'Karl' and four to six '10_11' samples were collected from the four blocks of the experiment, with each sample consisting of flag leaves from 8 to 10 plant shoots. Three samples of 'Karl' flag leaves (at 14 and 21 dpa) and three samples of '10_11' flag leaves (at 14 and 21 dpa) were randomly picked from the material stored at -80 °C for proteomic analyses.

Proteomic analysis - Frozen flag leaves were ground for 1 min in liquid nitrogen with a mortar and pestle. Samples were prepared for 2D-DIGE and for shotgun proteomic analysis, and were analyzed by 2D-DIGE and mass spectrometry essentially as previously described (Maaty et al., 2012). For shotgun analysis, LC-MS/MS was performed using a Bruker maXis Ultra High Resolution micrOTOF-Q (Bruker Corporation, Fremont, CA 94538, USA) mass spectrometer fitted to an UltiMate® 3000 Nano LC (Thermo Scientific Inc., Rockford, IL 61101). Samples were trapped and desalted on a ProntoSIL Eurobond capillary column (125 mm x 4 mm ID; packed with ProntoSIL 120-5-EuroBOND-C18; 5 μm particles, Bischoff). The peptides were then eluted and loaded onto the analytical Synergi™ 4 μm Fusion-RP 80 Å column (100 mm x 2 mm ID, packed with C18 particles, 4 μm particles, Phenomenex Inc.) in 5% acetonitrile / 0.1% formic acid delivered by an auxiliary pump at 4 μL min^{-1} in-line to the mass spectrometer with a flow of 600 nL min^{-1}. Peptides were eluted with a 5 to 90% acetonitrile gradient over 120 min. Data-dependent acquisition of collision induced dissociation tandem mass spectrometry was performed in Auto (MS/MS) mode over a mass range of 200 to 2,200 at 24,300 *m/z*-s. The acquired mass spectra were processed by Compass DataAnalysis 4.0 software (Bruker Daltonics) and exported as raw data files for analysis.

Data analysis and statistics - Not all details can be reported here for reasons of space. Briefly, for shotgun analysis, a theoretical peptide library was generated by performing an *in silico* tryptic digest on the barley protein sequence entries available in the UniProt database (http://www.uniprot.org; 52,289 sequences) and used for comparison with our MS data. A protein was 'identified' when two or more peptides were assigned. Members of this group possessing three or more peptides were designated 'quantifiable'. Protein abundances were determined for each sample type ('Karl' or '10_11') and time point (14 or 21 dpa) by averaging intact tryptic peptide ion intensities from three biological replicates of each sample. The resulting average intensity values were combined (sum of three 'Karl' and sum of three '10_11' precursor ion intensities) providing an abundance value for proteins in each sample. Fold-change ratios were determined by dividing the abundance values against one another ('10_11' *vs.* 'Karl'). A Student's *t* test was performed and *p* values are reported for each quantified ratio.

RESULTS AND DISCUSSION

Numbers of identified and differentially regulated proteins - Image analysis of 2D gels showed that 332 (14 dpa) and 323 (21 dpa) protein spots were present in both 'Karl' and '10_11'.
Among these, seven spots were significantly (≥1.5-fold, *p* value ≤0.05) more abundant in the early-senescing line '10_11' at 14 dpa, while ten were more abundant in line '10_11' than in variety 'Karl' at 21 dpa.

Considerably larger numbers of differentially regulated proteins were found using a shotgun proteomics approach (Table 1). In agreement with our previous transcriptomic analysis (Jukanti et al., 2008), more differences between 'Karl' and '10_11' were identified at 21 than at 14 dpa. Considering that protein degradation and remobilization are hallmarks of leaf senescence (Distelfeld et al., 2014), it is unsurprising that, among proteins with significant differences between 'Karl' and '10_11', a majority was higher-abundance in the late-senescing variety 'Karl' (Table 1). However, it may be argued that proteins bucking this trend, i.e. those which are more abundant in the earlier-senescing flag leaves of line '10_11', are enriched in functions necessary for senescence regulation and nutrient remobilization, and are therefore of particular interest.

Upregulation of defense functions in early-senescing line '10_11' as compared to late-senescing variety 'Karl' - The largest group of proteins upregulated in flag leaves of line '10_11' consisted of those with likely or putative functions in plant pathogen defense. These included membrane and intracellular receptors or co-receptors (LRR-RLKs, NBS-LRRs), enzymes involved in attacking pathogen cell walls (e.g. glucanases), enzymes with possible roles in cuticle modification, classical pathogenesis-related proteins, membrane transporters, and enzymes involved in DNA repair. Several or numerous transcripts belonging to all these functional groups, with the exception of DNA repair, were also identified as upregulated in our previous transcriptomic comparison of 'Karl' and '10_11' flag leaves at 14 and 21 dpa (Jukanti et al., 2008), indicating regulation of defense-related functions at both the transcript and protein levels. Induction of defense-related genes during developmental senescence has been observed in several studies (Guo and Gan, 2012; Quirino et al. 1999, Quirino et al., 2000). At the same time, *Senescence-Associated Genes* have been shown to be induced during plant defense against various pathogens (Lers, 2007), and a series of genes and proteins have been demonstrated to be involved in both senescence regulation and plant pathogen defense. The hypothesis has been forwarded that senescing plant organs are susceptible to opportunistic infections (e.g., Lers, 2007); upregulation of defense-related genes and proteins may therefore have evolved as part of the developmental senescence program to protect plant nutrients during their remobilization to surviving organs or seeds. An alternative explanation is based on the fact that both senescence and plant defense mechanisms (specifically the hypersensitive response) lead to cell death. However, the hypersensitive response occurs much faster than organ or whole-plant senescence and is limited to cells surrounding the infection site (Lers, 2007). Partial overlap between regulatory mechanisms and gene regulatory networks guiding both types of plant cell death is therefore conceivable.

Variety 'Karl' and line '10_11', which is near-isogenic to 'Karl', differ in the allelic state of a chromosome six locus containing the *Hv*NAM-1 gene. The role of several NAC transcription factors in senescence regulation is now well-established, but *NAC* genes are also important for plant adaptation to biotic stress (see introduction). Upregulation of the genes and proteins described in Jukanti et al. (2008) and in the present study therefore likely depend on allelic variation in the coding or control sequences of *HvNAM-1* (Distelfeld et al., 2008), but it cannot be excluded that linked genes also have some influence on the presented data.

Other functions that are upregulated in line '10_11' - Besides defense-related proteins, a second functional group identified as upregulated in line '10_11' consists of proteins involved in protein synthesis and protein degradation. This group contains a putative mitochondrial

import receptor, three ribosomal proteins, two chaperonins and a putative prolyl-tRNA synthetase. While overall/net proteolysis is predominant during senescence, it has long been acknowledged that senescence is associated with changes in gene expression, and with the synthesis of new proteins (Thompson et al., 2004). Enzymes and proteins involved in protein degradation include a family S8 serine protease (see Rawlings et al., 2016 for protease classification), a tripeptidyl peptidase, and several elements of the ubiquitin-proteasome system. The low number of significantly regulated proteases may be surprising compared to transcriptomic analyses of the senescence process including our own (e.g., Jukanti et al., 2008). Additional proteases were quantified and identified as upregulated in this study, but with *p* values >0.05. These included a calpain (family C2A cysteine peptidase), an additional (family S53) tripeptidyl peptidase, two family A1 aspartic peptidases or related proteins, and two metallopeptidases from families M41 and M48, indicating that functionally interesting information was eliminated by the statistical tests applied. Considering that peptidases, particularly cysteine and serine peptidases, are typically activated from precursor proteins through limited proteolysis, it will become important to apply additional proteomic methods such as activity-based protein profiling (Sanman and Bogyo, 2014) to fully characterize senescence-associated proteolysis and nitrogen remobilization.

Table 1. Shotgun proteomics data summary.

Protein category	Sampling time point (dpa)	
	14	21
	Number of protein groups	
Identified (≥2 peptides)	5055	5338
Quantifiable (≥3 peptides)	1176	1839
Significantly regulated total (*p* value ≤0.05; ≥1.5-fold)	65	347
Significantly *up*regulated in '10_11' vs. 'Karl' (*p* value ≤0.05; ≥1.5-fold)	9	49
Significantly *down*regulated in '10_11' vs. 'Karl' (*p* value ≤0.05; ≥1.5-fold)	56	298

dpa, days past anthesis

REFERENCES

Al-Daoud, F., and Cameron, R.K. 2011. ANAC055 and ANAC092 contribute non-redundantly in an EIN2-dependent manner to Age-Related Resistance in *Arabidopsis*. Physiol. Mol. Plant Pathol. 76:212-222.

Chen, X., Wang, Y., Lv, B., Li, J., Luo, L., Lu, S., Zhang, X., Ma, H., and Ming, F. 2014. The NAC family transcription factor OsNAP confers abiotic stress response through the ABA pathway. Plant Cell Physiol. 55:604-619.

Distelfeld, A., Avni, R., and Fischer, A.M. 2014. Senescence, nutrient remobilization, and yield in wheat and barley. J. Exp. Bot. 65:3783-3798.

Distelfeld, A., Korol, A., Dubcovsky, J., Uauy, C., Blake, T., and Fahima, T. 2008. Colinearity between the barley grain protein content (GPC) QTL on chromosome arm 6HS and the wheat *Gpc-B1* region. Mol. Breeding 22:25-38.

Fukushima, A., and Kusano, M. 2014. A network perspective on nitrogen metabolism from model to crop plants using integrated 'omics' approaches. J. Exp. Bot. 65:5619-5630.

Guo, Y., and Gan, S.S. 2012. Convergence and divergence in gene expression profiles induced by leaf senescence and 27 senescence-promoting hormonal, pathological and environmental stress treatments. Plant Cell Environ. 35:644-655.

Heidlebaugh, N.M., Trethewey, B.R., Jukanti, A.K., Parrott, D.L., Martin, J.M., and Fischer, A.M. 2008. Effects of a barley (*Hordeum vulgare*) chromosome 6 grain protein content locus on whole-plant nitrogen reallocation under two different fertilisation regimes. Funct. Plant Biol. 35:619–632.

Jukanti, A.K., and Fischer, A.M. 2008. A high-grain protein content locus on barley (*Hordeum vulgare*) chromosome 6 is associated with increased flag leaf proteolysis and nitrogen remobilization. Physiol. Plant. 132:426–439.

Jukanti, A.K., Heidlebaugh, N.M., Parrott, D.L., Fischer, I.A., McInnerney, K., and Fischer, A.M. 2008. Comparative transcriptome profiling of near-isogenic barley (*Hordeum vulgare*) lines differing in the allelic state of a major grain protein content locus identifies genes with possible roles in leaf senescence and nitrogen reallocation. New Phytol. 177:333-349.

Lers, A. 2007. Environmental regulation of leaf senescence. Annu. Plant Rev. 26:108-144.

Maaty, W.S., Selvig, K., Ryder, S., Tarlykov, P., Hilmer, J.K., Heinemann, J., Steffens, J., Snyder, J.C., Ortmann, A.C., Movahed, N., Spicka, K., Chetia, L., Grieco, P.A., Dratz, E.A., Douglas, T., Young, M.J., and Bothner, B. 2012. Proteomic analysis of *Sulfolobus solfataricus* during *Sulfolobus* Turreted Icosahedral Virus infection. J. Proteome Res. 11:1420-32.

McGrann, G.R.D., Steed, A., Burt, C., Goddard, R., Lachaux, C., Bansal, A., Corbitt, M., Gorniak, K., Nicholson, P., and Brown, J.K.M. 2015. Contribution of the drought tolerance-related stress-responsive NAC1 transcription factor to resistance of barley to *Ramularia* leaf spot. Mol. Plant Pathol. 16:201-209.

Quirino, B., Normanly, J., and Amasino, R.M. 1999. Diverse range of gene activity during *Arabidopsis thaliana* leaf senescence includes pathogen-independent induction of defense-related genes. Plant Mol. Biol. 40:267-278.

Quirino, B.F., Noh, Y.-S., Himelblau, E., and Amasino, R.M. 2000. Molecular aspects of leaf senescence. Trends Plant Sci. 5:278-282.

Rawlings, N.D., Barrett, A.J., and Finn, R. 2016. Twenty years of the MEROPS database of proteolytic enzymes, their substrates and inhibitors. Nucleic Acids Res. 44:D343-D350.

Sanman, L.E., and Bogyo, M. 2014. Activity-based profiling of proteases. Annu. Rev. Biochem. 83:249-273.

Thompson, J.E., Hopkins, M.T., Taylor, C., and Wang, T.-W. 2004. Regulation of senescence by eukaryotic translation initiation factor 5A: implications for plant growth and development. Trends Plant Sci. 9:174-179.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., and Dubcovsky, J. 2006. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298-1301.

The INUDFOOD Report

B. Meints[1]*, P. Batlle[2], L. Cistué[3], S. Fisk[4], L. Helgerson[4], K. Murphy[5], I. Romagosa[6], W. Thomas[7], and P. M. Hayes[4]
[1]Dept. of Crop and Soil Science, Washington State University, NWREC, Mount Vernon, 98273; [2]Semillas Batlle, Lleida, Spain; [3]Estación Experimental de Aula Dei, CSIC, Zaragoza, Spain; [4]Dept. of Crop and Soil Science, Oregon State University, Corvallis, OR 97331; [5] Dept. of Crop and Soil Science, Washington State University, Pullman, WA 99164; [6]Institut de Recerca i Tecnologia Agroalimentàries, Universitat de Lleida, 25196, Lleida, Spain; [7]The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland UK
*Corresponding author: PH: (541) 231-6747, Email: brigid.meints@wsu.edu

OBJECTIVES

To improve the agronomic performance of winter and facultative hull-less food barley and provide growers and consumers with a range of grain colors, flavors, textures, and processing attributes. The end-goal of this trial is to release varieties that are best adapted to the regions where the trial is being grown.

INTRODUCTION

In the last several millennia, barley consumption as a food product has fallen out of favor in much of the western world as white bread, oatmeal, and rice have replaced it in modern diets. In 2009, 147,412,740 metric tons of barley were produced worldwide (FAO-STAT, 2009). Of the total amount of barley produced, 4,911,061 metric tons were consumed as food in Africa and Asia (where barley remains a staple crop in many areas) and 1,326,479 metric tons were consumed as food in the Americas, Europe, and Oceania. Since health claims for barley have been approved in the US, Europe, and Canada (Ames and Rhymer, 2008; National Barley Foods Council, 2003; EFSA NDA Panel, 2011; http://www.hc-sc.gc.ca/fn-an/label-etiquet/claims-reclam/assess-evalu/barley-orge-eng.php) there has been a resurgence in food barley consumption and interest. However, most breeding programs have focused on breeding for low β-glucan, which is desirable for malt and feed barley. There is a need to breed high-yielding varieties with desirable food barley quality traits and to distinguish these lines from those developed for other end-uses.

The health claims are based on the capacity of barley foods to reduce coronary heart disease, due to the β-glucan, a soluble dietary fiber located in the subaleurone layers, endosperm adjacent to the subaleurone layers, and throughout the endosperm (Izydorczyk et al., 2008). There are qualitative and quantitative genetic components to grain β-glucan, with several known QTL contributing to high β-glucan (Islamovic et al., 2013). There is a pleiotropic effect of the waxy starch trait, which is controlled by a recessive mutation in the granule-bound starch synthase 1 (*GBSS1*) gene (*wx* allele) on β-glucan levels (Patron et al., 2002; Islamovic et al., 2013). Breeders can successfully select for higher grain β-glucan by targeting the recessive allele. Many breeding programs focus on breeding extremely high β-glucan (12-20%) for extraction purposes. Our breeding targets moderate β-glucan levels (4-8%) for whole-grain use.

The INUDFOOD (international naked food barley) trial represents the start of an international collaboration directed at the rapid development of diverse winter food barley germplasm resources. Working with a network of international collaborators, varieties with excellent

agronomic performance and high levels of winter hardiness were collected from Europe to broaden the germplasm base. In order to efficiently introgress this exotic germplasm into adapted backgrounds, we produced doubled haploids (using anther culture) from crosses of Oregon State University (OSU) food germplasm with selected German winter barleys. These doubled haploids were initially grown in Corvallis, Oregon and at multiple locations in Spain.

MATERIALS AND METHODS

After several years of phenotypic selection for agronomic and quality traits, selected lines were advanced to the INUDFOOD trial. There are 30 lines in this trial, 13 selected in Oregon, 14 selected in Spain, and three hulled check varieties selected for their adaptation to the Pacific Northwest of the USA, the UK, and Spain. The experimental germplasm is all doubled haploid and hull-less and includes waxy and non-waxy starch types with moderately high grain β-glucan content.

The INUDFOOD trial was planted in fall 2013 at four locations: Corvallis, OR, USA; Pullman, WA, USA; Lleida, Spain; and Dundee, Scotland, and in winter 2014 at Corvallis, OR, USA. The trial was planted again in fall 2014 at five locations: Corvallis, OR, USA; Pullman, WA, USA; Mount Vernon, WA, USA; Lleida, Spain; and Dundee, Scotland. Management of the trial was conducted based on local practice.

Agronomic traits and resistance to biotic and abiotic stresses were measured at each location based on regional relevance. β-glucan was measured at all locations. Malt quality traits were measured on the 2013-14 Corvallis, OR trial. Data will be statistically analyzed using ANOVA.

RESULTS AND DISCUSSION

We are currently in the process of analyzing the results of this trial across years and locations. Two years of data at multiple locations will allow us to begin selecting entries for variety release. A number of the hull-less entries compare with or out-yield the checks and have excellent agronomic and food quality ratings.

Additionally, this population will be used to look at genotype-by-environment interactions for β-glucan content across continents. Results to date have shown that across the population, the β-glucan levels vary by environment, with the highest levels being in Spain (hot and dry) and the lowest levels being in Scotland (cool and wet). This is not unexpected; Meints et al. (2015) reported differences in β-glucan levels across high-rainfall, irrigated, and dryland environments in the Pacific Northwest using a population of waxy and non-waxy food barley lines. Anker-Nilssen et al. (2008) reported that barley grown in hotter and drier climates tends to have higher β-glucan than barley grown in wetter climates. Chutimanitsakun et al. (2013) also found that β-glucan content was significantly higher with increased daytime temperatures, even under irrigated conditions.

Table 1. Winter-planted Corvallis, OR data (2014).

Entry	Name	Yield (kg/ha)	Test weight (kg/hL)	Heading (Days after Jan. 1)	Height (cm)	Stripe rust (%)	Leaf rust (%)	Lodging (%)	Brackling (%)	Protein (%)	β-glucan (% w/w)	Kernel hardness (SKCS units)
1	10.0656	3974	79	147.5	70.0	12.5	1.0	0.0	2.5	11.1	6.2	44.2
2	10.0691	4060	80	149.0	76.5	2.5	3.0	0.0	0.0	10.9	8.0	51.4
3	10.0883	3195	77	147.5	70.5	5.0	0.5	0.0	0.0	11.3	6.1	48.7
4	10.0969	4389	77	143.5	80.0	12.5	0.0	0.0	5.0	11.7	4.8	38.2
5	10.1151	5638	75	142.5	85.5	2.5	0.5	2.5	0.0	10.7	4.4	39.4
6	10.1154	4473	74	146.0	89.0	0.0	0.5	0.0	0.0	11.0	5.2	45.8
8	10.1332	5959	74	145.0	78.5	0.0	5.0	0.0	0.0	11.2	5.4	57.0
9	10.1661	3169	76	153.5	67.0	0.0	5.0	0.0	0.0	11.4	3.5	51.7
11	10.1985	3605	78	151.0	81.0	0.0	2.5	0.0	0.0	12.0	5.9	58.1
13	10.2393	4012	71	146.5	87.5	0.0	1.0	0.0	5.0	10.8	4.7	47.3
14	10.0655	4546	79	138.5	76.5	22.5	1.0	0.0	2.5	12.8	7.5	47.9
15	10.0662	4959	78	138.5	84.0	50.0	2.5	2.5	2.5	12.0	7.0	49.4
16	10.0686	2493	72	153.5	75.0	35.0	0.5	0.0	0.0	12.8	.	.
17	Food 12	5343	79	138.0	84.0	40.0	0.5	5.0	7.5	11.6	7.1	53.0
18	10.0964	5714	79	143.0	87.0	17.5	5.0	0.0	7.5	11.9	4.4	38.9
19	10.1119	6441	81	141.0	83.5	0.0	7.5	2.5	10.0	9.8	5.3	49.1
20	10.1135	6976	78	142.0	86.5	0.0	2.5	0.0	0.0	10.1	4.9	48.4
21	10.115	5576	74	147.0	81.0	2.5	3.0	0.0	0.0	10.3	4.2	55.0
22	Food 26	6521	79	144.0	84.0	35.0	15.0	7.5	12.5	9.0	5.7	41.5
23	10.1492	5130	75	149.0	77.0	0.0	15.0	0.0	0.0	10.4	5.0	53.5
24	10.1618	5044	76	149.0	85.5	35.0	3.0	0.0	2.5	9.8	5.1	42.6
25	10.2006	4665	74	151.5	87.0	40.0	0.0	0.0	0.0	9.5	4.7	47.6
26	10.2021	3208	74	155.0	65.0	0.0	1.0	0.0	0.0	12.0	4.8	56.3
27	10.2464	5477	70	149.5	85.0	0.0	12.5	0.0	2.5	10.6	4.3	47.8
28	Alba	6537	66	148.5	101.5	0.0	0.5	0.0	7.5	9.0	4.0	67.1
29	KWS Cassia	5108	66	152.0	76.0	7.5	0.0	0.0	0.0	10.4	4.5	68.0
30	Hispanic	7259	68	145.5	79.0	0.0	0.5	0.0	0.0	9.5	4.3	53.8
LSD (0.05)		1333	2	1.5	5.8	13.1	6.9	2.6	4.9	0.7	--	--
CV		13.1	1.3	0.5	3.5	53.6	101.3	172.8	96.4	3.1	21.4	15.0

Table 2. Dundee, Scotland data (2013-14).

Entry	Name	Yield (kg/ha)	Test weight (kg/hL)	Heading (Days after Jan. 1)	Height (cm)	Scald (1-9)	Frost damage (1-9)	Thousand kernel weight (g)	β-glucan (%w/w)
1	10.0656	4860	73	138.0	95.0	2.5	2.5	57.1	5.5
2	10.0691	5603	73	141.5	99.0	3.0	2.0	54.2	6.2
3	10.0883	5103	71	138.5	92.5	2.5	2.5	57.2	5.0
4	10.0969	5545	69	136.0	95.0	2.5	3.0	61.2	4.0
5	10.1151	5289	70	133.0	88.0	3.5	3.5	36.4	3.5
6	10.1154	5680	71	141.0	103.0	2.5	2.5	39.2	3.5
7	10.1328	6096	74	139.0	110.5	2.0	2.0	41.4	3.8
8	10.1332	5767	72	139.0	87.0	4.0	4.5	39.1	4.7
9	10.1661	6580	70	144.0	102.0	2.5	2.0	37.6	3.1
10	10.1934	6006	70	136.0	116.0	2.0	2.5	44.4	3.5
11	10.1985	5941	76	141.5	113.0	3.5	3.0	36.6	3.6
12	10.1986	6756	75	136.5	94.0	2.0	3.0	40.9	4.1
13	10.2393	6361	71	137.5	98.5	2.5	3.0	42.6	3.6
14	10.0655	5495	74	132.0	83.0	3.5	3.0	48.8	5.6
15	10.0662	5758	72	133.0	88.5	2.5	4.0	51.9	5.9
16	10.0686	5344	73	136.0	100.5	2.5	2.5	60.7	4.7
17	Food 12	4820	71	133.0	88.5	3.0	4.0	48.5	5.8
18	10.0964	6067	74	135.5	87.5	3.0	4.0	57.8	3.8
19	10.1119	5345	72	133.0	96.0	3.0	3.5	42.0	4.4
20	10.1135	5558	71	139.0	89.0	4.0	6.5	43.0	3.9
21	10.115	6035	69	138.0	103.0	2.0	2.5	43.3	3.6
22	Food 26	5622	68	142.5	96.5	3.5	5.0	42.0	4.5
23	10.1492	6231	70	138.5	95.0	2.0	2.0	41.5	3.9
24	10.1618	6099	70	137.5	102.5	3.0	3.0	36.6	4.1
25	10.2006	5466	73	136.0	98.5	4.0	6.0	37.7	4.0
26	10.2021	6257	73	138.5	106.5	2.5	2.5	36.0	3.2
27	10.2464	5404	72	141.0	98.5	3.0	4.5	43.0	4.2
28	Alba	9049	62	147.0	115.0	2.0	2.5	47.3	3.4
29	KWS Cassia	8710	61	141.5	90.0	2.0	2.5	60.0	3.7
30	Hispanic	7066	62	133.0	76.5	4.0	5.5	53.1	3.7
LSD (0.05)		695	3	2.2	7.9	0.9	1.6	2.3	0.4
CV		5.7	2.3	0.8	4.0	15.7	23.1	2.4	4.3

Table 3. Lleida, Spain data (2013-14).

Entry	Name	Yield (kg/ha)	β-glucan (% w/w)
1	10.0656	6540	6.5
2	10.0691	5535	8.8
3	10.0883	8527	10.9
4	10.0969	6500	6.5
5	10.1151	8125	7.1
6	10.1154	8820	4.4
7	10.1328	6990	5.4
8	10.1332	6745	5.9
9	10.1661	5550	5.5
10	10.1934	6590	5.1
11	10.1985	5505	6.2
12	10.1986	8730	6.7
13	10.2393	6990	5.3
14	10.0655	7360	7.2
15	10.0662	6330	8.4
16	10.0686	6914	9.5
17	Food 12	8600	9.9
18	10.0964	6474	8.4
19	10.1119	8200	5.8
20	10.1135	8984	6.1
21	10.115	9344	5.7
22	Food 26	6834	6.1
23	10.1492	7580	5.8
24	10.1618	7194	4.8
25	10.2006	7914	7.3
26	10.2021	8424	4.7
27	10.2464	7400	5.6
28	Alba	9565	5.5
29	KWS Cassia	8120	5.1
30	Hispanic	8007	5.5
LSD (0.05)		767	--
CV		5.0	25.0

REFERENCES

Ames, N.P., Rhymer, C.R., 2008. Issues surrounding health claims for barley. J. Nutr. 138, 1237S–1243S.

Anker-Nilssen, K., Sahlstrøm, S., Knutsen, S. H., Holtekjølen, A. K., & Uhlen, A. K. (2008). Influence of growth temperature on content, viscosity and relative molecular weight of water-soluble β-glucans in barley (*Hordeum vulgare* L.). J. Cereal Sci. 48(3), 670-677.

Chutimanitsakun, Y., Cuesta-Marcos, A., Chao, S., Corey, A., Filichkin, T., Fisk, S., Ross, A. S. (2013). Application of marker-assisted selection and genome-wide association scanning to the development of winter food barley germplasm resources. Plant Breeding. 132(6), 563-570.

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2011. Scientific Opinion on the substantiation of a health claim related to barley beta-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No. 1924/2006. EFSA J.; 9(12), 2471, 13 pp.

FAOSTAT, 2009. Production and trade. FAO, Rome, Italy. http://faostat3.fao.org/home/E (accessed 15.04.2016.).

Islamovic, E., Obert, D.E., Oliver, R.E., Harrison, S.A., Ibrahim, A., Marshall, J.M., Miclaus, K.J., Hu, G., Jackson, E.W., 2013. Genetic dissection of grain beta-glucan and amylose content in barley (*Hordeum vulgare* L.). Mol. Breed. 31, 15–25.

Izydorczyk, M.S., Dexter, J.E., 2008. Barley b-glucans and arabinoxylans: molecular structure,
physicochemical properties, and uses in food products – a review. Food Res. Int. 41, 850–868.
Meints, B., Cuesta-Marcos, A., Ross, A. S., Fisk, S., Kongraksawech, T., Marshall, J. M., Murphy, K. & Hayes, P. M. (2015). Developing Winter Food Barley for the Pacific Northwest of the US. Crop Sci. 55(4), 1563-1573.

National Barley Foods Council, 2003. Petition for unqualified health claim: barley beta-glucan soluble fiber and barley products containing beta-glucan soluble fiber and coronary heart disease. Submitted to the FDA on September 25, 2003.

Patron, N.J., Smith, A.M., Fahy, B.F., Hylton, C.M., Naldrett, M.J., Rossnagel, B.G., Denyer, K., 2002. The altered pattern of amylose accumulation in the endosperm of low-amylose barley cultivars is attributable to a single mutant allele of granule-bound starch synthase I with a deletion in the 59-noncoding region. Plant Physiol. 130, 190–198.

The Barley 'Nibblerome': Defining the Set of NB-LRR-Type R-Genes from a Diverse Collection of Barley

Authors: M. Moscou[1*] W. Jackson[1], I. Hernández-Pinzón[1]
The Sainsbury Laboratory[1], Norwich Research Park, Norwich, Norfolk, NR4 7UH, UK
[*]Corresponding Author: PH: +44 (0) 1603 450296, E-mail: matthew.moscou@sainsbury-laboratory.ac.uk

OBJECTIVES

The key objective of this study is to catalogue the NB-LRR (NLR) gene family complement in barley. NLR genes play an important role in the plant immune system, where they function as sensors of pathogen attack; and as such represent an important focus of research into developing sustainable methods for protecting crops against plant pathogens.

INTRODUCTION

Plant pathogens are a major impediment to sustainable food production, particularly in light of an increasing human population. One of the major forms of protection against plant pathogens are derived from the plant itself. Resistance genes (*R* genes) defend the plant in a pathogen-specific manner and is commonly associated with a hypersensitive response (HR) (Dangl, et al., 2013). The majority of *R* genes in plants encode NLRs, which are cytosolic proteins with a nucleotide-binding (NB) domain and several leucine-rich repeat (LRR) domains (Dangl, et al., 2013). NLR encoding genes exist in complex loci (Michelmore and Meyers, 1998), meaning that the correct assembly and annotation from short read next-generation sequencing data is an extremely difficult task. Several researchers have identified NLRs in approximately 40 different monocot and eudicot plant genomes (including barley) (Jupe, et al., 2012, Kroj, et al., 2016, Li, et al., 2010, Meyers, et al., 2003, Sarris, et al., 2016, Tan and Wu, 2012). These studies scanned publically available genomes and gene annotation sets to identify signatures of NLRs (identified from experimentally validated NLRs) using local alignment (BLAST suite), motif searches (MEME suite), domain prediction software (InterProScan) and gene clustering algorithms (TRIBEMCL).

Due to the complex nature of the barley genome (5.1 Gbp and ~80% repetitive), and the fragmented state of the current draft genome sequence (I.B.S. Consortium, 2012), we surmise that the annotation and physical anchoring of the complex NLR gene family is incomplete. To address these issues, we began by scanning the barley genome for signatures of NLRs and confirming or repairing existing annotations with *de novo* assembled transcripts from eight different tissues from the reference accession Morex (described in (I.B.S. Consortium, 2012)). Future work will include combining this technique with more extensive transcriptomic analysis and barley-specific NLR exome capture (RenSeq) to improve this initial assessment even further.

MATERIALS AND METHODS

Using a random selection of NLRs identified in rice and *Brachypodium distachyon* by (Li, et al., 2010) and (Tan and Wu, 2012) as a training set, we developed 20 key motifs that generally detect coiled-coil (CC), nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains (identified with MEME version 4.9.0 (Bailey, et al., 2009)). These motifs were used to scan the entire set of high and low confidence gene models (I.B.S. Consortium, 2012) of barley for signatures of NLRs using the Motif Alignment & Search Tool (MAST (also from the MEME suite version 4.9.0)). The motifs corresponded closely with those previously identified from NLRs of *Arabidopsis thaliana* by (Meyers, et al., 2003). Using the Geneious genome browser (version 9.1.1), predicted NLR candidates were

manually curated and subjected to domain structure prediction with Coils version 1.1.1 and InterProScan version 1.0.6 (http://www.geneious.com/; (Kearse, et al., 2012)).

We performed *de novo* assemblies of eight transcriptomes using RNAseq reads from eight different tissues from the reference accession Morex (described in (I.B.S. Consortium, 2012)), using Trinity version 2.0.6 (Grabherr, et al., 2011). After prediction of the best transcript ORFs with TransDecoder version 2.0.1 (Haas, et al., 2013), and removal of 100% redundancy with CD-HIT version 4.6.4 (Li and Godzik, 2006), the transcripts were aligned to the 419 candidate NLR genes using BLAST (Altschul, et al., 1990), and best hit alignments visualised and assessed in Geneious version 9.1.1 (http://www.geneious.com/ (Kearse, et al., 2012)).

RESULTS AND DISCUSSION

Using the Morex whole genome shotgun reference assembly (I.B.S. Consortium, 2012) as a starting point, we found that 419 putative NLRs are present in the annotated Morex genome. The NLRs are unequally distributed across the genome, with particular enrichment on chromosome arms: 1HS, 2HS, 6HL, and 7HS (Figure 1). Of the 419 genomic candidates, 172 had classical CC-NB-LRR domain structure, of which 12 contained predicted integrated decoy (ID) domains, confirming the recent results of (Kroj, et al., 2016) and (Sarris, et al., 2016). Example ID domains with predicted function include WRKY and B3 transcription factors, zinc-fingers, protein kinases, thioredoxin, and lectin binding domains (Figure 2). A total of 109 NB-LRR-type candidates were also retrieved from the barley genome, with 13 of these containing ID domains with similar functions to those found for CC-NB-LRRs. The remaining 138 gene models that harboured NLR-type signatures were made up of individual CC (4), NB (27) and LRR domains (11); CC-NBs (95); and one CC-LRR.

We were unable to conclude from the available genome assembly whether or not these 138 candidates were an accurate representation of correct gene model, or if they were fragmented due to assembly errors. Therefore, we turned to *de novo* transcriptome assemblies based on publically available RNAseq data as a means of both independently validating the genomic NLR annotation and in some cases improving and enhancing them. When non-redundant best ORFs from the 8 independent transcriptome assemblies were aligned to the set of 419 NLR candidates, 299 of the previously described gene models (MLOC and AK designations) were validated, meaning the gene model derived from the genome assembly agreed perfectly with the *de novo* assembled transcript. The remaining 120 NLR gene models require further investigation based on evidence for incomplete gene models based on fragmented genomic contigs, presence/absence variation between Morex and Haruna Nijo (the cultivar used for generating full length cDNAs), and incorrectly assembled genomic contigs (Figure 3).

To understand the natural variation in NLR repertoire, we have sequenced the leaf transcriptome from 38 diverse barley including elite, landrace, and wild accessions. Future work will involve the characterization of allelic and presence/absence variation, the identification of novel NLR-ID, and physical anchoring of all unmapped NLRs. In parallel, we have developed an exome capture (RenSeq) specifically targeting NLRs from barley and are currently assessing the results. The annotated NLRome will be a critical resource in the rapid identification of resistance genes and for the development of closely linked markers for molecular breeding.

FIGURES

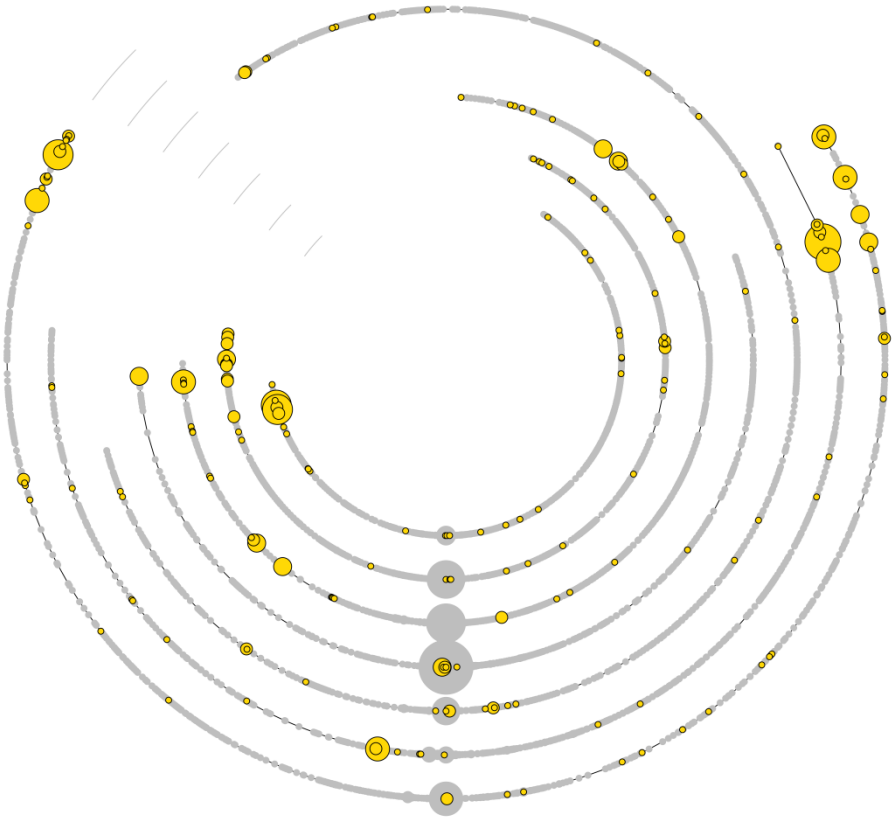


Figure 1. Anchoring of NLRs on the physical map of barley. Concentric rings correspond to chromosomes 1H (inner ring) to 7H (outer ring). Yellow circles indicate the number of NLRs at a locus ranging from 1 (smallest circle) to 6 (largest circle) and grey circles indicate the number of contigs present within a POPSEQ genetic bin (Mascher, et al., 2013). In total, 293 NLRs were physically anchored. Scale bars indicate 10 cM based on POPSEQ anchoring.

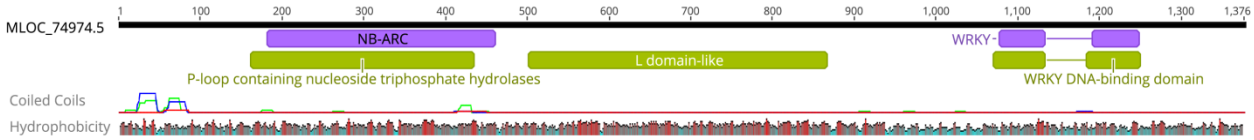


Figure 2. Gene model of a CC-NB-LRR fused with a WRKY domain. The bottom track indicates the prediction of a coiled-coil domain at the N-Terminus of the protein. Purple bars are domains predicted by the PFAM protein domain database, while green bars are predictions by the Superfamily database.

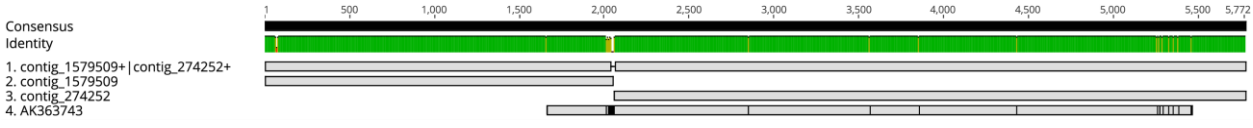


Figure 3. Example of repaired/extended NLR gene model. The black bar at the top denotes the consensus sequence, while the green bar indicates the alignment identity score. Sequence 1 is the merged sequence of sequences 2 and 3 (joined by Ns). The bottom sequence is a predicted NLR. At approximately 2000bp identity drops to zero where sequence 1 and sequence 2 have been merged together, but then returns to 100% identity.

REFERENCES

Altschul, S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403-410.

Bailey, T.L., M. Boden, F.A. Buske, M. Frith, C.E. Grant, L. Clementi, et al. 2009. MEME Suite: tools for motif discovery and searching. Nucleic Acids Res. 37: W202-W208. doi:10.1093/nar/gkp335.

Consortium, I.B.G.S. 2012. A physical, genetic and functional sequence assembly of the barley genome. Nature 491: 711-716.

Dangl, J.L., D.M. Horvath and B.J. Staskawicz. 2013. Pivoting the plant immune system from dissection to deployment. Science 341: 746-751.

Grabherr, M.G., B.J. Haas, M. Yassour, J.Z. Levin, D.A. Thompson, I. Amit, et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29: 644-652.

Haas, B.J., A. Papanicolaou, M. Yassour, M. Grabherr, P.D. Blood, J. Bowden, et al. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nature protocols 8: 1494-1512.

Jupe, F., L. Pritchard, G.J. Etherington, K. MacKenzie, P.J. Cock, F. Wright, et al. 2012. Identification and localisation of the NB-LRR gene family within the potato genome. BMC Genomics 13: 1.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649.

Kroj, T., E. Chanclud, C. Michel-Romiti, X. Grand and J.B. Morel. 2016. Integration of decoy domains derived from protein targets of pathogen effectors into plant immune receptors is widespread. New Phytol. 210: 618-626.

Li, J., J. Ding, W. Zhang, Y. Zhang, P. Tang, J.-Q. Chen, et al. 2010. Unique evolutionary pattern of numbers of gramineous NBS–LRR genes. Mol. Genet. Genomics 283: 427-438.

Li, W. and A. Godzik. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22: 1658-1659.

Mascher, M., G.J. Muehlbauer, D.S. Rokhsar, J. Chapman, J. Schmutz, K. Barry, et al. 2013. Anchoring and ordering NGS contig assemblies by population sequencing (POPSEQ). Plant J. 76: 718-727. doi:10.1111/tpj.12319.

Meyers, B.C., A. Kozik, A. Griego, H. Kuang and R.W. Michelmore. 2003. Genome-wide analysis of NBS-LRR–encoding genes in Arabidopsis. The Plant Cell 15: 809-834.

Michelmore, R.W. and B.C. Meyers. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. 8: 1113-1130.

Sarris, P.F., V. Cevik, G. Dagdas, J.D. Jones and K.V. Krasileva. 2016. Comparative analysis of plant immune receptor architectures uncovers host proteins likely targeted by pathogens. BMC Biol. 14: 1.

Tan, S. and S. Wu. 2012. Genome wide analysis of nucleotide-binding site disease resistance genes in *Brachypodium distachyon*. Comp. Funct. Genomics 2012.

Does the *Alt* Gene Influence the Agronomic Response of Barley?

B.H. Paynter
Department of Agriculture and Food Western Australia, 75 York Road, Northam WA 6401
PH: +61 8 9690 2115, E-mail: blakely.paynter@agric.wa.gov.au

OBJECTIVES

Determine if the agronomic response of barley to changes in crop management is altered on acidic soils if the barley cultivar was carrying the aluminium tolerance gene *Alt*.

INTRODUCTION

Soil acidity is a major production constraint in the south-west of Western Australia affecting more than 14.25 million ha of wheatbelt soils (Gazey et al., 2014). More than 70% of surface soils (0-10cm) and almost 50% of subsurface (10-30cm) soils in the Western Australian wheatbelt are below the target pH_{Ca} of ≥5.5 and ≥4.8, respectively (Gazey et al., 2014), required to maximise the productivity of barley. As soil pH drops aluminium (particularly Al^{3+}) is released into the soil solution. In most WA wheatbelt soils Al reaches toxic levels when subsurface pH_{Ca} falls below 4.8. High levels of Al in the soil can reduce plant water use in two ways. As barley roots are unable to effectively grow through acidic subsurface soil the plants are restricted in their access to stored subsoil water for grain filling, lowering its yield potential. Aluminium toxicity can also damage roots so that they are unable to absorb water efficiently, even under moist conditions.

The solution to improving barley's productivity on acidic soils is to apply lime. Improving the genetic tolerance of barley to low soil pH and Al toxicity does not replace the need for lime, it is a complimentary activity. Amelioration of soil acidity is a slow process, especially if the subsurface pH_{Ca} is below 4.8. Improving the genetic tolerance of barley offer growers the opportunity to sow a cereal other than wheat in the rotation and/or improve the yield stability of barley where there is spatial variability in the subsurface pH_{Ca} whilst they lime.

In Western Australia genetic improvement in the tolerance of barley to Al toxicity has to date focused on the *Alt* gene found in Al tolerant breeding lines like WB229 (O'Connor/Kaniere). The *Alt* gene improves the Al tolerance of barley through the release of citrate (up to 10-fold increase) from the root apices via the *HvAACT1* (*HvMATE*) transporter (Furukawa et al. 2007 and Wang et al., 2007). The exudated citrate reduces the toxicity of Al in the immediate area surrounding the root apex and allows the barley roots to increase their proliferation in the soil. Plants carrying the *Alt* gene can lift the yield of barley on Western Australian acidic soils by 10-40% compared to plants not carrying the gene (Paynter and Fettell 2010).

As low soil pH and Al toxicity are influencing rooting depth and access to key nutrients, it is possible that cultivars carrying the *Alt* gene could influence the agronomic response of barley on both acidic and marginally acidic soils with elevated levels of Al. This paper looked at the response of cultivars with and without the *Alt* gene to changes in nitrogen (N) application and seeding rate (plant density) on two soils with a subsurface pH_{Ca} below 4.8 over three years.

MATERIALS AND METHODS

A long term research site was prepared at two lower rainfall environments in Western Australia, Merredin and Wongan Hills, contrasting in their subsoil aluminium toxicity (Table 1). Both sites had aluminium concentrations above 4 μg/g, levels toxic to barley roots. At each location lime sand was top-dressed in 2008 at 3 t/ha in 20 m wide strips by 300 m long, replicated three times, alternating with an un-limed 20 m wide strip. For the three subsequent years (2009, 2010 and 2011) a different 100 m section of each research site was used.

Table 1. Long term growing season rainfall (GSR, April-October), soil type, soil pH_{Ca}, soil aluminium concentration and soil conductivity of un-limed soil to 30 cm depth at Merredin and Wongan Hills.

Location	**Merredin**			**Wongan Hills**		
GSR	**227 mm**			**284 mm**		
Soil type	**acid sandy earth**			**brown deep sand**		
Depth (cm)	**pH** (0.01 M $CaCl_2$)	**Al** (μg/g)	**Conductivity** (μS/cm)	**pH** (0.01 M $CaCl_2$)	**Al** (μg/g)	**Conductivity** (μS/cm)
0-10	4.8	3.2	151	4.9	1.4	79
10-20	4.1	15.0	69	4.3	5.6	51
20-30	4.0	18.7	65	4.1	7.2	47

Three two-row spring barley cultivars were sown in a cyclic randomised split-split-split plot design with seeding rate randomised with nitrogen (N) blocks within randomised cultivars across lime strips with three replicates of each treatment. Hamelin, a malting cultivar, was compared to two BC4 lines (WABAR2481 and WABAR2492) derived from Hamelin (WB229/4*Hamelin) but carrying the *Alt* gene from WB229. The barley was sown at three target densities, 75, 150 and 300 plants/m^2, and top-dressed at 3 – 4 weeks after seeding with 0, 20, 40 or 80 kg N/ha. The seed rate (kg/ha) to establish the target densities varied for each cultivar and year and was adjusted based on their kernel weight and germination per cent.

Plots were assessed for establishment (plants/m^2), leaf area index at stem elongation (Z31) and ear half emerged (Z55) (m^2/m^3), plant height at maturity (cm, base of ear), lodging score (9-0) close to harvest, grain yield (t/ha), kernel weight (mg, db), hectolitre weight (kg/hL), screenings (% < 2.5 mm), grain protein concentration (%, db) and grain brightness (Minolta 'L*').

At mid grain fill, soil cores in 10 cm increments to 1 m were removed (two per plot) from selected plots (20 N and 80 N treatments of 150 plants/m^2) of each cultivar. Gravimetric moisture (%) was determined on each 10 cm increment and used to estimate plant available water (mm). Soil cores were also taken from a sprayed out section in each strip (bare soil) at six sites from each trial site. At maturity the same selected plots were assessed for yield components (dry matter (kg/ha), tillers/m^2, grain number per ear and harvest index) from hand cuts combined from two sites per plot of the rows either side of a rod 1 m in length.

Data was analysed with Genstat (VSN International 2013) with a block structure of (colrep+rep/lime)/cultivar/Napplied/seedrate and a treatment structure of lime x cultivar x Napplied x seedrate.

RESULTS AND DISCUSSION

Table 2. Per cent of trials (out of six) with significance ($p<0.05$) in the ANOVA table for leaf area index (LAI), plant height at maturity, lodging at maturity and grain yield.

ANOVA	LAI - Z31	LAI - Z55	Plant height	Lodging	Grain yield
Lime (L)	33%	67%	17%	0%	50%
Cultivar (C)	100%	67%	67%	67%	100%
L x C	0%	0%	0%	0%	0%
N applied (N)	100%	100%	33%	50%	33%
L x N	33%	0%	0%	0%	0%
C x N	0%	0%	17%	0%	0%
L x C x N	17%	0%	0%	0%	17%
Seedrate (SR)	83%	67%	100%	50%	83%
L x SR	0%	0%	0%	0%	0%
C x SR	33%	0%	17%	17%	0%
N x SR	33%	0%	17%	17%	17%
L x C x SR	0%	17%	17%	0%	0%
L x N x SR	0%	17%	17%	0%	0%
C x N x SR	17%	0%	0%	0%	0%
L x C x N x SR	0%	0%	0%	0%	0%

Table 3. Per cent of trials (out of six) with significance ($p<0.05$) in the ANOVA table for kernel weight, hectolitre weight, screenings, grain protein and grain brightness.

ANOVA	Kernel weight	Hectolitre weight	Screenings (% < 2.5 mm)	Grain protein	Grain brightness
Lime (L)	17%	0%	0%	33%	17%
Cultivar (C)	67%	17%	100%	83%	100%
L x C	0%	0%	17%	0%	0%
N applied (N)	67%	33%	83%	100%	67%
L x N	0%	0%	0%	17%	17%
C x N	0%	17%	0%	0%	50%
L x C x N	0%	0%	0%	0%	0%
Seedrate (SR)	100%	67%	83%	67%	67%
L x SR	17%	0%	0%	0%	0%
C x SR	0%	17%	33%	17%	17%
N x SR	33%	17%	33%	33%	0%
L x C x SR	0%	0%	17%	17%	0%
L x N x SR	0%	0%	0%	0%	0%
C x N x SR	0%	0%	0%	0%	0%
L x C x N x SR	0%	0%	0%	0%	17%

Main effect – lime, cultivar, nitrogen and seed rate – As the lime sand had only been applied one to three years previous to the trial series being conducted, the impact of lime on soil pH_{Ca} was relatively small (< 1 unit) at the surface (0-10 cm) and negligible (<0.2 units) in the subsurface (10-30 cm) (data not shown). It was not until the second and third year after lime application that there was a positive grain yield response to lime application. At those sites the yield response was 23% at Merredin in 2010, 11% at Wongan Hills in 2011 and 3% at Merredin, in 2011.

Cultivar regularly (in at least two out of every three trials) had a significant impact on crop performance except for grain number per ear, harvest index and hectolitre weight (Tables 2 to 4). The two BC4 Hamelin based lines carrying the *Alt* gene out-yielded Hamelin at all six sites. The average yield improvement was 22%. The Al tolerant Hamelin's did this because they were able to extract more water from the profile, grew more tillers resulting in more grains per m^2 that were heavier than the grains produced by Hamelin (Table 5 and Figure 1).

At the three trials with a site mean yield below 1 t/ha (Wongan Hills 2010, Merredin 2009 and 2010), the yield advantage of the Al tolerant Hamelin's was 47% (or 0.29 t/ha). At the three trials with a site mean yield above 2 t/ha (Wongan Hills 2009 and 2011, Merredin 2011), the yield advantage was 16% (or 0.44 t/ha) (data not shown).

Both agronomic treatments, N and seed rate, regularly influenced crop performance, except plant height, lodging, grain yield and hectolitre weight for N and lodging for seed rate (Tables 2 and 3). Despite all sites showing biomass increases (based on LAI at Z31 and Z55) to increasing N (data not shown), only one in three sites were grain yield responsive to N. The yield response to N was only observed in 2009 and not in 2010 or 2011 (data not shown). Five of the six sites had a significant increase in grain yield with increasing seed rate (data not shown). The average increase in grain yield due to increasing seed rate was 5% across all six trials.

Table 4. Per cent of trials (out of six) with significance ($p<0.05$) in the ANOVA table for dry matter at maturity, tiller number, grain number per ear and per m^2 and harvest index.

ANOVA	Dry matter	Tiller number/m^2	Grain number/ear	Grain number/m^2	Harvest index
Lime (L)	17%	33%	50%	17%	17%
Cultivar (C)	83%	83%	17%	67%	50%
L x C	0%	0%	0%	0%	17%
N applied (N)	17%	33%	17%	17%	67%
L x N	0%	0%	0%	0%	17%
C x N	0%	0%	17%	0%	0%
L x C x N	0%	0%	0%	0%	0%

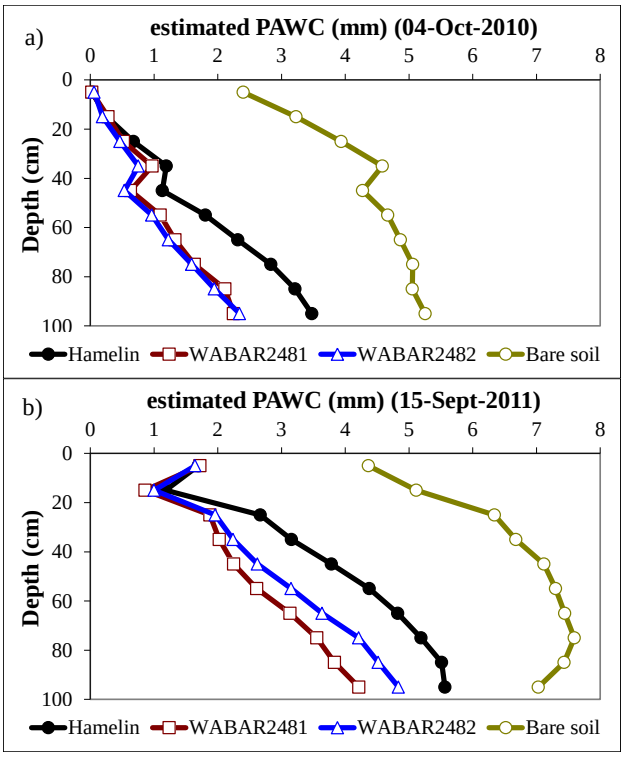


Figure 1. Estimated plant available water (based on gravimetric %) to 1 m depth under Hamelin, WABAR2481, WABAR2482 and bare soil during early grain fill for a) Wongan Hills in 2010 and b) Merredin in 2011 averaged over lime strips and two N rates at one seed rate.

Interaction with cultivar – Cultivar did not regularly influence the response of barley to lime application, increasing N rate or increasing seed rate in either 2-, 3- or 4-way interactions (Tables 2 and 3). The six trials demonstrated no evidence that barley cultivars carrying the *Alt* gene would respond differently to management inputs on acidic soils. In part this may have been due to the lack of overall yield response to N and low yield response to increasing seed rate.

Conclusions – In summary this study suggests that cultivars with the *Alt* gene will just be higher yielding in Western Australia, especially in years with a low yield potential, but will not require different management guidelines for the inputs – N and target plant density.

Table 5. Agronomic performance of Hamelin versus two BC4 Hamelin lines carrying the *Alt* gene when averaged over all six trials and treatments.

Trait	Hamelin	WABAR2481	WABAR2482
Plant establishment (plants/m^2)	159	160	162
LAI – Z31 (m^2/m^3)	1.7	2.0	2.0
LAI – Z55 (m^2/m^3)	2.2	2.7	2.7
Dry matter (t/ha)	5.45	6.73	6.69
Tiller number (tillers/m^2)	359	427	413
Grain number per ear (grains/ear)	20.7	20.7	21.3
Grain number per m^2 (grains/m^2)	7,756	9,151	9,151

Harvest index (0-1)	0.50	0.51	0.51
Plant height (cm)	47	52	52
Lodging (9-0)	8.0	7.7	7.5
Grain yield (t/ha)	1.68	2.08	2.01
Average grain weight (mg, db)	33.5	34.8	34.7
Hectolitre weight (kg/hL)	69.9	70.3	70.1
Screenings (% < 2.5 mm)	37.4	34.0	34.7
Grain protein (%, db)	13.9	13.5	13.4
Grain brightness (Minolta 'L*')	61.2	61.7	62.0

REFERENCES

Furukawa, J., Yamaji, N., Wang, H., Mitani, N., Murata, Y., and Sato, K, Katsuhara, M., Takeda, K., and Ma J. 2007. An aluminium-activated citrate transporter in barley. Plant Cell. Physiol. 48:1081–1091.

Gazey, C., Davies, S., and Master, R. 2014. Soil Acidity A guide for WA farmers and consultants 2ed. Bulletin 4858, Department of Agriculture and Food, Western Australia.

Paynter, B., and Fettell, N. 2010. Chapter 9 – Cultural Practices: Focus on Major Barley-Producing Regions - Australia, in Barley: Production, Improvement, and Uses (ed., S. E. Ullrich), Wiley-Blackwell, Oxford.

Wang, J., Raman, H., Zhou, M., Ryan, P., Delhaize, E., Hebb, D., Coombes, N., and Mendham, N. 2007. High resolution mapping of the *Alp* locus and identification of a candidate gene HvMATE controlling aluminium tolerance in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 115:265–276.

Modulation of integrated decoy R-genes/transcription factor assembly elicits wheat stem rust resistance responses in barley: *rpg4/Rpg5*-mediated *Ug99* resistance

Shyam Solanki, Jonathan Richards, Gazala Ameen and Robert Brueggeman*
Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA
*Corresponding author: PH: (701) 730-6102, E-mail: robert.brueggeman@ndsu.edu

OBJECTIVE

Elucidation of molecular mechanisms underlying *rpg4/Rpg5* mediated wheat stem rust resistance in barley.

INTRODUCTION

Plants are continuously challenged by an onslaught of potential pathogenic microbes. The majority of these pathogens are recognized by conserved pathogen associated molecular patterns (PAMPs). The conserved PAMP molecules are recognized by plant cell surface receptors known as pattern-recognition receptors (PRR's), which are capable of recognizing extracellular ligands (Zipfel, 2008). These receptors generally elicit a low amplitude defense response that results in callose deposition, reactive oxygen burst, PR gene activation and in some instances a tightly regulated programmed cell death (PCD) response. However, pathogens evolve effectors that suppress these early defense responses allowing them to become host specific pathogens. The second level of defense genes typically encodes cytoplasmically localized immunity receptors having nucleotide binding-leucine rich repeat (NLR) protein domain architecture. Functionally characterized NLRs perceive the pathogen effectors eliciting a strong PCD response known as the hypersensitive response (HR) resulting in localized cell death that arrests colonization by the biotrophic pathogens.

Recently, NLR's of different families located immediately adjacent to each other in a head to head architecture have been shown to function together to elicit defense responses. Limited functional analysis data suggests that one of the NLR partners acts as a sensor via direct binding with the pathogen effector and also suppresses the activation of the second NLR. The second NLR initiates the defense signaling responses upon pathogen perception once inhibition by its partner NLR is released (Cesari et al., 2013; Williams et al., 2014). Typically, in these dual NLR mechanisms the pathogen sensing NLRs were shown to contain a nontypical "integrated sensory domain (ISD)" that is targeted and manipulated by the pathogen effector/avirulence proteins directly. Recent genome analyses have suggested that these ISDs represent virulence effector targets that were integrated into the immunity receptors via translocation events providing the host with a more efficient surveillance mechanism (Kroj et al., 2016; Sarris et al., 2016). Mounting evidence suggests that these dual NLR/NLR-ISD pairs function in a guardee/decoy relationship representing a host immunity receptor complex with the ability to directly recognize pathogens through their intended virulence function (Cesari et al., 2014; Wu et al., 2015). There is strong evidence that has recently emerged via genome surveys of NLRs with these ISDs from many diverse genomes showing that a high proportion of these domains are effector triggered susceptibility targets, with the largest class of ISDs being the protein kinases. These data strongly suggest that the ISDs act as baits to trap the pathogen and this recognition elicits a strong ETI response.

The *rpg4*/*Rpg5* stem rust resistance locus in barley contains two NLR genes, *HvRga1* and *Rpg5*, and an actin depolymerization factor gene *HvAdf3* that are all required for

resistance against many races of *Puccinia graminis* f sp. *tritici* (*Pgt*) including the virulent race TTKSK (Wang et al., 2013; Arora et al., 2013; Steffenson et al., 2016). *Rpg5* is a typical NLR, but contains a C-terminal protein kinase domain. We hypothesize barley recognizes stem rust early in the infection process which elicits early PTI responses including the transcriptional regulation of *HvAdf3* which acts as a signaling intermediate between the PTI signaling and later *rpg4*/*Rpg5*-mediated ETI defense responses. The later responses rely on the Rpg5 kinase domain functioning as an "integrated sensory domain" that is manipulated by the haustorial expressed stem rust effector *Avr45*. Thus, the protein kinase domain acts as "bait" to trap the pathogen, which invokes an ETI resistance response post-haustoria formation. The research reported here begins to lay the ground-work for the spatial and temporal timing of the host pathogen recognition events, the molecular mechanisms, and components involved in this important stem rust resistance complex in barley.

MATERIALS AND METHODS

qRT PCR - Leaf tissue (~30 mg) were collected from the barley lines Q21861 (*rpg4/Rpg5* +) and Steptoe (*rpg4/Rpg5*-) from soltrol rust non-inoculated controls, and *Pgt* race QCCJ inoculated 10 day old seedlings at 6, 12, 24, 48, and 72 hour post inoculation (hpi). Total RNA was isolated from the samples using Qiagen RNA extraction kit following the manufacturers standard protocol. The RNA (~250 ng) was used to synthesize cDNA using the GoScript Reverse transcription system (Promega). The resulting cDNA was diluted 1:5 with ultrapure water (Ambion). The qPCR reactions for *HvAdf3* were performed using the BIO-RAD SsoFast Evagreen supermix using the manufacturer protocol on a CFX-96 Real Time PCR detection system (BIO-RAD). The barley GAPDH gene was used as a housekeeping gene to normalize the expression. Non-inoculated samples were used as control for expression analysis. Three biological replicates were analyzed using three experimental replicates per sample and the resulting data was analyzed using the BIORAD CFX Manager software.

Y2H library screening - A full-length *Rpg5* cDNA clone was used as template to amplify the protein kinase (PK) domain, which was cloned into the pENTER/D/TOPO vector. The Rpg5 PK cDNA was transferred into the gateway pDEST32 binary vector using LR recombination for use as the bait plasmid in yeast-two-hybrid (Y2H) experiments. A Y2H prey library was developed from ~30 μg of mRNA isolated from barley line Q21861 primary seedling leaves, 48 hpi with *Pgt* race QCCJ. A nano-quantity library was created using CloneMiner II cDNA library construction kit (ThermoFisher Scientific) according to manufacturer instructions. Approximately 2.4×10^6 primary entry clones were obtained which were further transferred into gateway pDEST22 prey plasmid using LR recombination. Chemically competent *Saccharomyces cerevisiae* MaV203 cells were used to co-transform with bait plasmid and prey library and plated on SC-Leu-Trp-His+30 mM 3AT auxotrophic selection plates to identify putative Rpg5 PK domain interacting proteins.

RESULTS AND DISCUSSION

Early recognition events connected with later ETI responses - The qPCR transcript analysis of *HvAdf3* (Fig.1) in resistance barley line Q21861 shows a five fold down regulation at time points 6 and 12 hpi with *Pgt* race QCCJ. The down regulation occurs through 72 hpi. Interestingly, the transcript analysis of susceptible barley cultivar Steptoe showed a 2.8-fold upregulation of the *HvAdf3* transcript at 6 hpi. In Steptoe we did not detect any significant differential *HvAdf3* transcript levels compared to the non-inoculated controls at the later time points. Preliminary DAB staining coupled with confocal microscopy experiments (data not

shown) detected a strong HR response in Q21861 that did not occur until 24-48 hpi at the regions of pathogen ingress, adjacent to or under stomata. No HR was detected in the susceptible cv Steptoe. Thus, collectively the results suggest that the transcriptional reprogramming of *HvAdf3* may be a consequence of an early recognition response possibly mediated through a PRR type of cell surface receptor. We hypothesize that this early recognition may prime the later *Rpg5*-mediated defense responses and that HvAdf3 may act in the intermediate signaling between the early PTI responses and the later *Rpg5*-mediated ETI responses.

The *rpg4/Rpg5*-mediated resistance has strong parallels with the *P. syringae-Arabidopsis* system that requires the NLR *R*-gene *RPS5*, the serine threonine protein kinase (PK) "decoy" protein *PBS1*, and the actin depolymerization factor *AtADF4* that functions as a signaling intermediate between PTI and ETI (Porter et al., 2012). This model resistance mechanism is elicited by pathovars of *P. syringae* carrying the effector, AvrPphB, a cysteine protease, which acts to block flg22 mediated PTI responses, but also manipulates the sensory "decoy", *PBS1*, which is guarded by the *RPS5* NLR *R*-gene. We have shown that the barley *rpg4/Rpg5*-mediated resistance requires *Rpg5*, with a NLR homologous to *Rps5* and a PK domain with homology to *PBS1*, a second unrelated NLR gene, *HvRga1*, and *HvAdf3*, with homology to AtAdf4 (Wang et al., 2013). Based on the recent studies suggesting that ISDs are decoys of the intended virulence effector host target proteins (Kroj et al., 2016; Sarris et al., 2016) we hypothesize that the effector/avirulence protein, *Avr45*, is expressed and secreted into barley cells via the haustoria where it manipulates the Rpg5 PK domain. The Rpg5 PK integrated domain manipulation by Avr45 reveals the pathogens presence invoking a strong HR response mediated by the dual NLR proteins.

Y2H identification of a transcription factor that interacts with Rpg5 and HvRga1 - We performed protein-protein interaction experiment between the three proteins required for resistance, Rpg5, HvRga1 and HvAdf3, and their separate modular domains using the Y2H approach and no interactions were detected between any homo or heterodimer combinations. These data suggested that the proteins may not interact in a resistance signaling complex. However, subsequent screening of a full scale cDNA library constructed from barley line Q21861 inoculated with *Pgt* race QCCJ with an Rpg5-PK bait construct identified four interacting clones. Sequence analysis of these putative interacting prey clones revealed two independent clones of the *Hordeum vulgare* orthalog of the *Arabidopsis AtVoz1* transcription factor (Mitsuda et al., 2004), which we designated *HvVoz1*. Mutants of *AtVoz1* were previously shown to be compromised for resistance to the fungal pathogen *Colletotrichum higginsianum* and the bacterial pathogen *Pseudomonas syringae* (Nakai et al., 2013). The *HvVoz1* prey clone was utilized to screen for Rpg5-LRR domain, HvRga1 LRR domain, and HvAdf3 and Rpg5 full-length protein interactions. Surprisingly, HvVOZ1 was found to interact with all of them except HvAdf3 (Fig.2). These preliminary results suggest that HvVOZ1 may act as a scaffold to hold the HvRga1/Rpg5 NLR-complex together in an inactive state until effector binding or manipulation of the Rpg5- PK integrated sensory domain. To validate our results, we are still in process of additional screening and are currently using virus induced gene silencing (VIGS) for the functional characterization of *HvVOZ1* in resistance against wheat stem rust, *Pgt* race QCCJ.

TABLES AND FIGURES

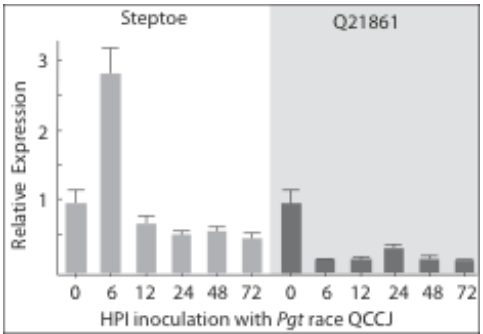
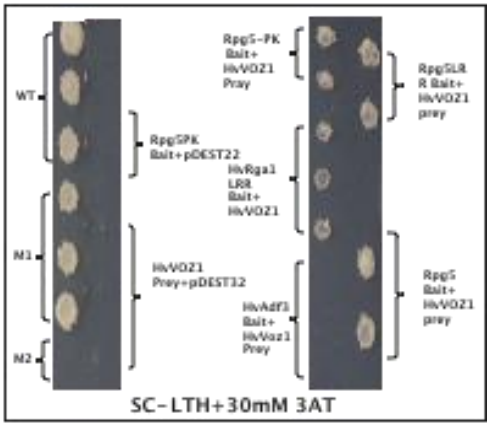


Fig1. Time course qPCR transcript analysis of *HvAdf3* in Steptoe inoculated with *Pgt* race QCCJ (left panel) and

Q21861 inoculated with QCCJ (right panel). Error bar depict SEM±1(n=3). Time point 0HPI (non-inoculated) was used as control sample for relative expression.

Fig.2 Y2H to test interaction of HvVOZ1 prey construct with different bait constructs consisting of Rpg5, Rpg5-LRR, Rpg5-PK, Rga1-LRR and HvAdf3. His auxotroph+30 mM 3AT growth plates were used for screening. Growth pattern depict the magnitude of interaction. Strong, moderate, weak, interaction controls are shown in the left panel.



REFERENCES

Arora, D., T. Gross, R. Brueggeman. 2013. Allele characterization of genes required for rpg4-mediated wheat stem rust resistance identifies Rpg5 as the R gene. Phytopathol. 103:1153-1161.

Cesari S., M. Bernoux, P. Moncuquet, T. Kroj, P.N. Dodds. 2014. A novel conserved mechanism for plant NLR protein pairs: the "integrated decoy" hypothesis. Front. Plant Sci. 5:1-10.

Cesari S., G. Thilliez, C. Ribot, V. Chalvon, C. Michel, A. Jauneau, S. Rivas, L. Alaux, H. Kanzaki, Y. Okuyama, J.B. Morel, E. Fournier, D. Tharreau, R. Terauchi, T. Kroj. 2013. The rice resistance protein pair RGA4/RGA5 recognizes the *Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 by direct binding. Plant Cell. 25:1463–1481.

Kroj, T., E. Chanclud, C. Michel-Romiti, X. Grand, J.B. Morel. 2016. Integration of decoy domains derived from protein targets of pathogen effectors into plant immune receptors is widespread. New Phytologist 210:618-626.

Mitsuda N., T. Hisabori, K. Takeyasu, M.H. Sato. 2004. VOZ; isolation and characterization of novel vascular plant transcription factors with a one-zinc finger from *Arabidopsis thaliana*. Plant Cell Physiol. 45:845-854.

Nakai, Y., Y. Nakahira, H. Sumida, K. Takebayashi, Y. Nagasawa, K. Yamasaki, M. Akiyama, M. Ohme-Takagi, S. Fujiwara, T. Shiina, N. Mitsuda. 2013. Vascular plant one-zinc-finger protein 1/2 transcription factors regulate abiotic and biotic stress responses in Arabidopsis. The Plant J. 73:761-775.

Nirmala, J., T. Drader, P.K. Lawrence, C. Yin, S. Hulbert, C.M. Steber, B. J. Steffenson, L. J. Szabo, D. von Wettstein, A. Kleinhofs. 2011. Concerted action of two avirulent spore effectors activates Reaction to Puccinia graminis 1 (Rpg1)-mediated cereal stem rust resistance. Proc. Natl. Acad. Sci. USA. 108:14676-14681.

Porter, K., M. Shimono, M. Tian, B. Day. 2012. Arabidopsis Actin-Depolymerizing Factor-4 links pathogen perception, defense activation and transcription to cytoskeletal dynamics. PLoS Pathog. 8(11): e1003006.

Sarris P.F., V. Cevik, G. Dagdas, J.D.G. Jones, K.V. Krasileva. 2016. Comparative analysis of plant immune receptor architectures uncovers host proteins likely targeted by pathogens. BMC Biol. 14:8.

Steffenson, B.J., S. Solanki, and R.S. Brueggeman. 2016. Landraces from mountainous regions of Switzerland are sources of important genes for stem rust resistance in barley. Alpine Botany. 126:23-33.

Wang X., J. Richards, T. Gross, A. Druka, A. Kleinhofs, B. Steffenson, M. Acevedo, R. Brueggeman. 2013. The rpg4-mediated resistance to wheat stem rust (*Puccinia graminis*) in barley (*Hordeum vulgare*) requires Rpg5, a second NBS-LRR gene, and an actin depolymerization factor. Mol Plant Microbe Interact. 26:407-418.

Williams S. J., K.H. Sohn, L. Wan, M. Bernoux, P.F. Sarris, C. Segonzac, V. Thomas, M. Yan, S.B. Saucet, D.J. Ericsson, L.W. Casey, T. Lonhienne, D.J. Winzor, X. Zhang, A. Coerdt, J.E. Pareker, P.N. Dodds, B. Kobe, and D.G. Jones. 2014. Structural basis for assembly and function of a heterodimeric plant immune receptor. Science. 344:299-303.

Wu C. H., K.V. Krasileva, M.J. Banfield, R. Terauchi, S. Kamoun. 2015. The "sensor domains" of plant NLR proteins: more than decoys? Front. Plant Sci. 6:134.

Zipfel C. 2008 Pattern-recognition receptors in plant innate immunity. 2008. Curr opin immunol. 20.1:10-16.

Molecular Genetic Characterization of Key Genes for Utilization of Barley as Human Food and Animal Feed

S. Taketa[1]*, T. Tonooka[2], E. Himi[1], and T. Kato[3]
[1]Institute of Plant Science and Resources, Group of Genetic Resources and Functions, Okayama University, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan. [2]NARO Kyushu Okinawa Agricultural Research Center, Chikugo, Fukuoka, 833-0041, Japan. [3]Tochigi Prefecture Agricultural Experiment Station, Utsunomiya, Tochigi, 320-0002, Japan.
*Corresponding author: PH: (81)86-434-1237, E-mail: staketa@rib.okayama-u.ac.jp

OBJECTIVES

Because barley is widely recognized as healthy food, our researches focus on morphology and quality of seed characteristics. Japanese barley landraces are characterized by a high frequency of naked barley, which has separable hulls by threshing. Naked grains are edible as whole grains or polished grains after pearling outer hard pericarp layers. Genes controlling naked caryopsis, grain color and soluble dietary fiber contents are described. Barley is also utilized as forages for animals. Long and stiff awns are harmful for use of whole barley crop as feed. Short awns appear to be suitable for utilization as forage. Molecular identification of a short awn gene in barley is described.

INTRODUCTION

Archeological records show that, about 2,000 years ago, barley was first introduced into Japan by way of China and Korean Peninsula. Since then, Japan has a long history of consuming barley as food. When the rice production was low, barley was an essential substitute to fill the food shortage. Currently Japan faces the overproduction of rice. Thus, production of food barley shrank in Japan, but barley has found a niche and is receiving attentions as healthy food. Here, we summarize genes controlling morphology and quality characteristics useful for food barley that were identified by our research groups. Four genes, (1) naked caryopsis (*nud*), (2) polyphenol oxidase (*PPO*), (3) proanthocyanidin-less 28 (*ant28*) and (4) (1:3;1:4)-beta-glucan less (*betaglucanless, bgl*) genes are summarized. Moreover, molecular identification of the short awn 2 (*lks2*) gene is also described. This short awn gene could be promising for better preference of barley as feed because it shorten and soften the awns.

MATERIALS AND METHODS

Of the five barley genes that have been molecularly characterized, positional cloning approaches were used for *nud* and *lks2*. The other three genes were identified by candidate gene approaches on the basis of rough mapping of respective gene in barley plus available genetic information in other plant species.

RESULTS AND DISCUSSION

Gene cloning- The following five genes were cloned and characterized.

Naked caryopsis gene, Naked caryopsis gene was fine mapped to a 0.18-cM interval on 7HL using 2,828 F_2 plants derived from two cross combinations between naked and hulled caryopsis lines. This interval was physically filled by five contiguous BAC clones. Their shot gun sequences revealed that only one transposon-unrelated gene is present in this region. Thus, we concluded that an ethylene response factor (ERF) family transcription factor gene

controls the covered/naked caryopsis phenotype. One hundred naked barley accessions from wide areas all shared a 17-kb deletion, suggesting the monophyletic origin of naked barley. The *Nud* gene has homology to the Arabidopsis WIN1/SHN1 transcription factor gene, whose deduced function is control of a lipid biosynthesis pathway. Staining with a lipophilic dye (Sudan black B) detected a lipid layer on the pericarp epidermis only in covered barley. We infer that, in covered barley, lipids secreted in the inner side of the hull, generates adhesion to the caryopsis surface (Taketa et al., 2008, 2013).

We also studied molecular variation of the marker sKT7 tightly linked to *nud*. Of 259 accessions studied, all 100 naked domesticated accessions and one wild barley (*Hordeum vulgare* subsp. *spontaneum*) accession were classified into type IV. DNA sequence variation analysis of selected type IV accessions showed that sub-type IVb was predominant and that there were two migratory routes of naked domesticated barley to Eastern Asia (Figure, Taketa et al., 2004).

PPO genes, PPOs are copper-containing metalloenzymes encoded in the nucleus and then transported into the plastids. Reportedly, PPOs cause time-dependent discoloration of end products of wheat and barley. PCR using a wheat PPO primer pair amplified two bands with different mobility in barley. Linkage analysis showed that the polymorphisms in the *PPO1* and *PPO2* genes cosegregated with the phenol reaction phenotype of awns that was evaluated by staining with the 1% phenol solution. RT-PCR experiments showed that *PPO1* was expressed in hulls and awns, and that *PPO2* was expressed in caryopses. Allelic variation of *PPO1* and *PPO2* was analyzed in 51 phenol reaction-negative barley accessions that were screened from about 8,000 lines. In *PPO1*, five types of amino acid substitutions affecting functionally important motif(s) or C-terminal region(s) were identified in 40 of the 51 accessions tested. In *PPO2*, only one mutant allele with a precocious stop codon as a result of 8-bp insertion in the first exon was found in three of the 51 phenol reaction-negative accessions (Taketa et al., 2010). From these observations, we concluded that *PPO1* is the *Phenol reaction* gene (*Phr*) reported by Takeda and Chang (1996). Use of *ppo1 ppo2* double recessive mutant does not appear to solve the browning problem of cooked barley.

ant28 gene, In barley, anthocyanin- and proanthocyanidin-free mutants (*ant* mutants) are classified into *Ant1* to *Ant30* through allelism tests. We isolated an R2R3 MYB gene of barley by PCR and named *Hvmyb10* because of its similarity to *Tamyb10* of wheat. Mapping showed that a polymorphism in *Hvmyb10* perfectly cosegregated with the *ant 28* phenotype on the distal region of the long arm of chromosome 3H. Barley lines with *ant28* mutation showed reduced browning after cooking, but were susceptible to preharvest sprouting (Himi et al., 2012).

bgl gene, (1,3;1,4)-ß-D-glucans are almost specifically found in grasses, and are particularly high in barley grains. Through chemical mutagen treatments, we isolated three independent *bgl* mutants of barley which completely lack (1,3;1,4)-ß-D-glucan in all tissues tested. The *bgl* phenotype cosegregates with the cellulose synthase like *HvCslF6* gene on chromosome arm 7HL. Each of the *bgl* mutants has a single nucleotide substitution in the coding region of the *HvCslF6* gene resulting in a change of a highly conserved amino acid residue of the HvCslF6 protein. Transient expression of the *HvCslF6* cDNAs in *Nicotiana benthamiana* leaves shows that the *bgl* mutants lack detectable (1,3;1,4)-ß-D-glucan synthase activity. These results demonstrate that, among the 10 *CslF* genes and one *CslH* gene present in the barley genome, *HvCslF6* has a unique role and is the key determinant controlling biosynthesis of (1,3;1,4)-ß-D-glucans. Natural allelic variation in the *HvCslF6* gene was found predominantly within introns among 29 barley accessions studied. Genetic manipulation of the *HvCslF6* gene may

be effective to control the levels of (1,3;1,4)-ß-D-glucans in accordance of the usages (Taketa et al., 2012).

lks2 gene, The awn, an apical extension from the lemma of the spikelet, is unique to grasses and assists seed dispersal and burial in the wild. In barley, the awn is an important organ for photosynthesis. Barley typically has long awns, but short-awn variants exist. The short awn 2 (***lks2***) gene, which produces awns about 50% shorter than normal, is a natural variant that is restricted to Eastern Asia. Positional cloning demonstrated that ***Lks2*** encodes a ***SHI***-family transcription factor. Allelism tests revealed that ***lks2*** is allelic to ***unbranched style 4*** (***ubs4***) and ***breviaristatum-d*** (***ari-d***), for which the phenotypes are severe with a quarter of awn length and sparse stigma hairs. The gene identity was validated by 25 mutant alleles with lesions in the ***Lks2*** gene. ***Lks2*** is highly expressed in awns and pistils. Histological observations of longitudinal awn sections showed that the ***lks2*** short-awn phenotype resulted from reduced cell number. Natural variants of ***lks2*** were classified into three types, but all shared a single-nucleotide polymorphism (SNP) that causes a proline-to-leucine change at position 245 in the IGGH domain. All three ***lks2*** natural variants were regarded as weak alleles, and therefore found a niche in high-precipitation areas of Eastern Asia. Natural variants of ***lks2*** found in the east of China and the Himalayas had considerably different sequences in the regions flanking the critical SNP, suggesting independent origins (Yuo et al., 2012).

TABLES AND FIGURES

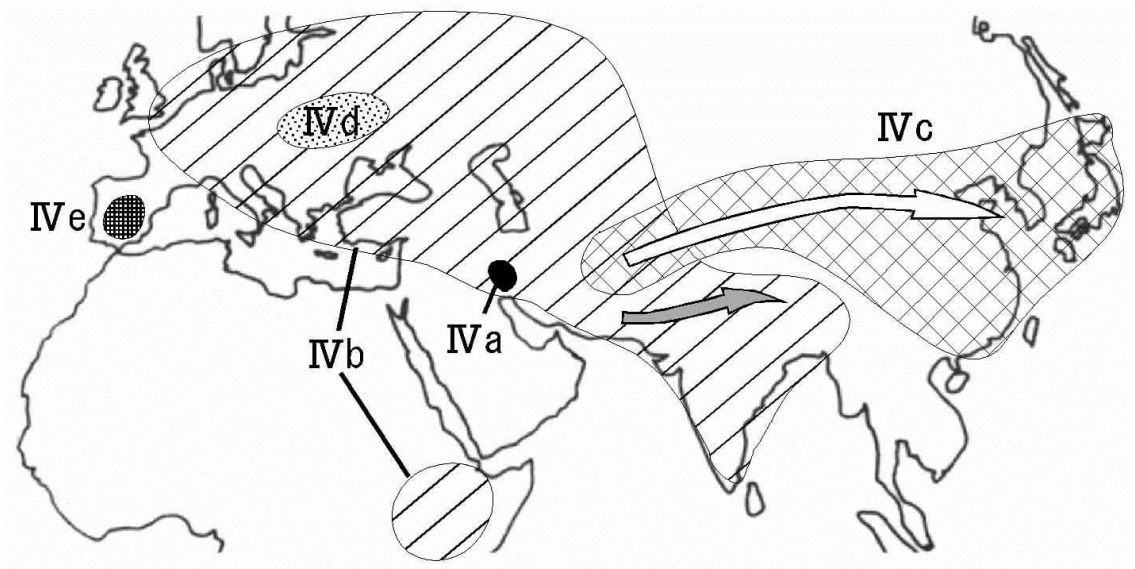


Fig. Geographical distribution of five haplotypes of allele IV at the sKT7 locus. Haplotype IVa was found only in a single wild barley accession, and the rest were found in naked domesticated barley. Arrows indicate the two migratory routes of naked domesticated barley to Eastern Asia.

REFERENCES

Himi, E., Y. Yamashita, N. Haruyama, T. Yanagisawa, M. Maekawa, and S. Taketa. 2012. *Ant28* gene for proanthocyanidin synthesis encoding the R2R3 MYB domain protein (Hvmyb10) highly affects grain dormancy in barley. Euphytica 188:141–151.

Takeda K, CL. Chang 1996. Inheritance and geographical distribution of phenol reaction-less varieties of barley. Euphytica 90:217–221.

Taketa, S., S. Kikuchi, T. Awayama, S. Yamamoto, M. Ichii and S. Kawasaki. 2004. Monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (*nud*). Theor Appl Genet 108:1236-1242.

Taketa, S., S. Amano, Y. Tsujino, T. Sato, D. Saisho, K. Kakeda, M. Nomura, T. Suzuki, T. Matsumoto, K. Sato, H. Kanamori, S. Kawasaki, K. Takeda. 2008. Barley grain with adhering hulls is controlled by an ERF family transcription factor gene regulating a lipid biosynthesis pathway. Proc. Natl. Acad. Sci. USA 105:4062–4067.

Taketa, S., K. Matsuki, K., S. Amano, D. Saisho, E. Himi, N. Shitsukawa, T. Yuo, K. Noda, and K. Takeda. 2010. Duplicate polyphenol oxidase genes on barley chromosome 2H and their functional differentiation in the phenol reaction of spikes and grains. J. Exp. Bot. 61:3983–3993.

Taketa, S., T. Yuo, T. Tonooka, Y. Tsumuraya, Y. Inagaki, N. Haruyama, O. Larroque, and S.A. Jobling. 2012. Functional characterization of barley betaglucanless mutants demonstrates a unique role for CslF6 in (1,3;1,4)-β-D-glucan biosynthesis. J. Exp. Bot. 63:381–392.

Taketa, S., T. Yuo, Y. Yamashita, M. Oozeki, N. Haruyama, H. Maejima, H. Kanamori, T. Matsumoto, K. Kakeda and Sato K. 2013. Molecular mechanisms for covered vs. naked caryopsis in barley. In G. Zang et al. (editors), Advances in Barley Sciences: Proceedings of 11th International Barley Genetics Symposium, Zhejiang University Press and Springer Science. p. 453-460.

Yuo, T., Y. Yamashita, H. Kanamori, T. Matsumoto, U. Lundqvist, K. Sato, M. Ichii, S.A. Jobling, and S. Taketa. 2012. A *SHORT INTERNODES* (*SHI*) family transcription factor gene regulates awn elongation and pistil morphology in barley. J. Exp. Bot. 63:5223–5232.

Imidazolinone tolerant barley: an Australian barley breeding success story

D. Moody[1]*, M. Materne[2], C. Pittock[3], S. Crane[4] and D. Liesegang[5]
[1]InterGrain Pty Ltd, Bibra Lake, Western Australia 6163
[2]GlobalGrainGenetics, Quantong, Victoria 3401
[3]Department of Economic Development, Jobs, Transport and Resources, Carlton, Victoria 3053
[4]SeedNet, Bendigo, Victoria 3551
[5]Agriculture Victoria Services Pty Ltd, Attwood, Victoria 3049
*Corresponding author: PH: (+61) 8 94198019, E-mail: dmoody@intergrain.com

OBJECTIVES

To develop and commercially deploy imidazolinone tolerant barley varieties to aid in the management of weed species in Australian cropping systems.

INTRODUCTION

The overall annual cost of weeds to Australian grain growers is estimated to be $3,300 million, equating to $146/ha in expenditure and losses (Llewellyn et al. 2016). Based on extent and cost, the most costly weeds nationally in terms of total yield loss are ryegrass, wild radish, wild oats and brome grass. For some weed species, notably the *Bromus* species, there has been increased prevalence since the adoption of no-till farming practices (Kon and Blacklow 1988). Changing climatic conditions within Australia, with less winter and spring rainfall but an increase in summer rainfall, has encouraged earlier sowing with growers on average now planting 4 weeks earlier than 30 years ago (Stephens, Anderson and Siddique, in press). Early sowing now often occurs into dry soil before any opportunity for pre-sowing weed management. These changes in farming systems have encouraged the evolution of herbicide tolerance in weed species which can only be mitigated using integrated weed management strategies. One component of these strategies is the rotation of herbicide groups used within a cropping cycle; crops species tolerant of alternate chemistry groups increase the range of herbicide options available for rotation.

A core requisite for improved weed management is the development of herbicide tolerance in a range of crop species. Knowledge of the genetic basis of imidazolinone (IMI) tolerance is well understood for a range of crop species (Tan et al. 2005). In Australia, Kleenan and Gill (2009) have described the effectiveness of using IMI herbicides for the control of rigid brome in IMI tolerant wheat varieties, noting that one of the limitations to the adoption of the technology was limited varietal choice. We report on the development of IMI tolerant barley and the success of the deployment of this technology in commercial barley varieties.

MATERIALS AND METHODS

Mutagenesis and selection

Seed (33.3kg or approximately 812,195 seeds) of the genotype VB0105 was soaked in 0.25% ethyl methane sulfonate (EMS) and dried using an air blower. Mutated seed was sown on 0.5ha land at Horsham, Victoria, Australia in June 2005 using standard management practices. At

maturity the sown area was harvested, with subsamples taken to form a representative composite of approximately 200kg. This 200kg sample of M2 seed (approx. 4.88 million seeds) was sown on 2 ha of land at Horsham, Victoria, Australia in June 2006 and sprayed post-emergent with ON DUTY® at a rate of 80g/ha (a.i. Imazapic 42g and Imazapyr 14g). Only twenty plants survived through to maturity and these were harvested individually by hand.

Of the twenty plants harvested, ten plants with the largest dry matter and seed yield were identified and further evaluated. Thirty seeds from each selection were sown in 10 pots (3 seeds per pot) in a glasshouse with half of these pots for each selection sprayed with ON DUTY at a rate equivalent to 50g/ha (a.i. Imazapic 26.25g and Imazapyr 8.75g); each selection was classified for tolerance to ON DUTY® based the reaction of progenies. Plants were deemed tolerant if they appeared unaffected by ON DUTY® (no visual symptoms). The original plants were classified as being either homozygous or heterozygous for IMI tolerance based on the performance of the progeny.

Genetic characterization

Phenotypic and genotypic assessment of single plant reselections taken from the original VB0105 indicated a high level of heterogeneity. A composite of reselections that were similar for morphological characteristics was created to form the basic seed of the variety Buloke. A single plant selection (VB0105*12) that was representative of the haplotypes and phenotypic characteristics of Buloke was retained as a reference sample for later genetic comparison with herbicide tolerant genotypes from the mutagenized VB0105. Seven of the selected IMI tolerant mutants were assayed for SNPs on a BOPA 1 array and compared for polymorphic differences with each other and against the reference reselection VB0105*12.

Using a collection of publicly available reference sequences of the aceto-hydroxy-acid synthase (AHAS) gene, sequences were obtained from a range of monocotyledonous species (including barley) and were used for PCR primer design to conserved regions of the gene to ensure optimal primer performance. Nested PCR was employed to reduce amplification product complexity and to increase reaction specificity. Using the nested PCR strategy, a region of the AHAS gene for Buloke (represented as VB0105*12), and 3 IMI tolerant mutants was uniquely amplified and subjected to direct sequencing. The sequences were aligned using Sequencher v3.7 and compared to other wild type sequences and known mutations.

Glasshouse, field and laboratory evaluation of the progenies of the initial ten plants occurred during the 2007 and 2008, with selected genotypes entered for evaluation in the Australian National Variety Trials (NVT) during 2009.

RESULTS AND DISCUSSION

The relatively low rate of EMS (0.25%) deployed during mutagenesis resulted in very high germination rates in the M1 population and presumably low mutation rates. We believe the relatively low rate of EMS, combined with the use of a very large population size, were key factors in allowing the direct release of a mutant from the mutagenized population, avoiding the requirement of backcrossing to reduce deleterious effects of background mutational events.

Heterogeneity existing within VB0105 was exploited to understand the origin of herbicide tolerant mutants; an understanding of this heterogeneity was also important in identifying an IMI tolerant genotype with equivalence to Buloke in phenotypic performance for all other traits. Of the 1536 SNP's on the BOPA 1 array, the number that were polymorphic between VB0105*12 and the seven tolerant mutants ranged from 5 to 241.

Three of the mutants (designated VBHT0805, VBHT0807 and VBHT0810) were monomorphic for all SNP on BOPA1 when compared with each other, and differed from VB0105*12 for only 40 SNPs. It was concluded that these mutants were either derived from a single M1 plant or represented independent mutations of the same haplotype within VB0105. Based on the relatively low level of SNP polymorphism of these 3 mutants compared to the VB0105*12, we believe that they could be considered to be derived from a haplotype that was representative of the parental population rather than derived from a mutation of contaminate within the original population. Two further mutants (VBHT0802 and VBHT0806), that differed from VB0105*12 at only 5 and 41 SNPs respectively, were considered to be derived from independent mutational events of different haplotypes within VB0105. VBHT0802 differed from VBHT0806 for 36 SNP, and differed from VBHT0805, VBHT0807 and VBHT0810 for 43 SNP. VBHT0806 differed from VBHT0805, VBHT0807 and VBHT0810 for 36 SNP.

The two other mutants assayed (VBHT0803 and VBHT0809), each differed from VB0105*12 by more than 300 SNP, and differed from each other by 333 SNP. These were considered as independent mutations of haplotypes within VB0105 that were not representative of the variety Buloke. When examined for morphological characteristics, both VBHT0803 and VBHT0809 differed from Buloke for awn colour and plant height.

Based on these analyses, there is strong evidence to indicate there were at least 5 independent mutations conferring IMI tolerance identified from within the mutagenized population.

Sequencing experiments identified for VBHT0805 and VBHT0806, around base coordinate 1742 as described in the wheat AHAS reference sequence, a G had been mutated to an A, leading to an amino acid substitution from serine to asparagine. The serine to asparagine change, referred to as the SER653 mutation, has been found to confer IMI herbicide tolerance in other plant species (Tan et al., 2005). In VBHT0802, no sequence change was identified around base coordinate 1742 and it is speculated that a mutation elsewhere in the AHAS gene, which was not sequenced in this series of experiments, is causal of IMI tolerance in this genotype.

Based on high level tolerance to IMI herbicides, performance in the NVT trials, and comparative disease resistance and quality assessment compared to Buloke, VBHT0805 was selected for release as the variety "Scope" in 2010 and as has continued in NVT in the period 2010-2015.

Accreditation and regulatory approvals

Scope was submitted for accreditation through the Australian malting barley quality accreditation program and, based on performance in commercial malting and pilot brewing trials, Barley Australia announced the accreditation of Scope as a malting variety in March 2013.

The Australian Pesticides and Veterinary Medicines Authority granted an initial permit for the use of IMI chemistry on Scope barley in 2010, with full approval granted in 2012. In 2012, BASF included Scope on the Intervix® (a.i. 33 g/L Imazamox, 15 g/L Imazapyr) label as part of the BASF Clearfield® production system for early post emergent weed control. This represented a significant deregulation milestone critical for adoption of the trait by the local barley industry.

To meet the safety assessment process for Plants with Novel Traits (PNTs), required by the Canadian Food Inspection Agency and Health Canada, an application for the unconfined release of plants possessing this trait into Canada was made in June 2016. The outcome of this application is pending and is critical to the deployment of this trait in North America.

Commercial variety adoption

Adoption of Scope by growers has been rapid following public release in 2011, with the national market share reaching approximately 17% in 2015 (Table 1). The reasons for the rapid rate of adoption are twofold. Firstly, there existed a clearly identifiable market gap for alternate crop species with herbicide tolerance, especially given the adoption of no-till farming systems that have increased the prevalence of brome grass. Secondly, the choice of Buloke as the parental material for mutagenesis meant that by the time of commercialization of Scope, a relatively seamless transition could occur from an established, successful variety (Buloke), with well understood agronomic characteristics and with established malting markets, to a variety that was essentially an IMI tolerant version of the same variety.

In contrast, the areas sown in 2015 to a range of IMI tolerant wheat (300,000ha) and canola (180,000ha) varieties represent only 2.4% and 6.2% of the national areas sown to these crops respectively (A. Simeonidis, BASF, Pers. Comm.), despite a longer breeding history for this trait. This suggests that, for growers, the trait value is greater in barley than in wheat and canola.

In comparison with current competitor barley varieties, Scope has some significant weaknesses. Scope has relatively weak straw, is prone to head loss, is prone to high screenings under harsh spring conditions and is significantly lower yielding than varieties such as Hindmarsh (Table 2). Average gross returns, calculated using trial yield and grain quality data from Western Australian NVT between 2010 and 2014, and using typical prices received for each of the varieties, indicated the average gross returns of Scope was over $70 per hectare lower than for Hindmarsh (Moody, 2015). To explain the rapid adoption of Scope, growers and their advisors must perceive the benefit of IMI tolerance in barley, primarily for long term weed management in their farming systems, at least negates this lower gross return estimated from NVT evaluation.

Future Developments

In 2016, Agriculture Victoria Services (AVS) entered into a breeding and commercialization license agreement with InterGrain allowing InterGrain to develop new IMI tolerant barley varieties and to produce and commercialize these new IMI tolerant barley varieties. The variety Spartacus CL (see Table 2), released in 2016 by InterGrain, is expected to be a major variety over the next 8 to 10 years with a forecast national market share of 25%.

In Australia, patent and PBR rights exist in relation to Scope CL and Spartacus CL for the production, reproduction, conditioning, sale, importation and exportation of any seed, plants and plant materials. The combination of these rights means that the sale and propagation of Scope CL and Spartacus CL and the use of Scope CL and Spartacus CL for breeding and commercial purposes without the permission of SeedNet (for Scope CL), InterGrain (for Spartacus CL) or AVS (for either variety) is unlawful. Internationally patents have been granted in the US and China, accepted and expected to be granted in 2016 in Canada, whilst patent examination is ongoing in Brazil and Europe.

Table 1. Market share (estimated from SeedNet grower surveys) of the variety Scope CL as a proportion of the Australian barley production area during the period since the variety's release.

Year	Area (ha) sown to Scope	% National Area sown to barley
2010	Nominal	-
2011	15,000	0.4%
2012	74,000	1.8%
2013	220,000	5.4%
2014	463,000	11.4%
2015	700,000	17.2%

Table 2. Relative yields of Scope versus competitor varieties, expressed as a percentage of site mean BLUPs averaged across sites within 4 yield environmental groupings, in Australian NVT trials during the period 2010 – 2015. Derived from data at http://www.nvtonline.com.au/.

Variety		> 4.5 t/ha	3 - 4.5 t/ha	1.5 – 3 t/ha	< 1.5 t/ha
	(No. Trials)	**(75)**	**(198)**	**(172)**	**(31)**
Scope	(452)	98	98	101	106
Buloke	(420)	99	99	102	106
Hindmarsh	(460)	104	108	113	142
Spartacus CL	(142)	106	109	114	140

REFERENCES

Kon, K.F., and Blacklow, W.M. 1988a. *Bromus diandrus* Roth and *B. rigidus* Roth. Pages 13–27 in The Biology of Australian Weeds, Volume 1. Melbourne, Australia: R.G. and F.J.Richardson.

Llewellyn R.S., Ronning D., Ouzman J., Walker S., Mayfield A., and Clarke, M. 2016. Impact of Weeds on Australian Grain Production: the cost of weeds to Australian grain growers and the adoption of weed management and tillage practices. Report for GRDC. CSIRO, Australia.

Kleemann, S.G.L. and Gill, G.S. 2009. The role of imidazolinone herbicides for the control of *Bromus rigidus* (rigid brome) in wheat in southern Australia. Crop Protection 28 (11): 913-916.

Moody, D.B. 2015. Breeding for imidazolinone tolerant barley varieties: industry issues and concerns. https://grdc.com.au/Research-and-Development/GRDC-Update-Papers/2015/02/

Tan, S., Evans R.R., Dahmer, M.L., Singh, B.K. and Shaner, D.L. 2005. Imidazolinone-tolerant crops: history, current status and future. Pest Management Science 61(3): 246-257.