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The effect of ACC deaminase (1-aminocyclopropane-1-carboxylate deaminase) in producing plant growth-promoting rhizobacteria on wheat under drought stress.

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Soil-borne microorganisms that help plants in promoting their growth and development are plant growth-promoting rhizobacteria (PGPR). They are abundantly present in the plant rhizosphere and survive on root exudates and lysates. Excessive production of ethylene by plants on exposure to certain biotic and abiotic stresses is known as 'stress ethylene'. Drought stress also has been suggested to alter tissue sensitivity to ethylene. In wheat, stress ethylene is increased by certain enzymes, such as benzyladenine (BA) and auxin, whereas ABA inhibits the biosynthesis of ethylene as in the unstressed condition. Thus, increased concentration of abscisic acid decreases ethylene in water-stressed wheat plants. A high level of ethylene slows down root elongation, root nodulation, and auxin transportation and encourages hypertrophies, paces up aging, and endorses the rate of senescence and abscission. For this reason, the severity of the infection from fungal or bacterial attack can significantly be reduced by inhibiting ethylene production. 1-aminocyclopropane-1-carboxylate deaminase (ACCD) was originally found inside a soil bacterium belonging to *Pseudomonas* sp. ACCD was recognized as an enzyme involved in the degradation of a cyclopropanoid amino acid (ACC) into α-ketobutyrate and ammonia. Ethylene and ACC (an intermediate) levels can be deceased up to 2–4 fold in wheat plants where the seedlings and roots are coated with ACCD-producing bacteria.

Collection, characterization for ACCD, and identification of bacterial strains. The bacterial strains were collected and characterized for the presence of ACCD. The ACCD-containing bacterial strains were identified by 16rRNA and were found to be of *Serratia* spp. and *Providencia* spp.

Sowing of wheat seeds coated with ACCD-producing bacteria. The experiment was conducted at the fields of Barani Agriculture Research Institute (BARI), Chakwal, which is considered a stress prone area. Field trial was conducted from October 2010 to May 2011. The experiment was laid out in randomized complete block design with four replications.

Root colonization assay. Twenty-five different bacterial isolates were collected from root rhizosphere and three were isolated from endosphere at 30 days postinoculation (Table 1). A total of 16 bacteria were isolated from root rhizosphere and three were isolated from endosphere. On the basis of their biochemical properties, the strains A, a, c, d, e, t, s, u, y, and z were selected for molecular characterization. ANOVA was applied to three bacterial isolates 30 days after inoculation and at harvest. The ACCD-producing *Serratia grimesii* strain z and *S. marcescens* strain t showed the best root colonization and persistence at 7.3 log CF U/g even at harvest. *Serratia grimesii* strain z also was found in the root endosphere at harvest. The root-colonizing ability of strains is given (Table 1).

Table 1. Statistical analysis of the root colonization assay for plant growth-promoting rhizobacteria. CFU = colony forming unit. All values are means of three replicates. Values with different letters are statistically different. LSD values at 0.05 = 0.2701).

Treatment	Log CFU
Cultivar	
Pasban-90	6.9 A
Sahar-2006	5.4 B
Inoculation	
Individual	7.5 C
Time	
30 days after sowing	6.7 E
Harvest	5.5 F
Strains	
A.1	7.3 G
A.2	7.3 G
F.11	3.8 H
Treatment interactions	
$V_1 I_1 T_1 S_1$	8.6 I
$V_1 I_1 T_1 S_2$	8.7 I
$V_1 I_1 T_1 S_3$	8.7 I
$V_1 I_1 T_2 S_1$	8.3 J
$V_1 I_1 T_2 S_2$	8.3 J
$V_1 I_1 T_2 S_3$	8.6 I
$V_1 I_2 T_1 S_1$	8.7 I
$V_1 I_2 T_1 S_2$	8.5 IJ
$V_1 I_2 T_1 S_3$	0.0 O
$V_1 I_2 T_2 S_1$	7.3 LM
$V_1 I_2 T_2 S_2$	7.1 M
$V_1 I_2 T_2 S_3$	0.0 O
$V_2 I_1 T_1 S_1$	7.5 KL
$V_2 I_1 T_1 S_2$	7.6 K
$V_2 I_1 T_1 S_3$	7.6 K
$V_2 I_1 T_2 S_1$	5.3 FN
$V_2 I_1 T_2 S_2$	5.3 FN
$V_2 I_1 T_2 S_3$	5.5 FN
$V_2 I_2 T_1 S_1$	7.5 KL
$V_2 I_2 T_1 S_2$	7.5 KL
$V_2 I_2 T_1 S_3$	0.0 O
$V_2 I_2 T_2 S_1$	5.3 FN
$V_2 I_2 T_2 S_2$	5.3 FN
$V_2 I_2 T_2 S_3$	0.0 O

Molecular characterization. PCR was by extracting DNA by boiling. After purification, the PCR product was sequenced and aligned (Fig. 1). The amplified 1,500-bp band was found to have close homology with two strains of *Serretia* spp. showing that the inoculated strain survived in the field.

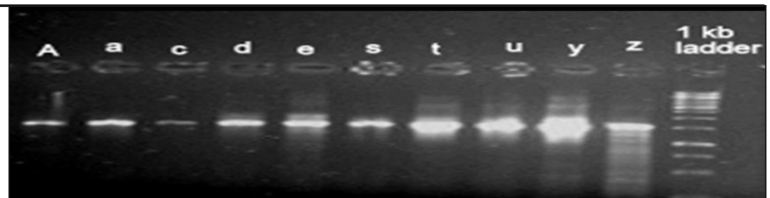

Fig. 1. The bright band at 1,500 bp shows the amplification of the 16srRNA gene of selected strains from a root rhizosphere and endosphere population.

Field evaluation. The following parameters were evaluated at harvest.

Plant height. ANOVA was used to deduce that plant height was not affected by any treatment. Differences in cultivar plant height could not be due to the effect of the treatment (Table 2).

1,000-kernel weight. ANOVA deduced that 1,000-kernel weight also was not affected by any of the treatments individually but it had affects in combination (Table 2) and also a cultivar effect. Pasban-90 and Sahar-2006 had higher grain weights.

Grain yield. ANOVA revealed that grain yield was higher in most of the treatments and highest in treatment 4, which was the combination of all three treatments. Yield was least in treatments 5 and 6 (Table 2).

Table 2. Statistical analysis of treatments given and their effect on different parameters (NS = not significant; all values are means of three replicates; values with different letters are significantly different; LSD values for plant height at 0.05 = 9.638, 1,000-kernel weight at 0.05 = 5.463, and for grain yield at 0.05 = 0.2701).

Parameter	Plant height (cm)	1,000-kernel weight (g)	Grain yield (t/ha)
Treatment			
T_1 : *Serratia grimesii* strain A2	103.5	45.65	1. 96 a
T_2 : *Serratia marcescens* strain A3	98.78	45.17	1.82 b
T_3 : *Serratia liquefaciens* strain F11	99.78	45.77	1.99 b
T_4 : Consortium	103.0	47.09	2.81 a
T_5 : ACCD-negative strain R9	97.42	47.54	2.20 a b
T_6 : Control	98.34 NS	44.78 NS	2.05 b
Cultivar			
Pasban-90	97.50 a	37.40 a	1.91 c
Sahar-2006	102.80 b	54.61 b	2.37 d
Treatment combination			
$T_1 V_1$	98.53 c d e	36.74 c d	1.90 f g
$T_2 V_1$	98.45 c d e	36.60 c d	1.90 f g
$T_3 V_1$	96.24 d e	36.06 e	1.95 f g
$T_4 V_1$	99.90 d e	36.85 d e	2.50 e f
$T_5 V_1$	89.99 e	41.80 d	1.65 g
$T_6 V_1$	101.9 c d	36.35 d e	1.60 g
$T_1 V_2$	99.06 c d e	54.56 c	2.02 f g
$T_2 V_2$	101.1 c d	53.75 c	1.74 g
$T_3 V_2$	100.4 c d	55.49 c	2.0 b g
$T_4 V_2$	106.1 c	57.33 c	3.11 e
$T_5 V_2$	104.8 c d	53.28 c	2.75 e
$T_6 V_2$	105.2 c d	53.22 c	2.50 e f

Other parameters. Other parameters, such as germination %, plant height, spike length, grains/spike, and canopy temperature, also were recorded (Table 3, pp. 61). None showed a significant effect when inoculated with PGPR.

Table 3. List of parameters used to evaluate the effect of plant growth-promoting rhizobacteria on two wheat cultivars.

Cultivar	Treatment	Replication	Germination (%)	Plant height (cm)	Spike length (cm)	Grains/spike	Canopy temperature (°C)
Pasban-90	1	1	80	96.62	9.0	38.6	16.1
	2	1	80	95.40	7.9	31.7	15.7
	3	1	80	86.25	8.9	42.3	15.4
	4	1	75	90.52	9.2	37.3	17.5
	5	1	90	92.35	8.9	46.0	17.1
	6	1	90	96.62	8.9	33.6	16.5
Sahar-2006	1	1	80	104.54	8.1	44.0	16.0
	2	1	80	111.86	8.5	41.0	16.5
	3	1	85	109.72	8.6	37.3	16.7
	4	1	85	104.54	8.8	41.0	16.5
	5	1	90	105.76	9.1	42.3	16.2
	6	1	90	106.68	7.7	35.0	16.7
Pasban-90	1	2	80	98.45	10.8	55.3	16.3
	2	2	85	101.49	7.3	20.3	16.2
	3	2	85	94.48	8.5	38.0	15.5
	4	2	80	91.44	7.5	33.3	16.4
	5	2	95	93.26	8.0	39.0	15.8
	6	2	95	104.54	8.0	52.3	15.7
Sahar-2006	1	2	70	97.53	7.7	37.0	17.2
	2	2	70	98.45	7.5	54.3	17.6
	3	2	85	100.58	9.2	45.0	16.0
	4	2	80	100.58	8.1	40.6	17.5
	5	2	95	102.41	7.5	28.0	16.0
	6	2	95	107.59	9.8	60.3	17.3
Pasban-90	1	3	85	105.76	8.0	38.0	16.3
	2	3	90	104.54	7.8	37.3	16.8
	3	3	85	111.86	9.5	39.4	15.4
	4	3	75	108.81	9.7	37.0	16.8
	5	3	85	90.22	8.2	37.3	16.8
	6	3	90	105.76	8.0	26.6	16.5
Sahar-2006	1	3	85	88.39	9.0	44.3	15.9
	2	3	85	90.52	7.5	34.0	16.3
	3	3	75	86.86	10.0	56.0	18.0
	4	3	90	109.72	9.4	42.0	16.0
	5	3	90	107.59	8.8	39.7	16.6
	6	3	90	94.48	8.2	37.6	18.3
Pasban-90	1	4	90	93.26	8.0	38.0	16.0
	2	4	75	92.35	8.7	39.0	15.8
	3	4	85	92.35	8.4	36.3	15.7
	4	4	80	108.81	10.0	40.6	15.5
	5	4	90	84.12	7.4	37.3	17.2
	6	4	95	100.58	11.0	59.3	15.4
Sahar-2006	1	4	80	105.76	8.8	38.0	16.0
	2	4	85	103.63	9.0	37.0	16.3
	3	4	85	104.54	8.6	39.7	15.9
	4	4	85	109.72	9.8	43.3	17.1
	5	4	85	103.63	9.1	40.0	16.8
	6	4	95	111.86	11.5	56.0	14.0

Discussion. Ethylene is the major plant phytohormone and is produced by all almost all plants. Microorganisms present in the soil that produce ACCD promote plant growth by not only sequestering the ACC but also by lowering ethylene. Thus, lower ACCD levels make wheat plants more tolerant and resistant to stress. Soil in close proximity of plant roots is rich in ACCD-containing bacteria and more than 50% of these PGPR belong to *Pseudomonas* spp. and similar bacteria such as *Enterobacter* spp. This study gives information that none of the ACCD-positive strains belonged to either species.

In vivo evaluation was in the field. A root-colonization assay was done in order to check the survival of inoculated strains. Because different strains have different ability to colonize root rhizopshere. Colonization of these PGPR also depends upon the cultivar of wheat. The root-colonizing ability of PGPR was greater in Pasban-90 compared to that for Sahar-2006 (Table 1, p. 59), which can be due to the difference in root exudate composition in the rhizosphere that may vary due to the differences in cultivars. Rhizospheric conditions also vary greatly between cultivars. Elevation of the pH in the root rhizosphere was indicated in Sahar-2006. A soil analysis at BARI, Chakwal, showed that the soil was basic, the elevation in pH making it more alkaline and reducing the rhizophere population. The root-colonization assay indicated that in treatment 4, which was combination of all strains, F11 was not found, but was present in treatment 3, which consists of F11. We deduced that other bacteria, A2 and A3, may kill F11, so it was not found. F11 also was not found in the sequencing results. Sequencing of the bacterial isolates obtained from the root-colonization assay showed that the inoculated strains were present until harvest, because a *Serratia* sp. was obtained in the result. We concluded that these PGPR not only improve plant growth but also competitively survive, surpassing other bacteria, including pathogens. Because they were found throughout the experiment, they give a indication of their powerful nature to survive in any soil condition.

During the course of the experiments, 1,000-kernel weight was not affected by any of the treatments (Table 2, p. 60). Although grain weight is considered to be an important yield-contributing factor, individual grain weight was increased, reduced, or unaffected by the treatments or it may have relation with the number of grains obtained and the degree of environmental stress. Moreover, several reports indicate an inverse relationship between grain number and grain weight. The treatments yielding higher grain number have less grain weight, as was the case for Pasban-90 and Sahar-2006. Pasban-90 had higher grain number and less grain weight, whereas Sahar-2006 had a lower number of grains and their weight was greater. We also observed that these PGPR have different effects on different wheat cultivars, because their root-colonizing ability and survival in the root rhizosphere and endopshere are is cultivar-specific.

The effect of PGPR on individual wheat cultivars was significant in plant height but was not significant in combinations (Table 2, p. 60, and Table 3, p. 61). Plant height was greater for Sahar-2006 compared to that of Pasban-90. Many report that the plant height of Sahar-2006 generally is greater than that of Pasban-90, therefore, the effect of PGPR on plant height is not significant.

Wheat grain yield was higher in most of the treatments and was the best in treatment 4, which was the combination of treatments 1, 2, and 3. Yield was least in treatments 5 and 6. The improvement in grain yield under different treatments was different because of the effect of PGPR inoculated. Second, rhizobacteria containing the ACCD gene inhibit ethylene synthesis thus enhancing the growth of primary roots, seedling roots, and shoot growth, which are the major factors contributing to increased grain yield. ACCD-producing rhizobacteria decrease seedling ethylene, reducing stress in the seedlings. Treatment 4 may be the best because the combination of all the strains may have competitive nature, whcih suppresses other pathogenic strains that harmful for the plant. As a result, treatment 4 increased the number of productive tillers/m^2 and the number of grain/spike.

Conclusion. This research looks at the response of rhizobacteria on root growth and hormone signalling to positively enhance plant yield, thus increasing the plant water usage and harvest index. *Serratia* spp. proved to increase all yield components. However, the fact that *Serratia grimesii* strain A2, *S. marcescens* strain A3, and *S. liquefaciens* strain F11 remained in the root rhizosphere and endopshere throughout the trial proved that they influenced both systemic and local hormone signaling. These microorganisms containing the ACCD enzyme can be used in agriculture to promote plant growth, because they are both environment friendly and also cost effective. Plants inoculated with ACCD-containing bacteria proved to environmentally sociable and also cost effective compared to other technologies employed in agriculture against drought, high salt concentration, and high metals.

**NATIONAL AGRICULTURAL RESEARCH CENTER (NARC), ISLAMABAD
WHEAT WIDE CROSSES AND CYTOGENETICS**

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The Wheat Wide Crosses Program in Pakistan.

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The Wheat Wide Crosses Program around applied targets has its seat in Mexico at CIMMYT since early 1970s and was executed from 1979 until late 2004 by the senior author of this lead article. Upon retirement in late 2004, the CIMMYT program underwent an evolution and several areas were moved to Pakistan to be carried on in this new location but remaining under the leadership A. Mujeeb-Kazi, who was recruited by the Government of Pakistan.

The program is targeted heavily around prebreeding to harness genomic diversity encompassing basic, strategic, and applied tangents by utilizing the diversity potential of all three Triticeae gene pools. The focus is to address major biotic and abiotic stresses complimented by quality trait analyses crucial for nutritive value and grain export advantage.

The abiotic stresses influenced by climate change include tolerance to heat, drought, and salinity/sodicity. On a minor level, at this stage, is the threat of low temperature on which breeders have their sights set. The biotic stresses are stem, yellow, and leaf rust; Karnal bunt; and to a lesser level the new emergence of spot blotch, with sporadic presence in limited acreages of powdery mildew and barley yellow dwarf virus (BYDV).

Stresses are influenced by new pathogen emergence and the cropping systems. The first and most glaring example is the concern Pakistan faces from stem rust entry from the west via Iran around the race Ug99 and its variants. Cropping systems that follow rice and cotton delay wheat planting causing terminal heat to become a significant production constraint. In general, climate change has led to rainfall fluctuations that erratically influence output production in the rainfed areas. Hence, national total yields have stayed around 25 to 30 maunds/acre when the potential of such widely adapted germ plasm can approach 85 to 90 maunds/acre. National mean productivity levels as of the 2012 summer May harvest are 24.5 x 10^6 tons at 2.6 tons/ha. The yield gap remains huge and, if managed well, can add significantly to self-sufficiency, which can be addressed via management practices and varietal uniqueness where genetic diversity has a distinct role because the base of national cultivars is quite narrow. We have a heavy reliance on introduced germ plasm and the better adaptabilty of entries built on winter/spring combinations that possess the T1BL·1RS translocation all possessing a pedigree that have four wheats involved, i.e., Kavkaz, Buho, Kalyansona, and Bluebird. A prudent way forward would to diversify an effort the Wide Crossing Program is addressing.

Our efforts are a shift from the adaptive breeding dominance to take on recombination breeding using genetic resources that are not used in such creativity output by a majority of the national programs. Where we also follow the conventional breeding to a smaller degree, new modes are used like limited backcrossing and selected modified bulk as an efficient process with sights set on integrating doubled haploidy to achieve homozygosity on F_3 selections. The genetic diversity so far has exploited the D, A, and to a limited extent the B(S) genomes of the wheat diploid progenitor species/accessions based upon stocks that were brought in from CIMMYT, Mexico, where they were produced (Mujeeb-Kazi et al. 2004).

The current program in Pakistan is targeted around the production constraints mentioned above having the following major components:
- Exploiting the D- and A-genome, synthetic hexaploid wheats of up to 1,400 derivatives.
- Categorizing the national land races for all major stresses and their incorporation in cultivar development.
- Breeding specifically for yield components that target 1,000-kernel weight, overall spike parameters with a focus on large spikes and multiple ovaries, stay green, prostrate habit, and exploiting intraspecific diversity that includes resistant durums, multiple accessions of *T. turgidum* subsps. *dicoccum*, *dicoccoides*, and *carthlicum*, and, at the bread wheat level, investigate the potential of *T. aestivum* subsp. *spelta*.

— Maintaining global genetic stocks with high focus on wheat/alien chromosome translocations where those already available are getting transferred into national elite wheats via recurrent backcrossing and, in addition, producing new translocations with the salt tolerant and stem rust resistance genetic potential of *Thinopyrum bessarabicum* receiving the main attention. Global partnerships have allowed links to study other resources where *Haynaldia villosa*, *Leymus racemosus*, and *Secale cereale* take priority.

Molecular areas relate to adding efficiency to our major biotic and abiotic stresses targets in the program so far include QTL mapping, association mapping, extensive phenotyping to harness genotyping data (DArT) available globally derived from synthetic hexaploid wheats, use of allele specific markers for cereal quality (HMW, LMW, and grain hardiness), biotic stresses including plant growth aspects, e.g., stay green, dwarfing, and coleoptile length. The present genetic holdings of greater significance are in Table 1.

Table 1. Genetic holdings of greater significance to the Wide Crosses Program, National Agricultural Research Center, Islamabad, Pakistan.

Germ plasm	Entries	Source
Landraces	1,012	Pakistan
A -genome synthetics	194	CIMMYT, Mexico
B-genome synthetics	20	CIMMYT, Mexico
D-genome spring synthetics	1,400	CIMMYT, Mexico
D genome winter synthetics	84	CIMMYT, Mexico
Wheat/alien translocations	24	Kansas State University
CS monosomic, telosomic, di-telosomic lines	All	University of Missouri
Wheat/ alien translocations (Pavon)	67	University of California
Wheat/alien addition lines (*Th. bessarabicum*)	8	CIMMYT and Pakistan
Mapping populations	9	CIMMYT and Pakistan
Wheat/alien amphiploids	25	CIMMYT and Pakistan
Wheat/alien BC_1 self-fertile	19	CIMMYT and Pakistan
Aegilops tauschii	137	US (Idaho)
Aegilops speltoides	75	US (Idaho)
T. monococcum subsps. *monococcum* and *aegilopoides* and *T. urartu*	1,923	US (Idaho)
T. turgidum subsp. *dicoccum*	700	US (Idaho)
T. turgidum subsp. *dicoccoides*	800	US (Idaho)
T. turgidum subsp. *polonicum*	70	US (Idaho)
T. turgidum subsp. *sphaerococcum*	30	US (Idaho)
T. turgidum subsp. *carthlicum*	78	US (Idaho)
T. aestivum subsp. *spelta*	1,300	US (Idaho)
Secale cereale	153	US (Idaho)

Program modus operandi.

The national partners associated with the Wide Crosses Program are spread across all the provinces of Pakistan and are based upon multidisciplinary ties that aid our efforts to combat the target major and minor wheat production constraints.

In Sindh, links are with groups in Sakrand, Tandojam, Jamshoro, and Karachi, with these collaborators planting at diverse sites within the province. Objectives covered are local stem rust races, salinity, heat and drought tolerance, and cereal quality with complete rheology coverage. Advanced pre-bred germ plasm also is evaluated for varietal release potential via wheat breeders for this province.

In the Punjab province, germp lasm is evaluated for stem rust, leaf rust, spot blotch, salinity/sodicity, drought, heat, Karnal bunt, and BYDV. This hub also has a focus on evaluating advanced pre-bred lines for their varietal potential through key breeding programs of the public and private sectors. Within this province, the wide crosses group has partnerships with molecular geneticists who interact in areas of QTL mapping, association/nested association mapping, and wheat transformation. Our program is located in this province and, thus, all wide crossing areas from basic, strategic, and applied sectors are conducted here with rapid flow of germ plasm to all partners nationally and internationally for stress testing. Specific areas are cytology, cytogenetics, molecular cytology, biochemical genetics, molecular genetics, tissue culture, interspecific/intergeneric/intraspecific hybridization, in vitro screening for abiotic stresses, micronutrient assays, recombination, and pre-breeding

In the Khyber Pakhtunkhwa province, pre-breeding ties are in place with the breeding partners with stress evaluations being conducted for yellow rust, BYDV, and drought tolerance.

Lastly, in Baluchistan, our focus is on sharing materials with partners for evaluation and utilization of wide cross products for varietal outputs aided by screening for yellow rust and drought tolerance.

Across all provinces are alliances with national universities aiding student and young human resource development through hands on experience and assist degree program research functions that lead to M.Phil. and Ph.Ds.

Brief write-ups from various national partners across their working disciplines reflect the strong, integrative ties that are vital for this nations wheat based food security 2050 vision. Our program has, in addition, alliances with international partners where CIMMYT plays a major role in sharing of expertise, training our young professionals, and providing user friendly germ plasm for rapid exploitation in breeding programs, and also direct cultivar releases via the adaptation/selection course. International alliances have been a major conduit to knowledge generation of which the recent major publications (Published or in Process) are supportive.

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QTL mapping of doubled haploids for physiological attributes under drought stress.

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Drought stress is one of the major environmental constraints to crop plants, including wheat, worldwide. Synthetic hexaploids can act as a vehicle for improving crop tolerance against various biotic and abiotic stresses. A doubled-haploid (DH) population consisting of 140 individuals derived from a cross between Opata and SH223 was used to identify genomic regions associated with various quantitative, physiological attributes. The DH mapping population was phenotyped for chlorophyll content, chlorophyll flouresence, osmotic adjustment, and proline and superoxide dismutase content under control and drought stress over two years. Genotyping utilized 261 polymorphic wheat microsatellites from Gaterslaben (Germany) and the Agriculture Research Center (Beltsville, MD USA) simple sequence repeats. The linkage map of the DH population comprised 19 linkage groups covering a map length of 2,626 cM was constructed using MapMaker software. Major and minor QTL associated with quantitative physiological traits were identified using QGene software. The phenotypic variation explained by QTL was under high control compared to those for drought stress depicting the complex genetics of drought. The identified QTL are important for high-resolution mapping in synthetic hexaploid wheat. The 'SH223/Opata' DH mapping population provides an excellent genetic stock for digesting genetically complex quantitative attributes under biotic and abiotic environmental constraints. Genomic synteny was observed in DHs with rice because of the occurrence of chlorophyll content QTL on chromosomes 2, 4, and 7, and with maize due to the presence of a chlorophyll fluorescence QTL on chromosome 7.

Major QTL for chlorophyll content (*QTc.wwc-1B-S11*) in the DH mapping population at anthesis during drought stress was mapped on chromosome 1B and exhibited 10.09% phenotypic variation at an LOD score of 5.5. The main allele for this QTL was from Opata. Two more QTL (*QTc.wwc-5B-S9* and *QTc.wwc-7B-S9*) for this trait were mapped on chromosomes 5B and 7B, respectively, under drought stress at anthesis and explained 10.09% and 12.30%, respectively, of the phenotypic variation. The positive allele for QTL on chromosome 5B was contributed by SH223, whereas the QTL on chromosome 7B was from Opata. Seven major and minor QTL for PCFK in the DHs were identified on chromosomes 1B, 7A, and 7D under control and drought stress conditions at anthesis. Only one minor QTL for osmotic adjustment in the DH mapping population was identified on chromosome 7A under drought stress at anthesis. The positive allele for this attribute was governed by SH223 and showed only 8.5% of phenotypic variation at LOD value of 2.5. QTL for chlorophyll content, osmotic adjustment, and chlorophyll fluorescence of the DHs were mapped on homoeologous group 7 under both control and drought stress conditions.

QTL mapping of doubled haploids for phenological attributes under drought stress.

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Drought, one of the major multidimensional environmental constraints to plant growth and productivity, impacts wheat yield in arid and semi-arid regions of the world. Synthetic hexaploids (SH) are considered a novel source of germ plasm under hostile environments because of their potential to cope with biotic and abiotic stresses. One hundred forty double haploids (DH) were evaluated for different phenological attributes at anthesis under drought stress (Table 2). Four minor and four major QTL for 1,000-kernel weight in a DH mapping population were identified on chromosomes 3D, 7B, 5A, 5B, and 3B under drought stress at anthesis. Six QTL for grain number in the DH mapping population were identified on chromosomes 1B, 2A, 3B, 5A, 7A, and 7B under preanthesis drought stress given over two years. Two minor and four major QTL for days-to-heading were identified in the population under drought stress at preanthesis. One major QTL (*QDh.wwc-5A-S9*) under drought stress explained 12.76% of the phenotypic variation at an LOD score of 4.54, flanking in the vicinity of *Xbarc10–Xwms71*. A second and third major QTL under drought stress were identified on chromosomes on 7A and 3B, respectively, and collectively explained 21.08% of the phenotypic variation.

Table 2. QTL identified in a doubled-haploid mapping populaton for phenological attributes under drought stress.

Parameter	1A	1B	1D	2A	2B	2D	3A	3B	3D	4A	4B	5A	5B	5D	6A	6B	6D	7A	7B	7D
1,000-kernel weight		X						X	X		X	X	X						X	
Grain number		X		X	X		X	X		X		X						X	X	
Spikelets/spike				X		X			X			X	X							
Days-to-heading		X		X				X				X		X				X	X	
Days-to-physiological maturity	X			X	X			X		X	X	X	X			X			X	
Spike length	X	X				X	X			X	X	X	X		X	X			X	
Plant height	X			X	X	X		X			X							X	X	
Total chlorophyll content		X			X			X		X			X					X	X	
Osmotic adjustment																		X		
Fo		X																		
Fv/Fo																		X		X
Fv/Fm																				X

The first major QTL for spike length (*QSl.wwc-2D-C9*) in the DH mapping population under controlled conditions was on chromosome 2D. This QTL explained 10.23% of the phenotypic variation at an LOD score of 6.5. Chromosomes 5A and 5B are considered important genomic regions for QTL for 1,000-kernel weight, plant height, physiological maturity, spike length, and days-to-heading under drought stress. QTL detected under control and drought stress conditions not only provide information for the polygenic inheritance and their interaction with the environment but also helps to identify molecular markers closely linked with quantitative traits.

Phenological evaluation and RGAP-based molecular characterization of some exotic wheat genetic stocks.

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Plants feed ~93% of the people of the world, two-thirds of which is contributed by the cereals (wheat, maize, rice, barley, sorghum, and millet). Approximately 80% of the global cereal production is wheat, maize, and rice. Among the cereals, wheat is the largest, being cultivated in 27 countries of the developing world. Of the two principle types of wheat, i.e., bread and durum, 90% is bread wheat. Wheat cultivation encompasses a major production area of 8.303 x 10^6 ha, thereby engaging 33% of the cultivated area of the country each year with a production around 21.7 x 10^6 tons in the last wheat season. The annual average volume of world wheat trade has been about 106 x 10^6 tons between 1999 and 2003. By 2020, the world's demand for wheat is expected to be 40% higher than that in 1990. The demand for wheat, based on production and stock changes, is estimated to increase from a current level of roughly 625 x 10^6 tons to around 813 x 10^6 tons in 2030 and to more than 900 x 10^6 tons by 2050.

Wheat production is threatened by various factors. Apart from abiotic factors, including scarcity of water, drought, and salinity, biotic factors, such as the rust diseases, are the oldest known to humans. Stripe or yellow rust is one wheat disease that has caused yield loss. These diseases of cereals cause low germination, slower growth, shorter height, foliar damage, reduced floret set, stumpy forage quality, and reduced grain yield. Like other developing countries, agriculture is the most important sector of Pakistan's economy and food. Wheat is the country's most important agricultural product, cultivated by 80% of the farmers and contributing to roughly a quarter of the total crop sector value added. Of the total wheat production area of 8.303 x 10^6 ha in Pakistan, 70% is prone to stripe rust, an area of approximately 5.8 x 10^6 ha. In Pakistan, wheat production is threatened due to rust diseases, especially stripe rust. Stripe rust is significant in southern Pakistan, the foothills, northern areas, and Baluchistan. Molecular markers are being used to characterize genetic diversity, population structure, phylogenetic relationships, and pathotypes in the pathogen populations and to study the disease epidemiology and verify an extensive effective control of the disease. A variety of molecular marker systems have been used to study the stripe rust pathogen. Genetic diversity between and within diverse populations of wheat has been analyzed using molecular markers such as isozymes, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple-sequence repeats (SSR), and resistant gene analogue polymorphism (RGAP). Among these, RGAP markers have been developed from conserved domains of plant resistance genes and distributed in the genome to assess their validity for determining genetic diversity and genetic relationships. In addition, the RGAP approach has been utilized successfully to identify markers associated with resistance genes in wheat for stripe rust. SSRs have the capability to differentiate among closely related individuals for diversity and allelic variation across a broad range of germ plasm and have the advantage, over other markers, to trace plants pedigrees. In this study, we identified ergonomically suitable land races of wheat based upon phenological parameters and applied SSR and RGAP markers on wheat stripe rust for genetic diversity (Table 3, pp. 68-70).

To spot molecular markers linked to rust resistance gene(s) in wheat, RGAP and SSR techniques were used determine genetic diversity. Seven SSR primers were screened; primers CFD-13 and CFD-81 showed 29% genetic diversity and polymorphism. CFD-13 showed a high level of polymorphism. The co-efficient ranged from 0.44 to 1.00 for the SSR cluster analysis. Genetic diversity also was accessed using five RGAP primers. Only one RGAP primer, XLRR, gave positive results and showed a 20% polymorphism. Seven genotypes showed 6.25% polymorphism. The co-efficient ranged from 0.55 to 1.00. The SSR markers showed more genetic diversity compared to the RGAP markers. Based upon morphological characteristics, nine genotypes are the most appealing based on their higher number of grains/spike, lower inter-node distance, normal height, and grain quality and color. Out of 112 landraces, 14 were highly polymorphic (12.5% polymorphism). A number of exceptional lines, 7, 14, 26, 44, 45, 85, 107, and 110, were identified on the basis of higher number of grains/spike and lower internode distances ranging from 0.3 to 0.4 cm. The height of these lines is normal, lower than 100 cm, with well-filled grains.

Stripe rust is considered the major constraint in meeting the challenges of sustainable production and can cause yield loss worldwide. Constant growing of cultivars with major genes increases vulnerability, so there is a need to construct a breeding program that uses major genes in combination with minor genes. The competent monitoring of disease and the expansion of genetic diversity may result in valuable control of the disease and reduce major grain losses resulting from the stripe rust pathogen. So, it is clear now that wider the genetic makeup, the wider will be the diversity, and, therefore, the possibility of the crop affected by the pathogen will be reduced.

Table 3. A phenological characterization of 112 landraces of wheat (pubescence lacking (–) and present (+)).

Line	Pubes-cence	Plant height (cm)	Awns	Spikes/ plant	Grains/ spike	Spike length (cm)	Seed color	Internode distance (cm)	1,000—kernel weight (g)
1	—	97.5	gold	12	33	10	amber	0.3	33
2	—	85	gold	15	32	10	brown	0.4	31
3	—	70	gold	12	40	5	amber	0.4	40
4	—	97.5	gold	13	39	10	gold	0.4	39
5	—	92.5	gold	11	44	10	gold	0.5	36
6	—	82.5	gold	14	33	7.5	brown	0.3	30
7	—	72.5	gold	15	67	7.5	amber	0.5	28
8	—	77.5	gold	17	39	5	amber	0.3	39
9	—	80	gold	20	33	7.5	amber	0.3	36
10	—	75	gold	17	20	5	amber	0.3	41
11	—	97.5	gold	20	35	7.5	gold	0.3	43
12	—	87.5	gold	17	24	10	brown	0.2	37
13	—	75	gold	23	25	7.5	amber	0.3	30
14	—	90	—	22	45	7.5	gold	0.3	32
15	—	102	—	12	33	9.5	amber	0.2	37
16	+	82.5	—	19	37	7.5	amber	0.3	36
17	+	95	—	25	32	5	brown	0.3	29
18	—	97.5	gold	11	34	10	gold	0.3	39
19	—	105	gold	18	49	5	amber	0.5	42
20	—	97.5	gold	17	42	7.5	brown	0.5	37
21	—	102.5	gold	16	23	10	brown	0.6	42
22	—	92.5	gold	14	42	7.5	amber	0.4	37
23	—	120	gold	18	33	10	amber	0.4	32
24	—	102.5	—	17	23	12.5	amber	0.4	35
25	—	95.5	—	18	31	8	amber	0.4	36
26	—	92.5	gold	16	48	7.5	amber	0.5	41
27	—	93	—	16	32	8	amber	0.4	31
28	—	97.5	—	16	37	7.5	amber	0.5	42
29	—	97.5	gold	30	21	7.5	amber	0.6	63
30	—	97.5	gold	16	35	7.5	amber	0.5	24
31	—	87.5	—	20	31	7.5	amber	0.4	45
32	—	77.5	gold	14	41	7.5	amber	0.5	32
33	—	88	—	24	33	8	amber	0.4	41
34	—	87.5	—	20	34	7.5	amber	0.4	35
35	—	100	gold	15	33	10	amber	0.4	43
36	—	92.5	gold	18	38	7.5	amber	0.3	20
37	—	85	gold	12	13	7.5	amber	0.4	38
38	—	83	gold	10	31	8	brown	0.5	22
39	—	92.5	gold	7	35	7.5	amber/ white	0.4	36
40	—	100	—	14	41	7.5	amber/ brown	0.5	28
41	—	85	—	4	24	7.5	amber	0.4	14
42	—	80	—	9	38	7.5	amber	0.3	34
43	—	77.5	—	11	36	7.5	amber	0.4	39
44	—	131.5	gold	11	51	11.5	amber	0.4	31
45	—	115	—	13	64	12.5	amber	0.5	46

Table 3. A phenological characterization of 112 landraces of wheat (pubescence lacking (–) and present (+)).

Line	Pubes-cence	Plant height (cm)	Awns	Spikes/ plant	Grains/ spike	Spike length (cm)	Seed color	Internode distance (cm)	1,000—kernel weight (g)
46	—	112.5	—	15	30	10	amber	0.5	56
47	—	87.5	—	17	34	7.5	amber/ white	0.4	36
48	—	95	—	13	34	7.5	amber	0.5	33
49	—	80	—	14	40	7.5	amber	0.6	33
50	—	78	—	10	43	8	amber	0.5	22
51	—	86	—	17	39	7.5	amber	0.4	38
52	—	97	gold	18	40	9.5	amber	0.4	38
53	—	73	—	14	30	7.5	amber	0.4	36
54	+	78	gold	7	32	8	amber	0.4	59
55	+	90	—	20	46	7.5	amber	0.5	31
56	+	77.5	—	14	37	7.5	amber	0.6	37
57	+	80	—	15	40	7.5	amber	0.4	34
58	+	85	—	16	32	7.5	white/ amber	0.4	36
59	+	113	—	24	36	7.5	white/ amber	0.5	48
60	+	85	—	17	31	7.5	amber	0.5	39
61	+	77	—	16	39	7	amber	0.3	36
62	+	83	gold	17	31	7.5	amber	0.4	43
63	+	87.5	gold	12	42	7.8	white/ amber	0.5	30
64	+	108	gold	16	36	12.5	amber	0.4	41
65	—	83	gold	10	37	7.5	amber	0.3	34
66	—	97.5	gold	16	32	7.5	amber	0.4	38
67	—	97.5	gold	14	37	7.5	amber	0.3	38
68	—	93	gold	10	37	7.5	white/ amber	0.4	33
69	—	85	gold	18	31	10	amber/ brown	0.6	31
70	—	82.5	gold	9	47	10	amber	0.4	35
71	+	87.5	gold	12	49	7.5	amber	0.4	41
72									
73	+	102.5	gold	12	45	7.5	white/ amber	0.4	48
74	+	92.5	gold	8	48	7.5	amber	0.2	38
75	+	75.3	gold	10	38	7.8	amber	0.2	52
76	+	76	gold	7	57	7.5	amber	0.4	46
77	+	97	—	15	34	9.5	amber	0.3	35
78	—	90	—	17	40	7.5	amber	0.3	36
79	+	80	—	9	41	7.5	amber	0.3	41
80	+	95	—	15	35	7.5	amber	0.4	40
81	+	77.5	—	16	26	7.5	amber	0.3	35
82	—	92.5	—	14	30	7.5	brown	0.3	40
83	+	83	gold	20	42	8	amber	0.4	29
84	+	87.5	—	11	35	7.5	amber	0.4	33
85	+	85	gold	13	60	7.5	amber	0.3	43

Table 3. A phenological characterization of 112 landraces of wheat (pubescence lacking (–) and present (+)).

Line	Pubes-cence	Plant height (cm)	Awns	Spikes/ plant	Grains/ spike	Spike length (cm)	Seed color	Internode distance (cm)	1,000—kernel weight (g)
86	+	93	—	12	41	8	white/ brown	0.3	34
87	+	107.5	—	11	52	10	white/ brown	0.5	40
88	+	100	—	17	46	10	amber	0.3	34
89	+	97.5	—	18	39	7.5	amber	0.4	37
90	+	97.5	gold	17	37	7.5	amber	0.3	47
91	+	98	—	18	33	10.5	amber	0.3	30
92	—	108	—	13	45	8	amber	0.4	36
93	—	115	gold	10	30	10	white/ amber	0.7	29
94	+	111.5	gold	19	27	11.5	amber	0.6	36
95	+	121.5	gold	17	29	11.5	amber	0.5	41
96	+	118	gold	9	56	11	amber	0.4	36
97	—	107.5	gold	16	19	12.5	amber	0.5	43
98	+	103	gold	11	19	10.5	amber	0.4	22
99	+	104	gold	10	30	15	amber	0.4	27
100	+	100	gold	13	33	10	amber	0.5	34
101	+	107.5	gold	17	17	7.5	amber	0.4	37
102	+	98.5	gold	12	37	8.5	amber	0.5	42
103	+	89	gold	9	55	8.5	amber	0.5	37
104	—	85	gold	14	34	7.5	amber	0.5	37
105	+	83	gold	14	36	7.5	amber	0.6	45
106	—	106	gold	10	45	12.5	amber	0.4	24
107	+	85	gold	12	51	10	amber	0.4	37
108	—	77.5	gold	16	32	7.5	amber	0.4	44
109	+	100	gold	13	36	7.5	amber	0.3	42
110	+	73	gold	8	68	10.5	white/ amber	0.4	35
111	—	110	gold	3	19	10	amber	0.5	38
112	—	112	gold	13	23	5	amber	0.4	33

Detection of QTL for drought tolerance in a doubled-haploid mapping population.

Sammer Fatima, Muhammad Arshad, Anna Maria Mastrangelo, Daniela Marone, Giovani Laido, Rahmatullah Qureshi, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

A doubled-haploid (DH) mapping population, with the pedigree 'Doy–1//*Aegilops tauschii* (458)/5/Opata', was planted under control (field, fully irrigated) and stress (rain shelter) conditions. The parents of mapping population, SH-349 (a D-genome based drought tolerant synthetic hexaploid wheat) and the bread wheat Opata M-85 (drought susceptible), also were grown individually in the field and shelter. A biochemical analysis included osmolyte determination, i.e., soluble sugar, proline, antioxidant superoxide dismutase, and cell membrane stability followed by molecular diagnostics, including DNA extraction, PCR, and capillary electrophoresis. Yield and yield components determined were days-to-heading, days-to-physiological maturity, plant height (cm), spike length (cm), number of spikelets/spike, 1,000-kernel weight (g), pubescence, awn color, and growth habit. The treatments were arranged in a complete randomized design, and comparison of means was by least significant difference. JoinMap4 and MapQTL5 were used for molecular diagnostics. Drought tolerant wheat lines were identified, and QTL controlling drought tolerance in the wheat mapping population were determined. Eight drought-tolerant lines were identified for different traits. A linkage map was constructed using SSR markers; 141 markers used to screen the Opata, drought-sensitive, and SH-349, drought-tolerant, parents. Seventy-nine polymorphic markers were identified and applied on the mapping population. We identified 61 markers that showed linkage and 16 linkage groups. The map covered 14 chromosomes. The length of genetic linkage map was 665.3 cM with a density of 10.91 cM/marker. Interval mapping and multiple QTL mapping were used to detect 120 QTL. Many major QTL were identified (Table 4).

Table 4. QTL detected by multiple QTL mapping in a 'synthetic hexaploid/ Opata' doubled-haploid mapping population.

Line	QTL name	Chromosome	Trait	Environment
1	*QDH.C.1.MQ.wwc-2D*	2D	DH-1st	field
2	*QDH.S.1.MQ.wwc-2D*	2D	DH-1st	rain shelter
3	*QDPM.C.1.MQ.wwc-2D*	2D	DPM-1st	field
4	*QDH.C.2.MQ.wwc-7D*	7D	DH-2nd	field
5	*QDH.S.2.MQ.wwc-6A*	6A	DH-2nd	rain shelter
6	*QDPM.C.2.MQ.wwc-7A*	7A	DPM-2nd	field
7	*QDPM.S.2.MQ.wwc-7D*	7D	DPM-2nd	rain shelter
8	*QPH.S.2.MQ.wwc-7A*	7A	PH-2nd	rain shelter
9	*QSp.L.C.1.MQ.wwc-4A*	4A	Sp-L-1st	field
10	*QSp.L.S.1.MQ.wwc-7A*	7A	Sp-L-1st	rain shelter
11	*QPub.C.2.MQ.wwc-1B*	1B	Pub-2nd	field
12	*QTGW.S.1.MQ.wwc-5A*	5A	TGW-1st	rain shelter
13	*QAC.C.1.MQ.wwc-1B*	1B	AC-1st	field
14	*QAC.S.1.MQ.wwc-2D*	2D	AC-1st	rain shelter
15	*QAC.C.2.MQ.wwc-7D*	7D	AC-2nd	field
16	*QAC.S.2.MQ.wwc-7D*	7D	AC-2nd	rain shelter
17	*QG/S.C.1.MQ.wwc-6A*	6A	G/S-1st	field
18	*QG/S.S.1.MQ.wwc-1B*	1B	G/S-1st	rain shelter
19	*QG/S.C.2.MQ.wwc-6A*	6A	G/S-2nd	field
20	*QG/S.S.2.MQ.wwc-6A*	6A	G/S-2nd	rain shelter
21	*QPros.C.1.MQ.wwc-7D*	7D	Pros-1st	field
22	*QPros.S.2.MQ.wwc-7D*	7D	Pros-2nd	rain shelter
23	*QRL.C.MQ.wwc-2D*	2D	RL-C	hydro
24	*QSL.S.MQ.wwc-7A*	7A	SL-S	hydro
25	*QChlro.S.MQ.wwc-2A*	2A	Chlro-S	hydro
26	*QCMS.MQ.wwc-2A*	2A	CMS	hydro
27	*QPhoto.C.MQ.wwc-7D*	7D	Photo-C	hydro
28	*QPhoto.S.MQ.wwc-7D*	7D	Photo-S	hydro
29	*QProlin.S.MQ.wwc-7A*	7A	Proline-S	hydro
30	*QSugar.S.MQ.wwc-4A*	4A	Sugar-S	hydro
31	*QSOD.C.MQ.wwc-6A*	6A	SOD-C	hydro

In conclusion, our results show that some drought-tolerant or resistant genes in both parents (Opata and SH-349) can be transferred to susceptible genotypes using marker-assisted selection. A variety of factors may affect the outcome of a QTL analysis; for instance, the selection of the cross, the population structure and size, the number of measured replications and environments, and the density of markers. The magnitude of the QTL effect and accurate chromosome map location also are important for verifying the identified QTL. Overall, many QTL were detected across multiple traits, and the wild or synthetic line contributed both positively and negatively to these traits. We have some indication that the wild species Ae. tauschii donated some alleles, because QTL for some traits were associated with the D genome and with the synthetic line. This study provided additional evidence that QTL strategy is useful and is able to enhance the performance of existing cultivars. Further QTL studies will assist in the contribution of positive allelic diversity in the future.

Grain morphology and quality characterization of synthetic hexaploids to enhance its breeding value.

Awais Rasheed, Abdul Aziz Napar, Hadi Bux, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Considerable genetic diversity resides in the wild progenitor species of bread wheat. A large collection of wild accessions of *Triticum turgidum* subsp. *dicoccum* and *Aegilops tauschii* has been captured in the primary synthetic wheats developed by the CIMMYT Wide Crosses Program. Diversity to various biotic (Mulki et al. 2012, Mol Breed 31:299-311) and abiotic stresses (Ogbonnaya et al. 2013, Plant Breed Rev 37:35-122) has been reported. Favorable genes for grain yield must be present in synthetic hexaploids, because several cultivars have been released in China, Spain, Ecuador, and Mexico from synthetic derivatives. However, no good strategy in place to identify and narrow down the wide array of synthetics based on grain quality and yield and attributes that enhance their true breeding value. The current study emphasizes a detailed genetic potential analysis at various grain quality loci and analyzing seed morphology of synthetic hexaploids using digital imaging technology.

Grain quality. The properties of processing quality were determined primarily by high- and low-molecular-weight gluten protein subunits in the flour that are associated with dough rheological properties determining bread-making quality. Polyphenol oxidase activity, yellow pigment content, grain hardiness (PINA, PINB, and PINBV2), lipoxygenase acitivity, zeta-carotene desaturase, and waxy protein have significant influence on the quality of end-use products. Several functional markers are available that cover the allelic variation at all contributing loci. A list of markers and the complete methodology is available at University of California–Davis website maintained by WheatCAP project (http://maswheat.ucdavis.edu/protocols/FunctionalMarkers/FM_quality.htm). We used these markers to elucidate allelic variation in 230 D-genome, synthetic hexaploids at major quality contributing loci.

Grain morphology. Useful and novel variation in untapped germ plasm needs state-of-the-art phenotypic characterization prior to their utilization. Maximizing yield is possible by enhancing the genetic gain from synthetic hexaploids by effective evaluation and exploitation. Grain yield primarliy depends on kernel weight, which is contributed to by several of the seed shape descriptors. The progress in phenotyping is not at par with high-throughput genotyping. More accurate, efficient, and high-throughput avenues for phenotyping need to be pursued in order to get maximum benefit from low-cost genotyping resources. In this scenario, photometric measurements or digital imaging provide more concise and cheaper phenotypic information and better elucidate the individual components of complex traits. The present study focuses on the state-of-the-art phenotyping of grain morphology using digital imaging to describe major kernel dimensions and elliptic fourier descriptots contributing to grain size and weight. A population comprised of 230 D-genome, synthetic hexaploids was genotyped for 1,500 DArT markers, facilitating a genome-wide association of kernel traits and the different kernel dimensions that contribute to size and weight. The population structure analysis and genetic diversity measurements, using DArT markers, indicated that these synthetics are diverse and will broaden the genetic base of crop if exploited for wheat genetic improvement. This technique also narrowed down the D-genome synthetic hexaploids as having the potential to improve grain yield, which will facilitate the targeted exploitation of these synthetics to improve grain yield practices in Pakistan under the umbrella of a wheat wide crosses program.

Comparative assessment of high-molecular-weight glutenin composition and their relationship with grain quality traits in bread wheat and synthetic derivatives.

Ahmad Ali, Muhammad Arshad, Abdul Aziz Napar, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Various synthetic hexaploid wheats (*T. turgidum* subsp. *durum*/*Ae. tauschii*) have resulted in significantly superior combinations for biotic/abiotic resistance/tolerances (Mujeeb-Kazi 2003). At the same time, baking industry exigencies and wide consumer preferences have driven wheat breeders to incorporate grain quality-related traits as an important preference in current research. Hence, the development of wheat cultivars with good bread-making quality is a challenging task in many wheat breeding programs (Oury and Godin 2007), particularly if adaptive to a stress environment. Gluten proteins are the key endosperm components that are mainly emphasized regarding end-use quality traits (Payne et al. 1987). The glutenins are long chains of polypeptides linked by disulfide bonds and comprised of low-molecular-weight (LMW-GS) and high-molecular-weight subunits (HMW-GS) (Payne and Lawrence 1983). The HMW-GSs designated as *Glu-A1*, *Glu-B1*, and *Glu-D1* are encoded by multi-allelic genes located on the long arms of chromosomes 1A, 1B, and 1D, respectively. The HMW-GSs constitute about 10% of the wheat endosperm storage proteins, compared to 40% LMW-GSs, but still have a major influence on the bread-making properties of flour (Payne et al. 1987). This study investigated a collection of drought-tolerant wheat genotypes, comprised of D-genome synthetic hexaploid derivatives and conventional bread wheat germ plasm, for key grain quality parameters and their genetic composition based on the HMW-GS profiles. The experimental germ plasm consisted of a core collection of 55 drought-tolerant wheats comprising three groups: i) D-genome synthetic hexaploid derivatives, ii) conventional bread wheat lines, and iii) elite check cultivars.

The frequency of HMW-GS alleles identified in the germ plasm is presented (Table 5, p. 73). *Glu-1* loci encoded 11 different alleles across the three genomes in these genotypes. At the *Glu-A1* locus, three alleles were observed, of which the Ax null allele was found predominantly in 46 (83.64%) genotypes. At the *Glu-B1* locus, subunit 17+18, encoded by *Glu-B1b*, was found in the most genotypes (54.44%). Similarly, *Glu-D1d*, which encodes the Dx5+Dy10 subunit, was observed in 63.64% of the genotypes, an important good quality subunit encoding a HMW-GS allele. Maximum allelic diversity was found at the *Glu-B1* (0.61) locus, followed by *Glu-D1* (0.50), and *Glu-A1* (0.29), primarily due to the allelic richness (five alleles) observed at the *Glu-B1* locus. However, *Glu-A1* and *Glu-D1* showed the same allelic

Table 5. Allelic variation at the *Glu-1* (high-molecular-weight subunit) loci in a core collection of 55 drought-tolerant wheats comprising three groups: i) D-genome synthetic hexaploid derivatives, ii) conventional bread wheat lines, and iii) elite check cultivars.

Locus	Allele	Subunit	Accessions	Frequency (%)	H (Nei's Index)
Glu-A1	A	1	3	5.45	
	B	2*	6	10.91	
	C	Null	46	83.64	0.29
Glu-B1	A	7	2	3.64	
	B	7 + 8	30	54.55	
	D	6 + 8	3	5.45	
	I	17 + 18	16	29.09	
	F	13 + 16	4	7.27	0.61
Glu-D1	A	2 + 12	17	30.91	
	D	5 + 10	35	63.64	
	Z	3 + 10	3	5.45	0.50

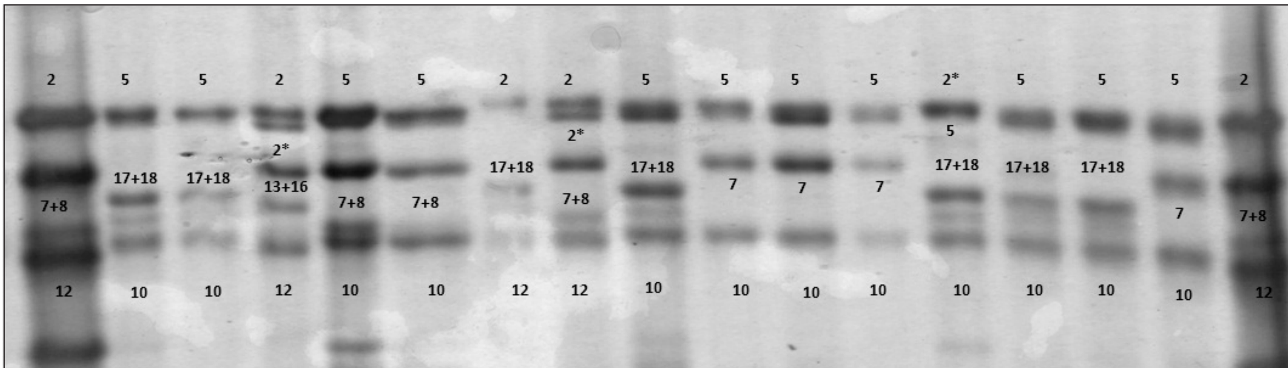

Fig. 1. High-molecular-weight glutenin subunit profile of germ plasm (from the left; lane 1, Chinese Spring (check); 2, AA11; 3, AA15; 4, AA37; 5, AA17, 6, AA18; 7, AA27; 8, AA31; 9, AA38; 10, AA22; 11, AA213; 12, C291 (check); 13, Pavon (check); 14, AA41; 15, AA45; 16, C591 (check); and 17, Chinese Spring (check).

richness (three alleles for both loci), but the distribution of allele frequencies at the *Glu-D1* locus contribute towards more diversity than that of alleles at the *Glu-A1* locus. The HMW-GS observed are presented (Fig. 1).

Results from previous studies on the relationship between glutenin subunits and end-use quality have confirmed that HMW-GS are highly correlated with bread baking quality (Payne et al. 1987). Glutenins and gliadins also were found to influence the bread-making quality (Payne et al. 1987) but with inconsistent results, mostly due to the use of different genetic materials with various genetic backgrounds in different studies. For example, when measuring the effect of 2* subunits on SDS-sedimentation volume, a value equal to 1 was observed from using the British-grown wheat cultivars (Payne et al. 1987), whereas a value greater than 1 was found by Mao et al. (1995) using different wheat cultivars, and a value of less than 1 was obtained by Liu et al. (2005) using 251cultivars and advanced lines.

The key quality parameters studied in this germ plasm include protein contents (%), SDS-sedimentation volume, and carotenoids and their values in individual genotypes are given (Table 6, pp. 74-75). Protein contents ranged from 11.2% to 19.9% with an average of 13.6%. The highest protein content was observed in AA53 (Chakwal-50) which is a rainfed cultivar released in Pakistan. The lowest protein content was found in AA55, which also is cultivated in Pakistan. Eighteen genotypes (32.7%) were found to have more than 14% protein content, which is significantly a promising result. The SDS sedimentation volume in the germ plasm ranged from 2.4 mL to 5.0 mL with an average 3.4 mL. Chakwal-50, which is known to have good glutenin composition and protein contents had SDS-sedimenation value of 5.0 mL. At the same time, other lines, including AA20 and AA48, which are synthetic derivatives, exhibited SDS-sedimenation volume of 4.3 mL and 4.1 mL, respectively. Carotenoids ranged from 4.4 ppm to 9.9 ppm with an average of 6.5 ppm. Results with NIR spectroscopy for grain quality traits of wheat were found promising and economical. Wheat flour SDS sedimentation volume, together with gluten strength, is correlated with dough rheology (Mondal et al. 2009). Because wheat is focused mainly from end-use quality, a better understanding of the genetics underlying specific quality parameters is essential to enhance selection during the breeding process (Carter et al. 2012). The results help us improve under-

Table 6. High-molecular-weight glutenin subunit and quality characteristics of the studied wheat genotypes (SBW = synthetic-derived bread wheat, CBW = conventional bread wheat, and CBW* = check cultivar).

Genotype (AA)	Type	High-molecular-weight glutenin subunit			Protein (%)	Carotenoid (ppm)	SDS-sedimentation (mL)
		Glu-1A	*Glu-1B*	*Glu-1D*			
1	CBW	Null	7+8	2+12	13.3	6.2	3.1
2	CBW	Null	13+16	2+12	13.3	8.1	3.5
3	CBW	Null	7+8	5+10	12.5	6.6	3.1
4	CBW	Null	17+18	5+10	13.2	8.1	3.2
5	SBW	Null	6+8	2+12	13.4	6.5	3.3
6	CBW	Null	7+8	5+10	13.0	8.3	3.3
7	CBW	Null	7+8	5+10	13.9	7.4	3.5
8	CBW	Null	7+8	5+10	12.3	4.5	3.0
9	CBW	Null	17+18	5+10	12.9	5.9	2.4
10	CBW	Null	7+8	2+12	12.0	7.2	2.9
11	CBW	Null	17+18	5+10	12.7	8.4	2.7
12	SBW	Null	13+16	3+10	13.5	9.9	3.6
13	SBW	Null	7+8	3+10	12.0	5.2	3.5
14	SBW	Null	7+8	5+10	12.5	5.5	3.1
15	CBW	Null	17+18	5+10	12.4	8.0	3.2
16	SBW	Null	7+8	2+12	13.7	5.5	3.9
17	SBW	Null	7+8	5+10	13.3	7.2	3.7
18	SBW	Null	7+8	5+10	15.0	8.3	3.4
19	SBW	Null	7+8	5+10	13.4	5.2	3.2
20	SBW	Null	7+8	5+10	14.5	6.1	4.3
21	CBW	Null	7+8	5+10	14.2	8.7	3.3
22	CBW	Null	7	5+10	13.6	8.5	3.4
23	CBW	Null	7	5+10	13.4	7.7	4.1
24	SBW	Null	7+8	2+12	13.7	7.0	3.2
25	CBW	Null	7+8	5+10	13.5	7.0	3.6
26	SBW	Null	6+8	2+12	14.9	5.7	3.6
27	SBW	Null	17+18	2+12	14.3	6.9	3.7
28	SBW	2*	17+18	5+10	15.3	8.2	3.6
29	SBW	Null	7+8	5+10	15.3	5.3	3.6
30	CBW	Null	17+18	2+12	15.3	5.9	3.4
31	SBW	2*	7+8	2+12	13.6	6.9	3.4
32	SBW	1	7+8	2+12	14.4	5.8	3.5
33	SBW	1	6+8	2+12	13.9	6.2	3.3
34	SBW	Null	7+8	5+10	12.7	6.2	2.9
35	CBW	Null	7+8	5+10	13.0	5.3	2.8
36	SBW	Null	7+8	5+10	12.0	6.6	3.3
37	CBW	Null	13+16	2+12	13.3	6.4	3.5
38	CBW	Null	17+18	5+10	12.9	6.5	3.8
39	SBW	2*	17+18	5+10	13.0	6.3	3.5
40	CBW	2*	17+18	2+12	12.7	5.1	3.0
41	SBW	Null	17+18	5+10	12.8	5.1	3.5
42	CBW	Null	7+8	5+10	13.5	6.0	3.0
43	CBW	Null	7+8	5+10	13.8	5.5	3.4
44	SBW	2*	17+18	5+10	14.8	7.7	3.6
45	SBW	Null	17+18	5+10	13.4	6.4	3.6
46	SBW	Null	7+8	2+12	13.8	5.9	3.4
47	SBW	Null	7+8	3+10	14.1	6.0	3.6

Table 6. High-molecular-weight glutenin subunit and quality characteristics of the studied wheat genotypes (SBW = synthetic-derived bread wheat, CBW = conventional bread wheat, and CBW* = check cultivar).

Genotype (AA)	Type	High-molecular-weight glutenin subunit			Protein (%)	Carotenoid (ppm)	SDS-sedimentation (mL)
		Glu-1A	*Glu-1B*	*Glu-1D*			
48	SBW	Null	13+16	2+12	14.6	6.0	4.1
49	CBW	Null	17+18	5+10	15.3	5.9	3.3
50	CBW	Null	7+8	5+10	14.1	7.0	4.1
51	CBW*	Null	17+18	2+12	14.0	6.7	3.5
52	CBW*	Null	7+8	5+10	14.7	6.0	3.2
53	CBW*	Null	7+8	5+10	19.9	5.1	5.0
54	CBW*	Null	17+18	5+10	12.2	4.4	3.0
55	CBW*	2*	7+8	5+10	11.2	5.3	3.2
Average					13.6	6.5	3.4
σ					1.28	1.18	0.41
CV (%)					9.37	18.04	11.89
Maxium					19.9	9.9	5.0
Minimum					11.2	4.4	2.4

standing of the relationships among glutenin compositions and grain quality traits (Tabasum et al. 2011). Quality traits and diversity for glutenin alleles were compared between conventional bread wheat germplasm and D-genome synthetic hexaploid derivatives (Table 7). In synthetic derivatives, more diversity for *Glu-A1* (0.38) and *Glu-D1* (0.59) was observed, whereas no comparison was found for *Glu-B1*. Similarly, protein content (13.8±0.9) and SDS-sedimentation volume (3.5±0.3) were slightly higher in synthetic derivatives compared to bread wheat cultivars. Carotenoids were slightly lower in synthetic derivatives, which is statistically not significant.

Table 7. Comparison of quality traits and glutenin diversity between D-genome synthetic derivatives (SBW) and conventional bread wheat (CBW).

Trait	SBW (n=26)	CBW (n=29)
Protein (%)	13.8 ± 0.90	13.5 ± 1.50
SDS sedimentation	3.5 ± 0.30	3.3 ± 0.40
Carotenoids	6.5 ± 1.10	6.6 ± 1.25
Diversity (H) at:		
Glu-A1	0.38	0.19
Glu-B1	0.59	0.60
Glu-D1	0.59	0.36

Conclusively, this germ plasm set, which is known to have drought-tolerant characteristics, possessed desirable glutenin alleles and grain quality characteristics. The diverse origin of the genotypes in this germ plasm set will enhance the genetic base of breeding programs and contribute to new alleles from D-genome synthetic hexaploids for both drought and grain quality.

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Comparative assessment of low molecular weight glutenin composition and their relationship with grain quality traits in bread wheat and synthetic derivatives.

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Common wheat, being one of the most important food crops worldwide, needs extensive research with major emphasis on yield improvement as well as its adaptation to various biotic and abiotic stresses. Because only a few accessions of the donor species were involved in the evolution of common wheat, genetic diversity was introduced into common wheat by the 'bridge' of a synthetic hexaploid (SH) wheat derived from artificial synthesis of hexaploid wheat (*T. turgidum*/*Ae. tauschii*) in a manner analogous to the evolution of hexaploid wheat (Mujeeb-Kazi et al. 1996). The quality and quantity of wheat gluten proteins give elasticity and extensibility necessary for bread making, contributes about 80–85% of the total flour protein (Shewry et al. 1995), and is comprised of two prolamine groups, gliadins, and glutenin. The glutenins, in turn, are comprised of high-molecular-weight (HMW-GS) and low-molecular-weight (LMW-GS) glutenin subunits. Utilization of wheat is highly dependent on its end-use quality, which, in turn, relies on protein content, carotenoid con-

Table 8. Low-molecular-weight glutenin subunits of wheat the genotypes (SBW = synthetic-derived bread wheat, CBW = conventional bread wheat, and CBW* = check cultivar).

Genotype (AA)	Type	Low-molecular-weight subunit		Genotype (AA)	Type	Low-molecular-weight subunit	
		Glu-A3	*Glu-B3*			*Glu-A3*	*Glu-B3*
1	CBW	b	J	29	SBW	c	f
2	CBW	b	J	30	CBW	f	J
3	CBW	b	g	31	SBW	c	h
4	CBW	d	f	32	SBW	b	e
5	SBW	d	i	33	SBW	b	h
6	CBW	c	h	34	SBW	b	h
7	CBW	f	h	35	CBW	c	h
8	CBW	c	h	36	SBW	b	b
9	CBW	c	i	37	CBW	f	d
10	CBW	f	J	38	CBW	c	e
11	CBW	g	d	39	SBW	b	b
12	SBW	d	i	40	CBW	b	i
13	SBW	b	f	41	SBW	c	h
14	SBW	g	J	42	CBW	b	d
15	CBW	b	d	43	CBW	f	h
16	SBW	c	i	44	SBW	c	J
17	SBW	f	c	45	SBW	c	i
18	SBW	c	h	46	SBW	c	e
19	SBW	c	h	47	SBW	c	h
20	SBW	c	f	48	SBW	b	i
21	CBW	b	J	49	CBW	c	e
22	CBW	a	d	50	CBW	c	j
23	CBW	c	f	51	CBW*	c	g
24	SBW	b	i	52	CBW*	g	g
25	CBW	a	d	53	CBW*	c	c
26	SBW	c	f	54	CBW*	c	c
27	SBW	f	e	55	CBW*	c	g
28	SBW	c	f				

tent, and SDS-sedimentation volume. Recently, Tang et al. (2008, 2010) suggested that grain quality improvement also is possible through the utilization of SHs in breeding programs. Our objective was to investigate a collection of drought-tolerant, wheat genotypes of D-genome SH derivatives and conventional bread wheat germ plasm for key grain quality parameters and their genetic composition based on the LMW-GS profiles.

Table 9. Allelic variation at the *Glu-3* (low-molecular-weight subunit) loci in a core collection of 28 D-genome synthetic derivatives (SBW) and conventional bread wheat (CBW).

Locus	Allele	Accessions	Frequency (%)	H (Nei's Index)
Glu-A3	a	2	3.64	0.70
	b	15	27.27	
	c	25	45.45	
	d	3	5.45	
	f	7	12.73	
	g	3	5.45	
Glu-B3	b	2	3.64	0.87
	d	6	10.91	
	e	5	9.09	
	f	7	12.73	
	g	4	7.27	
	h	12	21.82	
	i	8	14.55	
	j	8	14.55	

Results and discussion. Given the discrepancy reported in some studies about the correct identification of the alleles coded at LMW-GS loci using SDS-PAGE (Ikeda et al. 2008), we identified alleles using a PCR-based analysis. In effect, the recent development of allele-specific markers for *Glu-A3* (Wang et al. 2010) and *Glu-B3* (Wang et al. 2009) alleles have profoundly increased the efficiency, accuracy, and reduced the cost for allelic characterization in bread wheat germ plasm. The alleles present, their frequency, and the genetic diversity of alleles at each LMW-GS locus are reported (Tables 8, p. 76, and Table 9). The PCR profiles of LMW-GS alleles are presented (Fig. 2). Six different alleles at *Glu-A3* locus and eight alleles at *Glu-B3* locus were identified by allele-specific markers. At *Glu-A3*, the allele *Glu-A3c* was present in majority of the genotypes (45.45%), whereas *Glu-A3a* was present only in two genotypes (AA39 and AA41). The predominant frequency of *Glu-A3c* has been observed in Indian cultivars (Ram et al. 2011), and other studies also showed its presence in diverse wheat genotypes representing different regions (Wang et al. 2010; Zhang et al. 2004). The frequency of other alleles, *Glu-A3b*, *Glu-A3d*, *Glu-A3f*, and *Glu-A3g*, was found to be 27.27%, 5.45%, 12.73%, and 5.45%, respectively. The major

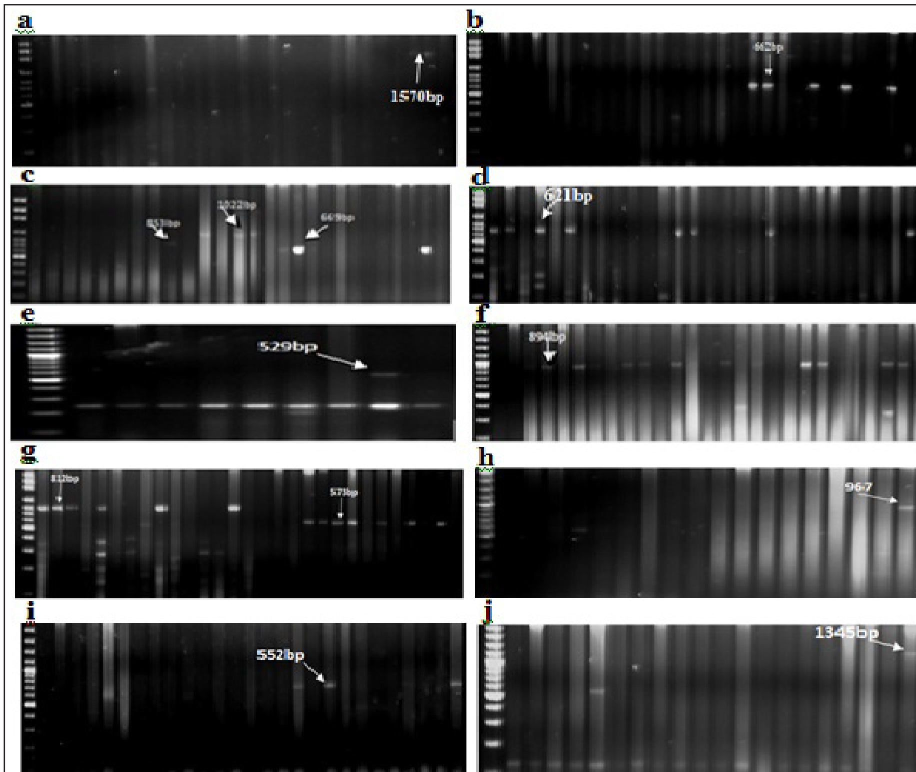

Fig. 2. Electrophoresis of some of the PCR products amplified from germ plasm on agarose gels using allele-specific markers for a, *gluB3b*; b, *gluB3d*; c, *gluB3g gluB3h gluB3e*; d, *gluB3i*; e, *gluA3a*; f, *gluA3b*; g, *gluB3f gluA3c*; h, *gluA3d*; i, *gluA3f*; and j, *gluA3g*. Materials used as PCR templates were (a) B3b- AA36, AA39; (b) B3d-Aa11, AA15, AA22, AA25, AA37; (c) B3g-AA29, B3h-AA18, AA19, AA31, B3e-AA27, AA32, AA46; (d) B3i- AA5, AA9, AA12, AA16, AA24, AA40, AA45, AA48; (e) A3a-AA22; (f) A3b-AA1, AA3, AA13, AA15, AA21, AA32, AA33, AA34, AA36, AA39, AA40, AA42, AA48; (g) B3f-AA4, AA13, AA20, AA26, AA28, AA29; A3c-AA16,.AA18, AA19, AA20, AA26, AA28, AA29, AA31; (h) A3d-AA30; (i) A3f-AA7, AA10, AA27, and (j) A3g-AA11.

allele found at the *Glu-B3* locus was *Glu-B3h*, which appeared in 12 genotypes (21.81%), followed by *Glu-B3i* and *Glu-B3j* (14.54%). Maximum allelic diversity was found at the *Glu-B3* (0.87) locus, followed by *Glu-A3* (0.70), which is primarily due to the allelic richness (eight alleles) observed at the *Glu-B3* locus. Many reports indicate different allelic frequencies representing the *Glu-B3* locus in genotypes from different regions. Among Indian cultivars, the frequency of *Glu-B3b* was the highest (29.3%), followed by *Glu-B3j* (27.1%) and *Glu-B3h* (13.8%).

Results from previous studies on the relationship between glutenin subunits and end-use quality have confirmed that LMW-GS are highly correlated with bread baking quality. Therefore, genotype selection based on HMW-GS and LMW-GS for bread-making quality trait is reliable and considered as a crucial analysis of the germ plasm (Xiyong et al. 2012).

Quality traits and diversity for glutenin alleles were compared between conventional bread wheat germ plasm and D-genome synthetic hexaploid derivatives (Table 10). In the synthetic derivatives, more diversity for *Glu-B1* (0.84) was observed but was equivalent to conventional wheats for *Glu-A3* (0.64). Similarly, protein contents (13.8±0.9) and SDS-sedimentation volume (3.5±0.3) were slightly higher in the synthetic derivatives compared to the bread wheat cultivars. Wheat flour SDS sedimentation volume together with gluten strength is correlated with dough rheology (Mondal et al. 2009).

Table 10. Comparison of quality traits and glutenin diversity between D-genome synthetic derivatives (SBW) and conventional bread wheat (CBW).

Trait	SBW (n=26)	CBW (n=29)
Protein (%)	13.8 ± 0.90	13.5 ± 1.50
SDS sedimentation	3.5 ± 0.30	3.3 ± 0.40
Carotenoids	6.5 ± 1.10	6.6 ± 1.25
Diversity (H) at:		
Glu-A3	0.64	0.64
Glu-B3	0.82	0.84

From the evaluation of ~200 synthetics, the *Ae. tauschii* parent appears to have a much larger influence on the quality of the synthetic hexaploid than the durum parent (Van Ginkel and Ogbonnaya 2007). About 20% of the SHs could be classified as attaining the Australian hard and Australian prime hard quality classification based on examination of the polymeric glutenins proteins (Van Ginkel and Ogbonnaya 2007). This study reports the glutenin diversity and good end-use quality profile of synthetic derivatives, which is the practical example for improving quality traits of bread wheat using SHs in breeding programs. Our results are consistent with those of Lage et al. (2006), who reported significant genetic variation among SHs for protein content and quality, grain weight, and plumpness. In recent studies at CIMMYT, synthetic derivatives carrying excellent bread-making quality can indeed be bred if the common bread wheat parent(s) in the cross has good bread quality traits. HMW- and LMS-GS profiles of the primary SHs (Rasheed et al. 2012) can be used to determine promising crosses and to identify the best quality lines in their progeny.

PCR-based, allele-specific markers were used to identify allelic variation at the *Glu-3* loci (LMW-GS), having a significant effect on visco-elastic properties of wheat dough. Several combinations of favorable LMW-GS alleles were observed at both *Glu-A3* and *Glu-B3* loci. Key quality parameters, such as protein, sedimentation volume and carotenoids, differed significantly within genotypes (Table 10). Higher values for desirable quality traits were found in synthetic derived genotypes as well as in conventional bread wheat varieties. Our results established significant variability in quality characteristics and glutenin composition among D-genome SH wheat derivatives as compared to conventional bread wheat germ plasm, suggesting their use in improving quality traits in bread wheat.

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Effect of pentosans on the peak viscosity and time to reach peak viscosity of wheat flour.

Saqib Arif, Qurrat ul ain Afzal, Mubarik Ahmed, Abid Hasnain, Uzma Sitara, Shazia Arif, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

The influence of added pentosans irrespective of cultivar was insignificant on peak viscosity (Fig. 3). The effects of cultivar differences, water-extractable pentosans (WEP), and water-unextractable pentosans (WUP) on the peak viscosity (PV) of hard white spring wheat (HWSP) flour was determined by an analysis of variance. The results indicate that cultivar differences, WEP, WUP, and the cultivar-by-WEP interaction contribute significantly in the variation of PV of HWSW flours

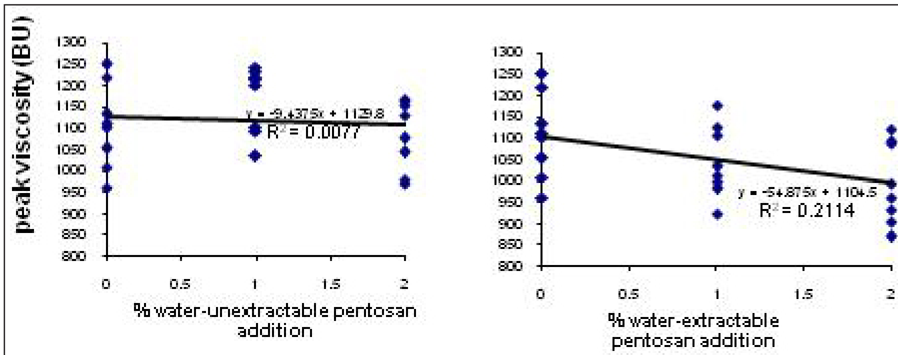

Fig. 3. Effect of pentosans on peak viscosity of wheat flour.

Table 11. Analysis of variance for peak viscosity of eight hard white spring wheat cultivars ($R^2 = 0.695$; adjusted $R^2 = 0.596$).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	1,605,866.975	39	41,176.076	7.009	0.000
Intercept	65,195,962.225	1	65,195,962.225	11097.453	0.000
Cultivar	385,704.775	7	55,100.682	9.379	0.000
Water-unextracted pentosan (WUP)	114,686.333	2	57,343.167	9.761	0.000
Water-extracted pentosan (WEP)	192,679.000	2	96,339.500	16.399	0.000
Cultivar x WUP	136,737.000	14	9,766.929	1.662	0.072
Cultivar x WEP	239,682.333	14	17,120.167	2.914	0.001
Error	704,983.000	120	5,874.858		
Total	189,146,590.000	160			
Corrected total	2310849.975	159			

(Table 11, p. 79). Lin and Czuchajowska (1997) also found a significant effect of cultivar on peak viscosity. Our results reveal that the supplementation level of pentosan influences the PV of wheat flour.

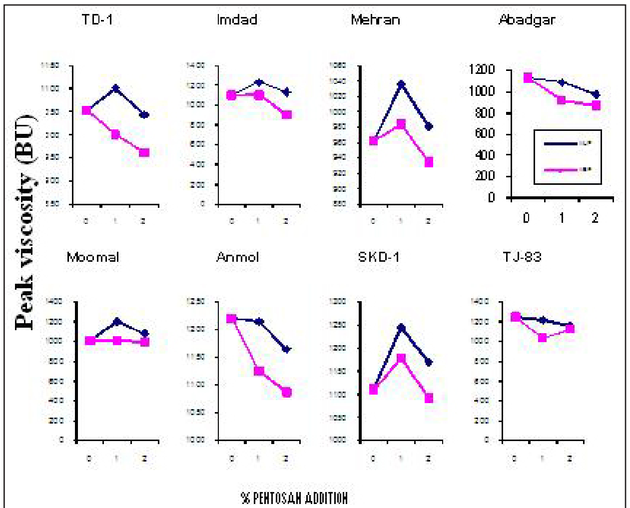

Fig. 4. The effect of water-extractable and water-unextractable pentosans on the peak viscosity of flours from different hard white spring wheat cultivars.

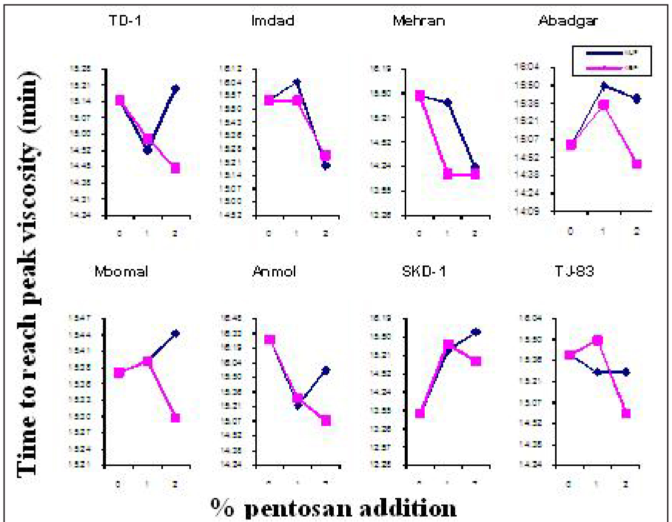

Fig. 5. The effects of water-extractable and water-unextractable pentosans on the time to reach peak viscosity of flours from different hard white spring wheat cultivars.

Because different cultivars were used in this study, we compared the influence of WEP and WUP on the PV of each cultivar at same concentration level (Fig. 4). At a concentration of 1%, both pentosans had the same influence (whether increase or decrease) on all cultivar flours except TD-1, but the magnitude of influence was different for the same cultivar. At a concentration of 2%, WEP had negative impact on all cultivars, whereas the influence of WUP was variable on PV. El-Wakeil et al. (1975) demonstrated a positive influence of pentosan content on amylograph peak viscosity and found greater effect with the addition of water-soluble pentosans than for water-insoluble pentosan.

The analysis of variance to determine the effects of varietal differences, WEP and WUP on time to reach peak viscosity (TPV) of HWSW flour (Table 12). Cultivar differences had highly significant and WEP imparted significant effect on TPV. Whereas, WUP caused negligible variation (F=0.360, P=0.698). 'Cultivar x WUP' and 'cultivar x WEP' interactions contributed significant variation in TPV.

Table 12. Analysis of variance for time to reach peak viscosity of eight hard white spring wheat cultivars ($R^2 = 0.479$; adjusted $R^2 = 0.310$)

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	43.387	39	1.112	2.831	0.000
Intercept	13,244.141	1	13244.141	33,705.178	0.000
Cultivar	12.928	7	1.847	4.700	0.000
Water-unextracted pentosan (WUP)	0.283	2	0.142	0.360	0.698
Water-extracted pentosan (WEP)	2.845	2	1.423	3.620	0.030
Cultivar x WUP	18.152	14	1.297	3.300	0.000
Cultivar x WEP	13.006	14	0.929	2.364	0.006
Error	47.153	120	0.393		
Total	37,053.900	160			
Corrected total	90.540	159			

The effect of both pentosans on TPV of each cultivar is shown (Fig. 5). Both pentosans had a different influence on TPV of all cultivar flours, and the influence of both pentosans was found to be inconsistent in terms of type and magnitude.

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El-Wakeil FA, Abdel-Akher M, Awad AA, and Morad MM. 1976. Wheat proteins and pentosans, their isolation and their effect on the rheological properties of dough and bread. Identification of wheat flour pentosans and their effect on dough rheology. Deutsche Lebensmittel-Rundschau 71(9):317-320.

The influence of pentosan type on the cold and hot paste viscosity of wheat flour.

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

An analysis of variance was used to determine the effects of cultivar differences, water-extractable pentosans (WEP), and water-unextractable pentosans (WUP) on cold-paste viscosity of hard white spring wheat (HWSW) flour (Table 13). Cultivar, WEP, WUP, and the 'cultivar x WEP' interaction significantly influence the CPV of HWSW flours. However, WEP contributed much greater variation in CPV of HWSW flours when compared to cultivar and WUP.

Table 13. Analysis of variance for cold paste viscosity of eight hard white spring wheat cultivars (R^2 = 0.896; adjusted R^2 = 0.863)

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	7,963,089.375	39	204,181.779	26.643	0.000
Intercept	40,010,000.625	1	40,010,000.625	5,220.699	0.000
Cultivar	695,599.975	7	99,371.425	12.966	0.000
Water-unextracted pentosan (WUP)	211,140.333	2	105,570.167	13.775	0.000
Water-extracted pentosan (WEP)	3,284,460.083	2	1,642,230.042	214.286	0.000
Cultivar x WUP	111,446.333	14	7,960.452	1.039	0.420
Cultivar x WEP	290,802.583	14	20,711.613	2.710	0.002
Error	919,647.000	120	7,663.725		
Total	45,394,512.000	160			
Corrected total	8,882,736.375	159			

The effects of WEP and WUP on CPV of each cultivar are shown (Fig. 6). We found that both pentosan fractions exhibited different impacts on CPV. The WEP considerably reduced the CPV of all flours, whereas WUP induced irregular changes in CPV values.

An analysis of variance determined the effects of varietal differences, WEP, and WUP on hot-paste viscosity of HWSW flour (Table 14, p. 82). Cultivar, WEP, WUP, and cultivar-by-WEP interaction significantly influenced the HPV of HWSW flours. However, WEP contributed much greater variation in HPV of HWSW flours when compared to cultivar and WUP.

Both pentosans had different impacts on HPV of same cultivar flours (Fig. 7, p. 82). WEP was found to reduce the HPV of all cultivar flours, whereas WUP induced irregular changes in HPV. The possible reason could be the hydrophilic nature of WEP that makes it capable of facilitating the rupturing of starch granules during isothermal phase.

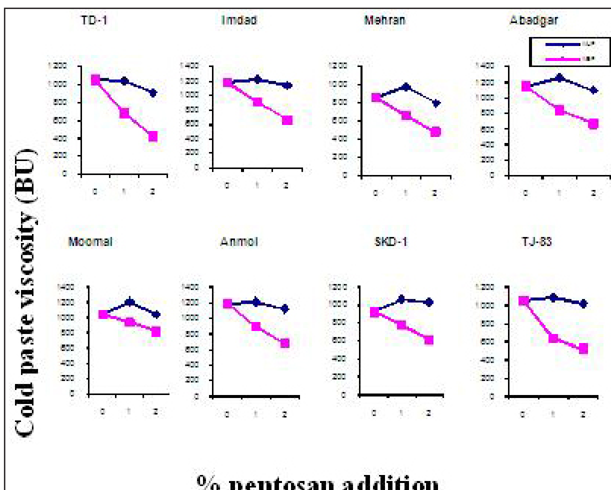

Fig. 6. The effect of water-extractable and water-unextractable pentosans on hot paste viscosity of wheat flours from different hard white spring wheat cultivar flours.

Table 14. Analysis of variance for hot paste viscosity of eight hard white spring wheat cultivars ($R^2 = 0.901$; adjusted $R^2 = 0.869$).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	4,922,296.775	39	126,212.738	28.083	0.000
Intercept	7,998,619.225	1	7,998,619.225	1,779.755	0.000
Cultivar	272,967.375	7	38,995.339	8.677	0.000
Water-unextracted pentosan (WUP)	189,022.583	2	94,511.292	21.029	0.000
Water-extracted pentosan (WEP)	1,993,657.583	2	996,828.792	221.802	0.000
Cultivar x WUP	69,742.083	14	4,981.577	1.108	0.357
Cultivar x WEP	232,213.083	14	16,586.649	3.691	0.000
Error	539,307.000	120	4,494.225		
Total	37,382,786.000	160			
Corrected total	5,461,603.775	159			

On the other side, WUP, being insoluble in water, failed to interact with the aqueous slurry.

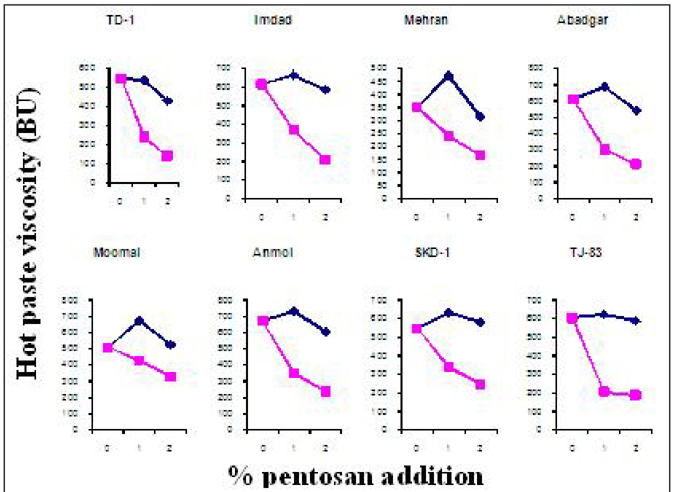

Fig. 7. Effect of water-extractable and water-unextractable pentosans on the cold paste viscosity of different hard white spring wheats cultivar flours.

Influence of water-unextractable pentosan concentration on the pasting temperature and the time required to reach the pasting temperature of wheat flours.

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

Pasting temperature (PT) is one of the most important parameters of wheat flour pasting properties. Moreover, the time required to reach peak viscosity also is important to determine. Many factors, including intrinsic and extrinsic, are known to determine the PT of wheat flour. The addition of pentosan (water unextractable in nature) to the flour may influence the PT of wheat flour. Our study was undertaken by adding water-unextractable pentosan (WUP) at two concentrations (1 % and 2%) to eight different wheat flours in order to examine the shifting of PT at different levels.

Pasting temperature. The effect of WUP at 1% and 2% was variable on the PT of flour. The influence of WUP, in terms of percent change (whether increase or decrease) in PT, from the respective control flour is shown (Fig. 8). Few cultivars showed an increase in PT, whereas others had a lower PT compared to their control flour. The largest reduction in PT was observed in the flours of the cultivar Abadgar, followed by SKD-1, at both 1% and 2% WUP concentration. The reduction is valuable in bread-making, because it implies an earlier start of starch gelatinization and, in turn, an increase in the availability of starch enzyme substrate during the baking period (Rojas et al. 1999). The PT of flour of Moomal remained unchanged in the presence

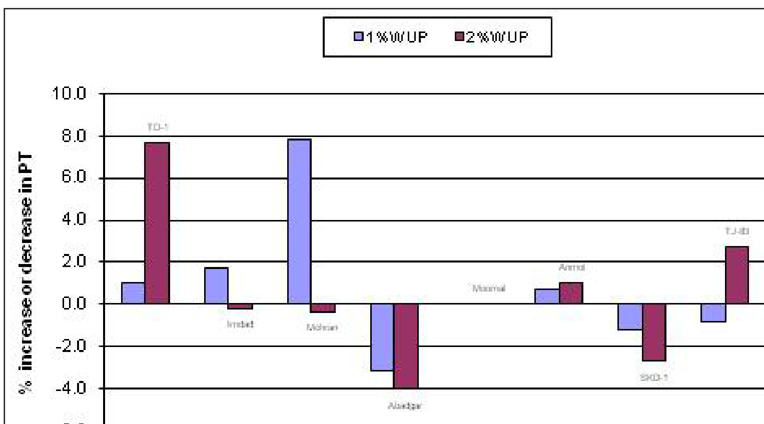

Fig. 8. The magnitude of increase or decrease (%) in pasting temperature of hard white spring wheat flours due to the addition of water-unsoluable pentosan (WUP).

of WUP. The greatest delay in PT was observed in the flour of Mehran at 1% WUP, but a slight decrease in PT was found when the WUP level increased to 2%. The PT of flour of TD-1 also was delayed at both levels of WUP, although the degree was higher at 2%.

Time to reach peak viscosity (TPV). For the same cultivar, both levels of supplementation of WUP imparted a similar type of effect (whether increase or decrease) with the exception of TD-1 and Imdad (Fig. 9). However, the magnitude of influence was different among all cultiarts except TJ-83. The maximum delay was found in TPV of SKD-1 upon addition of both concentration levels, whereas the greatest reduction (85 s) in TPV was found in the flour of Mehran.

Fig. 9. The increase or decrease in time to reach peak viscosity (TPV) of hard white spring wheat flours due to the addition of water-unextractable pentosan (WUP).

Reference.

Rojas JA, Rosell CM, and Bendito de Barber C. 1999. Pasting properties of different wheat flour-hydrocolloidal systems. Food Hydrocolloids 13:27-33.

Effects of water-extractable (WEP) and water-unextractable pentosan (WUP) on the setback viscosity of wheat flour.

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

An analysis of variance was used to determine the effects of cultivar differences, WEP, and WUP on setback viscosity (SB) of hard white spring wheat (HWSW) flour (Table 15). Cultivar, WEP, and WUP contributed significant variation in SB viscosity, but WEP caused a much greater effect on setback when compared to the cultivar and WUP.

Table 15. Analysis of variance for setback viscosity of eight hard white spring wheat cultivars (R^2 = 0.843; adjusted R^2 = 0.792)

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	1,504,984.000	39	38,589.333	16.503	0.000
Intercept	14,292,202.500	1	14,292,202.500	6,112.263	0.000
Cultivar	79,487.900	7	11,355.414	4.856	0.000
Water-unextracted pentosan (WUP)	63,643.083	2	31,821.542	13.609	0.000
Water-extracted pentosan (WEP)	561,932.583	2	280,966.292	120.159	0.000
Cultivar x WUP	42,667.583	14	3,047.685	1.303	0.215
Cultivar x WEP	36,482.750	14	2,605.911	1.114	0.352
Error	280,594.000	120	2,338.283		
Total	44,056,938.000	160			
Corrected total	1,785,578.000	159			

Both types of pentosan influence setback values of the same cultivar flour differently (Fig. 10, p. 80). WEP reduced the setback viscosity, whereas WUP increased the setback value of flour. The reduction in setback viscosities of flours indicates that WEP reduces the staling tendency of wheat flours. WEP, being hydrophilic in nature, possibly binds to the water molecules, which interacts with solubilized amylose chains to curtail the reassociaion tendency of amylose chains. Based on the low firmness values, pentosans reduce the starch retrogradation and bread staling (Kim and D'Appolonia 1997a, b; Jankiewicz and Michniewicz 1987). Schiraldi et al. (1996) explored the role of water-soluble pentosans besides other ingredients in bread staling through DSC and confirmed their indirect role anti-staling. Gul and Dizlek (2008) also reported that pentosan decreases the staling rate of bread by retarding the starch retrogradation.

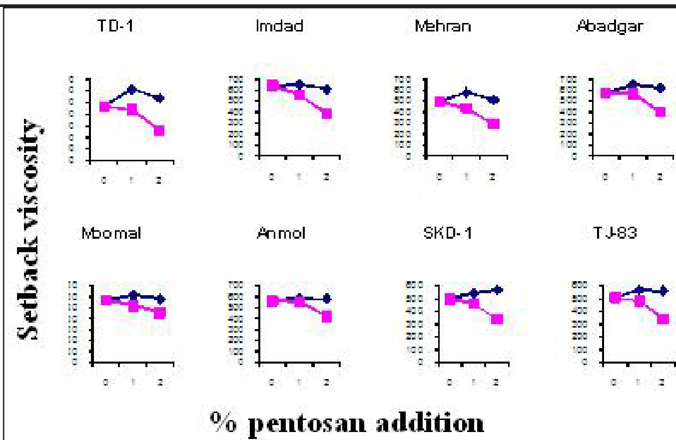

Fig. 10. The effects of water-extractable and water-unextractable pentosans on the setback viscosity of flours of different hard white spring wheat cultivars.

The relationship among pasting parameters in the presence of water-unextractable pentosan.

Saqib Arif, Qurrat ul ain Afzal, Mubarik Ahmed, Abid Hasnain, Shazia Arif, Ahmad Ali, Nosheen Shafqat, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Pasting parameters may relate differently with each other in the presence of added water-unextractable pentosan (WUP) in wheat flour. We checked this hypothesis by determining relationships among pasting parameters of WUP added flours. The relationship of pasting temperature (PT) with other pasting parameters upon the addition of WUP were analyzed by Pearson correlation coefficients (Table 16). The relationship was not in line with that of the control flours. The PT of WUP-substituted flours was weakly related with peak viscosity, time to reach peak viscosity, hot and cold past viscosity, breakdown, and setback of WUP-added flours.

Table 16. Correlation coefficients between pasting temperature and other pasting parameters of water-unextractable pentosan substituted flours.

	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Breakdown viscosity	Setback viscosity
Pasting temperature	–0.115	–0.282	–0.197	–0.306	0.103	–0.163

Peak viscosity of WUP-substituted flours had a significantly positive relationship with hot-paste viscosity (r=0.693**) and cold-paste viscosity (r=0.574**), whereas, peak viscosity did not relate with breakdown (r=0.219) or setback (r=0.102) values. The time to reach peak viscosity of WUP-substituted flours positively related with their hot-paste viscosity (r=0.404*), but did not relate with setback (r=0.214) or breakdown (r=-0.223). A strong relationship was found between cold-paste and host-paste viscosity in the presence of WUP (Table 17). Hot-paste viscosity moderately related with peak viscosity, time to reach peak viscosity, breakdown, and setback.

Table 17. Correlation coefficients between hot paste viscosity and other pasting parameters of water-unextractable pentosan substituted flours.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Cold paste viscosity	Breakdown viscosity	Setback viscosity
Hot paste viscosity	–0.197	0.693**	0.404*	0.919**	–0.552**	0.458**

Correlation coefficients between cold-paste viscosity and other pasting parameters in the presence of WUP indicate that cold paste viscosity relates almost in a similar manner in the presence of WEP with as in its absence (Table 18).

Table 18. Correlation coefficients between cold paste viscosity and other pasting parameters of water-unextractable pentosan substituted flours.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Breakdown viscosity	Setback viscosity
Cold paste viscosity	–0.306	0.574**	0.443*	0.919**	–0.580**	0.666**

The relationship between breakdown viscosity and other pasting parameters in the presence of WUP is shown (Table 19). Compared to the control flours, the WUP flours had similar pattern of relationship between breakdown viscosity and other pasting properties.

Table 19. Correlation coefficients between breakdown viscosity and other pasting parameters of water-unextractable pentosan substituted flours.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Setback viscosity
Breakdown viscosity	0.130	0.219	–0.223	–0.552**	–0.580**	–0.505**

Setback viscosity positively related with cold- and hot-paste viscosity and the time to reach peak viscosity, but was negatively related with breakdown viscosity in WUP-substituted flours (Table 20). Setback viscosity did not relate with pasting temperature or peak viscosity. However, the strength of relationships, as interpreted by their r values, was found to be weaker compared to WEP substituted flours.

Table 20. Correlation coefficients between setback viscosity and other pasting parameters of water-unextractable pentosan substituted flours.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Breakdown viscosity
Setback viscosity	–0.163	0.102	0.214	0.458**	0.666**	–0.502**

The influence of water-extractable and water-unextractble pentosans on the pasting temperature of wheat flour.

Qurrat ul ain Afzal, Saqib Arif, Mubarik Ahmed, Abid Hasnain, Uzma Sitara, Shazia Arif, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

The influence of added pentosans on pasting temperature (PT) of flours (irrespective of cultivar) is shown (Fig. 11). We found that substituting flours with pentosans up to 2% did not impart any significant change in the PT of wheat flour. The reason for insignificant changes in PT may probably be due to the reason that pentosan interacts with the wheat flour during later stage of heating cycle. The pasting temperature affects the availability of starch granules for amylolytic enzymes. Low pasting temperature is therefore considered desirable for yeast leavened breads (Rojas et al. 1999). Since substitution of pentosans did not affect pasting temperature, it is revealed that the biopolymer can be substituted up to a 2% level without affecting the availability of starch granules during bread making.

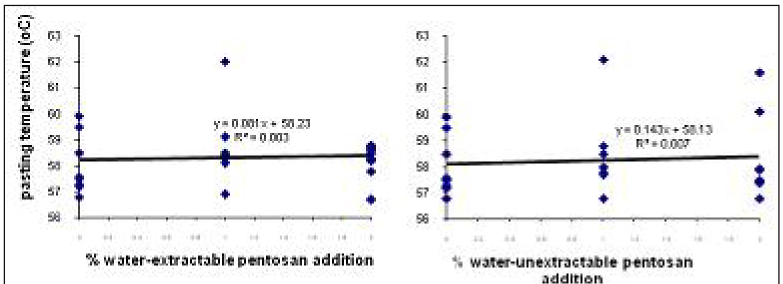

Fig. 11. The effect of pentosan addition on pasting temperature of wheat flour.

An analysis of variance to determined the effects of cultivar differences, water-extractable (WEP) and water-unextractable (WUP) pentosans on the PT of hard white spring wheat (HWSW) flour (Table 21, p. 86). The results indicated that neither WEP nor WUP had significant effect on PT of these flours. However, the significant variation in PT was caused by the cultivar differences and cultivar-by-WEP and cultivar-by-WUP interactions.

We observed slight differences in the effects of both pentosans (both in terms of type and magnitude) for the individual cultivars (Fig. 12, p. 86). We compared the influence (whether increase or decrease) of both types of pentosans at the same level of supplementation on the PT of each cultivar. The trend of both pentosans was similar only in the cultivars Imdad, Abadgar, and Anmol. In the other five, pentosans did not impart consistent impact and varied with the type.

Table 21. Analysis of variance for the effect of water-extractable (WEP) and water-unextractable (WUP) pentosans on the pasting temperature of eight hard white spring wheat cultivars (R^2 = 0.451; adjusted R^2 = 0.272).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	266.945	39	6.845	2.526	0.000
Intercept	436,388.175	1	436,388.175	161,056.086	0.000
Cultivar	86.823	7	12.403	4.578	0.000
Water-unextracted pentosan (WUP)	2.931	2	1.465	0.541	0.584
Water-extracted pentosan (WEP)	7.833	2	3.916	1.445	0.240
Cultivar x WUP	130.439	14	9.317	3.439	0.000
Cultivar x WEP	67.701	14	4.836	1.785	0.048
Error	325.145	120	2.710		
Total	544,671.040	160			
Corrected total	592.090	159			

Reference.

Rojas JA, Rosell CM, and Bendito de Barber C. 1999. Pasting properties of different wheat flour-hydrocolloidal systems. Food Hydrocolloids 13:27-33.

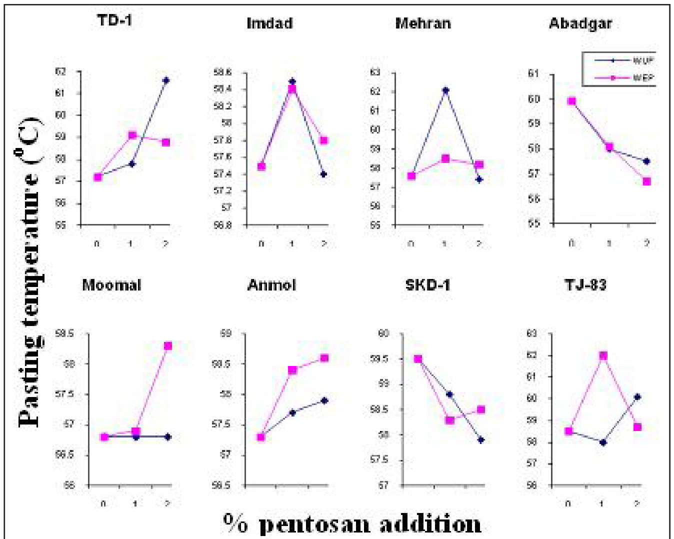

Fig. 12. The effects of water-extractable (WEP) and water-unextractable (WUP) pentosanson pasting temperature of different hard white spring wheat flours.

Comparing the influence of water-extractable pentosan and water-unextractable pentosan on the breakdown viscosity of wheat flour.

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

Two broad types of pentosans, water-extractable (WEP) and water-unextractable pentosan (WUP), exist in wheat flour. This study was to determine the different role of both pentosans in the breakdown viscosity of wheat flour. For this purpose, a portion of flours was replaced with pentosans at 1% and 2% levels in order to assess the influence of added WUP and WEP on breakdown viscosity of flour. WEP increased the breakdown viscosity of wheat flour (Fig. 13) but WUP did not. Sasaki et al. (2000) studied the influence of nonstarch polysaccharides on gelatinization properties of wheat starch. They found higher breakdown values in nonstarch polysaccharide starch compared to starch alone. The increase in breakdown values was evident with the addition of nonstarch polysaccharides (from rice and ragi) at levels of 0.25 and 0.5% to the wheat flour (Rao et al. 2007).

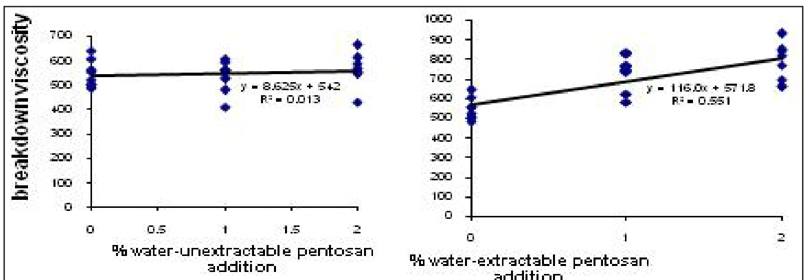

Fig. 13. The effect of pentosans on breakdown viscosity of wheat flour.

An analysis of variance determined the effects of cultivar differences, WEP, and WUP, on breakdown viscosity of hard white spring wheat flour (Table 21, p. 87). The cultivar and WEP contributed significant variation in breakdown viscosity of the flours. WUP and the cultivar-by-WEP, and cultivar-by-WUP interactions did not cause significant variation in breakdown viscosity.

Table 21. Analysis of variance for the effect of water-extractable (WEP) and water-unextractable (WUP) pentosans on the breakdown viscosity of eight hard white spring wheat cultivars ($R^2 = 0.809$; adjusted $R^2 = 0.748$).

Source	Type III sum of squares	df	Mean square	F	Significance
Corrected model	2,528,796.775	39	64,840.943	13.073	0.000
Intercept	27,458,147.025	1	27,458,147.025	5,536.075	0.000
Cultivar	335,671.575	7	47,953.082	9.668	0.000
Water-unextracted pentosan (WUP)	11,753.083	2	5,876.542	1.185	0.309
Water-extracted pentosan (WEP)	972,853.083	2	486,426.542	98.073	0.000
Cultivar x WUP	111,620.250	14	7,972.875	1.607	0.087
Cultivar x WEP	119,498.250	14	8,535.589	1.721	0.060
Error	595,183.000	120	4,959.858		
Total	67,302,602.000	160			
Corrected total	3,123,979.775	159			

The pentosans influenced the breakdown values of each cultivar flour differencly (Fig. 14). The influence of WEP was consistently positive, whereas WUP was variable depending on the concentration of WUP and genotype of wheat flour. Moreover, the degree of the effect of WEP was found to be greater (up to 63%) than that of WUP.

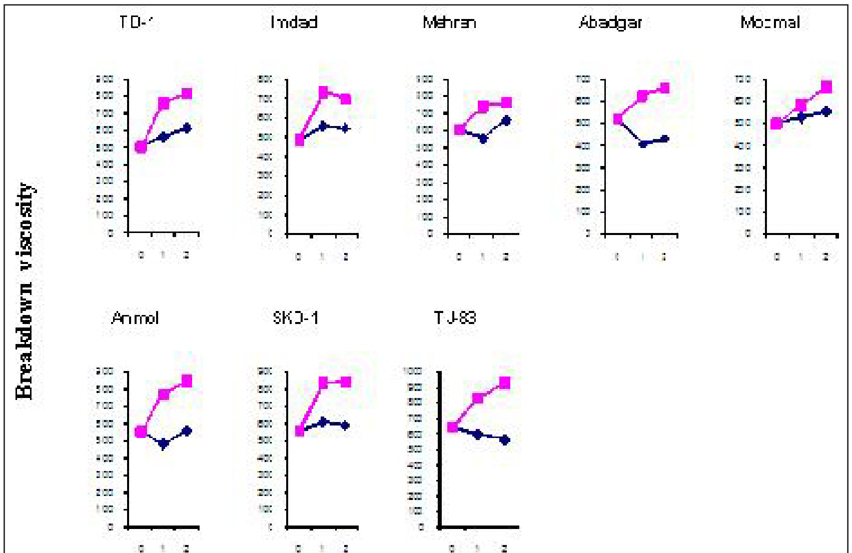

Fig. 14. The effect of water-extractable (WEP) and water-unextractable pentosan (WUP) on the breakdown viscosity of eight different hard white spring wheat cultivar flours.

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Cultivar and location-wise differences in arabinoxylan levels of wheat.

Qurrat ul ain Afzal, Saqib Arif, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Shazia Arif, Abdul Aziz Napar, Hadi Bux, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Eight wheat cultivars were analyzed to determine the differences in their arabinoxylan (AX) content. The study also included location-wise differences in total (TOAX) and water-unextractable (WEAX) and water-extractable (WUAX) arabinoxylan content (Fig. 15, p. 88).

Cultivar differences. Duncan's test was used to determine the mean separation of AX content among cultivars and across locations (Fig. 15, p. 88). The cultivar TJ-83 was found to be significantly ($P<0.05$) different in terms of TOAX, WEAX, and WUAX content from all the other cultivars. Higher AX content was found in SKD-1, followed by Anmol, Imdad, and Moomal. The values were insignificant ($P>0.05$) between the cultivars. Variation in WEAX fraction due to the genotype effect has been reported (Hong et al. 1989; Saulnier et al. 2007; Li et al. 2009). Cultivar variability in the total AX fraction also has been reported in durum wheat cultivars (Lempereur et al. 1997; Ciccoritti et al. 2011). The variability of WEAX contents in durum wheat was found to be higher compared to common wheat cultivars of Australia (Turner et al. 2008). Ramseyer et al. (2011) noted that cultivar influences WUAX content in wheat.

Location-wise differences. The AX content significantly (P<0.05) differed across the three cropping locations. The overall performance of cultivars in terms of AX content was found to be better in Hyderabad, followed by Dadu and Nawabshah. Location differences in WEAX content of barley has been reported (Henry 1986; Mikyska et al. 2002; Holtekjølen et al. 2006). The contribution of environmental differences in the variation of WUAX also has been reported for durum wheat (Ciccoritti et al. 2011).

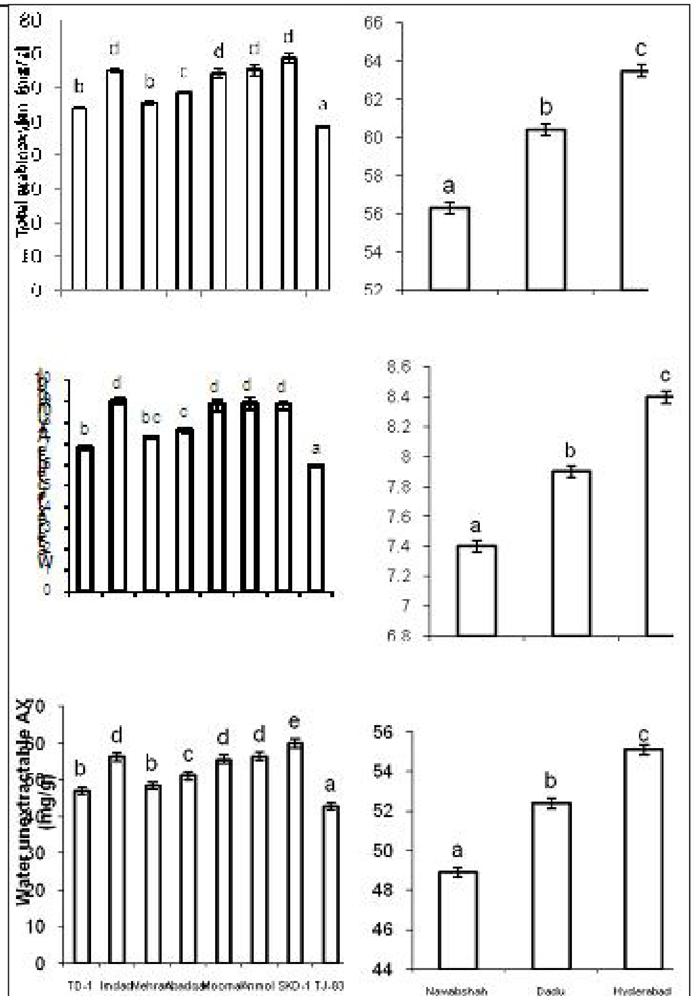

Fig. 15. Cultivar and location-wise differences in arabinoxylan (AX) content in wheat meal.

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Total arabinoxylan levels in whole meal flour of hard wheat cultivars.

Qurrat ul ain Afzal, Saqib Arif, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Shazia Arif, Sumaira Farrakh, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Arabinoxylans (AX) are the nonstarch polysaccharides present in wheat and are well- known for their impact on processing and product formulation. Eight prevalent Pakistani hard white spring wheat varieties (TJ-83, Abadgar, Anmol, TD-1, Moomal, Imdad, SKD-1, and Mehran) were selected for the study. These cultivars were grown at three different locations in the Sindh province (Nawabshah, Hyderabad, and Dadu) for three consecutive crop years (2005-06, 2006-07, and 2007-08). All wheat samples were ground into whole meal flour (referred to as meal) using a cyclone sample mill (UDY Corp, USA) fitted with a 0.5 mm screen. A modified colorimetric method was used to determine total arabinoxylans (TOAX) content in the meal samples (Table 22).

The TOAX contents irrespective of wheat cultivar, crop year, and location was found to range between 46.3 and 77.8 with an average of 59.7 mg/g. The maximum TOAX content was found in SKD-1 (68.7 mg/g) and the minimum in TJ-83 (48.7 mg/g). Three cultivars, Anmol, Imdad, and Moomal, had a mean TOAX above 60 mg/g. Three other cultivars, TD-1, Mehran, and Abadghar, had a TOAX between 50 and 60 mg/g. TJ-83 was the only cultivar that fell below 50 mg/g. The cultivars grown in Hyderabad expressed the maximum TOAX value (62.4 mg/g), irrespective of the crop year. The average minimum TOAX value in the cultivars grown in Nawabshah was 56.3 mg/g. The variation in TOAX content, in terms of percentage, ranged from -22.44 to + 30.32. The coefficient of variance (CV) among the cultivars was between 9.0% and 16.3% in the same year and between the three crop years. Within the cultivar, there was less variation (CV =2 .7 and 4.6%) in five of the cultivars, whereas three cultivars, Moomal, SKD-1, and Anmol, were less stable across locations and crop years (CV = 10.0-11.8%). The mean CV between the cultivars, irrespective of crop year and location, was 13.2% and 6.4% within the cultiar. Other studies also have found TOAX in wheat meal to range between 40 to 78 mg/g (Saulnier et al. 1995; Lempereur et al. 1997; Wang et al. 2006; Barron et al. 2007).

Table 22. Total arabinoxylan content in meal of Pakistani hard white spring wheat cultivars grown in three locations for three crop years. Values are expressed as mg xylose equivalents/g of sample and were the average of three replications ± SE. Different lowercase letters within the same column are significantly different at P < 0.05. Different capital letters within same rows are significantly different at P < 0.05.

		Cultivar									
Location	Year	TD-1	Imdad	Mehran	Abadgar	Moomal	Anmol	SKD-1	TJ-83	Mean	CV (%)
Nawabshah	2006	52.4±0.10 aA	63.0±0.12 aB	53.9±0.10 aA	57.5±0.10 aC	56.6±1.19 aC	57.8±0.07 aC	62.7±0.09 aB	47.8±0.17 aD	56.5	9.0
	2007	52.9±0.07 aA	61.8±1.55 aB	53.0±0.53 aA	56.0±1.01 aC	59.3±1.29 aB	58.5±0.01 aC	61.9±0.03 aB	46.3±0.52 aD	56.2	9.5
	2008	52.8±0.12 aA	64.0±0.32 aB	53.5±0.03 aA	56.7±0.07 aC	57.9±0.03 aC	56.9±1.33 aC	61.1±1.22 aB	47.2±0.07 aD	56.3	9.2
Hyderabad	2006	54.5±0.01 bA	67.0±0.16 bB	58.4±0.07 bC	61.7±0.22 bD	66.4±0.24 bB	67.3±1.58 bB	77.8±3.28 bE	50.8±0.10 bF	63.0	13.5
	2007	56.1±1.36 bA	68.9±3.38 bB	57.6±1.34 bA	62.3±1.09 bC	72.4±5.38 cD	75.5±4.45 cD	74.2±0.06 cD	51.6±0.09 bE	64.8	14.1
	2008	55.4±0.07 bA	65.1±0.16 cB	59.4±0.12 bC	60.0±0.24 bC	69.5±0.07 cD	71.4±0.10 dD	70.5±3.13 cD	49.8±0.71 bE	62.6	12.5
Dadu	2006	52.7±0.15 aA	65.0±0.21 cB	53.4±0.21 aA	56.6±0.12 aC	58.0±0.14 aC	67.5±0.54 bB	77.6±0.56 bD	47.4±0.31 aE	59.8	16.3
	2007	52.4±0.22 aA	68.7±0.83 bB	57.7±0.91 bC	57.3±0.28 aC	66.6±0.39 bB	57.9±0.20 aC	70.4±0.82 cB	48.0±0.29 aD	59.9	13.3
	2008	55.1±0.21 bA	63.3±0.49 aB	53.7±0.35 aA	60.3±0.39 bC	72.1±0.80 cD	75.2±0.41 cE	62.2±0.42 aB	49.4±0.62 bF	61.4	14.5
Data for three years across three location	Mean	53.8	65.2	55.6	58.7	64.3	65.3	68.7	48.7		
	CV (%)	2.7	3.9	4.6	4.1	10.0	11.8	10.0	3.7		

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Level of water-extractable and unextractable arabinoxylans in whole meal flour of hard wheat cultivars.

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

Arabinoxylans can be divided into two main groups, water extractable and water unextractable. Each group was analyzed for their content in Pakistani wheat cultivars.

Water-extractable arabinoxylan (WEAX). The WEAX content in meals of different wheat cultivars is given (Table 23). The results more or less reflect the same pattern as that for TOAX content; they have a highly significant correlation (r = 0.929**) with each other. WEAX ranged between 6.8 and 9.0 mg/g (= 7.87 mg/g) irrespective of cultivar, year, or location. The average minimum and maximum WEAX content of 7.4 and 8.3 mg/g were found in Hyderabad and Nawabshah, respectively. The mean CV values between the cultivars in different years at the two locations were found to be 18.0% and 11.4% at Hyderabad and Nawabshah, respectively The average maximum WEAX contents were 8.8–9.0mg/g in cultivars Imdad Anmol, Moomal, and SKD-1. Cultivars Mehran, Abadghar, and TD-1 showed values between 6.8 and 7.6 mg/g, the lowest value of 5.9 mg/g was found in TJ-83. The variation within the cultivar, irrespective of crop year and location, were the least in Imdad (3.0%) and Mehran (4.0%) and the greatest in Anmol (13.4%) and Moomal (12.7%). The mean CV between cultivars was 15.6% and within cultivars was 7.29%. WEAX content ranged from 4.75 to 9.19 mg/g in the meal of 25 hard spring wheat varieties grown under three different environmental conditions in the United States (Li et al. 2009).

Water-unextractable arabinoxylan (WUAX). WUAX is the larger proportion, consisting of about 75% of the total arabinoxylans. Some workers have reported the influence of WUAX on the functional properties of wheat flour. We determined the level of WUAX in wheat flour in various wheat cultivars (Table 24, p. 91).

WUAX constitutes the major portion of TOAX. The WUAX fraction was approximately 7-fold greater than that of the WEAX, which agrees with Finnie et al. (2006). Both of these arabinoxylan fractions showed a highly significant

Table 23. Water-extractable arabinoxylan content in meal of Pakistani hard white spring wheat cultivars grown in three locations for three crop years. Values are expressed as mg xylose equivalents/g of sample and were the average of three replications ± SE. Different lowercase letters within the same column are significantly different at P < 0.05. Different capital letters within same rows are significantly different at P < 0.05.

Location	Year	Cultivar									
		TD-1	Imdad	Mehran	Abadgar	Moomal	Anmol	SKD-1	TJ-83	Mean	CV (%)
Nawabshah	2006	6.4±0.12aA	8.7±0.03aB	7.0±0.16aC	7.7±0.21abD	7.2±0.89aC	7.7±0.03aD	7.9±0.21aD	5.7±0.21aE	7.3	12.8
	2007	6.7±0.27aA	8.5±0.39aB	7.1±0.18aC	7.2±0.65aC	7.9±0.21bD	7.7±0.15aD	8.1±0.03aB	6.5±0.21bA	7.5	9.3
	2008	6.5±0.07aA	8.8±0.19aB	7.0±0.03aC	7.3±0.03aC	7.5±0.03abC	7.8±0.51aBC	8.3±0.51aB	6.1±0.03bA	7.4	12.1
Hyderabad	2006	6.9±0.39aA	9.2±0.03aB	7.6±0.03bC	7.9±0.03bC	9.6±0.15cB	9.1±0.28bB	10.1±0.80bB	5.8±0.03aD	8.3	17.8
	2007	7.6±0.21bA	9.1±0.47aB	7.5±0.18bA	8.0±0.24bC	9.9±1.28cBD	10.7±0.71cD	9.4±0.03cB	6.2±0.09bE	8.6	17.2
	2008	7.4±0.03bA	9.3±0.38aB	7.7±0.19bA	7.8±0.27bA	9.7±0.03cB	9.3±0.03bB	8.8±0.12dC	5.5±0.21aD	8.2	16.9
Dadu	2006	6.6±0.21aA	9.2±0.28aB	6.9±0.12aA	7.4±0.07aC	7.8±0.20bC	9.2±0.21bB	10.0±0.73b	6.0±0.16bD	7.9	18.1
	2007	6.3±0.09aA	9.1±0.47aB	7.4±0.12abC	7.8±0.15bC	9.5±0.16cB	7.8±0.10aC	8.7±0.21dB	5.6±0.16aD	7.8	17.2
	2008	7.2±0.22bA	8.9±0.16aB	7.2±0.16abA	7.6±0.18abA	9.7±0.21cC	10.5±0.53cC	8.3±0.12aB	5.7±0.17aD	8.1	19.0
Data for three years across three location	Mean	6.8	9.0	7.3	7.6	8.8	8.9	8.8	5.9		
	CV (%)	6.8	3.0	4.0	3.7	12.7	13.4	9.2	5.5		

Table 24. Water-unextractable arabinoxylan content in meal of Pakistani hard white spring wheat cultivars grown in three locations for three crop years. Values are expressed as mg xylose equivalents/g of sample and were the average of three replications ± SE. Different lowercase letters within the same column are significantly different at P < 0.05. Different capital letters within same rows are significantly different at P < 0.05.

Location	Year	Cultivar									
		TD-1	Imdad	Mehran	Abadgar	Moomal	Anmol	SKD-1	TJ-83	Mean	CV (%)
Nawabshah	2006	46.0±0.14aA	54.3±0.09aB	46.9±0.12aA	49.8±0.18aC	49.4±0.48aC	50.0±0.03aC	54.8±0.12aB	42.1±0.30aD	49.2	8.6
	2007	46.2±0.21aA	53.3±1.21aB	46.0±0.40aA	48.8±0.50aC	51.4±1.50aD	50.8±0.15aD	53.8±0.15aB	39.8±0.05bE	48.8	9.5
	2008	46.2±0.09aA	55.2±0.41bB	46.4±0.03aA	49.3±0.03aC	50.4±0.05aC	49.1±1.19aC	52.8±0.94aD	41.0±0.03aE	48.8	8.9
Hyderabad	2006	47.6±0.39aA	57.8±0.17cB	50.8±0.06bC	53.7±0.19bD	56.8±0.36bB	58.2±1.30bB	67.7±2.52bE	45.0±0.07cF	54.7	13.0
	2007	48.6±1.21bA	59.7±3.00dB	50.1±1.48bC	54.3±0.91bD	62.5±4.11cE	64.8±3.77cF	64.8±0.03cF	45.4±0.06cG	56.3	13.7
	2008	48.0±0.10abA	55.8±0.33bB	51.7±0.31bC	52.2±0.36bC	59.7±0.03dD	61.5±0.07dE	61.8±3.24cE	44.3±0.64cF	54.4	11.8
Dadu	2006	46.1±0.21aA	55.8±0.27bB	46.5±0.10aA	49.2±0.07aC	50.2±0.30aC	58.3±0.53bD	67.6±0.22bE	41.4±0.24aF	51.9	16.1
	2007	46.1±0.21aA	59.6±0.83dB	50.3±0.98bC	49.6±0.18aC	57.1±0.42bB	50.1±0.10aC	61.8±0.78cB	42.4±0.4aD	52.1	13.0
	2008	47.9±0.43aA	54.4±0.37aB	46.5±0.26aA	52.7±0.28bB	62.4±0.60cC	64.7±0.21cC	53.9±0.33aB	43.7±0.70aD	53.3	13.9
Data for three years across three location	Mean	47	56.2	48.4	51.1	55.5	56.4	59.9	42.8		
	CV (%)	2.2	4.1	4.8	4.2	9.6	11.5	10.2	4.5		

correlation (r=0.899**) with each other. The average maximum and minimum content, irrespective of cultivar, crop year, or location, were 67.7 and 39.8 mg/g, respectively. Like TOAX, the maximum quantity of WEAX was found in cultivars grown in Hyderabad (53.7 mg/g), followed by those grown in Dadu (52.0 mg/g). Five cultivars had a WUAX content less than 50 mg/g. The CV values ranged from 8.6 to 16.1% (=12.05%) between the cultivars and 2.2 to 10.5% (=6.39%) within the cultivars.

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Li S, Morris CF, and Bettge AD. 2009. Genotype and environment variation for arabinoxylans in hard winter and spring wheats of the us pacific northwest. Cereal Chem 86(1):88-95.

The proportion of water-extractable arabinoxylans in total arabinoxylans and the ratio of water-extractable to water-unextractable arabinoxylans in wheat.

Qurrat ul ain Afzal, Saqib Arif, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Shazia Arif, Hadi Bux, Abdul Aziz Napar, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Water-extractable arabinoxylan (WEAX) is an important proportion of arabinoxylan. Soluble in water, WEAX has tremendous nutritional importance as a dietary fiber and is known to affect the functional properties of wheat flour. The proportion of WEAX in wheat flour is important for determining the behavior of dough during processing and assessing the quality of finished product. However, the proportion of WEAX varies with genotype and location. The variation in the amount of WEAX can be assessed with the values of percentage of WEAX in total arabinoxylan (TOAX) and the ratio of WEAX to WUAX (water-unextractable arabinoxylan). We determined the levels of WEAX in some of the wheat cultivars grown at different locations in southern Pakistan. Eight wheat cultivars grown at three locations for three crop years were tested for their WEAX levels. The tests were performed on wheat meal obtained by grinding wheat grains with a UDY cyclone mill.

The percent WEAX in TOAX and WEAX:WUAX ratio across the locations and crop years is presented (Fig. 16, p. 92). The percent WEAX in TOAX was found to be independent of TOAX; it was weakly related (r=0.384) with the amount of TOAX in the wheat meal. The water-extractable fraction ranged from 12.1 to 13.8%. A narrow range of percent WEAX in TOAX in meals is possibly because all the cultivars under study were hard white spring wheats. Li et al. (2009) found that the percent WEAX content ranged from 11.7 to 23% in TOAX in both spring and winter wheats. Faurot et al. (1995) found that WEAX, as a fraction of TOAX in wheat, was higher (up to 30%). A higher molecular weight WEAX fraction in TOAX may contribute to the desirable technological properties for dough and bread-making quality characteristics of wheat (Courtin and Delcour 2002).

The WEAX:WUAX ratio (also called the extractability ratio of AX) was narrow, ranging between 0.14 and 0.17 and was the same across all three locations and crop years. We also found that the WEAX:WUAX ratio did not depend on the AX content; the ratio had a weak relationship with TOAX (r=0.390) and WUAX (r=0.326). Others have also reported that the WEAX:WUAX extractability ratio is independent of the TOAX content (Lempereur et al. 1997; Ciccoritti et al. 2011).

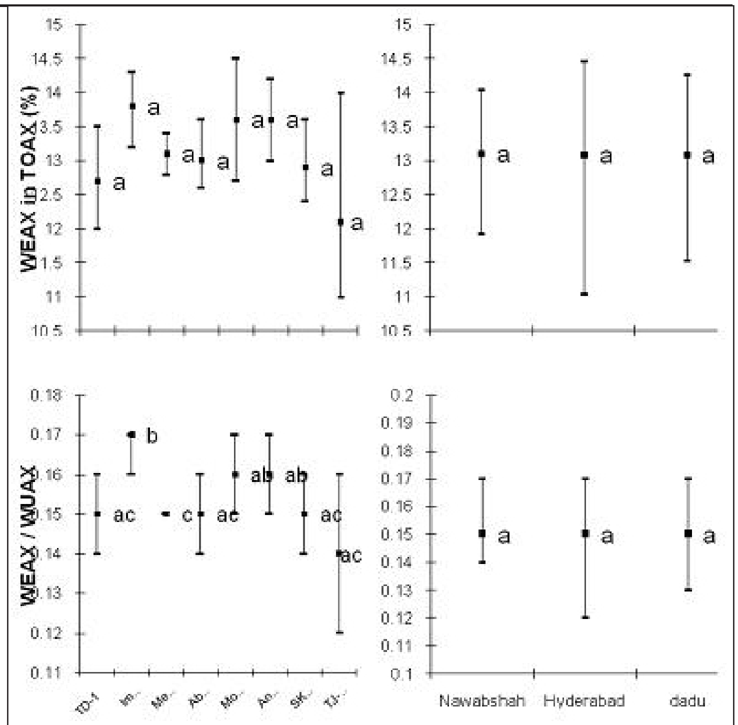

Fig. 16. Minimum, maximum, and mean values of % water-extractable arabinoxylan (WEAX) in total arabinoxylan (TOAX), and WEAX/ WUAX (water-unextractable arabinoxylan) ratio of wheat cultivars.

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Relationship of arabinoxylans with breakdown, setback, and other paste viscosities of wheat flour.

Saqib Arif, Qurrat ul ain Afzal, Mubarik Ahmed, Abid Hasnain, Uzma Sitara, Shazia Arif, Hadi Bux, Sumaira Farrakh, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

The relationship of arabinoxylan (AX) content (naturally present in flour) with the pasting properties of wheat flour was estimated through Pearson's correlation. The AX content, consisting of total arabinoxylan (TOAX), water-extractable arabinoxylan (WEAX), and water-unextractable arabinoxylan (WUAX), were negatively related with breakdown viscosity (BD) (Fig. 17). The percentage of WEAX in TOAX was moderately correlated with BD value.

The relationship of setback viscosity with AX content (naturally present) was esti-

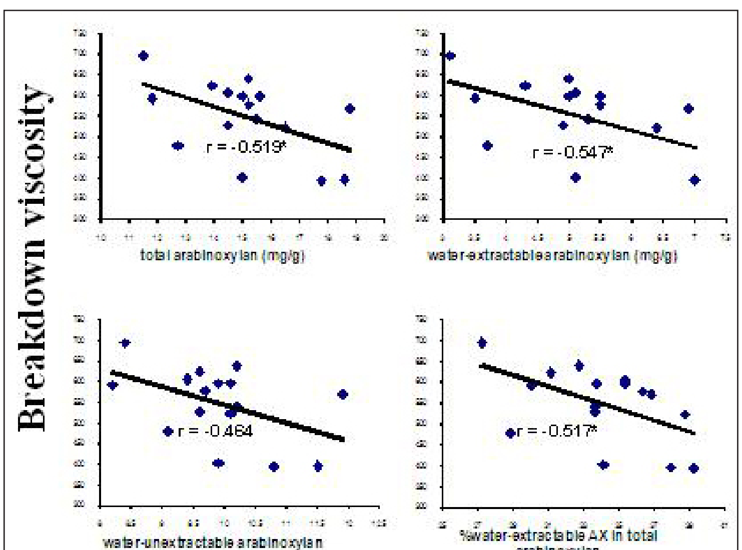


Fig. 17. Relationship of breakdown viscosity with arabinoxylans.

mated. The setback value positively relates with AX content and % WEAX in TOAX (Fig. 18). Flours containing a higher AX content have larger setback values and are more susceptible to bread staling. The flours investigated were extracted from sound wheats, because they had setback values less than 650BU.

The AX content naturally present in wheat flour does not relate with HPV. The correlation coefficients of HPV with TOAX, WEAX, WUAX, and %WEAX in TOAX are 0.172, 0.166, and 0.057, respectively, due to the low amount of AX (1.2-1.7%) in wheat flour. The AX content and % WEAX in TOAX weakly relate with peak viscosity (PV) of wheat flour. Iriki et al. (2003) found that AX content significantly correlated with the apparent viscosity of heat-treated flour paste. In our study, the relationship of PV is not statistically significant with TOAX (r=-0.262), WEAX(r=-0.289), WUAX(r=-0.203), or % WEAX in TOAX (r= -0.369).

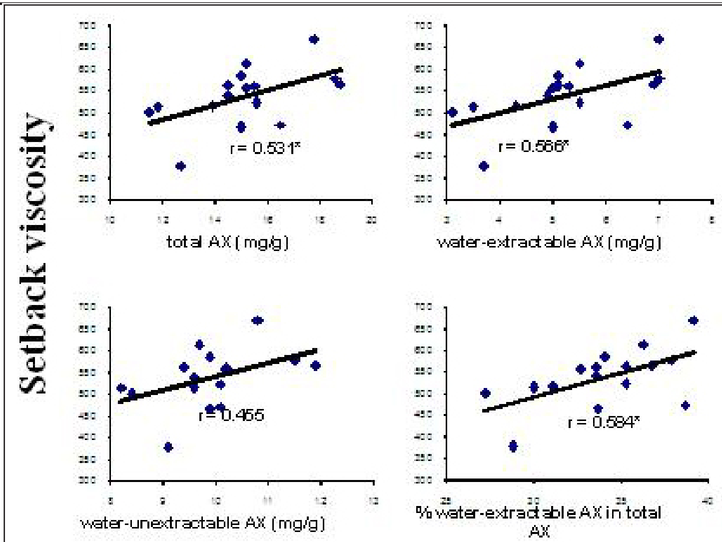


Fig. 18. The relationship between setback viscosity and arabinoxylans (AX).

In order to understand the dependence of time to reach peak viscosity (TPV) on native AX content, the relationship of TTP and AX was determined. TPV did not relate with TOAX (r=0.038), WEAX (r=-0.037), WUAX (r=0.132), or %WEAX in TOAX (r=-0.194).

Reference.

Iriki N, Yamauchi H, Takata K, Nishio Z, Ichinose Y, and Yoshihira T. 2003. Effects of genotype and growth consitions on apparent viscosity of heat-treated flour paste and their correlation with certain flour properties in wheat produced in Hokkaido. Food Sci Technol Res 9(1):104-109.

The impact of weather conditions on arabinoxylan content in wheat.

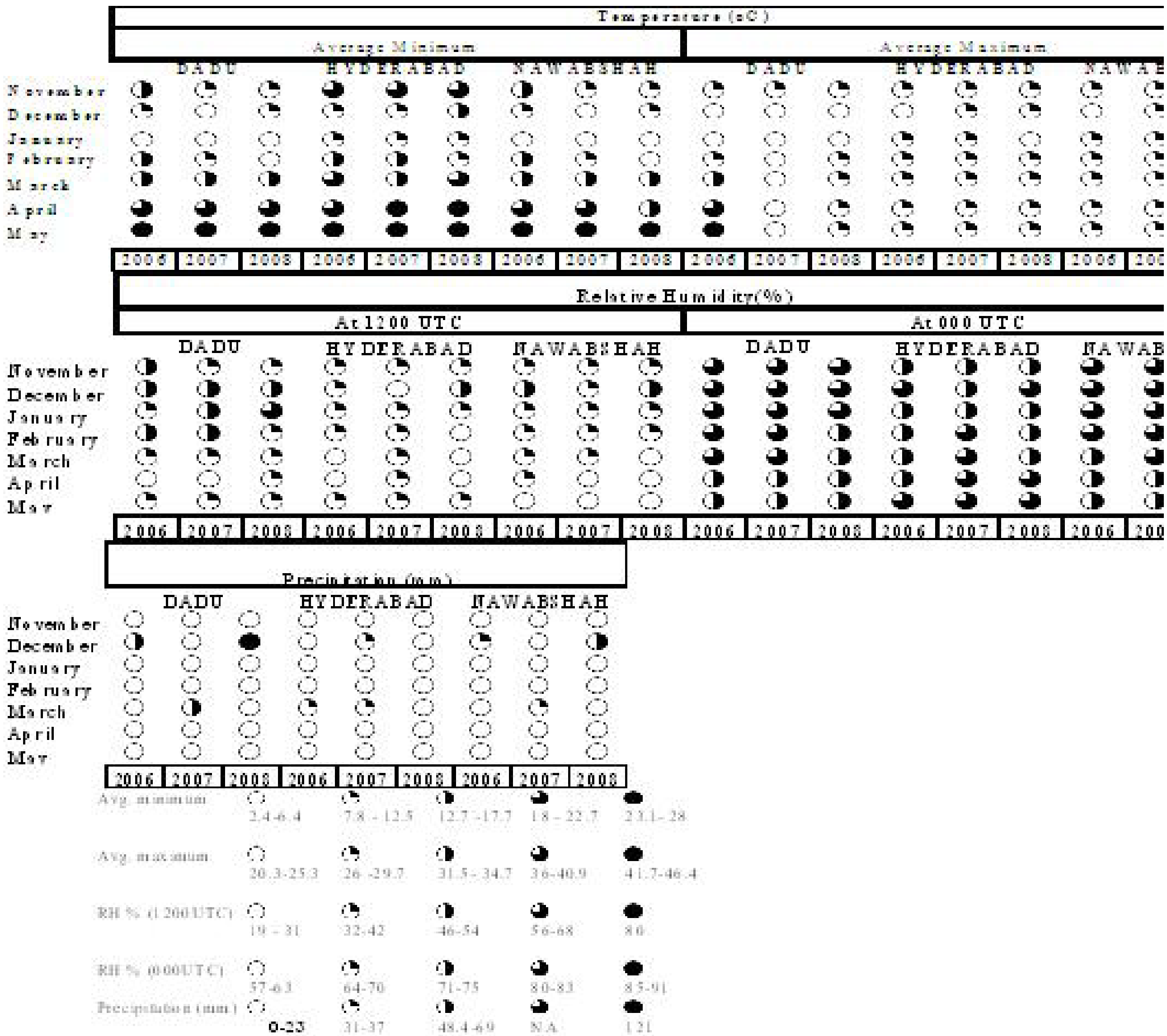
Qurrat ul ain Afzal, Saqib Arif, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Shazia Arif, Abdul Aziz Napar, Hadi Bux, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

The impact of weather conditions during growth period on arabinoxylan content of wheat was studied. The overall weather in all the crop producing areas was dry and the prime source of water was canal irrigation (Table 25, p. 94). Climatic conditions during the crop year and between heading and harvest were analyzed separately for their correlation with arabinoxylan (AX) fractions (Table 26, p. 95).

Temperature. The minimum temperature across the crop year was significant and positively correlated with AX content. The maximum temperature between heading and harvest showed a negative relationship with AX content. The average temperature recorded throughout the crop year had a moderately positive influence on the amount of AX in wheat meal. Shewry (1999) found that WEAX was negatively correlated with the average temperature during heading to harvest. This relationship was in the flour and bran fractions of winter wheat at heading-to-harvest temperatures between ~10-25oC. In our study, no relationship was found between the average temperature during heading-to-harvest in any of the AX fractions. The differences in findings may be the impact of different temperature values, the nature of samples, and the wheat fraction.

Relative humidity and precipitation. Our investigations relate to wheat cultivation under dry environment with less precipitation. The average precipitation during heading-to-harvest was a moderate negative relationship with % WEAX in TOAX and the WEAX:WUAX ratio. Li et al. (2009) indicated a negative relationship between rainfall and WEAX content and the amount of WEAX in TOAX. Other investigators reported positive relationship of rainfall with TOAX

Table 25. Temperature, relative humidity, and precipitation at the preharvest stage of wheat cultivation in different localities during 2006–08.



(Ciccoritti et al. 2011) and WEAX (Dornez et al. 2008). The amount of the AX fraction had a positive correlation with the average relative humidity during the crop year, but no relative humidity value between heading-to-harvest influenced the AX level. Thus, a humid environment during early growth period of the crop may increase the accumulation of AX in wheat grain.

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Table 26. Correlation of arabinoxylan parameters with weather conditions during the crop year and during heading-to-harvest (*correlation is significant at the 0.05 level (2-tailed); TOAX = total arabinoxylan; WEAX = water-extractable arabinoxylan; WUAX = water-unextractable arabinoxylan).

	TOAX	WEAX	WUAX	% WEAX in TOAX	WEAX:WUAX ratio
Across crop year					
Temperature maximum	–0.309	–0.281	–0.306	0.063	0.044
Temperature minimum	0.711*	0.684*	0.7178*	0.042	0.016
Temperature mean	0.477	0.465	0.484	0.058	0.030
Precipitation	0.313	0.305	0.309	0.044	0.054
Humidity at 0:00	–0.269	–0.211	–0.275	0.254	0.218
Humidity at 12:00	–0.186	–0.197	–0.186	–0.112	–0.144
Humidity average	0.653	0.679*	0.647	0.336	0.280
Across heading-to-harvest					
Temperature maximum	0.673*	–0.671*	–0.677*	–0.189	–0.145
Temperature minimum	0.426	0.398	0.426	–0.038	0.028
Temperature mean	–0.012	–0.034	–0.014	–0.133	–0.055
Precipitation	–0.008	–0.086	0.008	–0.466	–0.463
Humidity at 0:00	0.077	0.114	0.084	0.245	0.249
Humidity at 12:00	0.164	0.182	0.163	0.154	0.118
Humidity average	0.172	0.200	0.173	0.225	0.194

Effects of cultivar, location, and year on the arabinoxylan content of wheat.

Qurrat ul ain Afzal, Saqib Arif, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Shazia Arif, Hadi Bux, Abdul Aziz Napar, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

The amount of arabinoxylan in wheat is reported to be influenced by the cultivar and location. However, the extent of influence varied with the change of genotypes and locations and, therefore, it is important to analyze the effects of these factors on the arabinoxylan (AX) content of wheat grown in Pakistan. An analysis of variance (ANOVA) was used to analyze the sources of variation of AX fractions (Table 27, p. 96). The ANOVA model explained 58-96% of the total variation in AX content, %WEAX (water-extractable arabinoxylan) in TOAX (total arabinoxylan) and the WEAX:WUAX (water-unextractable arabinoxylan) (extractability ratio) in wheat meal due to the effects of cultivar, location, harvest year, and their interaction.

TOAX, WEAX, and WUAX varied significantly at $P<0.001$ by the both cultivar and location. The F-value for cultivar was 2-fold and ~1.4-fold greater than that for the location for TOAX and WUAX and WEAX, respectively. Li et al. (2009) reported environmental conditions as the main variance contributor for WEAX. Saulnier et al. (1995) and Finnie et al. (2006) have reported the genotypic variance as the primary source of variation. In our study, TOAX, WEAX, and WUAX content varied insignificantly ($P>0.05$) by harvest year. The effects of 'cultivar × location' and 'cultivar × year' were significant for TOAX, WEAX, and WUAX. Differing results in the variation in crop year can be attributed to the different agronomic practices during the different crop years but further study is required. The interaction of climate with agronomic inputs and soil type dictates variations in AX content (Gebruers et al. 2010). For breeding prospects, the crop year could be an important factor imparting variation in the genotypes. Significant variation was identified in TOAX and WUAX due to 'location × year' interaction, although it was insignificant for WEAX. TOAX, WEAX, and WUAX varied significantly due to the interaction of 'cultivar × location × year', but WEAX variation was significantly lower ($P< 0.01$). Although significant, the effect of 'cultivar × location × year' was found to be lower than the individual effects of cultivar and location. The results are inline with the those of Li et al. (2009).

Table 27. Analysis of variance of arabinoxylan content (TOAX (total arabinoxylan), WEAX (water-extractable arabinoxylan), and WUAX (water-unextractable arabinoxylan)) in the meal of different hard white spring wheat cutivars (F values used type-III mean squares; ns = nonsignificant; * = P<0.05; ** = P<0.01; and *** = P<0.001).

Source	TOAX	WEAX	WUAX	%WEAX in TOAX	WEAX:WUAX ratio
R^2	0.956	0.887	0.955	0.578	0.587
Corrected model *F* value	44.261***	15.923***	43.189***	2.774***	2.881***
Cultivar *F* value	308.958***	118.158***	295.313***	15.239***	16.313***
Location *F* value	221.269***	55.301***	225.980***	0.025 ns	0.055 ns
Year *F* value	1.377ns	0.978ns	1.251ns	0.342 ns	0.571 ns
Cultivar * location *F* value	12.239***	5.828***	11.849***	2.638**	2.789**
Cultivar * year *F* value	10.445***	2.627**	11.049***	1.035 ns	0.955 ns
Location * year *F* value	5.657***	1.997ns	5.779***	0.939 ns	1.109 ns
Cultivar * location * year *F* value	6.940***	2.303**	7.181***	1.229 ns	1.151 ns

Cultivar and the 'cultivar × location' interaction had a significant impact on %WEAX in TOAX and the WEAX:WUAX ratio. Location, 'cultivar × year', and the 'cultivar × location × year' interaction were insignigicant. In contrast, the environmental conditions had a greater influence on the quantity of WEAX in TOAX. A genotypic influence was found for both spring and winter wheat classes (Li et al. 2009). The influence of individual cultivars was much larger than the impact of interaction on AX content.

Our results show that the variance ratios of cultivar-to-location (σ2 cultivar/σ2 location) were 1.9, 4.6, and 1.7 for TOAX, WEAX, and WUAX, respectively. Higher values of 'σ2 cultivar/σ2 location' for WEAX revealed that it remained stable across the locations. The variance ratios of 4.4 for TOAX and 4.9 for WEAX has been reported (Lempereur et al. 1997). Hong et al. (1989) reported 'σ cultivar/ σ location' as 1.6 and 2.4 for WEAX and TOAX, respectively, which shows a greater variance ratio of TOAX than WEAX, in contrast to our results. The 'σ2 cultivar/σ2 location' values for %WEAX in TOAX and the WEAX:WUAX ratio are overestimates because there was insignificant variation at the different locations. Genotypic variance ratios (σ2 cultivar/σ2 culitvar + σ2 location + σ cultivar x location) were 0.66, 0.82, and 0.63 for TOAX, WEAX, and WUAX, respectively.

The percent contributions of variation in AX contents due to cultivar and location are illustrated (Fig. 19). The cultivar is the major variance contributor to TOAX, WEAX, and WUAX content. Percent WEAX in TOAX and the WEAX:WUAX ratio showed approximately 97% variation due to the cultivar effect.

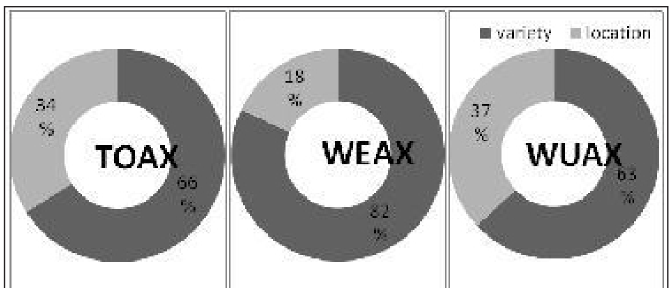

Fig. 19. Percent contribution of cultivar and location in the variability of arabinoxylan (TOAX (total arabinoxylan), WEAX (water-extractable arabinoxylan), and WUAX (water-unextractable arabinoxylan)) content in wheat.

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The effect of water-unextractable pentosans on peak viscosity and time to reach peak viscosity of hard wheat flours.

Saqib Arif, Qurrat ul ain Afzal, Mubarik Ahmed, Abid Hasnain, Shazia Arif, Hadi Bux, Abdul Aziz Napar, Nosheen Shafqat, Ahmad Ali, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Peak viscosity. Peak viscosity (PV) is one of the most important pasting attributes useful for distinguishing starch properties. This study investigates the changes in PV of different wheat flours after the addition of water-unextractable pentosan (WUP) (Fig. 20). Duncan's test was used to further analyze the impact of WUP on the PV of the flour of each cultivar. We found that the changes induced by WUP in PV were statistically insignificant.

The PV of 1% WUP-substituted flours varied between 1,036 and 1,245 BU, with the lowest value in the flour of cultivar Mehran and the highest in SKD-1 (Fig. 21). The PV of Mehran was significantly different from those of all the other cultivars except TD-1 and Abadgar. The PV of 2% WUP-substituted flours was between 973 and 1,170 BU. The differences were statistically insignificant among the PV of 2% WUP-substituted flours.

Time to reach peak viscosity. Time to reach peak viscosity (TPV) is an indication of the time required for cooking. Kuo et al. (2001) related the amylographic parameters with the eating quality of rice and found negative correlation between TPV and palatability score. The flour suspension took less time to reach peak viscosity as compared to starch suspension even when both were extracted from the same source of wheat (Mira et al. 2005).

The impact of WUP addition on the time to reach peak viscosity of flours from different wheat cultivars is shown (Fig. 22). WUP imparted insignificant changes in TPV of all cultivar flours. The reason may be due to the lack of interference in the extent of granule swelling. No doubt, WUP competes for water with other constituents of flour, but, possibly due to sufficient availability of water, WUP does not hinder the extent of granule swelling.

Insignificant differences were found among the TPV of 1% and 2% flours (Fig. 23, p. 98). The time taken by 1% WUP-substituted flours to reach their peak viscosity was found in the narrow range of 14:53–16:05 min. Flour of cultivar TD-1 took the lowest time, whereas

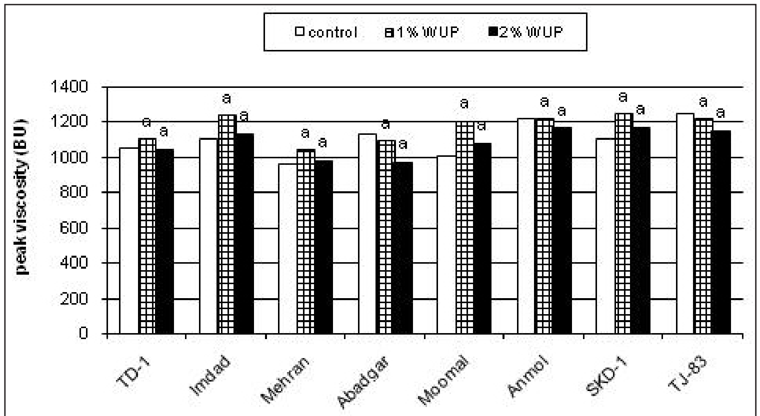

Fig. 20. Effect of water-unextractable pentosan (WUP) on the peak viscosity of flours from different hard white spring wheat cultivars. Bars labeled with an 'a' indicate an insignificant difference from the control at P<0.05.

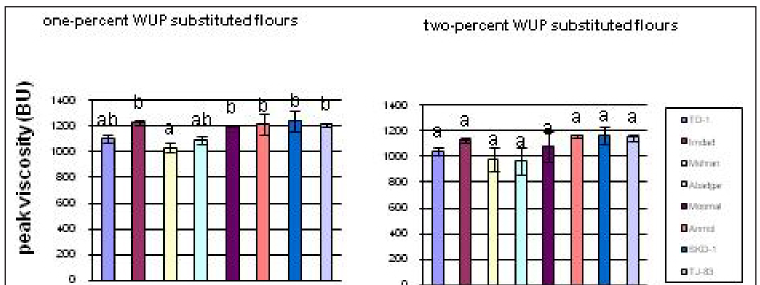

Fig. 21. Peak viscosities of water-unextractable pentosan (WUP)-substituted flours. Bars labeled with different letters are significantly different at P<0.05.

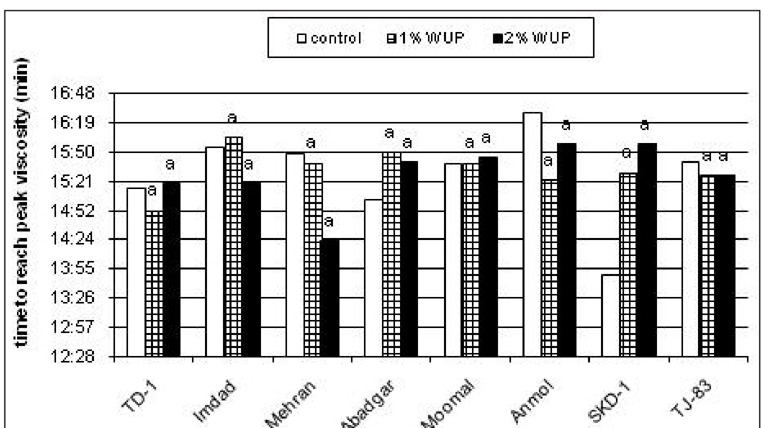

Fig. 22. The effect of water-unextractable pentosan (WUP) on the time to reach peak viscosity of flours from different hard white spring wheat cultivars. Bars labeled with a 'a' indicate an insignificant difference from their control at P<0.05.

more time was needed by the flour of cultivar Imdad to reach its maximum viscosity. With the exception of Mehran, 2% WUP-substituted flours of all cultivars took more than 15 min to reach their maximum viscosity ranging from 15:20 min to 15:58 min. Among the 2% WUP-substituted flours, the shortest time to reach maximum viscosity in all the cultivars was that of the flour of Mehran.

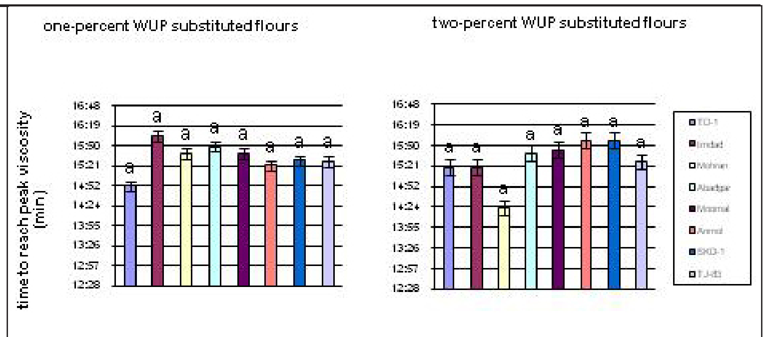

Fig. 23. Time to reach peak viscosity of water-unextractable pentosan (WUP)-substituted flours. Bars labeled with different letters are significantly different at P<0.05

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The effect of water-unextractable pentosans on pasting temperature of hard wheat flours.

Saqib Arif, Qurrat ul ain Afzal, Mubarik Ahmed, Abid Hasnain, Shazia Arif, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Pasting temperature (PT) is defined as the temperature when the first rise in viscosity is recorded by a viscoamylograph. The granules of starch undergo swelling and amylose leaching when the suspension is heated to more than the specific temperature in the presence of excess water. The temperature needed to begin the pasting process usually depends on the cooking conditions and the type of starch present in the suspension. Added components also may shift the PT of wheat flour, but lacks a comprehensive study. This study will determine the influence of water-unextractable pentosans (WUP) on the PT of wheat flour by of different wheat cultivars.

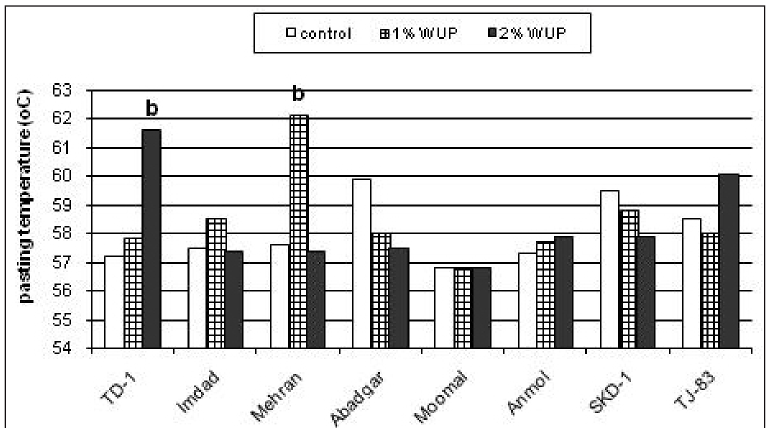

Fig. 24. The eEffect of water-unextractable pentosans (WUP) on the pasting temperature of different cultivar flours. The means of bars labeled with a 'b' differ significantly at P<0.05 from the control.

The effect of WUP on pasting temperature was found to be more inconsistent compared to the effect of water-extractabe pentosan (WEP) and varied from cultivar to cultivar (Fig. 24). WUP delayed the pasting temperatures of flours of cultivars TD-1, Anmol, and TJ-83, whereas the pasting temperature of flours of Imdad, Mehran, Abadgar, and SKD-1 were earlier in the presence of WUP. A lower PT means a faster swelling of granules. No change observed in the pasting temperature of the variety Moomal.

The pasting temperature of 15 1% and 2% WUP-substituted flours of all cultivars (except Mehran) ranged between 56.8 and 58.8°C (Fig. 25). Flour of Mehran exhibited an exceptionally higher (62.1°C) pasting temperature, which was found to be statistically different from all other cultivars except SKD-1. Higher

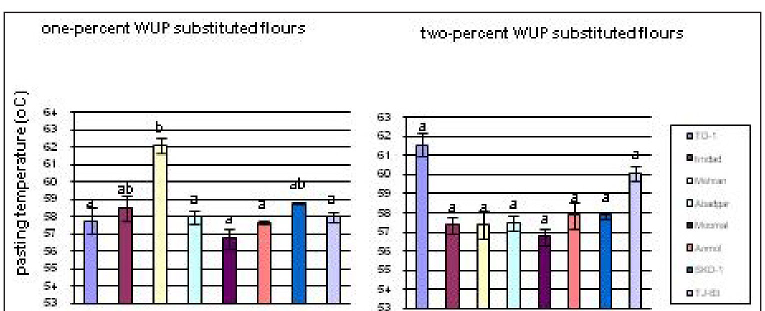

Fig. 25. Pasting temperatures of water-unextractable pentosans (WUP) added to the flours of hard white spring wheat cultivars. Bars labeled with different letters are significantly different at P<0.05.

temperature would be due to the presence of 1% WUP, which delays the swelling and amylose leaching of flour of in Mehran; a phenomenon that can not be generalized over all cultivars. For 2% WUP-substituted flours, the temperature needed to gelatinize starch granules was in a narrow range, 56.8–57.9°C for all cultivars except TJ-83 and TD-1. Flours of TJ-83 and TD-1 had PTs higher than that of all 25 WUP-substituted flours and was greater than 60°C. However, the differences in the 2% WUP-substituted flours were found to be statistically insignificant.

The effect of water-unextractable pentosans on hot paste and cold paste viscosities of hard wheat.

Saqib Arif, Qurrat ul ain Afzal, Mubarik Ahmed, Abid Hasnain, Shazia Arif, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Hot paste viscosity. Hot paste viscosity (HPV) is defined as the viscosity measured after a holding period of 10 mins at 95°C. A wheat starch slurry held at 95°C leads to a reduction in the pasting viscosity of the wheat flour slurry as it is subjected to mechanical stress. This isothermal phase eventually results in the rupture of swollen starch granules responsible for viscosity development. Our study shows the influence of water-unextractable pentosan (WUP) addition on the hot paste viscosity of flours of different hard white spring wheat cultivars (Fig. 26).

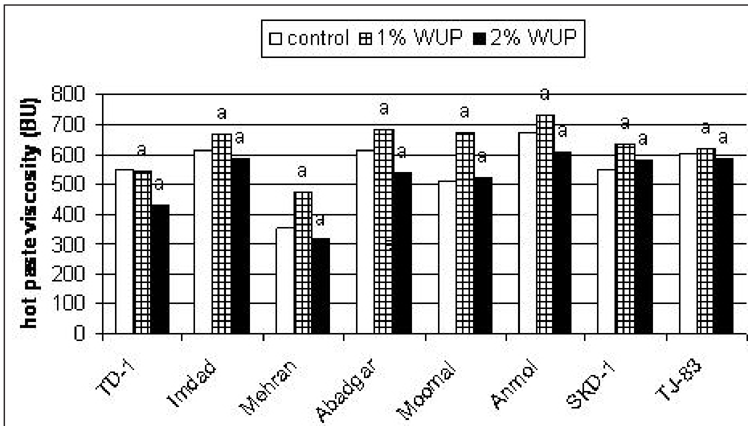

Fig 26. The effect of water-unextractable pentosans (WUP) on the hot paste viscosity (HPV) of flours from different hard white spring wheat cultivars. Bars labeled with an 'a' indicate an insignificant difference from control at P<0.05.

WUP did not induce significant changes in HPV. Moreover, the changes were inconsistent and varied with the cultivar and WUP concentration. The variable effect of WUP might be due to the insoluble nature of WUP even though WUP is expected to compete for water.

The hot paste viscosities of WUP substituted flours of different wheat varieties are shown (Fig. 27). Among the 1% flours, the least viscous hot paste was the flour of cultivar Mehran; the most viscous hot paste was that of Anmol. With the exception of Mehran and Anmol, the hot paste viscosities of flours of all cultivars ranged between 542 and 685 BU. Insignificant differences were found between the HPV of 2% WUP-substituted flours. The HPV of Mehran exhibited the lowest viscosity (315 BU) and the most viscous (606 BU) was the flour of Anmol. Hot paste viscosities of 2% WUP-substituted flours of all other cultivars (except Mehran and Anmol) varied between 431 and 587 BU.

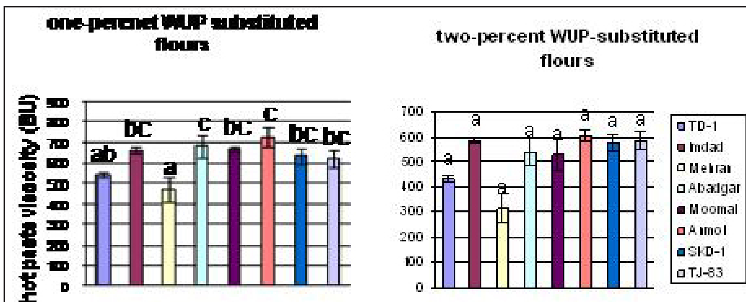

Fig 27. Hot paste viscosities of water-unextractable pentosans (WUP)-substituted flours of different hard white spring wheats.

Cold paste viscosity. Cold paste viscosity (CPV) is the viscosity measured after holding the slurry at 50°C for 10 min. The value of CPV serves as an indicator of paste stability after cooking. The paste- or gel-forming ability of starch after cooling corresponded well with CPV values (Shimelis et al. 2006). Moreover, CPV is a significant attribute in some food processing operations, such as canning (Beta et al. 2001), and predicts the starch property in the preparation of food items (such as instant soup, creams, and sauces) that require cold thickening capacity (Alves et al. 1999).

Duncan's test was used to determine the effect of WUP in the CPV of each cultivar (Fig. 28, p. 100). We found that within a cultivar, the addition of WUP to wheat flour did not make significant changes in CPV of all cultivar flours. WUP also made irregular changes in CPV and varied from cultivar to cultivar. The insignificant impact of WUP may reflect the less interference of WUP in reassociation of amylase molecules upon cooling after cooking. The WUP added flours can be used in the formulation of instant soups, creams etc without significantly affecting their cold thickening capacity.

Cold paste viscosities of 1% WUP-substituted flours ranged between 982 and 126 1BU (Fig. 28). Flours of cultivars TD-1, Mehran, SKD-1, and TJ-83 exhibited cold paste viscosities less than 1,100 BU. Cold paste viscosities of flours of Imdad, Abadgar, Moomal, and Anmol were greater than 1,200 BU. Cold paste viscosities of 2% WUP-substituted flours of all other cultivars varied between 916 and 1,124 BU (Fig. 28). Similar to hot paste viscosity, the cold paste viscosity of Mehran was the lowest viscosity. The most viscous cold paste was found to be the flour of Imdad (Fig. 29).

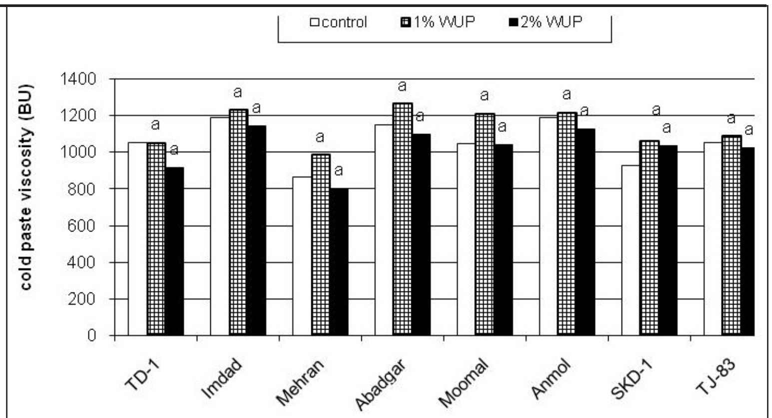

Fig. 28. The effect of water-unextractable pentosans (WUP) on the cold paste viscosity of flours from different hard white spring wheat cultivars. Bars labeled with an 'a' indicate a insignificant difference from the control at P<0.05.

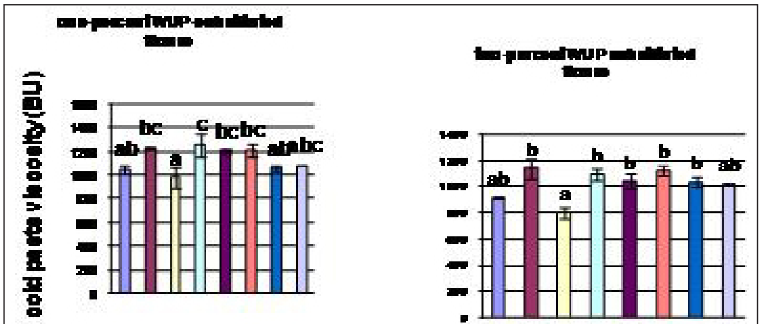

Fig 29. Cold paste viscosities of water-unextractable pentosan (WUP)-substituted hard white spring wheat flours. Bars labeled with the same letters are not significantly different at P<0.05.

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Effects of water-unextractable pentosans on breakdown and setback viscosities of hard wheat flours.

Saqib Arif, Qurrat ul ain Afzal, Mubarik Ahmed, Abid Hasnain, Shazia Arif, Ahmad Ali, Nosheen Shafqat, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Breakdown viscosity. Breakdown viscosity (BD) is the measure of fragility of starch granules. Higher breakdown viscosity indicates less tendency of starch granules to resist shear. The present findings depict the influence of water-unextractable pentosan (WUP) addition on the BD of wheat flour (Fig. 30). The addition of WUP to wheat flour induced insignificant change in BD values of all cultivar flours, suggesting that WUP does not influence the

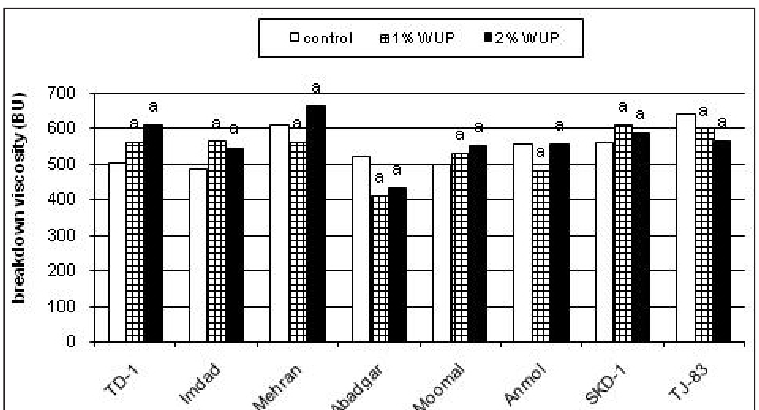

Fig. 30. Effect of water-unextractable pentosans (WUP) on the breakdown viscosity of flours from different hard wheat cultivars. Bars labeled with an 'a' indicate a significant difference from the control at P<0.05.

tendency of starch granules against shearing probably due to the insoluble nature of WUP. Moreover, the effect of WUP was irregular on BD values depending on the genotype of wheat flour and concentration of WUP.

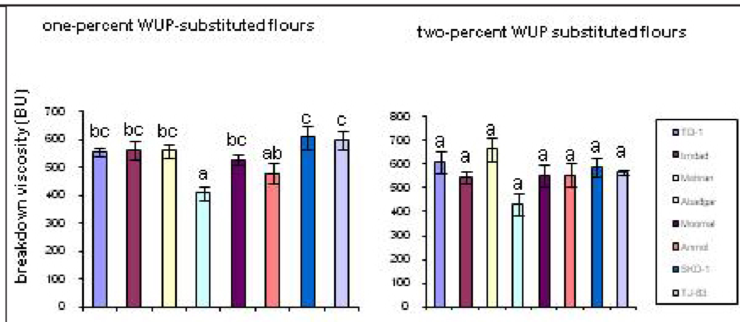

Fig. 31. Breakdown viscosities of water-unextractable pentosan (WUP)-substituted flours. Bars labeled with same letters are not significantly different at P<0.05.

Comparing the BD viscosities of 1% and 2% WUP-substituted flours. One-percent WUP, when added to the flour of cultivar Abadgar, was the most resistant to shearing as interpreted by the lowest BD values (Fig. 31). Pastes of all other cultivars exhibited BD viscosities ranging from 481 to 609 BU. The least tendency against shearing was found in the flour of variety SKD-1. Flour of the cultivar Mehran showed less tendency against shear. The most resistant flour was found to be of the cultivar Abadgar. The BD values of 2% WUP added flour of all cultivars varied insignificantly between 433 and 612BU.

Setback viscosity. Setback viscosity (SB) is the measure of retrogradation tendency of starch granules (Abd Karim et al. 2000). After gelatinization, the leached out linear amylose chains start reassociating on cooling, which subsequently results in increased the viscosity of flour pastes. Retrogradation of starch is a good indicator of bread staling. Other flour components such as gluten, lipids, and pentosans also may be involved in the process of staling (Martin et al. 1991; Shelton and D'Appolonia 1985).

Within cultivars, WUP insignificantly increased the setback viscosities of all cultivars except Imdad (Fig. 32). Only a 5% reduction in setback value was found at a 2% WUP substitution level in the cultivar Imdad. WUP possibly facilitated the reassociation of amylase chains upon cooling after gelatinization that subsequently caused slight increase in the viscosity of flour pastes. WUP itself might increase in viscosity upon cooling after cooking. The SB viscosity of wheat flour becomes greater when the nonstarch polysaccharides from rice and ragi are added up to the level of 0.5% (Rao et al. 2007).

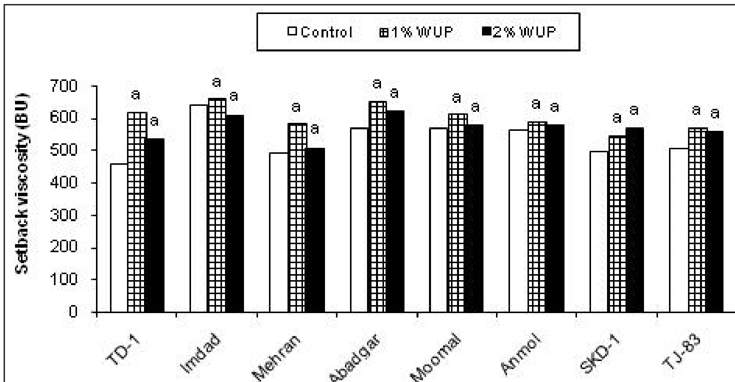

Fig. 32. The effect of water-unextractable pentosans (WUP) on the setback viscosity of flours from different wheat cultivars. Bars labeled with an 'a' indicate a significant difference from the control at P<0.05.

Setback viscosities of 15 WUP-substituted flours of all cultivars was between 543 and 657 BU (Fig. 33). The lowest retrograded valus were in the flour of SKD-1 and the highest was in Imdad. Setback viscosities of 2% WUP-substituted flours varied insignificantly, between 509 and 622 BU. The most retrograded flours was that of Abadgar and least in Mehran.

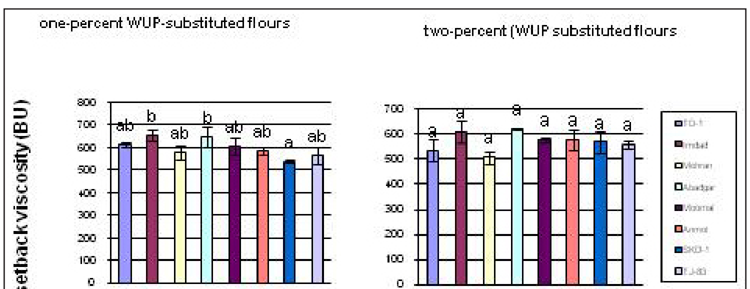

Fig. 33. Setback viscosities of water-unextractable pentosan (WUP)-substituted flours of different hard white spring wheat cultivars. Bars labeled with same letters are not significantly different at P<0.05

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Martin ML and Hoseney RC. 1991. A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. Cereal Chem 68:503-508.

Rao RSP, Manohar RS, and Muralikrishna G. 2007. Functional properties of water-soluble non-starch polysaccharides from rice and ragi: Effect on dough characteristics and baking quality. Food Sci Technol 40(10):1678-1686.
Shelton DR and D'Appolonia BL. 1985. Carbohydrate functionality in the baking process. Cereal Foods World 437-442.

The influence of water-unextractable pentosan concentration on paste viscosities of flours from different wheat cultivars.

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

The study was designed to determine the influence of water-unextractable pentosan (WUP) addition on the paste viscosities of flours of different wheat cultivars.

Peak viscosity. Changes were inconsistent at both levels of WUP supplementation and different types of effects (increasing or decreasing) were found on peak viscosity (PV) with increasing concentrations of WUP (Fig. 34). In addition to the type of WUP effect on PV, the degree of the effect varied widely among the cultivars. The maximum decrease in PV was in the cultivar TJ-83 (17.3% decrease) , followed by Abadgar (14.2% decrease). the maximum increase was in the PV of Moomal (18.9% increase), followed by SKD-1 (12.2% increase).

Hot paste viscosity (HPV). The effect of WUP was the opposite at 1% and 2% supplementation levels in all cultivars except TD-1, Moomal, and SKD-1 (Fig. 35). In general, WUP increased HPV at a 1% concentration, whereas HPV was reduced at a 2% concentration. The variability in magnitude of the WUP effect is reflected (Fig. 35). At a 15 concentration, the increase in HPV (3–35%) was found in all cultivars except TD-1 (only 1% decrease). The maximum increase was exhibited in Mehran. A reduction in HPV was found at a 2% concentration level in all cultivars except Moomal (3% increase) and SKD-1 (6% increase). The maximum reduction was found in TD-1 (22%). When the concentration of WUP increased, the amount of WUP component was enough to impart a similar impact as did the components of water-extractable pentosan. However, the mode of action might not be same. WUP possibly facilitates the process of rupturing of swollen starch granules without direct interaction with starch molecules.

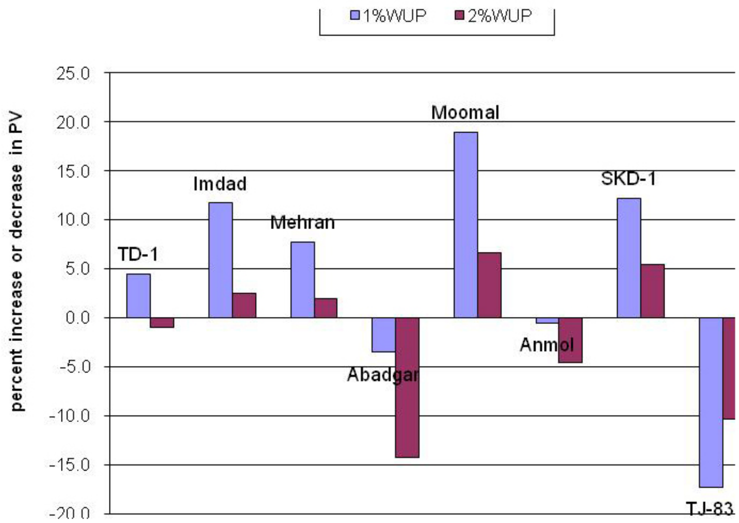

Fig. 34. The magnitude of increase or decrease (in %) in the peak viscosity (PV) of hard white spring wheat flours due to the addition of water-unextractable pentosan (WUP).

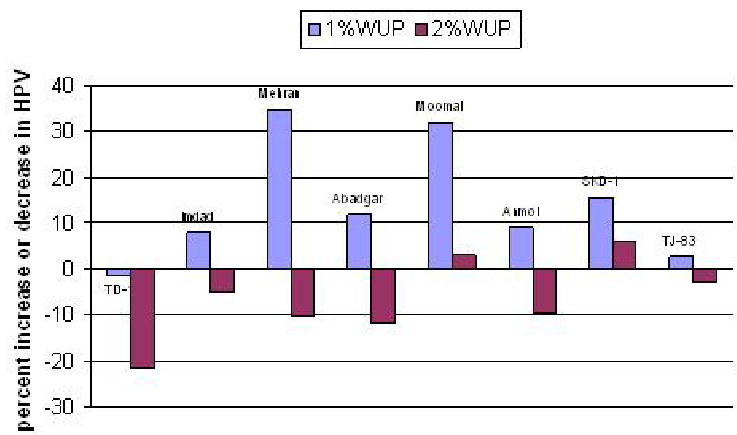

Fig. 35. The increase or decrease in hot paste viscosity (HPV) due to the addition of water-unextractable pentosan (WUP).

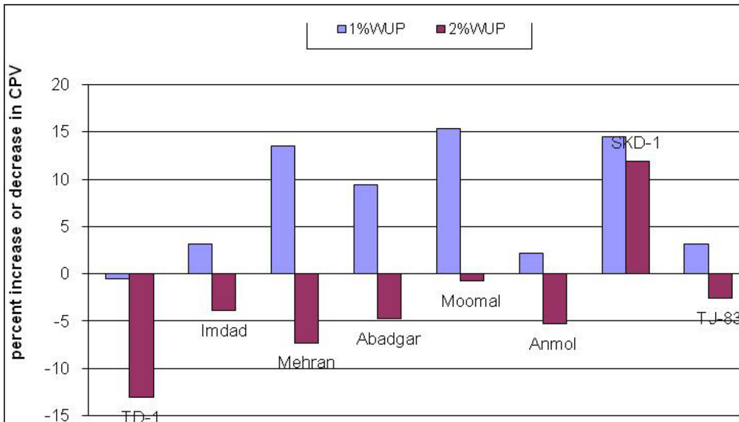

Fig. 36. Percent increase or decrease in the cole paste viscosity (CPV) with the addition of water-unextractable pentosan (WUP).

Cold paste viscosity (CPV). The effect of WUP was found variable on CPV of different varieties depending largely on the concentration of WUP (Fig. 36, p. 102). At the 1% and 2% concentration levels, the effect of WUP was the opposite on CPV of all cultivar flours except SKD-1 and TD-1. At a 1% concentration, the CPV of all cultivar flours except TD-1 increased (2–15%), whereas the CPV decreased between 1% and 13% with the addition of 2% WUP in all cultivar flours except SKD-1.

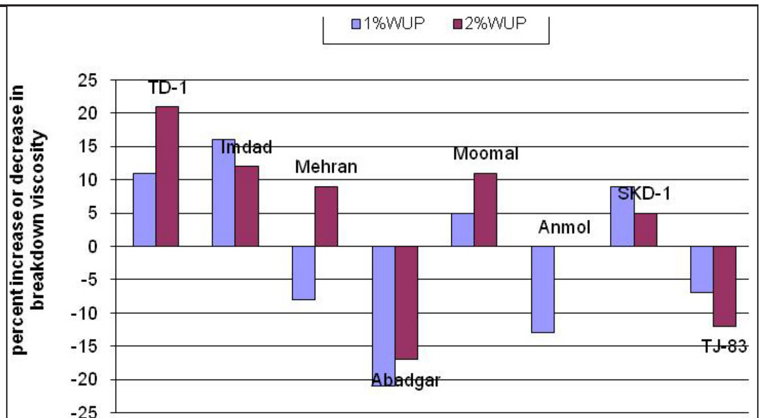

Fig. 37. Percent increase or decrease in the breakdown viscosity values of different hard white spring wheat cultivar flours.

Breakdown viscosity (BD). The supplementation level of WUP did not induce similar changes in BD of all cultivar flours (Fig. 37). Within cultivar, the same type of influence (increase or decrease) at both concentration levels and a similar type of WUP influence (increase or decrease) were found for each cultivar except Mehran. However, the magnitude of effect was variable at different WUP concentrations.

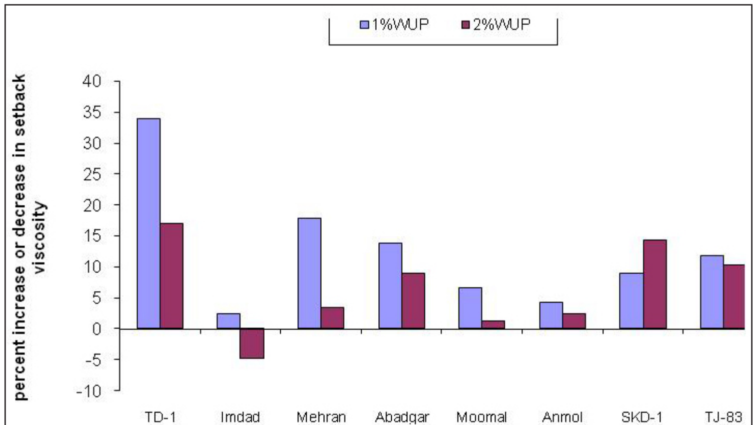

Fig 38. Percent decrease or increase in the setback viscosity of different hard white spring wheat cultivar flours due to the addition of water-unextractable pentosan (WUP) addition.

Setback viscosity (SB). Setback value did not linearly increase with increasing concentration of WUP (Fig. 38). The increase in SB values was variable at both 1% and 2% concentrations depending on the genotype of wheat flour. In fact, higher increases were noticed at 1% substitution in SB values of almost all cultivars, ranging between 2% and 34%. At a 2% concentration, the increase in setback value reached up to 17%.

Influence of water unextractable pentosan concentration on the pasting temperature and the time required to reach the pasting temperature of wheat flours.

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

Pasting temperature (PT) is one of the most important parameters of wheat flour pasting properties. Determining the time required to reach peak viscosity also is important. Many intrinsic and extrinsic factors are known to determine the PT of wheat flour. The addition of pentosan, i.e., water unextractable in nature, to the flour may influence the PT of wheat flour.Assuming this, our study added water-unextractable pentosan (WUP) at 1% and 2% concentrations to eight different wheat flours in order to examine the shifting of PT with at different concentrations.

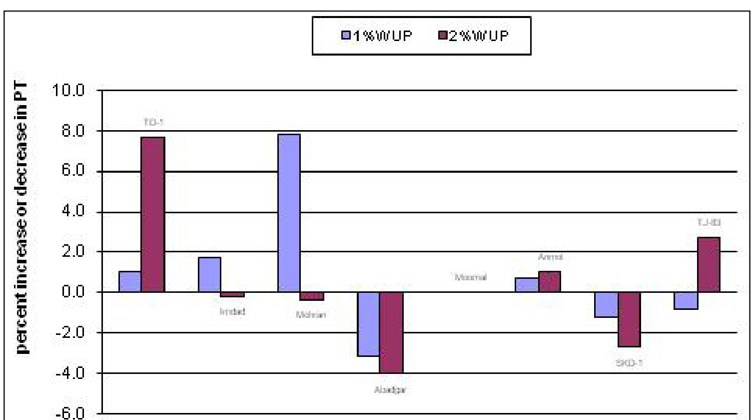

Fig. 39. The magnitude of increase or decrease (in %) in the pasting temperature (PT) of hard white spring wheat flour due to the addition of water-unextractable pentosan.

The effect of WUP at 1% and 2% addition was variable on the PT of flours (Fig. 39). Few cultivars showed an increase in PT whereas others had lower PT compared to the respective control flours. The largest reduction in PT was observed in the flour of Abadgar, followed by that of SKD-1 at both 1% and 2% levels of WUP. The reduction is valuable in bread-making because it implies an earlier beginning of starch gelatinization and, in turn, an increase in the availability of starch enzyme substrate during baking period (Rojas et al. 1999). The PT of flour of Moomal remained unchanged in the presence of WUP. The highest delay in PT was observed in the flour of cultivar Mehran at 1% WUP addition with a slight decrease when the WUP level increased to 2%. The PT of flour of TD-1 also was delayed at both levels of WUP, although to a higher degree at 2% WUP addition.

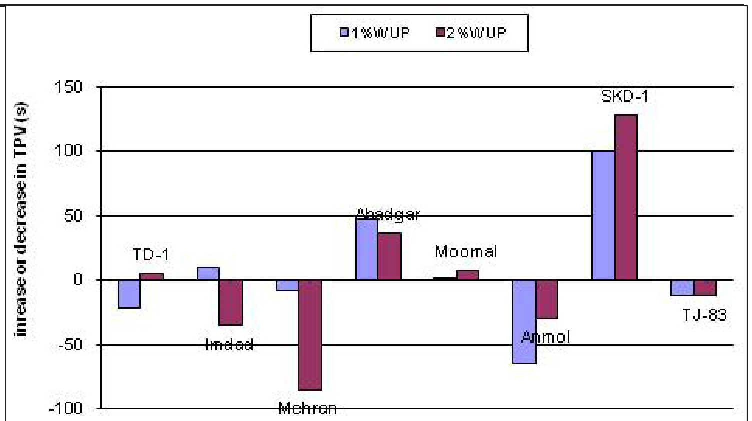

Fig. 40. Increase or decrease in time to reach peak viscosity due to the addition of water-unextractable pentosan (WUP).

Time to reach peak viscosity (TPV). Both levels of WUP supplementation imparted similar effects (whether increasing or decreasing) for each cultivar with the exception of TD-1 and Imdad (Fig. 40). However, the magnitude of the influence was different among all cultivars except TJ-83. The maximum delay was found in TPV of SKD-1 upon addition of both concentration levels, and the highest reduction (85 s) was in the flour of Mehran.

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Rojas JA, Rosell CM, and Bendito de Barber C. 1999. Pasting properties of different wheat flour-hydrocolloidal systems. Food Hydrocolloids 13:27-33.

The impact of a foliar application of manganese on various physiological and biochemical attributes of two contrasting wheat lines grown at different osmotic levels.

Mehmoona Ilyas, Abdul Waheed, Alvina Gul Kazi, Zaheer Ahmad, Rabia Farid, Noshin Shafqat, and Abdul Mujeeb-Kazi.

Salinity is one of the major problems in arid and semi-arid regions of the world where annual precipitation is less than evapo-transpiration and is major impediment to crop production. Saline conditions result in high ion cytotoxicity and low osmotic potential, which in turn reduces the uptake of micronutrients. This reduction in micronutrients is another factor limiting plant growth under saline conditions. Along with other micronutrients, absorption of manganese also is reduced under the low osmotic potential of the medium because of excessive accumulation of soluble salts. Exogenous application of manganese is used to improve the growth of wheat, because manganese is component of photosystem II and, thus, involved in photosynthesis.

Two genotypes and three levels of salinity, 0 (control), 150, and 300 molm^{-3} NaC were used to detect the effect of each level of salinity on two wheat cultivars tested with and without Mn applied through the roots and shoots. Different physiological and biochemical parameters, such as ionic balance, osmotic potential, leaf chlorophyll content, photosynthetic rate, soluble proteins, proline, free amino acids, and soluble sugar, were estimated to understand the physiological and biochemical mechanism of salt tolerance in contrasting wheat cultivars.

Increased sodium concentration due to salt stress reduced the fresh and dry weight of shoot and root of both Pasban 90 and PBW 343; however the depression of biomass was ameliorated by Mn application through the roots (Table 28, p. 105). Salinity induced through a Mn deficiency might have been responsible for the retarded growth in all treatments, but the impact was more pronounced at salt stress with Mn deficiency. Foliar application increased the growth of salt-stressed plants by increasing the Mn availability but not to an extent that was accomplished when Mn was available through the soil medium. Potassium concentration in the plant tissue was reduced and that of Na increased as the Na salinity in the root medium increased. Manganese application, through the medium or by foliar application, enhanced the uptake of K and decreased that of Na in the shoots of the tolerant cultivar Pasban 90, which might have helped it maintain a low Na:K ratio; an essential feature for plants to survive under saline conditions. Increasing salt

Table 28. Mean shoot and root fresh and dry biomass (g/plant) of two wheat cultivars grown under different salinity levels with and with out manganese application.

Cultivar	Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Pasban 90 (INIA-66/AG. DI.//INIA-66/3/ GENARO-81 [1388][2857])	T_0 (control)	47.7 A	10.15 B	21.6 A	4.69 B
	T_1 (-ive control)	33.6 B	7.87 C	16.6 B	2.86 C
	T_2 (NaCl 150 $molm^{-3}$)	30.4 C	5.05 D	15.2 B	2.41 D
	T_3 (NaCl 300 $molm^{-3}$)	24.2 D	3.57 E	7.8 EF	1.45 EFG
	T4 (-Mn +NaCl 150 $molm^{-3}$)	17.2 F	3.96 E	5.0 HI	0.97 HI
	T_5 (-Mn + NaCl 300 $molm^{-3}$)	7.6 I	1.15 H	4.6 I	0.84 I
	T_6 (-Mn + NaCl 150 $molm^{-3}$ + Mn foliar spray	20.0 EI	3.58 E	9.6 DE	1.39 EFG
	T_7 (-Mn + NaCl 300 $molm^{-3}$ + Mn foliar spray)	13.8 G	2.4 FG	6.6 FGH	1.22 FGH
PBW 343 (ND/VG9144// KAL/BB/3/ YACO/4/ VEE#5)	T_0 (control)	46.4 A	11.23 A	21.0 A	4.25 A
	T_1 (-ive control)	34.2 B	11.48 A	15.8 B	3.08 BC
	T_2 (NaCl 150 $molm^{-3}$)	20.0 E	3.30 EF	12.0 C	1.69 E
	T_3 (NaCl 300 $molm^{-3}$)	14.0 G	3.78 E	6.2 FGHI	1.4 EFG
	T4 (-Mn +NaCl 150 $molm^{-3}$)	14.4 G	3.22 EF	5.8 GHI	1.18 GH
	T_5 (-Mn + NaCl 300 $molm^{-3}$)	8.4 HI	1.87 GH	4.8 HI	1.13 GH
	T_6 (-Mn + NaCl 150 $molm^{-3}$ + Mn foliar spray	14.6 G	3.54 E	10.6 CD	2.35 D
	T_7 (-Mn + NaCl 300 $molm^{-3}$ + Mn foliar spray)	10.6 H	2.29 G	7.6 FG	1.64 EF
LSD Values	2.240	0.918	1.878	0.3601	

treatments consistently increased Cl and decreased the Mn content of shoots and roots of both wheat cultivars. The application of Mn significantly increased the shoot and root Mn content of both cultivars and application through roots had a significantly higher impact on Mn accumulation in shoots and roots of both cultivars. Manganese application, esspecially though the rooting medium, significantly enhanced the accumulation of organic osmotica such as soluble proteins, free amino acids, and proline. This, along with the uptake of inorganic osmotica might have helped to reduce the osmotic potential tolerant cultivar to produce turgidity. Increased salinity levels decreased chlorophyll a content and photosynthetic rate of both cultivars, and a foliar Mn application ameliorated this effect more effectively than a Mn application through roots.

Our results make it clear that Mn application ameliorated the negative consequences of salinity stress by enhancing the accumulation of inorganic and organic osmotica, chlorophyll content, and photosynthetic rate. In most cases, Mn application through the rooting medium showed more pronounced, positive impacts, except for chlorophyll content and photosynthetic rate, where Mn application through shoots enhanced the both parameters under salt stress. This study also shows that Mn application has partially ameliorated the hazardous effects of salinity stress by ionic balancing through reducing the uptake of Na and Cl and enhancing that of K and Mn; accumulating organic osmotica including soluble proteins, free amino acid, and proline to maintain better turgor; and increasing the chlorophyll content and photosynthetic rate. Manganese application through rooting medium had a more pronounced, positive effect on nearly all parameters except chlorophyll content and photosynthetic rate, whereas Mn application through shoots alleviated the negative consequences of salinity more effectively than Mn application through roots. To elucidate how this all happens, it will be necessary to work out the role of Mn on various enzymes involved in metabolism and photosystems.

Screening synthetic hexaploid wheat for drought tolerance at early growth stages.

Mehmoona Ilyas, Abdul Waheed, Alvina Gul Kazi, Husn-e-Sahar Zaide, and Abdul Mujeeb-Kazi.

Drought is one of the main agricultural menaces limiting the successful exploitation of land potential and consequently reducing crop productivity. Screening of available germ plasm of a crop for drought tolerance is of considerable value for utilizating drought-affected soil. During the early phase of this study, response of 116 wheat lines from a diverse gene pool was assessed at germination stage using polyethylene glycol (PEG 8000) at 10% and 20% in full strength Hoagland's nutrient solution (Table 29, pp. 106-107). Seedling growth was determined in an independent study. The

Table 29. Description of seed material of the synthetic hexaploid (SH) wheats used in the study to screen for drought tolerance.

Genotype	Original #	Source/Origin
Dh Drought-1	1	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (208)/5/Opata
	58	
	59	
	70	
	102	
	120	
Dh Drought-2	3	YAV-3/SCO//JO69/CRA/3/YAV79/4/*Ae. tauschii* (498)/5/Opata
	29	
	64	
	36	
	43	
Dh Drought-3	49	D67.2/P66-270//*Ae. tauschii* (257)/3/Opata
	50	
	60	
	61	
	70	
Dh Drought-4	6	GAN/*Ae. tauschii* (897)//Opata
	22	
	51	
	61	
	70	
Dh Drought-5	5	DOY1/*Ae. tauschii* (458)//Opata
	17	
	35	
	46	
	53	
SH DD Drought BV/02	10	D67.2/P66.270//*Ae. tauschii* (217) DVERD_2/*Ae. tauschii* (221) D67.2/P66.270//*Ae. tauschii* (257) CETA/AE. CETA/*Ae. tauschii* (1026) CETA/*Ae. tauschii* (1031)
ONMKDISH	1	Altar 84/*Ae. tauschii*//Opata
	2	CROC_1/*Ae. tauschii* (224)//Opata
	3	CROC_1/*Ae. tauschii* (224)//Opata
	4	CHEN/*Ae. tauschii*//2*Opata
	5	Altar 84/*Ae. tauschii*/2*Opata
MX101-02		
M11SAWYT	1	CROC_1/*Ae. tauschii* (205)//KAUZ/3/Sasia
	2	Dharwar Dry/Nesser
	3	Dharwar Dry/Nesser
	4	SUJATA/SERI
	6	PASTOR/3/MUNIA//CHEN/Altar 84/5/CNDO/RI43//ENTE/MEXI_2/3/*Ae. tauschii*/4/Weaver
	7	FILIN/Irena/5/CNDO/RI43//ENTE/MEXI_2/3/*Ae. tauschii*/4/Weaver
	18	URES/PRL//AV32
	20	Pastor/VAV92
	21	Irena/Babax//Pastor
	22	Irena/Babax//Pastor
	23	CROC_1/*Ae. tauschii* (224)//Opata/3/Pastor
	25	BJY/COC//PRL/BOW/3/Attila

Table 29. Description of seed material of the synthetic hexaploid (SH) wheats used in the study to screen for drought tolerance.

Genotype	Original #	Source/Origin
M11SAWYT	26	Parus/Pastor
	27	FILIN/3/CROC_1/*Ae. tauschii* (205)//KAUZ/4/FILIN
	28	FILIN/3/CROC_1/*Ae. tauschii* (205)//KAUZ/4/FILIN
	29	GEN*2//BUC/FLK/3/2*Pastor
	32	VEE/MJI//2*TUI/3/2*Pastor
	33	Milan/KAUZ//Babax/3/Babax
	35	Pastor/3/Altar 84/*Ae. tauschii*//Opata
	37	URES/JUN//KAUZ/3/Babax
Darwar		
Sitta		
Nesser		
Opata		
Veebli		
Inqalab-91		
Kohistan-97		
Baraine 83		
C-591		
C-271		
Rohtas 90		

Richardselection-3	Richard selection-16	Richard selection-25
Richard selection-4	Richard selection-17	Richard selection-26
Richard selection-6	Richard selection-18	Richard selection-27
Richard selection-8	Richard selection-19	Richard selection-28
Richard selection-9	Richard selection-20	Richard selection-29
Richard selection-13	Richard selection-21	Richard selection-30
Richard selection-14	Richard selection-22	Richard selection-32
Richard selection-15	Richard selection-23	Richard selection-33
Richard selection-34	Richard selection-52	Richard selection-75
Richard selection-35	Richard selection-53	Richard selection-76
Richard selection-41	Richard selection-57	Richard selection-77
Richard selection-42	Richard selection-58	Richard selection-79
Richard selection-46	Richard selection-62	L. sequia-8
Richard selection-47	Richard selection-66	L. sequia-11
Richard selection-48	Richard selection-69	L. sequia-12
Richard selection-49	Richard selection-70	L. sequia-13
Richard selection-50	Richard selection-71	
Richard selection-51	Richard selection-74	

study was designed across two treatments each with three replicates having three plants/pot. T_0 was the control treatment and T_1 was the drought evaluation treatment.

The lines M11SAWYT 25, 36, 21, and 34 showed significantly higher total germination percent in the PEG solutions and C-271 and the Richard's selections 29, 33, 34, 23, and 41 showed lower germination than the other lines. The M11SAWYT 32 line and Richard's selections 3 and 79 showed relatively poor performance for all the growth parameters measured at the seedling stage. No consistent relationship between tolerance was observed at the germination and seedling stages, however, the presence of a great amount of variability for drought tolerance in wheat observed in this study at each stage can be beneficial for improving drought tolerance through recurrent selection. Data for different growth parameters at the seedling stage showed that WEEBLI; Dh Drought 2/29, 3/60, 4/70, and 5/5; and ONMKDISH 1 produced significantly greater biomass in absolute terms, and these lines also were characterized as tolerant lines on the basis of their performance for number of tillers and relative water content under drought stress (Table 30). The good performance of these tolerant lines could be attributed to osmotic regulation, which might have enabled the maintenance of cell turgor that could assist plant survival under drought stress. High tiller numbers in these drought-tolerant lines could have played central role in their recovery from early season drought, and this character might have contributed to greater biomass production under water-deficient conditions. The prolific root system of these drought-tolerant lines could have improved

Table 30. Classification of 116 lines of wheat on the basis of their performance

Class	Range	Number	Genotypes
Root length (cm) after two month growth under drought stress			
I	36–40	1	Dh drought-4/6
II	31–35	9	Sh DD Drought; Dh Drought-3/60, -3/61, -3/77; ONMKDISH 1, 2, 3, Weebli, M11SAWYT 36
III	26–30	29	C-591; L. Sequia-8, -13; Kohistan-97, Opata, C-271, Dh Drought-2/29, -2/43, -3/49, -3/50, -4/22, -4/70, -5/5, -5/17; M11SAWYT 18v, 5, 6, 7, 20, 21, 33, 34, Richard selections-14, -48, -49, -51, -70, -75, -76
IV	21–25	70	ONMKDISH 5, Richard selections-3, -4, -6, -9, -13, -15, -16, -17, -18, -19, -20, -21, -22, -23, -25, -26, -27, -28, -29, -30, -32, -33, -34, -35, -41, -42 , -46, -47, -50, -52, -53, -57, -58, -62, -66, -68, -69, -71, -74, -77, -79; L. Sequia-11; Nesser Baranie-83; M11SAWYT 8, 10, 11, 22, 23, 25, 27, 28, 29; Dh drought-1/102, -1/1, -1/120, -1/58, -2/34, -2/36, -4/61, -4/51, -5/46, -5/35, -5/53; Rohtas-90, Inqalab-91; ONMKDISH 4, Darwar, L. Sequia-12
V	15–20	7	Dh drought 1/59, 1/70, 2/3; Sitta; M11SAWYT 26, 32; Richard selection-8
Relative water content after two month growth under drought stress			
I	91–100	7	Dh drought-1/59, -2/3, -2/43, -3/60; M11SAWYT 7; Weebli; Sitta
II	81–90	48	M11SAWYT 18v, 5, 6, 10, 11, 18v, 20, 21, 23, 25, 29, 32, 36; ONMKDISH 4; Nesser; M11SAWYT 6, 10, 25, 26, 32; C-271; ONMKDISH 1, 2, 3, 5; Richard selections-52, -58; Rohtas-90, Sh DD Drought, Inqalab-91; Darwar, Dh Drought-1/120, -1/1, -1/70, -1/58, -2/34, -2/29, -2/36, -3/50, -3/61, -3/77, -3/49, -4/51, -4/6, -4/70, -4/22, -4/61, -5/35, -5/53, -5/5, -5/17, -5/46, -5/5
III	71–80	42	C-591; M11SAWYT 8, 22, 27, 28, 33; Kohistan-97; L. Sequia-8, -11, -12; Baranie-83; Richard selections-3, -4, -6, -8, -9, -13, -14, -16, -18, -19, -22, -28, -29, -30, -32, -33, -35, -41, -46, -47, -48, -51, -53, -57, -69, -70, -71, -74, -76, -77, -79
IV	61–70	17	Opata; M11SAWYT 34; L. Sequia-13; Richard selections-15, -17, -20, -23, -25, -26, -27, -34, -42, -49, -50, -62, -66, -68
V	51–60	1	Richard selection-21
VI	40–50	1	Richard selection-75

the water uptake and this imperative character might have increased the above-ground, dry biomass. The tolerance of WEEBLI; Dh Drought-2/29, -3/60, -4/70, and -5/5; and Dh ONMKDISH 1 observed at the seedling stage was not conferred at the germination stage, because all these lines were ranked as moderately tolerant for germination percent.

The results presented here deal with the drought tolerance of 116 lines of wheats at two initial growth stages, germination and seedling. The tolerance observed in six lines (WEEBLI; Dh Drought -2/29, -3/60, -4/70, and -5/5; and Dh ONMKDISH 1) at the early growth stages may or may not be conferred at the adult stage. Nevertheless, the tolerance observed here is of great economic importance, because many workers have emphasized that the assessment of drought tolerance at every growth stage of a plant species is of considerable value in determining the magnitude of genetic diversity and the ultimate tolerance of the species.

Development of drought-tolerant wheat cultivars.

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Wheat is a staple diet of major population in Pakistan (Ahmad et al. 200; Ajmal et al. 2009) contributing 13.1% of the value added in agriculture and 2.7% to GDP (Anonymous 2011). Wheat supplies 72% of the calories and proteins in the average diet (Azam et al. 2007). Areas receiving rainfall above 350 mm annually are able to grow a wheat crop under rainfed conditions. The Barani areas have a great significance in wheat production, covering about 12.3% of the area under wheat and contributing about 7% of the wheat production in Punjab and 7.65% in Pakistan (Anonymous 2009). Rain fall distribution is, of course, very crucial for crop production. With considerable year to year variation in precipitation, uneven rainfall and crop yields are more frequent in the Rabi season than in Kharif (Fig. 41).

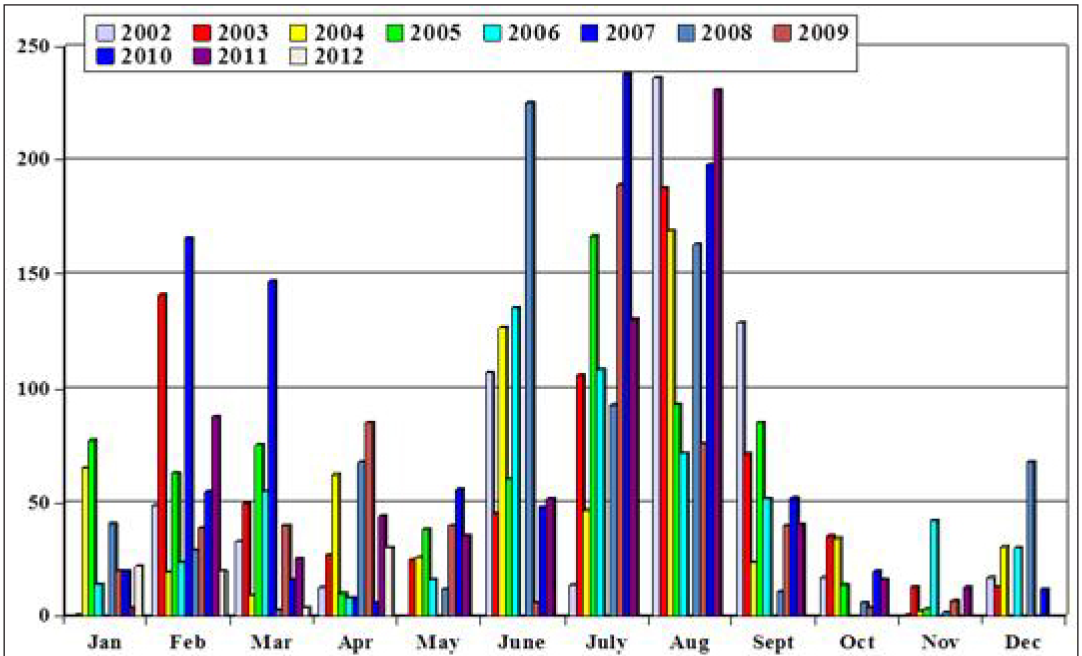

Fig. 41. Rainfall at Chakwal, Pakistan, 2002-2012.

Both biotic and abiotic stresses are major threats to the crop productivity. In Pakistan, brown and yellow rusts are among the serious diseases of wheat and, in most cases, wheat cultivars are replaced with newer cultivars due to susceptibility (Rattu et al. 2007). Wheat cultivars with narrow, erect to semi erect leaves and deep root systems that tolerate drought stress and leads to higher biomass and grain production are the most suitable. Presently, Chakwal-50, GA-2002, and Inqilab-91 are commonly grown in the rain-fed areas of the Punjab. Because these cultivar have become susceptible to rusts, it is necessary to develop new, disease-tolerant wheat cultivars suitable for the rainfed areas.

A team of devoted, highly qualified research Scientists is engaged at Barani Agricultural Research Institute, Chakwal, for this purpose. The wheat research team is working day and night to develop high-yielding cultivarss and production technology for wheat in the Barani area with the main objectives to

- collect and maintain germ plasm of wheat for utilization in the hybridization program,
- morphologically and physiologically characterize germ plasm,
- develop high-yielding, drought-tolerant and disease resistant wheat cultivars suitable for rainfed areas,
- collaborate with national and international organizations through exchange and evaluation of wheat material, and
- maintain genetic purity of wheat cultivars through BNS, pre-basic and basic seeds.

The Barani Agricutural Research Institute, Chakwal, received a set of 36 wheat genotypes from Dr. Abdul Mujeeb-Kazi during the 2011–12 season. This material was planted at the Barani Agricultural Research Institute in six 5-m rows and evaluated under rainfed conditions. Fertilizer was applied at 90:60:30 N:P:K. Morphological and disease data were recorded and nine high-yielding genotypes were selected based on this data. These nine genotypes are now included in crossing block and gene pool of Barani Agricultural Research Institute. These selected genotypes are included in a preliminary wheat yield trial in 2012–13 for further evaluation (Table 31, p. 110).

Table 31. Phenological parameters evaluated in a set of 36 wheat genotypes in the 2011–12 crop cycle at the Barani Agricutural Research Institute, Chakwal, Pakistan.

Genotype	Germination %	Plant height (cm)	Spike length (cm)	Tillers/m	Grains/spike	Yield (kg/ha)
K1	90	116	10.8	130	56.7	6,170
K2	87	116	11.7	141	57.0	5,740
K3	82	112	12.1	133	43.7	4,540
K9	82	117	10.5	142	51.3	2,140
K11	80	113	11.4	128	44.0	3,250
MK2	82	95	10.2	168	43.7	4,057
MK3	80	100	11.0	120	47.0	3,511
MK4	82	98	11.6	132	46.7	4,591
MK5	78	97	10.4	160	40.0	3,102
MK6	75	101	12.4	137	36.7	3,115
MK7	82	104	11.3	174	53.7	4,582
MK8	87	113	12.6	160	61.0	5,742
MK9	84	103	10.7	123	71.7	4,048
MK10	82	104	11.1	164	76.7	3,844
MK11	76	105	11.7	132	63.7	3,218
MK12	82	110	12.0	158	75.0	4,262
MK13	72	103	12.1	130	69.3	5,506
MK14	73	99	10.5	168	50.0	3,266
MK15	77	104	12.3	180	66.7	4,622
MK17	80	104	11.2	188	58.3	4,364
MK18	75	104	11.4	135	41.0	4,164
MK19	72	107	10.6	152	70.3	4,368
MK20	74	97	10.4	191	44.7	3,471
MK21	70	88	11.2	139	62.3	3,840
MK22	76	90	11.9	135	57.3	3,142
MK23	72	101	11.4	181	50.3	3,386
MK24	74	101	11.8	193	64.0	3,599
MK25	65	101	11.0	207	53.7	2,738
MK27	75	105	12.5	122	67.0	4,284
MK28	70	103	12.1	105	41.0	4,613
MK29	72	107	12.2	118	55.7	3,689
MK30	70	93	12.0	190	49.0	3,129
MK31	62	92	11.9	153	46.0	2,040
MK32	65	92	12.1	184	53.3	2,866
MK33	75	99	11.1	168	53.3	2,998
MK34	78	105	13.4	197	57.0	1,652
Selected wheat material						
K1	90	116	10.8	130	56.7	6,170
K2	87	116	11.7	141	57.0	5,740
MK8	87	113	12.6	160	61.0	5,742
MK13	72	103	12.1	130	69.3	5,506
MK15	77	104	12.3	180	66.7	4,622
MK28	70	103	12.1	105	41.0	4,613
K3	82	112	12.1	133	43.7	4,540
MK4	82	98	11.6	132	46.7	4,591
MK7	82	104	11.3	174	53.7	4,582

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Breeding of wheat genotypes for the semidwarf character and high grain yield.

Wheat is the basic staple food for most of the population and largest grain source of the country. The importance of wheat is always recognized when formulating agricultural policies. Wheat contributes 12.5% to the value added in agriculture and 2.6% to GDP. Wheat was cultivated in an area of 8.666 x 10^6 ha in 2011–12 (Table 1), a decrease of 2.6% over last year when the area of 8.901 x 10^6 ha. A production of 23.5 x 10^6 is estimated in 2011–12. The yield/ha posted a negative growth of 4.2% compared to 11% last year, which is due to the fact that sowing was delayed because of standing water and other climatic factors.

Table 1. Area, production, and yield of wheat grown in Pakistan between the 2007–08 and 2011–12 crop years (Source: Pakistan Economic Survey (2011–12).

	Area		Production		Yield	
Year	x 10^6 ha	% change	x 10^6 ha	% change	x 10^6 ha	% change
2007–08	8.550	–0.3	20.959	–10.0	2.451	–9.8
2008–09	9.046	5.8	24.033	14.7	2.657	8.4
2009–10	9.132	1.0	23.311	–3.0	2.553	–3.9
2010–11	8.901	–2.5	25.214	8.2	2.833	11.0
2011–12	8.666	–2.6	23.517	6.7	2.714	–4.2

Progress of cultivar NIA-Sunhari. A total of 5,000 kg of NIA-Sunhari pre-basic seed was produced from three acres during 2011 out of which 2,500 kg seed sold to growers and seed companies during 2011–12.

Candidate varietal material. Two candidate cultivars (NIA-22-03 and NIA-54-03) are being maintained for sending as entries to National Uniform Wheat Yield Trials (NUWYT).

Zonal Trial studies. Two genotypes (NIA-6-12 and NIA-CIM-04-10) were tested in zonal/regional trials in the Sindh province. The performance of NIA-CIM-04-10 was comparatively better for grain yield (kg/plot).

Trial I. This trial was conducted with 14 advanced lines with two check cultivars, NIA-Sunhari and Kiran. The trial was sown on 2 November, 2010. The trial had six rows of each genotype with a 4-m row length in three replicates. The results indicated that line (01) had the highest grain yield/plot (2.52 kg). Possible reasons could be that the line also had a higher number of spikelets/spike and increased main spike grain yield. Subsequent lines that had higher grain yield were 03 (2.37 kg), 08 (2.4 kg), Kiran (2.32 kg), 12 (2.23 kg), 11 (2.20 kg), and 02 (2.18 kg). Line 10 had the lowest grain yield (0.983 kg), possiblally because ths line possesses the lowest number of grains/main spike.

Trial II. This trial consisted of 16 genotypes including the two check cultivars NIA-Sunhari and Kiran. The trial had six rows of each genotype with a 4-m row length in three replicates. Sowing of the trial was completed on 2 November, 2010. The results showed that line 09 had the highest grain yield/plot (2.75 kg). Subsequent lines that had higher grain yield were Kiran (2.70 kg) and line 02 (2.65 kg). The possible reasons for higher grain yield in Kiran and line 02 could be due to increased main spike grain yield. Line 05 (1.65 kg) had the lowest grain yield per plot compared with other lines and cultivars.

Trial III (Isoline material). Thirty-six genotypes of isogenic material for Norin-10 (*Rht1*, *Rht2*, *rht*) were sown on 3, November, 2010. The trial had six rows of each genotype with a 4-m row length of four meters in three replicates. In this comparison, line 03 (2.433 kg) had the highest grain yield/plot. Subsequent lines with a higher grain yield were 17 (2.400 kg), 28 (2.383 kg), 26 (2.333 kg), 19 (2.283 kg), 30 (2.250 kg), 18 (2.233 kg), and 2 (2.217 kg). Line 08 (0.750 kg) had the tallest plant height with the lowest grain yield due to lodging.

Mutation breeding for drought tolerance. The material for drought consisted of 72 M_4 genotypes planted with two 4-m rows in three replicates. Lines 62 and 63 had produced the highest grain yield (388 g/plot) under zero/no irrigation. Line 64 had produced the highest grain yield (542 g/plot) under two irrigations. Line 58 had produced the highest grain yield (542 g/plot) under full irrigation conditions. Line 64 had also produced the highest grain yield (461 g/plot) average performance over three irrigation treatments.

Salinity tolerance. The salinity of soil ranged from 3.1 to 11.4 EC (1:2.5) ds/m. The same 72 M_4 progenies used for the drought studies also were grown in a saline soil. Line 9 (330 g/plot), from mutated progenies of the cultivar Bhittai, had the highest grain yield/plot than that of all other genotypes and check cultivars. Subsequent lines derived from Bhittai that had a higher grain yield were 17 (245 g), 15 (233 g), 30 (230 g), 10 (225 g), and 22 (215 g). The mutated line 10 (270 g) derived from the cultiar Kiran, had the highest grain yield/plot. Subsequent lines with higher grain yield/plot from the cultivar Kiran were 5 (237 g) and 23 (215 g). The Kiran check had 202 g grain yield/plot. Thirty mutant progenies were selected for next M_5 generation.

Mutation studies under normal conditions. The 72 mutated M_4 progenies used for drought studies also were grown in normal soil conditions. The lines that produced highest grain yield/plot were 14 (428 g), 15 (425 g), 21 (440 g), and 27 (437 g) from the cultivar Bhittai. However, the maternal Bhittai parent had a plot grain yield of 328 g. Lines that had the highest grain yield/plot were 11 (588 g), 15 (563 g), and 17 (588 g) from the cultivar Kiran. However, the maternal Kiran parent had a comparatively lower grain yield/ plot (430 g).

Introduction of *Rht8*. The semidwarfing gene *Rht8* was transferred from the Italian cultivar Mara. The material is in the F_5 generation.

Breeding material. The breeding material consisted of three crosses F_1 generation, four crosses from the F_4 generation, two crosses from the F_7, one cross from the F_8.

Germ plasm material. We are maintaining 140 lines with different characteristics for use in the crossing program.

Sanction of a new international project. A new IAEA/RCA/RAS/05/56 project entitled 'Supporting mutation breeding approaches to develop new crop varieties adaptable to climate change' has been sanctioned for the 2012 and 2015.

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