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ITEMS FROM PAKISTAN

NATIONAL AGRICULTURAL RESEARCH CENTER (NARC), ISLAMABAD
Wheat Wide Crosses and cytogenetics

ATTA-UR-RAHMAN SCHOOL OF APPLIED BIOSCIENCES (ASAB),
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Germplasm conservation prebreeding value addition for yield enhancement as a conduit to national food security for combating climate change and the 2050 vision.

A. Mujeeb-Kazi and Alvina Gul Kazi.

The importance of wheat as a food cereal is paramount, and the need to be on secure production grounds a national priority. Changing international scenarios around wheat production in light of productivity constraints and new sophisticated technologies necessitate that our researchers move with time and be proactive. This involves swift research program restructuring that generates outputs efficiently, is unique, and targets threats due to climate change that will impact food security issues in 2050, when a population surge touching 9.2 billion approximately 230 million of which will be Pakistan's share.

National wheat yields currently are 2.6 t/ha with annual productivity approaching 25 x 10^6 tones. Our 2015 goal is 30 x 10^6 tonnes and about 33 x 10^6 tones for 2030. Maximizing yields is a tall order, because a huge yield gap exists and the full varietal productivity potential near 9.0 t/ha is too distant. Despite cultivar releases that are high yielding, national yield levels remain stagnant, between 25–30 maunds/acre (2.5–3.0 t/ha) when the upper limit touchs about 8.4 t/ha, and close to 5 t/ha in irrigated and rainfed areas by progressive farmers. Policy setting and management play a vital role to counter this poor performance but, on the research horizon, stress constraints are a huge concern. The fear of seeing the migration of stem rust Ug99 in to Pakistan is one factor, another is the breakdown of yellow rust resistance that has occurred, and finally, the emergence of spot blotch in scattered locations in 2009 go hand in hand to motivate proresearchers to find solutions that can curb these new dangers. Exploiting genetic resources is a viable option and this taps the abundant genomic diversity of the annual and perennial Triticeae members across all three wheat gene pools.

Our Wide Cross Program is an offshoot of the CIMMYT program as Mujeeb-Kazi, who was the architect of the CIMMYT program and led it from 1979 until late 2004, has provided that program's basic outputs under the devolution concept that allowed CIMMYT to focus on their special tasks with our efforts to compliment the remaining wide cross activities. Over the years at CIMMYT, seed maintenance, viability, and distribution of stocks that earlier were major

gram mainstream activities have for some unknown reason weakened. Thus, we have increased our efforts in Pakistan to compliment the CIMMYT holdings so that stocks remain well authenticated and can be provided to researchers as user-friendly packages. Our efforts are categorized in this article by highlighting our holdings that have enormous national and international diversity exploitation value. We have gathered stocks from other sources, maintain them, and have them stored in the gene bank at the NARC. These stocks will, upon increase, become freely available under an MTA and shared after actual shipping costs are covered.

Our current focus during the on-going 2013–14 crop cycle is to have completed increase and storage of the germplasm (Table 1, p. 48) from which the NARC gene bank, after further increase, can distribute to users. The wide cross program will maintain a modest working collection.

Salient comments. The Wide Crossing Program covers various facets that embrace multidisciplinary aspects of wheat research and production geared to address food security issues that prevail within the country. Heavy reliance is given to harness novel genomic diversity that resides in the under-utilized Triticeae member resources. As a consequence, the program has embraced several multidisciplinary areas that can in a holistic manner deliver outputs that will augment wheat productivity specifics are as follows that are in force as a way forward:

Human resource development. This component is comprised of young professionals who are pursuing their academic degrees and conduct research on the programs genetic stocks, prebreding, and breeding materials aided by national support staff. The research directions currently are heavy on QTL and association mapping; digital imaging; application of allele-specific markers; utilizing genomic information from the WX germplasm; crop physiology targeted for heat, drought, and salinity tolerance traits; micronutrient profiles of iron, zinc, and phytic acid linked with phosphate uptake; and quality across complete rheology, but more on HMW (A-PAGE) and LMW (allele-specific markers).

Research area coverage. The multidisciplinary areas summarized above detail comprise cytology, cytogenetics, biochemical genetics, molecular genetics, doubled haploidy/tissue culture, crop physiology, prebreeding/breeding for major biotic and abiotic stresses, micronutrients, cereal quality, and the all-important water-use efficiency covering its availability and purity. Specific targets are to ensure that resistance/tolerance is available for all three rusts, Karnal bunt, heat/drought/salinity/sodicity blended with quality attributes across complete rheology parameters with a careful oversight that considers the lesser important stresses at this stage such as powdery mildew, spot blotch, and barley yellow dwarf virus, including aphids.

Keeping in view the current global scenario of technology development and availability of advances in genomic applications with availability of outsourcing opportunities around the 9K, 90K, and DArT genotyping platforms, our program focus has been intensified to concentrate on stringent phenotyping targets. This strategy has been adopted and partnerships are being established around relevant partners for relevant objectives.

Conclusions. The various articles that follow reflect our partners' contributions on various investigative areas that encompass the multidisciplinary research targets being evaluated.

Future awareness. After the emergence of the new stem rust race Ug 99 in 1999 in Uganda, its presence and anticipated spread became a serious threat to all neighboring countries and also for those in its migratory path. This led to the formation of the 'Global Rust Initiative' with the advocacy of late Dr. N.E. Borlaug. Variants of Ug99 developed and spread, but intensive global breeding efforts led to the release of numerous resistant cultivars to contain the pathogens possible damage on wheat productivity.

Recently, information has surfaced of stem rust infections in Ethiopia and Germany. This presence has been associated with climate change factors and, in Ethiopia, infections are not Ug99, whereas those in Germany are being studied. This presence will continue the vigilance by researchers to combat the new spread. Other minor diseases have an enormous potential to intensify and spread. Hence prebreeding, tapping the novel genomic diversity across all selected Triticeae members of the various gene pools, will be pivotal for maintaining yield output in the decades ahead and continuing our operating targets in anticipation that new alleles will be identified and pyramided to become a source of resistance that has durability.

Table 1. Germplasm maintained in NARC (2013–14), Islamabad, Pakistan

Germplasm	Detail	#	Germplasm	Detail	#
A-genome synthetics	2n=6x=42, AABBAA	194	Amphiploids		30
D-genome synthetics	2n=8x=42, AABBDD	1,014	Backcross 1	Self-fertile	19
Durums in synthetics	2n+4x=28, AABB	50	**Alien Addition Lines**		
Elite 1 DD synthetics	2n=6x=42, AABBDD	95	*Th. bessarabicum*	Complete, disomic	7
Elite 2 DD synthetics	2n=6x=42, AABBDD	34	*S. cereale*	Complete, disomic	7
DD Drought subset	2n=6x=42, AABBDD	23	*H. villosa*	disomic	6
DD Yellow Rust subset	2n=6x=42, AABBDD	40	**Translocation Stocks**		
DD Septoria subset	2n=6x=42, AABBDD	10	Seri 82 (T1BL·1RS and 1B)	10 of each	20
DD Scab subset	2n=6x=42, AABBDD	35	Bread wheat (T1BL·1RS and 1B)	17 wheats	34
DD Spot Blotch subset	2n=6x=42, AABBDD	34	Altar 84 (1B and T1BL·1RS)	10 of each	20
DD Salinity subset	2n=6x=42, AABBDD	13	Durum wheat (1B and T1BL·1RS)	6 wheats	12
DD synthetics (DArT)	2n=6x=42, AABBDD	241	**Genetic Stocks**		
DD Karnal subset	2n=6x=42, AABBDD	4	CS monosomics	Complete set	21
DD Waterlogging subset	2n=6x=42, AABBDD	4	Glennson monosomics	Complete set	21
Land Races : Pakistan	International Sub-Set	112	CS ditelocentrics		
Land Races : Core		200	CS nulli-tetrasomics		
Land Races : Iran			CS *phph*		
Land Races : Afghanistan			CS *PhPh*		
Pakistan's Historical set of Cultivars		115	CS *PhIPhI*		
Salinity tester set		24	Cappelli		
Wild Species			Cappelli *ph1cph1c*		
Ae. cylindrica			Solid stem		1
Ae. tauschii			Multiple ovaries		2
T. urartu			Long coleoptile		3
T. monococcum subsp. *aegilopoides*			Early flowering subset		8
T. monococcum subsp. *monococcum*			Crop Science Registrations		135
T. turgidum subsp. *dicoccum*			Wide cross bread wheat/synthetic		240
T. turgidum subsp. *dicoccoides*			Observation nursery	WXBWSHON	Varies yearly
T. aestivum subsp. *spelta*					
T. aestivum subsp. *sphaerococcum*					
T. turgidum subsp. *carthlicum*					
T. turgidum subsp. *polonicum*					
S. cereale					

Morphological and physiological characterization of BW/SH germplasm.

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For producing high-yielding wheat cultivars, the new D-genome diversity of synthetic wheats was utilized and improved advanced prebreding lines were selected. Fifty lines were studied further at the National Agricultural Research Center (NARC), Islamabad. Materials were observed for morphological and physiological parameters, such as canopy temperature, chlorophyll content, photosynthesis rate, transpiration rate, stomatal conductance (gs), internal CO_2, and water-use efficiency, that influence grain yield.

Seedling habit. Plant habit at growth stage GS:31 was observed according to Zadoks' scale (1974). Three different habits, erect (E), semi-erect (S), and prostrate (P), were noted.

Crop ground cover. The percentage of the soil surface enclosed by plant foliage is an imperative measure of crop establishment. Accessions with a high DGC are capable of capturing incident radiation, thereby escalating soil shading and declining soil evaporation, which elevates water-use efficiency and may improve competitiveness with weeds and potentially reduce soil erosion. DGC-based phenotyping was used on all 50 lines with the help of a DSC-S300 at a height of 1.5 m and Adobe Photoshop CS3 Extended software. Photoshop software includes the functionality required to perform and export the automated DGC used to determine DGC (Fig. 1).

Fig. 1. Original image before processing (top) and a black and white image of accession BW/SH 2 after Photoshop processing.

$$\%GC = (\text{Mean grey walue} / 255) \times 100$$

Leaf area. Leaf area was measured manually by measureing the length and width of flag leaf. The following formula was used for leaf area (Muller 1991):

$$\text{Leaf area} = \text{length} \times \text{width} \times 0.74$$

Waxiness. Existence of leaf and/or spike glaucousness is observed visually and recorded according to a 1–5 scale, where 1 = absent, 2 = low, 3 = medium, 4 = moderately high, and 5 = high waxiness (Rebetzke et al. 2012, Table 2, p. 50)

Digital imaging of seed. The phenology of seed was by digital analysis. Twenty-five seed from each accession were selected randomly and placed over a grid. Horizontal and vertical images were taken, and size, length, width perimeter, length-to-width ratio, and circularity calculated using the 'SMART GRAIN' software.

Physiological evaluation. ***Canopy temperature.*** Canopy temperature was measured during the boot stage on bright days during peak hours using an infrared thermometer MIKRON (I.R MAN) protocol (Pask et al. 2012).

Chlorophyll content measurement. Chlorophyll content at stage GS:32 was recorded by using a Minolta SPAD-502 instrument. Three readings were randomly recorded with arithmetic means.

Rate of photosynthesis, transpiration, internal CO_2, stomatal conductance from gas exchange, and water-use efficiency were recorded using an infrared gas analyzer (IRGA) on bright sunny days.

Multiple factor analysis. Morphological (plant habit, waxiness, digital ground cover percent, and leaf area), physiological (photosynthetic rate, rate of transpiration, internal CO_2, stomatal conductance, canopy temperature, and chlorophyll concentration index), and seed shape (area, perimeter, length, width, length/width ratio, circularity, and distance of intersection with center of gravity) parameters were analyzed as major factors along with sub-factors of each. We observed that all seed shape attributes have the same variability with leaf area and digital ground cover percent and are explained by multiple factor F1 (eigenvalue = 1.743). Variation in physiological characterization and plant habit are

explained by multiple factor F2 (eigenvalue = 1.093). Moreover, all the morphological, physiological, and seed shape attributes are explained with 38.668%, 23.289%, and 38.043%, respectively, by multiple factor F1 on the X-axis (Figs. 2 and 3).

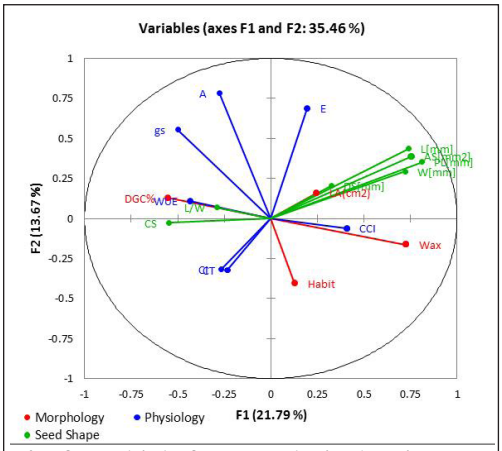

Fig. 2. Multiple factor analysis showing variability in all attributes analyzed.

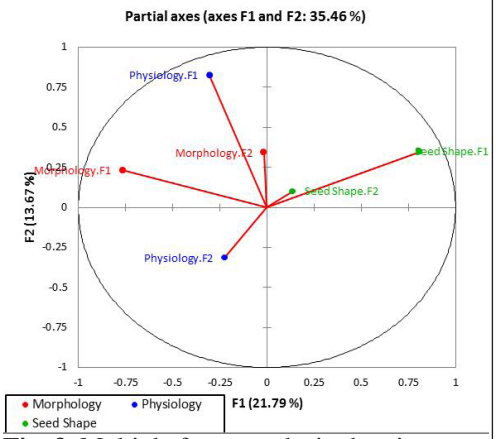

Fig. 3. Multiple factor analysis showing variability in subfactors.

Table 2. Pearson's correlation. Values in bold are different from 0 with a significance level of $\alpha = 0.05$. LA = leaf area (cm^2), Ci = internal CO_2, A = photosynthetic rate, E = transpiration rate, DI = gs = stomatal conductance from gas exchange, WUE = water-use efficiency, CT = canopy temperature, AS (mm^2) = seed area, PL = digital area of leaf (mm), L = seed length (mm), W = seed width (mm), L/W = length-to-width ratio, and CS = circularity, and DS = digital area of seed (mm).

	Wax	Habit	DGC%	LA (cm^2)	CCI	A	E	Ci	gs	WUE	CT	AS	PL	L	W	L/W	CS
Habit	0.203																
DGC%	–0.161	–0.091															
LA (cm^2)	0.024	–0.072	–0.242														
CCI	0.264	0.003	–0.179	–0.092													
A	–0.247	–0.038	0.158	–0.015	–0.138												
E	0.172	–0.100	0.063	0.114	–0.097	**0.476**											
Ci	–0.125	–0.119	0.083	–0.080	–0.034	**–0.515**	–0.222										
gs	**–0.349**	–0.151	0.240	–0.069	–0.112	**0.546**	**0.299**	0.397									
WEU	**–0.393**	0.051	0.085	–0.100	–0.017	**0.484**	**–0.522**	–0.246	0.280								
CT	–0.077	0.018	0.013	0.036	–0.058	–0.013	–0.233	–0.107	–0.162	0.175							
AS	**0.344**	–0.040	**–0.319**	0.083	**0.323**	0.057	0.230	–0.273	–0.140	–0.133	–0.276						
PL	**0.435**	–0.018	**–0.331**	0.085	**0.339**	0.023	0.222	–0.273	–0.175	–0.148	**–0.310**	**0.966**					
L	**0.381**	–0.084	**–0.291**	0.089	**0.349**	0.070	**0.309**	–0.190	–0.055	–0.188	**–0.327**	**0.937**	**0.952**				
W	**0.292**	–0.004	**–0.342**	0.152	0.230	0.010	0.114	**–0.280**	–0.209	–0.078	–0.216	**0.947**	**0.898**	**0.802**			
L/W	–0.019	–0.104	0.212	–0.151	0.047	0.089	0.197	0.227	**0.288**	–0.095	–0.050	**–0.409**	**–0.311**	–0.090	**–0.663**		
CS	**–0.480**	–0.052	0.198	–0.031	–0.228	0.118	–0.055	0.121	0.209	0.123	0.232	**–0.320**	**–0.552**	**–0.468**	–0.236	–0.187	
DS	0.240	**–0.339**	–0.060	0.204	0.199	–0.069	0.079	–0.058	–0.093	–0.106	–0.123	0.259	0.277	0.274	0.219	–0.022	–0.182

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Association analysis of agronomic traits in diverse wheat germplasm grown under water deficit conditions.

Ahmad Ali, M. Arshad, A. Rasheed, A.A. Napar, H. Sher, R. Ali, Alvina Gul Kazi, and A. Mujeeb-Kazi.

Crop yield losses under water shortage require a genetic solution, because irrigation or other management practices cannot be considered as sustainable options for drought. Harnessing new alleles from the wheat wild relatives through interspecific and intergeneric hybridization are vital to achieve this goal (Mujeeb-Kazi et al. 2013). Various synthetic hexaploid wheats (derivatives) have resulted in significantly superior combinations of biotic/abiotic resistance/tolerance (Mujeeb-Kazi 2003). Association mapping (AM), which shows association between marker alleles and phenotypic traits, is now used extensively as an alternative approach to overcome the shortcomings of pedigree-based quantitative trait loci (QTL) mapping. The main theme in AM is to carry out genome-wide searches through panels of accessions having medium-to-high linkage disequilibrium (LD) levels for chromosomal regions harboring loci regulating the expression of particular phenotypic traits (Abdurakhmonov and Abdukarimov 2008; Sorrells and Yu 2009; Yao et al. 2009). We conducted a marker–trait association study based on the polymorphisms present at 101 SSR loci, each locus with at least two major alleles. We identified MTAs influencing agronomic performance of bread wheat germplasm, comprised of synthetic-derived (SBW) and conventional bread (CBW) wheat along with check cultivars (CCT), and grown across different soil moisture conditions. A series of chromosome areas significantly affected the variability of traits related to plant drought response, yield components (1,000-kernel weight), and grain yield are reported.

Comparisons among the three wheat groups are shown (Table 3). The SBW lines performed relatively much better than the corresponding check cultivars for most of the studied traits across both years. To sum up, we can say that SBW and CBW, collectively, were better in performance both under control irrigated and field-induced drought stress than the corresponding check cultivars. Furthermore, allelic introgression from *Ae. tauschii* into bread wheat did improve the expression of grain yield and related components. Therefore, synthetic-derived wheats are a promising source for improved yield and yield related traits. Water stress (drought), especially at anthesis, is considered the most important environmental stress factor in agriculture, resulting in decreased wheat yield. Drought stress affects the physiological processes in plants, including respiration, photosynthesis, and carbohydrate metabolism, ultimately reducing growth, grain set, and grain fill, resulting in reduced grain yield (Ji et al. 2010).

Table 3. Comparison of means among check cultivars (CCT) and synthetic-derived (SBW) and conventional (CBW) bread wheat under irrigated and drought stress.

Treatment	Germ-plasm	Plant height	Days-to-flowering	Days-to-physiological maturity	Spikes/ plant	Spike length (cm)	Grain/ spike	1,000-kernel weight (g)	Grain yield (g)
Irrigated	SBW	95.24	114.00	144.02	12.90	12.59	52.28	43.97	30.00
	CBW	93.92	116.85	146.15	12.24	12.35	53.35	43.16	27.98
	CCT	84.47	116.10	146.10	13.53	11.35	41.10	36.27	20.10
Stress	SBW	83.86	105.75	135.75	11.11	11.20	44.90	40.55	20.71
	CBW	82.22	108.58	137.95	9.97	11.14	45.84	38.93	17.71
	CCT	76.07	108.30	138.50	11.63	10.68	34.13	32.47	13.06
LSD for treatment		1.10***	0.83***	0.89***	0.47***	0.27***	1.45***	0.72***	1.32***
LSD for group		1.64***	1.25***	1.31***	0.60***	0.34**	1.81***	0.87***	3.10***
LSD for treatment*group		1.78***	1.39***	1.51***	0.80***	0.46***	2.36***	1.11***	3.10 NS

We compared the two model approaches for all studied traits (Table 4, p. 52). Higher MTA numberss (total 122) were found with the general linear model (GLM), whereas the mixed linear model (MLM) approach detected only 40 MTAs at $p < 0.05$. Consequently, the MLM approach detected 42.6% less MTAs than the corresponding GLM approach. Similarly, the number of MTAs at $p < 0.01$ was 40 and 27 with GLM and MLM approachs, respectively. Some markers

were associated specifically either to only one trait or to more traits. The number of significant MTAs that were similar in both approaches was 61 at $p > 0.05$ and 25 at $p < 0.01$. Some unique associations were found either only in the GLM or the MLM approach. The number of such unique MTAs at $p < 0.05$ was 61 (50.0%) for GLM and 9 (12.9%) for the MLM approach. At $p < 0.01$, the percentage of these specific MTAs in GLM was 37.5% (total 15), whereas MLM exhibited 7.4% (only two MTAs). At the same time, the effect was variable for individual studied traits and, in some instances, no prominent effects were observed for some of the studied traits. Similar findings have been discussed by Neuman et al. (2011) in their GWAS by genotyping a core collection (96) of wheat lines with DArT markers.

These results provide a stimulus for wheat breeders to exploit the germplasm for crop improvement. Developing improved cultivars with sufficient tolerance to drought stress and identifying osmotic stress related molecules and their roles in biochemical, physiological, and gene regulatory networks is necessary. Precise phenotyping is vital for screening larger core collection/mapping populations for exploring QTL and candidate genes to be used in plant breeding. This study adds strength for the genomic involvement capabilities to the A and B (S) resources via direct or synthetic hexaploids include tetraploids via pentaploid breeding and exploit the tertiary gene pool in a holistic manner for delivering practical outputs that are durable and an optimistic solution to address food security (Mujeeb-Kazi et al. 2013). Selection for high grain yield based on direct selection for grain weight is generally not desirable because of the limitations imposed by spike number/plant and grains/spike. Although some sort of compensation will always be there in any selection method, the major objective is to decrease it by finding the most appropriate yield component. Because synthetic-derived wheats maintain a comparatively high grain weight, especially under drought treatment, higher gain from selection would be expected when selecting for other components. Linkage mapping analysis in populations designed specifically for appropriate objectives will point out to what degree the AM identified regions play a role in particular genetic backgrounds.

Table 4. Comparison of the general linear model (GLM) and the mixed linear model (MLM) for calculation of associations between markers and traits.

Trait	No significant GLM at p ≤		No significant MLM at p ≤		Significant GLM and MLM at p ≤		GLM unique at p ≤		MLM unique at p ≤		MLM unique (%) at p ≤		GLM unique (%) at p ≤	
	0.05	**0.01**	**0.05**	**0.01**	**0.05**	**0.01**	**0.05**	**0.01**	**0.05**	**0.01**	**0.05**	**0.01**	**0.05**	**0.01**
Plant height	21	9	10	4	9	4	12	5	1	0	57.1	55.6	10.0	0.0
Days-to-flowering	8	2	6	1	3	1	4	1	2	0	50.0	50.0	33.3	0.0
Days-to-physiological maturity	21	7	17	8	16	7	5	0	1	1	23.8	0.0	5.9	12.5
Spikes/plant	14	3	8	4	8	3	7	0	1	1	50.0	0.0	12.5	25.0
Spike length	15	2	6	2	5	2	10	0	1	0	66.7	0.0	16.7	0.0
Grains/spike	8	3	5	2	4	2	4	1	1	0	50.0	33.3	20.0	0.0
1,000-kernel weight	20	11	10	4	8	4	12	7	2	0	60.0	63.6	20.0	0.0
Grain yield/plant	15	3	8	2	8	2	7	1	0	0	46.7	33.3	0.0	0.0
Total	**122**	**40**	**70**	**27**	**61**	**25**	**61**	**15**	**9**	**2**	**50.0**	**37.5**	**12.9**	**7.4**

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Available options to overcome food security issues in Pakistan in times of increasing water scarcity: I.

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Wheat is recognized as the conduit to food security, and its ample production is significantly linked with water availability (Ali et al. 2013). Nearly all regions globally face water scarcity due to increasing population demands. Decline in this precious and limited resource is a major threat to the survival of mankind in terms of food security. Water resources are unevenly distributed globally; some regions face serious water shortages, whereas others have adequate water to sustain agricultural activities at an optimum (Pereira et al. 2002). Pakistan is a water-stressed country with an alarmingly low per capita water availability i.e. < 1,000 m^3 (Martin et al. 2006). Agriculture consumes most of Pakistan's available fresh water supplies (96%) for 86% of the total cultivable area (irrigated area) (Ali et al. 2014). The remaining 14% of the cultivable area is entirely rain-fed. Pakistan wheat production, targeting good agronomic practices, are hampered by water scarcity in irrigated and particularly in rain-fed agricultural areas. To ensure national food and fiber requirements by 2025 in connection with rapidly growing population, around 28–37 x 10^6 acre feet (MAF) of additional water will be required. Ensuring food security by maximizing wheat yield under declining available water assets is a challenging target that behooves national institutions to explore alternate water resources, e.g., reclamation of wastewater and rain water harvesting.

The Pakistan Agricultural Research Council (PARC), being an apex organization in rhw agriculture sector, was highly concerned about the gravity of water scarcity and the reclamation of wastewater for agricultural usage. Therefore, PARC initiated and developed a municipal wastewater reclamation facility at National Agricultural Research Center (NARC), Islamabad, to overcome water scarcity owing to its location in the rain-fed region. The municipal wastewater treatment facility (Bioremediation Orchard) is comprised of inter-connected constructed wetland and six detention ponds to cyclically treat wastewater through biological means under national environmental quality standards. Two large sedimentation ponds were constructed before the wastewater treatment facility to remove solid wastes. After sedimentation, wastewater was moved to the constructed wetland, which contained aquatic macrophytes, i.e., *Vetiver zizanoides*, *Phragmites australis*, and *Typha latifolia*, on top, and physical substrates beneath, i.e., gravel, boulders, brick pieces, sand, and crush. Wastewater treatment through a constructed wetland was a combination of physical and bioremediation processes. Effective microbe consortia (EM) also were applied in trickling filters of constructed wetland to support the wastewater treatment processes. Detention ponds contained floating aquatic plants, i.e., *Ceratophyllum demersum*, *Hydrocotyle umbellata*, *Pistia stratiotes*, *Lemna minor*, and *Eichhornia crassipes*, with phytoremediation being the dominant process for wastewater treatment. Some glimpses of this facility are illustrated (Fig. 4).

Fig. 4. The municipal wastewater treatment facility (Bioremediation Orchard) constructed wetlands (top left) with aquatic macrophytes *Pistia stratiotes* in a (upper left), *Hydrocotyle umbellata* (lower left), and *Lemna minor* (lower right) in detention ponds.

Water movement from sedimentation ponds to constructed wetland to detention ponds is under gravity with zero electrical or mechanical input. The whole bioremediation facility was underlined with UV stabilized polyethylene plastic sheets (500 microns thick) to avoid wastewater percolation to deeper soil profiles and the underground aquifer. This wastewater reclamation facility was spread over an area of 2.83 hectares operating at a hydraulic retention time

of 7 days. Treated water was approximately 0.67 x 10^6 gallons/day and irrigated 97.12 hectares through flood irrigation and 194.24 hectares through a high efficiency irrigation system. This technology was completely based on the indigenous resources and proved cost effective as well as environment friendly to overcome water scarcity within NARC premises (Farid et al. 2014). Comparison of untreated and treated wastewater characteristics are described (Table 5). Water quality in the reclaimed/treated water significantly improved with respect to the total coliforms, fecal coliforms, total hardness, chemical oxygen demand, biological oxygen demand, cadmium, and bicarbonate levels rendering this municipal wastewater fit for agricultural usage especially for wheat production in NARC with limited human and environmental health risks. Successful application of this wastewater reclamation technology at NARC and its subsequent utilization in wheat production has opened ways to overcome national water scarcity in rain-fed and irrigated areas. The adopted technology befits national agro-climatic regions and can greatly boost the area under cultivation particularly the rain-fed locations which will further help in ensuring food security in times of increasing water scarcity.

Table 5. Comparison of untreated and treated wastewater for wheat production at the municipal wastewater reclamation facility at the National Agricultural Research Center, Islamabad (NEQ = National Environmental Quality Standard).

Parameter	Unit	NEQ	Untreated wastewater	Treated wastewater
pH		6.5–8.5	8.4	7.5
Electrical conductivity	dS/m	0–3	1.206	0.740
Total dissolved solids	mg/L	0–2,000	884	560
Turbidity	FTU		201.00	19.03
Salinity	mg/L		0.9	0.3
Chloride (Cl^{-1})	mg/L	0–1,065	536.00	74.85
Calcium (Ca^{+2})	mg/L	200	84.10	42.02
Magnesium (Mg^{+2})	mg/L	150	67.00	32.19
Free CO_2	mg/L		0	0
Carbonate (CO_3^{-2})	mg/L	0–3	0.15	0.0
Bicarbonate (HCO_3^{-1})	mg/L	0–610	640	130
Nitrate (NO_3^{-2})	mg/L	0–30	12	6
Sulfate (SO_4^{-2})	mg/L	0–960	48.18	25.45
Chromium (Cr)	mg/L	1	0.10	0.04
Cadmium (Cd)	mg/L	0.1	0.09	0.03
Total coliforms	MPN/100 mL	0–1,000	≥1,600	110
Fecal coliforms	MPN/100 mL	0–1,000	≥1,600	80
Chemical oxygen demand	mg/L	150	192	45
Biological oxygen demand	mg/L	80	127	25

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Available options to overcome food security issues in Pakistan in times of increasing water scarcity: II.

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Variations in hydraulic retention time (HRT), type of constructed wetland, substrates/aquatic plants used in wetlands, and detention ponds can greatly influence the treatment efficiency of the bioremediation system (Ali et al., pp. 53-54, this publication; Ali et al. 2013; Farid et al. 2014). After successful completion of the municipal wastewater treatment project that generated 0.67 x 10^6 gallons/day, another bioremediation facility (The Bioremediation Garden) was designed

and developed to harvest wastewater generated by the NARC offices and its residential colony. The purpose of establishing this facility was to bring more acreage under an irrigated regime within the NARC research station in Islamabad. The engineering design for this facility varied from the earlier models, and contained only five detention ponds preceding the constructed wetland (Fig. 5). Detention ponds contained only *Pistia stratiotes* and *Eichhornia crassipes*, whereas the constructed wetland contained physical substrates coupled with *Phragmites australis* and *Typha latifolia*. This facility was over an area of 0.16 hectares treating 0.053 x 10^6 gallons/day and operated at an HRT of 5 days. The treated water is continuously utilized in the safe irrigation of the wheat crop within our NARC premises. Comparison of untreated and treated wastewater is shown (Table 6) and all investigated parameters are within national environmental quality standards in the treated water.

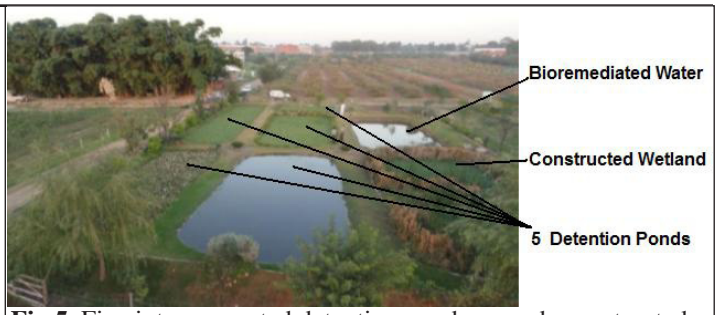

Fig 5. Five interconnected detention ponds precede constructed wetlands at the he Bioremediation Garden, National Agricultural Research Center, Islamabad.

Table 6. Comparison of untreated and treated wastewater for wheat production at the municipal wastewater reclamation facility at the National Agricultural Research Center, Islamabad (NEQ = National Environmental Quality Standard).

Parameter	Unit	NEQ	Untreated wastewater	Treated wastewater
pH		6.5–8.5	8.96	7.50
Electrical conductivity	dS/m	0–3	0.821	0.532
Total dissolved solids	mg/L	0–2,000	639	324
Turbidity	FTU		150.0	43.5
Chloride (Cl^{-1})	mg/L	0–1,065	236	47
Calcium (Ca^{+2})	mg/L	200	75	65
Magnesium (Mg^{+2})	mg/L	150	50.00	22.19
Free CO_2	mg/L		0	0
Carbonate (CO_3^{-2})	mg/L	0–3	0	0
Bicarbonate (HCO_3^{-1})	mg/L	0–610	450	320
Nitrate (NO_3^{-2})	mg/L	0–30	6.5	2.1
Sulfate (SO_4^{-2})	mg/L	0–960	41.0	30.5
Chromium (Cr)	mg/L	1	0.10	0.04
Cadmium (Cd)	mg/L	0.1	0.09	0.03
Total coliforms	MPN/100 mL	0–1,000	≥1,600	220
Fecal coliforms	MPN/100 mL	0–1,000	≥1,600	50
Chemical oxygen demand	mg/L	150	119	56
Biological oxygen demand	mg/L	80	98	66

Diverse, local aquatic flora is continuously monitored for phytoremediation potential and introduced in the existing detention ponds to optimize the bioremediation processes. Aptness of this wastewater treatment technology for national water scarce/limited regions is tremendous and urges ready devolution of this technology in our provincial areas such as Cholistan (Punjab) and Tharparkar (Sindh). These areas are truly rain-fed and currently suffering from famine like conditions leading to precious life losses. Increased wheat production in these areas only can be reached through increased fresh water supplies which currently are not feasible; however, biological reclamation of municipal wastewater may serve the purpose well. In Pakistan, 23 x 10^6 acre feet of wastewater is generated per annum, which, if treated wisely, can meet the national wheat crop irrigation demands. Allied research on wastewater quality, its biological treatment, and adequate utilization in wheat production can help meeting escalating population demands and ensuring food security issues imminent due to progressively declining water availability (Ali et al. 2013).

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Utilization and optimization of fourteen wheat quality standards for allocation of HMW glutenin subunits.

Hidayatullah, H. Ahmad, A. Ali, S.S. Lodhi, M. Jamil, Alvina Gul Kazi, and A. Mujeeb-Kazi.

Common wheat is a globally important agriculture crop that contributes significantly to human diet and is consumed in many diverse forms. Wheat is a staple food for about 40% of the world's population (Goyal and Prasad 2010; Peng et al. 2011). The wheat endosperm contains a major class of storage protein (glutenin) that plays a major role in bread making quality. These proteins are the foremost cause for the unique viscoelastic properties of wheat flour and dough. The polymeric glutenin proteins are separated into two subunit groups: high molecular weight glutenin subunits (HMW–GS) and low-molecular-weight glutenin subunits (LMW–GS), according to their motilities during sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE).

HMW–GS have the largest effect on bread-making quality despite preserving only 10% of the total storage proteins, compared to the LMW–GS, which contribute about 40% of the total flour protein. The HMW–GS are encoded by dominant genes at the *Glu-1* locus, *Glu-A1*, *Glu-B1*, and *Glu-D1*, located on the long arms of chromosomes 1A, 1B, and 1D, respectively (Payne et al. 1984). Each locus contains two tightly linked genes (*Glu-1-1* and *Glu-1-2*) encoding two different types of subunits, one large (80–88 KDa) x-type and one small (67–73 KDa) y-type subunits (Mackie et al. 1996).

Bread wheat generally exhibits three to five HMW–GS that are separated by using SDS-PAGE (Payne et al. 1984). Variation in the number of these subunits results from some specific gene silencing. Apart from the silencing of specific genes that leads to variation in the subunit number, allelic variation in these subunits encoded by active genes results in proteins with different electrophoretic motilities (Payne 1987; Shewry et al. 2001). Such allelic variation composition at each locus was found to be strongly associated with the various HMW–GS.

Consequently, different subunits have been identified and characterized, and the significant association between some subunits and quality traits found (Gianibelli 2001). This information has been used for screening because these subunits are highly polymorphic in nature and not environmentally affected (Payne et al. 1981). The identification of specific HMW-GS alleles is, therefore, as an important target for improving wheat quality (Gale 2005).

An important area of our Wheat Wide Crosses and Cytogenetics program at the NARC, Islamabad, is the identification of specific HMW–GS alleles for quality improvement and selection of high quality genotypes for the major wheat breeding program. We made electrophoretic analyses on diverse wheat genotypes for optimizing and utilizing 14 different wheat standards to identify HMW–GS allelic variation (Table 7, Figs. 6 and 7, p. 57). These quality standards were used for allocating and optimizing allelic designations at the *Glu-1* loci using 7.5% gels. This base line standard array will be beneficial in our characterizing the large genomic stocks to be evaluated later (Figs. 6 and 7).

Table 7. Wheat standards used for allocating allele designations of high-molecular-weight glutenin subunits.

#	Stand cultivar	Subunit composition	Source
1	ACA 303	2* 7+8 5+10	Liu et al. 2008
2	Chinese Spring	N 7+8 2+12	Das et al. 2001
3	Amadina	1 7+9 5+10	Liu et al. 2008
4	Blue Sky	2* 7+8 5+10	Cornish 2005
5	Darius	2* 7+8 2+12	
6	Opata M-85	2* 13+16 2+12	Rabinovich et al. 2000
7	Gabo	2* 17+18 2+12	Branlard et al. 2003
8	Glenlea	2* 7+8* 5+10	Ng and Pogna 1989
9	Pavon	2* 17+18 5+10	Liu et al. 2008
10	Insignia	1 20 5+10	Branlard et al. 2003
11	Halberd	1 20 5+10	Anonymous 1998
12	Marquis	1 7+9 5+10	Graybosh 1992
13	Norin 61	2* 7+8 2.2+12	Hua et al. 2005
14	Seri M-82	1 7+9 5+10	Payne and Peña 2006

Seeds of these standards were obtained from CIMMYT, Mexico (Dr. R.J. Peña). These seeds have been planted in the Wheat Wide Crosses and Cytogenetics screen house NARC during the current growing cycle (2013–14) for increase and possible distribution at the national and international level.

Endosperm was used for the subunit assays with the corresponding embryos germinated and respective seedlings used to produce pure seed for future studies. All 14 standards, except Darius, matched their subunit

designation. We will maintain the entries in our wheat program. A 5-g sample of each entry will be supplied for storage in the NARC gene bank. Requests for 10 seed/sample will be sent upon covering shipping and handling costs.

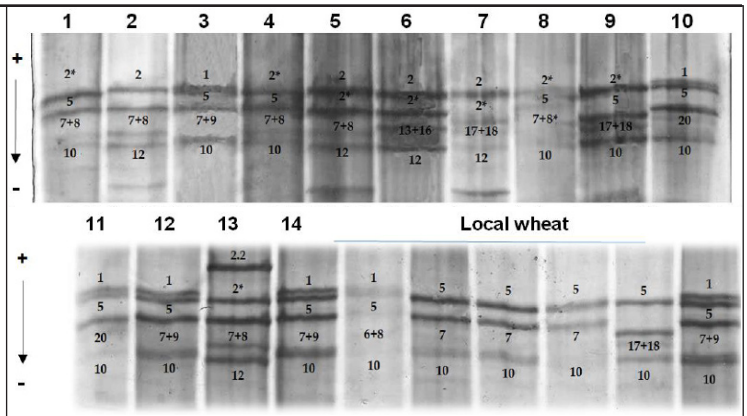

Fig. 6. Polyacrylamide gel electrophoresis profiles of high-molecular-weight glutenin subunit alleles of standard wheat cultivars.

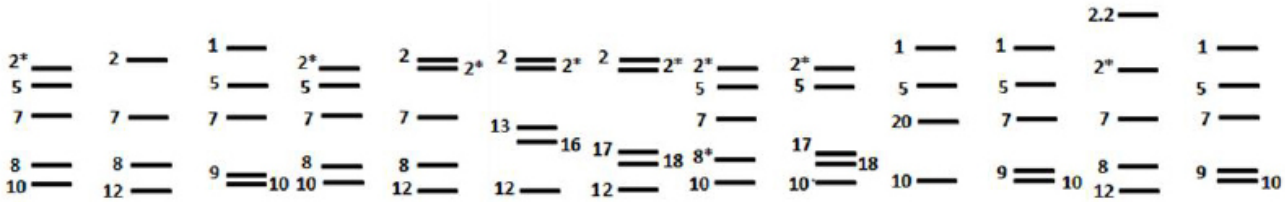

Fig. 7. Polyacrylamide gel electrophoresis profile of high-molecular-weight glutenin subunit alleles of standard wheat cultivars at the three *Glu-1* loci (L–R standards 1-14; because standards 10 and 11 are similar, cultivar 11 is not included.

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Genetic variation in diverse wheat germplasm for yield and yield-contributing traits.

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Wheat is the staple food for 35% of world's population (Ogbonnaya et al. 2013) and is, therefore, a key component of global food security (Mujeeb-Kazi et al. 2013). High wheat production may be achieved either by bringing more area under cultivation or enhancing yield per acre. Increasing the area under cultivation may possibly increase production but is not a feasible option, because global agricultural area is constantly decreasing due to urbanization, industrialization, soil salinization, and drought. Therefore, yield improvement is a plausible approach to enhance wheat production. Exploring the available genetic diversity can potentially lead to finding elite sources for inclusion in breeding for yield improvement. This study evaluated the extent of genetic diversity for individual yield traits in a diverse wheat germplasm.

The germplasm, consisting of 75 lines (Table 8), was evaluated under field conditions at National Agricultural Research Center (NARC), Islamabad, during the 2012–13 crop cycle in an augmented design. The material was hand-drilled in November 2012 in '6-m x 6-row' blocks with a row-to-row distance of 30 cm. The plant population/square meter square (plants/m^2) was 115. Standard cultural practices were followed to raise the crop to maturity. Data on days-to-maturity, plant height, tillers/plant, grains/spike, and spike length were recorded in March–April 2013 after physiological maturity. After harvesting, 1,000-kernel weight was recorded. Plant height was taken from the ground to the tip of top-most spike on three, randomly selected plants and averaged. Spike length was the average of three randomly selected plants. The number of spikes was taken from the three selected plants. Yield (g/m^2) was calculated by a formula ((yield (g/m^2) = number of plants/m^2 x number of tillers/plant x xumber of grains/spike x 1,000-kernel weight). Yield estimates for each genotype were g/m^2, using the number of plants/m^2 equal to 115 for all genotypes. The resulting data were extrapolated to t/ha. Data were analyzed in Excel 2010 for Summary Statistics. Traits showing considerably high genetic diversity were identified from the standard deviation.

Table 8. Germplasm type and number of genotypes evaluated for yield and yield-contributing traits. Source of germplasm is the CIMMYT and NARC Wheat Wide Crosses Programs.

Germplasm	# of genotypes
12x2	4
1x2	15
9x1	17
9x12	5
9x2	7
Ehydral	2
Kingbird	1
Mayoor/Ciano	2
Mayoor/Flycatcher	1
Mayoor/Opata	2
Nepal-AL	1
Sabuf/Ciano	4
Turaco/BCN	2
Turaco/Ciano	2
Turaco/Flycatcher	10
Total	**75**

Considerable genetic diversity was observed in almost all the quantitative traits under study (Table 9). The most notable variation was for plant height, grains/spike, grain yield, days-to-maturity, and 1,000-kernel weight. The higher the standard deviation of a trait, the higher was the genetic diversity of the material. This germplasm possesses enormous variation for these traits, which potentially can be utilized in future plant breeding. Decreased plant height is a desirable trait for abiotic stresses under irrigated planting, whereas reduced days-to-maturity is particularly important under terminal heat stress, which is vital under our conditions due to late wheat planting influenced by the cropping systems. Thousand-kernel weight supports overall yield potential of a cultivar.

Table 9. Variation in some quantitative traits of 75 wheat genotypes.

Variable	Mean	Minimum	Maximun	SD
Days-to-maturity	170.3	157.0	178.0	4.5
Plant height (cm)	99.9	68.0	131.0	9.3
Tillers/plant	9.9	7.0	14.0	1.5
Spike length (cm)	10.6	8.0	13.0	1.5
1,000-kernel weight (g)	37.3	28.0	50.0	4.3
Spikelets/spike	20.8	16.0	30.0	2.0
Grains/spike	62.3	48.0	90.0	6.1
Grain yield (g/m^2)	2,619.8	1,639.44	4,140.00	475.50
Grain yield (t/ha)	26.2	16.4	41.0	4.8

Recent findings on local wheat cultivars in Pakistan pinpoints the extent of variability and mean values obtained for a diverse set of wheat cultivars (Nawaz et al., 2013). Comparing our results to this study, we observed that our germplasm had promising wheat lines that outperformed. For example, cultivars Faisalabad-83 and SA-42 were outperformed by 46% for tillers/plant and 21.6% for grains/spike in our germplasm. Other traits, such as spikelets/plant, 1,000-kernel weight, and spike length, the best performing lines from our study out-performed the best (Nawaz et al. 2013) by 35%, 14%, and 11%, respectively.

Most notable results are those of grain yield (t/ha), which showed a remarkably high mean value compared to the current world record for wheat yield, i.e., 15.64 t/ha in the winter wheat cultivar Einstein, set by Mike Solari, a New Zealand farmer (http://www.farmersguardian.com/home/arable/arable-news/nz-farmer-beats-crop-world-record/31468).

The novel genetic diversity studied here is invaluable for breeding high yielding wheat cultivars for Pakistan, in particular, and globally via free germplasm exchange. The presence in the exploited germplasm has diversity from *Aegilops tauschii* (DD) and *Thinopyrum curvifolium* ($E^1E^1E^2E^2$), with several lines being resistant to other biotic stresses such as spot blotch, head scab, Karnal bunt, and *Septoria tritici*, making these lines a major parental source for recombination breeding around multiple stress resistance.

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Yield parameters of synthetic derivatives (bread wheat/synthetic hexaploid).

Farrukh Iqbal, M. Jamil, A.A. Napar, Z. Khan, S. Hussain, Alvina Gul Kazi, and A. Mujeeb-Kazi.

Synthetic derivatives of 269 lines were sown in the field at National Agricultural Research Center (NARC), Islamabad, during the 2012–13 crop cycle. Each line was grown in single, 1-m rows with a standard spacing. The rust-susceptible check cultivar Morocco, was sown after every 20 lines. Normal growing conditions were given and data was collected during May and after harvest. Only 260 of the 269 lines were successfully grown and data recorded for the following:

– Days-to-maturity was recorded when 50% of the plant color had turned golden brown.
– Fertile tillers of three plants were counted and averaged.
– Plant height (cm) was measured in centimeters from the ground to the tip of the spike (average for three spikes).
– Spike length (cm) of the main tiller of selected plants was measured from the base to the tip, excluding awns, and averaged.
– Spikelets/spike were counted from plants selected for spike length and averaged.
– Grains/spike of the main tiller were counted for each genotype after manual threshing.
– 1,000-kernel weight for each accession was determined by weighing 1,000-kernel samples from each line using an electronic balance.
– Karnal Bunt data was recorded by visual observation according to a scale of 1–5.

Data from 260 lines were recorded; 27 were selected on the basis of 1,000-kernel weight above 45 g (Table 10, p. 60). These 27 lines also are high yielding and will be used in further breeding programs. Entry number 23 has the highest weight and no rust, indicating resistance to biotic stress. Descriptive statistics were applied on 27 selected lines showing mean, standard error, mode, median, standard deviation, sample variance, range, minimum, maximum and sum of the data (Table 10). All selected lines had adult yellow rust scores ranging from 0 to 10 MRMS; only one plant was 60 MS.

Table 10. Descriptive statistics for in bread wheat/synthetic hexaploid derivatives.

Description	Mean	Standard Error	Median	Mode	Standard deviation	Sample variance	Range	Minimum	Maximum	Sum
Spike length (cm)	10.04	0.25	10	10	1.28	1.65	6	7	13	271
Plant height (cm)	90.22	1.28	91	82	6.68	44.71	23	79	102	2,436
Days-to-maturity	161.74	0.26	162	162	1.35	1.81	5	159	164	4,367
Tillers/plant	8.41	0.40	8	8	2.06	4.25	7	5	12	227
Spiklets/spike	20.15	0.28	20	19	1.43	2.05	5	18	23	544
Seeds/spike	58.11	1.65	61	61	8.59	73.87	30	44	74	1,569
1,000-kernel weight (g)	48.52	0.37	48	47	1.91	3.64	7	46	53	1,310

Exploitation of new genetic resources for water use efficiency and spot blotch (Cochliobolus sativus) resistance in bread wheat.

Muhammad Jamil, A. Ali, S. Hussain, A.A. Napar, K.F. Akbar, S. Asad, Alvina Gul Kazi, and A. Mujeeb-Kazi.

The food security vision for 2050 has put wheat production in the front line as the major conduit to provide for a global populace of 9.2 x 10^9. Thus, maximum yield is crucial and extremely challenging because the threat of climate change is significant. These changes can augment diminishing yield returns versus the influence of various abiotic (environmental) and biotic stresses. The latter encompass pathogen virulence shifts and migrations that lead to new stresses. One biotic stress that flared up in 2008–09 in parts of Pakistan was spot blotch. Two provinces of the country affected were the Upper Sindh and Lower Punjab. A major cultivar, Bhakkar, was consequently banned from subsequent cultivation and efforts to screen materials for resistance were initiated. We report our effort to assemble a wide germplasm array, including novel genomic diversity, as a first step to phenologically characterize a subset for future practical exploitation in the breeding targets.

One hundred lines of wheat diverse germplasm from CIMMYT nurseries 2012–13 were sown in three replicates then artificially inoculated with aggressive spore culture provided by the Crop Disease Research Institute at the National Agricultural Research Centre, Pakistan. This randomized complete block design and inoculation procedure followed that of Kumar et al. (2009). Data for plant habit was taken at growth stage GS:31 (Zadoks et al. 1974). At GS:32, the chlorophyll concentration index (CCI) was recorded using a Minolta SPAD-502 chlorophyll meter, and canopy temperature was measured by infrared thermometer MIKRON (IR-MAN) according to the set protocols by Pask et al. (2012). Using an infra-red gas analyzer, the rate of photosynthesis, transpiration rate, internal CO_2, and stomatal conductance was estimated.

Our results so far have enabled us to prescreen our germplasm on the basis of water use efficiency and chlorophyll concentration index. The purpose of the prescreening is to focus on broad-spectrum resistance regarding biotic and abiotic stress interactions. Because some master regulators relate biotic and abiotic stress responses in plants, a holistic approach must be used to identify and utilize broad spectrum stress tolerant genetic stocks (Atkinson and Urwin 2012).

For the phenological characters (Table 11, p. 61), we observed that the synthetic wheat line with *Ae. tauschii* has biotic and abiotic stress resistance in its pedigree; PBW343 and the back-cross derived line with Pastor are all abiotic stress tolerant, exhibit high water-use efficiency, and are candidate entries to be given high priority for biotic stress resistance utilization.

Significant correlation has been observed among rates of photosynthesis and transpiration, internal CO_2, and water-use efficiency (Table 12, p. 61). Plant habit also causes significant variation in stomatal conductance (Table 13, p. 61). Principal component and factor analysis for each generation are given (Table 14, p. 61).

In this ongoing study, the way forward will be disease scoring and genotyping through molecular marker application so that changes in these phenological characters during biotic stress can be mapped to explore the marker trait associations integrated with spot blotch resistance.

Table 11. Genotype from a set of 100 selected for eight with prominent attributes, especially water use efficiency.

Pedigree	#	Habit	Chlorophyll concentration index	Canopy temperature (°C)	Photosynthetic rate (μmol/m^2/s)	Transpiration rate (μmol/m^2/s)	Substomatal CO_2	Stomatal conductance	Water-use efficiency (μm CO_2/μm H_2O)
WBLL1*2/Kuruku//Heilo	45	2	49.03	20	14.31	1.30	27	0.10	11.01
PRL/2*Pastor	44	1	42.67	21	14.22	1.35	97	0.13	10.53
ROLF07*2/3/Prinia/Pastor//Huites	46	2	44.80	22	15.33	1.65	64	0.13	9.29
Kachu #1/Kiritati//Kachu	47	1	42.83	21	12.52	1.36	18	0.18	9.21
PBW343	43	1	43.30	22	9.82	1.11	101	0.08	8.85
D67.2/Parana 66.270//*Ae. tauschii* (320)/3/ Cunningham/4/VORB	16	1	43.27	20	13.35	1.54	53	0.10	8.67
Tacupetof 2001*2/Brambling//WBLL1*2/ Brambling	50	2	50.53	20	16.78	2.01	21	0.12	8.35
ROLF07*2/Diamondbird	65	2	44.07	20	13.71	1.65	56	0.08	8.31

Table 12. Correlation matrix (Pearson) for chlorophyll concentration index (CCI), canopy temperature (CT; °C), Photosynthetic rate (A; μmol/m^2/s), transpiration rate (E; μmol/m^2/s), substomatal CO_2 (C_I), and stomatal conducatance of H_2O (gs). Values in bold are different from 0 with a significance level alpha = 0.05.

Variable	Habit	CCI	CT	A	E	C_I	gs
CCI	0.119						
CT	–0.005	–0.098					
A	0.023	0.026	0.126				
E	0.048	–0.003	0.194	**0.566**			
C_I	–0.050	–0.066	–0.139	**–0.562**	–0.009		
gs	**0.257**	–0.136	0.127	0.066	0.093	0.000	
WUE	–0.033	0.038	–0.027	**0.569**	**–0.330**	**–0.620**	–0.019

Table 13. Single factor analysis of variance showing highly significant variation between habit and stomatal conductance

SOV	SS	MS	F	p
Habit	39.4139	19.70693	**15.25565**	**0.000002**
Error	125.3026	1.29178		
Total	164.7165			

Table 14. Principal component and factor analysis with eigenvalues and variability explained by each factor.

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	2.218	1.555	1.197	1.130	0.865	0.649	0.370	0.015
Variability (%)	27.725	19.441	14.968	14.123	10.807	8.117	4.627	0.192
Cumulative %	27.725	47.166	62.134	76.257	87.064	95.181	99.808	100.000

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Seedling evaluation for salt tolerance in D-genome synthetic hexaploid wheats.

Zeeshan Khan, M. Jamil, A.A. Napar, F. Iqbal, J. Qazi, Alvina Gul Kazi, and A. Mujeeb-Kazi.

Common bread wheat is a hexaploid (AABBDD) originating by natural hybridization of *Triticum turgidum* (AABB) and *Aegilops tauschii* (DD) (Dvorak et al. 1998). To enhance diversity, D-genome, synthetic hexaploid wheats (SH) were developed (Ogbonnaya et al. 2013). Several superior SH combinations for both abiotic/biotic stress resistance/tolerance have been reported (Mujeeb-Kazi 2003). For diversity to salinity, heat and drought have surfaced as vital parameters to target for wheat varietal development due to prevalent environmental changes and need to maximize yield for tackling global food security issues.

Salinity tolerance in wheat varies greatly at different growth stages. Therefore, assessment of wheat salinity tolerance preferably should be carried out at important growth stages. This integrated approach will allow us to identify the wheat growth stage that greatly reduces the crop production. Salinity can cause a significant reduction in development of spike (Maas and Grieve 1990), tillering ability, spikelets/spike, and grains/spike (Grattan and Grieve 1992; Akram et al. 2002). Wheat yield starts to decline at 6–8 dS/m (Maas and Hoffman 1977).

In this initial study, a set of primary SH entries were screened. The germplasm used a set of 231 synthetic hexaploid wheats that have been genotyped with 2000 DArT markers, and the phenotypic data will be used for association mapping. This germplasm was sown at the Bio-Saline Agriculture Research Station, Pakka Anna, near Faisalabad, in the 2013–14 crop cycle and was planted in November 2013 (Fig. 8). This station is an ideal site for salinity screening. The water used for irrigation at this site also is saline, with an ECw range from 3–4 dS/m. The seed were sown in a randomized complete block design with three replications, a row-to-row distance of 30 cm, and a 1-m row length.

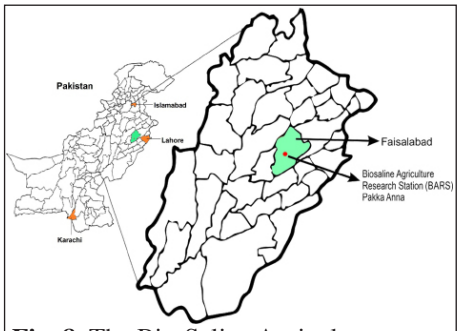

Fig. 8. The Bio-Saline Agriculture Research Station, Pakka Anna, near Faisalabad, Pakistan (longitude 73°05'E, latitude 31°24'N; elevation 190 m).

In vitro seedling growth was determined in an independent study carried out at Wheat Wide Crosses and Cytogenetic laboratory in NARC, Islamabad. Three treatments were used, one control (0 mM) and 2 NaCl treatments (75 mM and 150 mM). The top 19 genotypes are listed (Table 15, p. 63). The variability in frequency distribution is indicated in the histograms for height (Fig. 9, p. 63) and seedling weight (Fig. 10, p. 63) along with salt trait tolerance index (STTI). Pasban-91 and S-24 were used as tolerant checks and showed 17.91% and 41.37% STTI, respectively. PBW-343, with an STTI of 1.51%, was the susceptible check.

The centeral tendancy observed regarding seedling weight and height is that the treatments vary with the salt conentration.

An analysis of variance (Table 16, p. 64) for seedling height in 229 genotypes shows a highly significant variation in treatments and in genotypes along with their interaction, whereas the seedling weight was not significant.

Seedling height and the salt trait tolerance index show an 0.87 positive correlation coefficient, so regression highlights the relationship between seedling height and salt tolerance index with an $R^2 = 0.77$ variability explained.

Table 15. The top 19 of 226 genotypes with respect to the highest salt trait tolerance index (STTI %) for seedling height. * = *Aegilops tauschii* accession number of the WX working collection at CIMMYT, Mexico.

#	Germplasm	Pedigree	Genotype	Seedling height	STTI %
1	S-24	Local Check	2	2.00	41.37934
2	AUS30288	CROC_1/*Ae. tauschii* (466)*	12	5.83	35.71429
3	AUS34263	ARLIN_1/*Ae. tauschii* (225)	158	5.50	29.46429
4	AUS34262	ALTAR 84/*Ae. tauschii* (224)	157	5.00	27.77778
5	AUS34242	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (208)	137	4.50	26.73267
6	AUS34444	CROC_1/*Ae. tauschii* (210)	214	4.33	25.24272
7	AUS34264	GAN/*Ae. tauschii* (236)	159	4.50	24.54545
8	AUS33393	DVERD_2/*Ae. tauschii* (507)	95	2.50	23.07692
9	AUS30671	RASCON/*Ae. tauschii* (314)	76	3.67	21.56863
10	AUS34273	LARU/*Ae. tauschii* (309)	168	3.00	20.93023
11	AUS30664	GARZA/BOY//*Ae. tauschii* (281)	69	2.33	20.89552
12	AUS33398	CETA/*Ae. tauschii* (170)	100	2.50	20.83333
13	AUS34433	DOY1/*Ae. tauschii* (447)	203	3.50	20.00000
14	AUS34411	SORA/*Ae. tauschii* (211)	181	3.33	19.60784
15	AUS34443	CROC_1/*Ae. tauschii* (784)	213	3.16	19.58763
16	AUS34270	GARZA/BOY//*Ae. tauschii* (286)	165	3.00	19.14894
17	AUS34455	D67.2/P66.270//*Ae. tauschii* (213)	225	2.50	18.98734
18	AUS33405	GAN/*Ae. tauschii* (285)	107	3.16	18.44660
19	Pasban-90	Local check	1	2.00	17.91044

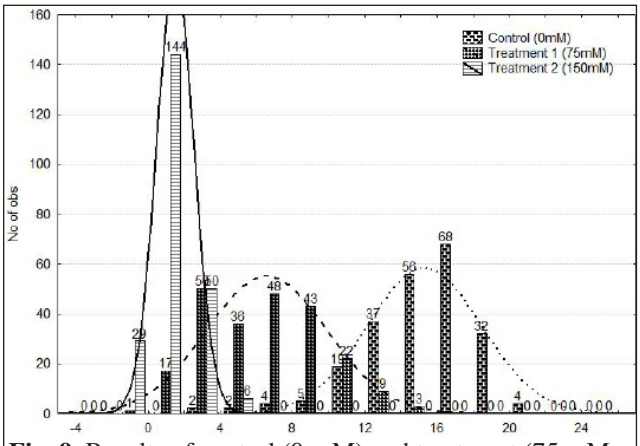

Fig. 9. Results of control (0 mM) and treatment (75 mM and 150 mM) effects on seedling height. In the control, (56+37) 93 genotypes ranged from 12 to 16 cm and (68+32) 100 genotypes ranged from 16 to 20 cm. In the 75 mM treatment (36+48) 84 genotypes ranges between 4–8 cm and (43+22) 65 genotypes ranged between 8–12 cm. In the 150 mM treatment, (144+50) 194 genotypes ranged from 0 to 4 cm and six genotypes were above 4 cm.

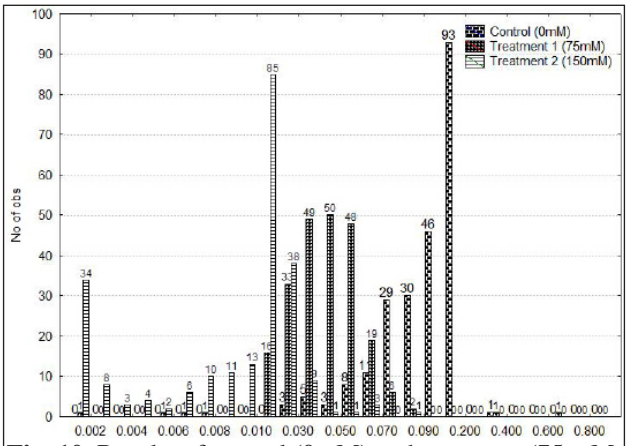

Fig. 10. Results of control (0mM) and treatment (75 mM and 150 mM) effects on seedling weight. In the control (29+30) 59 genotypes ranged between 0.07–0.09 g and (46+93) 139 genotypes ranged between 0.09–0.20 g. In the 75 mM treatment, (49+50) 99 genotypes ranged from 0.03–0.05 g and (48+19) 67 genotypes ranged from 0.05–0.07 g. In the 150 mM treatment, (85+38) 123 genotypes ranged between 0.01–0.03 g and nine genotypes are above 0.03 g.

The model equation for the salt trait tolerance index is:
STTI = 8.79+5.20*SH (cm)

From the equation obtained after regression analysis, we concluded that seedling height is directly proportional to the salt trait tolerance index (Fig. 11, p. 64).

Table 16. Analysis of variance highlighting significance in height of seedling; weight is nonsignificant.

		Seedling height (cm)				Seedling weight (g)			
	n	SS	MS	F	*p*	SS	MS	F	*p*
Genotype	228	8,771.7	38.5	16.26	0.00	1,150.93	5.04796	1.000689	0.487663
Treatment	2	66,218.4	33,109.2	13,991.82	0.00	11.26	5.63204	1.116476	0.327728
Genotype*treatment	456	6,206.0	13.6	5.75	0.00	2,300.45	5.04484	1.000072	0.494527
Error	1,374	3,251.3	2.4			6,931.12	5.04448		
Total	2,060	84,447.4				10,393.76			

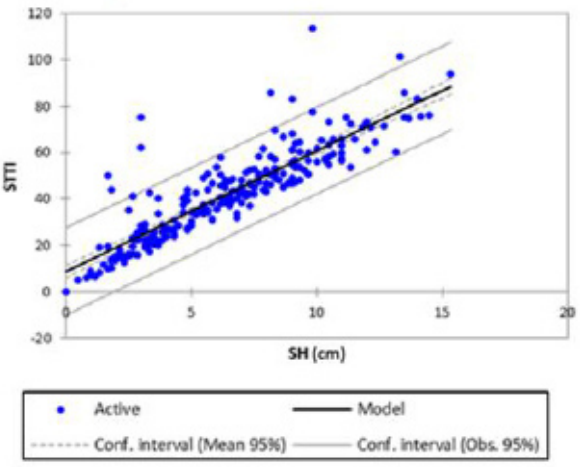

Fig. 11. Regression of salt trail tolerance index by seedling height (cm) ($R^2 = 0.770$).

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Digital imaging of selected landraces of Pakistan.

Sumaira S. Lodhi, Namra Haq, Peter John, Attya Bhatti, Alvina Gul Kazi, S. George, M. Jamil, and A. Mujeeb-Kazi.

Wheat improvement is based on harnessing genetic resource diversity that resides in the closely or distantly relatives of cereals within the primary, secondary, and tertiary gene pools. Detailed coverage on integrating such variability in wheat breeding programs was recently reviewed (Mujeeb-Kazi et al. 2013; Ogbonnaya et al. 2013). Germplasm within the primary gene pool is of high priority, because genetic affinity is high and recombination via homologous association is at a maximum. Landraces fall in this category and have been underutilized in wheat improvement. Reports from ICARDA showed the potential for barley landrace improvement, and we gain impetus from that effort to exploit the resources for wheat. This study covers a major segment of breeding value that targets seed size and shape.

Seed shape and size are one of the most important agronomic traits due to their effect on yield, eating quality, and market price. Plant research in genetics, functional analysis, and genomics can also benefit from quantitative evaluation of seed shape (Tanabata et al. 2012). In general, seed shape is scored in either manually or with computational methods. The manual method simply involves measuring seed length and width with callipers, but this method has a number of disadvantages. Computational methods, on the other hand, use digital imaging technology that enables one to automatically measure a variety of shape parameters of very small size in high-resolution images (Brewer et al. 2006; Bylesjö et al. 2008; Weight et al. 2008; French et al. 2009; Wang et al. 2009).

The progress in phenotyping is not at the same level as compared to high-throughput genotyping. More accurate, efficient, and high-throughput phenotyping in order to get maximum benefit from low-cost genotyping resources are needed (Houle et al. 2010). Photometric measurements or digital imaging provide briefer and cheaper phenotypic numbers and better elucidate the separate components of complex traits. Digital imaging is the process that converts images of plant organs into quantitative data. This process can increase the process of phenotyping, thus helping

to swiftly generate a large set of quantitative data. This method converts photographs into quantitative data based upon a measure of axes or pixel count. Digital imaging has the ability to demonstrate the dimensions of grain morphology contributing to grain weight and size. In wheat, these measurements might relate to traits such as yield or milling quality, which, in other ways, are quite costly to evaluate. Based upon this information, wheat seed shape might influence the possible endosperm to bran ratio. Thus, this process is important in providing us with the opportunity to evaluate the phenotypic and genetic components of seed, such as the wheat kernel.

We collected 211 landraces from different regions of Pakistan for this study (Table 17, pp. 65-69). All the accessions were photographed using a Nikon D5100 digital camera. Twenty-five sound and well developed seeds of each genotype were visually selected. Seeds were placed horizontally and vertically on black paper to provide color contrast (Figs. 12 and 13). Two photographs were taken at ~40 pixels/mm. All photographs were named according to accession and serial number.

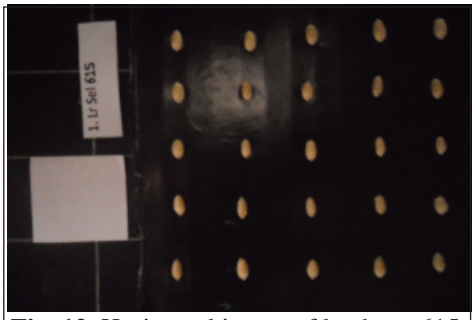

Fig. 12. Horizontal image of landrace 615.

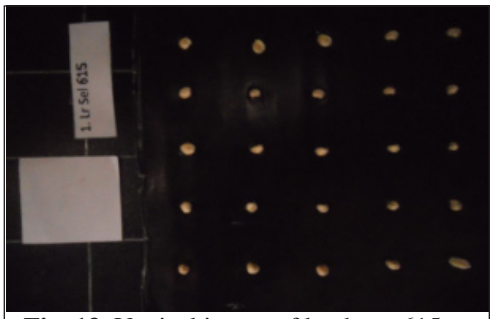

Fig. 13. Vertical image of landrace 615.

Table 17. Detail of landrace germplasm evaluated by digital imaging for seed shape and size.

#	Name	Detail
1	LRSEL 615	018836 *T. aestivum* PARC/MAFF 004290(01) 6/3/2001 Pakistan NWFP Swat Marghazar 1200
2	LRSEL 626	018849 *T. aestivum* PARC/MAFF 004297(04) 6/4/2001 Pakistan NWFP Chitral Bradam 1980
3	LRSEL 404	012103 *T. aestivum* PARC/JICA 003313(01) 6/1/1994 Pakistan NWFP Abbottabad Biroth 0920
4	LRSEL 406	012105 *T. aestivum* PARC/JICA 003315(01) 6/1/1994 Pakistan AJK Muzaffarabad Chattar 0750
5	LRSEL 620	018842 *T. aestivum* PARC/MAFF 004294(01) 6/4/2001 Pakistan NWFP Dir Darorah 1070
6	LRSEL 437	012219 *T. aestivum* PARC/JICA 003839(02) 7/1/1996 Pakistan NWFP Chitral Awilasht 2225
7	LRSEL 332	012012 *T. aestivum* PARC/JICA 003203(01) 6/1/1994 Pakistan NWFP Abbottabad Tarhanagala 1240
8	LRSEL 411	012116 *T. aestivum* PARC/ICARDA 003045(02) 5/1/1993 Pakistan Balochistan Kharan Karki/Kirichi 1250
9	LRSEL 619	018842 *T. aestivum* PARC/MAFF 004294(01) 6/4/2001 Pakistan NWFP Dir Darorah 1070
10	LRSEL 340	012021 *T. aestivum* PARC/JICA 003212(01) 6/1/1994 Pakistan NWFP Mansehra College Durai 0975
11	LRSEL 30	012010 *T. aestivum* PARC/JICA 003201(01) 6/1/1994 Pakistan NWFP Abbottabad Nawan Shehar 1135
12	LRSEL 321	011855 *T. aestivum* PARC/KUJ 002255(07) 10/1/1989 Pakistan AJK Muzaffarabad Pataka 0800
13	LRSEL 327	011931 *T. aestivum* PARC/NIAR 002827(02) 10/18/1991 Pakistan N. A. Gakuch Hatoon 1930
14	LRSEL 193	011521 *T. aestivum* PARC/IBPGR 001188(02) 10/1/1985 Pakistan Balochistan Awaran Jibri 1040
15	LRSEL 612	018833 *T. aestivum* PARC/MAFF 004288(06) 6/3/2001 Pakistan NWFP Swat Allahabad 1000
16	LRSEL 627	018850 *T. aestivum* PARC/MAFF 004297(05) 6/4/2001 Pakistan NWFP Chitral Bradam 1980
17	LRSEL 396	012079 *T. aestivum* PARC/JICA 003285(01) 6/1/1994 Pakistan F. A. Islamabad Pind begwal 0570
18	LRSEL 614	018835 *T. aestivum* PARC/MAFF 004289(02) 6/3/2001 Pakistan NWFP Swat Mena 1120
19	LRSEL 398	012081 *T. aestivum* PARC/JICA 003287(01) 6/1/1994 Pakistan F. A. Islamabad Pind begwal 0570
20	LRSEL 405	012104 *T. aestivum* PARC/JICA 003314(01) 6/1/1994 Pakistan AJK Muzaffarabad Kahala 0680
21	LRSEL 409	012114 *T. aestivum* PARC/ICARDA 003044(03) 5/1/1993 Pakistan Balochistan Kharan Chadman 1380
22	LRSEL 401	012084 *T. aestivum* PARC/JICA 003290(01) 6/1/1994 Pakistan F. A. Islamabad Karor 0990
23	LRSEL 394	012077 *T. aestivum* PARC/JICA 003283(01) 6/1/1994 Pakistan F. A. Islamabad Sary Chowk 0600
24	LRSEL 397	012080 *T. aestivum* PARC/JICA 003286(01) 6/1/1994 Pakistan F. A. Islamabad Pind begwal 0570
25	LRSEL 469	012284 *T. aestivum* PARC/JICA 003853(01) 7/1/1996 Pakistan N. A. Diamer Dazar 1595
26	LRSEL 455	012244 *T. aestivum* PARC/JICA 003691(01) 5/1/1996 Pakistan NWFP Buner Guswanda 0890
27	LRSEL 462	012269 *T. aestivum* PARC/JICA 003716(01) 6/1/1996 Pakistan NWFP Kohistan Patan 0820
28	LRSEL 634	018858 *T. aestivum* PARC/MAFF 004301(01) 6/4/2001 Pakistan NWFP Chitral Korepine 1500
29	LRSEL 402	012085 *T. aestivum* PARC/JICA 003291(01) 6/1/1994 Pakistan F. A. Islamabad Karor 1230
30	LRSEL 395	012078 *T. aestivum* PARC/JICA 003284(01) 6/1/1994 Pakistan F. A. Islamabad Pind begwal 0570
31	LRSEL 400	012083 *T. aestivum* PARC/JICA 003289(01) 6/1/1994 Pakistan F. A. Islamabad Karor 0990
32	LRSEL 485	012321 *T. aestivum* PARC/JICA 003892(01) 7/1/1996 Pakistan N. A. Ghanche Hushe 3280
33	LRSEL 457	012249 *T. aestivum* PARC/JICA 003697(01) 6/1/1996 Pakistan NWFP Swat Bekar 1430

Table 17. Detail of landrace germplasm evaluated by digital imaging for seed shape and size.

#	Name	Detail
34	LRSEL 459	012258 *T. aestivum* PARC/JICA 003705(01) 6/1/1996 Pakistan NWFP Swat Guza Sala 1200
35	LRSEL 625	018848 *T. aestivum* PARC/MAFF 004297(01) 6/4/2001 Pakistan NWFP Chitral Bradam 1970
36	LRSEL 236	011590 *T. aestivum* PARC/ICARDA/OSU 001306(01) 7/1/1986 Pakistan N. A. Gilgit Chalt 1660
37	LRSEL 314	011805 *T. aestivum* PARC/NIAR 002460(01) 5/1/1990 Pakistan N. A. Gilgit Masoth 1940
38	LRSEL 635	018859 *T. aestivum* PARC/MAFF 004301(03) 6/4/2001 Pakistan NWFP Chitral Korepine 1500
39	LRSEL 623	018846 *T. aestivum* PARC/MAFF 004296(01) 6/4/2001 Pakistan NWFP Dir Kotke 1280
40	LRSEL 6	011155 *T. aestivum* PARC/SVP 000197(01) 5/1/1981 Pakistan Balochistan Quetta Miangundi 1750
41	LRSEL 316	011807 *T. aestivum* PARC/NIAR 002461(01) 5/1/1990 Pakistan N. A. Gilgit Ali Abad 2180
42	LRSEL 581	018799 *T. aestivum* PARC/MAFF 6/2/2001 Pakistan NWFP 004277(01) Swat Shangla Pass 1940
44	LRSEL 244	011599 *T. aestivum* PARC/ICARDA/OSU 001315(05) 7/1/1986 Pakistan N. A. Gilgit Moor Khund 2480
45	LRSEL 461	012266 *T. aestivum* PARC/JICA 003714(01) 6/1/1996 Pakistan NWFP Dir Tormang 0950
46	LRSEL 638	018863 *T. aestivum* PARC/MAFF 004301(08) 6/4/2001 Pakistan NWFP Chitral Korepine 1500
48	LRSEL 195	011529 *T. aestivum* PARC/ICARDA 001210(02) 6/14/1986 Pakistan Balochistan Mastung Rangi Took 1860
49	LRSEL 308	011799 *T. aestivum* PARC/NIAR 002459(01) 5/1/1990 Pakistan N. A. Skardu Hanuchal 1420
50	LRSEL 163	011435 *T. aestivum* PARC/IBPGR 000610(07) 6/1/1982 Pakistan AJK Muzaffarabad Chibar 1550
52	LRSEL 309	011800 *T. aestivum* PARC/NIAR 5/1/1990 Pakistan N. A. 002459(02) Skardu Hanuchal 1420
53	LRSEL 307	011798 *T. aestivum* PARC/NIAR 002458(01) 5/1/1990 Pakistan N. A. Gilgit Parri 1350
54	LRSEL 145	011393 *T. aestivum* PARC/IBPGR 000593(04) 4/1/1982 Pakistan NWFP D.I. Khan Yarik 0300
55	LRSEL 151	011423 *T. aestivum* PARC/IBPGR 000604(03) 6/1/1982 Pakistan AJK Muzaffarabad Sharian 1250
56	LRSEL 310	011801 *T. aestivum* PARC/NIAR 002459(03) 5/1/1990 Pakistan N. A. Skardu Hanuchal 1420
57	LRSEL 311	011802 *T. aestivum* PARC/NIAR 002459(04) 5/1/1990 Pakistan N. A. Skardu Hanuchal 1420
58	LRSEL 389	012072 *T. aestivum* PARC/JICA 6/1/1994 Pakistan NWFP 003274(01) Nowsehra Pubbai 0290
59	LRSEL 392	012075 *T. aestivum* PARC/JICA 003277(01) 6/1/1994 Pakistan NWFP Nowsehra Pubbai 0290
60	LRSEL 385	012068 *T. aestivum* PARC/JICA 003268(01) 6/1/1994 Pakistan NWFP Malakand Durgai 0370
61	LRSEL 380	012063 *T. aestivum* PARC/JICA 003258(01) 6/1/1994 Pakistan NWFP Dir Rany 0830
62	LRSEL 190	011505 *T. aestivum* PARC/IBPGR 000936(06) 8/1/1983 Pakistan N. A. Gilgit Gulmit 2550
63	LRSEL 383	012066 *T. aestivum* PARC/JICA 003263(01) 6/1/1994 Pakistan NWFP Malakand Bat Khela 0670
64	LRSEL 384	012067 *T. aestivum* PARC/JICA 003266(01) 6/1/1994 Pakistan NWFP Malakand Durgai 0490
65	LRSEL 393	012076 *T. aestivum* PARC/JICA 003282(01) 6/1/1994 Pakistan F. A. Islamabad Sary Chowk 0600
66	LRSEL 312	011803 *T. aestivum* PARC/NIAR 002459(05) 5/1/1990 Pakistan N. A. Skardu Hanuchal 1420
67	LRSEL 408	012108 *T. aestivum* PARC/JICA 003320(01) 6/1/1994 Pakistan NWFP Abbottabad Turmothian 1280
68	LRSEL 152	011424 *T. aestivum* PARC/IBPGR 000605(01) 6/1/1982 Pakistan AJK Muzaffarabad Kucha 1010
69	LRSEL 379	012062 *T. aestivum* PARC/JICA 003256(01) 6/1/1994 Pakistan NWFP Dir Tamargarah 0770
70	LRSEL 386	012069 *T. aestivum* PARC/JICA 003270(01) 6/1/1994 Pakistan NWFP Mardan Rashakai 0320
71	LRSEL 387	012070 *T. aestivum* PARC/JICA 003271(01) 6/1/1994 Pakistan NWFP Mardan Rashakai 0320
72	LRSEL 388	012071 *T. aestivum* PARC/JICA 003272(01) 6/1/1994 Pakistan NWFP Mardan Rashakai 0320
73	LRSEL 334	012014 *T. aestivum* PARC/JICA 003205(01) 6/1/1994 Pakistan NWFP Abbottabad Tarhanagala 1240
74	LRSEL 362	012045 *T. aestivum* PARC/JICA 6/1/1994 Pakistan 003236(01) NWFP Swat Jaray 1230
75	LRSEL 685	018979 *T. aestivum* PARC/MAFF 004416(01) 4/28/2002 Pakistan NWFP Lakki Marwat Darra Jang 0470
76	LRSEL 331	012011 *T. aestivum* PARC/JICA 003202(01) 6/1/1994 Pakistan NWFP Abbottabad Nawan Shehar 1160
77	LRSEL 375	012058 *T. aestivum* PARC/JICA 003252(01) 6/1/1994 Pakistan NWFP Dir Talash 0890
78	LRSEL 358	012041 *T. aestivum* PARC/JICA 003231(01) 6/1/1994 Pakistan NWFP Swat Charbagh 1030
79	LRSEL 651	018880 *T. aestivum* PARC/MAFF 6/5/2001 Pakistan NWFP Chitral 004307(05) Green Lasht 1810
80	LRSEL 220	011571 *T. aestivum* PARC/ICARDA/OSU 001276(01) 7/22/1986 Pakistan N. A. Gilgit Hurmay 1850
81	LRSEL 246	011601 *T. aestivum* PARC/ICARDA/OSU 001318(01) 7/1/1986 Pakistan N. A. Gilgit Shakyot 1540
82	LRSEL 675	018926 *T. aestivum* PARC/MAFF 004360(01) 4/21/2002 Pakistan NWFP Lakki Marwat Basti Sultan Abad 0510
83	LRSEL 295	011783 *T. aestivum* PARC/NIAR 002598(07) 11/1/1989 Pakistan Balochistan Mastung Haji Ka 1720
84	LRSEL 333	012013 *T. aestivum* PARC/JICA 003204(01) 6/1/1994 Pakistan NWFP Abbottabad Tarhanagala 1240
85	LRSEL 319	011810 *T. aestivum* PARC/NIAR 002463(02) 5/1/1990 Pakistan N. A. Gilgit Rahimabad 1740
86	LRSEL 376	012059 *T. aestivum* PARC/JICA 003253(01) 6/1/1994 Pakistan NWFP Dir Talash 0890
87	LRSEL 245	011600 *T. aestivum* PARC/ICARDA/OSU 001317(01) 7/1/1986 Pakistan N. A. Gilgit Nafora Baseem 1320
88	LRSEL 496	013176 T. sp PARC/IBPGR 001189(04) 10/1/1985 Pakistan Balochistan Awaran Aladam 1100
89	LRSEL 234	011587 *T. aestivum* PARC/ICARDA/OSU 001295(01) 7/1/1986 Pakistan N. A. Baltistan Hajigam 2160
90	LRSEL 518	018688 *T. aestivum* PARC/PGRI 004104(01) 4/30/1999 Pakistan Punjab Chakwal Pind Kattha 0290
91	LRSEL 523	018693 *T. aestivum* PARC/PGRI 004109(01) 5/1/1999 Pakistan Punjab Chakwal Chakwal 0790

Table 17. Detail of landrace germplasm evaluated by digital imaging for seed shape and size.

#	Name	Detail
92	LRSEL 609	018830 *T. aestivum* PARC/MAFF 004288(03) 6/3/2001 Pakistan NWFP Swat Allahabad 1000
93	LRSEL 655	018884 *T. aestivum* PARC/MAFF 004308(03) 6/5/2001 Pakistan NWFP Chitral Kuragh 1910
94	LRSEL 616	018837 *T. aestivum* PARC/MAFF 004291(01) 6/3/2001 Pakistan NWFP Swat Islamapur 1090
95	LRSEL 621	018843 *T. aestivum* PARC/MAFF 004295(01) 6/4/2001 Pakistan NWFP Dir Chukiatan 1180
96	LRSEL 622	018844 *T. aestivum* PARC/MAFF 004295(04) 6/4/2001 Pakistan NWFP Dir Chukiatan 1180
97	LRSEL 242	011597 *T. aestivum* PARC/ICARDA/OSU 001314(03) 7/1/1986 Pakistan N. A. Gilgit Khaibar 2450
98	LRSEL 502	018667 *T. aestivum* PARC/MAFF 004271(01) 6/2/2001 Pakistan NWFP Swat Shangla 1600
99	LRSEL 357	012040 *T. aestivum* PARC/JICA 003230(01) 6/1/1994 Pakistan NWFP Swat Charbagh 1030
100	LRSEL 522	018692 *T. aestivum* PARC/PGRI 004108(01) 4/30/1999 Pakistan Punjab Chakwal Buchal Kalan 0680
101	LRSEL 247	011602 *T. aestivum* PARC/ICARDA/OSU 001319(02) 7/1/1986 Pakistan N. A. Gilgit Gitch 1660
102	LRSEL 438	012222 *T. aestivum* PARC/JICA 003841(01) 7/1/1996 Pakistan NWFP Chitral Hircheen 2830
103	LRSEL 342	012023 *T. aestivum* PARC/JICA 003214(01) 6/1/1994 Pakistan NWFP Mansehra Kotli Bala 0960
104	LRSEL 444	012228 *T. aestivum* PARC/JICA 003850(01) 7/1/1996 Pakistan NWFP Chitral Wadoos 1910
105	LRSEL 343	012025 *T. aestivum* PARC/JICA 003214(03) 6/1/1994 Pakistan NWFP Mansehra Kotli Bala 0960
106	LRSEL 674	018925 *T. aestivum* PARC/MAFF 004359(01) 4/21/2002 Pakistan NWFP Tank Pathan Colony 0490
107	LRSEL 443	012227 *T. aestivum* PARC/JICA 003849(01) 7/1/1996 Pakistan NWFP Chitral Gorapon 1685
108	LRSEL 417	012123 *T. aestivum* PARC/ICARDA 003066(04) 5/1/1993 Pakistan Balochistan Mastung Kolpur 1780
109	LRSEL 418	012144 *T. aestivum* PARC/SVP 000236(02) 6/1/1981 Pakistan Balochistan Ziarat Zandra 2100
110	LRSEL 281	011766 *T. aestivum* PARC/NIAR 002516(06) 10/1/1989 Pakistan N. A. Skardu Keris 2200
111	LRSEL 413	012119 *T. aestivum* PARC/ICARDA 003050(04) 5/1/1993 Pakistan Balochistan Kharan Baront 1250
112	LRSEL 359	012042 *T. aestivum* PARC/JICA 003232(01) 6/1/1994 Pakistan NWFP Swat Charbagh 1030
113	LRSEL 288	011776 *T. aestivum* PARC/NIAR 002591(02) 11/1/1989 Pakistan Balochistan Mastung Kahnak 1500
114	LRSEL 640	018865 *T. aestivum* PARC/MAFF 004302(02) 6/4/2001 Pakistan NWFP Chitral Chitral 1520
115	LRSEL 639	018864 *T. aestivum* PARC/MAFF 004302(01) 6/4/2001 Pakistan NWFP Chitral Chitral 1520
116	LRSEL 305	011796 *T. aestivum* PARC/NIAR 002456(01) 5/1/1990 Pakistan N. A. Chilas Ganni 1110
117	LRSEL 414	012120 *T. aestivum* PARC/ICARDA 003056(03) 5/1/1993 Pakistan Balochistan Kalat Jangan Wal 1830
119	LRSEL 210	011561 *T. aestivum* PARC/ICARDA/OSU 001264(01) 7/20/1986 Pakistan N. A. Gilgit Rai Kot 0950
122	LRSEL 381	012064 *T. aestivum* PARC/JICA 003260(01) 6/1/1994 Pakistan NWFP Dir Khal 0880
123	LRSEL 449	012233 *T. aestivum* PARC/JICA 003683(01) 5/1/1996 Pakistan NWFP Abbottabad Banda Sahib Khan 0910
124	LRSEL 645	018873 *T. aestivum* PARC/MAFF 004305(03) 6/5/2001 Pakistan NWFP Chitral Kosht 1600
125	LRSEL 617	018838 *T. aestivum* PARC/MAFF 004291(02) 6/3/2001 Pakistan NWFP Swat Islamapur 1090
126	LRSEL 399	012082 *T. aestivum* PARC/JICA 003288(01) 6/1/1994 Pakistan F. A. Islamabad Pind begwal 0570
127	LRSEL 679	018932 *T. aestivum* PARC/MAFF 004367(01) 4/21/2002 Pakistan NWFP Lakki Marwat Sarai Naurang 0560
128	LRSEL 296.	011787 *T. aestivum* PARC/NIAR 002450(01) 5/1/1990 Pakistan NWFP Malakand Malakand 0700
129	LRSEL 302	011793 *T. aestivum* PARC/NIAR 002452(04) 5/1/1990 Pakistan NWFP Swat Abuha 0810
130	LRSEL 652	018881 *T. aestivum* PARC/MAFF 004307(06) 6/5/2001 Pakistan NWFP Chitral Green Lasht 1810
131	LRSEL 636	018860 *T. aestivum* PARC/MAFF 004301(04) 6/4/2001 Pakistan NWFP Chitral Korepine 1500
132	LRSEL 328	011932 *T. aestivum* PARC/NIAR 002828(03) 10/20/1991 Pakistan NWFP Chitral Laspur Brook 2740
133	LRSEL 656	018885 *T. aestivum* PARC/MAFF 004308(04) 6/5/2001 Pakistan NWFP Chitral Kuragh 1910
134	LRSEL 301	011792 *T. aestivum* PARC/NIAR 002452(02) 5/1/1990 Pakistan NWFP Swat Abuha 0810
135	LRSEL 303	011794 *T. aestivum* PARC/NIAR 002453(01) 5/1/1990 Pakistan NWFP Swat Kandaray 1300
136	LRSEL 306	011797 *T. aestivum* PARC/NIAR 002457(01) 5/1/1990 Pakistan N. A. Chilas Governer Farm 1180
137	LRSEL 416	012122 *T. aestivum* PARC/ICARDA 003062(01) 5/1/1993 Pakistan Balochistan Quetta Hanna Lake 2000
138	LRSEL 658	018776 *T. aestivum* PARC/MAFF 004265(02) 6/1/2001 Pakistan NWFP Abbottabad Ferozabad 1240
139	LRSEL 153	011425 *T. aestivum* PARC/IBPGR 000605(02) 6/1/1982 Pakistan AJK Muzaffarabad Kucha 1010
140	LRSEL335	012016 *T. aestivum* PARC/JICA 003207(01) 6/1/1994 Pakistan NWFP Abbottabad Nika Pani 1195
141	LRSEL 135	011338 *T. aestivum* PARC/SVP 000431(01) 6/1/1981 Pakistan Balochistan Chagai Kitgai 0550
142	LRSEL 204	011547 *T. aestivum* PARC/ICARDA 001230(01) 6/17/1986 Pakistan Balochistan Pinhin Malang Abad 1380
143	LRSEL 351	012034 *T. aestivum* PARC/JICA 003224(01) 6/1/1994 Pakistan NWFP Mansehra Basi 1270
144	LRSEL 576	018794 *T. aestivum* PARC/MAFF 004273(01) 6/2/2001 Pakistan NWFP Swat Banjaar 1060
145	LRSEL 637	018861 *T. aestivum* PARC/MAFF 004301(06) 6/4/2001 Pakistan NWFP Chitral Korepine 1500
146	LRSEL 580	018798 *T. aestivum* PARC/MAFF 004276(01) 6/2/2001 Pakistan NWFP Swat Shangla Pass 2020
147	LRSEL 339	012020 *T. aestivum* PARC/JICA 003211(01) 6/1/1994 Pakistan NWFP Abbottabad Kalandar Abad 1250
148	LRSEL 320	011813 *T. aestivum* PARC/NIAR 002464(02) 5/1/1990 Pakistan N. A. Gilgit Juglote 1300
149	LRSEL 113	011312 *T. aestivum* PARC/SVP 000395(01) 5/1/1981 Pakistan Balochistan Chagai Lashkar Aab 0900

Table 17. Detail of landrace germplasm evaluated by digital imaging for seed shape and size.

#	Name	Detail
150	LRSEL 650	018879 *T. aestivum* PARC/MAFF 004306(08) 6/5/2001 Pakistan NWFP Chitral Burnes 1800
151	LRSEL 344	012027 *T. aestivum* PARC/JICA 003215(01) 6/1/1994 Pakistan NWFP Mansehra Rattar 1410
152	LRSEL 647	018875 *T. aestivum* PARC/MAFF 004306(01) 6/5/2001 Pakistan NWFP Chitral Burnes 1800
153	LRSEL 129	011332 *T. aestivum* PARC/SVP 000426(03) 6/1/1981 Pakistan Balochistan Ziarat Spinzandi 2280
154	LRSEL 348	012031 *T. aestivum* PARC/JICA 003218(01) 6/1/1994 Pakistan NWFP Mansehra Lilishan 1530
155	LRSEL 453	012238 *T. aestivum* PARC/JICA 003687(01) 5/1/1996 Pakistan NWFP Mansehra Hansherian 0850
156	LRSEL 578	018796 *T. aestivum* PARC/MAFF 004274(02) 6/2/2001 Pakistan NWFP Swat Rahimabad 1280
157	LRSEL 589	018809 *T. aestivum* PARC/MAFF 004280(02) 6/2/2001 Pakistan NWFP Swat Madyan 1340
158	LRSEL 25	011185 *T. aestivum* PARC/SVP 000220(01) 5/1/1981 Pakistan Balochistan Kharan 0800
159	LRSEL 72	011249 *T. aestivum* PARC/SVP 000261(05) 6/1/1981 Pakistan Balochistan Quetta Spezand 1710
160	LRSEL 587	018807 *T. aestivum* PARC/MAFF 004279(05) 6/2/2001 Pakistan NWFP Swat Khawaza Khala 1100
161	LRSEL 73	011252 *T. aestivum* PARC/SVP 000264(02) 6/1/1981 Pakistan Balochistan Quetta Near Lakh pass 1740
163	LRSEL 15	011171 *T. aestivum* PARC/SVP 000211(02) 5/1/1981 Pakistan Balochistan Kalat Halizai 1630
164	LRSEL 144	011392 *T. aestivum* PARC/IBPGR 000594(04) 4/1/1982 Pakistan NWFP D.I. Khan 0305
165	LRSEL 201	011543 *T. aestivum* PARC/ICARDA 001225(04) 6/17/1986 Pakistan Balochistan Quetta Baleli 1400
166	LRSEL 591	018811 *T. aestivum* PARC/MAFF 004281(01) 6/2/2001 Pakistan NWFP Swat Bahrain 1400
167	LRSEL 86	011274 *T. aestivum* PARC/SVP 000362(01) 5/1/1981 Pakistan Balochistan Pishin Sabura Post 2200
168	LRSEL 38	011200 *T. aestivum* PARC/SVP 000229(01) 5/1/1981 Pakistan Balochistan Pishin Barozai 1570
169	LRSEL 505	018671 *T. aestivum* PARC/PGRI 004082(01) 4/28/1999 Pakistan NWFP Chakwal Talial 0700
170	LRSEL 575	018793 *T. aestivum* PARC/MAFF 004270(03) 6/1/2001 Pakistan NWFP Mansehra Chappargram 1100
171	LRSEL 649	018877 *T. aestivum* PARC/MAFF 004306(03) 6/5/2001 Pakistan NWFP Chitral Burnes 1800
172	LRSEL 16	011172 *T. aestivum* PARC/SVP 000211(03) 5/1/1981 Pakistan Balochistan Kalat Halizai 1630
173	LRSEL 28	011188 *T. aestivum* PARC/SVP 000223(01) 5/1/1981 Pakistan Balochistan Chagai Nushki 1250
174	LRSEL 58	011229 *T. aestivum* PARC/SVP 000247(02) 6/1/1981 Pakistan Balochistan Loralai 1820
175	LRSEL 454	012240 *T. aestivum* PARC/JICA 003688(01) 5/1/1996 Pakistan NWFP Mansehra Khar Mang Bala 1300
176	LRSEL 594	018814 *T. aestivum* PARC/MAFF 004282(02) 6/3/2001 Pakistan NWFP Swat Rahmet 1540
177	LRSEL 143	011391 *T. aestivum* PARC/IBPGR 000595(02) 4/1/1982 Pakistan NWFP D.I. Khan 0360
178	LRSEL 212	011563 *T. aestivum* PARC/ICARDA/OSU 001266(01) 7/21/1986 Pakistan N. A. Gilgit Sakowar 1400
179	LRSEL 26	011186 *T. aestivum* PARC/SVP 000221(01) 5/1/1981 Pakistan Balochistan Kharan 1070
180	LRSEL 138	011341 *T. aestivum* PARC/SVP 6/1/1981 Pakistan Balochistan 000436(03) Chagai Kitaka 0950
181	LRSEL 586	018806 *T. aestivum* PARC/MAFF 004279(04) 6/2/2001 Pakistan NWFP Swat Khawaza Khala 1100
182	LRSEL 663	018894 *T. aestivum* PARC/MAFF 004311(01) 6/6/2001 Pakistan NWFP Chitral Bamburet 2010
183	LRSEL 664	018896 *T. aestivum* PARC/MAFF 004312(02) 6/6/2001 Pakistan NWFP Chitral Bamburet 1900
184	LRSEL 205	011549 *T. aestivum* PARC/ICARDA 001236(03) 6/18/1986 Pakistan Balochistan Pinhin Gawal 1570
185	LRSEL 76	011256 *T. aestivum* PARC/SVP 000265(05) 6/1/1981 Pakistan Balochistan Quetta 1720
186	LRSEL 95	011290 *T. aestivum* PARC/SVP 000377(01) 5/1/1981 Pakistan Balochistan Pishin Sheikh Wasil 1550
187	LRSEL 105	011303 *T. aestivum* PARC/SVP 000390(01) 5/1/1981 Pakistan Balochistan Chagai Peeshok 0850
188	LRSEL 7	011156 *T. aestivum* PARC/SVP 000198(01) 5/1/1981 Pakistan Balochistan Mastung Bolan 1630
189	LRSEL 209	011560 *T. aestivum* PARC/ICARDA 001261(01) 6/24/1986 Pakistan Balochistan Qila Saifullah – 1380
190	LRSEL 648	018876 *T. aestivum* PARC/MAFF 004306(02) 6/5/2001 Pakistan NWFP Chitral Burnes 1800
191	LRSEL 642	018868 *T. aestivum* PARC/MAFF 004303(03) 6/5/2001 Pakistan NWFP Chitral Kari 1510
192	LRSEL 666	018913 *T. aestivum* PARC/MAFF 004353(03) 4/20/2002 Pakistan NWFP D.I. Khan Darahan 0420
193	LRSEL 590	018810 *T. aestivum* PARC/MAFF 004280(03) 6/2/2001 Pakistan NWFP Swat Madyan 1340
195	LRSEL 583	018801 *T. aestivum* PARC/MAFF 004278(01) 6/2/2001 Pakistan NWFP Swat Sherai 1150
196	LRSEL 391	012074 *T. aestivum* PARC/JICA 003276(01) 6/1/1994 Pakistan NWFP Nowsehra Pubbai 0290
197	LRSEL 570	018788 *T. aestivum* PARC/MAFF 004269(01) 6/1/2001 Pakistan NWFP Mansehra Chattar Plain 1560
198	LRSEL 572	018790 *T. aestivum* PARC/MAFF 004269(03) 6/1/2001 Pakistan NWFP Mansehra Chattar Plain 1560
199	LRSEL 657	018886 *T. aestivum* PARC/MAFF 004309(01) 6/5/2001 Pakistan NWFP Chitral Cherun 1900
200	LRSEL 114	011313 *T. aestivum* PARC/SVP 000396(01) 5/1/1981 Pakistan Balochistan Chagai Isa Chah 0930
201	LRSEL 451	012235 *T. aestivum* PARC/JICA 003684(02) 5/1/1996 Pakistan NWFP Abbottabad Tanan Gali 1240
202	LRSEL 103	011301 *T. aestivum* PARC/SVP 000388(01) 5/1/1981 Pakistan Balochistan Chagai Cheattar 0850
203	LRSEL 62	011236 *T. aestivum* PARC/SVP 000253(01) 6/1/1981 Pakistan Balochistan Sibi Korjk 0190
204	LRSEL 111	011310 *T. aestivum* PARC/SVP 000394(03) 5/1/1981 Pakistan Balochistan Chagai Peeshuk 0910
205	LRSEL 510	018679 *T. aestivum* PARC/PGRI 004093(02) 4/29/1999 Pakistan Punjab Chakwal Chabbar 0765
206	LRSEL 511	018680 *T. aestivum* PARC/PGRI 004094(01) 4/29/1999 Pakistan Punjab Chakwal Buchal Khurd 0890

Table 17. Detail of landrace germplasm evaluated by digital imaging for seed shape and size.

#	Name	Detail
207	LRSEL 646	018874 *T. aestivum* PARC/MAFF 004305(04) 6/5/2001 Pakistan NWFP Chitral Kosht 1600
208	LRSEL 200	011537 *T. aestivum* PARC/ICARDA 001222(01) 6/16/1986 Pakistan Balochistan Quetta – 1540
210	LRSEL 659	018888 *T. aestivum* PARC/MAFF 004310(02) 6/5/2001 Pakistan NWFP Chitral Booni 2080
211	LRSEL 139	011342 *T. aestivum* PARC/SVP 000437(03) 6/1/1981 Pakistan Balochistan Chagai Kachao 1250

Smart Grain software (version 12) was used to analyze the seed images. This software helped us to calculate area, perimeter length, length, width, length-to-width ratio, circularity, and distance between IS and CG. To find out the appropriate combinations of studied attributes, principal component and bi-plot analyses were made using mean values. The Pearson correlation co-efficient revealed that highest correlation was observed between area and perimeter (89.9%) followed by perimeter and length (89.2%), area and length (85.9%), area and width (82.7%). Area was positively co-related with perimeter, length, and width, i.e., the greater the seed area, the greater the perimeter, length, and width. The Pearson correlation among the different seed variables is shown (Table 18).

Table 18. Pearson correlation matrix recorded for 200 Pakistani wheat landraces.

	Area	Perimeter	Length	Width	Length:width	Circularity	Distance
Area							
Perimeter	0.899						
Length	0.859	0.892					
Width	0.827	0.677	0.461				
Length:width	–0.067	0.121	0.428	-0.600			
Circularity	–0.109	–0.527	–0.350	0.063	–0.398		
Distance	0.063	0.174	0.267	–0.219	0.481	–0.299	1.000

The vector length shows the extent of variation explained by respective traits in the principal component analysis. The first two components showed 80.57% of the total variation. Considering PC1 and PC2, most of the components, such as area, perimeter, and length, contribute positively to PC1 (Fig. 14). The correlation matrix indicated that area, perimeter, and length were highly correlated, thus, they belonged to the same group. The length-to-width ratio, distance, and circularity form a group of negatively correlated entries.

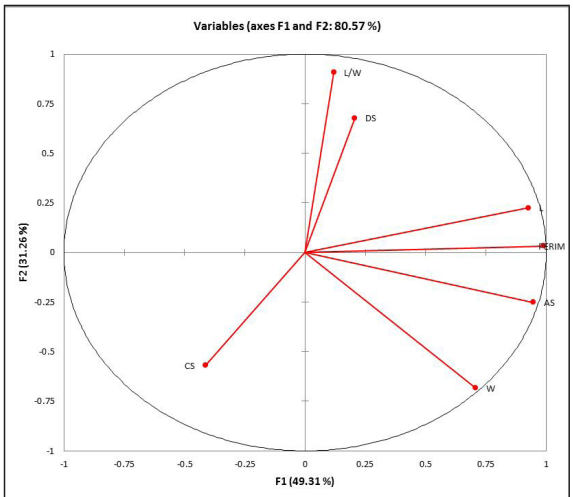

Fig. 14. Principal component analysis bi-plot of area (AS), perimeter (PERIM), length (L), width (W), the length-to-width ratio (L/W), circularity (CS), and distance (DS) on the first two principle components.

References.

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Exploring the genetic diversity for yellow rust resistance and yield contributing traits in wheat landraces.

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With increasing population growth, wheat production is crucial. To accomplish this, additional genetic diversity for major biotic and abiotic stresses is needed. Hence, cultivar improvement with new allelic genetic diversity is an approach that has global interest (Mujeeb-Kazi et al. 2013; Ogbonnaya et al. 2013). Landrace diversity has been underutilized in wheat improvement but holds high value and also is receiving our attention. The performance of Pakistani wheat landraces for grain yield contributing traits in relation to yellow rust diversity is of interest and is a facet that has previously delivered many novel genes for resistance to diseases of wheat. (Repellin 2001).

This ongoing study was envisioned to characterize and evaluate for stripe rust resistance and yield contributing traits in a set of 174 wheat landraces that included some historical set of Pakistan's wheat cultivars. During the 2012–13 wheat growing season, a study conducted at Wheat Wide Crosses Program, National Agricultural Research Center (NARC), Islamabad, Pakistan, to assess genetic diversity for stripe rust resistance and yield-contributing traits. The germplasm was comprised of 174 Pakistani wheat landraces, including historical wheat cultivars acquired from the Plant Genetic Resources Programme, NARC, Islamabad. All accessions were planted in 1-m rows with a 30-cm row-to-row distance. The entire trial was bordered by a rust susceptible spreader (Morocco). Recommended cultural practices were uniformly followed.

Percent severity and reaction of each accession was estimated according to a Modified Cobb's Scale (Peterson et al. 1948; Roelfs et al. 1992). Stripe rust was scored at heading, when the susceptible spreader exhibited maximum disease severity. Data was recorded on plant height, number of spikes/plant, spike length, number of days-to-maturity, and 1,000-kernel weight. A coefficient of infection (CI) was computed by multiplying disease severity (DS) with constant values of infection type (IF). Constant values for infection types were used based on the following: resistance = 0.1, moderate resistance = 0.25, medium = 0.5, moderate susceptibility = 0.75, susceptibility = 1.0 (Pathan et al. 2006) (Table 19). Data on yield-contributing traits were then statistically analyzed for mean, standard deviation, standard error, and Pearson's correlation. Data was streamlined by transforming number of correlated variables into a smaller number of variables through principal component analysis.

Rust severity on the screened genotypes describes the resistance behavior. To calculate coefficient of infection (CI) the data on disease severity and host reaction was combined according to (Sajid et al. 2007). Genotypes with CI values of 0–20%, 21–40%, and 41–60% were considered as having high, moderate, and low levels of adult-plant resistance, respectively. The results revealed that disease stress was noticeably high, as shown by CI of susceptible spreader (Table 19, pp. 70-71). In first group, 102 accessions have CI values of 0–20%, and are considered resistant to relatively better resistance for stripe rust severity. Within the first group, the lowest CI values (0.5–1.0%) for six accessions (49, 58, 65, 71, 72, and 165) were resistant to rust. Forthy-four ccessions in the second group, with CI values of 21–40%, were estimated to be moderately resistant. The last group was comprised of nine accessions with CI values of 41–60%. Nineteen accessions showed stripe rust severity of more than 60% and were rated susceptible. Similarly,

Table 19. Adult-plant infection type and coefficient of infection in a subset of Pakistani landraces. For Reaction, R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible.

Genotype	Reaction	CI	Genotype	Reaction	CI	Genotype	Reaction	CI	Genotype	Reaction	CI
49	R	0.5	20	MR-MS	15.0	69	MS	22.5	158	MS-S	50.0
58	R	0.5	23	MR-MS	15.0	75	MS	22.5	77	S	50.0

Table 19. Adult-plant infection type and coefficient of infection in a subset of Pakistani landraces. For Reaction, R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible.

Genotype	Reaction	CI	Genotype	Reaction	CI	Genotype	Reaction	CI	Genotype	Reaction	CI
65	R	0.5	12	MR-MS	15.0	91	S	20.0	143	S	50.0
72	R	0.5	25	MR-MS	15.0	102	S	20.0	145	S	50.0
71	R	1.0	27	MR-MS	15.0	29	MR-MS	25.0	146	S	50.0
165	R	1.0	33	MR-MS	15.0	32	MR-MS	25.0	37	S	60.0
NARC-09	MR	1.25	40	MR-MS	15.0	34	MR-MS	25.0	96	S	60.0
NARC-11	MR	1.25	45	MR-MS	15.0	35	MR-MS	25.0	105	S	60.0
4	MR	1.25	61	MR-MS	15.0	67	MR-MS	25.0	106	S	60.0
14	R-MR	1.25	62	MR-MS	15.0	111	MR-MS	25.0	108	S	60.0
38	MR	2.5	63	MR-MS	15.0	125	MR-MS	25.0	112	S	60.0
80	MR	2.5	70	MR-MS	15.0	134	MR-MS	25.0	159	S	60.0
131	MR	2.5	79	MR-MS	15.0	135	MR-MS	25.0	170	S	60.0
142	MR	2.5	85	MR-MS	15.0	157	MR-MS	25.0	176	S	60.0
149	MR	2.5	92	MR-MS	15.0	184	MR-MS	25.0	182	S	60.0
150	MR	2.5	114	MR-MS	15.0	188	MR-MS	25.0	109	S	70.0
151	MR	2.5	116	MR-MS	15.0	199	MR-MS	25.0	178	S	70.0
153	MR	2.5	120	MR-MS	15.0	200	MR-MS	25.0	187	S	70.0
168	MR	2.5	127	MR-MS	15.0	43	MR-MS	30.0	104	S	80.0
15	MR-MS	2.5	129	MR-MS	15.0	177	MS-S	30.0	122	S	80.0
18	MR	5.0	154	MR-MS	15.0	22	S	30.0	44	S	100.0
56	MR	5.0	161	MR-MS	15.0	55	S	30.0	119	S	100.0
132	MR	5.0	173	MR-MS	15.0	88	S	30.0	133	S	100.0
141	MR	5.0	174	MR-MS	15.0	94	S	30.0	156	S	100.0
144	MR	5.0	183	MR-MS	15.0	100	S	30.0	Morocco	S	100.0
189	MR	5.0	186	MR-MS	15.0	107	S	30.0			
16	MR-MS	5.0	190	MR-MS	15.0	128	S	30.0			
41	MR-MS	5.0	194	MR-MS	15.0	137	S	30.0			
42	MR-MS	5.0	196	MR-MS	15.0	140	S	30.0			
66	MR-MS	5.0	9	MR-MS	20.0	197	S	30.0			
117	MR-MS	5.0	11	MR-MS	20.0	13	MS-S	40.0			
123	MR-MS	5.0	17	MR-MS	20.0	21	S	40.0			
162	MR-MS	5.0	19	MR-MS	20.0	50	S	40.0			
1	MR-MS	10.0	26	MR-MS	20.0	54	S	40.0			
2	MR-MS	10.0	39	MR-MS	20.0	64	S	40.0			
30	MR-MS	10.0	60	MR-MS	20.0	95	S	40.0			
31	MR-MS	10.0	76	MR-MS	20.0	99	S	40.0			
46	MR-MS	10.0	83	MR-MS	20.0	110	S	40.0			
47	MR-MS	10.0	103	MR-MS	20.0	118	S	40.0			
48	MR-MS	10.0	115	MR-MS	20.0	130	S	40.0			
87	MR-MS	10.0	124	MR-MS	20.0	136	S	40.0			
121	MR-MS	10.0	163	MR-MS	20.0	138	S	40.0			
126	MR-MS	10.0	166	MR-MS	20.0	148	S	40.0			
160	MR-MS	10.0	169	MR-MS	20.0	152	S	40.0			
167	MR-MS	10.0	181	MR-MS	20.0	155	S	40.0			
180	MR-MS	10.0	198	MR-MS	20.0	175	S	40.0			
185	MR-MS	10.0	52	S	20.0	84	S	50.0			
193	MR-MS	10.0	53	S	20.0	89	S	50.0			
3	MR-MS	15.0	82	S	20.0	113	S	50.0			
7	MR-MS	15.0	86	S	20.0	139	S	50.0			

Mirza et al. (2000) and Sajid et al. (2009) also conducted field evaluations of quantitative resistance to stripe rust, finding that the resistance level established on disease severity went from very low to very high among the evaluated genotypes.

Grain yield and yield-contributing traits are important for assessing yield potential (Zamarud et al. 2007). These traits are complex characteristics and known to be the cumulative outcome of different physiological processes. Principal component and factor analyses (Fig. 15) revealed that 57.07% of the variability was explained by the F1 and F2. Moreover, the F1, with an Eigenvalue of 2.089 explained the variation of days-to-maturity, spike length, and plant height, and the F2, with Eigenvalue of 1.335, described the variation in the coefficient of infection, spikes/plant, and 1,000-kernel weight (Table 20). According to Saif et al. (2013), 1,000-kernel weight was an important yield-contributing trait. The coefficient of infection is negatively correlated with all yield components (Table 21). Days-to-phenotypic-maturity was highly correlated with plant height and spike length. These results agree with those of Sunderman and Wise (1964).

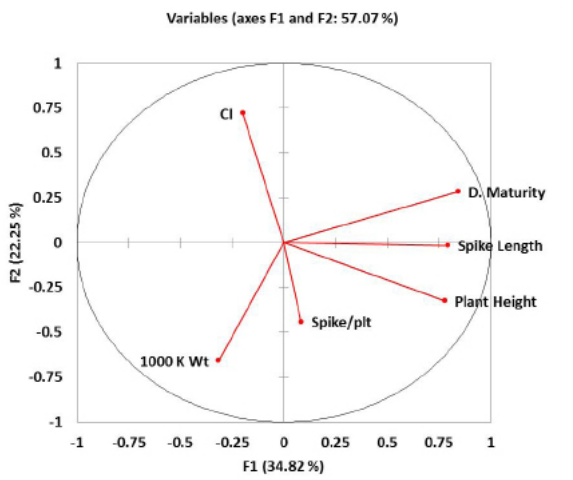

Fig. 15. Principal component analysis of the coefficient of infection (CI), days-to-maturity, spike length, plant height, spikes/plant, and 1,000-kernel weight in a subset of Pakistani landraces.

Table 20. Factors with Eigenvalues and % variability explained.

	F1	F2	F3	F4	F5	F6
Eigenvalue	2.089	1.335	1.081	0.708	0.470	0.316
Variability (%)	34.816	22.252	18.018	11.803	7.841	5.270
Cumulative (%)	34.816	57.068	75.086	86.889	94.730	100.000

Table 21. Pearson's correlation matrix (CI = coefficient of infection and DM = days-to-maturity).

Variable	Plant height	Spikes/ plant	Spike length	CI	DM
Spikes/plant	0.266				
Spike length	0.443	–0.070			
CI	–0.205	–0.073	–0.160		
DM	0.503	–0.097	0.546	0.012	
1,000-kernel weight	–0.030	–0.027	–0.103	–0.208	–0.328

These findings show that the genotypes had diversity to stripe rust resistance, ranging from resistant to moderately resistant and moderately resistant to susceptible genotypes. About 3% of the evaluated germplasm exhibited resistance, 59% were moderately resistant, and 33% were susceptibe. Landraces 49, 58, 65, 71, 72, and 165 were resistant and expected to have resistance genes. Landraces 4, 18, 38, 56, 80, 131, 132, 141, 142, 144, 149, 150, 151, 153, 168, and 189 may have different degrees of slow rusting. These genotypes can be used for upcoming breeding for stripe rust resistance after the ongoing confirmatory studies. Our Wheat Wide Crosses Program at the NARC has already acquired wheat landraces of different origins to carryout studies regarding genetic diversity for yellow rust resistance and yield-contributing traits. Our contention is that novel landraces may be an arsenal of unique genetic diversity for other new traits that need to be analyzed so multiple-value components can be blended swiftly. We are searching for resources that are rich in micronutrient profiles (Fe, Zn, and Phytase) and relate to PO_4 use efficiency with importance in place to climate change parameters (heat, drought, and soil variation to salinity/sodicity). Exploitation of landraces in wheat improvement will add to the in-place activities that embrace intraspecific, interspecific, and intergeneric programs.

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Analysis of genetic diversity and trait association under stripe rust pressure in 7th elite bread wheat yield trial (EBWYT) under agro-climatic conditions.

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The impact of CIMMYT's Wheat Breeding Program has been significant (Rajaram 1999). This study analyzed the genetic diversity, trait association and response against stripe rust in 24 genotypes of Elite Bread Wheat Yield Trial (EBWYT) contributed by CIMMYT (Table 22) with NARC-2009 as the commercial check. The experiment was

Table 22. Genotypes in 7th Elite Bread Wheat Yield Trial.

Entry	Pedigree
503	FRET2/Tukuru//FRET2/3/Munal #1
504	Kachu//WBLL1*2/Brambling
505	Kachu/Kiritati
507	Thelin#2/Tukuru//Kiritati
510	WBLL4/Kukuna//WBLL1/3/WBLL1*2/Brambling
511	FRET2*2/Brambling/3/FRET2/WBLL1//Tacupeto F2001/4/WBLL1*2/Brambling
512	Altar 84/*Ae. tauschii* (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/ Kachu/6/Kiritati//PBW65/2*SERI.1B
513	FRNCLN*2/Tecue #1
514	Milan/S87230//BAV92*2/3/Akuri
515	Kachu/Kinde
516	MUU/FRNCLN
517	MUU/KBIRD
518	Becard #1/4/Kiritati/3/2*SERI.1B*2//KAUZ*3/BOW
519	Becard/FRNCLN
520	WBLL1/Kukuna//Tacupeto F2001/4/Whear/Kukuna/3/C80.1/3*Batavia//2*WBLL1
521	Whear/Kukuna/3/C80.1/3*Batavia//2*WBLL1/4/Quaiu
522	WBLL1*2/Brambling//CHYAK
523	Becard//ND643/2*WBLL1
524	Attila/3*BCN*2//BAV92/3/Kiritati/WBLL1/4/Danphe
525	Attila/3*BCN//BAV92/3/Pastor/4/Tacupeto F2001*2/Brambling/5/Pauraq
526	Attila/3*BCN//BAV92/3/Pastor/4/Tacupeto F2001*2/Brambling/5/Pauraq
527	Kachu/Becard//WBLL1*2/Brambling
528	PFAU/SERI.1B//AMAD/3/Waxwing*2/4/Tecue #1
530	ND643//2*Attila*2/Pastor/3/WBLL1*2/Kuruku/4/WBLL1*2/Brambling

conducted in randomized complete block design with three replications at the National Agricultural Research Centre, Islambad, Pakistan, during winter 2012–13 crop season. Each plot consisted of four 4-m rows with a 30-cm row-to-row distance. The entire trial was bordered by a rust susceptible spreader (Morocco), which also was also planted after every 20th entry. The trial was subjected to constant recommended cultural practices. Percent severity of rust infection was taken according to a Modified Cobb's Scale (Paterson et al. 1948), and the reaction referred to the infection type on each genotype was estimated following Roelf et al. (1992) and Mcintosh et al. (1995). Rust notes for severity and infection type were taken together, with severity first. Stripe rust was scored at the heading stage when the susceptible spreader exhibited maximum disease severity. The coefficient of infection (CI) was calculated by multiplying disease severity (DS) with constant values for infection type (IF). Constant values for infection types were used based on the following scale: resistant = 0.1, moderately resistant = 0.25, medium = 0.5, moderately susceptible = 0.75, and susceptible = 1.0 (Pathan et al. 2006). Data was recorded on plant height (cm), spikes/plant, spike length (cm), days-to-maturity, 1,000-kernel weight (g), and and grain yield (kg/m^2).

Data on yield components was statistically analyzed and mean, standard deviation, standard error, and Pearson's correlation were estimated. Traits were analyzed by cluster and principal component analyses by using XLSTAT 3.06 software. A principal component analysis was used to streamline the data by transforming the number of correlated variables into a smaller number of variables. Cluster analysis classifies variables that are further grouped in to core groups and subgroups. Basic statistics also was computed for accessions in each cluster.

Variation in quantitative traits can be seen through descriptive statistics (Table 23). Thousand-kernel weight with a mean value of 42.0, ranges from 37.0 to 45.3 g and has a variance of 5.46. A narrow range with high variance indicates a greater variability among the genotypes. The mean value for the CI for stripe rust was 6.26, with high variability within the genotypes, and is low, highlighting most of the genotypes as stripe rust resistant. Days-to-maturity also showed a high level of variance, indicating the presence of early maturing genotypes. Selection can be made for heat-tolerant, early maturing lines. Spikelets/spike, spike length, and grain yield had low variance, describing the constant expression of the germplasm. High-yielding genotypes can be selected based on these characteristics as advocated by Ramzan et al. (1994). Such a substantial range of variation allows for a good foundation for a yield-maximization breeding target.

Table 23. Descriptive atatistics for six quantitative traits of 24 wheat genotypes. Traits are *Yr* CI = stripe rust coefficient of infection, DM = days-to-maturity, SPP = spikelets/plant, SPL = spike length (cm), TKW = 1,000-kernel weight (g), and GY = grain yield (kg/m^2). SE = standard error and SD = standard deviation.

Trait	Mean	SE	Median	Mode	SD	Variance	Range	Min	Max	Level (95%)
Yr CI	6.26	1.62	1.46	0	7.93	62.87	23.33	0.00	23.3	3.35
DM	164.90	0.81	166.20	168	3.98	15.80	12.67	156.00	169.0	1.68
SPP	9.10	0.15	9.00	9	0.75	0.56	3.00	8.00	11.0	0.31
SPL	10.15	0.19	10.00	10	0.95	0.91	3.33	8.33	11.7	0.40
TKW	41.97	0.48	42.33	44	2.34	5.46	8.33	37.00	45.3	0.99
GY	1.40	0.03	1.40	1.23	0.17	0.03	0.63	1.10	1.73	0.07

Days-to-maturity have a significantly positive correlation with spike length and a significantly negative correlation with CI for stripe rust (Table 24). Spikes/plant, spike length, 1,000-kernel weight, and grain yield showed positive correlations with the CI, presumably due to rust resistance and the slow-rusting behavior of the genotypes.

Table 24. Correlation coefficient (Pearson's Correlation) matrix shown for estimated traits. Traits are *Yr* CI = stripe rust coefficient of infection, DM = days-to-maturity, SPP = spikelets/plant, SPL = spike length (cm), TKW = 1,000-kernel weight (g), and GY = grain yield (kg/m^2).

Variable	*Yr* CI	DM	SPP	SPL	TKW
DM	–0.451				
SPP	0.081	0.015			
SPL	0.081	0.547	0.175		
TKW	0.369	–0.490	0.188	–0.128	
GY	0.234	0.217	0.281	0.144	–0.201

In the future, new, wide diversity is required that is present in wheat wild relatives (Mujeeb-Kazi et al. 2007). We have not identified any line with excellent yield potential, but within the best from the test group (Table 22, p. 85), few were selected for recombination stocks. New genomic diversity, coupled with multivariate cluster analysis of germplasm, might be helpful for identifying promising lines with respect to higher yield in the future.

Principal component and factor analysis (Table 25) revealed that factor F1 (Eigenvalue = 2.06) highlights the trend of variability in days-to-maturity, spike length, and 1,000-kernel weight and F2 (Eigenvalue = 1.53) depicts the extent of change in spikes/plant, grain yield, and the coefficient of infection of stripe rust (Fig. 16).

Table 25. Principal component and factor analysis.

	F1	F2	F3	F4	F5	F6
Eigenvalue	2.060	1.538	0.933	0.865	0.415	0.189
Variability (%)	34.329	25.634	15.553	14.413	6.922	3.148
Cumulative %	34.329	59.964	75.516	89.929	96.852	100.000

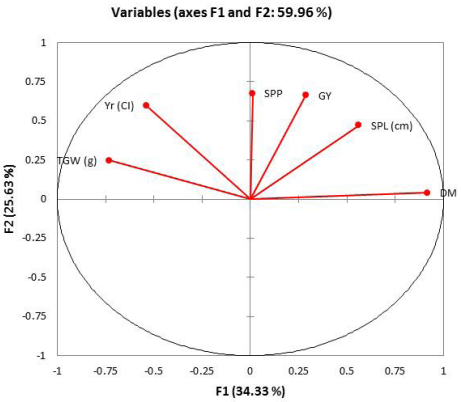

Fig 16. Principal component and factor analysis (TGW = 1,000-kernel weight, Yr (CI) = coefficient of variation for stripe rust, SPP = spikes/plant, GY = grain yield, SPL (cm) = spike length, and DM = (days-to-maturity).

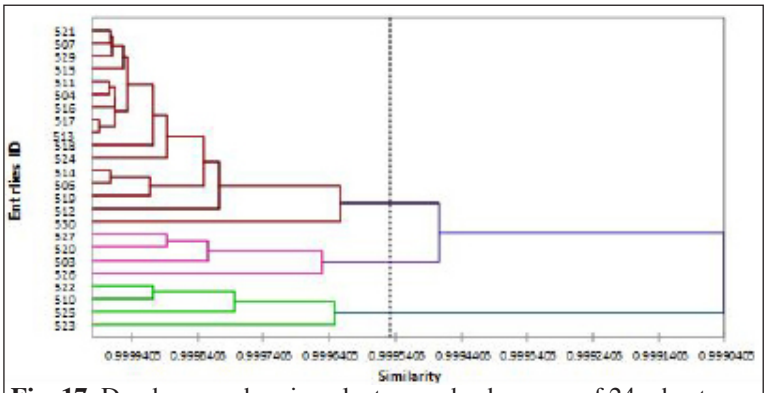

Fig. 17. Dendogram showing clusters and subgroups of 24 wheat genotypes.

Table 26. Cluster grouping of genotypes based on six quantitative traits.

Cluster	Frequency	Entries
1	4	503, 520, 526, 527
3	16	504, 505, 507,511, 512, 513, 514, 515, 516, 517, 518, 519, 521, 524, 529, 530
2	4	510, 522, 523, 525

The cluster analysis divides the 24 genotypes into three clusters showing high similarity within a cluster and high heterogeneity between clusters (Jaynes et al. 2003) (Fig. 17). Cluster one is composed of four genotypes with a medium stripe rust CI, a high number of spikes/plant, and a larger spike length (Table 26). The mean grain yield also is high in 16 genotypes of cluster 2 (Table 27). Cluster 3 is comprised of four genotypes having the lowest mean CI value for stripe rust. This cluster analysis indicates tremendous variation among genotypes of the three different clusters. Future selections from this germplasm can be made by keeping these three clusters in view. The applied value for breeders via clustering of germplasm by a multivariate approach appears to be the ability to select from specific clusters for breeding programs.

Table 27. Arithmetic means with standard deviation of each cluster for coefficient of variation for stripe rust (*Yr* (CI)), days-to-maturity (DM), spikes/plant (SPP), spike length (SPL (cm)), 1,000-kernel weight (TKW (g)), and grain yield (GY (kg/m^2)).

Cluster	*Yr* (CI)	DM	SPP	SPL	TKW (g)	GY (kg/m^2)
1	11.66+1.36	164.25+2.98	9.50+0.79	10.58+1.10	43.83+1.75	1.45+0.12
2	20.83+1.66	161.33+5.01	9.00+0.27	10.00+1.12	43.33+1.05	1.45+0.23
3	1.27+2.11	165.95+3.56	9.02+0.81	10.08+0.91	41.16+2.32	1.37+0.16

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Response of D-genome penetrance and expressivity on synthetic wheats for phenotypic traits and seed image analysis.

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As early as 1987, combining *Aegilops tauschii* accessions with *Triticum turgidum* cultivars was an effort launched by A. Mujeeb-Kazi at CIMMYT, Mexico. As a consequence, over the last few decades, synthetic wheats (SH) were developed that are beset with extensive user-friendly diversity for numerous biotic/abiotic stresses (Ogbonnaya et al. 2013). Over 1,000 synthetic wheats are available that harbor a genomic wealth that can unravel the basic information. We have focused on using SH-wheats that will elucidate how *Ae. tauschii* accessions are contributing to gene penetration and expressivity. We have selected material categorized as the same durum/different *Ae. tauschii* accessions to unravel how the D genome from different accessions has modified trait expressivity in a uniform AABB genomic profile. Initial data forms the basis of this brief study and will be expanded in the future on a larger scale.

Plant material. One-hundred seventeen, D-genome SH wheats developed from the combination of three durum wheats, Altar, Ceta, and D67.2, and different *Ae. tauschii* accessions; three original durum parents; four local checks, Pasban-90, S-24, Shorawaki, and PBW-343, were used. All the traits were recorded at different growth stages (Zadoks et al. 1974) (Table 28, p. 77). Traits studied were:

Plant habit. Recorded at GS-31 as prostrate, erect, and semi erect.

Anthocyanin pigment. Anthocyanins are pigmented compounds in plants that play a protective role under different stress conditions. Anthocyanin content tends to increase under drought, cold, UV-B irradiation, the presence of toxic metals in soils, and pathogen attack, and are found in different organs such as culm, leaf, auricle, pericarp, and coleoptiles. Recorded as either present or absent at GS-26 for all genotypes.

Leaf area index (LAI). The photosynthetic capacity of the crop is related to the total leaf area and the length of time that this area is maintained. Maintenance of the leaf area is essential for the production of the carbohydrates used for grain filling. Leaf area is influenced by the rates of leaf appearance, tiller production, and leaf expansion. Leaf area is measured as LAI. The LAI of a crop determines water-use efficiency. The higher the LAI, the more water used during the vegetative growth stage. In dryland crops, a large area of leaf in early vegetative growth may use water needed for flowering and grain fill. The leaf area of five random leaves from each genotype was measured. LAI has been calculated by the following formula of Muller (1991) at GS-41:

Leaf area index (cm^2) = Leaf length (cm) × leaf width (cm) × 0.74.

Table 28. Germplasm description used to study the response of D-genome penetrance and expressivity on synthetic wheats.

#	Entry	Pedigree	#	Entry	Pedigree
1	433	Altar 84/*Ae. tauschii* (1012)–	64	854	D67.2/P66.270//*Ae. tauschii* (634)
2	908	Altar 84/*Ae. tauschii* (1068)	65	855	D67.2/P66.270//*Ae. tauschii* (635)
3	1010	Altar 84/*Ae. tauschii* (1094)	66	260	D67.2/P66.270//*Ae. tauschii* (646)
4	3	Altar 84/*Ae. tauschii* (178)	67	823	D67.2/P66.270//*Ae. tauschii* (657)
5	5	Altar 84/*Ae. tauschii* (188)	68	861	D67.2/P66.270//*Ae. tauschii* (658)
6	8	Altar 84/*Ae. tauschii* (191)	69	261	D67.2/P66.270//*Ae. tauschii* (659)
7	12	Altar 84/*Ae. tauschii* (192)	70	865	D67.2/P66.270//*Ae. tauschii* (665)
8	17	Altar 84/*Ae. tauschii* (193)	71	866	D67.2/P66.270//*Ae. tauschii* (666)
9	20	Altar 84/*Ae. tauschii* (198)	72	867	D67.2/P66.270//*Ae. tauschii* (668)
10	23	Altar 84/*Ae. tauschii* (205)	73	875	D67.2/P66.270//*Ae. tauschii* (709)
11	33	Altar 84/*Ae. tauschii* (211)	74	803	D67.2/P66.270//*Ae. tauschii* (731)
12	48	Altar 84/*Ae. tauschii* (219)	75	804	D67.2/P66.270//*Ae. tauschii* (741)
13	49	Altar 84/*Ae. tauschii* (220)	76	884	D67.2/P66.270//*Ae. tauschii* (788)
14	52	Altar 84/*Ae. tauschii* (221)	77	885	D67.2/P66.270//*Ae. tauschii* (791)
15	57	Altar 84/*Ae. tauschii* (223)	78	887	D67.2/P66.270//*Ae. tauschii* (796)
16	64	Altar 84/*Ae. tauschii* (224)	79	888	D67.2/P66.270//*Ae. tauschii* (797)
17	918	Altar 84/*Ae. tauschii* (237)	80	889	D67.2/P66.270//*Ae. tauschii* (828)
18	464	Altar 84/*Ae. tauschii* (244)	81	895	Ceta/*Ae. tauschii* (1085)
19	80	Altar 84/*Ae. tauschii* (291)	82	440	Ceta/*Ae. tauschii* (1024)
20	551	Altar 84/*Ae. tauschii* (319)	83	962	Ceta/*Ae. tauschii* (683)
21	96	Altar 84/*Ae. tauschii* (328)	84	927	Ceta/*Ae. tauschii* (418)
22	97	Altar 84/*Ae. tauschii* (328)	85	930	Ceta/*Ae. tauschii* (442)
23	318	Altar 84/*Ae. tauschii* (333)	86	825	Ceta/*Ae. tauschii* (615)
24	923	Altar 84/*Ae. tauschii* (380)	87	955	Ceta/*Ae. tauschii* (680)
25	419	Altar 84/*Ae. tauschii* (502)	88	903	Ceta/*Ae. tauschii* (373)
26	572	Altar 84/*Ae. tauschii* (539)	89	578	Ceta/*Ae. tauschii* (1055)
27	993	Altar 84/*Ae. tauschii* (793)	90	449	Ceta/*Ae. tauschii* (166)
28	187	Altar 84/*Ae. tauschii* (JBANGOR)	91	448	Ceta/*Ae. tauschii* (1042)
29	186	Altar 84/*Ae. tauschii* (Y86-87 S401)	92	516	Ceta/*Ae. tauschii* (1043)
30	607	D67.2/P66.270//*Ae. tauschii* (1009)	93	517	Ceta/*Ae. tauschii* (1046)
31	608	D67.2/P66.270//*Ae. tauschii* (1015)	94	450	Ceta/*Ae. tauschii* (172)
32	610	D67.2/P66.270//*Ae. tauschii* (1017)	95	446	Ceta/*Ae. tauschii* (1030)
33	906	D67.2/P66.270//*Ae. tauschii* (1032)	96	477	Ceta/*Ae. tauschii* (371)
34	785	D67.2/P66.270//*Ae. tauschii* (1054)	97	454	Ceta/*Ae. tauschii* (200)
35	771	D67.2/P66.270//*Ae. tauschii* (1057)	98	483	Ceta/*Ae. tauschii* (445)
36	892	D67.2/P66.270//*Ae. tauschii* (1068)	99	513	Ceta/*Ae. tauschii* (1036)
37	894	D67.2/P66.270//*Ae. tauschii* (1074)	100	511	Ceta/*Ae. tauschii* (1031)
38	896	D67.2/P66.270//*Ae. tauschii* (1085)	101	515	Ceta/*Ae. tauschii* (1038)
39	899	D67.2/P66.270//*Ae. tauschii* (1090)	102	452	Ceta/*Ae. tauschii* (184)
40	909	D67.2/P66.270//*Ae. tauschii* (1093)	103	919	Ceta/*Ae. tauschii* (310)
41	584	D67.2/P66.270//*Ae. tauschii* (185)	104	921	Ceta/*Ae. tauschii* (345)
42	34	D67.2/P66.270//*Ae. tauschii* (211)	105	897	Ceta/*Ae. tauschii* (1090)
43	37	D67.2/P66.270//*Ae. tauschii* (213)	106	600	Ceta/*Ae. tauschii* (416)
44	44	D67.2/P66.270//*Ae. tauschii* (217)	107	429	Ceta/*Ae. tauschii* (540)
45	47	D67.2/P66.270//*Ae. tauschii* (218)	108	460	Ceta/*Ae. tauschii* (235)
46	50	D67.2/P66.270//*Ae. tauschii* (220)	109	1008	Ceta/*Ae. tauschii* (1093)
47	53	D67.2/P66.270//*Ae. tauschii* (221)	110	786	Ceta/*Ae. tauschii* (356)
48	614	D67.2/P66.270//*Ae. tauschii* (1039)	111	573	Ceta/*Ae. tauschii* (541)
49	59	D67.2/P66.270//*Ae. tauschii* (223)	112	640	Ceta/*Ae. tauschii* (299)
50	590	D67.2/P66.270//*Ae. tauschii* (239)	113	655	Ceta/*Ae. tauschii* (408)
51	223	D67.2/P66.270//*Ae. tauschii* (257)	114	485	Ceta/*Ae. tauschii* (450)
52	593	D67.2/P66.270//*Ae. tauschii* (260)	115	622	Ceta/*Ae. tauschii* (199)
53	781	D67.2/P66.270//*Ae. tauschii* (288)	116	479	Ceta/*Ae. tauschii* (391)
54	594	D67.2/P66.270//*Ae. tauschii* (301)	117	673	Ceta/*Ae. tauschii* (519)
55	224	D67.2/P66.270//*Ae. tauschii* (308)	118	Check	Pasban-90
56	595	D67.2/P66.270//*Ae. tauschii* (320)	119	Check	Shorawaki
57	596	D67.2/P66.270//*Ae. tauschii* (368)	120	Check	PBW-343
58	782	D67.2/P66.270//*Ae. tauschii* (400)	121	Check	S-24
59	599	D67.2/P66.270//*Ae. tauschii* (416)	122	Durum	Altar
60	603	D67.2/P66.270//*Ae. tauschii* (448)	123	Durum	Ceta
61	605	D67.2/P66.270//*Ae. tauschii* (497)	124	Durum	D67.2
62	847	D67.2/P66.270//*Ae. tauschii* (629)			
63	853	D67.2/P66.270//*Ae. tauschii* (633)			

Chlorophyll concentration index (CCI). Three measurements using a SPAD-502 leaf chlorophyll meter (Konica-Minolta, Osaka, Japan) from the random leaf blade were recorded. The crop ground cover, or the percentage of soil surface enclosed by plant foliage, is an important observation of crop establishment and early vigor. Genotypes with expansive early ground cover are able to better capture incident radiation, thus escalating soil shading and declining soil evaporation, which elevates the water-use efficiency and may have improved the competitiveness with weeds and potentially diminish soil erosion. Photographs of the ground cover of each genotype were taken at GS-32 with a digital camera and processed by using Adobe Photoshop CS3 Extended software (Fig. 18). The percent ground cover (%GC) has been calculated by the following formula:

Fig. 18. Crop ground cover images showing the prostrate foliar expanse.

$$\% \text{ GC} = (\text{mean grey value} / 255) \times 100$$

Digital imaging (DI) of seed. Seed shape and size are among the most important agronomic traits due to their higher effect on yield and grain quality. The DI technique was used to generate high-throughput photometric traits explaining various magnitudes of grain size and shape. DI has the ability to display the dimensions of grain morphology that are contributing to grain weight and size. Photograph of 25 well-developed seeds of each entries were taken. Seeds were placed horizontally with equal distances on black background to provide color contrast. Area size (mm^2), perimeter length (mm), length (mm), width (mm), length-to-width ratio, circularity, and distance between IS and CG were made and computed by the Smart Grain software version 1.1.

Statistical analysis and data interpretation indicate that the different *Ae. tauschii* accessions show their higher penetrance effect in the D-genome SH as observed via the digital imaging behavior of seed, plant habit, anthocyanin pigment, leaf area index, ground cover percentage (GC%), and CCI. The leaf area of Altar 84, Ceta, and D67.2 were 32.56, 25.53, and 35.96, respectively. The SHs ranged from 17.30 to 64.75, due to contributions from the D genome. The seed area of Altar 84, Ceta, and D67.2 were computed as 36 mm^2, 31 mm^2, and 32 mm^2, respectively, whereas the SHs varied from 19 mm^2 to 47 mm^2 (Fig. 19).

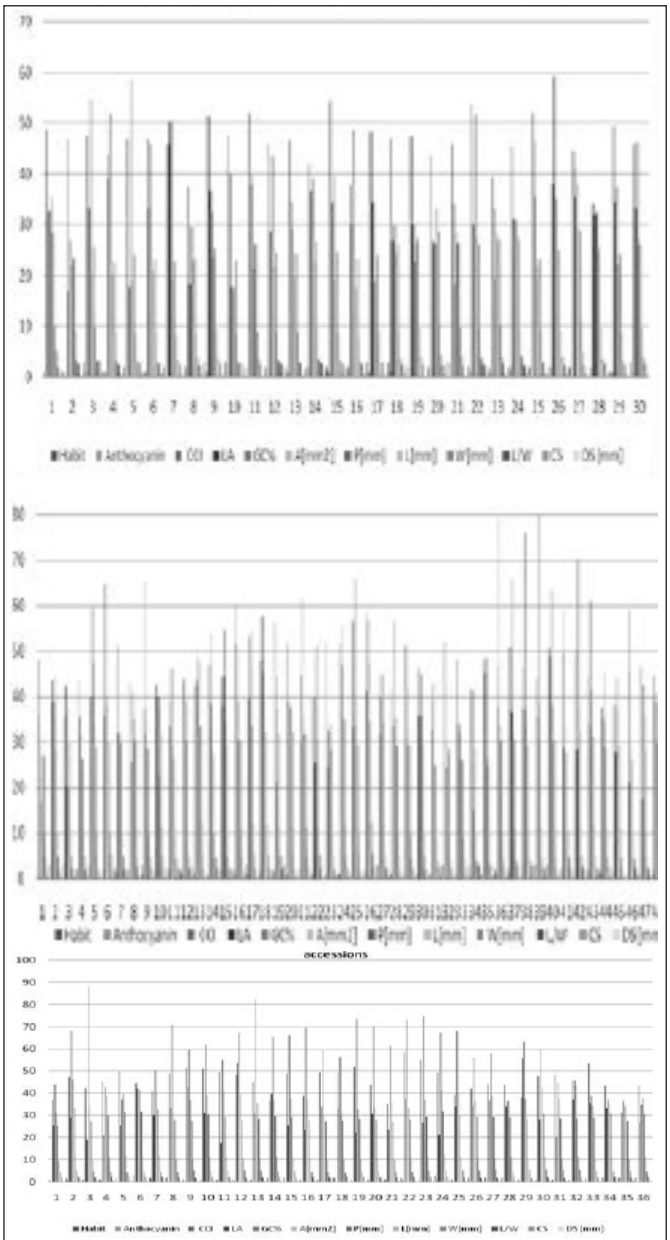

Fig. 19. Parameters showing genetic penetrance and expressivity in synthetic hexaploid wheat developed from the same durum cultivars (Altar 84 (top), D67.2 (middle), and Ceta (bottom) with different *Ae. tauschii* accessions.

The multiple factor analysis shows that the durum parents with all the attributes have different patterns of variability as expressed through partial axes. Subfactors of Ceta and Altar 84 are explained by the multiple factor partial axis F1 and subfactors of D67.2 are explained by F2 (Fig. 20).

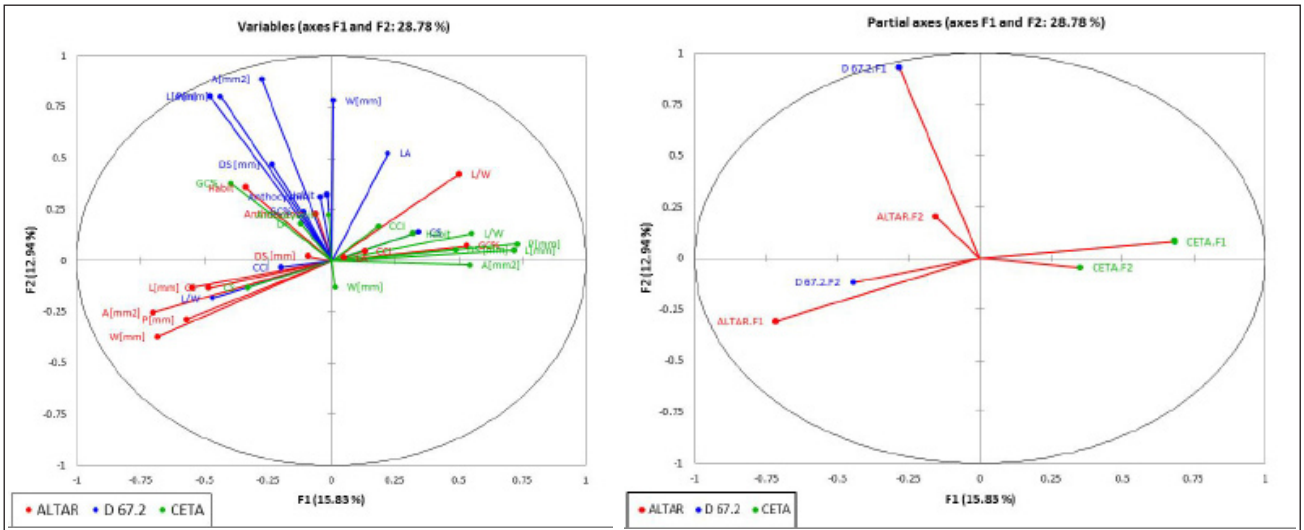

Fig. 20. Multiple factor analysis of different variables for Altar 84 and Ceta (left; axis F1 and F2: 28.78%) and PD67.2 (right; axis F1 and F2: 28.78%).

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Assessment of diverse bread wheat germplasm for drought tolerance via osmotic stress imposed during early seedling growth.

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Our annual wheat yields are severely affected by variable rain patterns that penalize or favor crop yields. Water shortages and climate change are two major factors that have forced us to maximize what productivity. Drought-tolerant wheat cultivars are paramount for providing food security in the future.

Drought stress is one of the most important abiotic stresses, generally accompanied by heat stress during the dry season. Drought is a global problem seriously influencing crop productivity. As a result of a possible climatic shift in the Indo-Gangetic Plains, which currently contributes 15% of the global wheat production, as much as 51% of the area is classified as heat stressed. This area could represent a significant reduction in wheat yield unless appropriate cultivars tolerant to abiotic stresses are adopted by farmers of this region. In Pakistan, this problem is multifaceted, because 20% of the cultivated area is rain-fed and is under the direct, severe threat of drought and heat. The remaining irrigated area is facing severe water availability problems. Coping with the consequences of climate change on agriculture requires unprecedented efforts toward the development of improved genetic resources more resilient to weather vagaries, particularly drought (Semenov and Halford 2009). Developing stress-tolerant cultivars is the most important goal of several breeding programs. However, success has been limited due to inadequate screening techniques, and clearly explains the lack of trait responsive tolerant genotypes. Yield has been the foremost criteria for such programs and is a very complex trait in terms of number of genes controlling it. Yield also is largely influenced by many environmental factors that cause selection for such traits to be less effective. Another problem is that, due to major environmental factors, the heritability of yield and its components also is very low (Ludlow and Muchow 1990). Drought-related traits

can, however, effectively be measured in off-season or in controlled laboratory conditions in early generations, which could be a cost-effective and user-friendly potential approach. Seedling emergence is a sensitive growth stage that is susceptible to water-deficit. Seed germination, seedling vigor, and coleoptile and root shoot length are well recognized prerequisites for the successful stand establishment of crop plants. Rauf et al. (2007) observed that in semiarid regions, low moisture is a restrictive factor during germination, hence, the rate and degree of seedling growth are extremely important in defining both yield and time of maturity. The availability of soil moisture has a major effect on germination and emergence. Besides reduction in total germination, relatively low soil moisture also causes a delay in seed emergence.

Selection for drought tolerance at an early stage in seedlings is most commonly carried out using chemical desiccators such as polyethylene glycol (PEG-6000). PEG also can be used to modify the osmotic potential of nutrient solution cultures and induce plant water-deficit in a relatively controlled manner (Lagerwerff et al. 1961). Rauf et al. (2007) evaluated 16 spring wheat genotypes at four PEG-6000 levels and noted a significant reduction in germination percentage, germination rate index, shoot length, root length, the fresh and dry weights of shoots and root, and the root:shoot ratio in all treatments except the control; the greatest decrease in these traits occurred with a 33% PEG concentration. Sayar et al. (2008) observed that a 25% PEG concentration decreased the germination by 9% in sensitive cultivars, whereas at 30% PEG, only a few seeds from drought-tolerant cultivars germinated. Bayoumi et al. (2008) assessed nine *Triticum aestivum* cultivars by using PEG at 0%, 15%, and 25%. PEG considerably reduced the shoot, root biomass, and coleoptile length in susceptible genotypes, whereas the reduction was less in the drought-tolerant genotypes.

Drought escape, due to a profound root system, enhances the ability of a plant to capture water from deeper layers of the soil and is a fundamental adaptation mechanism to drought. Root system characteristics are of fundamental importance for soil exploration and below-ground resource acquisition, and are strongly related to plant adaptation to suboptimal conditions, such as drought stress.

Our study was targeted to elucidate a rapid and upfront technique for screening wheat genotypes for drought tolerance in early seedling growth stages using PEG-6000 and also compute the associations between drought-related seedling traits. The study was in a growth room of the Wheat Wide Crosses and Cytogenetics laboratory, National Agricultural Research Centre (NARC), Islamabad, during early 2014. The experiment was laid-out in split-plots arranged in randomized complete blocks with three replications. Osmotic stresses were considered as the main plots and cultivars as subplots. Thirteen spring wheat genotypes, LC-2, LC-3, LC-4, LC-5, LC-6, LC-7 DL-1, DL-3 DL-6, SH.DR-24, SH.DR-42, NARC-09, and Durum-7, were studied to see the effect of osmotic stress imposed by using PEG-6000. Solutions were prepared according to weight-by-volume (w/v) as followe: T1 (control with distilled water), T2 (7.5% PEG solution), T2 (15% PEG solution), and T3 (25% PEG solution). For 15% PEG, 150 g of PEG was dissolved with stiring in 1,000 mL of distilled water. Similarly, for 25% PEG, 250 g of PEG was dissolved in 1,000 mL of distilled water. The temperature in growth room was kept at 25±20°C with 60% relative humidity. Five seeds were placed in Whatman No.1 filter paper in Petri dishes measuring 90 mm. After placing the seeds in petri dishes, 10 mL of the PEG solution or distilled water was pipetted in to the petri dishes. Thereafter, 5 mL of distilled water was added to the 7.5%, 15%, and 25% PEG treatments everyday; the same amount of distilled water was added to the control petri dishes to keep them moist and maintain the evaporation losses. The seeds were considered germinated when the radicle (coleorhizae) measured at least 5 mm; germination percentage and other measurements were recorded after 10 days of treatment. Data were recorded on the seedlings of each cultivar. The observations were taken on germination percentage, shoot length (cm), root length (cm), fresh weight of shoot (g), fresh weight of root (g), root/shoot ratio, and seed vigor index (calculated by multiplying the sum of the root and shoot lengths by the germination percentage). The data collected were statistically analyzed by using XLSTAT 2010 software to determine significant differences among the genotypes as affected by PEG osmotic stress. A least significant difference test was applied at the 5% probability level to compare mean differences. Pearson's simple correlation coefficient between different traits at seedling stage also was computed with the software.

Table 29. Mean squares from the ANOVA for shoot length (cm; SL), root length (cm; RL), shoot fresh weight (g; SFW), root fresh weight (g; RFW), shoot dry weight (g; SDW), root dry weight (RDW), and the root:shoot ratio (R:S).

Source of variation	df	SL	RL	SFW	RFW	SDW	RDW	R:S
Genotype	11	32.98***	29.87***	0.005286	0.0027*	0.000189	0.000053	0.297**
Treatment	3	153.63***	479.45***	0.035900**	0.0442***	0.000189	0.000214*	3.415***
Genotype x treatment	33	3.05**	15.57***	0.007387	0.0025**	0.000158	0.000069	0.218**

Mean square values form the ANOVA were calculated and observed. Genotypes showed highly significant variation in shoot length, root length, and root:shoot ratio. the root and shoot fresh or dry weight could not cause significant variation in the genotypes. In the treatments and the 'genotype x treatment' interaction, the significance was similar (Table 29, p. 80). From the LSDs, SHD-42, SHD-25, and DRL-1 were significantly different among the genotypes with respect to shoot and root length (Table 30). From the correlation table, seedling vigor index was found to be highly correlated with root and shoot length. Root and shoot weights were highly correlated with their lengths (Table 31, pp. 81-82).

Table 30. Least significant differences among the genotypes for shoot length (cm, SL), root length (cm, RL), root fresh weight (g; RFW), and the root:shoot ratio (RL:SL).

Genotype	Shoot length (cm)	Root length (cm)	Root fresh weight (g)	Root:shoot ratio
LC2	14.87 ab	9.7 b	0.11 b	0.95 e
LC3	14.98 ab	8.04 cd	0.11 bc	1.33 ab
LC4	14.58 ab	9.29 bc	0.11 b	1.18 bcde
LC5	12.85 c	9.66 b	0.11 bc	1.27 abc
LC6	12.54 cd	10.00 b	0.11 bc	1.22 bc
DRL-1	11.83 d	9.16 bc	0.12 ab	1.52 a
DRL-4	12.62 cd	7.41 de	0.11 bc	1.31 abc
DRL-6	14.22 b	10.00 b	0.13 ab	1.07 cde
SHD-25	15.29 a	10.33 b	0.11 b	1.21 bcd
SHD-42	14.95 ab	12.66 a	0.15 a	1.22 bcd
NARC-09	9.75 e	6.54 e	0.08 c	1.16 bcde
DURUM-7	13.08 c	10.37 b	0.11 bc	0.96 de
LSD Value	1.003	1.414	0.028	0.256

Table 31. Pearson's correlation coefficient for shoot length (cm; SL), root length (cm; RL), root:shoot ratio (RL:SL), shoot fresh weight (g, SFW), root fresh weight (g, RFW), shoot dry weight (g, SDW), root dry weight (g, RDW), and root:shoot dry weight ratio (RW:SW).

	SL	RL	RL:SL	SFW	RFW	SDW	RDW	RW:SW
Root length								
Control	0.296444							
7.50%	0.524969							
15.50%	0.706094							
22.70%	0.646982							
Root length:shoot length ratio								
Control	–0.271280	0.837365						
7.50%	0.256590	0.954407						
15.50%	0.216458	0.841235						
22.70%	–0.124670	0.670838						
Shoot fresh weight								
Control	–0.652080	–0.06776	0.326883					
7.50%	0.050850	0.208231	0.243353					
15.50%	0.779498	0.479115	0.068941					
22.70%	0.800233	0.737985	0.188666					
Root fresh weight								
Control	0.389142	0.719547	0.480178	–0.407660				
7.50%	0.507273	0.642640	0.528976	–0.121930				
15.50%	0.711619	0.939642	0.745960	0.592305				
22.70%	0.418570	0.657584	0.460609	0.572542				

Table 31. Pearson's correlation coefficient for shoot length (cm; SL), root length (cm; RL), root:shoot ratio (RL:SL), shoot fresh weight (g, SFW), root fresh weight (g, RFW), shoot dry weight (g, SDW), root dry weight (g, RDW), and root:shoot dry weight ratio (RW:SW).

	SL	RL	RL:SL	SFW	RFW	SDW	RDW	RW:SW
Shoot dry weight								
Control	0.359071	0.769049	0.564575	–0.163900	0.579765			
7.50%	0.472504	0.360522	0.236320	–0.071750	0.102192			
15.50%	0.702091	0.274264	–0.142740	0.894353	0.400084			
22.70%	0.279932	0.276042	0.089990	0.357253	0.357536			
Root dry weight								
Control	0.290032	0.221029	0.056403	0.060100	0.048613	0.188440		
7.50%	0.381970	0.456905	0.390496	–0.192770	0.659155	–0.218360		
15.50%	0.331981	0.730418	0.718320	0.383083	0.711905	0.222157		
22.70%	–0.103580	0.236161	0.473584	–0.004170	–0.047590	–0.135380		
Root dry weight:shoot dry weight ratio								
Control	–0.514590	0.055699	0.355197	0.421415	–0.045750	–0.31213	–0.289220	
7.50%	–0.209140	–0.091050	–0.027750	–0.148560	0.184877	–0.75811	0.719706	
15.50%	–0.322810	0.348964	0.683387	–0.337010	0.264258	–0.55766	0.666709	
22.70%	–0.165510	–0.259040	–0.212630	–0.359020	–0.086160	–0.71671	–0.101230	
SVI								
Control	0.429525	0.900982	0.664442	–0.147920	0.648923	0.767162	0.341680	–0.135680410
7.50%	0.781241	0.869703	0.735728	0.226182	0.664196	0.449724	0.438831	–0.212110630
15.50%	0.750181	0.756623	0.466283	0.624931	0.642574	0.501977	0.628624	0.106637384
22.70%	0.772956	0.720521	0.171480	0.770152	0.342675	0.144443	0.188704	0.007968453

Varied genotypic response to PEG treatment is important for plant breeders, because genotypes can be screened initially and tagged at the seedling stage before extensive and expensive field tests are conducted. Water stress, due to drought, is undoubtedly the most significant abiotic factor limiting plant and also crop growth and development. Drought stress *in vitro* is physiologically related, because of the induced osmotic stress and most of the metabolic responses that it affects, which are similar to some extent to *in vivo* performance. Water deficit negatively affects seed germination and plant growth of seedlings. Our results suggest that the SHD-42, SHD-25, and DRL-1 performed well under osmotic stress regarding seed germination percentage, root and shoot length, and root and shoot weight. Tthese lines can be utilized further in a breeding program for drought tolerance.

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High-throughput SNP and functional marker-based genotyping to assist marker-based breeding activities in the Wheat Wide Crosses Program.

Awais Rasheed, M. Jamil, A.A. Napar, Alvina Gul Kazi, and A. Mujeeb-Kazi.

High-density molecular markers are powerful tools for studying genomic patterns of diversity, the relationship between individuals in populations, and marker-trait associations in mapping experiments. Similarly, functional markers, designed from the gene of interest, aid in selecting superior progeny. These two genotyping resources are currently under exploitation that will be beneficial in following three disciplines:

– Identify new loci for the traits of economic interest.
– Slect superior genotypes with superior alleles for grain quality and yield.
– Unravel patterns of linkage disequilibrium and population structure in advanced progenies derived from wheat wide crosses.

Given the mandate of wheat wide crosses to enrich the wheat gene pool by exploiting available historical genetic diversity (cultivars and landraces) and novel germplasm developed from new Triticeae genomic resource (e.g. synthetic hexaploids), the main activities in progress are:

– High-throughput, DArT-based genotyping and phenotyping led to the identification of new loci for grain weight and size in synthetic hexaploids. Based on these findings, a chromosomal region on 3D is the future target for fine mapping and subsequent gene cloning for grain size and weight (Rasheed et al. 2014; BMC Plant Biol; in press). This initiative was funded by the GRDC, Australia, and Bioversity International, Italy, under a Vavilov-Frankel Fellowship in 2013.
– We genotyped 182 synthetic hexaploids in an Illumina iSelect SNP 9K assay (Cavanagh et al. 2013; Proc Natl Acad Sci USA) and several new loci were identified for stripe rust resistance (F.C. Ogbonnaya, personal communication). This set of synthetic hexaploids is under phenotyping experiments for grain Fe, Zn, Ca, and phytate contents, which are expected to unravel new genetic loci to enhance nutritional value of wheat.
– Illumina iSelect SNP 90K genotyping for 230 historical wheat cultivars and landraces from Pakistan is expected to unravel the footsteps of wheat domestication in Pakistan and will aid trait discovery.
– We developed 210 advanced lines from crossing elite Pakistan wheat cultivars and synthetic hexaploids. These advanced lines show promising agronomic features over the years in field experiments. This germplasm set is now in progress for genotyping using an Illumina iSelect SNP 90K assay that will highlight the important genomic regions from synthetic hexaploids retained in the advanced lines and influencing abiotic stress tolerances.
– The 210 advanced bread wheat/synthetic hexaploid lines are being investigated to identify novel haplotype variations for two important drought-tolerance genes TaDREB-D1 and TaCWI-D2. Important SNPs influencing drought tolerance will be targeted to design KASP-based, allele-specific markers for future diagnostic in germplasm derived from synthetic hexaploids. This initiative is partially funded by Food Security Center, Germany, and the CIMMYT-CAAS Wheat Improvement Center, China.
– Allelic variations at 24 loci in economically important traits were identified using functional markers in historical wheat cultivars and landraces from Pakistan. The results identified several genotypes carrying combinations of superior alleles for grain quality and yield which can be important parental candidates for future wheat breeding in Pakistan.

All germplasm mentioned in the above subsection are currently being phenologically observed, under seed increase, maintained in the Wheat Wide Crosses Program working collection. Five grams of each entry will be deposited in the NARC (Islamabad) gene bank from which seed can be requested under an MTA when shipping costs to be provided.

Thinopyrum bessarabicum for wheat improvement: current status.

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Bread wheat cultivation in Pakistan and globally is under irrigated and rainfed conditions. In irrigated areas with inorganic fertilizer input, salinity build-up is a common abiotic stress that limits crop productivity causing breeders to develop

cultivars with salinity tolerance. Conventional salinity diversity is very limited, with three sources receiving recognition so far, the cultivars Chinese Spring, Kharchia 65, and Shorawaki. Improved breeding material has been cultivars Lu26S, Pasban 90, KRL 1-4, KRL 19, the unreleased, advanced derivative S-24, plus a handful of others. Even after more than two decades since its release, Pasban 90 stands as the national salt-tolerance standard in Pakistan. *Thinopyrum distichum* (2n=4x=28) is in the pedigree of this cultivar. Several reports have associated alien allelic variation as a potent means from which to incorporate salt tolerance into wheat, including *Elytrigia pontica* (a decaploid), via use of the *Phl* cytogenetic manipulation system developed from *Th. junceiforme* (a tetraploid), salt-tolerant, wheat stocks (Wang, personal communication).

Numerous, global research efforts utilizing alien diversity are limited, but on-going activities appear promising as the value of using carefully selected alien species have a distinct advantage for addressing complex traits such as salinity. Hence, diploid alien sources are a priority in wheat improvement irrespective of their gene pool location. Well recognized is the D-genome diploid *Aegilops tauschii* (2n=2x=14) of the primary gene pool (Kazi 2011), a very effective source of salinity tolerance source with enormous diversity. Another diploid, *Th. bessarabicum* (2n=2x=14, E^bE^b) of the tertiary gene pool, has demonstrated superior tolerance potential and remains under study even today, after earlier promise was shown a few decades ago. At the diploid level, another resource, *Lophopyrum elongatum*, whose cytogenetic stocks are available in bread wheat and durum wheat backgrounds, has yet to be fully exploited.

Our current progress status with *Th. bessarabicum* on genetic stocks produced and maintained through continued efforts by A. Mujeeb-Kazi, first at CIMMYT, Mexico, and now in Pakistan collaborating with the parent wide cross program in CIMMYT, Mexico, are the following:

– A 2n=8x=56, AABBDDE^{b}E^b Chinese Spring/*Th. bessarabicum* amphiploid
– A complete set of seven *Th. bessarabicum* disomic chromosome addition lines (1E^b to 7E^b)
– Amount of the above stocks after selfing of the amphiploid and the seven disomic addition lines in the 2012–13 crop cycle are

Amphiploid (2n=8x=56)	225 seed
1E^{b}E^b (2n=6x=42+2)	750 seed
2 E^{b}E^b (2n=6x=42+2)	750 seed
3 E^{b}E^b (2n=6x=42+2)	750 seed
4 E^{b}E^b (2n=6x=42+2)	750 seed
5 E^{b}E^b (2n=6x=42+2)	750 seed
6 E^{b}E^b (2n=6x=42+2)	750 seed
7 E^{b}E^b (2n=6x=42+2)	750 seed

At the start of 2000, experiments were initiated at CIMMYT, Mexico, to produce wheat/*Th. bessarabicum* homoeologous translocation lines using the amphiploid/*phph*-based strategy. A wide array of translocations were generated around in the test materials. Restoring the *PhPh* composition by backcrossing, translocation homozygotes resulted and were increased. Detection and validation of euploid products was done by fluorescent in situ hybridization and Giemsa C-banding (Kazi et al. 2011) and further studies are elucidated in this update (Table 32).

Table 32. Seven euploid derivatives from the effort involving Pakistan/CIMMYT.

Entry	Detail	Chromosome #	Seed #
WWX-1	T7DS·7DL-4J	Euploid 42	250
WWX-2	T6BS·6BL-6J	Euploid 42	250
WWX-3	T6JS·7DL	Euploid 42	250
WWX-4	T5JS-5DS·5DL	Euploid 42	250
WWX-5	T1DS·1JS	Euploid 42	250
WWX-6	T1AS·1AL-1JL	Euploid 42	250
WWX-7	T3JS·3BL	Euploid 42	250

Targeted wheat/alien chromosome translocation production. From the disomic additions produced and maintained, biotic and abiotic stress screening aspects are in place. Our focus is on yellow rust, stem rust (local race), Karnal bunt, digital imaging for seed profile, and salinity/drought/heat tolerance. Interesting characters that also are observed for wheat stock development are solid stem (group-3 chromosomes), blue aleurone (group 4), and club-shaped spike (group 5). To effect these possible exchanges, we have initiated crosses of each disomic addition line with the Chinese Spring *phph* stock. We will select derivatives in the future that will possess translocations of each *Th. bessarabicum* chromosome with its homoeologous wheat group, i.e., 1E^b with 1A or 1B, or 1D through 7E^b with 7A, 7B, or 7D.

Practicality for food security and wheat productivity. We face two major concerns regarding wheat production. These are salinity and stem rust. Addition line $5E^bE^b$ was identified to contribute, and the role of chromosome additions $3E^b$, $4E^b$, and $7E^b$ also were positive. associated Ug99 stem rust resistance to the amphiploid and some addition lines. We have several *Th. bessarabicum* chromosomes contributing resistance to these stresses. The solid-stem characteristic on group 3 also is of breeding interest because, in our efforts to maximize yield with a high 1,000-kernel weight between 55 to 65 g from the D-genome synthetic hexaploids, tiller solidity will be valuable. We have in place a long-term program that is actively exploiting the translocations from the amphiploids through *phph* manipulation and also using individual disomic addition lines for targeted group transfers.

Germplasm sharing. The germplasm from the CIMMYT Wide Cross Program has been shared with various global programs and partners. Of notable mention is the USDA–ARS, Logan, Utah, (R.C-C. Wang) and Steven Xu in North Dakota. The disomic additions also were provided to Rafiq Islam at the Waite University, Adelaide, Australia. In 2011, a student from Leicester, UK, (Pat Heslop-Harrison's group) took the seven euploid translocations from the CIMMYT program with the major objective of marker designation for each of the earlier produced homozygous translocation stocks 1160, 1164, 1168, 1172, 1176, 1180, and 1184. These lines are similar to the translocation euploids mentioned earlier as WWX1 to WWX 7.

The disomic addition lines and all translocation lines are currently the subject of extensive field trials in a saline environment at Pakka Anna in the Punjab and are being studied for various morpho-physiological traits after which seed will be freely shared globally.

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Total arabinoxylan content in flour of selected wheat cultivars.

Qurrat ul ain Afzal, Saqib Arif, Mubarik Ahmed, Zia ul Hasan, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Arabinoxylans (AXs) are an important component of wheat having nutraceutical value and functional properties, which influence the processing and baking of wheat-based products. Arabinoxylans are generally classified as water-extractable arabinoxylan (WEAX) or water-unextractable arabinoxylan (WUAX). This study was conducted on flour of different wheat varieties of Pakistan to determine the total arabinoxylan (TOAX) content and WEAX.

The TOAX and WEAX of the eight hard white spring wheat cultivars grown in different years in various locations are summarized (Table 33A and Table 33B, p. 86). The highest TOAX level across the years was in cultivars SKD-1 (15.1–20.4 mg/g, = 17.1 mg/g) and Anmol (14.6–20.3 mg/g = 17.1 mg/g), followed by that of Imdad (15.2–18.0 mg/g = 17.1 mg/g) (Table 33A). The minimum AX contents were found in the cultivar TJ-83 (11.3-12 mg/g, =11.7 mg/g). These results also agree with those of Finlay et

Table 33A. Total arabinoxylan content in the flour of eight Pakistani hard white spring wheat cultivars grown in three locations for three crop years. The values are expressed as mg xylose equivalents/g of sample and were the average of triplicates ± SE.

Location	Year	Cultivar								Mean	CV (%)
		TD-1	Imdad	Mehran	Abadgar	Moomal	Anmol	SKD-1	TJ-83		
Nawabshah	2006	12.3±0.20	15.8±0.03	13.9±0.47	14.8±1.08	14.3±1.53	15.0±0.03	15.1±0.46	11.7±0.21	14.1	10.1
	2007	13.0±0.50	16.5±1.21	13.9±0.59	15.3±1.29	14.7±1.28	14.6±0.50	15.4±0.01	11.3±0.12	14.3	11.3
	2008	12.7±0.07	15.2±0.44	13.9±0.03	15.0±0.03	14.5±0.03	15.5±0.93	15.6±1.29	11.5±0.03	14.2	10.3
Hyderabad	2006	14.4±0.24	17.9±0.03	14.6±0.06	15.5±0.07	18.2±1.27	17.3±0.32	20.4±1.97	11.9±0.03	16.3	16.5
	2007	14.5±0.27	17.9±1.21	14.2±1.48	15.8±0.27	18.8±2.68	20.3±2.10	18.5±0.09	12.0±0.32	16.5	17.1
	2008	14.5±0.03	17.8±0.86	15.0±0.55	15.2±0.60	18.6±0.07	18.8±0.03	16.5±0.07	11.8±1.48	16.0	14.9
Dadu	2006	12.9±0.40	17.7±0.69	13.7±0.26	14.9±0.16	14.5±0.06	17.4±0.39	20.1±1.77	11.6±0.10	15.4	18.4
	2007	13.0±0.31	18.0±1.15	14.5±0.39	14.7±0.98	18.3±1.17	15.1±0.10	16.4±0.24	11.8±0.36	15.2	14.9
	2008	14.2±0.41	15.8±0.06	13.8±0.53	15.4±0.60	18.7±0.62	20.2±0.84	15.5±0.10	11.7±0.21	15.7	17.2
Mean		13.5	17.0	14.2	15.2	16.7	17.1	17.1	11.7		
CV (%)		6.5	6.6	3.1	2.4	12.7	13.1	12.1	1.8		

al. (2007), who reported that pentosans range between 1.65% and 2.08% (16.5–20.8 mg/g) in flours of various wheat genotypes. The percentage of AX in flours of various cultivars with respect to TOAX content showed only minor differences (24.02–26.07%) among the cultivars. The WEAX in cultivar TJ-83 was the lowest among all. The highest WEAX was found in Imdad (6.4±0.7 mg/g), followed by that of SKD-1 (6.3±0.8 mg/g), Moomal (6.2±1.0 mg/g), and Anmol (6.0±1.0 mg/g). The level of WEAX in flour significantly ($P < 0.01$, $r = 0.941$) corresponded to the level of TOAX in flours (Table 33B). Water-extractable arabinoxylan was the major fraction in the TOAX of meal and flour. The proportion of WEAX in TOAX in the flour was larger than in the meal. On average, around 33% of AX in flour was in the form of WEAX. The larger extraction of WEAX from flour may be due to its presence in the outside of the cell wall (Courtin and Delcour 2002). A greater level of WEAX also increased the levels of TOAX in the flour.

The results also show that a larger coefficient of variation exists among the cultivars that range between 10.1–18.4%, compared to the variation within a cultivar (1.8–13.1%) (Table 33A, p. 85). The largest variation observed was in 2006 in the cultivar Dadu (18.4%), and the minimum in Nawabshah (10.1%) also in 2006. These findings clearly indicate that TOAX is largely dependent on the varietal composition and is less influenced by the location.

Larger coefficients of variation were expressed among different cultivars, ranging from 4.3–17.5% (Table 33B). The coefficient of variation has a broader range than TOAX content in the flour. Among the different cropping environments, which include different cropping location and years, the coefficients of variation ranged from 4.7–6.0%. Variation in WEAX content in the flour is largely due to cultivar differences.

Table 33B. Water-extractable arabinoxylan content in the flour of eight Pakistani hard white spring wheat cultivars grown in three locations for three crop years. The values are expressed as mg xylose equivalents/g of sample and were the average of triplicates ± SE.

Location	Year	Cultivar								Mean	CV (%)
		TD-1	Imdad	Mehran	Abadgar	Moomal	Anmol	SKD-1	TJ-83		
Nawabshah	2006	3.7±0.30	5.7±0.03	4.1±0.49	5.2±0.27	5.1±0.70	4.9±0.03	5.5±0.12	3.3±0.21	4.7	18.7
	2007	3.7±0.24	5.9±0.59	4.4±0.20	5.1±0.60	5.1±0.44	4.7±0.19	5.5±0.01	2.9±0.09	4.7	20.8
	2008	3.7±0.03	5.5±0.06	4.3±0.03	5.1±0.03	5.1±0.03	5.3±0.79	5.5±0.42	3.1±0.03	4.7	19.2
Hyderabad	2006	4.7±0.27	7.0±0.03	5.1±0.03	5.3±0.03	7.5±0.75	6.3±0.15	7.6±0.71	3.6±0.01	5.9	24.4
	2007	5.1±0.33	7.0±0.68	5.3±0.24	5.6±0.27	6.6±0.84	7.4±0.90	6.9±0.03	3.7±0.27	6.0	20.8
	2008	4.9±0.03	7.0±0.44	5.0±0.12	5.0±0.32	7.0±0.03	6.9±0.06	6.4±0.10	3.5±0.03	5.7	22.7
Dadu	2006	3.6±0.20	7.1±0.21	4.1±0.16	5.2±0.10	5.2±0.10	6.4±0.10	7.5±0.66	3.0±0.06	5.3	31.0
	2007	3.6±0.09	6.9±0.24	5.1±0.18	5.3±0.21	6.7±0.76	5.0±0.09	6.3±0.12	3.2±0.10	5.3	25.7
	2008	4.9±0.12	5.8±0.10	4.5±0.33	4.8±0.20	7.3±0.52	7.2±0.59	5.7±0.16	3.7±0.16	5.5	23.2
Mean		4.2	6.4	4.7	5.2	6.2	6.0	6.3	3.3		
CV (%)		15.8	10.7	10.0	4.3	16.7	17.5	13.5	9.2		

Effect of variety, location, and crop year on the arabinoxylan content in wheat flour.

Qurrat ul ain Afzal, Saqib Arif, Mubarik Ahmed, Zia ul Hasan, Alvina Gul Kazi, Abdul Aziz Napar, Hadi Bux, and Abdul Mujeeb-Kazi.

The arabinoxylan (mainly endosperm arabinoxylan) of flour are known to differ in extractability due to differences in molecular structure compared to the arabinoxylan accumulated in aleurone layers (Wang et al. 2006). We wanted to determine the sources of variation in arabinoxylan present in flour in addition to meal.

An analysis of variance (ANOVA) was used to analyze the effects of cultivar, growing location, and crop year on total (TOAX), water-extractable (WEAX), and water-unextractable (WUAX) arabinoxylan content of hard white spring wheats. The ANOVA models described the variation in arabinoxylan content in flour (Tables 34, 35, and 36, p. 87). The cultivar, location, and 'cultivar × location' interaction showed a significant influence on the total arabinoxylan content of wheat flour ($P < 0.001$) (Table 34, p. 87). Crop year caused negligible variation ($F = 0.118$, $P = 0.888$). The influence of the 'cultivar × year' interaction was less significant ($P < 0.01$) compared to that of the 'cultivar × location' interaction. The 'cultivar × location × year' interaction was the least significant ($P < 0.05$) source of variation in relation to TOAX content in flour. The 'location × year' interaction

was found to have an insignificant (F = 0.834, P = 0.506) effect on the TOAX in flour.

The cultivar, location and 'cultivar × location' interaction caused significant (P < 0.001) variation in the WEAX content of flour, compared to crop year (F = 0.015, P = 0.985) and the 'location × year' interaction (F = 1.049, P = 0.384), which did not seem to make any significant influence (Table 35). The 'cultivar × year' and 'cultivar × location × year' interactions led to significant (P < 0.01) effects on the WEAX content of flour.

Cultivar and location significantly (P < 0.001) influenced WEAX (Table 36), whereas, the year showed an insignificant (F = 0.192, P = 0.826) influence. The 'cultivar × location' and 'cultivar × year' interactions also had significant (P < 0.01) effects on WEAX in flour. The 'location × year' and 'cultivar × location × year' interactions did not influence significantly (P > 0.05).

Table 34. Analysis of variance for total arabinoxylan content in the flour of eight hard white spring wheat cultivars (R^2 = 0.824 (adjusted R^2 = 0.737)).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	1,162.889	71	16.379	9.500	0.000
Intercept	50,583.332	1	50,583.332	29,339.679	0.000
Cultivar	773.674	7	110.525	64.107	0.000
Location	149.524	2	74.762	43.364	0.000
Year	0.408	2	.204	0.118	0.888
Cultivar x location	74.811	14	5.344	3.099	0.000
Cultiavar x year	71.348	14	5.096	2.956	0.001
Location x year	5.748	4	1.437	0.834	0.506
Cultivar x locatiokn x year	87.375	28	3.121	1.810	0.013
Error	248.264	144	1.724		
Total	51,994.485	216			
Corrected total	1,411.153	215			

Table 35. Analysis of variance for total arabinoxylan content in the flour of eight hard white spring wheat cultivars (R^2 = 0.873 (adjusted R^2 = 0.810)).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	346.622	71	4.882	13.882	0.000
Intercept	6,053.668	1	6,053.668	17,214.221	0.000
Cultivar	247.559	7	35.366	100.566	0.000
Location	48.432	2	24.216	68.861	0.000
Year	1.037 E^{-2}	2	5.185 E^{-3}	0.015	0.985
Cultivar x location	14.911	14	1.065	3.029	0.000
Cultiavar x year	13.586	14	0.970	2.759	0.001
Location x year	1.476	4	0.369	1.049	0.384
Cultivar x locatiokn x year	20.648	28	0.737	2.097	0.003
Error	50.640	144	0.352		
Total	6,450.930	216			
Corrected total	397.262	215			

Table 36. Analysis of variance for total arabinoxylan content in the flour of eight hard white spring wheat cultivars (R^2 = 0.693 (adjusted R^2 = 0.541)).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	276.583	71	3.896	4.569	0.000
Intercept	21,639.018	1	21,639.018	25,380.021	0.000
Cultivar	154.484	7	22.069	25.885	0.000
Location	27.814	2	13.907	16.311	0.000
Year	0.327	2	0.164	0.192	0.826
Cultivar x location	26.741	14	1.910	2.240	0.009
Cultiavar x year	31.097	14	2.221	2.605	0.002
Location x year	2.100	4	0.525	0.616	0.652
Cultivar x locatiokn x year	34.020	28	1.215	1.425	0.093
Error	22038.375	216	0.853		
Total	399.358	215			
Corrected total	1,411.153	215			

Reference.

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Effect of pentosans on dough properties of wheat.

Saqib Arif, Qurrat ul Ain Afzal, Najmus Sahar, Mubarik Ahmed, Abid Hasnain, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Keeping in view the importance of hard wheats and pentosans as an alternate source of dietary fiber, we studied the effects of pentosans on the farinographic properties of hard white spring wheats. The results and discussion for the effect of pentosans on each of the farinographic parameters of water absorption, dough-development time, dough stability, and mixing tolerance index are presented.

Effect of pentosans on the water absorption of wheat flour. At same absorption level, the farinograms of the control flours were compared with those of pentosans added flours of different hard white spring wheata cultivars (Fig. 21). These farinograms were developed on the water absorption of the control flours and the drifts were measured in BU. The addition of water-unextractable (WUP) and water-extractable (WEP) pentosans tend to shift the farinogram upward without change in the pattern. However, the drift varied with the change of cultivar. Furthermore, the drift was increased by increasing the level of pentosan from 1% to 2%. The flours of all cultivars showed greater shifts when supplemented with WEP.

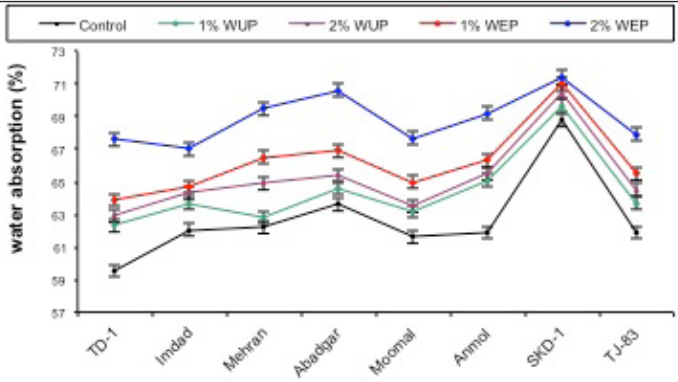

Fig 21. The water absorption of control flours and water-unextractable (WUP) and water-extractable (WEP), pentosan-supplemented flours of hard white spring wheat cultivars.

An analysis of variance determined the effects of cultivar differences, WEP, and WUP on water absorption of hard white spring wheat flour (Table 37). The results indicate that cultivar differences, WEP, and WUP contributed significantly ($P < 0.001$) in the variation of water absorption of the flour. However, WEP had a much greater influence than WUP and cultivar on the water-absorption capacity of the flour. The 'cultivar × WUP' interaction also was found to have significant influence on water absorption ($P < 0.01$) but to a much lesser magnitude compared to the individual effects of these components. The 'cultivar × WEP' interaction had an insignificant influence ($P > 0.05$) .

Table 37. Analysis of variance of water-unextractable (WUP) and water-extractable (WEP) pentosans on water absorption in the flour of eight hard white spring wheat cultivars ($R^2 = 0.911$ (adjusted $R^2 = 0.883$)).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	1,377.660	39	35.325	31.686	0.000
Intercept	260,854.801	1	260,854.801	233,985.470	0.000
Cultivar	169.355	7	24.194	21.702	0.000
WUP	103.968	2	51.984	46.629	0.000
WEP	608.191	2	304.095	272.772	0.000
Cultivar x WUP	15.499	14	1.107	0.993	0.465
Cultivar x WEP	44.996	14	3.214	2.883	0.001
Error	133.780	120	1.115		
Total	686,903.840	160			
Corrected total	1,511.440	159			

Kim and D'Appolonia, (1977) confirmed the impact of pentosans on water absorption. Denli and Ercan (2000) reported that water absorption highly increased with the addition of pentosan fractions obtained from wheat and rye. Moreover, the role of pentosans in end-use quality of flour is mainly due to its higher water-absorbing capacity (Finnie et al. 2006; Du et al. 2009).

Effect of pentosans on the dough-development time of wheat flour. Pentosan-added flours took a longer time to develop optimum dough compared to the control flours (Fig. 22, p. 89). The difference in the dough-development time of the control flours and 1% WUP-added flours varied between 0.3 and 5.4 min, with the minimum and maximum differences shown by the cultivar Moomal and Imdad. Larger differences were observed in the control flours, and 2%

WUP-added flours. The minimum (0.7 min) and maximum (10.5 min) differences were exhibited in SKD-1 and Imdad, respectively. Dough-development time of WEP-added flours of all cultivars, except Abadgar and TJ-83, were longer compared to that of the control flours.

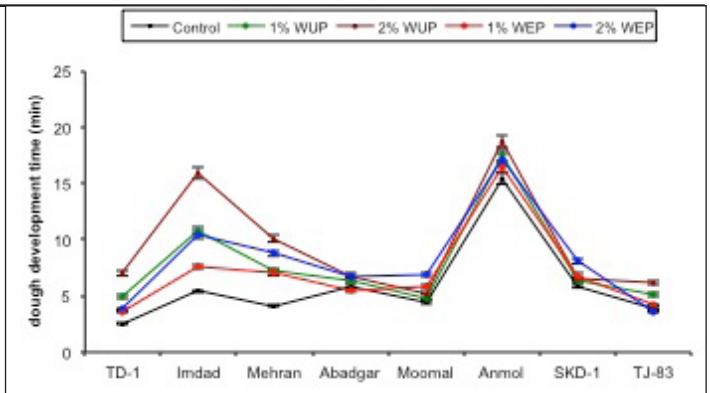

Fig 22. The dough-development times of control flours and water-unextractable (WUP) and water-extractable (WEP), pentosan-suppplemented flours of hard white spring wheat

An analysis of variance determined the effects of cultivar differences, WEP, and WUP on dough-development time in hard white spring wheat flour (Table 38). Cultivar differences, WEP, and WUP had a significant ($P < 0.001$) influence on dough-development time. We also found that the 'cultivar x WUP' interaction had a significant ($P < 0.001$) influence, but the 'cultivar x WEP' interaction did not ($P > 0.05$). Micniewicz et al. (1991) studied the effect of pentosans on dough and gluten attributes. They found a remarkable effect of pentosans on dough-development time. Pentosans have a greater capacity to absorb water compared to other flour constituents (Brennan and Cleary 2007; Rao et al. 2007). Thus, the higher amount of arabinoxylan reduced the water availability for other components like gluten and ultimately delayed the development of dough (Autio 2006).

Table 38. Analysis of variance of water-unextractable (WUP) and water-extractable (WEP) pentosans on dough-development time in the flour of eight hard white spring wheat cultivars ($R^2 = 0.897$ (adjusted $R^2 = 0.864$)).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	2,895.044	39	74.232	26.894	0.000
Intercept	4,583.881	1	4,583.881	1,660.726	0.000
Cultivar	1,044.007	7	149.144	54.034	0.000
WUP	212.816	2	106.408	38.551	0.000
WEP	82.066	2	41.033	14.866	0.000
Cultivar x WUP	156.564	14	11.183	4.052	0.000
Cultivar x WEP	47.954	14	3.425	1.241	0.255
Error	331.220	120	2.760		
Total	12,737.320	160			
Corrected total	3,226.264	159			

Effect of pentosans on the dough stability of wheat flour.
The stability of WUP-added dough of all cultivars (except Anmol and Mehran) was greater than that of the control dough (Fig. 23). On the other hand, WEP-added dough of all cultivars had greater stability than that of the control dough. The difference in dough-stability time of 1% WEP-added dough and that control dough varied between 0.4 and 3.3 min. The difference increased when the control dough was compared with that of the 2% WEP-added dough and ranged between 0.5 and 8.0 min.

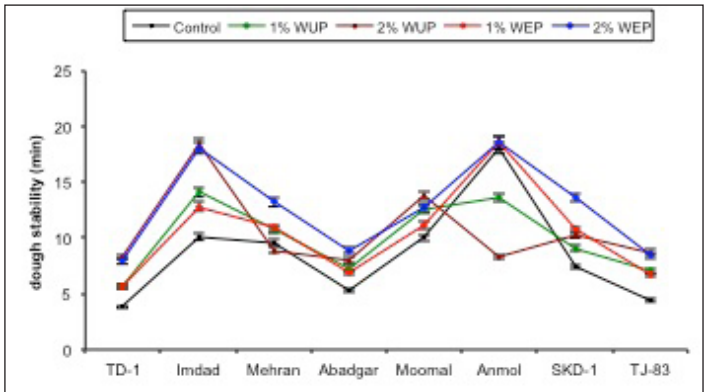

Fig 23. The dough-stabilityt times of control flours and water-unextractable (WUP) and water-extractable (WEP), pentosan-suppplemented flours of hard white spring wheat cultivars.

An analysis of variance determined the effects of cultivar, WEP, and WUP on dough stability of the hard white spring wheat flours (Table 39, p. 90). The cultivar, WEP, and WUP were found to have significant ($P < 0.001$) influence on the dough stability of wheat flour. The influence of WEP was larger than that of WUP and the cultivar. The interaction between culitvar and WUP significantly influenced the dough stability of wheat flour ($P < 0.001$). However, the 'cultivar x WEP' interaction was not a significant ($P > 0.05$) influence on dough stability. The increased stability of dough may be due to the interaction of hydroxyl groups present in hydrocolloids (Park et al. 1997; Rosell et al. 2001, 2009; Collar et al. 1999).

Effect of pentosans on the mixing-tolerance index of wheat flour. The mixing-tolerance index of control and pentosan-added dough was lower than that of the control dough (Fig. 24). However, the mixing-tolerance index value of WUP-added dough of Anmol was greater than that of the control dough.

Table 39. Analysis of variance of water-unextractable (WUP) and water-extractable (WEP) pentosans on dough stability in the flour of eight hard white spring wheat cultivars ($R^2 = 0.841$ (adjusted $R^2 = 0.789$)).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	2,479.220	39	63.570	16.286	0.000
Intercept	7,708.952	1	7,708.952	1,974.924	0.000
Cultivar	547.822	7	78.260	20.049	0.000
WUP	67.286	2	33.643	8.619	0.000
WEP	231.181	2	115.590	29.613	0.000
Cultivar x WUP	411.541	14	29.396	7.531	0.000
Cultivar x WEP	71.026	14	5.073	1.300	0.217
Error	468.410	120	3.903		
Total	20,132.800	160			
Corrected total	2,947.630	159			

An analysis of variance to determine the effects of cultivar, WEP, and WUP on the mixing-tolerance index (Table 40). The influence of WEP and WUP was highly significant on dough mixing-tolerance index values ($P < 0.001$). However, the effect of WEP was greater in magnitude compared to cultivar and WUP. The interaction between variety and WUP was a less significant source of variation ($P < 0.05$). The 'cultivar x WEP' interaction did not influence significantly the mixing-tolerance index values ($P > 0.05$). The effects of individual components were far greater than the interaction effects on mixing-tolerance index.

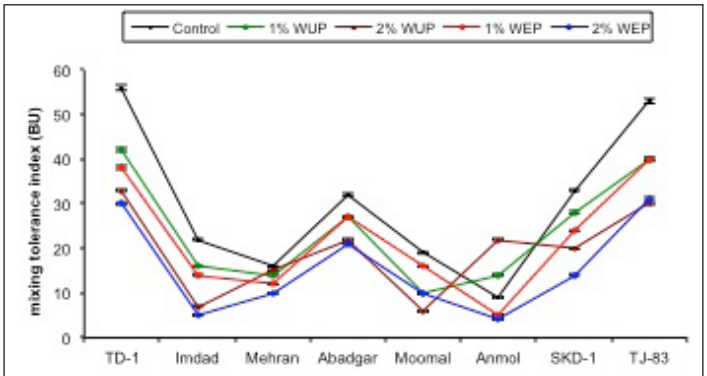

Fig 24. The mixing-tolerance index of control flours and water-unextractable (WUP) and water-extractable (WEP), pentosan-suppplemented flours of hard white spring wheat cultivars.

Table 40. Analysis of variance of water-unextractable (WUP) and water-extractable (WEP) pentosans on the mixing-tolerance index in the flour of eight hard white spring wheat cultivars ($R^2 = 0.769$ (adjusted $R^2 = 0.694$)).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	26,415.100	39	677.310	10.247	0.000
Intercept	16,080.100	1	16,080.100	243.269	0.000
Cultivar	4,142.300	7	591.757	8.952	0.000
WUP	1,820.333	2	910.167	13.770	0.000
WEP	3,320.333	2	1660.167	25.116	0.000
Cultivar x WUP	2,023.667	14	144.548	2.187	0.012
Cultivar x WEP	912.333	14	65.167	0.986	0.472
Error	7,932.000	120	66.100		
Total	111,260.000	160			
Corrected total	34,347.100	159			

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Relationship of dough parameters with arabinoxylan fraction and flour quality attributes.

Saqib Arif, Qurrat ul Ain Afzal, Mehwish Iqbal, Mubarik Ahmed, Abid Hasnain, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

The relationship between water absorption and arabinoxylan fractions. We found that WA has slightly positive relationship with water-extractable arabinoxylan but does not relate with total and water unextractable arabinoxylan fractions (Table 41). Water absorption of the hard white spring wheat flours does not relate with dough parameters. The correlation coefficients of water absorption with dough-development time, dough stability, and mixing-tolerance index are 0.113, –0.086, and –0.049, respectively.

Table 41. Correlation of water absorption with arabinoxylan fractions and dough parameters.

	Total arabinoxylan	Water extractable arabinoxylan	Water unextractable arabinoxylan	Dough-development time	Dough stability	Mixing-tolerance index
Water absorption	0.194	0.235	0.140	0.113	–0.086	–0.049

The relationship between dough-development time and arabinoxylan fraction. All arabinoxylan fractions are positively related with dough-development time. However, the relationship of dough-development time is slightly greater with water-unextractable arabinoxylan ($r = 0.561$, $P < 0.05$) as compared to total ($r = 0.482$, $P > 0.05$) and water-extractable arabinoxylan ($r = 0.403$, $P > 0.05$). Biliaderis et al. (1995) recorded that water-soluble pentosans of different molecular weights imparted a significantly positive influence on dough-development time.

The relationship between dough-stability time and arabinoxylan fractions. The relationship between arabinoxylan fractions and dough-stability time was determined (Fig. 25). We found that dough stability is significantly ($P < 0.05$) and positively correlated with total arabinoxylan ($r = 0.556$), water-extractable arabinoxylan ($r = 0.502$), and water-unextractable

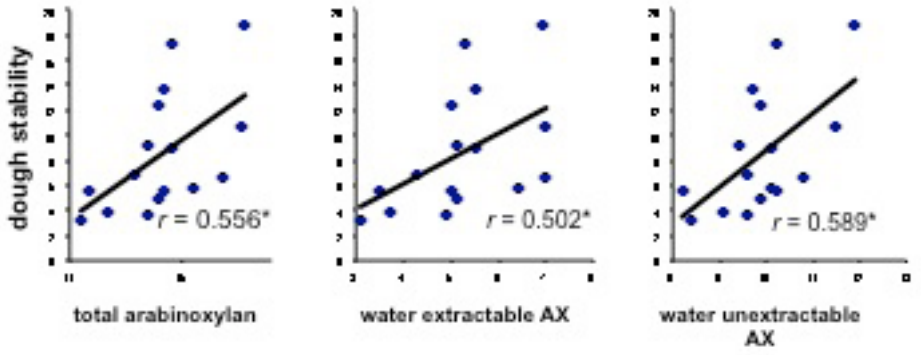

Fig. 25. The relationship between dough stability and arabinoxylan fraction.

arabinoxylan (r = 0.589). Thus, flours with higher arabinoxylan content may form dough with greater stability compared to the flours of lower arabinoxylan content. Stojceska and Ainsworth (2008) indicated that the dietary fiber significantly ($P < 0.001$) and positively influence dough stability.

The relationship between mixing-tolerance index and arabinoxylan fractions. Conversely to that of the dough-development and dough-stability times, the relationship of the mixing-tolerance index was found to be moderately negatively correlated with all arabinoxylan fractions. The correlations of mixing-tolerance index with water-unextractable arabinoxylan ($r = –0.458$, $P > 0.05$) was found to be slightly higher in magnitude than that of the water-extractable arabinoxylan ($r = –0.396$, $P > 0.05$) and total arabinoxylan ($r = –0.435$, $P > 0.05$).

The relationship between flour quality attributes and the water absorption capacity of wheat flour. The correlation coefficients between arabinoxylan and flour quality attributes are presented (Table 42). We found that water absorption did not relate with most of the flour quality attributes. Shogren et al. (1987) also found no relationship between water absorption and protein. The water absorption of flours in this study was only found to weakly positively relate with wet gluten (r = 0.316) and negatively relate with moisture (r = –0.428) contents. Adeyeye and Aye (1998) studied yam bean flour and confirmed the protein functionality in water-retention capacity of food products such as soups, dough, and baked product. The damage to starch during milling is capable of absorbing a much greater (10 times) amount of water compared to intact starch and, therefore, the amount of damaged starch is generally positively related with the water absorption of flour (Catteral 1995; Rasper and Walker 2000). In this study, all flours had a narrow range (20.9–23.1 UCD) of starch damage, because all wheats were milled by the same procedure, which possibly may be the reason that water absorption did not relate with starch damage.

Table 42. Correlation of water absorption and flour quality attributes.

	Starch damage	Falling number	Ash	Protein	Wet gluten	Dry gluten	Gluten index	Moisture
Water absorption	0.013	0.020	–0.152	0.089	0.316	–0.007	0.009	–0.428

The relationship between flour quality attributes and dough-development time. The relationship of flour quality attributes and dough-development time was determined. All wheats were milled at the same extraction rate to yield straight grade flour. The variation in ash content (0.52–0.72%) may be due to varietal differences. We found that the ash content of flour positively correlated (r = 0.30) with dough-development time. Vetrimani et al. (2005), Orth and Mander (1975), and Haridas and Rao (1991) have investigated the relationship of dough-development time with the extraction rate of flour and all reported that the higher extraction flours have longer dough-development times compared to straight-grade flours. The higher amount of bran in high-extraction flour hinders the hydration and quick development of dough (particularly gluten) resulting in a higher dough-development time. Rao et al. (1983) and Corbellini et al. (1999) also indicated that whole-meal flour has a higher dough-development time compared to that of straight-grade flour.

Dough-development time also was found to be significantly and positively correlated with falling number ($r = 0.521$, $P < 0.05$). All the flours were sound, as interpreted by a greater falling number (> 420s) and peak viscosity (950 BU). The positive relationship between falling number and dough-development time shows that the flours with greater falling number take longer for dough development. Dough-development time was weakly, but positively, corelated with protein content (r = 0.222) and gluten index (r = 0.269) but did not correlate with wet gluten (r = 0.042) and dry gluten (r = 0.041). Lin et al. (2003) observed a significant and positive relationship between protein and dough rheological properties. Cuniberti et al. (2003) recorded a positive correlation between percentage of polymeric proteins in protein and dough-development time.

The relationship between flour quality attributes and dough stability. Gluten strength is one important parameter that determines the appropriate use of wheat and is well interpreted by gluten-index values. Gelinas and McKinnon (2011) suggested that gluten index, in combination with dry gluten, is a useful test for early breeding lines. We found that dough stability is significantly ($P < 0.01$) and positively correlated with gluten index (Fig. 26, p. 93). Flours with stronger gluten may develop a more stable dough compared to weak gluten flours.

A positive correlation ($r = 0.350$, $P > 0.05$) is found between ash content and dough stability. Flours with higher ash content may form dough of greater stability. Koppel and Ingver (2010) suggest that a positive correlation between

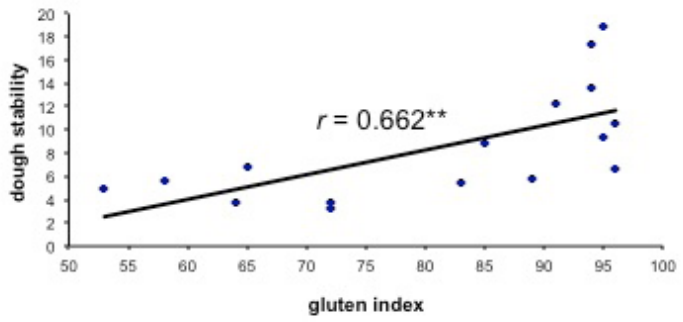

Fig. 26. The relationship between dough stability and gluten index.

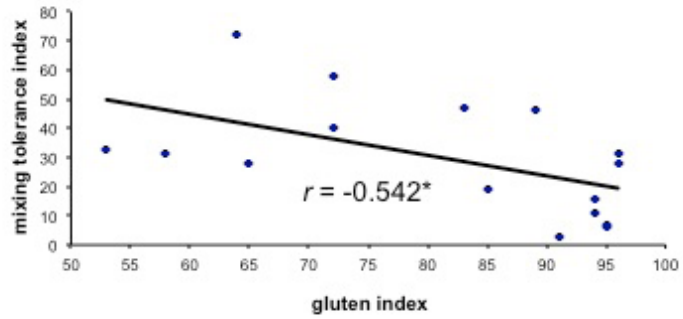

Fig. 27. The relationship between mixing-tolerance index and gluten index.

protein and dough-stability time. In this study, the protein did not correlate (r = 0.173) with dough-stability time, and also that dough-stability time exhibited no relationship with starch damage (r = 0.075), falling number (r = 0.245), wet gluten (r = –0.186), dry gluten (r = 0.004), and moisture (r = 0.044).

Relationship between flour quality attributes and mixing-tolerance index. The relationship between mixing-tolerance index and flour quality attributes was determined. The strength of gluten has a direct influence on dough properties. Gluten index is a good reflection of gluten strength, and the extent of dough tolerance after development can be seen from mixing-tolerance index values. We have found a significant but inverse relationship (r= –0.542, P < 0.05) between the mixing-tolerance and gluten indicies (Fig. 27), which indicates that stronger flour has a greater tendency to tolerate against mixing after development.

We found that the protein is negatively correlated (r = –0.321, P > 0.05) with the mixing-tolerance index. Sadeghi and Bhagya (2008) studied the effect of a mustard protein isolate fraction on pasta dough properties and indicated that the mixing-tolerance index was reduced with an increasing fortification level up to 10%. We found that the mixing-tolerance index is weakly correlated with ash (r = –0.253), dry gluten (r = –0.200), and moisture (r = 0.245), whereas it did not relate with starch damage (r = 0.077), falling number (r = 0.070), or wet gluten (r = –0.039).

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High-molecular-weight glutenin subunit variation in a spring wheat collection.

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The composition of high-molecular-weight glutenin subunits (HMW-GS) and allele frequencies in 26 Pakistani cultivars and 47 CIMMYT wheat accessions were identified. Sixteen different *Glu-1* alleles were found, three at the *Glu-A1* locus, nine at the *Glu-B1* locus, and four at the *Glu-D1* locus (Figs. 1 and 2). Those loci in the CIMMYT accessions, but not in the local cultivars, included four alleles (8, 13+19, 14+15, and 20) at *Glu-B1* and two (5+11 and 2+11) at *Glu-D1*. No Pakistani cultivars had alleles that were absent in the CIMMYT accessions (Figs. 1 and 2). These sixteen alleles at *Glu-A1*, *Glu-B1*, and *Glu-D1* loci resulted in 28 allelic HMW-GS combinations (Table 1). Of these 28 combinations in both groups, five were present only in the Pakistani cultivars (Table 1, p. 95), whereas 12 combinations were present only in CIMMYT accessions (Table 1). Ten different alleles were identified in all the Pakistani cultivars; three corresponding to the *Glu-A1* locus, five to *Glu-B1*, and two to *Glu-D1* (Fig. 1). All of the commonly found allelic variants at the *Glu-A1* locus (null, 1, and 2*) were found in the germplasm. The frequency of HMW-GS (Table 2, p. 107) and the percent partial frequency (Fig. 2) of the 2* allele were 12 and 46.15%, respectively, and were high among all the alleles at the *Glu-A1*locus. At the *Glu-B1* locus, five different HMW-GS combinations (17+18, 13+16, 7+9, 7+8, and 7) appeared in the Pakistani cultivars. Among these, subunits 7+9 and 17+18 were more frequent with a frequency and partial frequency of 9 and 34.62% (Fig. 1). Subunit 7 occurred

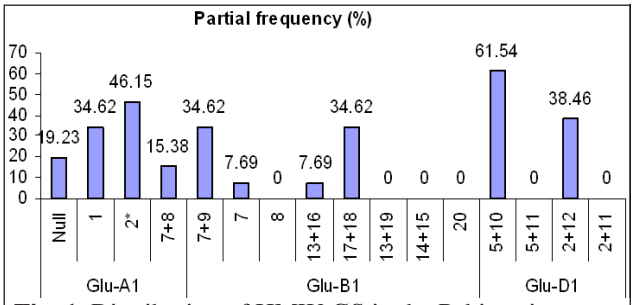

Fig. 1. Distribution of HMW-GS in the Pakistani genotypes for the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci.

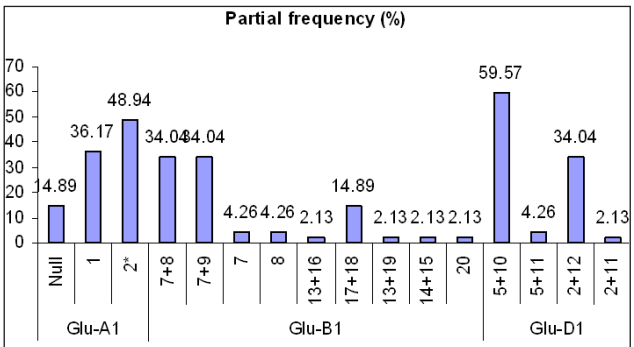

Fig. 2. Distribution of HMW-GS in the CIMMYT genotypes for the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci.

Table 1. Combination of *Glu-1* alleles and quality scores in Pakistani wheat cultivars.

Glu-A1	*Glu-B1*	*Glu-D1*	Frequency	Pakistani cultivar	Quality score
1	7+8	5+10	1	Kohsar-95	10
1	13+16	5+10	1	SA-75	10
1	17+18	5+10	1	Zamindar-80	10
2*	17+18	5+10	1	Zardana-89	10
1	7+9	5+10	3	Lu-26, Punab-76, Punjab-85	9
2*	7+9	5+10	4	Karwan, Manthar, Pasina-90, Satluj-86	9
Null	17+18	5+10	2	Naeem-82, Pavon-76	8
2*	7+8,	2+12	2	Fareed-6, Shahkar-95	8
2*	17+18	2+12	4	Bulbul, Parwaz-94, Punjab-81, Punjab-96	8
2*	7	5+10	1	MH-97	8
1	13+16	2+12	1	Ufaq	8
1	7	5+10	1	Pari-73	8
1	7+9	2+12	1	Kohinoor-83	7
Null	17+18	2+12	1	SA-42	6
Null	13+16	2+12	1	Shalimar-88	6
Null	7+9	2+12	1	Blue Silver	5

Table 2. Combination of *Glu-1* alleles and quality scores in CIMMYT wheats lines and cultvars.

Glu-A1	*Glu-B1*	*Glu-D1*	Frequency	Pakistani cultivar	Quality score
1	7+8	5+10	5	Hoosam-3, KAUZ/STAR, PBW65/ROEK, SW89-5277/BOR95, Milan	10
1	17+18	5+10	2	SNI/PBW65, TE173	10
2*	17+18	5+10	3	TOBA97/BAV-92, Pastor//2*Milan/KAUZ, HUW234/LR 34	10
2*	7+8	5+10	3	PVN/YACO, F60314.76, VEE/PVN	10
1	7+9	5+10	6	NG 8201/KAUZ, RL6043/4*NAC, JUP/ZP//COC, SW91-4903/Circus, Opata//SOR, Johara-14	9
1	7+9	5+11	1	AMSEL/2*BAU	9
1	7+9	5+11	1	AMSEL/2*BAU	9
2*	7+9	5+11	1	SKAUZ	9
2*	7+9	5+10	1	Mango	9
Null	17+18	5+10	1	Crow ‘S’	8
2*	7+8	2+12	6	SHA7/VEE5, Girwill-7, Gamdow-6, Local White, BAYA ’S’, Kanchan	8
2*	17+18	2+12	1	Myna ’S’	8
2*	7	5+10	1	Attila/Pastor	8
2*	20	5+10	1	Weaver/TSC	8
2*	13+19	2+12	1	Weaver/VEEP	8
2*	7+8	2+11	1	HD29/2*Weaver	8
1	7	5+10	1	PBW343*2/Kuruku	8
Null	7+8	5+10	1	KAUZ/TSC	8
Null	7+9	5+10	0	KAUZ ’S’	7
Null	14+15	5+10	1	ESDA/SHWA	7
2*	7+9	2+12	4	SLVS/Pastor, NG8201/KAUZ/Pastor, PBW343*2/Chapio, V763.2312	7
1	8	5+10	2	KAUZ*2/MNV, Kasyon	6
Null	7+8	2+12	1	HXL8246	6
Null	7+9	2+12	2	Opata/Rayon/KAUZ, HP-1744	5

with the least frequency (2) and lease partial frequency (7.69%) (Fig. 2, p. 94). Two alleles (5+10 and 2+12) were found at the *Glu-D1* locus. Allele 5+10 had a higher frequency (16) and partial frequency (61.54%).

Sixteen HMW-GS combinations were observed in the Pakistani cultivars (Table 1, p. 95). Subunit compositions of 2*, 7+9, 5+10 and 2*, 17+18, 2+12 were the most frequent and each was observed in four of the 26 Pakistani cultivars. The second most frequent combination was 1, 7+9, 5+10 found in three of the 26 cultivars. Subunit compositions 2*, 7+8, 2+12 and N, 17+18, 5+10 were present in two cultivars. Eleven other subunit combinations had low frequencies (1) and were present in only one cultivar.

The HMW-GS quality scores for the individual cultivars ranged from 5 to 10 (Table 1). According to the quality score, Kohsar-95, SA-75, Zamindar-80, and Zardana-89 had the highest scores of 10. Seven cultivars (Lu 26, Karwan, Manthar, Pasina 90, Punjab-85, Satluj-86, and Punjab-76) had a quality score of 9. Blue Silver had the lowest score of 5.

Sixteen different alleles at the *Glu-1* locus were identified in the 47 CIMMYT accessions, three corresponding to the *Glu-A1* locus, nine to *Glu-B1*, and four to *Glu-D1* (Table 2, p. 95). At the *Glu-A1* locus, three HMW-GS (Null, 1, and 2*) were present. The frequency and partial frequency of the 2* allele were 23 and 48.94%, respectively, were among the highest of all the alleles at the *Glu-A1* locus (Fig. 2, p. 94). Subunit 1 was second most frequent with a frequency and partial frequency of 17 and 36.17%, respectively (Fig. 2). At the *Glu-B1* locus, nine different subunit combinations (17+18, 13+16, 7+9, 7+8, 7, 8, 13+19, 14+15, and 20) appeared in the CIMMYT accessions. Of these subunits, 7+8 and 7+9 were the most frequent, each with a frequency and partial frequency of 16 and 34.04%, respectively (Fig. 2). Subunits 13+16, 13+19, 14+15, and 20 occurred with the least frequency (1) and partial frequency of (2.13%) (Fig. 2). Four subunits were found at the *Glu-D1* locus (5+10, 5+11, 2+12, and 2+11). Subunits 5+10 had the highest frequency (28) and partial frequency (59.57%), and subunit combination 2+11 was the least frequent (1) and partial frequency (2.13%) (Fig. 2).

Twenty different allelic combinations of *Glu-A1*, *Glu-B1*, and *Glu-D1* were found (Table 1, p. 95). Each of the combinations, 1, 7+9, 5+10 and 2*, 7+8, 5+10, was of the highest frequency of 6 (Table 1). Combination 1, 7+8, 5+10 had the next highest frequency of 5. Fourteen different subunit combinations were the lowest in frequency at 1 and each combination was found in only one accession.

Thirteen accessions, SW89-5277/BOR95, Milan, TOBA97/BAV-92, Astor//2*Milan/KAUZ, HUW234/LR34, PVN/Yaco, SNI/PBW65, VEE/PVN, Hoosam-3, KAUZ/Star, TE173, F60314.76, and PBW65/ROEK had the maximum score of 10, indicating their use to improve bread-making quality. The second highest score (9) for HMW-GS was observed in NG-8201/KAUZ, SW91-4903/Circus, RL6043/4*NAC, OPTA//SOR JUP/ZP//COC/3, AMSEL/2*BAU, Skauz, Mango, and Johara-14. Thirteen accessions, including Crow 'S', SHA7/VEE5, Girwill-7, Gamdow-6, Local White, Baya 'S', Kamcjam, Myna 'S', Attila/Pastor, Weaver/TSC, Weaver//VEEP, HD29/2*Weaver, PBW343*2/ Kuruku, and KAUZ/TSC had a quality score of 8. Only two CIMMYT accessions, Opata/Rayon/KAUZ and HP-1744 had the lowest quality score of 5 (Table 1, p. 95).

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Phenotype-based genetic diversity in spring wheat germplasm for wheat yield improvement.

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Diverse genetic resources are necessary for adequate food production in changing environments. Germplasm is often exploited to develop improved culitvars for changing needs and challenges. Variability for economic traits in the working germplasm is very important for rewarding utilization following recombination breeding and selection. Selecting genetically diverse parents for recombination breeding in a self-pollinated species such as wheat to produce transgressive segregants has been repeatedly emphasized. Assessing the genetic diversity can also be invaluable for analyzing genetic

variability in cultivars and introgressing desirable traits from diverse germplasm into the available genetic base. A collection of 500 cultivars and breeding lines of spring bread wheat was evaluated to determine (i) the magnitude of variability in the germplasm for twelve quantitative traits, (ii) the grouping pattern of the genotypes, and (iii) identify genetically diverse and agronomically promising genotypes for exploiting in breeding programs to improve grain yield potential of wheat.

Our analysis of variance (ANOVA) revealed that the genotypes included in the study had significant variation for most of the traits. In 2008–09, spike density and chlorophyll content had no workable diversity in the germplasm. Chlorophyll content was the only trait that showed no significant variation among the genotypes in 2009–10. Spike density was significant in 2009–10. The means of all traits decreased from 2008–09 to 2009–10. The range for grain yield was 10.5–71.0 g/plant in 2008–09 and 10.3–66.8 g/plant in 2009–10. The number of kernels/plant ranged from 253 to 1,812 in 2008–09 and 246 to 1,764 in 2009–10. The minimum and maximum kernel size for the year 2008–09 was 25.6 and 68.6 g/1,000 kernels, respectively. The range in kernel size in 2009–10 was 24.7–66.4 g/1,000 kernels. The number of spikes/plant was 6.3–55.2 in 2008–09 and 6.1–53.7 in 2009–10. The range for the number of spikelets/spike was 16.7–27.2, maximum number of fertile florets/spikelet was 3–508, spike dry weight was 2.3–5.9 g, plant height was 62.1–129.4 cm, spike length was 8.1–16.8 cm, awn length was 0.0–13.6 cm, spike density was 1.5–2.2 spikelets/cm, and chlorophyll content was 36.9–65.8 in 2008–09. In 2009–10, the number of spikelets/spike was 16.2–26.5, maximum fertile florets/spikelet was 2.9–5.6, spike dry weight was 1.8–5.6 g, plant height was 60.9–126.8 cm, spike length was 7.8–16.0 cm, awn length was 0.0–13.2 cm, spike density was 1.4-4.7 spikelets/cm, and chlorophyll content was 35.5–63.2.

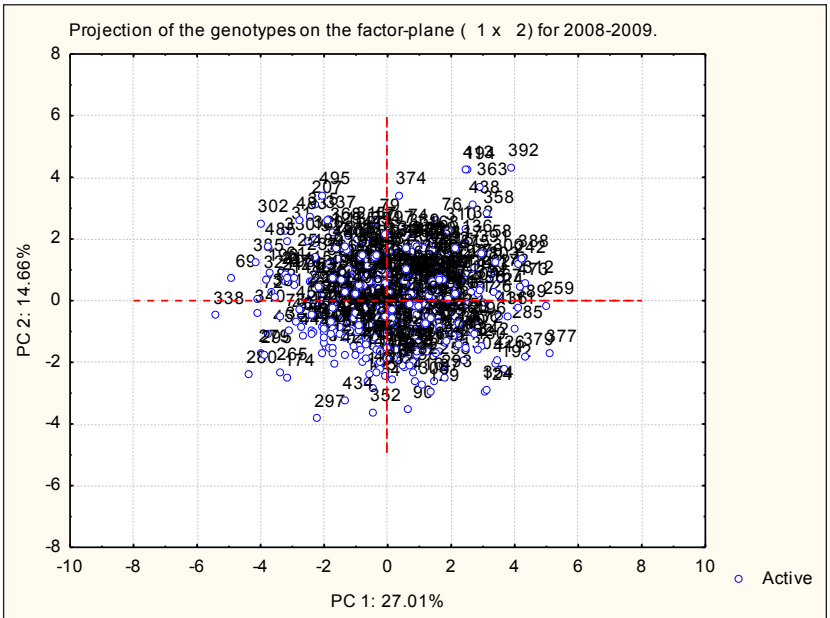

Fig. 3. Two-dimensional ordination of 500 wheat genotypes on prinicpal components PC1 and PC2 in 2008–09.

Of the 12 principal components (PCs), the first four had eigen values (latent root) > 1 (significant) in 2008–09 and 2009–10. The other eight PCs explained a nonsignificant amount of variation and were not worth interpreting. For 2008–09 and 2009–10, the first four PCs showed 62.03% and 64.47% variation, respectively, in the germplasm. The first PC accounted for 27.01% of the variance, the second for 14.66%, the third for 11.77%, and the fourth for 8.59% in 2008–09 (Fig. 3). The first PC showed 27.76% of the total variance, the second for 16.28%, the third for 11.86%, and the fourth for 8.57% in 2009–10 (Fig. 4).

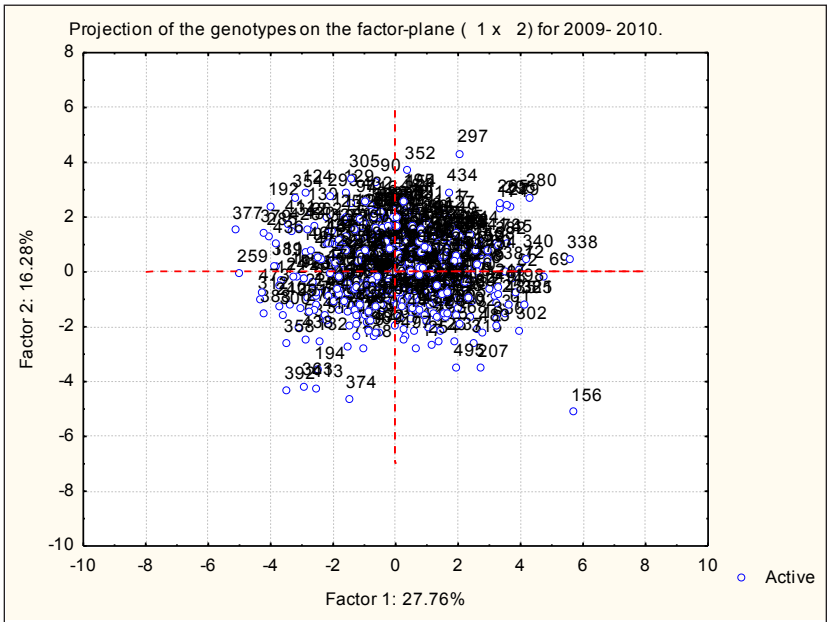

Fig. 4. Two-dimensional ordinations of 500 wheat genotypes on PC1 and PC2 in 2009–10.

The importance of a trait coefficient for each significant principal component was determined. The first PC was highly related to grain yield, number of kernels/plant, spike dry weight, and

spike length for both years, implying that PC1 is a weighted average of these four traits. The number of spikes/plant, 1,000-kerne. weight, and the number of kernels/plant was of significant importance in PC2 in 2008–09. The important traits in PC2 in 2009–10 were the number of spikes/plant, 1,000-kernel weight, number of kernels/plant, and spike density. The third PC was related to the number of spikelets/spike and PC4 to 1,000-kernel weight and the maximum number of fertile florets/spikelet in 2008–09. The significant trait in PC3 was the number of spikelets/spike and in PC4 were 1,000-kernel weight and spike length in 2009–10. The projection of traits on PC1 and PC2 revealed that the number of kernels/plant, the number of spikes/plant, and the number of spikelets/spike were positively related to grain yield in both years. Spike density was opposite grain yield and other yield contribution traits on PC1 in both years and, therefore, it had a negative correlation with all other traits. Becaise the variation in chlorophyll content wasa not significant among the genotypes, it was not projected significantly on the first two PCs by the principal component analysis. The projection pattern of the traits on first two PCs for both years indicated that the key yield-contributing traits were the number of kernels/plant, spike dry weight, and spike length. The projection of genotypes on the first two PCs was useed to identify diverse groups of parents for better transgressive segregation. The projection of genotypes on PC1 and PC2 showed a population structure in both years (Figs. 3 and 4). For 2008–09, the following hetrotic groups were identified: 192, 363, 392, 414, and 488 (Kambara-1, KAUZ//TFAU/VEE#5, V-97100, and Jauhar-78) were opposite to 174, 297, 265, and 280 (Local White, Condor 'S'/ANA75//Condor 'S'/MUS 'S', and Abadgar-93) (Fig. 3). Genotype 338 (Qafzah-21) was in contrast to 259 (Goshawk 'S'). Genotype 374 (Hibara-3) had the maximum diversity from 352 (Bolsena 'S'). Genotypes 69, 385, and 485 (Oasis AGA/3*YR, IZAZ-1, and Abadgar-93) were opposite to 124, 192, 334, and 377 (Webelli/Kambi, Kambara-1, Crow 'S'/BOW#1, and Qafzah-18) (Fig. 3). In 2009–10, the most diverse parents were 280 (BLS/KLT 'S') vs. 392 (KVZ/3/TOB/CFTN//BB/4/BLO 'S'/5/VEE#5/6/BOW 'S'/3/YDING 'S'//BB/CHA), 352 (Bolsena 'S') vs. 374 (Hubara-3), and 302 (Parula) vs. 307 (BUC 'S'/BJY 'S'/3CNDR 'S'/ANA//CNDR 'S'/MUS 'S') (Fig. 2, p. 94).

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Association mapping identifies drought-tolerance QTL on wheat chromosome 2A.

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Most of the association mapping studies so far have been reported under favorable conditions. In the previous studies, a number of QTL for agronomic traits (spike length, spikelets/spike, grains/spike, plant height, spikelet density, and 1,000-kernel weight) were identified on different chromosomes. For complex traits, such as drought tolerance and yield, the knowledge of the interaction between the marker/QTL and the environment are necessary to efficiently utilize marker-assisted selection in plant breeding. Comparing the performance of the same genotype across environments, the best-suited environment for the expression of a particular QTL, can be identified, but hundreds of markers are required for sufficient mapping resolution covering the whole genome of wheat. Therefore, targeting an individual linkage group is a quite reasonable strategy. This study identified the population structure among a collection of 108 germplasm lines, the extent of LD decay on chromosome 2A, and the association of SSR markers with drought-tolerance traits on chromosome 2A.

Association mapping analysis was performed on a panel of 108 diverse wheat accessions to dissect the genetic background of drought-adaptive traits. These genotypes were characterized with 25 SSR loci on chromosome 2A. Sixteen agronomic traits were evaluated under well-irrigated and drought-stress conditions. The population structure and kinship were inferred on the basis of 30 unlinked SSR loci covering all 21 wheat chromosomes, which enhanced the mapping strength by eliminating spurious associations.

The admixture, model-based analysis with the STRUCTURE software was used to investigate the genetic relationship among the population. An optimal number of subpopulations was identified when K was set to three, because the likelihood peaked at K = 3 in the range of 2–20 subpopulations. The number of genotypes assigned to each inferred subpopulation was 33, 37, and 36. The FST values between all subpopulations were significant ($P < 0.001$), confirming a real difference among these subpopulations and supporting the prevalence of genetic structure.

A mixed, linear model approach was used to determine marker–trait associations (MTAs) for the 16 phenotypic traits and 28 SSR loci on chromosome 2A. Subpopulations were used as covariates for association mapping analysis. A total of 10 MTAs were identified for five morphological traits under drought-stress conditions at a probability level of 0.001 with the range of 3.63 to 29.16% of the phenotypic variation (Table 3). Under well-irrigated conditions, only four MTAs were found, and the r^2 range was 3.55 to 10.04 (Table 4). The highest number of associations was observed for awn length under drought stress. Other traits that showed significant associations under drought include days-to-heading, glaucausness, shoot length, and the stress susceptibility index. Under well-irrigated conditions, significant marker trait associations were found for 1,000-kernel weight, shoot length, and coleoptile length. Nineteen SSR markers showed significant association with morphological traits, 11 (*Xcfa2121*, *Xwmc382*, *Xwmc407*, *Xgwm122*, *Xwmc445*, *Xwmc552*, *Xbarc212*, *Xgwm445*, *Xwmc445*, *Xbarc5*, and *Xgwm558*) were associated only with one trait and, therefore, can be considered as trait-specific MTAs. Markers *Xcfa2099*, *Xcfa2043*, *Xcfa2263*, and *Xgwm95* were associated with two traits, and markers *Xcfa2086*, *Xbarc124*, *Xgwm312*, and *Xgwm265* were associated with three traits.

Table 3. Association (r^2) of the SSR markers with drought-related traits under stress conditions in wheat.

Trait	QTL	Flanking marker/s	R^2 (%)	P (Q+K)
Days-to-heading	*QDth.uaf.2A.3*	*Xcfa2099*	4.35	0.0063
	QDth.uaf.2A.2	*Xcfa2121*	8.1	0.0000
Awn length	*QAl.uaf.2A.2*	*Xwmc407*	12.16	0.0000
	QAl.uaf.2A.2	*Xcfa2099*	29.16	0.0000
	QAl.uaf.2A.2	*Xcfa2263*	5.82	0.0001
Glaucausness	*QGla.uaf.2A.2*	*Xwmc445*	8.75	0.0038
	QGla.uaf.2A.2	*Xcfa2043*	5.41	0.0005
	QGla.uaf.2A.2	*Xbarc212*	5.0	0.0084
Shoot length	*QSl.uaf.2A.2*	*Xcfa2263*	6.75	0.0003
Stress-susceptibility index	*QSsi.uaf.2A.2*	*Xgwm265*	4.93	0.0031

Table 4. Association (r^2) of the SSR markers with morphological traits under normal conditions in wheat.

Trait	QTL	Flanking marker/s	r^2 (%)	P (Q+K)
1,000-kernel weight	*Qgw.uaf.2A.3*	*Xwmc455*	10.04	0.002
Shoot length	*QSl.uaf.2A.3*	*Xbarc5*	4.17	0.007
	QSl.uaf.2A.3	*Xgwm265*	4.84	0.003
Coleoptile length	*QCl.uaf.2A.1*	*Xbarc124*	4.35	0.006

Under drought stress conditions, *Xcfa2099* and *Xcfa2121* showed a significant association with number of days to heading (Table 3). Awn length, which showed the maximum number of MTAs, was associated with primers wmc382, wmc407, cfa2043, cfa2086, cfa2099, cfa2263, barc124, gwm95, gwm312, and gwm122. Only three primers, wmc 382, wmc 407, and gwm312, showed non-overlapping associations. Marker–trait associations for glacousness were found with primers wmc445, wmc552, cfa2043, cfa2086, barc212, gwm265, and gwm445. Overlapping associations for glacausness were observed only for cfa2043, cfa2086, and gwm445. Shoot length was associated with cfa2263, barc124, gwm95, and gwm312, and the stress-susceptibility index with cfa2086 and gwm265. Under favorable conditions, only six marker–trait associations were found, and shoot length showed a maximum number of three MTAs. Markers *Xbarc5*, *Xgwm265*, and *Xgwm312* showed a significant association with shoot length under well-irrigated conditions, and gwm312 was associated with shoot length under both well-irrigated and drought environments (Table 4). Coleoptile length also showed two MTAs with *Xbarc124* and *Xgwm558*. Only one MTA was found for 1,000-kernel weight with wmc445 under favorable conditions.

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Association mapping identifies yield QTL on wheat chromosome 3A.

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Association mapping, which has been widely used in human and animal genetics where large segregating populations are not possible to develop, has been validated in plants. Association mapping has many advantages over the traditional FBL-based, QTL mapping including higher QTL resolution, higher allele coverage, and readily available mapping populations in the form of natural germplasm. Initially, the presence of a population structure within the mapping population was not statistically handled, causing spurious associations, compared to FBL mapping, but the recent statistical developments, such as Q and K estimates, substantially reduced false associations due to population structure. These matrices take into account population structure and combine it to covariate in association tests. Gene mapping studies in wheat using the assoication mapping approach are still few. Most of these studies have focused on individual chromosomes, where QTL have been previously identified by linkage analysis, or on monogenic traits. Grain yield increment is the most important challenging objective faced by plant breeders. Several yield-related QTL on chromosome 3A were identified using this RICL population. Covering the whole genome of hexaploid wheat with sufficient mapping resolution requires hundreds of SSR probes, therefore, targeting individual linkage group is a reasonable strategy. Marker–trait associations for grain yield traits on chromosome 3A of hexaploid spring wheat were determined. We analyzed population structure, linkage disequilibrium, and associations of SSR markers with 12 yield-related traits.

A panel of 94 diverse hexaploid wheat accessions was used to map QTL underlying the yield-related traits on chromosome 3A. Population structure and kinships were estimated using unlinked SSR markers from all 21 chromosomes, which eliminated spurious associations and enhanced mapping strength. Analysis of variance revealed significant difference among accessions; however, the 'genotype × year' interaction was not significant for most yield-related traits.

An admixture, model-based analysis with the STRUCTURE software identified an optimal number of subpopulations when K was set at 7, because likelihood peaked at K = 7 in the range of 2–20 subpopulations. The number of genotypes assigned to each inferred subpopulation ranged from 8 to 16. The FST values between all subpopulations were significant ($P < 0.001$), confirming the real difference among these subpopulations and supporting the prevalence of genetic structure.

The unlinked markers used to detect population structure also were used to determine background LD (unlinked LD). The background LD in the genome, caused by the genetic structure, was used to set a critical value of LD for markers on chromosome 3A. The unlinked r^2 value ranged from 0.000 to 0.1303 for all unlinked loci pairs, with an average of 0.0014. The 95th percentile of the distribution of unlinked r^2 (0.015) was used as a population-specific threshold for this parameter as an evidence of LD because of linkage. The syntenic r^2 was obtained from the analysis of 23 SSRs on chromosome 3A. The value of the pairwise syntenic r^2 ranged from 0.0007 to 0.167 with an average of 0.016, significantly higher than the average of the unlinked r^2.

A decay scatterplot of the syntenic LD values of r^2 in population was made. The extent of LD on chromosome 3A was ~40 cM, with a critical value of 0.015. About 40% of the LD pairs were found with an $r^2 < 0.015$. The chromosomal region where all pairs of flanking loci were in LD, were referred to as an LD block. One LD block was observed on chromosome 3A between 37–46 cM, including markers *Xbarc45*, *Xgwm2*, *Xgwm674*, and *Xgwm30*.

We used a mixed linear model approach to determine marker–trait associations for 12 yield traits and 23 SSRs on wheat chromosome 3A. The results were similar for both years. A mixed linear model approach identified six QTL for four traits that individually accounted for 10.7–17.3% of the phenotypic variability (Table 5). Primers cfa2134 (51 cM) and gwm369 (14 cM) were significantly associated with maximum number of fertile florets/spikelet. Grain yield/plant, plant height, and spike length were significantly associated gwm155 (85 cM), wmc527 (53 cM), gwm155 (85 cM), and gwm369 (14 cM).

Table 5. Association (r^2) of SSR markers with yield traits in wheat. Map position (cM) was based on the consensus map Ta-SSR-2004; r^2 indicates the percentage of the total variation explained at the loci significant at a level of $P < 0.01$.

Trait	QTL	Flanking marker/s	r^2 (%) 2008–09	P (Q+K) 2008–09	r^2 2009–10	P (Q+K) 2009–10
Grain yield	*QGyld.uaf.3A.3*	*Xgwm155–Xwmc215*	17.3	0.0020	16.9	0.0030
	QGyld.uaf.3A.2	*Xwmc527–Xwmc269*	16.1	0.0023	15.3	0.0029
Plant height	*QPht.uaf.3A.3*	*Xgwm155–Xgwm215*	10.7	0.0093	10.7	0.0090
Spike length	*QSpl.uaf.3A.1*	*Xgwm369–Xcfd79*	16.8	0.0062	14.9	0.0013
Fertile florets/spikelet	*QMff.uaf.3A.2*	*Xgwm369–Xcfd79*	15.3	0.0070	13.6	0.0090
	QMff.uaf.3A.1	*Xcfa2134–Xwmc527*	15.4	0.0098	14.3	0.0016

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