Nondestructive, high-throughput phenotyping to study cereals under stress conditions.

Bettina Berger ¹ and M. Tester ^{1,2}.

¹ The Plant Accelerator, The University of Adelaide and ² Australian Centre for Plant Functional Genomics, Waite Campus, Urrbrae, Australia.

Genetics and genomics are powerful tools for gene discovery. However, the ability to phenotype at high-throughput plant growth and function is forming a bottleneck in discovering the role of genetic loci in plants. Increasingly, efficient transgenic technologies are generating large numbers of GM crop plants; and the genotyping of mapping and mutant populations is now highly efficient. However, the ability to quantitatively phenotype these populations is limiting progress in plant science. The increasing power of digital imaging and computational technologies offers the opportunity to relieve this phenotyping bottleneck. The Plant Accelerator® is a new, 4,500-m² growth facility that provides -omic-scale shoot phenotyping of large populations of plants using automated plant handling and digital imaging. Current projects use the phenotyping capacity to analyze cereals under various stress conditions, including water deficit and salinity. First results and the experimental approach taken will be discussed.

POSTER ABSTRACTS

Poster 1. The International Wheat Genome Sequencing Consortium (IWGSC).

The International Wheat Genome Sequencing Consortium. IWGSC, 5207 Wyoming Road, Bethesda, MD 20816, USA.

Bread wheat, the staple food for 35% of the world's population and the most widely produced crop, is one of the most important crop species. Genomics offers powerful tools for understanding the molecular basis of phenotypic variation as well as accelerating gene cloning, marker-assisted selection, and more efficient exploitation of genetic diversity. In 2005, a group of growers, breeders, and plant scientists launched the International Wheat Genome Sequencing Consortium (IWGSC) with the goal of securing a high quality, reference sequence of the bread wheat genome. The IWGSC facilitates and coordinates research projects and funding efforts at the national and international levels; develops and supports the design of research proposals; provides a framework for the establishment of common guidelines, protocols, and resources; and organizes scientific meetings and workshops. The IWGSC is governed by six co-chairs, a Coordinating Committee, and an executive director. General membership is open to any individual, laboratory, or entity with an active interest in meeting IWGSC objectives. The mission, goals, organizational structure, projects, and online membership registration are available at http://www.wheatgenome.org. IWGSC activities are guided by a milestone-based strategy coupled with short- and long-term roadmaps designed to provide breeders access to an increasing array of tools and resources without having to wait for the completed sequence. To reduce the complexity of the allohexaploid, highly repetitive, 17-Gb bread wheat genome, the IWGSC follows a chromosome-specific approach to develop physical maps, low coverage sequencing, and high quality sequencing of the Minimum Tiling Paths before moving towards a gold standard reference sequence. Physical maps have been completed or are underway for all 21 chromosomes of the reference cultivar, Chinese Spring. To facilitate anchoring, marker development, and to gain a first insight into the gene space composition, the IWGSC launched an internationally coordinated survey sequencing initiative that is providing breeders with survey sequences and the virtual gene order for all 21 chromosomes. High quality, BAC-by-BAC sequencing of chromosome 3B was completed in 2011 and sequencing of other chromosomes is under way. IWGSC activities and results will be presented.

Poster 2. The IWGSC Chromosome-Based Survey Sequencing Initiative.

The International Wheat Genome Sequencing Consortium. IWGSC, 5207 Wyoming Road, Bethesda, MD 20816, USA.

Applying advanced genomics to wheat breeding will play a central role in securing affordable and nutritious food. Bread wheat (*Triticum aestivum*) has one of the largest (~17 Gb) and most complex (allohexaploid, 6n=42, AABBDD) genomes. Although genome size varies in grasses, gene order, generally, is conserved along large chromosomal segments enabling comparative methods between related species. The IWGSC aims to establish a high quality, reference sequence

of the wheat genome (cv. Chinese Spring) that is anchored to the genetic and phenotypic maps to provide high resolution linkages between the traits and the underlying variations in sequence and polymorphisms. A first goal has been to obtain physical maps of the individual chromosomes/arms. To anchor the physical contigs, survey sequences were achieved using NGS technologies for a majority of the Chinese Spring chromosomes. The IWGSC then launched a short-term initiative to provide survey sequences of all 21 chromosomes. Sponsored by industry and government partners, the aim is to generate sequence and virtual order for most wheat genes. The sequences generated for each chromosome arm are assembled using the latest software tools. A first pass annotation is implemented over the draft assembly sequences where comparative genomics and colinearity with other grass genomes is used to derive a virtual gene order with an account of non-syntenic genes and pseudogenes. As sequences are generated independently for each chromosome, a second aim of this project is to characterize genomic variation between gene homoeologues (ie orthologous genes placed in different subgenomes), regulatory elements, and repeat content. An update of this initiative will be presented.

Poster 3. TriAnnot: a high performance pipeline for the automated structural and functional annotation of plant genomes - new developments.

Philippe Leroy ¹, Nicolas Guilhot ¹, Isabelle Lesur-Kupin ⁴, Patricia Faivre ⁵, Sébastien Theil ¹, Frédéric Choulet ¹, Hiroaki Sakai ³, Michael Alaux ², Takeshi Itoh ³, Hadi Quesneville ², Christophe Plomion ⁴, and Catherine Feuillet ¹.

¹ INRA-UBP UMR1095, Clermont-Ferrand, France; ² INRA UR1164, Versailles, France; ³ NIAS, Tsukuba, Japan; ⁴ INRA UMR1202, Cestas, France; and ⁵ INRA UMR1165, Evry, France.

A versatile, easy-to-use, online, automated annotation pipeline, TriAnnot (Leroy et al. 2012. Frontiers in Plant Sciences 3:1-14; http://www.clermont.inra.fr/triannot), has been developed under the umbrella of the International Wheat Genome Sequencing Consortium, the TriticeaeGenome, and 3BSEQ projects to obtain a reference sequence of the bread wheat genome. Its modular architecture allows for the annotation and masking of transposable elements, the structural and functional annotation of protein-coding genes with an evidence-based quality indexing, and identification of conserved noncoding sequences and molecular markers. The performance of TriAnnot was evaluated in terms of sensitivity and specificity using curated reference sequence sets from rice (IRGSP build5, August 2010) and wheat (Choulet et al. 2010. Plant Cell 22:1686-1701). In less than 8 hours, TriAnnot was able to predict more than 83% of the 3,748 CDS from rice chromosome 1 with a fitness of 67.4%. On a set of 12 reference Mb-sized contigs from wheat chromosome 3B, TriAnnot predicted and annotated 93.3% of the genes among which 54% were perfectly identified in accordance with the reference annotation. It also allowed the curation of 12 genes based on new biological evidences, increasing the percentage of perfect gene predictions to 63%. TriAnnot systematically showed a higher fitness than other annotation pipelines that are not improved for wheat. The TriAnnot pipeline is parallelized on a 712 CPU computing cluster that can run a 1-Gb sequence annotation in 26 hours. It is accessible through a web interface for small-scale analyses and/or through a server for large scale annotations. For the later, the pipeline is launched automatically using a PERL script. After completion, the structural and functional annotation can be viewed through an online GBrowse and can be manually curated using Artemis and GenomeView graphical editors. As it is easily adaptable to the annotation of other plant genomes, TriAnnot is currently improved for other plant genomes annotations such as barley, rice, maize, and oak species, and should become a useful resource for the annotation of large and complex genomes in the future. Release 3.5 is on-line since January 2012, and a new release 3.6 is underway.

Poster 4. Current status of physical mapping on wheat chromosome 6B.

F. Kobayashi ¹, S. Katagiri ¹, W. Karasawa ¹, Y. Hanawa ¹, S. Kaneko ², S. Nasuda ², K. Hayakawa ³, H. Fujisawa ¹, Y. Ito ¹, Y. Mukai ¹, J. Dolezel ⁴, T. Matsumoto ¹, J. Wu ¹, and H. Handa ¹.

¹ National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; ² Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Kyoto, Japan; ³ Nisshin Flour Milling Inc, Tsukuba, Ibaraki, Japan; and ⁴ Institute of Experimental Botany, Olomouc, Czech Republic.

For the purpose of better understanding the genome structure in wheat and accelerating the development of DNA markers for gene isolations and future breeding, the Japanese research consortium, as a member of IWGSC, is now conducting a project for the physical mapping and genomic sequencing of Chinese Spring chromosome 6B (914 Mb). With a