ITEMS FROM PAKISTAN

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Diversity and distribution of rust diseases of wheat (Puccinia spp.) in southern Pakistan.

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Pakistan is an agricultural country. A large portion of the population is directly or indirectly dependent on agriculture. Presently, a burgeoning population is demanding more food, which only can be accomplished when the production of cereal crops in the country is increased. To achieve this, sustainable control of biotic and abiotic constraints is needed. Among the biotic constraints, rust diseases (stem, leaf, and stripe) caused by *Puccinia spp*. are important fungal diseases of the wheat crop attributed to substantial grain losses (Chen 2005; Bux et al. 2012).

Leaf, stem, and yellow rust prevail in all the wheat-growing areas of southern Sindh, spanning from Thatta to Sanghar and the Nawabshah area. Leaf rust is common in all the areas and occurs regularly. Yellow rust is sporadic and prevails when the climatic conditions are favorable for the pathogen. Stem rust is a regularly occurring and most dangerous rust occurring in the southern wheat-growing areas of Sindh. These diseases effect the wheat crop by damaging the respiratory system, killing foliage, stunting growth, and most importantly reducing grain yield by shriveling grain, reducing weight, and effecting quality (Chen 2005). Grain losses of 10–70% caused by these devastating pathogens have been reported. In severe disease epidemics, the grain damage may be up to 100% (Chen 2005).

These destructive fungal pathogens are controlled by fungicides and host-genetic resistance through developing resistant wheat cultivars. The pathogens are dynamic, changing their genetic make-up over time to become more aggressive and devastating. Therefore, for sustainable control, studies on the diversity of the pathogen and identifying genetic resources through molecular technology are needed. To achieve this, developing efficient molecular markers for the genes conferring resistance are indispensible (Bux et al. 2011).

We carried out a survey of wheat rust diseases across Sindh province, southern Pakistan. Occurrence of leaf, stem, and yellow rust is limited to particular areas or overlap each other. In 2012, leaf rust was common and observed across the wheat tract in the Sindh province. We observed a high incidence of the disease in Kunri, Umar kot, Petaro, Jamshoro, Tandom Adam, Mirpur Matrhelo, Kandiaro, and other areas. Stem rust was limited to a few areas. During our survey, we observed a few pustules of stem rust on wheat growing around Matiari and Sanhgar. Yellow rust was completely absent in the southern parts of Sindh province. However, a few diseased leaves were observed in Ghotki wheat-growing regions lying on the border of Sindh–Punjab provinces.

All three rust diseases are damaging the rural economy in Sindh. To control these diseases sustainably, monitoring the pathogen's prevalence is necessary before applying the appropriate control measures. However, sustainable control can be accomplished by increasing the genetic diversity of existing wheat cultivars. Furthermore, agricultural extension services will assist in combating the pathogen by creating awareness among the farmers. Research on modern lines, in parallel with those in developed countries, will help resolve the problem and devise new avenues for sustainable control.

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Relationship of pasting parameters with gluten and some other attributes of flour.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Awais Rasheed, Alvina Gul Kazi, Abdul Aziz Napar, and Abdul Mujeeb-Kazi.

Pearson's correlation coefficients were used to analyze the relationships between pasting parameters and quality attributes of hard white spring wheat flours. The relationships between pasting parameters and flour quality attributes are given in Table 1. A significant positive relationship was found between pasting temperature and gluten content (wet and dry). Addo et al. (2001) addressed the requirement for higher temperature by high-gluten flours over other components, such as prime starch, tailing starch, and their mixtures.

Table 1. Physio-chemical attributes and arabinoxylan contents of some wheat cultivars (SD = starch damage; FN = falling number; PC = protein content; WG = wet gluten; DG = dry gluten; GI = gluten index; MC = moisture content; TOAX = total arabinoxylan content; WEAX = water extractable arabinoxylan content; and WUAX = water unextractable arabinoxylan content).

Cultivar	SD (UCD)	FN (s)	Ash (%)	PC (%)	WG (%)	DG (%)	GI	MC (%)	TOAX (mg/g)	WEAX (mg/g)	WUAX (mg/g)	%WEAX in TOAX
TD-1	23.0	594	0.54	11.0	30.4	9.7	68	15.1	13.6	4.3	9.4	31.2
Imdad	23.1	566	0.72	11.2	29.5	9.6	95	15.1	16.5	6.3	10.3	37.8
Mehran	22.0	902	0.56	12.2	31.0	10.6	78	14.7	14.5	4.7	9.8	32.4
Abadgar	22.4	586	0.66	13.0	38.2	11.0	56	12.7	15.1	5.1	10.1	33.4
Moomal	22.9	582	0.69	11.1	27.8	9.1	96	14.7	16.6	6.1	10.5	36.6
Anmol	22.1	505	0.71	11.5	28.4	8.7	95	14.7	17.2	6.1	11.1	35.2
SKD-1	22.5	844	0.52	11.3	33.0	9.1	87	14.5	16.1	6.0	10.1	37.0
TJ-83	20.9	583	0.60	9.4	28.5	8.3	78	14.5	11.7	3.3	8.3	28.6

Regarding the soundness of wheat kernels, the cultivars used in this study were found to have very low α-amylase activity as interpreted by higher values for falling number (> 450s) and peak viscosity (> 960 BU), because falling number is inversely related with α-amylase activity (D'Appolonia et al. 1982; Moot and Every 1990) and peak viscosity (D'Appolonia et al. 1982). But when the α-amylase activity is too low, as in our study, the resultant falling number will exclusively be the function of flour viscosity instead of α-amylase. Therefore, the falling number was found to strongly corelate with amylograph peak viscosity. Vijayakumar et al. (2009) also found a positive relationship between falling number and PV in composite flours. Noda et al. (2003) includes sprouted wheat (high α-amylase activity) in their study and displayed the curvilinear relationship between peak viscosity and α-amylase activity.

The arabinoxylan (AX) content and percentage of water-extractable AX in total AX weakly related with peak viscosity of wheat flour. In heat-treated flour paste, the AX contents significantly correlated with apparent viscosity (Iriki et al. 2003).

A significantly positive relationship was found between time to reach peak viscosity and cold paste viscosity (r = 0.626**, Table 2, p. 135). The relationship revealed that the flours with long peak-paste time had a greater ability to form a paste or gel after cooling. Wiesenborn et al. (1994) suggested that flours exhibiting longer time to reach their maximum viscosity are more resistant to mechanical damage. Moreover, the flours having good cold thickening capacity are suitable for use in recipes for instant soup, cream, and sauces (Alves et al. 1999).

Hot paste viscosity was strongly and positively related with cold paste viscosity (r = 0.857**, Table 2, p. 135). Kaur et al. (2007) also showed a significantly positive relationship between hot paste viscosity and cold paste viscosity in potato cultivars.

Breakdown viscosities were moderately related with peak viscosity (r = 0.433, Table 2), suggesting that flours having a greater peak viscosity can be broken down rapidly resulting in weak gels or pastes. These flours are suitable in food applications where low thickening power is required such as in pastries. Tsakama et al. (2010) also reported a significant positive relationship between peak viscosity and the breakdown viscosity of sweet potato starches.

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Table 2. Correlation coefficients for wheat flour pasting parameters and quality attributes (PT, pasting temperature; PV, peak viscosity; TPV, time to reach peak viscosity; HPV, hot paste viscosity; CPV, cold paste viscosity; BD, breakdown viscosity; SB, setback viscosity; SD, damaged starch; FN, falling number; AC, ash content; PC, protein content; WG, wet gluten; DG, dry gluten; GI, gluten index; MC, moisture content; TOAX, total arabinoxylan; WEAX, water–extractable arabinoxylan; WUAX, water–unextractable arabinoxylan; and %WEAX, percent water–extractable arabinoxylan in total arabinoxylan (*0.05 and **0.01 level of significance.).

	PT	PV	TPV	HPV	CPV	BD	SB	SD	FN	ASH	PC	WG	DG	GI	MC	TOAX	WEAX	WUAX
PV	0.041																	
TPV	–0.542*	0.309																
HPV	0.164	0.66**	0.418															
CPV	–0.031	0.470	0.626**	0.857**														
BD	–0.156	0.433	–0.124	–0.390	–0.447													
SB	–0.097	0.36	0.192	0.289	0.502*	–0.30												
SD	–0.168	–0.354	–0.192	–0.005	–0.021	–0.427	0.116											
FN	–0.008	0.901**	0.365	0.568*	0.371	0.427	–0.014	–0.458										
AC	–0.187	0.171	0.291	0.343	0.516*	–0.187	0.614*	0.202	0.209									
PC	0.512*	–0.102	–0.110	0.029	0.198	–0.148	0.021	–0.154	–0.220	0.14								
WG	0.633**	0.124	–0.211	0.165	0.209	–0.40	–0.098	–0.254	–0.101	–0.12	0.88**							
DG	0.504**	–0.133	–0.13	–0.076	0.112	–0.068	–0.088	–0.108	–0.288	–0.035	0.947**	0.864**						
GI	–0.337	–0.086	0.223	0.008	0.10	–0.109	0.239	0.172	0.071	0.236	–0.265	–0.543*	–0.373					
MC	–0.60	–0.442	0.014	0.046	0.083	–0.612*	0.303	0.526*	–0.343	0.044	–0.213	–0.420	–0.178	0.149				
TOAX	–0.150	–0.262	0.038	0.172	0.200	–0.519*	0.531*	0.567*	–0.238	0.504*	–0.036	–0.273	–0.196	0.486	0.426			
WEAX	–0.102	–0.289	–0.037	0.166	0.171	–0.547*	0.566*	0.588*	–0.278	0.456	–0.069	–0.290	–0.226	0.542*	0.457	0.983**		
WUAX	–0.203	–0.203	0.132	0.189	0.244	–0.464	0.455	0.518*	–0.173	0.542*	0.02	–0.220	–0.137	0.380	0.356	0.971**	0.911**	
%WEAX	0.004	–0.369	–0.194	0.057	0.060	–0.517*	0.584*	0.573*	–0.388	0.310	–0.037	–0.242	–0.179	0.561*	0.461	0.891**	0.955**	0.764**

Effect of water-extractable pentosan on the cold paste viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Awais Rasheed, Alvina Gul Kazi, Abdul Aziz Napar, and Abdul Mujeeb-Kazi.

Breakdown viscosity (BD) is the measure of fragility of starch granules. A high breakdown viscosity indicates fewer tendencies of starch granules to resist shear. The BD viscosities of flours varied insignificantly between 487 BU to 644 BU. Singh et al. (2010) relates climatic conditions with breakdown viscosity and found a decrease in breakdown viscosity under rain-fed conditions. Flour from the cultivar Imdad showed the maximum resistance to shear as interpreted by its least BD viscosity amongs cultivars. The higher breakdown viscosity found in the flour of cultivar TJ-83 was followed by those of Mehran, SKD-1, and Anmol. The addition of water-extractable pentosan (WEP) was found to increase the BD viscosity of the flour of all cultivars, suggesting that the presence of hydrophilic WEP exerts more stress on wheat starch granules resulting in a rapid decline in the viscosity of wheat flour suspensions. A statistically significant increase was found in the BD viscosity of cultivars TD-1, Imdad, Anmo, SKD-1, and TJ-83 (Fig. 1).

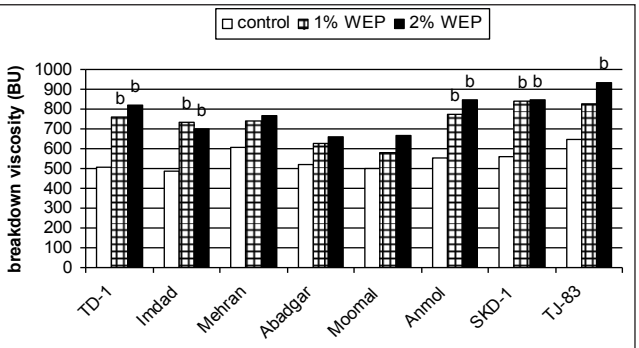

Fig. 1. The eEffect of water-extractable pentosan (WEP) on breakdown viscosity of flour from different wheat cultivars. Bars labeled 'b' indicate a significant difference from the control at $P < 0.05$.

The effect of WEP concentration. An increase in WEP concentration did increase the BD values of all flours, but the extent of the increase varied from cultivar to cultivar. The magnitude of increase (in terms of percentage) in BD viscosities of different cultivar flours with the substitution of WEP is presented in Fig. 2. At a 1% concentration, the increase in BD varied between 16% and 51% of the BD value of control flours. The BD values further increased and reached 63% (maximum) of the BD value of control flours when WEP was added up to the 2% level.

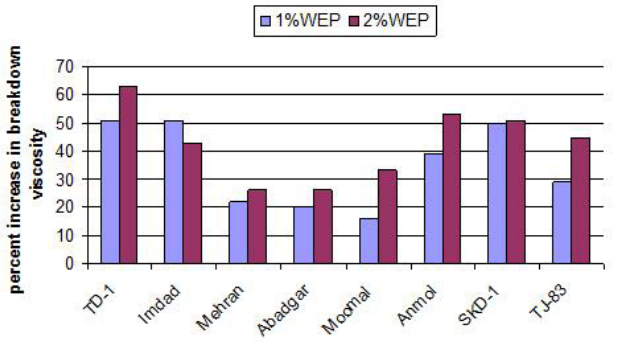

Fig. 2. Percent increase in breakdown viscosity values of different cultivar flours due to the addition of water-extractable pentosan (WEP).

Cultivar differences in the breakdown viscosities in WEP-substituted flours. Breakdown viscosities in 1% WEP-substituted flours of all cultivar (except SKD-1, TJ-83, and Moomal) varied between 624 and 774BU. Starch granules in the flour of SKD-1 and TJ-83 showed a low tendency against shearing in the presence of WEP (Fig. 3 (left)), whereas the highest tendency to resist shear was in the flour of Moomal. The lowest tendency was exhibited by the starch granules in flour of TJ-83 to resist shear in the presence of 2% WEP (Fig. 3 (right)). The breakdown viscosities of two-percent WEP substituted flours of all other varieties ranged between 660 and 850BU.

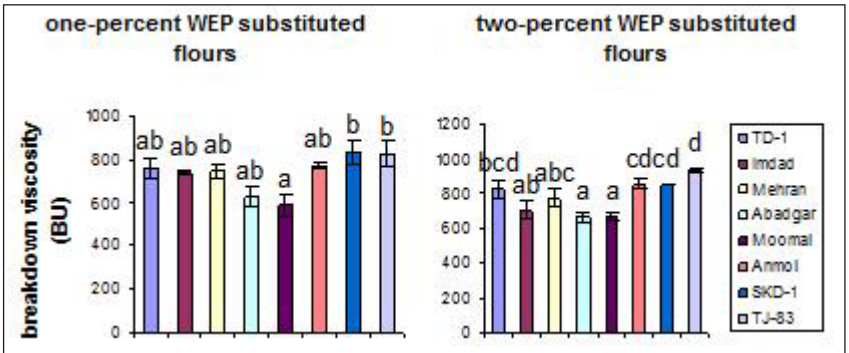

Fig. 3. Breakdown viscosities of water-extractable pentosan (WEP)-substituted flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

The relationship between breakdown viscosity and other pasting parameters in presence of WEP. Correlation coefficients between BD and other pasting parameters in presence of WEP are given in Table 3. We found that WEP did not influence the relationship of BD with other pasting parameters. BD viscosity had a similar pattern of relationship with other pasting parameters in the presence or absence of WEP.

Table 3. Relationships between BD viscosity and other pasting parameters in presence of water-extractable pentosan (WEP).

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Setback
Breakdown viscosity	0.191	0.637**	–0.052	–0.335	–0.350*	–0.264

Reference.

Singh S, Gupta AK, Gupta SK, and Kaur N. 2010. Effect of sowing time on protein quality and starch pasting characteristics in wheat (*Triticum aestivum* L.) genotypes grown under irrigated and rain-fed conditions. Food Chem 122(3):559-565.

Effect of water-extractable pentosan on cold paste viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Awais Rasheed, Alvina Gul Kazi, Abdul Aziz Napar, and Abdul Mujeeb-Kazi.

Cold paste viscosity (CPV) is the viscosity measured after holding the slurry at 50°C for 10 minutes. The value of CPV serves as an indicator of paste stability after cooking. The paste or gel-forming ability of starch after cooling corresponds well through CPV values. Moreover, CPV is a significant attribute in some food-processing operations, such as canning, and predicts the starch property in the preparation of food items such as instant soup, creams, and sauces that require cold thickening capacity. All cultivar flours varied in their CPV ranging between 865 BU to 1187 BU. The differences among cultivars in CPV might be due to different flour composition.

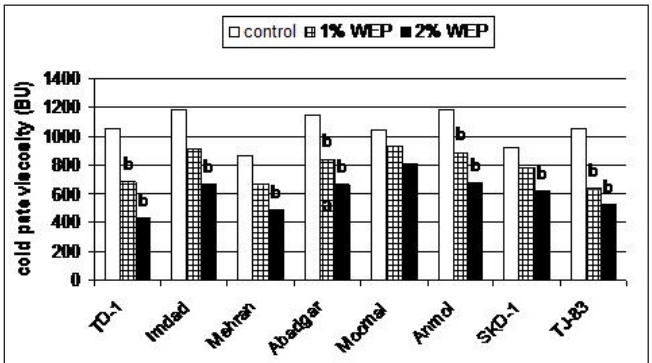

Fig. 4. Effect of water-extractable pentosan (WEP) on cold paste viscosity of flour from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

The addition of water-extractable pentosan (WEP) to flour induced significant reduction in CPV of all cultivar flours (Fig. 4), which may be a result of hydrophilic nature of WEP that weakens the gelling tendency of wheat flour possibly by hindering the reassociation of amylose molecules while they aggregate on cooling. Rao et al. (2007) reported an increase in CPV values when the water-soluble, nonstarch polysaccharides obtained from rice and ragi were added at 0.5% to a wheat flour sample. This is in contrast to present study in which pentosans were isolated from a hard-type wheat flour and were substituted at 1% and 2% into eight different genotypes of hard white spring wheats. The differences in the pentosan source, the supplementation level and the number and quality of wheat flour samples analyzed might be the possible reasons for contrary results.

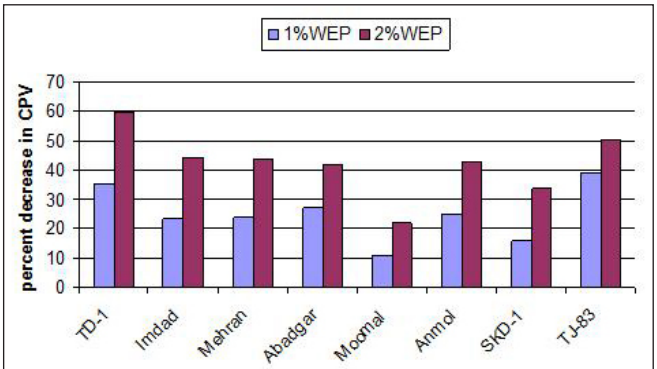

Fig. 5. Percent decrease in cold past viscosity of different cultivar flours due to the addition of water-extractable pentosan (WEP).

Effect of WEP concentration. The concentration of WEP did not influence the type of effect because CPV decreased with the substitution of WEP but the magnitude of decrease was found to be variable depending on the genotype of wheat flour. The decrease in CPV was estimated in terms of percent decrease from their control (Fig. 5). The CPV of all cultivar flours was nonlinearly

reduced with increasing concentration of WEP. The reduction varied from cultivar to cultivar at the same concentration level. At a concentration of 1%, the reduction varied between 11% and 39% with the highest reduction in CPV in the culivar TJ-83. A further decrease (22–60%) in CPV was found when the WEP concentration was increased upto 2%. At a concentration of 2%, the highest decrease was found in the CPV of cultivar TD-1, followed by TJ-83. The reduction with increasing WEP concentration of WEP showed further weakening in paste stability after cooking. A greater amount of WEP molecules could more strongly hinder the reassociation of amylose molecules upon cooling resulting in the further reduction in CPV.

Relationship between CPV and other pasting parameters in the presence of WEP. The Pearson's correlation coefficients between CPV and other pasting parameters in presence of WEP are given in Table 4. We found that CPV related in a similar fashion with other pasting parameters in the presence or absence of WEP.

Table 4. Relationship between cold pasting viscosity and other pasting parameters in presence of water-extractable pentosan.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Breakdown	Setback
Cold paste viscosity	–0.234	0.426*	0.517**	0.910**	–0.350*	0.883**

Cultivar differences in CPV of WEP-substituted flours. A wide range (638-937BU) of cold paste viscosities was observed in one-percent WEP substituted flours (Fig. 6). The least and most viscous cold paste was formed with the flours of varieties TJ-83 and Moomal respectively. Amongst two-percent WEP substituted flours, all varieties had viscosities varied insignificantly between 426 and 816BU. The most viscous cold paste belonged to the variety of Moomal.

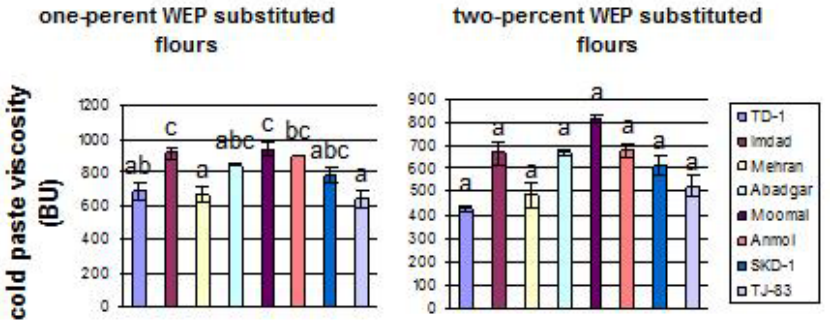

Fig. 6. Cold paste viscosities of water-extractable pentosan (WEP)-substituted flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

Reference.

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-LEB 40(10):1678-1686.

Effect of water-extractable pentosan on hot paste viscosity of some wheat cultivars with with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Awais Rasheed, Alvina Gul Kazi, Hadi Bux, and Abdul Mujeeb-Kazi.

Hot paste viscosity (HPV) is defined as the viscosity measured after a holding period of 10 minutes at 95°C. Holding a wheat starch slurry at 95° C leads to a reduction in pasting viscosity, because it is subjected to mechanical stress during this isothermal phase that eventually results in the rupture of swollen starch granules that are responsible for viscosity development. Hot paste viscosity of flours of all cultivars (except Mehran) was found to range between 510BU to 671BU. The hot paste of flour of Mehran had an exceptionally low viscosity (351BU).

The addition of water-extractable pentosan (WEP) at both levels of supplementation induced a significant reduction in HPV of all cultivar flours (Fig. 7, p. 139), which may be a result of the hydrophilic nature of WEP that increases the rupturing tendency among starch granules during the isothermal phase or under induced mechanical stress while they are suspended in flour. Rao et al. (2007) reported an increase in HPV values when the water-soluble, nonstarch polysaccharides obtained from rice and ragi were added at 0.5% to a wheat flour sample. In our study, pentosans were isolated from a hard-type wheat flour and were substituted at 1% and 2% levels in to eight different hard white spring wheat

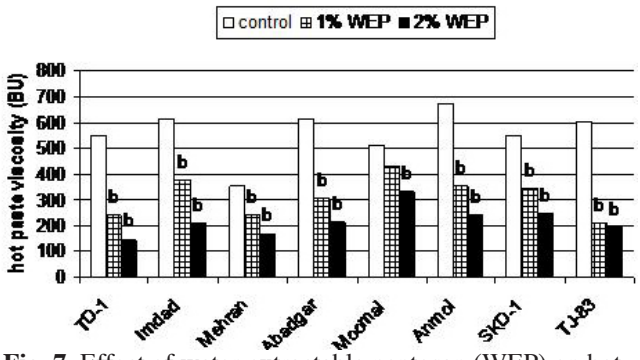

Fig. 7. Effect of water-extractable pentosan (WEP) on hot paste viscosity of flour from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

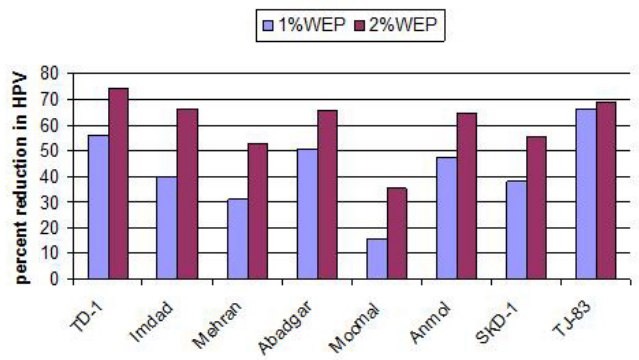

Fig. 8. Percent decrease in hot past viscosity of different cultivar flours due to the addition of water-extractable pentosan (WEP).

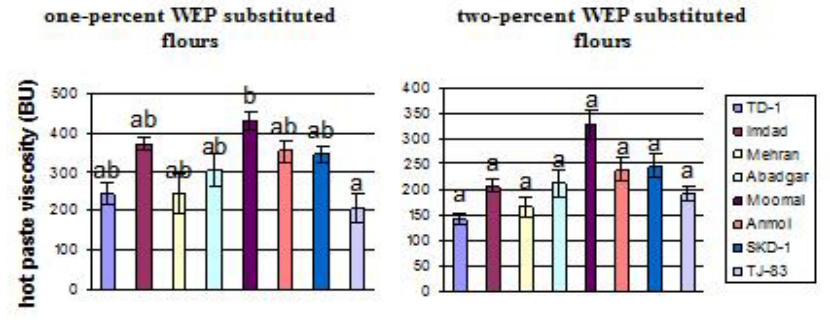

Fig. 9. Hot paste viscosities of water-extractable pentosan (WEP)-substituted flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

genotypes. The differences in pentosan source, supplementation level, and the number and quality of wheat flour samples analyzed might be the possible reasons for different results.

The type of WEP effect, that is, decreasing the viscosity, on HPV was found to be same for all cultivars, but the magnitude of WEP varied from cultivar to cultivar.

Effect of WEP concentration. The HPV of all flours was found to be inversely related with the concentration of WEP. However, HPV of all cultivars reduced nonlinearly with increasing concentration of WEP. The decrease in HPV was larger from control to 1% WEP (79-398BU) than from 1% to 2% (16-165BU) in all cultivars except Moomal. Moomal flour was the least affected having the lowest reduction observed.

The magnitude of reduction in terms of percent decrease upon the addition of 1% and 2% of WEP to flours of each cultivar is in Fig. 8. In terms of percentage, the reduction in HPV ranged between 15% and 66% at 1% concentration, and a further reduction noticed when WEP concentration increased to 2%. At a concentration of 1%, the highest reduction was observed in TJ-83 flour, followed by TD-1 and Abadgar, with the percent decrease of 66%, 56%, and 51%, respectively. Further reductions (35–74%) were found with 2% WEP; the maximum reduction in the HPV of TD-1 flour.

Cultivar differences in the HPV of WEP-substituted flours. The hot paste of 1% WEP-substituted flours of all cultivars exhibited viscosities ranging between 206 and 431 BU (Fig. 9). The least and most viscous hot pastes belonged to cultivars TJ-83 and Moomal, respectively. Hot paste viscosities of 2% WEP-substituted flours varied between 142 and 330BU, however, the differences were not statistically significant.

Relationship between HPV and other pasting parameters in presence of WEP. Similar to the control flours, HPV strongly and positively corelated with CPV upon the addition of WEP in flour (Table 5). However, HPV was moderately related with setback, peak viscosity, and time to reach peak viscosity and weakly related with pasting temperature and breakdown viscosity.

Table 5. Relationship between hot pasting viscosity and other pasting parameters in presence of water-extractable pentosan.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Cold paste viscosity	Breakdown	Setback
Hot paste viscosity	–0.370*	0.512**	0.453**	0.910**	–0.335	0.698**

Reference.
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-LEB 40(10):1678-1686.

Effect of water-extractable pentosan on the pasting temperature of some wheat cultivars with with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Awais Rasheed, Alvina Gul Kazi, Hadi Bux, 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 a specific temperature in the presence of excess water. The pasting temperatures of flours from eight different wheat cultivars (TD-1, Imdad, Mehran, Abadgar, Moomal, Anmol, SKD-1, and TJ-83) were determined using a viscoamylograph. Pasting temperatures of these cultivars were found to be narrow, ranging from 56.8–59.9°C. The highest temperature to gelatinize the starch was in flour of Abadgar, followed by those of cultivars SKD-1 and TJ-83.

Water-extractable pentosan (WEP) was found to marginally increase the pasting temperature of all cultivar flours except those of Abadgar and SKD-1 (Fig. 10). The shift in PT was found not statistically significant in any cultivar. In the presence of WEP, the onset of the pasting process took place earlier (at lower temperatures) in Abadgar and SKD-1 flour and was delayed (took place at higher temperatures) in all the other cultivar flours. WEP facilitates the process of granule swelling–amylose leaching in the flours of Abadgar and SKD-1, but also hindered the same process, resulting in a delay in pasting because they required higher temperature for onset.

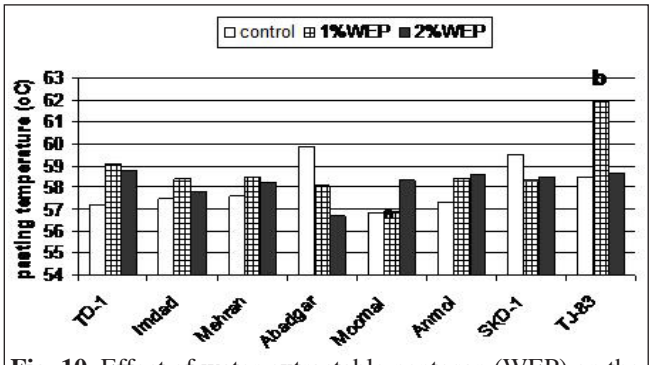

Fig. 10. Effect of water-extractable pentosan (WEP) on the pasting temperature of different hard white spring wheat flours from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

Effect of WEP concentration. WEP, substituted at 1% and 2% were studied to reveal their effect on PT in the different cultivar flours. Results showed that the concentration of WEP did not influence the type of effect (whether increasing or decreasing) but induced variable changes in the magnitude of the effect on PT on the cultivar.

The PT of all cultivars (except two) was delayed in the presence of WEP up to the 2% level (Fig. 11). The magnitude of increase (0.2–0.6%) or decrease (1.7–5.3%) varied from cultivar to cultivar at the same WEP concentration. The maximum increase at 1% was in TJ-83 flour and maximum decrease at 2% was in Abadgar flour.

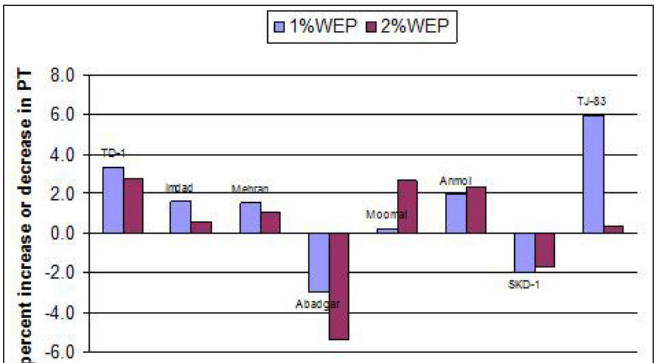

Fig. 11. Magnitude (% increase or decrease) of the effect of water-extractable pentosan (WEP) on the pasting temperature of different hard white spring wheat flours.

Relationship between PT and other pasting parameters in presence of WEP. Pasting temperature was found to relate differently with other pasting parameters upon the addition of WEP to flour (Table 6). Pasting properties of flour are different in the presence of WEP. A moderately negative correlation was found between PT and hot paste viscosity in the presence

Table 6. Relationship between pasting temperature and other pasting parameters in presence of water-extractable pentosan.

	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Breakdown	Setback
Pasting temperature	–0.129	0.088	–0.370*	–0.234	0.191	0.061

of WEP. With other pasting parameters, there was no linear correlation.

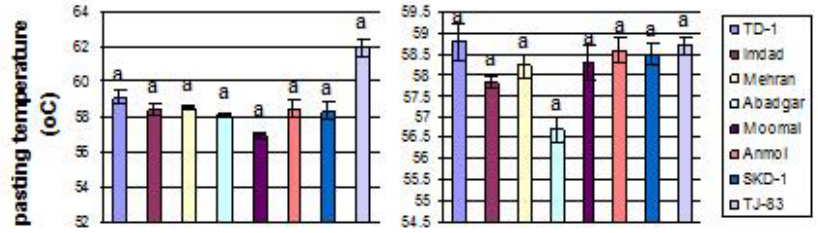

Fig. 12. Pasting temperatures of water-extractable pentosan (WEP)-substituted, hard white spring wheat flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

Cultivar differences in the PT of WEP-substituted flours. The pasting temperature of WEP-substitute flours from different cultivars at the same supplementation level was not found to be statistically different (Fig. 12). The pasting temperatures of 1% WEP-substituted flours ranged between 56.9° and 62°C, whereas 2% WEP varied from 56.7° to 58.8C. The greatest temperature needed to trigger the starch swelling–amylose leaching process was in TJ-83 flour in the presence of 1% WEP. In the 2% WEP-substituted flours, the lowest temperature needed to gelatinize starch granules was in the flour of Abadgar and the highest in TD-1 flour. The pasting temperature of flour of Anmol, SKD-1, and TJ-83 were only 2°C, 3°C, and 1°C, respectively, lower than that of TD-1.

Effect of water-extractable pentosan on peak viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Awais Rasheed, Alvina Gul Kazi, Hadi Bux, and Abdul Mujeeb-Kazi.

Peak viscosity (PV) is one of the most important pasting attributes that is useful for distinguishing starch properties. Peak viscosity is the equilibrium point between swelling and rupture of starch granules (Newport Scientific 1995). The viscosity of a wheat flour slurry reaches peak when there is the maximum number of swollen intact granules present. The value of PV corresponds to the extent of granule swelling. The PV value of the flour from different cultivars varied between 962 and 1,252 BU. The highest PV was in the flour of TJ-83, followed by Anmol and Abadgar. The flour of these cultivars could be a good thickening agent. The least viscous flour was found in the cultivar Mehran.

Water-extractable pentosan (WEP) appears to be one of the significant sources of variation in PV of hard white spring wheat flours. Yin and Walker (1992) found a significant affect of pentosan on RVA peak viscosity of starch and reported that the PV of starch decreased with the addition of WEP. They used only one commercial starch sample, whereas our study used flour samples from several cultivars. Moreover, the pasting properties of starch may not necessarily correspond to that of flour because differences have been found in the PV pattern of starch and flour from same wheat (Lin and Czuchajowska et al. 1997). In another study, the increase in the PV of wheat flour was reported with the addition of water-soluble, nonstarch polysaccharide from other sources, such as rice and ragi (Rao et al. 2007). In our study, the pentosans were isolated from wheat flour. Hence, slight differences in results would be due to different analytical techniques for pasting properties, the nature of the sample, the pentosan source and isolation procedure, and the number of samples analyzed. We found that WEP, at two levels of supplementation, made insignificant changes in the PV of each cultivar, except Abadgar at the 2% level (Fig. 13).

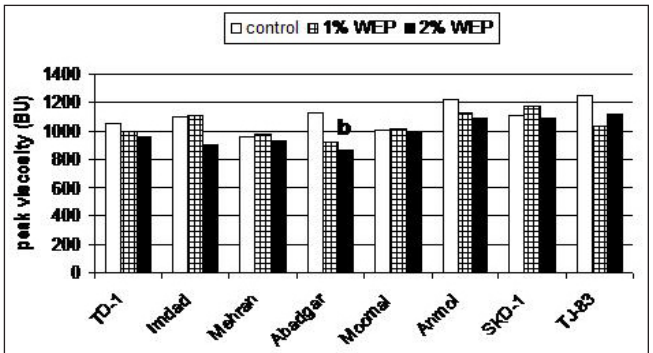

Fig. 13. Effect of water-extractable pentosan (WEP) on the peak viscosity of the flour from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

Effect of WEP concentration. An increasing concentration of WEP did not impart a uniform influence on the PV of all flours. WEP concentration did affect the PV, varied in type and magnitude, and largely depended on the genotype of the wheat flour. However, the addition of WEP at a concentration of 2% reduced the PV of all cultivar flours between 1.6%

and 23.3% (Fig. 14). The maximum decrease in PV was found in the flour of Abadgar.

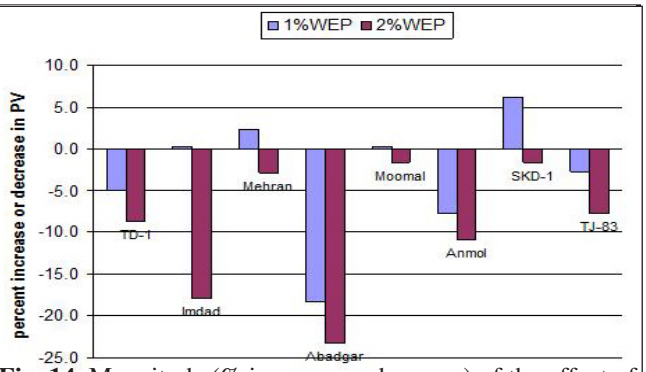

Fig. 14. Magnitude (% increase or decrease) of the effect of water-extractable pentosan (WEP) on the peak viscosity of different hard white spring wheat flours.

Varietal differences in PV of WEP-substituted flours. The peak viscosity exhibited by 1% WEP-substituted flours varied between 926 and 1,179 BU (Fig. 15). However, differences in PV among cultivars were not found to be statistically significant. With 2% WEP-substituted flours, the highest PV, 1,123 BU, was in TJ-83 flour and the lowest, 870 BU, was in Abadgar flour.

Relationship between PV and other pasting parameters in presence of WEP. A moderate relationship was found between PV and other pasting parameters with the addition of WEP in flour. Peak viscosity was positively correlated with hot paste viscosity (r = 0.512**), cold past viscosity (r = 0.426**), breakdown (r = 0.637**), and setback (r = 0.331).

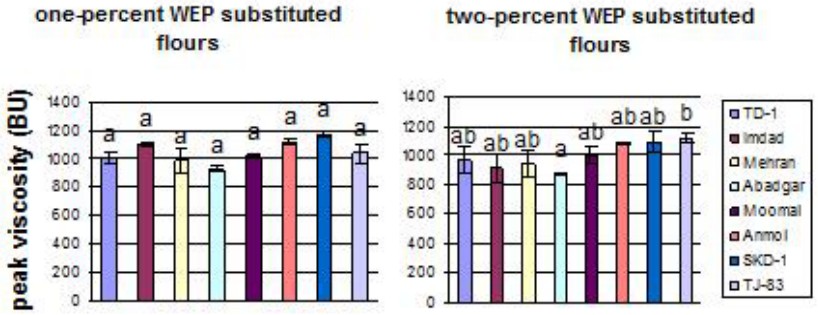

Fig. 15. Peak viscosity of water-extractable pentosan (WEP)-substituted, hard white spring wheat flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

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Lin P-Y, and Czuchajowska Z. 1997. Starch properties and stability of club and soft white winter wheats from the pacific northwest of the United States. Cereal Chem 74(5):639-646.

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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-LEB 40(10):1678-1686.

Effect of water-extractable pentosan on setback viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Awais Rasheed, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Setback viscosity (SB) measures the retrogradation tendency of starch granules (Abd Karim et al. 2000). After gelatinization, the leached out linear amylose chains start reassociating with each other on cooling, which subsequently results in increased 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). The setback value of all cultivar flours (except Imdad) varied between 459 and 571 BU. Flour of Imdad had an SB viscosity of 641 BU, exceptionally higher than that of all other cultivars.

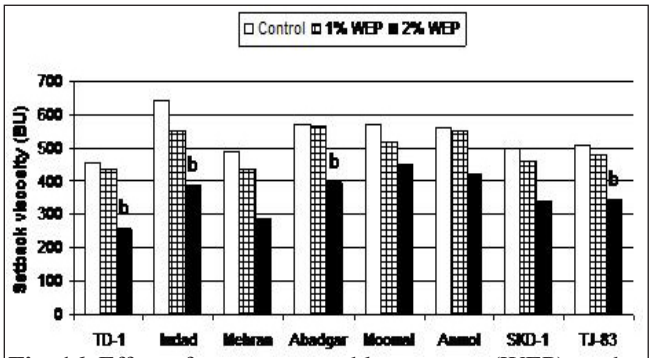

Fig. 16. Effect of water-extractable pentosan (WEP) on the setback viscosity of the flour from different wheat cultivars. Bars labeled with a 'b' indicate a significant difference from the control at $P < 0.05$.

The addition of water-extractable pentosan (WEP) reduced the SB viscosity of all cultivar flours (Fig. 16). Because WEP is hydrophilic in nature, bound water molecules may interact with solubilized amylose chains

and curtail the tendency of the amylose chains to reassociate. Based on low firmness values, previous reports indicate that pentosan reduces starch retrogradation and bread staling (Kim & D'Appolonia 1997 a, b; Jankiewicz and Michniewicz 1987). The addition of 1% WEP did not significantly reduce SB values; a significant reduction was found in TD-1, Imdad, Abadgar, and TJ-83 at 2% WEP.

Effect of WEP concentration. The concentration of WEP did influence the magnitude of the reduction in SB values. The extent of reduction was higher at 2% WEP compared to 1% (Fig. 17). The reduction in SB values was found to vary from cultivar to cultivar at same level of WEP supplementation. At a 1% concentration level, the reduction in setback value varied between 2% and 14%. The reduction degree increased with increasing pentosan concentration (2% level) and the setback values reduced up to 44%. Further decreases in setback viscosity were possibly because WEP reduces the amount of starch components available for crystallization. The maximum reduction in setback value was found in the flour of TD-1, followed by Mehran and Imdad. Differences in flour composition may have causede this, because flour components, including gluten, pentosans, and lipids, are involved in bread staling (Martin et al. 1991). The flours used in this study were found to have different compositions and differences in setback values were found among the cultivars. Moreover, setback viscosity largely depends on the amylose content of starch. Variation in the amylose-to-amylopectin ratio also could be responsible for the differences in setback viscosities of flour and their subsequent interaction with WEP.

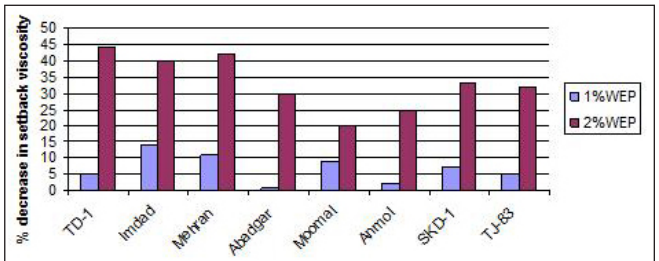

Fig. 17. Percent decrease in setback viscosity of the flour from different wheat cultivars after the addition of water-extractable pentosan (WEP).

Varietal differences in setback viscosity of WEP-substituted flours. No statistically significant differences were found among the 1% and 2% WEP-substituted flours. We found a narrow range in the setback viscosity (438–567) in 1% WEP-substituted flours (Fig. 18). The least retrograded flours were those of TD-1 and Mehran and the highest setback viscosities were found in the flour of Abadgar. All setback viscosities of the 2% WEP-substituted flours were between 258 and 454 BU. The least and most retrograded flours were in the flour of TD-1 and Moomal, respectively.

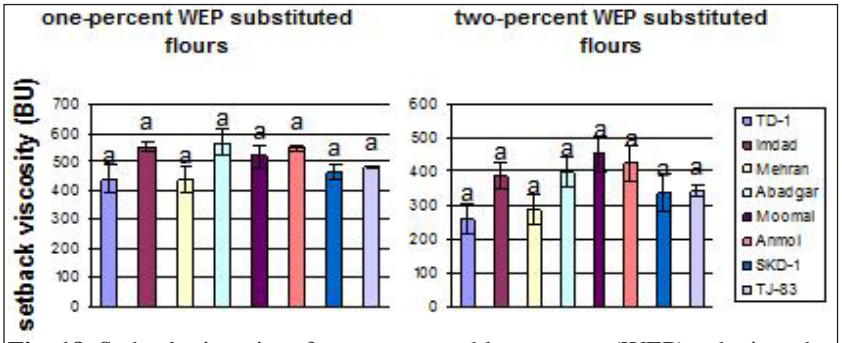

Fig. 18. Setback viscosity of water-extractable pentosan (WEP)-substituted flour from different wheat cultivars. Bars labeled with the same letter are not significantly different at $P < 0.05$.

Relationship between setback viscosity and other pasting parameters in presence of WEP. The relationship between SB viscosity and other pasting parameters in WEP-substituted flours are given (Table 7). Setback value was positively related with cold past viscosity, hot paste viscosity, time to reach peak viscosity, and peak viscosity, but negatively correlated with breakdown viscosity. Setback did not relate with PT; SB viscosity related more with other pasting parameters in the presence of WEP.

Table 7. Relationship between setback viscosity and other pasting parameters in presence of water-extractable pentosan.

	Pasting temperature	Peak viscosity	Time to reach peak viscosity	Hot paste viscosity	Cold paste viscosity	Breakdown viscosity
Setback viscosity	0.061	0.331	0.530*	0.698**	0.883**	–0.264

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Effect of water-extractable pentosan on time to reach peak viscosity of some wheat cultivars with high-molecular-weight glutenin subunits 5+10.

Qurrat ul ain Afzal, Saqib Arif, Tahira Mohsin Ali, Mubarik Ahmed, Abid Hasnain, Akhlaq Ahmed, Awais Rasheed, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Time to reach peak viscosity (TPV) is an indication of the time required for cooking. Flours of all the cultivars, except that of SKD-1, were found to require more than 15 min to reach their maximum viscosity. Flour of SKD-1 took 13:50 min to reach its maximum viscosity. The less time required to reach peak viscosity indicates that more energy is required to gelatinize starch granules, which ultimately decreases energy costs. Thus, SKD-1 flour may give a lower energy cost compared to the other flours.

Water-extractable pentosan (WEP) was found to be one of the significant sources of variation in the TPV of wheat flour. However, WEP did not induce a significant change in the TPV of all the cultivars tested (Fig. 19). The insignificant influence of WEP on peak viscosity confirmed the weak interference of WEP in the process of granule swelling, which may be the reason that the time needed by flour paste to gain the maximum number of swollen granules did not shift drastically with the addition of WEP.

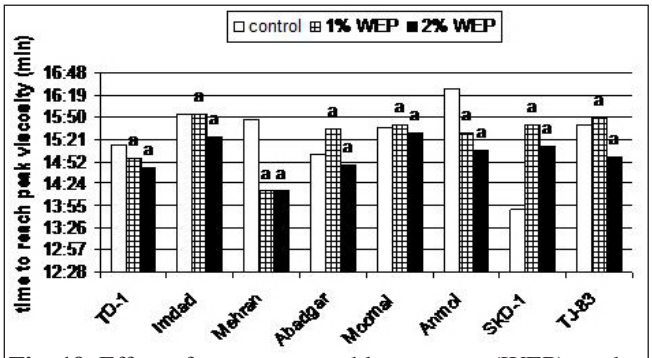

Fig. 19. Effect of water-extractable pentosan (WEP) on the time to reach peak viscosity of the flour from different hard white spring wheat cultivars. Bars labeled with an 'a' are not significantly different from the control at $P < 0.05$.

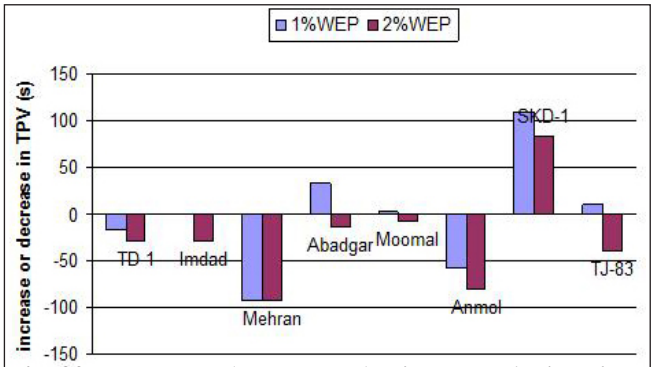

Fig. 20. Increase or decrease on the time to peak viscosity of the effect of water-extractable pentosan (WEP) on different hard white spring wheat flours.

Effect of WEP concentration. The difference between TPV (in seconds) in WEP-substituted flours (1% and 2%) and control flours is shown in Fig. 20. The addition of 1% and 2% WEP to flour induced similar effects, whether an increase or decrease) on the TPV of TD-1, Mehran, Anmol, and SKD-1. However, the difference in magnitude was not similar among all the cultivars except Mehran. At a 2% supplementation level, the TPV of all cultivars, except SKD-1, decreased from the control; the maximum reduction was found in the flour of Mehran.

Varietal differences in TPV of WEP substituted flours. The time for 1% WEP-substituted flours to reach their maximum viscosities ranged between 14:15 and 15:55 min (Fig. 21). The shortest time was in the flour of Mehran

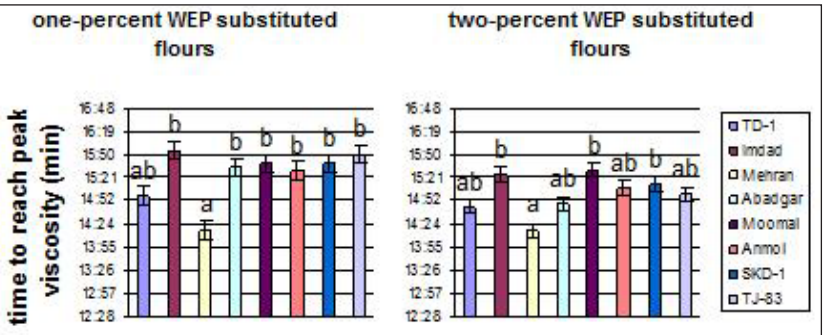

Fig. 21. Time to reach peak viscosity of water-extractable pentosan (WEP)-substituted flours. Bars labeled with the same letter are not significantly different at $P < 0.05$.

and the longest in the flour of Imdad. At 2% WEP-substituted flours, the TPV for all cultivars was 14:15–15:30 min. The shortest time was in the flour of Mehran and the longest in the flour of Moomal.

Relationship between TPV and other pasting parameters in presence of WEP. WhenWEP is added to flour, the TPV was found to have a positive effect on all pasting parameters except breakdown (r = –0.052). The relationship between TPV with hot paste viscosity (r = 0.453**), cold paste viscosity (r = 0.517**), and setback (r = 0.532**) were statistically significant.

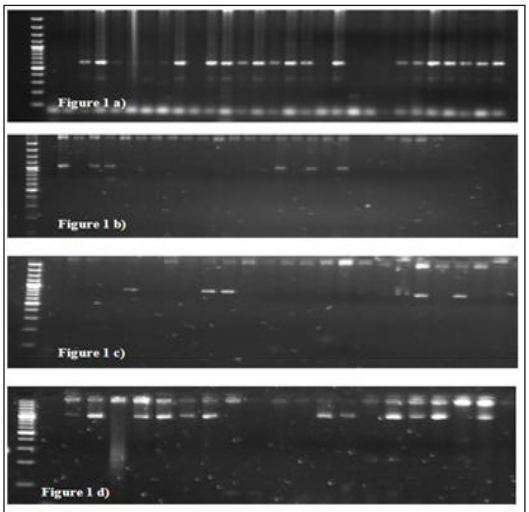

Fig. 22. Amplicons based on STS markers for low-molecular-weight glutenin subunit alleles, a) *Glu-A3c*, 573bp; b) *Glu-B3h*, 1022 bp; c) *Glu-B3g*, 853 bp; and d) *Glu-A3d*, 967bp.

Identification of allelic variation at the Glu-A3 and Glu-B3 loci using allele-specific PCR markers.

Mah-Jabeen Tariq, Kausar Nawaz Shah, Awais Rasheed, Alvina Gul Kazi, and Abdul Mujeeb-Kazi.

Glutenin proteins are the major factors responsible for the unique viscoelastic characteristics of wheat dough. They determine rheological properties and bread-making performance. The polymeric glutenin proteins, with molecular weights ranging from less than 300 to more than 1,000 kDa, are composed of two groups of subunits. These subunits include the low-molecular-weight glutenin subunits (LMW-GS), which are similar in size and structure to the γ- gliadins (30–40 kDa), and the high-molecular-weight glutenin subunits (HMW-GS), which range in molecular mass from ~65 to 90 kDa. The LMW-GS represent about one-third of the total seed protein and ~60% of the total glutenins, and are essential in determining dough properties, such as dough extensibility and gluten strength. Hence, characterizing allelic variation among cultivars and investigating their relationship with end-use quality has been a key area of research on quality improvement during the last 15 years and is the basis for the success of using specific LMW-GS alleles in breeding programs. Various methods are available for detecting LMW-GS in wheat, including SDS-PAGE, 2-D gel electrophoresis, MALDI-TOF-MS, and PCR-based identification of alleles. In this study, allele-specific, PCR markers developed by Wang et al. (2009; 2010) were used to characterize the LMW-GS composition in 27 wheat cultivars (Fig. 22). The presence of various alleles of *Glu-A3* and *Glu-B3* is given in Table 8.

Table 8. *Glu-A3* and *Glu-B3* alleles detected in wheat genotypes using STS markers.

Genotype	*Glu-A3*	*Glu-B3*
1x2-46	d	h
1x2-99	c	h
1x2-121	d	h
12x2-11	c	h
12x2-29	d	h
12x2-163	c	h
LLR-30	c	i
LLR-32	c	d
LLR-33	c	d
LLR-36	c	d
LLR-38	c	i
Bhakkar-2000	d	g
Chakwal-50	c	i
Miraj-2008	c	g
Abadghar-93	c	–
Seher-2006	c	–
Parvaz-94	d	–
Bhittai	d	–
Inqilab-91	g	–
Jauhar-78	g	–
Kirman	d	–
Zindad-2000	g	–
Shafaq	c	–
Faislabad-2008	c	g
Zamindar-80	d	i
Lasani	b	g
Pak-81	b	h

References.

Wang LH, Li GY, Peña RJ, Xia XC, and He ZH. 2010. Development of STS markers and establishment of multiplex PCR for *Glu-A3* alleles in common wheat (*Triticum aestivum* L.). J Cereal Sci 51:305-312.

Wang LH, Zhao XL, He ZH, Ma W, Appels R, Peña RJ, and Xia XC. 2009. Characterization of low-molecular-weight glutenin subunit *Glu-B3* genes and development of STS markers in common wheat (*Triticum aestivum* L.). Theor Appl Genet 118:525-539.

Tolerance to boron toxicity in synthetic hexaploid wheats.

Mohammad Ilyas, Tariq Mahmood, Awais Rasheed, Alvina Gul-Kazi, and Abdul Mujeeb-Kazi.

Wheat is one of the most important food crops of the world, supporting 35% of the world population, but production is limited by several abiotic stress factors. Boron toxicity can cause serious loss in wheat production in different parts of the world. In synthetic hexaploid wheats (SH), the D-genome donor is one source for bringing in genetic variability in to common, hexaploid wheat. Forty-five SH wheats were screened for their tolerance to boron toxicity at seedling and adult stages. At the seedling stage, genotypes were screened in two different boron treatments; BO1 at 0.01M and BO2 at 0.025M along with a control using filter paper. Root growth suppression was expressed as a percent of the control was used as the selection criterion for tolerance to boron toxicity. The RGS % varied between 15.61% for SH-113 and 87.45% for SH-447. In addition to the RGS %, shoot growth suppression (SGS), expressed as a percent of the control, also was measured and varied between 1.84% for SH-117 and 94.74% for SH-385. The SGS % data further confirmed the results from the RGS % data for tolerant genotypes. At the adult-plant stage, genotypes were screened in a soil-assay experiment. Two different boron treatments, BO1 (25 mg/kg) and BO2 (50 mg/kg), along with control (no boron), were used and symptom data were taken 45 days after sowing. Using RGS %, SGS %, and symptom data, the plants were divided into four groups, tolerant, moderately tolerant, moderately susceptible, and susceptible. Genetic diversity for the respective genotypes was calculated using 10 SSR primers specific to 7D chromosome. The data was further subjected to cluster analysis using the NTSYS program. Simple sequence repeat primers amplified a total of 38 alleles with an average of 3.8 allele/primer. The polymorphism information content value was calculated and ranged from 0.34 (BARC214) to 0.69 (BARC53) with an average of 0.56. The nutritional quality of the respective genotypes was assessed by calculating iron (Fe) and Zinc (Zn) content for tolerant genotypes using an atomic absorption spectrophotometer, in order to use tolerant genotypes for breeding purposes without compromising on nutritional quality. A high level of variability was found among genotypes in response to different boron treatments at the seedling stage, adult-plant stage, and the molecular level. We concluded that genotypes SH-505, SH-380, SH-117, SH-131, SH-361, and SH-618 have the best potential for tolerance to boron toxicity that can be used in breeding program.

High-molecular-weight glutenin subunit composition of synthetic hexaploids derived from the durum wheat cultivar Decoy.

Madiha Khalid, Tariq Mahmood, Awais Rasheed, Alvina Gul-Kazi, and Abdul Mujeeb-Kazi.

Characterizing high-molecular-weight glutenin subunits is a fundamental approach for categorizing genotypes with good bread-making quality. Allelic variation at the *Glu-D^{1}1* locus is major determinant of end-use quality of bread wheat. In synthetic hexaploids (SHs), the D genome encodes numerous allelic variants of HMW-GS that require appropriate identification prior to their exploitation for bread wheat improvement. This study was conducted to identify allelic variation at *Glu-D^{1}1* loci of 47 accessions of D-genome SHs derived from crossing durum wheat Decoy with different accessions of *Ae. tauschii*. Biochemical (SDS-PAGE) and molecular-marker techniques were used to identify allelic variation at *Glu-D^{1}1* locus (Table 9, p. 147). Ten different alleles at *Glu-D^{1}1* were observed, which formed 16 different subunit combinations. The frequency of inferior quality encoding allele, 1Dx2+1Dy12, is low (19.14%) compared to the frequency of superior quality encoding allele, 1Dx5+1Dy10, (21.27%). A large allelic diversity was observed at *Glu-D^{1}1* with improved frequency of occurrence. This attribute makes the SHs able to be utilized for improving bread-making quality. Codominant molecular markers were used to validate the *Glu-A1c* (null), *Glu-D1d* (1Dx5+1Dy10), *Glu-D1a* (1Dx2+1Dy12), and *Glu-D1-1g* (1Dx2.1) alleles. The high number of glutenin subunits observed in the SHs indicated narrow genetic base for the D-genome-encoding glutenin subunits in bread wheat, which can be broaden by using synthetic hexaploids and inferior alleles in the D genome can be replaced with other better allelic variants from *Glu-D1* locus of SHs that are inherited from *Ae. tauschii*.

Table 9. Allelic identification and nomenclature of *Glu-D1* alleles in 47 sunthetic hexaploid (SH) wheats derived from Decoy durum wheat.

SH line	*Glu-D1*	Alleles
SH-6	2.1 + 10.5	*Glu-D1ai*
SH-43	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-115	1.5 + 10.5	*Glu-D1-1l + Glu-D1-2p*
SH-117	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-123	1.5 + 10.5	*Glu-D1-1l + Glu-D1-2p*
SH-128	1.5 + 10	*Glu-D1ah*
SH-129	1.5 + 12/10	*Glu-D1ah/Glu-D1aj*
SH-131	5 + 10	*Glu-D1d*
SH-302	3/4 + 10	*Glu-D1z/Glu-D1ac*
SH-323	3 + 12.2	*Glu-D1y*
SH-326	2 + 12.2	*Glu-D1x*
SH-330	2 + 12.2	*Glu-D1x*
SH-341	1.5 + 10.5	*Glu-D1-1l + Glu-D1-2p*
SH-349	5 + 10	*Glu-D1d*
SH-361	3 + 10	*Glu-D1z*
SH-363	2 + 10	*Glu-D1e*
SH-373	2 + 12	*Glu-D1a*
SH-377	2 + 12	*Glu-D1a*
SH-380	2.1 + 10.5	*Glu-D1ai*
SH-381	5 + 10	*Glu-D1d*
SH-385	5 + 10	*Glu-D1d*
SH-395	5 + 10	*Glu-D1d*
SH-396	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-398	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-401	2/2.1 + 12.2	*Glu-D1x/Glu-D1ae*
SH-403	1.5 + 10.5	*Glu-D1-1l + Glu-D1-2p*
SH-409	2.1 + 10.5	*Glu-D1-ai*
SH-422	5 + 10	*Glu-D1d*
SH-424	2 + 12	*Glu-D1a*
SH-426	5 + 10	*Glu-D1d*
SH-434	2 + 12	*Glu-D1a*
SH-441	5 + 10	*Glu-D1d*
SH-444	2 + 12	*Glu-D1a*
SH-447	2.1 + 12	*Glu-D1-1g + Glu-D1-2a*
SH-500	1.5 + 10	*Glu-D1ah*
SH-505	3 + 12.2	*Glu-D1y*
SH-509	3 + 12.2	*Glu-D1y*
SH-618	1.5 + 12.2	*Glu-D1ag*
SH-638	2 + 12	*Glu-D1a*
SH-648	2 + 12	*Glu-D1a*
SH-649	1.5 + 12	*Glu-D1aj*
SH-672	2 + 12	*Glu-D1a*
SH-675	1.5 + 12	*Glu-D1aj*
SH-677	2 + 12	*Glu-D1a*
SH-678	2/2.1 + 12	*Glu-D1a/Glu-D1-1g + Glu-D1-2a*
SH-852	5 + 10	*Glu-D1d*
SH-907	5 + 10	*Glu-D1d*

Optimizing a PCR marker-based technique to identify low-molecular-wheat s in wheat.

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Characterizing allelic variation among cultivars and investigating their relationships with end-use quality has been a key area of research for quality improvement during the last 15 years and is the basis for the success of using specific low-molecular-weight glutenin subunit (LMW-GS) alleles in breeding programs. The genes coding for LMW-GS are located on the short arms of homoeologous group-1 chromosomes at the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci. Identification of LMW glutenins through SDS-PAGE does not allow differentiating several of alleles with certainty. On the other hand, 2-D gel electrophoresis is not generally recommended for use in breeding programs, due to its time consuming procedure, high costs, and skill requirements. Recently, a simple, rapid, and sensitive PCR approach has proven to be a very useful tool for identifying LMW-GS composition in common wheat. The PCR markers developed by Wang et al. (2010) for the *Glu-A3* alleles and by Wang et al. (2009) for the *Glu-B3* alleles were optimized in Wheat Wide Crosses and Cytogenetics Lab for their further utilization in identification of alleles in breeding material (Fig. 23, p. 148). The list of cultivars optimized as standards based on earlier literature is given in Table 10.

Table 10. Cultivars used as standards to optimize a marker-assisted selection strategy for *Glu-A3* and *Glu-B3* alleles.

Locus	Allele	Cultivar
Glu-A3	*a*	Chinese Spring
	b	Pavon, King Bird
	c	Waxwing
	d	Pastor
	e	Kiriati
	f	—
	g	Inquilab-91
Glu-B3	*a*	Chinese Spring
	b	Pitic
	c	—
	d	—
	e	—
	f	—
	g	PRL*2/Paston
	h	Pavon, King Bird
	i	Kiriati

References.

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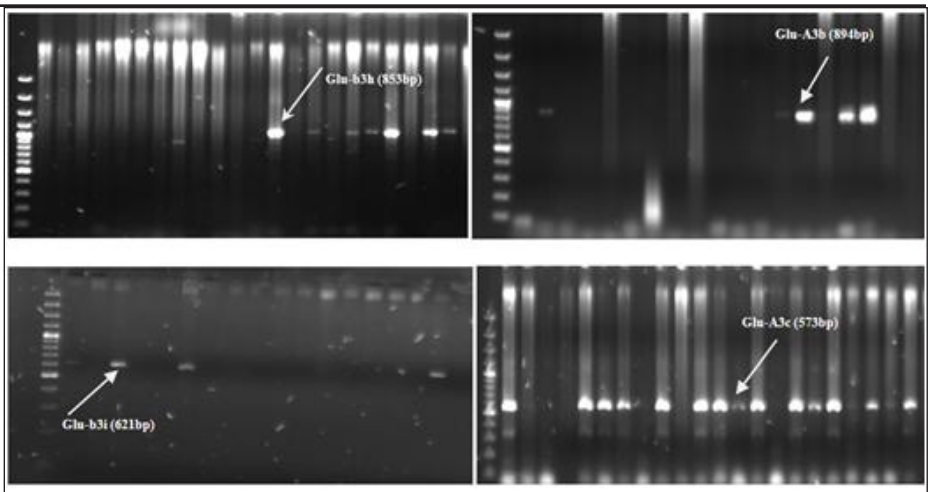

Fig. 23. Identification *Glu-3* alleles in wheat genotypes using allele specific markers for *Glu-B3h*, *Glu-A3b*, *Glu-B3i*, and *Glu-A3c*.

Drought tolerance studies in diverse wheats involving land races, local cultivars, and novel exotic germ plasm.

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Breeding for drought tolerance has been recognized as an important target in Pakistan's wheat improvement because, out of a total cultivated area of 20.9 mha, 4.8 mha (24.4%) is rainfed. To utilize this area efficiently and get high yields, the main emphasis is to develop drought-tolerant wheat cultivars that are able to survive under conditions of limited water. To complement the conventional variability, other options have been identified globally and also within Pakistan, including land races of wheat and species within the Triticeae gene pools. Around this material, conventional wheats, land races, and the progenitor species, we tried to identify sources of for drought, a key abiotic stress drought that leads to improving our wheat cultivars through recombination breeding. The evaluation parameters involved *in vitro* and *in vivo* studies conducted under laboratory, controlled screen houses, and field conditions, and the best drought-tolerant cultivars were identified.

The research material was comprised of 51 entries in three categories; local land races (29), local cultivars (12), and exotic germ plasm (10 genotypes) (Table 11, p. 148-149). This germ plasm was studied and investigated for drought tolerance under *in vivo* and *in vitro* conditions at the National Agriculture Research Centre (NARC), Islamabad, during the November 2009–May 2010 crop cycle. *In vivo* parameters were days-to-heading, days-to-maturity, plant height, spike length, number of grains/spike, and 1,000-kernel weight. *In vitro* parameters on wheat seedlings were proline content, chlorophyll content, protein content, sugar content and superoxide dismutase (SOD) content. For statistical analysis of all field and laboratory data, analysis of variance (ANOVA) was computed by using MINITAB software, and the treatment means were compared by Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) test at a probability level of 0.05 by using MSTATC software.

Table 11. List of selected local Landraces and cultivars of Pakistan and exotic germ plasm (synthetic derivatives) used in the drought studies.

ID	Parent/pedigree	ID	Parent/pedigree
Landraces		**Local cultivars**	
Landrace 1	T1 (*T. durum* subsp. *durum*)	Inqilab 91	
Landrace 2	T2 (*T. durum* subsp. *durum*)	Baviacora	
Landrace 3	T3 (*T. durum* subsp. *durum*)	Opata M85	
Landrace 5	T5 (*T. aestivum* subsp. *sphaeorcoccum*)	Suleman 96	
Landrace 7	T7 (*T. aestivum* subsp. *sphaeorcoccum*)	Sitta	
Landrace 8	T8 (*T. aestivum* subsp. *aestivum*)	Weebill	

Table 11. List of selected local Landraces and cultivars of Pakistan and exotic germ plasm (synthetic derivatives) used in the drought studies.

ID	Parent/pedigree	ID	Parent/pedigree
Landraces		**Local cultivars**	
Landrace 9	T9 (*T. aestivum* subsp. *aestivum*)	Nesser	
Landrace 12	T12 (*T. aestivum* subsp. *aestivum*)	Dharwar	
Landrace 14	T14 (*T. aestivum* subsp. *aestivum*)	Zarghoon	
Landrace 15	T15 (*T. aestivum* subsp. *aestivum*)	Chakwal 86	
Landrace 16	T16 (*T. aestivum* subsp. *aestivum*)	Margalla 99	
Landrace 17	T17 (*T. aestivum* subsp. *aestivum*)	Marwat	
Landrace 18	T18 (*T. aestivum* subsp. *aestivum*)	**Exotic germ plasm**	
Landrace 20	T20 (*T. aestivum* subsp. *aestivum*)	F4 719	SH DR#45/Sehar
Landrace 24	T24 (*T. aestivum* subsp. *aestivum*)	F4 786	S.RIC-62/NR-26
Landrace 26	8A (Selection)	F4 826	DR.MP.1-95/NN(L)R1-4
Landrace 27	D-9 (Barani Selection)	F4 834	L.SEQ.15/N(N)17R1
Landrace 28	C-217 (C-516/C-591)	F4 841	S.RIC-10/NN(L)R2-48
Landrace 29	C-288 (Hard Federation/9D)	F4 883	DR.MP.2-26/NNR1-2
Landrace 30	C-245	F4 922	S.RIC-75/Wafaq
Landrace 31	C-247	F4 925	S.RIC-51/Pastor 68
Landrace 32	C-248 (Lr28, 14A)	F4 1992	F1460 Seq.3/Seq.4-36//Wafaq
Landrace 33	C-250 (Hard Federation/9D) (Lr30, 14A)	F4 2011	F1484 Seq.4-78/IBWSN152//NN(L) R1-8
Landrace 34	C-256 (Lr10, 23, 30)		
Landrace 35	C-258		
Landrace 36	C-269 (Lr2a, 18)		
Landrace 37	C-271(C-220/IP165)		
Landrace 39	C-288		
Landrace 40	C-518 (T9/8A)		

Significant differences were obtained between all field parameters, days-to-heading, days-to-maturity, plant height, spike length, number of grains/spike, and 1,000-kernel weight at $P < 0.05$ for interaction between the control and drought stress of different wheat genotypes along with the genotype, treatment, and replication.

Days to heading significantly decreased in most wheat genotypes under stress conditions compared to the control but increased significantly in other genotypes. In drought conditions, the percent reduction in late-heading genotypes was 0.8% in landrace 15, 5.6% in Chakwal 86, 7.0% in Inqilab-91, 11.0% in landrace 11, and 5.0% in landrace 3 compared to the control (Table 12, p. 150). In the early-heading genotypes, the percent reduction was 1.8% in F4 834, 3.5% in landrace 37, 6.0% in landrace 35, 6.8% in landrace 34, 8.0% in F4 826, and 10.0% in F4 786, compared to the control (Table 12, p. 150). Various studies have already revealed that drought stress decreases the number of days to heading, as we also observed in most wheat genotypes, and this decrease is due to water stress. Heading days increased in F4 841, F4 883, F4 925, F4 2011, and landrace 27 compared to the control. Early heading genotypes also may give higher yields. In our study, genotype F4 2011 had increased heading days and high yield compared to other genotypes.

Days to maturity significantly decreased under stress conditions compared to the control in most wheat genotypes except a few in which it significantly increased. Maximum days to maturity were reduced in landrace 15 (4.8%), Inqilab-91 (9.5%), Baviacora (11.3%), and Opata M 85 (10.2%) under drought condition compared to the control. The minimum number of days to maturity were reductions of 12.0% in Nesser, 12.3% in landrace 33, 16.0% in landrace 39, and 13.8% in landrace 20 compared to control (Table 13, p. 150). Studies on drought stress revealed that it reducs the days to maturity in different wheat cultivars, in sunflower, and in common bean cultivars. Some genotypes had reduction in maturity days were F4 826, F4 1992, and F4 719, whereas late maturity or increase days compared to control were F4 2011, F4 925, F4 922, F4 883, F4 841, and F4 834. These genotypes differed in their response for days to maturity may be because of the different genetic makeup in different wheat cultivars.

The maximum plant height reduction was observed in line F4 2011 (3.9%), F4 841 (7.9%), and landrace 34 (11.4%) under drought condition in comparison to control. Minimum plant height reductions were observed in Opata M 85 (10.3%), Baviacora (16.0%), and Chakwal 86 (38.6%) compared to the control (Table 14, p. 151). These results are supported by other studies indicating that plant height significantly decreased under water stress and is significantly effects wheat cultivars differently.

Table 12. Means for days-to-heading of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 1.610, LSD (0.05) of genotypes (G) = 1.138, and LSD (0.05) of treatments (T) = 0.224, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	112.0 PQ	111.0 QR	111.5 TU	Landrace 7	119.0 IJ	115.0 MN	117.0 JKL
F4 786	119.0 IJ	107.0 UV	113.0 RS	Landrace 8	118.0 JK	116.0 LM	117.0 JKL
F4 826	114.0 NO	104.0 W	109.0 WX	Landrace 9	123.0 F	115.0 MN	119.0 FGH
F4 834	111.0 QR	109.0 ST	110.0 VX	Landrace 12	118.0 JK	113.0 OP	115.5 MNO
F4 841	107.0 UV	110.0 RS	108.5 XY	Landrace 14	120.0 HI	116.0 LM	118.0 HIJ
F4 883	104.0 W	111.0 QR	107.5 Y	Landrace 15	122.0 FG	121.0 GH	121.5 DE
F4 922	115.0 MN	113.0 OP	114.0 PQR	Landrace 16	117.0 KL	112.0 PQ	114.5 OPQ
F4 925	106.0 V	111.0 QR	108.5 XY	Landrace 17	123.0 F	116.0 LM	119.5 FG
F4 1992	115.0 MN	110.0 RS	112.5 ST	Landrace 18	119.0 IJ	117.0 KL	118.0 HIJ
F4 2011	111.0 QR	114.0 NO	112.5 ST	Landrace 20	119.0 IJ	112.0 PQ	115.5 MNO
Inqilab-91	127.0 D	118.0 JK	122.5 CD	Landrace 24	121.0 GH	115.0 MN	118.0 HIJ
Baviacora	120.0 HI	110.0 RS	115.0 NOP	Landrace 26	120.0 HI	117.0 KL	118.5 GHI
Opata M 85	125.0 E	115.0 MN	120.0 F	Landrace 27	115.0 MN	117.0 KL	116.0 LMN
Sitta	122.0 FG	111.0 QR	116.5 KLM	Landrace 28	115.0 MN	112.0 PQ	113.5 QRS
Suleman 96	123.0 F	113.0 OP	118.0 HIJ	Landrace 29	118.0 JK	111.0 QR	114.5 OPQ
Weebill	125.0 E	114.0 NO	119.5 FG	Landrace 30	120.0 HI	116.0 LM	118.0 HIJ
Nesser	122.0 FG	112.0 PQ	117.0 J-L	Landrace 31	118.0 JK	117.0 KL	117.5 IJK
Dharwar	125.0 E	115.0 MN	120.0 F	Landrace 32	122.0 FG	115.0 MN	118.5 GHI
Zarghoon	128.0 D	117.0 KL	122.5 CD	Landrace 33	117.0 KL	111.0 QR	114.0 PQR
Chakwal 86	125.0 E	118.0 JK	121.5 DE	Landrace 34	117.0 KL	109.0 ST	113.0 RS
Margalla 99	128.0 D	110.0 RS	119.0 FGH	Landrace 35	115.0 MN	108.0 TU	111.5 TU
Marwat	127.0 D	116.0 LM	121.2 E	Landrace 36	119.00 IJ	113.00 OP	116.0 LMN
Landrace 1	134.0 B	118.0 JK	126.0 B	Landrace 37	113.0 OP	109.0 ST	111.0 UV
Landrace 2	130.0 C	117.0 KL	123.5 C	Landrace 39	117.0 KL	110.0 RS	113.5 QRS
Landrace 3	143.0 A	121.0 GH	132.0 A	Landrace 40	121.0 GH	112.0 PQ	116.5 KLM
Landrace 5	134.0 B	116.0 LM	125.0 B	Mean	119.9 A	113.4 B	

Table 13. Means for days-to-maturity of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 2.540, LSD (0.05) of genotypes (G) = 1.796, and LSD (0.05) of treatments (T) = 0.353, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	143.6 S-X	143.0 T-Z	143.3 RS	Landrace 7	164.0 B-D	140.3 Y-f	152.1 F-K
F4 786	146.0 Q-T	141.6 W-c	143.8 RS	Landrace 8	164.6 BC	143.6 S-X	154.1 B-F
F4 826	141.3 W-d	141.3 W-d	141.3 TU	Landrace 9	163.6 B-E	144.0 S-X	153.8 B-G
F4 834	140.3 Y-f	141.0 X-e	140.6 U	Landrace 12	158.0 I-M	140.0 Z-g	149.0 M-P
F4 841	139.0 b-h	143.0 T-Z	141.0 U	Landrace 14	160.0 G-K	142.0 N-b	151.0 I-M
F4 883	141.0 X-e	145.3 R-V	143.1 R-T	Landrace 15	161.3 D-H	148.3 PQ	154.8 B-D
F4 922	143.3 S-Y	150.3 P	146.8 Q	Landrace 16	162.3 B-H	138.0 e-h	150.1 K-N
F4 925	138.6 c-h	145.6 Q-U	142.1 S-U	Landrace 17	157.3 J-N	138.6 c-h	148.0 O-Q
F4 1992	150.3 P	150.3 P	150.3 J-N	Landrace 18	163.6 B-E	144.0 S-X	153.8 B-G
F4 2011	142.6 U-Z	153.3 O	148.0 O-Q	Landrace 20	159.3 H-L	137.3 f-i	148.3 N-Q
Inqilab-91	163.3 B-F	147.6 P-R	155.5 AB	Landrace 24	162.3 B-H	141.6 W-c	152.0 G-K
Baviacora	165.0 B	146.3 Q-S	155.6 AB	Landrace 26	160.3 F-J	143.0 T-Z	151.6 H-L
Opata M 85	162.6 B-G	146.0 Q-T	154.3 B-E	Landrace 27	157.0 K-N	143.3 S-Y	150.1 K-N
Sitta	160.6 E-I	145.3 R-V	153.0 D-I	Landrace 28	158.3 I-M	140.0 Z-g	149.1 M-P
Suleman 96	163.3 B-F	142.0 W-b	152.6 E-I	Landrace 29	159.3 H-L	141.3 W-d	150.3 J-N
Weebill	163.6 B-E	141.0 X-e	152.3 E-J	Landrace 30	158.3 I-M	142.3 V-a	150.3 J-N
Nesser	154.6 NO	135.0 i	144.8 R	Landrace 31	155.3 M-O	140.3 Y-f	147.8 O-Q
Dharwar	161.6 C-H	139.0 b-h	150.3 J-N	Landrace 32	156.0 M-O	140.0 Z-g	148.0 O-Q
Zarghoon	164.0 B-D	142.6 U-Z	153.3 C-H	Landrace 33	156.3 L-N	137.0 ghi	146.6 Q
Chakwal 86	169.0 A	141.6 W-c	155.3 A-C	Landrace 34	159.3 H-L	139.3 a-h	149.3 M-P
Margalla 99	167.6 A	142.6 U-Z	155.1 A-C	Landrace 35	158.0 I-M	138.3 d-h	148.1 N-Q
Marwat	160.6 E-I	142.6 U-Z	151.6 H-L	Landrace 36	157.0 K-N	142.6 U-Z	149.8 L-O
Landrace 1	169.0 A	142.6 U-Z	155.8 AB	Landrace 37	155.6 M-O	139.3 a-h	147.5 PQ
Landrace 2	163.6 B-E	144.3 S-W	154.0 B-G	Landrace 39	162.3 B-H	136.3 hi	149.3 M-P
Landrace 3	169.6 A	144.3 S-W	157.0 A	Landrace 40	158.3 I-M	138.3 d-h	148.3 N-Q

Table 14. Means for plant height of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 3.720, LSD (0.05) of genotypes (G) = 2.631, and LSD (0.05) of treatments (T) = 0.517, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	90.21 K-N	81.35 R-U	85.78 E-I	Landrace 7	82.75 Q-T	61.77 jkl	72.26 U
F4 786	80.51 S-V	80.48 S-V	80.49 M-Q	Landrace 8	92.36 I-M	70.12 a-e	81.24 K-O
F4 826	84.95 P-S	73.59 X-b	79.27 O-R	Landrace 9	96.53 E-I	62.13 i-l	79.33 O-R
F4 834	91.46 J-M	80.31 S-V	85.89 E-I	Landrace 12	93.56 H-L	72.80 Y-c	83.18 I-N
F4 841	91.57 J-M	84.25 P-S	87.91 B-E	Landrace 14	93.19 H-L	70.61 a-e	81.90 J-O
F4 883	81.30 R-U	80.92 S-V	81.11 L-P	Landrace 15	97.53 D-H	71.22 Z-d	84.38 F-K
F4 922	79.43 T-N	74.46 X-a	76.95 RS	Landrace 16	100.93 B-E	80.89 S-V	90.92 AB
F4 925	98.62 C-G	81.49 R-U	90.06 A-C	Landrace 17	101.29 B-D	75.03 N-Z	88.16 B-E
F4 1992	85.79 O-R	77.88 U-X	81.83 J-O	Landrace 18	106.02 A	77.98 U-X	92.00 A
F4 2011	90.21 K-N	86.61 N-Q	88.41 B-E	Landrace 20	97.12 D-H	75.91 W	86.51 D-H
Inqilab-91	89.45 L-O	63.75 h-l	76.59 RS	Landrace 24	86.30 N-Q	65.34 f-j	75.82 ST
Baviacora	70.85 a-e	59.45 lm	65.15 VW	Landrace 26	94.78 G-K	71.47 Z-d	83.13 I-N
Opata M 85	66.53 e-i	59.66 lm	63.09 W	Landrace 27	93.06 H-L	70.18 a-e	81.62 J-O
Sitta	91.77 J-M	72.19 Y-c	81.98 J-O	Landrace 28	102.33 BC	64.38 g-k	83.35 I-M
Suleman 96	70.47 a-e	60.62 kl	65.55 VW	Landrace 29	104.63 AB	74.35 X-a	89.49 A-D
Weebill	69.50 b-f	61.21 jkl	65.35 VW	Landrace 30	101.79 B-D	73.03 X-b	87.41 C-F
Nesser	71.38 Z-d	59.86 lm	65.62 VW	Landrace 31	100.32 B-E	70.83 a-e	85.57 E-I
Dharwar	71.98 Z-d	62.81 i-l	67.39 V	Landrace 32	99.11 C-F	66.51 e-i	82.81 I-N
Zarghoon	69.84 b-f	60.48 kl	65.16 VW	Landrace 33	94.72 G-K	73.37 X-b	84.05 G-L
Chakwal 86	90.43 K-N	55.44 m	72.94 U	Landrace 34	95.73 F-J	84.73 P-S	90.23 A-C
Margalla 99	99.25 C-F	68.25 c-g	83.75 H-L	Landrace 35	92.93 I-M	63.39 h-l	78.16 P-S
Marwat	84.47 P-S	70.73 a-e	77.60 Q-S	Landrace 36	83.72 Q-T	80.71 S-V	82.23 J-O
Landrace 1	76.27 V-Y	70.66 a-e	73.47 TU	Landrace 37	92.52 I-M	76.75 V-Y	84.64 F-J
Landrace 2	88.19 M-P	72.14 Y-c	80.16 N-Q	Landrace 39	93.95 H-L	79.85 T-W	86.91 D-G
Landrace 3	72.86 Y-c	72.80 Y-c	72.83 U	Landrace 40	101.35 B-D	79.24 T-W	90.30 A-C
Landrace 5	77.28 U-X	67.17 d-h	72.23 U	Mean	88.88 A	71.60 B	

Table 15. Means for spike length of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 0.826, LSD (0.05) of genotypes (G) = 0.584, and LSD (0.05) of treatments (T) = 0.114, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	12.23 B-D	11.22 E-J	11.72 C	Landrace 7	7.73 i-l	5.63 m	6.68 X
F4 786	10.28 I-Q	10.03 L-T	10.16 J-M	Landrace 8	10.13 K-S	8.28 a-j	9.20 O-T
F4 826	10.40 I-P	8.87 W-g	9.63 M-R	Landrace 9	10.28 I-Q	7.23 kl	8.76 S-V
F4 834	12.20 B-E	9.50 P-Y	10.85 F-I	Landrace 12	10.39 I-P	7.95 f-l	9.17 O-T
F4 841	11.06 G-L	9.88 N-W	10.47 G-K	Landrace 14	9.19 R-b	8.42 a-j	8.81 S-V
F4 883	10.69 G-O	9.03 T-e	9.85 K-P	Landrace 15	11.03 G-L	9.79 O-X	10.41 H-L
F4 922	10.14 K-S	9.55 P-Y	9.85 K-P	Landrace 16	10.21 I-R	7.93 g-l	9.07 Q-T
F4 925	11.51 D-H	8.00 e-l	9.76 L-Q	Landrace 17	12.14 B-E	11.24 E-I	11.69 CD
F4 1992	12.72 A-C	8.58 Y-i	10.65 F-J	Landrace 18	10.90 G-N	9.59 P-Y	10.25 I-M
F4 2011	13.48 A	11.59 D-H	12.54 B	Landrace 20	12.09 B-E	10.11 E-I	11.11 C-G
Inqilab-91	13.42 A	11.68 D-G	12.55 B	Landrace 24	10.98 G-N	8.21 b-k	9.59 M-R
Baviacora	9.53 P-Y	7.81 h-l	8.67 T-V	Landrace 26	8.39 a-j	8.10 d-l	8.23 V
Opata M 85	10.20 J-R	9.54 P-Y	9.87 K-O	Landrace 27	11.04 G-L	7.73 i-l	9.38 N-S
Sitta	13.00 A-C	9.05 T-d	11.03 D-H	Landrace 28	10.09 K-S	7.21 kl	8.65 T-V
Suleman 96	9.60 P-Y	8.97 U-f	9.28 N-T	Landrace 29	10.64 H-O	9.21 R-b	9.93 K-N
Weebill	12.16 B-E	10.30 I-Q	11.23 C-F	Landrace 30	10.35 I-P	7.60 i-l	8.98 R-U
Nesser	12.02 C-F	9.98 M-U	11.01 E-H	Landrace 31	10.68 H-O	8.94 V-g	9.81 K-P
Dharwar	10.86 G-N	10.35 I-P	10.61 F-J	Landrace 32	7.58 i-l	7.42 jkl	7.50 W
Zarghoon	12.30 B-D	9.95 M-V	11.13 C-G	Landrace 33	9.21 R-b	8.34 a-j	8.78 S-V
Chakwal 86	13.06 AB	10.18 K-R	11.62 C-E	Landrace 34	9.28 Q-a	9.00 T-e	9.14 P-T
Margalla 99	11.06 G-L	10.23 I-R	10.65 F-J	Landrace 35	11.08 F-K	8.14 b-l	9.61 M-R
Marwat	13.48 A	13.36 A	13.42 A	Landrace 36	9.13 S-c	8.34 a-j	8.74 S-V
Landrace 1	9.47 P-Z	8.93 V-g	9.20 O-T	Landrace 37	9.91 N-V	8.78 X-h	9.35 N-T
Landrace 2	8.37 a-j	8.36 a-j	8.37 UV	Landrace 39	9.59 P-Y	9.21 R-b	9.41 N-S
Landrace 3	9.87 N-W	9.51 P-Y	9.69 M-Q	Landrace 40	8.47 Z-i	8.07 d-l	8.27 V
Landrace 5	7.14 l	5.37 m	6.25 X	Mean	10.56 A	9.07 B	

Table 16. Means for grains/spike of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 1.308, LSD (0.05) of genotypes (G) = 0.925, and LSD (0.05) of treatments (T) = 0.182, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	52.33 MN	50.67 O	51.50 E-G	Landrace 7	51.33 NO	50.67 O	51.00 FG
F4 786	51.00 NO	36.67 V	43.83 K	Landrace 8	51.33 NO	36.67 V	44.00 K
F4 826	46.33 Q	40.33 T	43.33 K	Landrace 9	52.33 MN	24.67 ef	38.50 O
F4 834	53.33 LM	42.33 S	47.83 I	Landrace 12	62.33 F	11.67 k	37.00 P
F4 841	57.67 HI	48.67 P	53.17 D	Landrace 14	47.33 PQ	25.67 de	36.50 PQ
F4 883	39.67 TU	29.67 Za	34.67 R	Landrace 15	57.33 HI	24.67 ef	41.00 L
F4 922	52.67 MN	43.33 RS	48.00 I	Landrace 16	52.00 MN	28.33 ab	40.17 LM
F4 925	58.33 GH	36.67 V	47.50 IJ	Landrace 17	69.67 B	23.67 f	46.67 J
F4 1992	56.33 IJ	44.67 R	50.50 G	Landrace 18	70.33 A	32.33 Y	51.33 FG
F4 2011	67.67 C	56.33 IJ	62.00 A	Landrace 20	57.67 HI	16.67 ij	37.17 P
Inqilab-91	51.33 NO	35.67 VW	43.50 K	Landrace 24	51.67 NO	30.67 Z	41.17 L
Baviacora	38.67 U	26.67 cd	32.67 S	Landrace 26	61.33 F	36.67 V	49.00 H
Opata M 85	51.33 NO	34.67 WX	43.00 K	Landrace 27	59.67 G	27.67 bc	43.67 K
Sitta	58.33 GH	42.67 S	50.50 G	Landrace 28	57.33 HI	21.67 g	39.50 MN
Suleman 96	65.33 DE	54.33 KL	59.83 B	Landrace 29	64.67 E	38.33 U	51.50 EFG
Weebill	56.33 IJ	47.67 PQ	52.00 EF	Landrace 30	52.33 MN	28.67 ab	40.50 LM
Nesser	40.33 P	15.67 j	28.00 U	Landrace 31	52.33 MN	33.67 XY	43.00 K
Dharwar	40.33 P	19.67 h	30.00 T	Landrace 32	52.33 MN	28.67 ab	40.50 LM
Zarghoon	55.33 JK	48.67 P	52.00 EF	Landrace 33	53.33 LM	32.67 Y	43.00 K
Chakwal 86	61.00 F	51.67 NO	56.33 C	Landrace 34	50.33 O	35.67 VW	43.00 K
Margalla 99	65.00 DE	52.67 N	58.83 B	Landrace 35	47.67 PQ	15.67 j	31.67 T
Marwat	61.00 F	50.67 O	55.83 C	Landrace 36	61.67 F	33.67 XY	47.67 I
Landrace 1	29.33 Za	8.67 l	19.00 W	Landrace 37	66.33 CD	38.67 U	52.50 DE
Landrace 2	54.33 KL	17.67 i	36.00 Q	Landrace 39	47.67 PQ	26.67 cd	37.17 P
Landrace 3	38.67 U	15.00 j	26.83 V	Landrace 40	51.67 NO	26.67 cd	39.17 NO
Landrace 5	43.33 RS	26.67 cd	35.00 R	Mean	54.34 A	33.71 B	

Table 17. Means for 1,000-kernel weight of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 0.455, LSD (0.05) of genotypes (G) = 0.322, and LSD (0.05) of treatments (T) = 0.063, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	30.72 R	29.24 S	29.98 TU	Landrace 7	33.02 O	20.11 a	26.56 b
F4 786	30.58 R	29.81 S	30.19 ST	Landrace 8	31.28 Q	27.24 U	29.31 WX
F4 826	34.24 N	30.30 R	32.27 O	Landrace 9	32.01 P	27.72 U	29.87 TU
F4 834	45.99 D	24.19 X	35.09 J	Landrace 12	25.72 W	24.84 X	25.28 e
F4 841	50.18 B	28.11 T	39.15 C	Landrace 14	31.18 Q	24.91 X	28.05 Z
F4 883	51.83 A	31.63 Q	41.74 A	Landrace 15	26.00 V	24.19 X	25.09 e
F4 922	35.22 M	30.41 R	32.81 MN	Landrace 16	38.16 J	26.97 V	32.56 NO
F4 925	43.40 E	31.08 Q	37.24 F	Landrace 17	30.05 R	19.24 b	24.65 f
F4 1992	41.00 G	33.20 O	37.10 F	Landrace 18	31.54 Q	22.47 Z	27.00 a
F4 2011	39.61 I	36.98 L	38.29 D	Landrace 20	32.43 P	19.64 b	26.04 d
Inqilab-91	34.16 N	25.25 W	29.70 UV	Landrace 24	30.01 R	27.52 U	28.77 Y
Baviacora	41.00 G	26.24 V	33.62 K	Landrace 26	30.34 R	27.69 U	29.02 XY
Opata M 85	33.36 O	28.31 T	30.84 R	Landrace 27	26.43 V	26.01 V	26.22 cd
Sitta	36.39 L	31.05 Q	33.72 K	Landrace 28	31.11 Q	25.54 W	28.32 Z
Suleman 96	32.87 P	25.19 W	29.03 XY	Landrace 29	37.45 K	26.25 V	31.85 P
Weebill	32.02 P	24.41 X	28.22 Z	Landrace 30	36.16 L	35.26 M	35.71 I
Nesser	41.09 G	31.51 Q	36.29 GH	Landrace 31	30.58 R	30.26 R	30.42 S
Dharwar	40.15 H	35.60 M	37.88 E	Landrace 32	30.41 R	26.99 V	28.69 Y
Zarghoon	41.44 G	23.11 Y	32.28 O	Landrace 33	40.13 H	25.74 W	32.93 LM
Chakwal 86	48.03 C	24.23 X	36.13 H	Landrace 34	31.23 Q	27.76 U	29.49 VW
Margalla 99	48.52 C	33.45 O	36.48 G	Landrace 35	35.37 M	19.26 b	27.31 a
Marwat	40.01 H	32.08 P	36.05 H	Landrace 36	31.13 Q	25.36 W	28.25 Z
Landrace 1	41.11 G	38.53 J	39.82 B	Landrace 37	35.01 M	31.30 Q	33.16 L
Landrace 2	32.33 P	30.03 R	31.18 Q	Landrace 39	42.16 F	27.67 U	34.92 J
Landrace 3	40.71 H	37.69 K	39.19 C	Landrace 40	28.43 T	24.62 X	26.52 bc
Landrace 5	35.46 M	28.43 T	31.94 P	Mean	35.63 A	27.99 B	

The maximum spike length was found in genotypes Marwat (13/36 cm, 0.8% reduction compared to the control), Inqilab-91 (11.68 cm, 12.9% reduction), and F4 2011 (11.59 cm, 14.0% reduction) under drought conditions. The minimum spike length was observed in landraces 5 (24.0% reduction) and 7 (27.0% reduction) compared to the control. No significant difference in the spike length of Marwat and landrace 2 compared to the control (Table 15, p. 151).

The maximum number of grains/spike was observed in landrace 7 (1.3% reduction from the control) and F4 719 (3.1% reduction). Lines Chakwal 86 (15.2% reduction), F4 2011 (16.7% reduction), Suleman 96 (16.8% reduction), Margalla 99 (18.9% reduction), Marwat (16.9% reduction) under drought conditions. The greatest reduction was in landraces 1, 3, 12, 20, and 35 and in the cultivar Nesser (Table 16, p. 152).

Under drought conditions, the maximum 1000-kernel weight was observed in landrace 30 (2.4% reduction from the control) followed by landrace 1 (6.2% reduction), F4 2011 (6.6% reduction), landrace 3 (7.4% reduction), Dharwar99, Margalla 99 (15.3% reduction), F4 1992 (19.0% reduction) and Marwat (19.8% reduction) . The minimum grain weights were observed in landraces 7, 15, 17, 18, 20, and 35 (Table 17, p. 152).

The *in vitro* parameters proline, chlorophyll, protein, sugar, and SOD content revealed significantly different results at $P < 0.05$ for genotypes, the treatments, and the interaction between genotype and treatment. The amount of proline, protein, sugar, and SOD content significantly increased under drought conditions in all wheat genotypes compared to the control; chlorophyll content significantly decreased under drought stress.

The highest proline content in drought conditions was in genotype F4 922 (a 96.1% over the control), followed by F4 1992 (91.6% increase), F4 719 (89.8% increase), F4 826 (86.1% increase), F4 2011 (78.9% increase), Marwat (61.3% increase), Margalla 99 (59.8% increase), and Suleman 96 (59.3% increase). The lowest proline content was observed in landraces 15, 20, and 35 (Table 18). Plants accumulate different amounts of proline in response to abiotic stress, which protect the plant by reducing the oxidative damage created by osmotic stress. This proline accumulation may create tolerance and protect the plant from oxidative stress.

Table 18. Means for proline content of different wheat genotypes tested for drought *in vivo* (the LSD (0.05) of interaction (G×T) = 640.3, LSD (0.05) of genotypes (G) = 452.8, and LSD (0.05) of treatments (T) = 78.9, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	297.1 Z-i	2,925.0 AB	1,611.0 A-C	Landrace 7	88.8 ghi	655.5 S-i	372.1 OP
F4 786	288.4 Z-i	1,847.0 E-L	1,067.7 E-J	Landrace 8	431.1 X-i	686.0 S-i	558.6 L-P
F4 826	323.4 Y-i	2,330.6 B-F	1,327.0 B-E	Landrace 9	85.9 ghi	1,213.4 K-V	649.6 J-P
F4 834	227.2 c-i	1,360.5 J-T	793.8 F-O	Landrace 12	1,009.4 M-Z	1,423.1 I-R	1,216.3 C-G
F4 841	171.9 d-i	1,876.1 E-K	1,024.0 E-L	Landrace 14	1,053.1 M-X	1,335.7 J-T	1,194.4 C-G
F4 883	263.6 a-i	1,669.3 F-N	966.5 E-M	Landrace 15	256.3 b-i	419.5 X-i	337.9 OP
F4 922	118.0 f-i	3,054.6 A	1,586.3 A-D	Landrace 16	552.0 V-i	1,491.6 H-Q	1,021.8 E-L
F4 925	486.5 W-i	2,053.9 D-I	1,270.2 C-F	Landrace 17	103.4 ghi	419.5 X-i	289.8 P
F4 1992	183.5 d-i	2,206.8 C-G	1,195.1 C-G	Landrace 18	471.9 W-i	1,360.5 J-T	916.2 E-N
F4 2011	640.9 T-i	3,041.5 A	1,841.2 A	Landrace 20	224.3 c-i	418.0 X-i	321.1 OP
Inqilab-91	938.1 O-c	1,194.4 K-W	1,066.2 E-J	Landrace 24	756.0 R-i	1,707.2 F-M	1,231.6 C-F
Baviacora	834.6 P-f	1,535.3 G-P	1,185.0 C-H	Landrace 26	186.4 d-i	954.1 O-b	570.3 K-P
Opata M 85	719.6 R-i	1,621.2 G-O	1,170.4 C-I	Landrace 27	131.1 f-i	747.2 R-i	439.1 N-P
Sitta	145.6 e-i	5,95.7 U-i	370.7 OP	Landrace 28	74.3 hi	866.7 P-e	470.5 N-P
Suleman 96	1,009.4 M-Z	2,482.1 A-E	1,745.8 AB	Landrace 29	69.9 hi	598.6 U-i	334.3 OP
Weebill	303.0 Y-i	782.2 Q-h	542.6 L-P	Landrace 30	809.9 Q-g	1,136.2 M-X	973.0 E-M
Nesser	225.7 c-i	1,997.0 D-J	1,111.4 D-J	Landrace 31	151.5 e-i	1,166.7 L-W	659.1 J-P
Dharwar	100.5 ghi	678.8 S-i	389.6 OP	Landrace 32	578.3 U-i	1,691.1 F-M	1,134.7 C-J
Zarghoon	948.2 O-b	1,159.5 L-W	1,053.8 E-K	Landrace 33	604.5 U-i	1,698.4 F-M	1,151.4 C-I
Chakwal 86	212.6 d-i	1,171.1 L-W	691.9 I-P	Landrace 34	37.8 i	1,331.3 J-T	684.6 I-P
Margalla 99	1,089.6 M-X	2,713.7 A-C	1,901.6 A	Landrace 35	160.2 e-i	419.5 X-i	289.8 P
Marwat	1021.1 M-Y	2,639.4 A-D	1830.3 A	Landrace 36	319.0 Y-i	1076.4 M-X	697.7 H-P
Landrace 1	174.8 d-i	895.8 P-d	535.3 M-P	Landrace 37	568.1 U-i	2166.0 C-H	1367.0 B-E
Landrace 2	1286.2 K-U	1871.8 E-K	1579.0 A-D	Landrace 39	474.9 N-i	980.3 N-a	727.6 G-P
Landrace 3	85.9 ghi	642.3 T-i	364.1 OP	Landrace 40	677.3 S-i	1369.2 J-S	1023.3 E-L
Landrace 5	1160.9 L-W	1187.1 K-W	1174.0 C-I	Mean	453.6 B	1431.1 A	

Chlorophyll content significantly decreased in all wheat genotypes under drought conditions compared to the control. The maximum chlorophyll content under drought was observed in landrace 40 (a 35.7% reduction from the control) followed by Baviacora (18.1% reduction), landrace 39 (14.2% reduction), landrace 37 (14.1% reduction), and Marwat (0.9% reduction). The minimum chlorophyll content was observed in lines F4 834, F4 719, and F4 786 (Table 19).

Table 19. Means for chlorophyll content of different wheat genotypes tested for drought *in vitro* (the LSD (0.05) of interaction (G×T) = 0.3387, LSD (0.05) of genotypes (G) = 0.2395, and LSD (0.05) of treatments (T) = 0.0471, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	0.62 O-b	0.19 c	0.41 U-X	Landrace 7	0.42 V-c	0.41 W-c	0.41 U-X
F4 786	0.43 U-c	0.19 c	0.31 X	Landrace 8	0.55 P-c	0.36 Y-c	0.45 S-X
F4 826	0.52 R-c	0.26 a-c	0.39 V-X	Landrace 9	0.41 W-c	0.32 Z-c	0.36 WX
F4 834	0.43 U-c	0.18 d	0.31 X	Landrace 12	1.17 B-M	0.81 I-W	0.99 C-L
F4 841	0.61 O-c	0.31 Z-c	0.46 S-X	Landrace 14	1.09 C-N	0.96 F-R	1.03 B-J
F4 883	0.55 P-c	0.31 Z-c	0.43 T-X	Landrace 15	0.97 E-R	0.59 P-c	0.78 I-Q
F4 922	0.57 P-c	0.37 X-c	0.47 R-X	Landrace 16	0.93 F-S	0.87 G-T	0.90 D-O
F4 925	0.54 Q-c	0.20 bc	0.37 WX	Landrace 17	1.08 C-N	0.85 H-V	0.97 C-M
F4 1992	0.82 I-W	0.57 P-c	0.70 L-T	Landrace 18	1.22 A-I	0.77 K-Y	0.99 C-L
F4 2011	1.06 C-N	0.83 I-W	0.95 C-N	Landrace 20	1.39 A-E	0.86 G-U	1.12 A-F
Inqilab-91	0.89 G-T	0.67 N-a	0.78 I-Q	Landrace 24	1.28 A-H	0.51 S-c	0.90 D-O
Baviacora	1.21 A-J	0.99 D-P	1.10 A-G	Landrace 26	0.66 N-a	0.58 P-c	0.62 O-W
Opata M 85	1.29 A-G	0.82 I-W	1.06 B-I	Landrace 27	0.93 F-S	0.76 L-Y	0.84 F-P
Sitta	0.90 G-T	0.74 M-Z	0.82 G-P	Landrace 28	1.03 D-O	0.71 N-Z	0.87 E-P
Suleman 96	1.22 A-I	0.77 K-Y	0.99 C-L	Landrace 29	1.19 B-L	0.72 N-Z	0.95 C-N
Weebill	1.06 C-N	0.76 L-Y	0.91 D-O	Landrace 30	1.61 A	0.68 N-a	1.14 A-E
Nesser	1.35 A-F	0.85 H-V	1.10 A-G	Landrace 31	0.74 M-Z	0.72 N-Z	0.73 K-S
Dharwar	0.89 G-T	0.56 P-c	0.73 K-S	Landrace 32	0.93 F-S	0.65 N-a	0.79 H-Q
Zarghoon	0.69 N-a	0.66 N-a	0.67 N-V	Landrace 33	1.51 AB	0.82 I-W	1.17 A-D
Chakwal 86	1.09 C-N	0.92 G-S	1.01 C-K	Landrace 34	0.99 D-P	0.72 N-Z	0.86 E-P
Margalla 99	0.73 N-Z	0.66 N-a	0.70 L-T	Landrace 35	1.35 A-F	0.71 N-Z	1.03 B-J
Marwat	1.08 C-N	1.07 C-N	1.08 B-H	Landrace 36	0.89 G-T	0.62 O-b	0.75 J-R
Landrace 1	0.97 E-R	0.78 J-Y	0.87 E-P	Landrace 37	1.48 A-C	1.27 A-H	1.37 A
Landrace 2	0.98 D-Q	0.66 N-a	0.82 G-P	Landrace 39	1.40 A-D	1.20 A-K	1.30 AB
Landrace 3	0.55 P-c	0.47 T-c	0.51 Q-X	Landrace 40	1.51 AB	0.97 E-R	1.24 A-C
Landrace 5	0.85 H-V	0.35 Y-c	0.60 P-W	Mean	0.95 A	0.65 B	

Highest protein content was observed in Landrace 31 followed by landrace 27 (68.7% increase), F4 2011 (48.0% increase), F4 922 (22% increase), and F4 719 (7.5% increase) under drought condition compared to the control. The lowest content was observed in landrace 26, followed by landraces 34 and 28 (Table 20, p. 155). Different studies have revealed that drought-resistant cultivars of wheat a specific type of protein, along with heat-shock protein, increased in a stress environment, which we observed also. Proteins probably function by protecting cells from dehydration, such as the enzymes required for the biosynthesis of various osmoprotectants and detoxification enzymes.

Under drought conditions, the highest sugar content was observed in F4 922 (51.6% increase over the control) followed by F4 1992 (48.2 % increase), F4 925 (47.6% increase), Marwat (41.1% increase), Margalla 99 (45.7% increase), F4 826 (41.7% increase), and F4 719 (38.4% increase). The lowest sugar contents were observed in landraces 20 and 40 (Table 21, p. 155). The complex, essential role of soluble sugars in plant metabolism is well known as products of hydrolytic processes, substrates in biosynthesis processes, and energy production. Under drought stress conditions, even sugar flux may be a signal for metabolic regulation.

The maximum SOD content was observed in Marwat followed by Margalla 99, F4 2011, F4 719, F4 922, F4 826 and F4 1992 with 24, 11.6, 2.4, 24, 21.7, 4.4 and 10.9 percent increase respectively under drought condition in comparison to control while minimum SOD content was observed in Landrace 20, Landrace 35 (Table 22, p. 156). Antioxidative enzymes (e.g., superoxide dismutase, catalase and ascorbic peroxidases) have been found to be related with water deficiency and considered as the main components of anti-oxidative machinery for drought resistance in higher plants. These antioxidants efficiently scavenge active oxygen species and prevent damaging effects of free radicals. Thus an antioxidant system was induced in drought stress in different wheat varieties as observed in this study.

Table 20. Means for protein content of different wheat genotypes tested for drought *in vitro* (the LSD (0.05) of interaction (G×T) = 258.5, LSD (0.05) of genotypes (G) = 182.8, and LSD (0.05) of treatments (T) = 35.9, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	1,143.1 D-P	1,473.8 A-D	1,308.5 A-F	Landrace 7	1,203.3 B-P	1,229.5 B-P	1,216.4 A-G
F4 786	1,222.0 B-P	1,379.4 B-L	1,300.7 A-F	Landrace 8	1,153.0 C-P	1,160.2 C-P	1,156.6 C-H
F4 826	1,303.5 B-M	1,424.9 B-I	1,364.2 A-D	Landrace 9	1,207.8 B-P	1,326.9 B-L	1,267.4 A-F
F4 834	1,104.4 H-Q	1,157.0 C-P	1,130.7 D-I	Landrace 12	1,210.0 B-P	1,270.9 B-O	1,240.4 A-G
F4 841	1,050.4 L-Q	1,102.2 H-Q	1,076.3 F-I	Landrace 14	921.1 P-S	1,395.3 B-K	1,158.2 C-H
F4 883	1,072.5 J-Q	1,335.6 B-L	1,204.1 A-G	Landrace 15	1,114.8 G-P	1,163.7 C-P	1,139.2 D-I
F4 922	1,302.7 B-M	1,461.2 B-E	1,381.9 A-C	Landrace 16	1,191.2 C-P	1,358.9 B-L	1,275.1 A-F
F4 925	1,051.4 L-Q	1,224.1 B-P	1,137.7 D-I	Landrace 17	1,200.3 B-P	1,372.5 B-L	1,286.4 A-F
F4 1992	1,213.4 B-P	1,416.1 B-I	1,314.8 A-E	Landrace 18	1,240.4 B-P	1,359.7 B-L	1,300.0 A-F
F4 2011	1,374.9 B-L	1,487.4 A-C	1,431.2 A	Landrace 20	1,145.0 D-P	1,305.7 B-M	1,225.3 A-G
Inqilab-91	1,265.8 B-O	1,342.0 B-L	1,303.9 A-F	Landrace 24	1,064.0 K-Q	1,234.0 B-P	1,149.0 C-H
Baviacora	1,224.1 B-P	1,275.7 B-N	1,249.9 A-F	Landrace 26	355.7 V	668.8 R-U	512.2 L
Opata M 85	1,251.9 B-P	1,358.1 B-L	1,304.9 A-F	Landrace 27	790.4 Q-T	1,535.1 AB	1,162.7 B-H
Sitta	568.4 T-V	942.3 N-S	755.4 JK	Landrace 28	459.0 UV	729.6 R-U	594.3 KL
Suleman 96	1,327.8 B-L	1,454.3 B-F	1,391.1 AB	Landrace 29	714.4 R-U	1,133.9 E-P	924.1 IJ
Weebill	1,307.0 B-M	1,378.1 B-L	1,342.5 A-E	Landrace 30	1,351.4 B-L	1,405.1 B-J	1,378.2 A-C
Nesser	1,279.2 B-M	1,336.1 B-L	1,307.7 A-F	Landrace 31	547.2 T-V	1,750.9 A	1,149.1 C-H
Dharwar	1,243.1 B-P	1,301.4 B-M	1,272.2 A-F	Landrace 32	1,257.8 B-O	1,261.3 B-O	1,259.5 A-F
Zarghoon	1,155.1 C-P	1,321.4 B-L	1,238.3 A-G	Landrace 33	1,230.8 B-P	1,330.8 B-L	1,280.8 A-F
Chakwal 86	938.9 O-S	975.2 M-R	957.1 HI	Landrace 34	662.7 S-U	717.4 R-U	690.0 KL
Margalla 99	1,349.8 B-L	1,450.8 B-G	1,400.3 A	Landrace 35	1,046.9 L-Q	1,404.8 B-J	1,225.9 A-G
Marwat	1,296.3 B-M	1,428.1 B-H	1,362.2 A-D	Landrace 36	1,138.6 D-P	1,179.2 C-P	1,158.9 B-H
Landrace 1	1,128.1 E-P	1,205.1 B-P	1,166.7 B-H	Landrace 37	1,269.8 B-O	1,452.7 B-F	1,361.3 A-D
Landrace 2	1,216.4 B-P	1,297.9 B-M	1,257.1 A-F	Landrace 39	1,136.2 D-P	1,166.4 C-P	1,151.3 C-H
Landrace 3	975.8 M-R	1,057.9 K-Q	1,016.8 G-I	Landrace 40	1,104.6 H-Q	1,120.1 F-P	1,112.4 E-I
Landrace 5	939.7 O-S	1,088.8 I-Q	1,014.3 G-I	Mean	1,098.4 B	1,268.7 A	

Table 21. Means for sugar content of different wheat genotypes tested for drought *in vitro* (the LSD (0.05) of interaction (G×T) = 290.6, LSD (0.05) of genotypes (G) = 205.5, and LSD (0.05) of treatments (T) = 40.4, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	1,367.6 B-F	2,222.0 A	1,794.8 A	Landrace 7	614.3 O-f	640.8 N-f	627.6 K-R
F4 786	627.8 N-f	774.0 I-b	700.9 J-P	Landrace 8	657.8 N-f	742.5 J-e	700.2 J-P
F4 826	830.4 I-Y	1,424.8 B-D	1,127.6 C-E	Landrace 9	338.0 e-f	681.6 L-e	509.8 N-R
F4 834	726.2 J-d	745.0 J-d	735.6 I-O	Landrace 12	488.7 V-f	604.1 P-f	546.4 M-R
F4 841	712.0 K-e	1,350.8 B-F	1,031.4 D-G	Landrace 14	555.7 R-f	617.3 O-f	586.5 L-R
F4 883	641.1 N-f	765.5 I-c	703.3 J-P	Landrace 15	855.9 I-V	907.4 H-T	881.7 F-K
F4 922	999.3 G-N	2,065.6 A	1,532 5 B	Landrace 16	449.0 Z-f	548.9 R-f	499.0 O-R
F4 925	836.4 I-Y	1,597.4 B	1,216.9 CD	Landrace 17	1,023.1 F-M	1,039.1 F-L	1,031.1 D-G
F4 1992	824.7 I-Z	1,594.9 B	1,209.8 CD	Landrace 18	415.7 b-f	522.9 U-f	469.3 P-R
F4 2011	835.7 I-Y	1,396.6 B-E	1,116.1 C-F	Landrace 20	368.3 d-f	470.2 X-f	419.2 R
Inqilab-91	660.3 M-f	673.8 L-f	667.1 J-R	Landrace 24	672.0 L-f	693.5 L-e	682.8 J-Q
Baviacora	492.9 V-f	786.7 I-b	639.8 J-R	Landrace 26	666.8 L-f	717.3 J-d	692.0 J-Q
Opata M 85	450.7 Z-f	961.1 G-Q	705.9 J-P	Landrace 27	474.9 W-f	596.4 P-f	535.6 M-R
Sitta	534.9 T-f	594.4 P-f	564.6 L-R	Landrace 28	568.9 R-f	777.5 I-b	673.2 J-R
Suleman 96	873.2 I-U	1,402.3 B-E	1,137.8 CD	Landrace 29	618.3 O-f	689.6 L-e	653.9 J-R
Weebill	688.3 L-e	826.4 I-Z	757.4 H-N	Landrace 30	459.4 Y-f	632.8 N-f	5,46.1 M-R
Nesser	773.2 I-b	849.4 I-W	811.3 G-L	Landrace 31	589.3 Q-f	798.2 I-a	693.8 J-Q
Dharwar	861.9 I-U	923.9 H-R	892.9 E-J	Landrace 32	1,031.3 F-M	1,074.8 E-K	1,053.0 C-F
Zarghoon	707.3 K-e	915.6 H-S	811.5 G-L	Landrace 33	605.3 O-f	690.3 L-e	647.8 J-R
Chakwal 86	856.2 I-V	1,089.5 D-J	972.9 D-I	Landrace 34	725.3 J-d	809.9 I-Z	767.6 H-M
Margalla 99	810.0 I-Z	1,493.5 B-C	1,151.8 CD	Landrace 35	555.1 R-f	635.3 N-f	595.2 L-R
Marwat	954.6 H-R	1,621.2 B	1,287.9 C	Landrace 36	982.3 G-O	1,236.9 C-H	1,109.6 C-F
Landrace 1	838.0 I-X	1,120.0 D-I	979.0 D-H	Landrace 37	970.9 G-P	1,307.1 B-G	1,139.0 CD
Landrace 2	370.5 d-f	547.4 R-f	459.0 P-R	Landrace 39	430.2 a-f	534.4 T-f	482.3 O-R
Landrace 3	388.2 c-f	537.1 T-f	462.7 P-R	Landrace 40	409.2 b-f	472.7 a-f	441.0 QR
Landrace 5	302.5 f	539.6 S-f	421.1 R	Mean	676.3 B	926.7 A	

Table 22. Means for SOD contentof different wheat genotypes tested for drought *in vitro* (the LSD (0.05) of interaction (G×T) = 8.624, LSD (0.05) of genotypes (G) = 6.098, and LSD (0.05) of treatments (T) = 1.200, genotypes with different letters are significantly different).

Genotype	Control	Drought	Mean	Genotype	Control	Drought	Mean
F4 719	30.13 C-T	39.69 B-D	34.91 B-D	Landrace 7	17.65 X-b	24.69 K-a	21.17 L-Q
F4 786	28.70 D-X	32.18 C-P	30.44 D-J	Landrace 8	18.87 U-b	20.80 Q-b	19.84 M-R
F4 826	37.24 B-H	38.96 B-F	38.10 A-C	Landrace 9	30.39 C-T	32.20 C-P	31.29 C-I
F4 834	21.81 N-b	26.10 I-Z	23.96 I-Q	Landrace 12	33.82 B-M	34.50 B-L	34.16 B-E
F4 841	26.94 H-Y	34.44 B-L	30.69 D-J	Landrace 14	21.25 O-b	29.43 D-V	25.34 G-P
F4 883	20.14 Q-b	25.30 J-a	22.72 K-Q	Landrace 15	20.83 Q-b	21.75 N-b	21.29 L-Q
F4 922	30.59 C-S	39.07 B-E	34.83 B-D	Landrace 16	27.57 G-Y	32.57 C-N	30.07 D-K
F4 925	20.85 Q-b	21.12 P-b	20.98 L-R	Landrace 17	19.75 S-b	19.83 R-b	19.79 M-R
F4 1992	32.47 C-N	36.48 B-I	34.48 B-E	Landrace 18	32.91 B-N	35.12 B-K	34.01 B-F
F4 2011	39.54 B-D	40.53 A-C	40.03 AB	Landrace 20	13.39 b	14.31 ab	13.85 R
Inqilab-91	25.41 I-a	27.87 G-Y	26.64 F-N	Landrace 24	28.41 E-X	36.44 B-J	32.43 C-G
Baviacora	32.11 C-P	32.59 C-N	32.35 C-G	Landrace 26	24.75 K-a	26.70 H-Y	25.72 G-O
Opata M 85	23.78 L-b	26.54 H-Y	25.16 G-P	Landrace 27	25.33 I-a	26.61 H-Y	25.97 G-O
Sitta	19.57 S-b	25.67 I-Z	22.62 K-Q	Landrace 28	16.74 Y-b	20.67 Q-b	18.70 O-R
Suleman 96	19.19 T-b	22.47 N-b	20.83 L-R	Landrace 29	27.31 G-Y	29.50 D-U	28.41 D-L
Weebill	18.26 V-b	20.22 Q-b	19.24 N-R	Landrace 30	23.86 L-b	25.33 I-a	24.60 H-P
Nesser	25.04 K-a	25.82 I-Z	25.43 G-P	Landrace 31	19.51 S-b	28.17 E-X	23.84 I-Q
Dharwar	28.98 D-W	29.12 D-V	29.05 D-K	Landrace 32	22.98 M-b	23.76 L-b	23.37 J-Q
Zarghoon	32.67 C-N	34.78 B-L	33.73 B-F	Landrace 33	17.89 W-b	18.80 U-b	18.35 O-R
Chakwal 86	31.08 C-Q	32.33 C-O	31.71 C-H	Landrace 34	24.76 K-a	29.28 D-V	27.02 E-M
Margalla 99	38.33 B-G	43.37 AB	40.85 AB	Landrace 35	14.98 Z-b	18.78 U-b	16.88 QR
Marwat	37.48 B-H	49.37 A	43.43 A	Landrace 36	25.06 K-a	30.98 C-R	28.02 D-L
Landrace 1	23.67 L-b	31.18 C-Q	27.42 D-M	Landrace 37	27.92 F-Y	34.82 B-L	31.37 C-I
Landrace 2	15.24 Z-b	20.55 Q-b	17.89 P-R	Landrace 39	24.55 K-a	26.83 H-Y	25.69 G-O
Landrace 3	17.49 X-b	27.94 F-Y	22.72 K-Q	Landrace 40	19.43 S-b	30.98 C-R	25.20 G-P
Landrace 5	22.02 N-b	29.37 D-V	25.70 G-O	Mean	25.07 B	29.13 A	

Genotypes that performed best under drought (rainout shelter), field, and laboratory conditions were F4 2011, F4 719, F4 922, Margalla 99, Marwat, F4 826, F4 1992, landrace 37, Suleman 96, F4 841, and F4 786. These lines gave high yield in both field and laboratory tests and exhibited a high amount of proline, protein, sugar, and SOD content. Thus, having better osmoregulation mechanisms to tolerate water-deficit conditions. These genotypes also provided wide genetic diversity, because they have diverse alleles compared to the local cultivars.

In vitro studies evaluating elite D-genome synthetic hexaploid wheat for drough tolerance.

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Abstract. Pakistan falls into arid and semi-arid regions of climatic conditions. Areas receiving a rainfall less than 100 to 250 mm to less than 500 mm are called semi-arid lands. These lands constitute about 88% of the country's total geographic area of 79.6 x 10^6 ha and are prone to occasional drought resulting in low or no production. Drought is one key constraint that influences our national wheat yield. For breeding targets to be achieved, unique diversity is paramount. Synthetic hexaploid (SH) wheats may provide this diversity. Earlier, international screening under reduced irrigation conditions led to the identification of 53 SH wheats. Twenty-seven of these SH wheats have been tested now under Pakistani conditions for the first time along with three standard, global, drought-tolerant check wheat cultivars. The germ plasm evaluation was done under *in vitro* conditions by giving a drought stress of 30% PEG to the SH wheats and checks. Their proline, protein, sugar, chlorophyll, and SOD content were assessed by comparing with the control lines. We observed that the degree of drought tolerance varied in all 27 SH wheats and thr checks, because of their genetic variability. SHDR 17, SHDR 18, SHDR 45, SHDR 46, SHDR 49 SHDR 50, SHDR 51, SHDR 52, and SHDR 53 were drought tolerant, whereas three lines, SHDR 29, SHDR 43, and SHDR 21, were sensitive.

Introduction. Drought stress remains an ever increasing problem that severely limits crop production by preventing crop plants from expressing their full genetic potential (Rampino et al. 2006; Karmer 1980) and can cause yield losses greater

than any other environmental factor, particularly in arid and semi-arid areas. Of the total area of Pakistan, 75% is arid and semi-arid with average temperature ranging between 2°C–50°C. An extensive, canal irrigation system was introduced for use in the vast agricultural area. Nevertheless, a large part of the country is still rain fed (Ashraf and McNeilly 1988).

Depending on the duration and extent of drought stress, a range of plant processes occurring at molecular, biochemical, cellular, and whole-plant levels may be altered. Plant adaptation to drought stress can involve avoidance mechanisms such as morphological changes in the roots and shoots. In addition to avoidance mechanisms, plant response to water shortage can involve changes in biochemical pathways and the expression of genes encoding proteins that contribute to drought adaptation. Such changes can bring about drought tolerance, whereby plants continue to function at the low water potentials caused by water deficit. Osmotic adjustment is one of the most effective physiological mechanisms underlying plant resistance to water deficit (Turner and Jones 1980; Morgan 1984; Blum 1988). With 50% of the world's arable land being arid or sensitive to drought, developing drought-tolerant wheat cultivars is an important aim of plant scientists (Ruivenkamp and Richards 1994) with better adaptation to water deficits and augmenting the productivity of rain fed wheat (Rajaram 2002).

Because of land limitations, enhanced wheat production must come from higher absolute yields, which can only be met by the concerted efforts in plant breeding (Braun et al. 1998). Synthetic hexaploid wheat is a relatively new germ plasm obtained by artificially crossing durum wheat, *Triticum turgidum* subsp. *durum* (2n = 4x = 28, AABB) and *Aegilops tauschii* (2n = 14, DD). This germ plasm has proven to be very useful as a source of resistance to diseases and pests, as well as for tolerance to environmental stresses (Gorham 1990; Limin and Fowler 1993). Synthetic hexaploids are routinely crossed and backcrossed with common wheat (2n = 6x = 42, AABBDD) to achieve acceptable agronomic types. In defining a strategy for wheat breeding under drought stress, Rajaram et al. (1996) suggested that simultaneous evaluation of the germ plasm should be carried out under both near-optimum conditions (to utilize high heritabilities and identify genotypes with high yield potential) and stress conditions (to preserve alleles for drought tolerance).

Table 23. Mean chlorophyll a (mmol/g fresh weight) and chlorophyll b (mmol/g fresh weight) content of synthetic hexaploid wheat populations grown at control (T_0) and drought (30% PEG (T_1)) conditions. Means with same letters in each column do not differ significantly.

Population	Chlorophyll a		Chlorophyll b	
	T_0	T_1	T_0	T_1
SHDR 5	3.183 A-G	2.546 A-M	0.977 B-J	0.778 C-K
SHDR 6	3.105 A-H	2.564 A-L	1.312 A-C	0.762 D-K
SHDR 7	3.382 AB	3.352 AB	1.342 AB	1.255 A-D
SHDR 9	2.611 A-L	3.354 AB	0.757 D-K	1.117 B-F
SHDR 11	2.297 D-N	2.992 A-J	0.632 E-K	0.939 B-K
SHDR 13	2.320 D-N	2.268 E-N	1.016 B-I	0.763 D-K
SHDR 14	3.266 A-E	2.220 F-N	1.338 AB	0.684 E-K
SHDR 16	3.227 A-F	2.432 B-N	1.036 B-H	0.753 D-K
SHDR 17	2.888 A-K	1.228 O-Q	0.921 B-K	0.374 K
SHDR 18	3.121 A-H	2.374 B-N	1.143 B-F	0.676 E-K
SHDR 19	3.384 AB	1.751 L-Q	1.086 B-G	0.643 E-K
SHDR 21	3.297 A-D	1.471 N-Q	1.177 B-E	0.521 G-K
SHDR 23	3.095 A-H	1.031 Q	0.934 B-K	0.452 I-K
SHDR 24	3.226 A-F	1.991 I-Q	0.905 B-K	0.682 E-K
SHDR 29	3.005 A-I	2.247 E-N	0.709 D-K	0.781 C-K
SHDR 32	2.946 A-J	1.823 L-Q	0.596 F-K	0.649 E-K
SH DR 33	3.210 A-G	1.984 J-Q	1.168 B-E	0.689 E-K
SHDR 37	3.113 A-H	2.049 I-O	0.959 B-J	0.705 D-K
SHDR 38	3.332 A-C	1.810 L-Q	0.765 D-K	0.661 E-K
SHDR 43	3.442 A	1.094 PQ	1.743 A	0.506 H-K
SHDR 45	1.897 K-Q	1.533 M-Q	0.534 G-K	0.440 JK
SHDR 46	2.374 B-N	2.259 E-N	0.688 E-K	0.661 E-K
SHDR 49	2.408 B-N	2.263 E-N	0.702 D-K	0.596 F-K
SHDR 50	2.562 A-L	1.977 J-Q	0.808 B-K	0.574 F-K
SHDR 51	2.203 G-O	2.407 B-N	0.618 E-K	0.761 D-K
SHDR 52	2.542 A-M	2.063 I-P	0.746 D-K	0.589 F-K
SHDR 53	2.340 C-N	2.079 I-O	0.703 D-K	0.609 E-K
Baviacora	2.324 C-N	2.270 E-N	0.676 E-K	0.728 D-K
Nesser	2.301 D-N	1.897 K-Q	0.673 E-K	0.529 G-K
Marwat	2.159 H-O	2.158 H-O	0.616 E-K	0.630 E-K
LSD 1%	0.822		0.453	

Results and discussion. According to Saadalla (2001), drought-tolerant genotypes of wheat showed higher chlorophyll a/b contents and dry weight than drought-susceptible genotypes. We found this assumption to be true as SHDR 9, SHDR 11, SHDR 51, SHDR 53, SHDR 52, SHDR 46, SHDR 45, SHDR 50, and

SHDR 9 showed a higher amount of chlorophyll a than the susceptible lines, and SHDR 9, SHDR 11, SHDR 29, SHDR 32, and Baviacora showed a higher chlorophyll b content after drought stress (Table 23, p. 157). Similarly, Nikolaeva et al. (2010) found that the chlorophyll content in wheat leaves at the beginning of a drought treatment (3 days) increased insignificantly with respect to that in untreated plants. As water limitation becomes more pronounced (5 days), the chlorophyll content decreased but the chlorophyll a/b ratio remained unchanged. According to Garg et al. (1998), chlorophyll a and b and chlorophyll a/b ratios are not affected by drought stress; we found similar results (Table 23, p. 157). In some lines, SHDR 5 (2.546 mmol/g fresh weight), SHDR 6 (2.564), SHDR 7 (3.352), SHDR 9 (3.354), SHDR 13 (2.268), SHDR 29 (2.247), SHDR 37 (2.049), SHDR 45 (1.533), SHDR 53 (2.079), and SHDR 52 (2.063), no significant change in chlorophyll content was found. The check lines, Baviacora, Marwat, and Nesser, also showed no significant change in chlorophyll content after drought stress. All these results contrast the assumption that photosynthesis is sensitive to heat and drought stresses and it is often the first process affected by stress. Photosynthetic activity was reduced by water stress and SHDR 14 (2.220 mmol/g fresh weight), SHDR 17 (1.228), SHDR 19 (1.751), SHDR 21 (1.471), SHDR 23 (1.031), SHDR 24 (1.991), SHDR 32 (1.823), SHDR 33 (1.984), SHDR 38 (1.810), and SHDR 43 (1.094) showed significantly low chlorophyll a contents under drought stress (Table 23). These results agree with those of Balouchi (2010), who found a marked reduction in chlorophyll and carotenoid content under water stress in all cultivars. Sairam et al. (1998) also found that total chlorophyll and carotenoid contents showed a decreasing trend with age, under both control and stress conditions.

The limitation of photosynthesis by water stress, especially when it is combined with conditions of high temperature and light, may cause photo-oxidative damage to the photosynthetic apparatus if the plant does not avoid or utilize the excess excitation energy (Asada 1999). However, if the photoprotective mechanisms are insufficient, the leaves are protected from stress-induced oxidative damage by several antioxidant systems (Foyar et al. 1994). Carotenoids, such as β-carotene, are key scavengers of reactive oxygen species, such as singlet oxygen, and so protect thylakoid membranes from oxidative damage (Young 1991). The carotenoids serve at least two important functions in photosynthesis, namely light harvesting and photoprotection and, hence, their comparative levels in a genotype will determine its relative tolerance. Lines SHDR 51 (1.152 mmol/g fresh weight), SHDR 6 (0.203), SHDR 7(0.2631), SHDR 11 (0.196), and SHDR 14 (0.175) showed an increase in carotenoid content after drought stress compared to control lines (Table 24). These results agree with those of Rahman et al. (2003), Kraus et al. (1995), and Sairam et al. (1998), who reported higher carotenoid levels in tolerant genotypes. The remaining 25 lines did not have any significant change in their carotenoid content. Price and Hendry (1991) also reported unchanged carotenoid concentration in wheat leaves under drought. Lines SHDR 24 (0.063 mmol/g fresh weight) and SHDR 29 (0.072 mmol/g fresh weight) were significantly lowest in their carotenoid content of all germ plasm tested, as confirmed by Balouchi (2010) and Tas and Tas (2007).

Table 24. Mean carotenoid (mmol/g fresh weight) content of synthetic hexaploid wheat populations grown at control (T_0) and drought (30% PEG (T_1)) conditions. LSD 1% = 0.09557 Means with same letters in each column do not differ significantly.

Population	Carotenoid content		Population	Carotenoid content	
	T_0	T_1		T_0	T_1
SHDR 5	0.181 A-K	0.167 A-K	SHDR 32	0.197 A-H	0.089 F-K
SHDR 6	0.142 B-K	0.203 A-G	SHDR 33	0.168 A-K	0.080 H-K
SHDR 7	0.152 A-K	0.263 A	SHDR 37	0.146 A-K	0.066 JK
SHDR 9	0.237 A-C	0.231 A-E	SHDR 38	0.144 A-K	0.101 F-K
SHDR 11	0.155 A-K	0.196 A-H	SHDR 43	0.075 I-K	0.064 JK
SHDR 13	0.113 D-K	0.112 E-K	SHDR 45	0.090 F-K	0.084 G-K
SHDR 14	0.102 F-K	0.175 A-K	SHDR 46	0.107 F-K	0.095 F-K
SHDR 16	0.184 A-J	0.179 A-K	SHDR 49	0.174 A-K	0.147 A-K
SHDR 17	0.172 A-K	0.123 C-K	SHDR 50	0.116 D-K	0.101 F-K
SHDR 18	0.232 A-D	0.205 A-F	SHDR 51	0.159 A-K	0.152 A-K
SHDR 19	0.203 A-G	0.140 B-K	SHDR 52	0.170 A-K	0.147 A-K
SHDR 21	0.155 A-K	0.070 JK	SHDR 53	0.173 A-K	0.124 C-K
SHDR 23	0.155 A-K	0.104 F-K	Baviacora	0.195 A-I	0.155 A-I
SHDR 24	0.248 AB	0.063 K	Nesser	0.164 A-K	0.125 C-K
SHDR 29	0.240 AC	0.072 JK	Marwat	0.174 A-K	0.110 F-K

One biochemical response to dehydrative stress is the accumulation of a family of proteins called dehydrins, which are believed to protect membranes and macromolecules against denaturation. Previous studies demonstrated the

Table 25. Mean protein content (mg/g fresh weight) of synthetic hexaploid wheat populations grown at control (T_0) and drought (30% PEG (T_1)) conditions. LSD 1% = 0.1352; means with the same letters in each column do not differ significantly.

Population	Protein content		Population	Protein content	
	T_0	T_1		T_0	T_1
SHDR 5	1.058 H-L	1.060 H-L	SHDR 32	1.067 H-L	1.063 H-L
SHDR 6	1.030 KL	1.053 I-L	SHDR 33	1.060 H-L	1.035 J-L
SHDR 7	1.061 H-L	1.069 H-L	SHDR 37	1.042 J-L	1.046 J-L
SHDR 9	1.048 J-L	1.067 H-L	SHDR 38	1.008 L	1.046 J-L
SHDR 11	1.063 H-L	1.059 H-L	SHDR 43	1.103 H-L	1.074 H-L
SHDR 13	1.078 H-L	1.079 H-L	SHDR 45	1.107 G-L	1.331 A-E
SHDR 14	1.093 H-L	1.101 H-L	SHDR 46	1.219 C-I	1.369 A-C
SHDR 16	0.999 L	1.042 J-L	SHDR 49	1.200 D-J	1.343 A-D
SHDR 17	0.992 L	1.065 H-L	SHDR 50	1.288 A-F	1.382 AB
SHDR 18	0.992 L	1.030 KL	SHDR 51	1.280 A-F	1.368 A-C
SHDR 19	1.076 H-L	1.069 H-L	SHDR 52	1.256 A-G	1.362 A-C
SHDR 21	1.063 H-L	1.061 H-L	SHDR 53	1.151 F-L	1.362 A-C
SHDR 23	1.051 J-L	1.078 H-L	Baviacora	1.292 A-F	1.397 A
SHDR 24	1.035 J-L	1.086 H-L	Nesser	1.225 B-H	1.308 A-E
SHDR 29	1.065 H-L	1.050 J-L	Marwat	1.178 E-K	1.376 A-C

accumulation of dehydrins in drought-stressed wheat (Lopez et al. 2003). These proteins have been assumed to function not as enzymes but to protect the plant cell during dehydration. Under drought conditions, all wheat lines accumulated soluble proteins except for SHDR 45 (1.331 mg/g fresh weight) and SHDR 53 (1.362), where the accumulation was highly significant (Table 25). Marwat (1.376), SHDR 46 (1.369), SHDR 49 (1.343), SHDR 52 (1.362), Baviacora (1.397), and SHDR 50 (1.382) had protein accumulation that was better than the control lines, and so can be considered as drought tolerant. Bensen et al. (1988) reported that drought stress resulted in an increase of some soluble proteins and a decrease of others. Lines SHDR 43, SHDR 29, SHDR 33, and SHDR 32 accumulated the lowest amount of soluble proteins and can be considered as sensitive.

Compatible solutes are overproduced under osmotic stress aiming to facilitate osmotic adjustment (Hasegawa et al. 2000; Shao et al. 2005; Zhu 2000). In this study, all germ plasm accumulated a significant amount of proline after stress (Fig. 24). The beneficial roles of proline in conferring osmotolerance have been widely reported (Bajji et al. 2000). Proline has been shown to have a key role in stabilizing cellular proteins and membranes in presence of high concentrations of osmoticum (Errabii et al. 2006). Tatar and Gevrek (2008) showed that wheat dry matter production, relative water content decreased and proline content increased under drought stress. Higher proline content in wheat plants after water stress was reported by Vendruscolo et al. (2007). Our results are similar; proline content in all wheat lines increased significantly (Fig. 24). Line SHDR 53 accumulated the maximum amount of proline (91%) during drought exceeding the check lines, whereas SHDR 18 (88%), Baviacora (85%), Nesser (64%), SHDR 32 (80%) SHDR 38 (88%), SHDR 45 (78%), SHDR 49 (83%), SHDR 50 (79%), SHDR 17 (72%), SHDR 9 (68%), SHDR 52 (64%), and SHDR 51 (67%) accumulated the highest proline content and can be considered as tolerant. However, Johari-Pireivatlou et al. (2010) also advocated that proline content increases significantly with stress compared to the control. The lowest proline accumulation was in SHDR 29 (23%) and SHDR 43 (38%); these lines can be considered as sensitive.

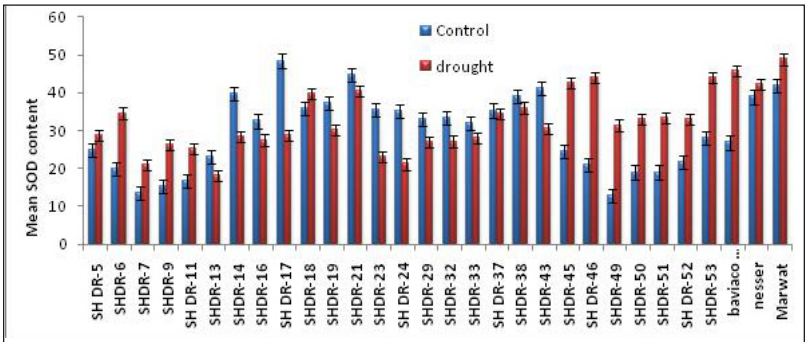

Fig. 24. Proline content (mg/g fresh weight) of synthetic hexaploid wheat lines grown at control (T_0) and drought (30% PEG (T_1)) conditions.

The increase in soluble sugars under water stress has been reported (Johari Pireivatlou et al. 2010). A higher amount of soluble sugars and a lower amount of starch were found under stress by Mohammad Khani and Heidari (2008). In both studies, sugars are suggested to play a role in osmotic adjustment. Therefore, the accumulation of glucose and fructose in drought may be involved in a signal transduction pathway or an increase in osmotic pressure leading

to drought stress tolerance. We observed a similar increase (Fig. 25) in lines SHDR 16 (30%), SHDR 17 (38%), SHDR 18 (39%), SHDR 49 (52%), SHDR 45 (57%), SHDR 50 (56%), SHDR 52 (38%), SHDR 53 (37%), and Baviacora (59%) after drought. These lines can be considered drought tolerant; they performed well under drought stress by accumulating soluble sugars. Lines SHDR 21 (7%), SHDR 32 (2%), SHDR 29 (15%), and SHDR 43 (9%) accumulated a minimum amount of soluble sugars (Fig. 25) and can be considered sensitive. Soluble sugar content proved to be a better marker for selecting improvement to drought tolerance in durum wheat than proline content (Al Hakimi et al. 1995).

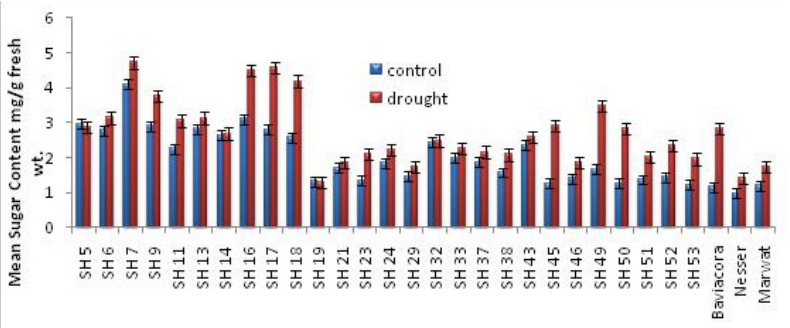

Fig. 25. Soluble sugar content (mg/g fresh weight) of synthetic hexaploid wheat population grown at control (T_0) and drought (30% PEG (T_1)) conditions.

Drought stress induced oxidative stress in plants that exhibited high H_2O_2 and oxidized ascorbate levels in relation to the prolonged drought period. A weak antioxidant enzymes response leading to enhanced membrane damage during severe drought stress was indicated by the accumulation of malondialdehyde. Suproxide dismutase, catalase, and andascorbic peroxidises have been related with water deficiency and are considered the main components of anti-oxidative machinery for drought resistance in higher plants (Bergmann et al. 1999). Tian and Lei (2007) advocated that the activities of superoxide dismutase increased under drought. Lines SHDR 6 (42%), SHDR 7 (36%), SHDR 9 (41%), Baviacora (41%), SHDR 46 (52%), SHDR 53 (36%), SHDR 45 (42%), SHDR 49 (60%), SHDR 50 (42%), SHDR 51 (43%), and SHDR 52 (34%) showed a significant increase in SOD after drought stress (Fig. 26). These lines have the good, anti-oxidative machinery for drought resistance and can be considered tolerant, which was confirmed in Al-Ghamdi (2009).

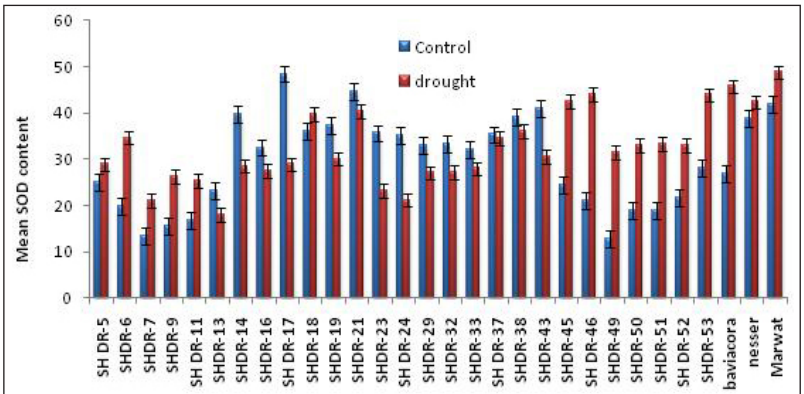

Fig. 26. Superoxide dismutase content (μ units/mg fresh weight) of synthetic hexaploid wheat population grown at control (T_0) and drought (30% PEG (T_1)) conditions.

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Evaluation of molecular mapping population from a 'wheat/synthetic hexaploid' cross for drought tolerance.

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In Pakistan, wheat production is very low in the rain-fed areas because of an uneven and unpredictable rainfall. Therefore, the production of high-yielding and drought tolerant cultivars is the main challenge for the plant breeders. QTL mapping, identifying groups of genes by mapping regions of the genome responsive to a particular stress, is used to enhance the efficiency of plant breeding through the use of molecular markers. Genotyping and phenotyping are the two categories of QTL mapping. The combined use of molecular, morphological, and physiological markers is proven to be one of the best approaches for coping with major abiotic stresses such as drought. This study is a morphological and physiological characterization of a double-haploid-based, molecular mapping population (bread wheat/synthetic hexaploid (SH) wheat) for various phenotypic parameters for drought tolerance. Our main objectives were to (a) evaluate and phenotypically characterize a double-haploid mapping population under *in vivo* conditions for drought tolerance and (b) evaluate and phenotype the mapping population under *in vitro* conditions.

The germ plasm studied was a molecular mapping population for drought tolerance produced by the cross 'Opata (susceptible bread wheat cultivar)/SH (tolerant D67.2/P66.270//*Ae. tauschii* 257)'. The F_1s were crossed with maize to produce haploids, which after a colchicine application, produced double haploids (DH). Of the original 142 individuals, 128 entries were evaluated under *in vivo* and *in vitro* conditions. The data recorded were subjected to an analysis of variance technique at probability level of 0.05 for all the parameters recorded in the field, tunnel, and for PEG-induced stress studies to observe the level of variation in all accessions.

In vivo* evaluation. *Plant height (cm). Plant height under controlled conditions ranged from 54.5 to 117.2 cm with an average height of 95.1 cm. Under drought conditions, height ranged from 46.9 to 115.0 cm with an average plant height of 85.0 cm; a decreasing trend with an average decrease of 10.6% compared to the average plant height of the control. Mapping population entries 126, 80, 125, 42, 89, and 145 had a decreased height by 2.0, 5.3, 3.7, 14.0, 30.0, and 33.1% respectively, when compared with control (Table 26, continued on p. 163).

Table 26. Mean values for plant height (cm) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Plant height			Plant height			Plant height			Plant height	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	107.3	82.7	45	95.6	79.8	85	96.6	92.4	119	99.9	83.1
4	106.3	96.1	46	93.4	64.2	86	92.57	78.4	120	101.5	94.2
5	102.3	90.6	47	92.4	63.1	87	111.7	105.1	121	103.1	81.4
7	101.3	92.4	48	95.7	90.3	88	97.6	87.4	122	106.3	105.0
8	104.1	84.5	49	96.7	90.2	89	69.1	48.7	123	106.3	99.2
9	92.6	80.0	51	80.7	76.9	90	100.6	82.6	124	106.6	103.2
12	98.8	88.5	53	66.1	57.2	91	97.4	80.4	125	110.4	106.3
13	91.3	70.5	55	97.9	94.5	92	92.1	76.2	126	117.2	115.7
14	94.5	83.7	57	96.7	90.1	93	81.5	77.2	127	100.5	95.3
15	85.4	72.2	58	63.3	57.8	94	85.6	85.1	128	107.3	97.4
16	80.4	74.6	59	102.5	94.3	95	103.9	102.4	130	80.7	71.7
17	90.2	82.5	60	98.9	87.7	97	77.8	73.1	132	87.5	77.3
18	87.3	83.1	61	90.1	88.8	98	96.5	83.9	133	104.7	100.2
19	82.6	78.5	62	93.5	93.3	99	95.6	92.2	134	108.1	101.3
21	96.4	78.3	63	112.9	99.9	100	98.8	92.2	135	100.4	93.6
22	95.6	89.7	64	81.8	62.4	101	95.4	90.5	136	112.2	81.4
23	100.6	93.6	67	86.2	76.5	102	97.0	86.2	137	101.1	92.3
24	97.8	96.4	68	96.2	95.3	103	96.6	80.6	138	95.7	86.4
26	96.3	98.6	69	105.3	101.2	104	86.2	68.6	139	102.9	87.4

Table 26. Mean values for plant height (cm) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Plant height			Plant height			Plant height			Plant height	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
27	100.9	96.8	71	91.2	86.5	105	104.1	91.1	140	102.9	76.3
28	100.4	90.7	72	89.6	76.6	106	75.60	57.6	141	75.0	73.4
30	61.6	55.4	73	100.3	98.6	108	95.83	87.5	142	103.1	81.2
31	78.3	69.9	74	100.2	98.1	109	103.0	91.4	143	81.5	55.9
32	87.3	65.3	75	94.3	84.7	110	102.3	94.4	144	93.7	68.6
33	95.4	89.2	76	103.3	81.1	111	103.3	92.0	145	74.0	49.5
35	83.4	65.5	77	101.5	92.5	112	99.2	88.2	146	99.5	93.1
37	96.5	89.2	78	94.5	88.5	113	98.6	94.1	147	94.5	88.5
39	109.7	100.5	79	104.4	94.6	114	93.3	74.5	148	94.5	84.5
40	105.6	99.1	80	113.7	107.7	115	85.6	71.2	149	92.4	83.6
41	97.5	59.2	81	117.2	103.0	116	81.5	72.3	150	100.7	97.4
42	54.5	46.9	82	110.2	104.4	117	102.3	99.1	151	101.0	97.1
43	61.4	50.3	84	88.4	82.4	118	99.7	98.9	152	103.6	95.0

Days-to-flowering. The data regarding mean values for days-to-flowering showed a range of 107 to 149 days with an average of 127 days under field conditions (Table 27). Entry 31 took 107 days to flower and was the earliest of all the genotypes studied in the field, followed by entries 26 and 151 with 109 and 111 days.

Table 27. Mean values for days-to-flowering under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Plant height			Plant height			Plant height			Plant height	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	122.0	105.7	45	144.0	121.7	85	121.0	115.0	119	111.0	109.7
4	114.7	109.0	46	137.0	134.7	86	125.0	121.0	120	138.0	126.0
5	132.7	110.0	47	131.0	103.3	87	123.0	112.0	121	130.0	114.0
7	132.0	130.0	48	132.0	127.3	88	129.0	114.0	122	140.0	129.0
8	123.0	128.7	49	119.0	112.7	89	130.0	110.0	123	124.0	121.0
9	125.7	115.7	51	119.0	118.3	90	126.0	112.0	124	129.0	117.7
12	138.7	130.3	53	125.0	113.3	91	126.0	117.0	125	135.0	117.7
13	127.3	125.3	55	121.0	105.7	92	124.0	121.0	126	134.0	120.7
14	113.3	111.7	57	119.0	107.0	93	130.0	128.3	127	137.3	122.3
15	121.0	109.7	58	135.7	124.7	94	132.0	129.0	128	121.0	109.0
16	122.3	121.0	59	116.0	113.0	95	135.0	130.3	130	139.0	126.0
17	121.0	120.7	60	118.0	113.7	97	123.0	120.7	132	122.0	115.7
18	119.7	118.3	61	115.0	113.3	98	130.0	120.7	133	134.0	117.0
19	130.3	121.0	62	120.0	112.3	99	133.0	120.7	134	137.0	118.0
21	116.7	112.3	63	112.0	105.0	100	134.0	124.0	135	140.0	121.0
22	125.3	112.7	64	131.0	113.7	101	129.0	125.3	136	143.0	122.0
23	125.3	107.3	67	131.0	129.7	102	136.0	131.0	137	130.0	127.0
24	127.0	117.0	68	115.0	109.3	103	135.0	128.3	138	142.0	123.3
26	109.0	118.3	69	121.0	110.0	104	133.0	129.7	139	118.0	105.0
27	120.0	110.3	71	129.0	121.3	105	132.0	127.3	140	120.0	117.0
28	132.0	121.0	72	139.0	121.7	106	135.0	131.0	141	121.0	113.0
30	127.0	122.7	73	149.0	117.3	108	138.0	125.0	142	132.0	106.3
31	107.0	106.7	74	120.0	117.7	109	140.0	131.0	143	118.0	117.0
32	114.0	107.0	75	121.0	106.0	110	131.0	108.0	144	112.0	107.3
33	134.0	123.3	76	116.0	105.0	111	126.0	110.0	145	131.0	109.7
35	129.3	111.0	77	140.0	122.0	112	112.0	119.0	146	112.0	107.3
37	145.0	134.3	78	136.0	115.0	113	123.0	116.0	147	115.0	113.0
39	131.0	129.0	79	124.0	123.0	114	122.0	113.0	148	112.0	107.0
40	124.0	107.7	80	115.0	109.0	115	141.0	130.0	149	116.0	106.0
41	130.0	120.0	81	133.0	115.0	116	139.0	126.0	150	128.0	109.0
42	132.0	117.7	82	140.0	123.3	117	120.0	113.7	151	111.0	107.3
43	133.0	122.0	84	135.0	134.3	118	120.0	101.7	152	134.0	119.0

In the tunnel, under drought conditions, entry 118 was the earliest of all the genotypes studied with mean value of 101.7 days, followed by entry 47 (103.3 days), and 2, 55, 63, 76, and 139 all with 105 days-to-flowering. Under drought conditions, days-to-flowering ranged from 101.7 to 134.5 days with an average of 118 days.

Days-to-physiological maturity. The average number of days to physiological maturity was 154 for the genotypes sown under control conditions, the range was 139.0–168.7 days. The minimum number of days to plant maturity under field conditions were recorded in genotypes 23 and 26 (138.7 days), followed by 149 (139.7) and 73 (139.0). Entry 118 was the earliest maturing genotype, taking 124 days to mature under drought conditions. The mean value of days-to-physiological maturity ranged from 124 to 155 days, with an average of 138 days under drought stress. A decrease in days to physiological maturity was observed in genotypes 67 (11 days earlier); 12 (12 days earlier than the control); 46, 93 and 109, (13 days earlier), 68 (14 days earlier); 95 (15 days earlier); 76 and 118 (19 days earlier); 151 (22 days earlier), and 90 (23 days earlier) compared to entries grown under control conditions (Table 28).

Table 28. Mean values for days-to-flowering under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Days-to-physiological maturity			Days-to-physiological maturity			Days-to-physiological maturity			Days-to-physiological maturity	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	147.7	127.3	45	164.0	140.0	85	153.0	134.3	119	140.7	129.0
4	145.7	128.3	46	168.7	155.0	86	156.7	140.0	120	164.3	146.3
5	152.3	129.0	47	168.0	142.3	87	145.7	132.0	121	155.7	134.7
7	161.0	148.3	48	162.7	148.7	88	156.3	132.3	122	163.7	150.3
8	156.3	141.3	49	148.0	132.7	89	155.0	129.3	123	155.0	141.7
9	153.0	131.3	51	147.3	135.7	90	149.7	126.7	124	152.7	137.0
12	163.7	151.0	53	153.0	133.3	91	152.7	134.0	125	152.7	135.0
13	160.3	144.3	55	146.7	127.7	92	150.3	142.7	126	151.3	138.3
14	146.7	132.0	57	149.0	129.7	93	164.0	151.0	127	155.7	138.7
15	152.0	137.7	58	161.0	141.0	94	159.3	149.3	128	150.0	136.3
16	145.3	137.0	59	147.3	130.3	95	166.0	151.0	130	160.7	143.0
17	149.7	137.7	60	141.3	133.0	97	148.3	131.3	132	151.0	137.0
18	149.3	142.7	61	143.7	131.0	98	159.0	141.7	133	149.3	135.7
19	161.0	148.7	62	147.3	133.3	99	163.7	145.7	134	157.3	140.7
21	145.0	139.7	63	147.3	128.0	100	161.0	146.0	135	159.0	139.7
22	144.7	138.3	64	154.0	131.0	101	165.0	147.3	136	168.0	144.7
23	138.7	126.0	67	162.3	151.0	102	167.0	150.3	137	157.7	147.3
24	149.3	135.3	68	140.0	126.7	103	164.0	147.7	138	157.0	145.7
26	138.7	129.3	69	146.0	129.3	104	164.7	149.0	139	146.3	127.7
27	151.7	136.7	71	160.3	144.0	105	161.7	146.7	140	152.0	141.3
28	156.0	147.0	72	160.7	139.0	106	163.3	152.3	141	150.0	138.7
30	152.7	143.7	73	139.0	130.3	108	161.0	146.0	142	154.0	128.0
31	141.3	131.7	74	151.0	136.3	109	164.3	151.0	143	145.7	132.3
32	141.7	127.7	75	151.3	129.3	110	152.0	132.3	144	142.3	130.0
33	157.7	142.3	76	144.7	125.7	111	147.3	131.0	145	149.0	129.0
35	154.7	127.3	77	162.3	145.3	112	151.0	138.7	146	142.3	127.3
37	167.3	150.3	78	156.3	132.7	113	149.0	131.0	147	152.3	132.7
39	154.7	141.0	79	153.7	136.7	114	146.0	127.3	148	146.7	127.7
40	152.3	133.7	80	144.7	129.3	115	157.7	148.7	149	139.7	127.0
41	155.3	135.3	81	156.0	135.3	116	159.7	145.3	150	150.7	129.7
42	160.7	138.0	82	163.0	144.0	117	146.0	132.0	151	147.7	125.3
43	156.3	137.0	84	166.3	155.0	118	143.0	124.0	152	150.3	144.7

Spike length. The maximum spike length of 13.30 cm was observed in 99 and 63 followed by entries 103 and 144 with spike length of 12.83 and 12.73 cm respectively. The data recorded for spike length under control condition ranged from 6.20 to 13.30 cm with an average spike length of 9.83 cm. Spike length under drought conditions ranged from 5.55 to 11.20 cm with an average value of 8.55 cm. Maximum spike length of 11.20 cm was recorded in entry 87 followed by genotypes 80, 78 with common value of 10.97 cm and 108 with 10.87 cm of spike length (Table 29, p. 165).

Table 29. Mean values for spike length (cm) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Spike length			Spike length			Spike length			Spike length	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	11.20	10.47	45	10.43	9.30	85	10.27	8.40	119	9.60	8.60
4	10.10	10.13	46	9.43	8.60	86	9.13	8.73	120	10.50	9.73
5	8.80	6.66	47	10.63	9.13	87	12.27	11.20	121	8.83	6.86
7	8.73	6.93	48	11.60	10.57	88	9.53	8.83	122	11.37	10.43
8	9.40	7.76	49	11.67	9.30	89	7.13	5.66	123	10.37	7.53
9	9.76	8.50	51	12.27	10.50	90	8.60	7.76	124	9.26	8.63
12	10.20	9.36	53	9.90	7.80	91	8.30	8.46	125	8.80	8.50
13	8.50	7.60	55	11.40	10.07	92	9.83	8.10	126	9.10	8.30
14	11.20	10.53	57	10.33	10.13	93	9.66	7.66	127	11.10	10.50
15	8.56	7.86	58	6.20	5.55	94	7.56	7.80	128	10.47	9.26
16	9.73	7.60	59	10.30	7.76	95	9.30	8.30	130	8.60	7.76
17	11.13	10.13	60	8.50	7.06	97	9.60	8.63	132	9.40	8.60
18	10.43	8.50	61	8.10	8.10	98	9.66	8.40	133	8.23	7.73
19	7.60	5.66	62	10.70	8.20	99	13.30	9.50	134	10.43	9.80
21	10.50	9.86	63	13.30	10.63	100	8.73	6.70	135	10.37	9.66
22	10.30	8.30	64	12.13	10.07	101	9.16	8.70	136	11.37	8.80
23	10.87	10.57	67	8.73	7.50	102	9.90	9.03	137	10.57	8.60
24	8.63	7.03	68	9.66	8.70	103	12.83	10.53	138	9.50	8.63
26	8.76	7.56	69	8.33	7.63	104	10.50	9.23	139	9.36	7.16
27	7.20	5.60	71	12.63	9.50	105	10.73	10.57	140	8.86	7.43
28	7.73	7.43	72	8.20	7.36	106	8.20	5.83	141	9.03	7.23
30	8.76	7.23	73	9.83	9.20	108	11.67	10.87	142	9.33	8.50
31	9.36	6.76	74	9.03	8.20	109	9.53	6.66	143	8.90	7.13
32	8.86	8.20	75	8.26	7.50	110	11.30	7.40	144	12.73	9.73
33	10.20	9.36	76	10.53	9.83	111	10.53	9.73	145	9.13	6.06
35	8.73	7.60	77	9.53	8.53	112	10.40	10.20	146	10.70	10.10
37	8.33	7.50	78	11.43	10.97	113	9.73	8.43	147	11.47	9.43
39	10.30	8.56	79	9.36	8.63	114	10.13	9.13	148	9.40	9.26
40	11.17	10.17	80	12.13	10.97	115	8.50	7.73	149	9.73	8.60
41	9.70	7.30	81	10.43	9.43	116	8.76	7.66	150	11.57	10.40
42	8.86	6.26	82	11.57	9.70	117	10.73	9.00	151	9.50	8.20
43	7.30	5.93	84	7.93	7.00	118	11.80	10.70	152	9.30	9.46

Number of grains/spike. The range of values of the drought mapping population for the number of grains/spike under control and stress conditions were 21.86–64.03 and 17.63–51.93, respectively (Table 30, p. 166). The average number of grains/spike was 42.87 under control conditions and 34.13 under drought stress. The highest mean value was recorded for entry 144, with mean value of 51.93, followed by genotypes 57 (51.40) and 150 (50.50).

1,000-kernel weight. Mean values for 1,000-kernel weight ranged from 25.56 to 60.66 g under control conditions and 15.60 to 50.53 g under drought. The maximum 1,000-kernel weight under control conditions of 60.66 g was for entry 63, followed by entries 108 (59.06 g) and 146 (58.90 g). Entry 148 had the highest 1,000-kernel weight (50.53 g) among all the genotypes under stress conditions (Table 31, pp. 166-167).

Pubescence. Only 14 entries, 4, 12, 16, 17, 18, 62, 84, 93, 99, 121, 123, 142, 147, and 151, were pubescent (Table 32, p. 167).

Waxiness. Of the drought mapping population, lines 4, 12, 17, 21, 37, 45, 46, 49, 99, 102, 121, 137, 142, 143, and 147 were waxy on their leaves. Genotypes 4, 12, 21, 37, 99, 121, 137, 142, and 147 were both waxy and pubescent (Table 32, p. 167).

***In vitro* evaluation.** The *in vitro* parameters included coleoptile length, proline content, chlorophyll content, and superoxide dismutase.

Coleoptile length. We had significant results for the mean value of the coleoptile length at four different treatment levels, control, 7.5% PEG, 15.0% PEG, and 22.5% PEG. Coleoptile length was 2.53–4.50 cm (3.18 cm average) in the control

Table 30. Mean values for number of grains/spike under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Grains/spike			Grains/spike			Grains/spike			Grains/spike	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	46.13	34.43	45	53.96	47.26	85	41.60	39.50	119	39.26	36.30
4	38.23	35.23	46	32.26	17.66	86	49.30	27.56	120	37.60	26.13
5	32.96	28.06	47	27.46	18.56	87	50.73	44.26	121	41.00	21.66
7	31.40	22.53	48	64.03	40.36	88	44.46	26.96	122	56.50	50.03
8	35.36	28.06	49	38.73	26.36	89	30.73	18.33	123	45.83	29.13
9	36.46	29.26	51	49.16	44.16	90	45.20	29.40	124	40.13	29.13
12	31.16	26.13	53	32.46	27.43	91	44.53	28.56	125	38.73	30.83
13	40.33	33.20	55	51.10	48.33	92	54.30	24.23	126	42.06	31.46
14	53.26	49.40	57	56.23	51.40	93	45.73	44.46	127	47.36	29.30
15	39.03	33.40	58	36.40	19.60	94	42.43	40.36	128	48.93	35.73
16	55.13	35.13	59	33.40	31.56	95	33.26	17.63	130	49.60	42.10
17	60.90	49.26	60	42.36	36.23	97	44.00	42.50	132	45.50	32.46
18	53.76	49.40	61	41.90	36.03	98	40.20	33.40	133	46.40	42.16
19	41.06	20.46	62	43.30	29.40	99	53.73	28.43	134	39.26	34.13
21	40.73	35.66	63	48.26	43.50	100	40.56	31.56	135	41.16	33.76
22	40.00	33.23	64	40.90	32.26	101	39.36	28.76	136	43.43	34.63
23	54.20	48.30	67	36.30	32.00	102	45.56	30.76	137	37.43	41.70
24	38.23	26.33	68	42.33	41.93	103	45.40	36.23	138	36.46	29.00
26	39.00	32.46	69	40.10	38.50	104	53.66	35.23	139	46.43	41.86
27	36.53	20.60	71	48.06	41.23	105	37.70	29.66	140	43.40	36.13
28	49.13	48.30	72	43.40	33.73	106	35.13	19.56	141	40.53	39.06
30	31.20	20.30	73	49.23	42.86	108	51.86	45.96	142	38.93	25.60
31	46.16	35.63	74	41.33	37.53	109	25.26	18.66	143	41.20	38.16
32	38.23	31.63	75	37.56	33.36	110	47.06	31.20	144	53.46	51.93
33	40.10	29.16	76	43.13	35.66	111	21.86	18.43	145	30.00	18.63
35	39.06	30.53	77	41.36	34.66	112	44.63	40.36	146	52.76	41.43
37	36.36	22.96	78	48.30	32.30	113	38.36	29.40	147	42.23	39.06
39	37.36	31.60	79	45.50	41.50	114	43.63	38.23	148	49.36	45.56
40	52.20	47.50	80	53.36	42.13	115	51.53	38.60	149	48.73	37.83
41	41.50	31.10	81	50.43	41.20	116	50.50	44.56	150	53.13	50.50
42	29.26	19.43	82	45.43	36.16	117	40.30	40.26	151	28.46	26.96
43	27.56	21.50	84	43.43	35.06	118	52.20	49.40	152	44.50	23.63

Table 31. Mean values for 1,000-kernel weight (g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	1,000-kernel weight			1,000-kernel weight			1,000-kernel weight			1,000-kernel weight	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	51.23	23.33	45	57.03	46.70	85	40.13	33.10	119	41.26	31.96
4	49.56	25.46	46	41.10	29.86	86	36.70	30.20	120	43.30	25.50
5	43.53	22.53	47	33.10	16.93	87	44.43	28.56	121	42.30	30.96
7	38.43	27.86	48	42.50	19.70	88	38.50	25.46	122	53.16	47.03
8	38.66	22.43	49	34.36	34.36	89	33.96	19.26	123	43.16	31.80
9	42.20	35.46	51	51.73	44.70	90	44.46	39.40	124	43.23	23.23
12	41.86	21.46	53	35.43	24.30	91	42.13	30.00	125	44.33	35.46
13	32.46	29.50	55	57.36	49.50	92	44.56	26.53	126	37.43	30.70
14	51.00	48.03	57	35.56	32.60	93	30.40	25.13	127	39.30	29.43
15	39.63	31.23	58	32.16	20.20	94	31.83	15.60	128	47.70	39.60
16	37.53	33.56	59	42.23	40.86	95	42.33	22.00	130	41.43	23.53
17	51.76	45.46	60	41.66	35.43	97	39.63	28.26	132	39.13	20.16
18	38.56	29.73	61	40.36	30.23	98	33.60	26.36	133	43.53	32.40
19	37.33	28.93	62	42.33	31.40	99	33.63	31.26	134	36.56	26.50
21	39.00	33.40	63	60.66	47.60	100	28.46	25.30	135	33.76	26.56
22	34.70	32.33	64	38.80	28.06	101	28.63	20.70	136	39.16	34.53
23	55.26	47.83	67	32.46	31.06	102	39.43	21.56	137	48.63	28.66
24	38.53	33.43	68	37.40	23.23	103	28.66	19.66	138	36.56	29.33

Table 31. Mean values for 1,000-kernel weight (g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	1,000-kernel weight			1,000-kernel weight			1,000-kernel weight			1,000-kernel weight	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
26	42.76	34.56	69	39.60	34.43	104	25.56	18.20	139	50.60	28.76
27	36.83	17.36	71	43.06	24.30	105	30.76	28.43	140	34.50	21.60
28	28.63	21.73	72	40.13	25.00	106	32.53	16.63	141	41.56	25.93
30	40.43	18.93	73	40.46	34.26	108	59.06	46.03	142	46.70	32.46
31	41.70	28.00	74	39.76	27.36	109	32.83	16.16	143	30.30	19.56
32	41.36	31.43	75	39.50	34.40	110	45.23	37.16	144	39.63	30.76
33	35.70	28.36	76	41.33	34.16	111	32.16	21.00	145	33.56	17.46
35	36.20	28.40	77	39.46	33.56	112	51.76	33.16	146	58.90	48.23
37	37.30	23.53	78	51.26	29.43	113	42.50	33.43	147	41.16	38.36
39	37.10	33.56	79	31.80	23.56	114	39.50	29.16	148	54.43	50.53
40	56.26	46.70	80	46.30	45.50	115	38.50	24.43	149	41.73	32.36
41	29.26	27.70	81	44.06	31.43	116	36.26	26.70	150	50.60	47.63
42	31.36	18.40	82	53.13	47.43	117	44.16	43.60	151	55.76	41.43
43	29.90	16.50	84	34.66	22.26	118	54.13	46.13	152	49.53	40.10

Table 32. Observations (+ = presence and – = absence) for leaf pubescence (PUB) and waxiness (WAX) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

Entry	PUB	WAX	Entry	PUB	WAX	Entry	PUB	WAX	Entry	PUB	WAX
2	–	–	45	–	+	85	–	–	119	–	–
4	+	+	46	–	+	86	–	–	120	–	–
5	–	–	47	–	–	87	–	–	121	+	+
7	–	–	48	–	–	88	–	–	122	–	–
8	–	–	49	–	+	89	–	–	123	+	–
9	–	–	51	–	–	90	–	–	124	–	–
12	+	+	53	–	–	91	–	–	125	–	–
13	–	–	55	–	–	92	–	–	126	–	–
14	–	–	57	–	–	93	+	–	127	–	–
15	–	–	58	–	–	94	–	–	128	–	–
16	+	–	59	–	–	95	–	–	130	–	–
17	+	+	60	–	–	97	–	–	132	–	–
18	+	–	61	–	–	98	–	–	133	–	–
19	–	–	62	+	–	99	+	+	134	–	–
21	–	+	63	–	–	100	–	–	135	–	–
22	–	–	64	–	–	101	–	–	136	–	–
23	–	–	67	–	–	102	–	+	137	–	+
24	–	–	68	–	–	103	–	–	138	–	–
26	–	–	69	–	–	104	–	–	139	–	–
27	–	–	71	–	–	105	–	–	140	–	–
28	–	–	72	–	–	106	–	–	141	–	–
30	–	–	73	–	–	108	–	–	142	+	+
31	–	–	74	–	–	109	–	–	143	–	+
32	–	–	75	–	–	110	–	–	144	–	–
33	–	–	76	–	–	111	–	–	145	–	–
35	–	–	77	–	–	112	–	–	146	–	–
37	–	+	78	–	–	113	–	–	147	+	+
39	–	–	79	–	–	114	–	–	148	–	–
40	–	–	80	–	–	115	–	–	149	–	–
41	–	–	81	–	–	116	–	–	150	–	–
42	–	–	82	–	–	117	–	–	151	+	–
43	–	–	84	+	–	118	–	–	152	–	–

plants, 1.36–3.16 cm (2.42 cm average) in the 7.5% PEG treatment, 0.83–2.50 (1.70 cm average) in the 15.0% PEG treatment, and 0.0–1.83 cm (0.57 cm average) in the 22.5% PEG treatment. Entry 77 had the best performance, the highest among the genotypic means with a coleoptile length of 2.97 cm, followed by genotypes 14 (2.76 cm) and 122 (2.71 cm). The data for all four levels of treatment showed that coleoptile length decreased under artificially induced stress (Table 33, continued on p. 169). The minimum reduction was observed at 7.5% PEG with an average decrease of 28.89% in coleoptile length compared to the average mean of the control; the maximum reduction was at 22.5% PEG, where the average decrease was 82.07% compared to the treatment mean of the control. At 15.0% PEG, a decrease of 46.54% was inbetween that of 7.5% and 22.5%, showing a continued decrease from lower to higher PEG concentrations. The genotypes showing a response under the maximum stress (22.5%) are more tolerant to drought and those higher mean values for coleoptiles length under all three PEG treatments are good for drought conditions.

Table 33. Mean values for coleoptile length (cm) at 0.0%, 7.5%, 15.0%, and 22.5% polyethylene glycol for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Coleoptile length (cm)						Coleoptile length (cm)				
Entry	Control	7.5% PEG	15.0% PEG	22.5% PEG	Mean	Entry	Control	7.5% PEG	15.0% PEG	22.5% PEG	Mean
2	3.16	2.50	1.43	0.86	1.99	86	3.03	2.70	1.83	0.00	1.89
4	3.56	2.66	1.70	1.23	2.29	87	2.86	2.23	1.63	0.00	1.68
5	3.06	2.40	1.70	1.16	2.08	88	3.16	2.50	1.66	1.76	2.27
7	2.96	2.40	1.26	0.00	1.65	89	3.56	2.73	1.96	0.00	2.06
8	2.96	2.60	1.73	0.00	1.82	90	3.06	2.60	1.80	0.00	1.86
9	3.73	2.56	1.76	0.00	2.01	91	3.16	2.43	1.80	0.00	1.85
12	3.00	2.16	1.43	0.83	1.85	92	3.43	2.66	1.60	0.00	1.92
13	3.00	2.20	1.63	1.00	1.95	93	3.16	2.40	1.70	0.00	1.81
14	4.06	3.06	2.23	1.70	2.76	94	3.33	2.53	1.80	0.00	1.91
15	2.90	2.03	1.36	0.00	1.57	95	3.60	2.80	1.83	0.00	2.05
16	3.00	2.26	1.53	0.00	1.70	97	2.86	2.23	1.60	0.00	1.67
17	3.80	2.96	2.36	1.40	2.63	98	3.33	2.33	1.73	0.00	1.85
18	2.96	2.43	1.73	1.20	2.08	99	3.70	3.03	2.10	0.00	2.20
19	3.13	2.43	1.63	1.23	2.10	100	3.50	2.90	1.93	0.00	2.08
21	2.96	2.50	1.73	1.33	2.13	101	3.43	2.83	1.96	0.00	2.05
22	3.06	2.40	1.53	0.96	1.99	102	3.06	2.33	1.76	0.00	1.79
23	3.33	2.76	2.13	1.16	2.35	103	3.33	2.60	1.80	0.00	1.93
24	3.03	2.33	1.80	1.33	2.12	104	2.86	1.96	1.36	0.00	1.55
26	3.43	2.46	1.73	1.23	2.21	105	3.00	2.43	1.60	0.00	1.75
27	2.53	1.83	1.13	0.00	1.37	106	3.00	2.43	1.73	0.00	1.79
28	3.53	2.60	1.83	0.00	1.99	108	3.26	2.30	1.93	0.00	1.87
30	3.03	2.40	1.66	1.33	2.10	109	3.70	2.93	1.80	0.00	2.10
31	3.03	2.53	1.73	1.23	2.13	110	2.96	2.30	1.93	0.00	1.80
32	3.16	2.53	1.80	1.20	2.17	111	3.00	1.73	0.90	0.00	1.40
33	3.16	2.50	1.73	1.40	2.20	112	3.03	2.13	1.93	0.00	1.77
35	3.36	2.76	2.13	1.46	2.43	113	2.63	1.80	1.03	0.00	1.36
37	2.96	2.10	1.63	1.20	1.97	114	3.06	2.13	1.63	0.00	1.70
39	3.56	2.56	1.83	1.43	2.35	115	3.40	2.33	1.56	0.00	1.82
40	3.13	2.26	1.80	1.13	2.08	116	3.03	2.46	1.70	0.00	1.80
41	2.90	2.26	1.50	1.03	1.92	117	3.23	2.46	1.86	0.00	1.89
42	2.90	1.80	1.10	0.00	1.45	118	3.50	2.76	2.00	0.00	2.06
43	2.93	1.73	1.03	0.00	1.42	119	3.36	2.83	2.00	0.00	2.05
45	2.86	2.33	1.66	1.23	2.02	120	3.26	2.50	2.03	0.00	1.95
46	3.33	2.63	2.00	1.56	2.38	121	3.40	2.50	1.66	0.00	1.89
47	2.56	1.60	1.03	0.00	1.30	122	3.76	3.00	2.50	1.60	2.71
48	3.00	2.60	1.73	1.43	2.19	123	3.06	2.46	2.03	1.43	2.25
49	3.50	2.80	1.93	1.16	2.35	124	2.96	1.36	0.83	0.00	1.29
51	3.43	2.60	1.83	1.36	2.30	125	3.13	2.26	2.00	1.53	2.23
53	3.46	2.63	1.90	1.33	2.33	126	3.00	1.96	1.43	1.00	1.85
55	3.06	2.50	1.66	0.00	1.80	127	3.13	2.33	1.66	0.00	1.78
57	2.96	2.63	1.86	0.00	1.86	128	2.86	1.90	1.30	0.00	1.51
58	2.86	2.40	1.83	1.00	2.02	130	3.30	2.33	1.73	1.30	2.16
59	2.86	2.30	1.60	0.00	1.69	132	3.26	2.40	1.86	1.30	2.20
60	3.06	2.53	1.80	1.40	2.20	133	3.70	2.90	2.06	1.63	2.57

Table 33. Mean values for coleoptile length (cm) at 0.0%, 7.5%, 15.0%, and 22.5% polyethylene glycol for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Coleoptile length (cm)						Coleoptile length (cm)				
Entry	Control	7.5% PEG	15.0% PEG	22.5% PEG	Mean	Entry	Control	7.5% PEG	15.0% PEG	22.5% PEG	Mean
61	3.16	2.76	2.03	1.46	2.35	134	3.56	2.96	2.16	1.60	2.57
62	4.10	3.16	2.43	0.00	2.42	135	3.10	2.26	1.60	1.13	2.02
63	3.20	2.50	1.86	0.00	1.89	136	2.90	1.76	1.10	1.03	1.70
64	3.00	2.53	1.83	1.23	2.15	137	3.23	2.43	1.80	1.33	2.20
67	3.03	2.53	1.90	0.96	2.10	138	3.16	2.26	1.70	0.00	1.78
68	3.13	2.50	1.83	1.13	2.15	139	3.23	2.36	1.70	1.20	2.12
69	3.43	2.60	1.86	0.00	1.97	140	3.16	2.36	1.70	0.00	1.80
71	2.86	1.70	1.03	0.00	1.40	141	2.96	2.23	1.06	0.00	1.56
72	3.06	2.40	1.66	0.00	1.78	142	3.00	2.23	1.33	0.00	1.64
73	2.96	2.46	1.53	0.00	1.74	143	2.90	2.20	1.50	0.96	1.89
74	2.80	1.76	0.90	0.00	1.36	144	3.03	2.10	1.56	1.40	2.02
75	3.23	2.53	1.73	0.00	1.87	145	3.30	2.40	1.73	0.00	1.85
76	2.96	2.20	1.60	1.06	1.95	146	3.06	2.40	1.53	1.00	2.00
77	4.50	3.06	2.50	1.83	2.97	147	2.86	1.90	1.13	0.56	1.61
78	3.20	2.33	1.76	1.46	2.19	148	3.40	2.36	1.36	1.00	2.03
79	3.00	2.43	1.80	0.00	1.80	149	3.23	2.43	1.90	0.00	1.89
80	3.70	3.00	2.00	0.00	2.17	150	3.53	2.93	1.83	1.33	2.40
81	3.33	2.53	1.93	0.00	1.95	151	3.10	2.26	1.50	1.20	2.01
82	3.20	2.90	2.13	0.00	2.05	152	3.16	2.46	1.50	0.56	1.92
84	3.00	2.40	1.80	0.00	1.80	Mean	3.19	2.42	1.70	0.57	—
85	3.33	2.50	1.86	0.00	1.92						

Physiological laboratory tests. ***Analysis of proline content.*** Proline content ranged from 84.10 to 5324.00 μg/g with an average value of 949.22 μg/g under control conditions. The maximum proline content accumulation under controled conditions was in entry 128 (5,324.00 μg/g), followed by entries 14 (5,016 μg/g) and 17 (4,958 μg/g). The highest proline accumulation under drought conditions was 9,277.30 μg/g in genotype 14, followed by entries 17 (8,837.90 μg/g) and 152 (8,199.80 μg/g). Proline content ranged from 399.00 to 9,277.30 μg/g with an average of 3,626.79 μg/g under water-stress (Table 34, p. 170).

Analysis of chlorophyll content. Examination of photosynthetic activity, including various other physiological parameters, is a useful approach. The germ plasm under study was measured for chlorophyll a, chlorophyll b, and total chlorophyll content.

Chlorophyll a content. The chlorophyll a content 0.293–1.396 mg/g under control and 0.115–1.190 mg/g under drought conditions with average values of 0.697 mg/g and 0.472 mg/g, respectively (Table 35, p. 171). The maximum chlorophyll a content was in entry 152 (1.396 mg/g), followed by entries 151 (1,180 mg/g) and 148 (1.168 mg/g). Under drought conditions, the maximum mean value was 1.190 mg/g for entry 152, followed by genotypes 89 (0.909 mg/g) and 49 (0.893 mg/g). When the entries of the mapping population with the highest and lowest means (drought tolerant and drought susceptible) under drought were compared to the control means, a decreasing trend in chlorophyll a content was observed for most of the genotypes. A decrease in chlorophyll a content compared to their mean values under controled conditions were noted in entries 152 (14.75%), 89 (18.83%), 149 (16.22%), 99 (61.66%), 100 (74.67%), and 75 (67.99%). Entries 152, 89, and 149 were drought tolerant; the percent reduction was less. A significant decrease in chlorophyll a content was observed in genotypes 99, 100, and 75, showed their poor performance under drought because lower chlorophyll content directly imposes negative effects on photosynthetic activity.

Chlorophyll b content. The maximum value for chlorophyll b content under control conditions was observed in entry 152 (0.665 mg/g), followed by entries 89 (0.562 mg/g) and 151 (0.545 mg/g). Chlorophyll b means were 0.158–0.655 mg/g with an average mean value of 0.331 mg/g for the control conditions (Table 36, p. 170). Under drought conditions, the maximum mean value for chlorophyll b content was in genotype 82 (0.656 mg/g), followed by genotypes 86 (0.623 mg/g) and 152 (0.561 mg/g). Comparing the mapping population entries with the high and low mean values under drought conditions to the control conditions revealed that the chlorophyll b content decreased under stress, except in entries 82 (65.00% increase) and 86 (70.46% increase). This increasing mean value from the general decreasing trend in other genotypes for mean chlorophyll b content may suggest that these genotypes will perform better under drought con-

Table 34. Mean values for proline content (μg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Proline content			Proline content			Proline content			Proline content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	4,492.0	5,894.8	45	1,578.0	4,913.6	85	1,715.0	3,613.2	119	761.6	3,572.7
4	1,062.0	2,839.2	46	3,134.0	7,006.8	86	1,090.0	3,683.3	120	1,086.0	3,401.4
5	1,532.0	4,127.2	47	286.8	444.7	87	1,037.0	4,539.9	121	637.0	2,312.8
7	756.9	4,268.3	48	1,216.0	4,857.6	88	1,070.0	455.5	122	1,780.0	6,091.5
8	485.9	6,959.3	49	658.8	3,680.2	89	3,576.0	5,827.6	123	574.7	453.8
9	543.5	4,529.0	51	1,019.0	3,614.7	90	593.4	5,517.9	124	345.4	4,398.2
12	1,803.0	3,272.1	53	870.6	1,932.7	91	1,715.0	4,186.3	125	903.3	2,926.4
13	638.5	3,751.8	55	753.8	3,258.1	92	725.8	3,141.3	126	565.3	1,384.5
14	5,016.0	9,277.3	57	467.2	3,251.8	93	517.1	3,624.1	127	845.7	5,510.1
15	1,059.0	7,017.7	58	531.1	3,991.7	94	552.9	3,730.0	128	5,324.0	7,248.9
16	191.6	640.1	59	467.2	3,991.6	95	448.5	4,222.2	130	652.6	1,370.5
17	4,958.0	8,837.9	60	352.0	1,389.2	97	496.8	4,667.6	132	487.5	1,537.2
18	394.0	1,348.7	61	426.7	2,766.0	98	1,109.0	3,289.3	133	610.5	5,765.5
19	250.7	1,168.1	62	576.2	2,404.6	99	704.0	3,547.8	134	465.7	4,381.0
21	319.3	974.9	63	3,926.0	4,309.0	100	825.4	2,817.4	135	336.4	2,818.9
22	422.1	1,585.4	64	465.7	2,963.8	101	443.9	2,230.2	136	411.2	4,315.6
23	4,900.0	6,044.1	67	380.0	2,382.8	102	118.4	819.2	137	529.5	5,740.6
24	542.0	411.8	68	856.6	2,748.8	103	119.9	1,002.9	138	456.3	6,200.1
26	183.8	730.4	69	345.7	2,431.1	104	84.1	543.5	139	342.6	6,234.3
27	239.2	971.8	71	359.8	594.0	105	95.0	942.2	140	894.0	7,153.2
28	311.5	669.7	72	291.2	2,794.0	106	154.2	1,264.6	141	404.9	6,033.4
30	241.4	399.0	73	451.7	4,402.8	108	87.2	6,450.2	142	478.1	8,020.7
31	232.1	1,094.9	74	570.0	4,810.8	109	95.0	3,198.9	143	372.2	5,405.8
32	292.8	769.4	75	462.6	3,111.7	110	193.1	4,578.8	144	403.4	4,486.9
33	574.7	1,060.6	76	352.0	3,063.4	111	767.8	1,784.8	145	580.9	6,039.7
35	1,726.0	4,130.3	77	383.1	3,236.3	112	922.0	1,350.3	146	325.5	6,402.5
37	2,402.0	3,754.9	78	319.3	2,912.4	113	341.3	411.4	147	344.2	6,742.0
39	1,880.0	4,963.5	79	640.1	1,094.9	114	1,104.0	1,348.7	148	447.0	6,888.5
40	4,306.0	7,575.6	80	390.9	2,426.5	115	1,036.0	642.4	149	363.8	439.8
41	3,221.0	4,893.4	81	602.7	3,554.0	116	1,335.0	3,026.0	150	417.4	6,924.3
42	369.8	3,645.9	82	627.6	5,991.5	117	996.7	4,102.2	151	587.1	7,416.4
43	4,224.0	481.4	84	146.4	3,142.9	118	881.5	6,290.4	152	336.4	8,199.8

Table 35. Mean values for chlorophyll a content (mg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Chlorophyll a content			Chlorophyll a content			Chlorophyll a content			Chlorophyll a content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	0.380	0.289	45	0.624	0.418	85	0.582	0.551	119	0.863	0.831
4	0.365	0.225	46	0.868	0.407	86	1.000	0.486	120	0.505	0.433
5	0.312	0.247	47	0.730	0.519	87	0.642	0.309	121	0.898	0.790
7	0.362	0.306	48	1.067	0.476	88	0.804	0.490	122	0.964	0.498
8	0.357	0.270	49	0.785	0.622	89	1.120	0.909	123	0.479	0.314
9	0.424	0.274	51	0.754	0.281	90	0.695	0.194	124	0.730	0.542
12	0.374	0.293	53	0.942	0.654	91	0.794	0.614	125	0.796	0.587
13	0.353	0.237	55	0.791	0.581	92	0.517	0.419	126	0.596	0.481
14	0.381	0.285	57	0.764	0.406	93	0.664	0.459	127	0.745	0.529
15	0.595	0.336	58	0.619	0.424	94	0.661	0.568	128	0.908	0.753
16	0.658	0.497	59	0.519	0.303	95	0.631	0.413	130	0.802	0.657
17	0.751	0.639	60	0.659	0.466	97	0.934	0.634	132	0.919	0.669
18	0.861	0.675	61	0.473	0.268	98	0.293	0.169	133	0.970	0.520
19	0.961	0.712	62	0.757	0.324	99	0.300	0.115	134	0.852	0.731
21	0.719	0.393	63	0.661	0.233	100	0.538	0.135	135	1.100	0.724
22	0.794	0.518	64	0.660	0.263	101	0.438	0.228	136	1.090	0.768
23	0.628	0.290	67	0.521	0.217	102	0.574	0.348	137	0.846	0.696

Table 35. Mean values for chlorophyll a content (mg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Chlorophyll a content			Chlorophyll a content			Chlorophyll a content			Chlorophyll a content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
24	0.716	0.578	68	0.366	0.154	103	0.371	0.306	138	0.843	0.728
26	0.592	0.473	69	0.821	0.187	104	0.585	0.331	139	0.598	0.428
27	0.613	0.424	71	0.613	0.369	105	0.901	0.370	140	0.604	0.518
28	0.673	0.643	72	0.782	0.232	106	0.873	0.479	141	0.809	0.646
30	0.744	0.468	73	0.663	0.399	108	0.760	0.455	142	0.798	0.530
31	0.763	0.418	74	0.300	0.476	109	0.586	0.516	143	0.855	0.819
32	0.591	0.272	75	0.453	0.145	110	0.640	0.515	144	0.670	0.425
33	0.691	0.452	76	0.360	0.175	111	0.759	0.447	145	1.046	0.875
35	0.476	0.328	77	0.509	0.438	112	0.638	0.516	146	0.992	0.596
37	0.514	0.338	78	0.435	0.367	113	0.580	0.366	147	1.038	0.867
39	0.582	0.410	79	0.324	0.219	114	0.678	0.401	148	1.168	0.686
40	0.898	0.856	80	0.653	0.469	115	0.655	0.451	149	1.066	0.893
41	0.823	0.592	81	0.637	0.458	116	0.405	0.178	150	1.101	0.862
42	0.749	0.622	82	0.835	0.491	117	0.612	0.316	151	1.180	0.866
43	0.704	0.648	84	0.773	0.520	118	0.673	0.375	152	1.396	1.190

Table 36. Mean values for chlorophyll b content (mg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Chlorophyll b content			Chlorophyll b content			Chlorophyll b content			Chlorophyll b content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	0.307	0.216	45	0.296	0.217	85	0.330	0.285	119	0.422	0.227
4	0.265	0.205	46	0.421	0.239	86	0.184	0.623	120	0.272	0.235
5	0.226	0.219	47	0.344	0.241	87	0.168	0.447	121	0.420	0.377
7	0.290	0.231	48	0.454	0.222	88	0.199	0.491	122	0.496	0.280
8	0.262	0.217	49	0.331	0.269	89	0.562	0.380	123	0.268	0.214
9	0.317	0.239	51	0.336	0.123	90	0.383	0.152	124	0.389	0.261
12	0.271	0.273	53	0.303	0.244	91	0.430	0.341	125	0.448	0.262
13	0.332	0.305	55	0.338	0.235	92	0.293	0.236	126	0.280	0.237
14	0.195	0.163	57	0.326	0.209	93	0.335	0.255	127	0.398	0.238
15	0.239	0.242	58	0.267	0.165	94	0.342	0.311	128	0.459	0.299
16	0.283	0.228	59	0.204	0.149	95	0.307	0.224	130	0.385	0.328
17	0.335	0.291	60	0.287	0.218	97	0.412	0.307	132	0.518	0.363
18	0.372	0.303	61	0.207	0.189	98	0.198	0.108	133	0.441	0.311
19	0.435	0.303	62	0.334	0.203	99	0.219	0.104	134	0.442	0.291
21	0.315	0.173	63	0.283	0.125	100	0.317	0.111	135	0.454	0.384
22	0.294	0.238	64	0.275	0.164	101	0.279	0.163	136	0.465	0.207
23	0.337	0.150	67	0.227	0.107	102	0.284	0.187	137	0.402	0.357
24	0.347	0.267	68	0.167	0.069	103	0.203	0.150	138	0.476	0.344
26	0.239	0.208	69	0.356	0.122	104	0.193	0.179	139	0.308	0.265
27	0.266	0.175	71	0.270	0.191	105	0.431	0.204	140	0.293	0.275
28	0.336	0.208	72	0.335	0.096	106	0.332	0.166	141	0.437	0.341
30	0.309	0.179	73	0.271	0.169	108	0.346	0.219	142	0.395	0.259
31	0.265	0.199	74	0.275	0.084	109	0.298	0.255	143	0.455	0.429
32	0.236	0.100	75	0.196	0.090	110	0.318	0.270	144	0.384	0.199
33	0.283	0.166	76	0.191	0.104	111	0.383	0.254	145	0.497	0.352
35	0.241	0.173	77	0.272	0.249	112	0.338	0.264	146	0.417	0.364
37	0.228	0.154	78	0.214	0.177	113	0.295	0.204	147	0.475	0.392
39	0.268	0.156	79	0.161	0.126	114	0.357	0.186	148	0.500	0.307
40	0.417	0.247	80	0.371	0.289	115	0.344	0.234	149	0.509	0.400
41	0.367	0.276	81	0.222	0.406	116	0.158	0.113	150	0.515	0.376
42	0.318	0.261	82	0.224	0.656	117	0.312	0.197	151	0.545	0.355
43	0.303	0.264	84	0.406	0.299	118	0.365	0.226	152	0.655	0.561

ditions. Chlorophyll content decreases with water deprivation, such as under drought conditions, and dehydration occurs in the plant cell.

Total chlorophyll content. Entry 152 had the maximum total chlorophyll content with a mean value of 2.043 mg/g, followed by genotypes 151 (1.717 mg/g) and 89 (1.675 mg/g). Total chlorophyll content under control conditions was 0.483–2.043 mg/g with an average value of 1.023 mg/g. Total chlorophyll content values under drought were 0.219–1.751 mg/g with an average of 0.719 mg/g. Entry 152 had the highest mean value, 1.751 mg/g, under drought conditions, entry 149 had 1.293 mg/g and 89 had 1.289 mg/g. Chlorophyll content under drought conditions in most of the mapping population entries decreased with the exception of very few genotypes (Table 35–37, pp. 170-172). Drought affects photosynthesis negatively and, thus, lowers the yield of the cultivars in drought-prone environments. In this mapping population, lines 152, 89, 149, 99, and 68 showed better performance with a low percent decrease in chlorophyll content and can be used in breeding wheat for improved photosynthetic activity.

Superoxide dismutase activity. The maximum superoxide dismutase activity under controled conditions was observed in entry 74 (49.97 units/g), followed by entries 109 (49.91 u/g) and 103 (49.59 u/g) Mean values show that SOD activity under normal conditions was 12.86–49.97 u/g with an average of 32.44 u/g (Table 38, p. 173). SOD mean values were high under the stress condition in most lines, ranging from 18.85 to 51.88 u/g with an average of 38.00 u/g. The maximum SOD activity under drought was in entry 103, with mean value of 51.88 u/g, followed by entries 106 (49.92 u/g) and 68 (49.71 u/g). When genotypes with highest and lowest mean values under stress were compared with the control, both increasing (4.41%, 1.24%, and 25.48 % increases in genotypes 103, 106, and 68, respectively) and decreasing (15.81%, 53.75%, and 55.62% decreased in entries 149, 111, and 124, respectively) trends were observed. The degree

Table 37. Mean values for total chlorophyll content (mg/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	Total chlorophyll content			Total chlorophyll content			Total chlorophyll content			Total chlorophyll content	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	0.684	0.505	45	0.916	0.635	85	0.908	0.836	119	1.280	1.058
4	0.627	0.430	46	1.284	0.646	86	1.028	1.108	120	0.774	0.669
5	0.535	0.467	47	1.070	0.760	87	0.786	0.756	121	1.314	1.167
7	0.649	0.537	48	1.516	0.699	88	1.001	0.981	122	1.454	0.778
8	0.616	0.487	49	1.112	0.892	89	1.675	1.289	123	0.744	0.528
9	0.737	0.513	51	1.086	0.404	90	1.074	0.346	124	1.115	0.803
12	0.642	0.566	53	1.241	0.898	91	1.219	0.955	125	1.239	0.849
13	0.682	0.542	55	1.125	0.816	92	0.807	0.655	126	0.873	0.718
14	0.574	0.448	57	1.086	0.615	93	0.995	0.715	127	1.138	0.767
15	0.831	0.578	58	0.882	0.589	94	0.998	0.879	128	1.362	1.052
16	0.938	0.725	59	0.690	0.452	95	0.935	0.638	130	1.182	0.985
17	1.082	0.930	60	0.942	0.684	97	1.341	0.940	132	1.430	1.032
18	1.229	0.978	61	0.677	0.457	98	0.490	0.278	133	1.405	0.831
19	1.391	1.016	62	1.088	0.527	99	0.517	0.219	134	1.290	1.022
21	1.031	0.566	63	0.940	0.358	100	0.851	0.246	135	1.548	1.109
22	1.084	0.756	64	0.932	0.427	101	0.714	0.391	136	1.558	0.976
23	0.961	0.440	67	0.746	0.324	102	0.854	0.535	137	1.243	1.054
24	1.059	0.845	68	0.531	0.223	103	0.572	0.456	138	1.314	1.072
26	0.828	0.681	69	1.172	0.309	104	0.823	0.510	139	0.902	0.694
27	0.875	0.599	71	0.879	0.560	105	1.328	0.573	140	0.894	0.794
28	1.006	0.852	72	1.113	0.328	106	1.201	0.645	141	1.241	0.987
30	1.049	0.647	73	0.930	0.568	108	1.101	0.674	142	1.189	0.788
31	1.025	0.617	74	0.572	0.561	109	0.880	0.772	143	1.304	1.248
32	0.824	0.373	75	0.646	0.235	110	0.953	0.784	144	1.050	0.624
33	0.971	0.618	76	0.548	0.279	111	1.138	0.702	145	1.537	1.227
35	0.714	0.500	77	0.778	0.680	112	0.972	0.780	146	1.404	0.960
37	0.740	0.492	78	0.646	0.544	113	0.872	0.571	147	1.506	1.259
39	0.847	0.567	79	0.483	0.344	114	1.031	0.587	148	1.661	0.993
40	1.311	1.104	80	1.019	0.758	115	0.995	0.686	149	1.569	1.293
41	1.186	1.104	81	0.864	0.857	116	0.561	0.291	150	1.609	1.239
42	1.063	0.883	82	1.147	1.055	117	0.918	0.514	151	1.717	1.221
43	1.003	0.912	84	0.819	1.175	118	1.034	0.601	152	2.043	1.751

of oxidative stress and antioxidant activity seems to be closely associated with the tolerance/susceptibility of a genotype to water stress, therefore, the reduced SOD activity in genotypes 140, 111, and 124 indicates their high susceptibility to drought.

Table 38. Mean values for superoxide dismutase (SOD) activity (units/g) under control and drought conditions for the members of an 'Opata (drought susceptible bread wheat cultivar)/SH (drought tolerant D67.2/P66.270//*Ae. tauschii* 257)' mapping population.

	SOD activity			SOD activity			SOD activity			SOD activity	
Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought	Entry	Control	Drought
2	26.09	41.43	45	35.67	35.89	85	25.03	26.48	119	39.88	42.94
4	32.27	48.15	46	40.85	36.69	86	30.30	28.66	120	48.55	48.30
5	27.21	45.07	47	41.49	21.44	87	33.88	37.28	121	39.97	40.92
7	17.55	31.02	48	28.18	32.02	88	35.88	36.10	122	37.15	47.90
8	21.70	45.28	49	42.39	42.48	89	24.03	31.18	123	35.73	47.76
9	19.39	47.89	51	27.11	46.14	90	34.24	34.82	124	43.88	19.47
12	24.24	49.25	53	38.18	39.76	91	34.36	43.07	125	38.39	35.89
13	26.42	49.48	55	36.21	44.25	92	23.91	28.41	126	33.18	35.07
14	28.00	45.22	57	30.33	43.15	93	27.39	27.66	127	33.09	40.48
15	38.94	35.23	58	32.76	45.48	94	22.82	23.46	128	32.73	33.69
16	45.94	44.30	59	24.91	45.25	95	31.21	30.07	130	37.33	38.79
17	37.09	48.41	60	32.67	40.76	97	24.82	27.17	132	38.70	37.46
18	38.52	38.87	61	31.46	40.82	98	24.85	39.00	133	24.03	30.46
19	32.97	32.74	62	31.64	40.53	99	44.82	46.59	134	24.18	25.56
21	27.97	30.12	63	40.94	49.56	100	33.09	41.02	135	28.85	31.20
22	32.97	38.10	64	34.52	40.71	101	28.94	38.05	136	21.21	30.41
23	35.64	46.48	67	26.49	35.51	102	49.27	48.64	137	27.03	26.48
24	46.45	47.21	68	37.03	49.71	103	49.59	51.88	138	26.24	29.94
26	35.55	40.10	69	33.70	38.71	104	44.76	42.74	139	32.73	36.07
27	12.86	21.50	71	15.99	21.56	105	43.49	49.10	140	29.15	32.82
28	26.33	29.12	72	29.12	41.46	106	49.30	49.92	141	33.39	37.74
30	15.20	20.50	73	30.21	46.77	108	48.85	46.00	142	37.94	41.00
31	27.15	30.94	74	49.98	39.94	109	49.91	49.46	143	36.51	39.00
32	38.36	36.97	75	40.73	47.20	110	48.30	49.61	144	32.70	29.20
33	42.36	42.64	76	32.73	43.95	111	41.39	19.14	145	28.12	31.43
35	35.70	35.05	77	34.39	36.41	112	15.44	48.07	146	31.82	48.07
37	39.97	39.17	78	29.39	43.89	113	49.27	49.64	147	25.42	23.58
39	32.67	36.23	79	34.76	44.20	114	23.76	48.35	148	26.15	25.71
40	24.55	45.15	80	23.36	31.30	115	16.38	21.04	149	22.39	18.85
41	25.03	29.28	81	27.94	39.64	116	32.09	42.79	150	21.79	24.61
42	32.61	34.51	82	26.73	38.87	117	31.27	49.30	151	30.21	47.01
43	18.31	21.08	84	34.58	33.18	118	35.91	48.10	152	24.12	29.35

Conclusion. To enhance the breeding efficiency in stress-prone environments, several molecular mapping populations have been generated globally. Our focus was to phenotype a mapping population derived from an 'Opata/Synthetic hexaploid' cross combination for various phenological and physiological parameters to evaluate for drought tolerance under *in vivo* and *in vitro* conditions. The combined use of morphological and physiological markers is one of the best approaches to evaluate genotypes for their potential tolerance to abiotic and biotic stresses.

The data from the phenological parameters showed that genotypes 87, 80, 78, 108, 118, 63, 105, 23, 48, 103, 14, 51, 127, and 122 had a very good spike length under drought conditions, which is directly related to higher yield performance of the plants; entries 144, 57, 150, 122, 14, 18, 118, 28, 55, 23, 40, 17, 45, and 108 were found to be good for number of grains/spike. Genotypes with excellent performance for the most important and key parameter, 1,000-kernel weight, which is a direct measure of the yield, include 148, 55, 146, 14, 23, 150, 63, 82, 118, 45, 41, and 17. These genotypes can be used in wheat breeding programs of Pakistan.

Genotypes under *in vitro* conditions also showed better defense mechanisms against drought. Entries 14, 17, 152, 142, 40, 151, 128, 118, 15, 46, 103, 106, 68, 12, 4, 9, 23, 105, 102, 75, 73, 63, 109, 110, 117, 149, 89, 147, 150,

145, 151, 121, and 82 had a high accumulation of proline and had more antioxidant activity under stress conditions. These lines can be used in any breeding program targeted to improve wheat against drought stress.

The best lines of mapping population, based on both morphological and physiological evaluation, are 14, 17, 23, 55, 108, 118, 122, 150, and 152. These genotypes performed the best equally under both *in vivo* and *in vitro* testing. This research study based on morphological and physiological evaluation suggests that unique genetic diversity from *Ae. tauschii* can be harnessed for increased yield by improving the existing cultivars against biotic and abiotic stresses. Such a targeted approach to incorporate novel genes for tolerance against drought can revolutionize wheat production in water-stressed environments.

Molecular validation and utilization of T1BL·1RS wheat and its isogenic lines in wheat breeding.

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Wheat occupies 220 million hectare or 17% of the total cultivated land in the world and supports nearly 35% of the world's population. One of the biggest adaptabilities of wheat is that it can grow in a variety of environments, ranging from fully irrigated to high rainfall and drought regions; it also faces a wide range of biotic and abiotic stresses. The wheat crop needs more focus on improvement in each area for higher production and to fulfill consumer demand.

Wheat, being an ancient crop of Asia, is the most important crop of Pakistan and occupies a central position in agriculture and economy. Pakistan is the 6th largest wheat producer, contributing about 3.2% of world's wheat production. As the leading crop in Pakistan, wheat contributes 14.4% to the value added in agriculture and 3.1% to the GDP. Wheat was cultivated on area of 9.042 x 10^6 ha and production was 23.864 x 10^6 tons during 2010 (Government of Pakistan, 2009–10).

In wheat improvement programs, two major problems are the targets regarding quantity and quality that are biotic and abiotic constraints. Of the biotic stresses, rusts are a major problem; abiotic stresses include drought, salinity, and heat. In order to meet the rapidly growing demand for food, genetic resistance or tolerance of these stresses has become important for increasing production.

Wheat, the world's leading food crop, is mainly grown on rainfed lands, so low moisture is a primary constraint on wheat production in the semiarid areas of developing countries. Worldwide, 37% of the semiarid, wheat-growing areas are effected by drought. Pakistan falls in the arid and semiarid climatic conditions, 14% of the rainfed wheat growing area is under drought stress and almost 15 x 10^6 ha of cultivated land is affected Government of Pakistan, 2009–10). Therefore, drought is a major factor in Pakistan that is detrimental to wheat production significantly depressing the wheat yield.

In order to increase grain yield/ha, wheat genotypes with high yield potential and resistance to biotic and abiotic stresses have been developed. Wheat genotypes having alien chromosomes are highly adapted with higher yield potential. Among them, the T1BL·1RS wheat genotype is a successful example. This translocation was first found in the winter wheat cultivar Kavkaz and was transferred to spring bread wheat at CIMMYT from rye sources. Other sources of T1BL·1RS are Buho, Kalyansona, and Blue Bird. From Kalyansona, Veery S, an advanced line of bread wheat, was derived at CIMMYT and, from this line, various sister lines were released as Seri 82, Ures 81, Genaro 81, Glennson 81, and V 10. By selection from Seri 82, the cultivar Pak 81 was released in Pakistan.

The homozygous chromosome T1BL·1RS involves short arm of *S. cereale* chromosome 1R and the long arm of chromosome 1B of *T. aestivum*. Genes for leaf rust (*Lr26*), yellow rust (*Yr9*), stem rust (*Sr31*), and powdery mildew (*Pm8*) have been associated with the rye chromosome arm 1RS. The T1BL·1RS lines have more vigorous root growth, but generally have inferior dough and breadmaking quality, mainly dough stickiness, and poor mixing tolerance. The T1BL·1RS wheats exhibit a yield advantage over normal wheat, and this positive yield advantage is expressed more under a water deficit. Worldwide, wheat cultivars with T1BL·1RS occupy 5 x 10^6 ha of cultivated area. Keeping in view the effect of T1BL·1RS on yield components and other parameters, this study was designed to focus on the following:

– check for the presence of T1BL·1RS in an experimental set of Seri 82 lines,
– find the importance of T1BL·1RS isogenic lines for wheat production in Pakistan under drought conditions via evaluation tests conducted under a rain shelter, and,

– determine the yield advantage of T1BL·1RS and test the response of isogenic lines under varying drought conditions through some standard *in vitro* tests.

Germplasm. The experimental material was comprised of bread wheat isogenic lines of Seri 82, which is a T1BL·1RS cultivar. By crossing with a 1B cultivar, Pavon 76, and several backcrosses to Seri 82, isogenic lines were obtained. Twenty isogenic lines of Seri 82 were studied, ten were T1BL·1RS and ten were normal chromosomes from Pavon 76. This set of 20 isogenic lines were validated and stringently used to define the contribution of the translocated chromosome under simulated drought conditions at National Agriculture Research Centre, Islamabad. Genetic material was obtained from Wheat Wide Crosses and Cytogenetics Program, National Agriculture Research Centre, Islamabad. Details of the germ plasm are given in Table 39.

Table 39. List of derived wheat isogenic lines from Seri 82.

Pedigree	Origin
SERI/PVN//8*SERI	02-515
SERI/PVN//8*SERI	02-516
SERI/PVN//8*SERI	02-517
SERI/PVN//8*SERI	02-518
SERI/PVN//8*SERI	02-519
SERI/PVN//8*SERI	02-520
SERI/PVN//8*SERI	02-521
SERI/PVN//8*SERI	02-522
SERI/PVN//8*SERI	02-523
SERI/PVN//8*SERI	02-524
SERI/PVN//8*SERI	02-525
SERI/PVN//8*SERI	02-526
SERI/PVN//8*SERI	02-527
SERI/PVN//8*SERI	02-528
SERI/PVN//8*SERI	02-529
SERI/PVN//8*SERI	02-530
SERI/PVN//8*SERI	02-531
SERI/PVN//8*SERI	02-532
SERI/PVN//9*SERI	02-533
SERI/PVN//9*SERI	02-534

Molecular validation. A rye-specific, SSR marker was used to validate the presence or absence of the 1RS arm in all the 20 isogenic lines. The protocol involved standard DNA analysis using a RYE–NOR marker that was rye chromosome specific. The oligonucleotide sequence of the RYE–NOR forward primer was GCATGTAGCGACTAACTCATC and reverse primer was CCCAGTTTTCCATGTCGC.

***In vitro* screening for drought tolerance.** Drought tolerance was investigated under *in vitro* conditions conducted in a moisture-controlled (rain shelter) tunnel; the control was grown under field conditions in the research area of Wheat Wide Crosses and Cytogenetics Program, NARC, Islamabad. Test parameters included plant growth development culminating in a final measure of standard yield.

PEG treatment. The *in vitro* tests were for root-growth parameters involving coleoptile emergence. To identify drought-tolerant, Seri 82 isogenic lines at the seedling stage, the treatments were control (0% PEG), treatment 1 (20% PEG, 200 g/800 mL dH_2O), treatment 2 (30% PEG, 300 g/700 mL dH_2O), and treatment 3 (40% PEG, 400 g/600 mL dH_2O).

Seeds of all isogenic lines were initially treated with solution of sodium hypochlorite for ten minutes. Residual chlorine was eliminated by washing seeds with distilled water. Five seeds of each line were placed on the moist filter papers under osmotic potentials of 0, –4, –10, and –17 bars induced by a polyethylene glycol solution (PEG-6000). Distilled water (2 mL) was added to each petri dish in the control treatment and 2 mL of each PEG solution (20%, 30%, and 40%) was added to the each petri plate under osmotic stress conditions every two days. All the petri plates were placed in a growth chamber for ten days at an average temperature of 22°C±2°C and 50% relative humidity. Data were recorded when the radicals reached at least 2 mm in length up to the tenth day after sowing. Data for coleoptile length, shoot length, and root length were recorded at four different moisture stress levels from five seedlings of each line.

The maximum germination and seedling emergence was observed at 0% PEG but markedly decreased at 20%, 30%, and 40% PEG concentrations due to PEG-induced drought stress. PEG concentration and time of application affect growth, and these concentrations also had lethal effects. At 0% and 20% PEG, all 20 lines germinated and had seedling emergence, but at no seedling emergence was shown at 30% and 40% PEG. The mean values of coleoptile, shoot, and root length of the 20 isogenic lines under four different levels of PEG-6000 are given (Table 40, p. 176).

Considerable variation was observed for coleoptile, shoot, and root length in the 0% and 20% PEG treatments. The highest coleoptile, shoot, and root lengths were observed at 0% PEG. Data recorded for various parameters were subjected to analysis of variance, which indicated a considerable amount of variability for all the parameters at 0% and 20% PEG. Analysis of variance indicated that the difference among the treatments for seedling traits was highly significant and that the genotypes were significantly different from each other for all seedling traits except root length, which was not significantly different (Table 41, p. 176).

Coleoptile length. The osmoregulation capability of the 20 Seri 82 isogenic lines was examined for coleoptile length under four different levels of PEG. Considerable variation for coleoptile length was observed under 0% and 20% PEG,

Table 40. Mean values for coleoptile length (CL), shoot length (SL), and root length (RL) of Seri 82 isogenic lines under different treatments of polyethylene glycol (PEG-6000).

Line	0% PEG (control)			20% PEG			30% PEG			40% PEG		
	CL	SL	RL	CL	SL	RL	CL	SL	RL	CL	SL	RL
02-515	1.70	8.33	15.50	1.10	2.23	4.23	0	0	0	0	0	0
02-516	1.83	10.00	14.83	0.90	0.87	1.83	0	0	0	0	0	0
02-517	2.00	9.17	13.00	0.00	0.00	1.33	0	0	0	0	0	0
02-518	2.13	9.07	8.57	1.83	2.23	6.20	0	0	0	0	0	0
02-519	2.50	12.73	13.27	1.33	2.03	3.57	0	0	0	0	0	0
02-520	1.67	7.33	9.60	1.40	3.03	4.90	0	0	0	0	0	0
02-521	1.90	9.97	16.30	1.33	2.87	4.40	0	0	0	0	0	0
02-522	1.87	7.70	10.10	0.93	1.07	2.23	0	0	0	0	0	0
02-523	1.87	9.33	12.57	0.83	0.73	2.50	0	0	0	0	0	0
02-524	1.93	9.73	13.23	1.17	1.80	3.20	0	0	0	0	0	0
02-525	1.73	9.60	8.83	1.07	1.17	3.63	0	0	0	0	0	0
02-526	2.60	10.47	17.53	2.23	7.30	7.97	0	0	0	0	0	0
02-527	1.80	8.03	10.37	1.03	2.10	2.00	0	0	0	0	0	0
02-528	1.87	10.07	11.57	1.20	2.10	3.20	0	0	0	0	0	0
02-529	1.97	9.50	14.30	1.40	1.10	3.67	0	0	0	0	0	0
02-530	2.33	11.77	12.00	1.83	2.77	5.33	0	0	0	0	0	0
02-531	2.37	10.73	15.10	1.37	1.30	1.70	0	0	0	0	0	0
02-532	2.10	10.57	11.33	1.53	2.57	4.87	0	0	0	0	0	0
02-533	2.03	9.90	14.07	1.57	2.07	3.87	0	0	0	0	0	0
02-534	2.17	9.93	15.23	2.03	1.77	3.07	0	0	0	0	0	0

and both treatments were significantly different from each other. The maximum coleoptile emergence was at 0%, least at 20%, and no emergence in the 30% and 40% PEG treatments (Table 40).

At 0% PEG (control), the coleoptile length was 1.67–2.60 cm (Table 40). The greatest coleoptile length was in isogenic line 02-526 and the shortest in line 02-520. Coleoptile length was 0.00–2.23 cm at 20% PEG (Table 40); the greatest length was in line 02-526 and the shortest in 02-517. At 30% and 40% PEG, coleoptile emergence was not observed in any isogenic line. The greatest reduction was noted for coleoptile length under osmotic stress.

Table 41. Mean square values of Seri isogenic lines for coleoptile, shoot, and root length under control and drought-stress conditions (* significant at the 5% level of probability; ** significant at the 1% level of probability).

Parameter	df	Coleoptile length	Shoot length	Root length
Genotype	19	0.726*	6.670*	14.603
Treatment	1	14.560**	1,759.502**	2,528.172**
G x E	19	0.288	4.715	13.900
Error	80	0.398	3.592	9.224

An analysis of variance revealed that there was significant difference among all lines for coleoptile length under 0% and 20% PEG (Table 41). The LSD determines the level of significance among all isogenic lines. On the basis of significance level, all of the 20 isogenic lines were divided in different ranges. Line 02-526 was significantly different from all the lines except 02-518, 02-519, 02-530, 02-531, 02-532, and 02-533, and 02-534. The highest mean coleoptile length was 2.146 cm at 0% and 20% PEG. the lowest mean coleoptile lenght of 1 cm was in line 02-517 under both 0% and 20% treatments, and it was significantly different from all other lines except 02-515, 02-516, 02-520, 02-521, 02-522, 02-523, 02-524, 02-525, 02-527, 02-528, and 02-529.

Shoot length. Shoot emergence was observed only in the 0% (control) and 20% PEG treatments. Emergence did not take place in 30% and 40% PEG treatments. The maximum shoot length was in the 0% treatment, ranged from 7.33 to 12.73 cm and the minimum was at 20% PEG, ranging from 0.00 to 7.30 cm (Table 40). At 0% PEG, the greatest shoot length was in line 02-519 and the shortest in line 02-520. At 20% PEG, isogenic line 02-526 had the greatest shoot length and line 02-517 the shortest.

The analysis of variance of shoot length showed that both treatments were significantly different from each other and all lines were found to be significantly different under both treatments with minute variation (Table 41). The LSD of the Seri 82 isolines for shoot length showed a significant difference among lines. Isogenic line 02-526 was not significantly different from 02-519 and 02-530 but was significantly different from all other lines, with the highest mean

value of 8.883 cm at 0% and 20% PEG. Isogenic line 02-522 was significantly different for shoot length from lines 02-519, 02-526, 02-530, and 02-532, with the lowest mean value of 4.383 cm under both PEG treatments.

Root length. Root emergence was observed in the isogenic lines subjected only to 0% and 20% PEG. Root length of the lines at 0% PEG were 8.57–17.53 cm (Table 40, p. 176). The longest root length was in line 02-526 and the shortest in 02-518. At 20% PEG, root length was 1.33–7.97 cm (Table 40, p. 176), with the longest shown in line 02-526 and the shortest in 02-517. Root emergence was not observed in any isogenic line of the 30% and 40% PEG treatments.

The analysis of variance showed that all lines were not significantly different from each other for root length, but both treatments were significantly different from each other (Table 41, p. 176). The level of significance between the lines was determined by an LSD test, and all lines showed almost same root length with minute variations. Isogenic line 02-526 had the highest mean value of 12.75 cm for root length at 0% and 20% PEG, which was significantly different from all other lines except 02-515 and 02-521. Line 02-522 was significantly different from isogenic lines 02-515, 02-521, and 02-526, with the lowest mean value of 6.166 cm. All other lines were not significantly different from each other at 0% and 20% PEG.

Correlation coefficients computed between the coleoptile, shoot, and root lengths which revealed that the three traits were strongly, positively correlated with each other with the highest correlation values in the 0% (control) and 20% PEG treatments. A strong correlation of 0.902 was shown by shoot length with root length at 0% and 20% PEG. Lines with a greater coleoptile length also showed greater shoot and root lengths and, therefore, performed better under drought conditions.

These results indicate that Seri 82 isogenic line 02-526 performed best under conditions of moisture availability and moisture stress with the longest coleoptile length (2.416 cm), shoot length (8.883 cm), and root length (12.75 cm). Other best performing lines were 02-519, 02-521, 02-524, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534. These lines have a good genotypic potential for drought tolerance.

Morphological evaluation. Data on eight morphological parameters were recorded for the 20 isogenic lines under both field (control) and rain-shelter conditions (moisture-stress conditions). Five plants of each entry were used for data and the arithmetic mean calculated. The correlation of Seri 82 isogenic lines data set was computed with the objective to determine the effect of various morphological and phenological traits (pubescence, waxiness, days-to-heading, days-to-physiological maturity, plant height, and spike length) on yield parameters (grains/spike and 1,000-kernel weight) under irrigated and drought-stressed conditions and the inherent association among the parameters. (Table 42, p. 178).

Seri 82 isogenic lines 02-522, 02-524, 02-525, 02-527, 02-528, 02-529, 02-530, 02-533, and 02-+534 performed well for all the morphological parameters and yield attributes under controlled and moisture-stress conditions. These lines are agronomically good, with high yield potential. The morphological analysis showed that there was a little diversity among the entries under controlled and stressed environments. Lines 02-519, 02-522, 02-526, 02-528, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534 had the 1RS translocation and were drought tolerant. These lines were validated for 1RS through molecular and cytological analysis and were selected on the basis of their root growth and morphological parameters, having the longest coleoptile, shoot, and root lengths under *in vitro* conditions and performed well for days-to-heading, days-to-physiological maturity, plant height, spike length, grains/spike, and 1,000-kernel weight under moisture stressed conditions.

Molecular diagnostics. Characterization and validation of chromosome arm 1RS in the Seri 82 isogenic lines at the DNA level used microsatellite markers or SSRs that were chromosome specific. The SSR-marker technique helped to characterize 1RS in wheat genotypes. SSRs are valuable and widely used molecular markers in plant species, especially wheat, for marker-assisted selection, because they are variable, co-dominant, genome specific, and need less DNA.

Chromosome arm 1RS of rye is intensively used in wheat breeding because of presence of many useful genes. The sequence-specific, SSR marker RYE–NOR, known to be specific for 1RS, was used for the molecular validation of the 1RS translocation in the isogenic lines. This primer amplified polymorphic bands in sizes ranged from 400 bp to 800 bp. The maximum number of scorable bands was three and the minimum was two.

The RYE–NOR primer amplification profile of the 20 Seri 82 isogenic lines detected scorable bands lines 02-519, 02-522, 02-523, 02-526, 02-528, 02-529, 02-530, 531, 02-532, 02-533, and 02-534, which ranged from 400 bp to 800 bp. Two had 1RS translocations. A maximum of three scorable bands were detected in lines 02-519, 02-526, 02-528,

Table 42. Mean values for morphological parameters of the Seri 82 isogenic lines under control (field) and stress (rain shelter) conditions.

Line	Pubescence	Waxiness	Days–to–heading	Days–to–physiological maturity	Plant height (cm)	Spike length (cm)	Grains/spike	1,000–kernel weight (g)
Control								
02-515	–	+	123	159	50.00	9.22	51	33.1
02-516	–	–	122	157	50.96	10.38	47	37.4
02-517	–	–	120	156	56.24	11.02	63	36.2
02-518	–	–	120	156	64.62	12.40	67	43.6
02-519	–	–	120	155	67.82	9.96	52	31.0
02-520	–	–	128	159	64.50	11.78	75	37.6
02-521	–	–	126	160	72.96	11.38	69	29.3
02-522	–	–	125	160	75.84	11.40	66	34.5
02-523	–	–	122	154	71.56	9.90	39	16.5
02-524	–	–	121	154	75.02	10.00	53	34.9
02-525	–	+	120	151	70.02	10.86	53	37.4
02-526	–	–	112	135	60.30	9.70	47	29.3
02-527	–	+	114	150	71.32	10.94	50	28.9
02-528	+	–	118	152	67.28	10.90	54	30.1
02-529	–	–	120	151	65.42	10.52	55	25.8
02-530	–	–	120	151	63.40	9.98	56	29.9
02-531	–	–	110	149	74.82	9.70	43	27.8
02-532	–	+	120	151	71.64	10.48	47	29.5
02-533	–	–	118	149	59.92	9.84	59	30.8
02-534	–	–	118	151	61.54	10.98	67	23.6
Drought stress								
02-515	–	+	118	151	60.06	8.62	23	21.8
02-516	–	–	111	151	71.00	9.66	29	20.0
02-517	–	–	116	147	69.56	9.84	29	22.7
02-518	–	–	117	152	68.92	11.06	39	25.6
02-519	–	–	116	147	61.68	11.24	44	13.5
02-520	–	–	118	148	68.30	10.60	38	19.5
02-521	–	–	118	152	71.50	11.36	37	11.9
02-522	–	–	116	151	72.56	11.54	49	23.5
02-523	–	–	113	143	68.02	12.06	48	22.8
02-524	–	–	113	145	69.48	12.38	48	26.8
02-525	–	+	111	147	63.28	11.68	41	23.2
02-526	–	–	115	138	62.50	9.00	43	28.5
02-527	–	+	118	144	63.84	11.74	49	32.6
02-528	+	–	111	145	58.90	12.22	48	31.2
02-529	–	–	118	147	59.26	12.00	40	26.5
02-530	–	–	118	145	60.20	11.82	43	23.0
02-531	–	–	121	149	49.06	11.44	46	30.0
02-532	–	+	111	149	59.20	12.52	57	30.5
02-533	–	–	111	149	57.24	10.98	43	26.8
02-534	–	–	111	146	70.48	11.06	48	28.6

02-529, 02-530, 02-531, 02-532, 02-533, and 02-534. Two bands were detected in lines 02-522 and 02-523 with band sizes ranging from 400 bp to 800 bp. Only one band was detected in isogenic line 02-527. No amplification was reported in 02-515, 02-516, 02-517, 02-518, 02-520, 02-521, 02-524, and 02-525.

The data of RYE–NOR was recorded in terms of the presence or absence of 2–3 bands within the range of 400–800 bp. The presence of bands within this range was recorded as positive (+), indicating the presence of rye chromatin (1RS), and the absence negative (–), no rye chromatin (Table 43, p. 179). Primer RYE–NOR showed the highest polymorphism in translocated lines.

Cytological diagnostics. Cytology of selected germ plasm was through validation by mitotic analysis and C-banding, which permit confirmation of T1BL·1RS. Routine mitotic analysis was done by staining the root tips with a 2% solution of aceto-orcein and visualizing the chromosomes under a microscope. The C-banding patterns were used to visualize the 1RS rye chromosome segment in the isogenic lines.

Mitotic analysis. For further validation of selected germ plasm, somatic cells of 20 isogenic lines were analyzed mitotically. In normal bread wheat, the short arms of chromosomes 1B, 6B, and 5D, have secondary constrictions, or satellites, but the resolutioj of the 5D satellites was inconsistent. In translocated wheat, T1BL1RS, a prominent satellite is associated with 6BS and 1RS replaces 1BS. Initial identification of 1B·1B and T1BL·1RS was by observing the satellites in somatic cells and through chromosome counts at metaphase. In all 20 lines, 42 chromosomes were counted. Four prominent satellites of 1B and 6B were observed in lines 02-515, 02-516, 02-517, 02-518, 02-520, 02-521, 02-524, 02-525, and 02-527, and two satellites of 6B were observed in isogenic lines 02-519, 02-522, 02-523, 02-526, 02-528, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534 at metaphase from several root tips, which confirmed the presence of T1BL·1RS. In various preparations, 5D satellites also were observed. The translocation status of 20 isogenic lines is given in Table 43.

Table 43. Molecular and cytological validation of Seri 82 isogenic lines for T1BL·1RS (For molecular marker and C-banding results, a + indicates presence and a – absence of the translocation. For cytology, the number in parentheses indicates the presence of the translocation, 2 indicates presence and 4 indicates absence).

Isoline	Molecular marker diagnostic for 1RS	Cytology diagnostic for 1RS	C-banding diagnostic for 1RS
02-515	–	1B·1B, 6B·6B (4)	–
02-516	–	1B·1B, 6B·6B (4)	–
02-517	–	1B·1B, 6B·6B (4)	–
02-518	–	1B·1B, 6B·6B (4)	–
02-519	+	6B·6B (2)	+
02-520	–	1B·1B, 6B·6B (4)	–
02-521	–	1B·1B, 6B·6B (4)	–
02-522	+	6B·6B (2)	+
02-523	+	6B·6B (2)	+
02-523	+	6B·6B (2)	+
02-524	–	1B·1B, 6B·6B (4)	–
02-525	–	1B·1B, 6B·6B (4)	–
02-526	+	6B·6B (2)	+
02-527	–	1B·1B, 6B·6B (4)	–
02-528	+	6B·6B (2)	+
02-529	+	6B·6B (2)	+
02-530	+	6B·6B (2)	+
02-531	+	6B·6B (2)	+
02-532	+	6B·6B (2)	+
02-533	+	6B·6B (2)	+
02-534	+	6B·6B (2)	+

C-banding analysis. The C-banding technique characterized the T1BL·1RS and 1B·1B chromosomes through prominent bands on terminal and subterminal regions. The 1BL·1RS C-banded chromosomes are characterized by prominent bands on the centromeric, terminal, and subterminal sites of the short arm indicating the presence of 1RS and the terminal band on the end of the long arm of chromosome 1B. The C-banded homozygous 1B chromosome is characterized by a banding pattern on both the short (1BS) and long (1BL) arms.

We used C-banding for additional validation of T1BL·1RS in the Seri 82 isogenic lines. The C-banding pattern of lines 02-515, 02-516, 02-517, 02-518, 02-520, 02-521, 02-524, 02-525, and 02-527 showed prominent bands on the short and long arms of chromosome 1B, confirming the absence of T1BL·1RS. In lines 02-519, 02-522, 02-523, 02-526, 02-528, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534, a positive banding pattern was observed at the centromeric, terminal, and subterminal regions of 1B short arm and terminal bands on 1B long arm confirmed the presence of 1RS in these lines (Table 43).

The relationship of T1BL·1RS wheat and rye have been validated more clearly through conventional cytological techniques, because these techniques helped to understand the homoeologous relationship among the chromosomes of cultivated wheat and rye. Using the conventional cytological technique for root tips not only reduces the possibilities of misinterpretation but allows larger numbers of plants to be cytologically examined. The C-banded 1RS arm of rye is totally different from that of 1BS arm of wheat. Good metaphase chromosome preparations from root tip cells result in clear resolution of chromosomes 1B and 6B, which carry secondary constrictions on their short arms. The 1B and 6B satellites were observed in the present study and found to be more conspicuous in good preparations; less conspicuous secondary constrictions were observed for chromosome 5D in only a few preparations.

Rye chromosomes were first time identified through C-banding. Through preferential staining, wheat and rye chromosomes stain differently and are distinguished easily. Using the landmark bands on the short and long arms of chromosomes, the Seri 82 isogenic lines were characterized as T1BL·1RS or nontranslocated 1B·1B chromosomes (Table 43). Molecular and cytological techniques enable the characterization and validation of 1RS in Seri 82 isogenic lines of wheat. Through these techniques, Seri 82 isogenic lines 02-519, 02-522, 02-523, 02-526, 02-528, 02-529, 02-530, 02-531, 02-532, 02-533, and 02-534 have T1BL·1RS, giving the same results with both techniques, and can be recommended for transferring 1RS to other wheat genotypes.

Cytological studies of genetic stocks from a Triticum aestivum/Thinopyrum bessarabicum intergeneric hybrid for wheat improvement.

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Wild species in the genus *Thinopyrum* constituting the tertiary gene pool represent a vast reservoir of useful agronomic traits for wheat and forage improvement. Chromosome engineering from *Thinopyrum* species into wheat has been practiced for 70 years. Approximately 30 economically important traits have been transferred from *Thinopyrum* species into wheat. Genetic transfer is usually realized through chromosome engineering, using *Triticum–Thinopyrum* partial amphiploids or chromosome addition, substitution, or translocation lines obtained from wide hybridization. The transfer and identification of desirable chromosome or chromosomal segments from *Thinopyrum* in wheat has benefited greatly from new developments in cytogenetics and molecular diagnostic techniques. Traditionally, the introduction of alien chromatin segments into wheat was confirmed by chromosome counting and pairing studies and/or with banding techniques. Although the conventional means are avenue to access the variability, newer technologies for the extraction of variability are needed to ameliorate the current cultivars. The study was conducted on a *T. aestivum* cv. Chinese Spring/*Th. bessarabicum* intergeneric hybrid, their disomic addition lines, and derived translocation stocks with their phenological characterization.

Our objectives were the cytological validation of the genetic stocks derived from bread wheat/*Th. bessarabicum* crosss as alien disomic addition (2x=6x=44) and translocation lines using conventional mitotic/meiotic analyses and giemsa C-banding and the development of fully characerized germ plasm with selections made for 56-chromosome amphiploid; 44-chromosome, disomic, addition lines; and full documentation of the translocation lines.

Experimental material. Germ plasm used included an intergeneric amphiploid of Chinese Spring bread wheat with *Th. bessarabiccum* that is an octaploid (2n=8x=56, AABBDDE^{b}E^b), seven disomic chromosome addition lines (2n=6x=42 + 1E^{b}E^b to 7E^{b}E^b) produced through backcrossing the amphiploid with wheat and cytologically identifying the complete set of seven additions, and wheat/alien chromosome translocation lines produced from backcrossing the amphiploid with a *phph* gene stock to facilitate alien introgressions leading to translocations through *ph*-based manipulation (Table 44). Seeds were germinated in petri dishes; 4,000 seeds of addition lines were planted. After germination, root tips were collected from individual seedling across all categories. Twenty-five seedlings of each amphiploid, addition, and translocation line were used for cytological studies. Individual seedlings were transplanted into Jiffy-7 peat pots. After 10 days growth, the seedlings of the amphiploid and *phph* stock were transplanted into pots filled with a soil:sand:leaf manure (2:1:1) mix and grown in a screen house. Chinese Spring and the seven addition lines were planted in the field.

Table 44. Chromosom status of wheat germ plasm with parentage.

Germ plasm	Parentage	Chromosome constitution
CS	Chinese Spring	42 (ABD)
Amphiploid	CS *Th. bessarabiccum*	56 (ABDE^b)
Addition line 1E^b	CS/ *Th. bessarabiccum*//Gen81	42+1E^{b}1E^b
Addition line 2E^b	CS/ *Th. bessarabiccum*//CS	42+2E^{b}2E^b
Addition line 3E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+3E^{b}3E^b
Addition line 4E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+4E^{b}4E^b
Addition line 5E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+5E^{b}5E^b
Addition line 6E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+6E^{b}6E^b
Addition line 7E^b	CS/ *Th. bessarabiccum*//2*Gen81	42+7E^{b}7E^b
phph stock	CS/ *Th. bessarabiccum*//*ph*	49 (ABDE^b)

Cytological characterization. ***Mitotic analysis.*** Mitotic analysis was conducted to purify the genetic stocks. Somatic counts of mitotic chromosomes of the amphiploid, the seven addition lines, and the translocation stock focused on selecting plants with 56, 44, and 49 chromosomes, respectively, to establish seed purity. Cytology confirmed the status of amphiploid, addition lines, and translocation stock.

In Chinese Spring, the secondary constrictions and satellites were observed at metaphase. The secondary constrictions are present on the short arms of chromosomes 1B, 6B, and 5D (Fig. 27, p. 181). The secondary constriction of chromosome 5D of wheat was observed in some preparations. The secondary constriction of 5D frequently can be observed by shortening the pretreatment time.

The somatic cytology of the seven addition lines focused on selecting plants with 44 chromosomes (Fig. 28). In the addition lines, a variable number of chromosomes was observed, i.e., 41, 42, 43, and 44. Secondary constrictions, satellites, and telocentrics were observed. Telocentrics were detected only in some preparations. The presence of telocentrics indicates a whole-arm translocation and a chromosome number up to 45. For intergeneric hybrid, plants with 56 chromosomes, and in *phph* stock, plants with 49 chromosomes, were selected. Variable somatic counts of 49, 50, 51, and 53 were found in the translocation stock.

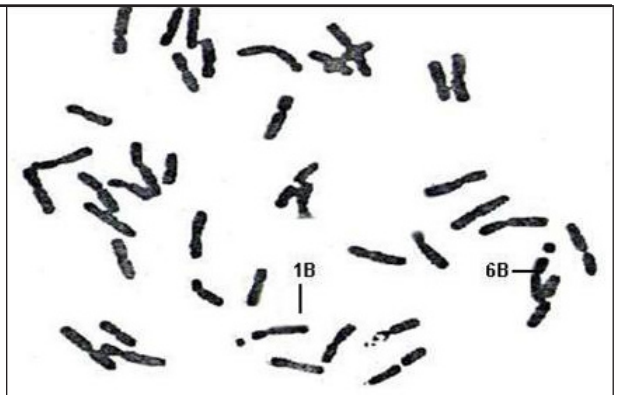

Fig. 27. Somatic chromosomes of wheat cultivar Chinese Spring (2n=6x=42).

Aceto-orcein binds with chromatin and stains chromosome nicely for optical microscopy. With traditional dyes, individual chromosomes in a complement can be identified only by chromosome size, the position of the centromere, and location of secondary constrictions. The pretreatment time for all samples was three hours, which seemed to be the optimum as inferred by the size of chromosomes at metaphase. The heat, pressure, and amount of the acetic acid determine the chromosomal spread, which aids in precise counting of the chromosomes and in observing the position of the centromere and secondary constrictions.

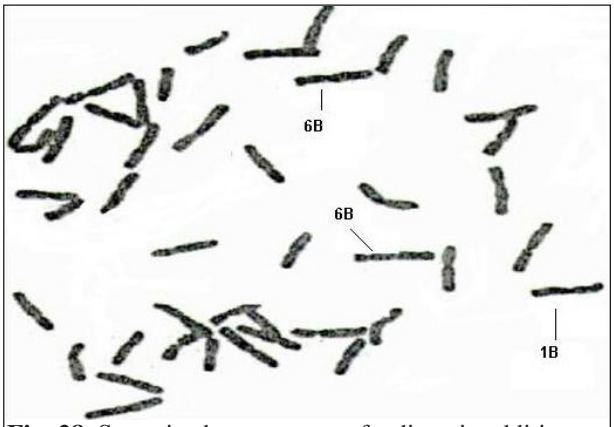

Fig. 28. Somatic chromosomes of a disomic addition line (2n=6x=44).

Cytological observation of each seed of the germ plasm was essential in order to maintain a normal euploid status, which aids in discerning hypo/hyperploidy. Cytological screening enabled the purification of the genetic stocks to give a wide array of plants with 56 (amphiploid), 44 (addition lines), and 49 (*phph* stock) chromosomes. Hybrid confirmation was obtained by a root-tip counts carried out either before or after the transplanting to peat pots or soil. Mitotic counts are essential for maintaining the normal euploid status of the seed stocks.

Quality, cytological procedures are essential for applied, alien transfers. Cytogenetic tools also are instrumental for studying the taxonomic relationships based upon karyotypic analysis. Metaphase chromosome spreads with optimum chromosome contraction and a high mitotic index are instrumental in the preparation of cytological preparations for use in chromosome banding.

Constitutive heterochromatin banding (C-banding). C-banding patterns of wheat/*Th. bessarabicum* amphiploid, the seven addition lines, and the translocation stock were determined by Giemsa C-banding. C-banding detects the presence of alien chromosomes to confirm *Th. bessarabicum* chromosome in the Chinese Spring background. *Thinopyrum bessarabicum* chromosomes exhibit prominent ,terminal heterochromatic regions and can be easily identified (Fig. 29). Distinct bands for each of the seven chromosomes of *Th. bessarabicum* were detected in the germ plasm. The C-banded karyotype distinctly identifies individual *Th. bessarabicum* chromosomes from those of *T. aestivum*.

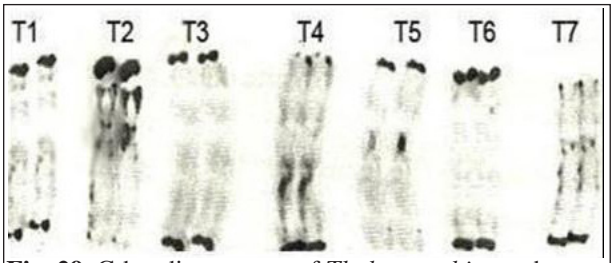

Fig. 29. C-banding pattern of *Th. bessarabicum* chromosomes.

C-banding of the CS–*Th. bessarabicum* showed that 14 of the 56 chromosomes were from *Th. bessarabicum* and the remaining 42 were wheat. In each of the seven addition lines, two of the 44 chromosomes were of *Th. bessarabicum* in $1E^b$ to $7E^b$ addition lines. In the *phph* translocation stock, seven of the 49 chromosome were *Th. bessarabicum* and the remaining were wheat. Due to an inactive *PhPh* locus, translocations were observed in this stock that need to be characterized further.

The bands present in the *Th. bessarabicum* chromosomes were medium to large and terminal (Fig. 29, p. 181). Chromosomes $1E^b$ (T1) and $2E^b$ (T2) had satellites on its short arms. Chromosomes $1E^b$, $3E^b$, (T3) and $6E^b$ (T6) had terminal bands on both the long and short arms. Terminal banding sites on the short arms were observed in chromosome $2E^b$ and $5E^b$ (T5). Chromosomes $4E^b$ (T4) and $7E^b$ (T7) had terminal bands on the long arms and variable terminal sites on the short arms. The banding details of *Th. bessarabicum* chromosomes confirm those reported earlier. C-banding is used to identify individual alien chromosomes in wheat background. The unique C-banding patterns of *Th. bessarabicum* chromosomes served as diagnostic markers for determining the chromosomes involved in translocations.

Meiotic analysis. Although the somatic evidence was adequate to validate the hybrid status, meiotic data reconfirms hybrids and provides a practical basis for advancing the F_1 hybrids. Chromosome pairing analysis in hybrids is based on the scoring of meiotic configurations i.e., univalents, rod and ring bivalents, trivalents, on metaphase I cells. The level of meiotic recombination between two chromosomes depends on the extent of chromosome pairing and chiasmata formation. Rod bivalents have one chiasmata, and ring bivalents have minimum two and maximum four chiasmata. Five chiasmata also are possible in ring bivalents, but it depends on personal observation. The extent of intergenomic recombination between the two parental species is determined by counting chromosomal arm associations. Meiotic studies of those plants were conducted, which showed a somatic count of 56 chromosomes (amphiploid), 44 chromosomes (addition lines), and 49 chromosomes (tanslocation stock).

Meiotic analysis of amphiploid showed a regular meiotic behavior. No multivalent or unpaired chromosomes were observed (Table 45). The amphilploid had the expected 28 bivalents. The amphiploid had a 2n=8x=56 chromosome composition with a mean association of 6.12 II rod bivalents, 21.89 II ring bivalents, and a mean chiasmata frequency of 64.12/cell. The high chiasmata frequency per cell supports the inactive 5B locus.

Table 45. Mean values for metaphase I chromosome associations in genetic stocks derived from *Th. bessarabicum*.

Line	Univalents	Bivalents		Trivalents	Chiasmata frequency per cell
		Rod	Ring		
Chinese Spring	–	18.20	2.80	–	56.60
Amphiploid	–	21.89	6.12	–	64.12
$1E^b$	–	18.84	3.15	–	56.92
$2E^b$	–	17.30	4.70	–	51.27
$3E^b$	–	18.26	3.74	–	49.70
$4E^b$	–	20.18	1.89	–	53.93
$5E^b$	–	19.67	2.34	–	52.50
$6E^b$	–	18.84	3.17	–	51.17
$7E^b$	–	19.11	2.88	–	55.55
phph stock	2.80	12.60	4.40	4.20	71.17

Cytological analysis of disomic addition lines provided evidence for normal meiotic metaphase I chromosome associations with 22 bivalents, as expected, and no multivalents or unpaired chromosomes (Fig. 30). The chromosome $1E^b$ addition line was 2n=6x=42 + $1E^b$ $1E^b$ or 44 chromosomes with a mean chromosome pairing of 18.84 II ring bivalents and 3.15 II rod bivalents and a chiasmata frequency of 56.92/cell. The chromosome $2E^b$ addition line was 2n=6x=42 + $2E^b$ $2E^b$ or 44 chromosomes with a mean chromosome pairing association of 17.30 II ring bivlalents, 4.70 II rod bivalents, abd a chiasmata frequency of 51.27/cell. The chromosome $3E^b$ disomic addition line was 2n=6x=42 + $3E^b$ $3E^b$ or 44 chromosomes with a mean chromosome pairing relationship of 18.26 II ring bivalents, 3.74 II rod bivalents, abd a chiasmata frequency of 49.70/cell. The $3E^b$ addition line showed a good meiotic index. The chromosome $4E^b$ addition line was 2n=6x=42 + $4E^b$ $4E^b$ or 44 chromosomes with a mean chromosome association of 20.18 II ring bivalents, 1.89 II rod bivalents, and a chiasmata frequency of 53.93/cell. At booting stage, eight florets/spikelet were observed in the $4E^b$ addition line whereas normally five florets are present in a spikelet. This addition line also had a high meiotic index.

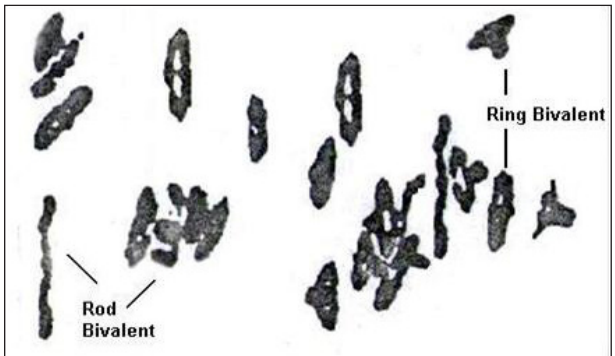

Fig. 30. Meiotic metaphase I associations in disomic addition lines 2n=6x=44 showing 22 ring and rod bivalents.

The chromosome $5E^b$ disomic addition line was 2n=6x=42 + $5E^b$ $5E^b$ or 44 chromosomes with a mean chromosome pairing of 19.67 II ring bivalents, 2.34 II rod bivalents, and a chiasmata frequency of 52.50/cell. The chromosome $6E^b$ disomic addition line had 2n=6x=42 + $6E^b$ $6E^b$ or 44 chromosomes with a mean chromosome pairing of 18.84 II ring

bivalents, 3.17 II rod bivalents, and a chiasmata frequency of 51.17/cell. The chromosome $7E^b$ disomic addition line was 2n=6x=42 + $7E^b$ $7E^b$ or 44 chromosomes with a mean chromosome pairing of 19.11 II ring bivalents, 2.88 II rod bivalents, and a chiasmata frequency of 55.55/cell.

The *phph* translocation stock was 2n=7x=49, AABBDDE^{b}E^b. This BC_1 progeny will become the source of BC_2 seed, which will have derivatives with 42 wheat chromosomes and zero to seven *Th. bessarabicum* chromosomes. This translocation stock showed 2.80 I univalents, 12.60 II ring bivalents, 4.40 II rod bivalents, and 4.20 III trivalents, with a chiasmata frequency of 71.17/cell.

Table 46. Phenotype of the genetic stocks derived from *Thinopyrum bessarabicum*. For grain color, BB = blackish brown, LB = light brown, and B = brown; for awn color, CW = creamy white.

Line	Days-to-heading	Days-to-physiological maturity	Plant height (cm)	Tillers/plant	Spike length (cm)	Grains/spike	Grains/plant	1,000-kernel weight (g)	Grain color	Awn color	Awns
Chinese Spring	123	174	120.00	–	10.20	67	315	24.40	B	–	Awnless
Amphiploid	134	163	70.70	6	13.20	19	32	–	LB	CW	Awnlets
Translocation stock	136	170	62.60	4	8.80	15	65	–	LB	CW	Awnlets
$1E^b$ addition line	79	110	67.00	12	11.20	41	384	19.50	B	CW	Awnlets
$2E^b$ addition line	78	110	70.20	14	10.60	35	316	17.30	LB	CW	Awnlets
$3E^b$ addition line	79	113	81.50	7	13.00	35	304	24.80	LB	-	Awnless
$4E^b$ addition line	80	113	77.70	10	12.70	26	305	22.80	BB	CW	Awned
$5E^b$ addition line	78	109	57.50	11	9.90	33	427	23.40	LB	CW	Awnlets
$6E^b$ addition line	80	104	55.00	2	6.90	17	35	–	LB	CW	Awnlets
$7E^b$ addition line	80	116	57.70	10	11.20	37	100	33.40	B	CW	Awned

The *phph* stock eliminates or suppresses the activity of the *Ph* gene to promote pairing between alien and wheat chromosomes to accelerate the process of gene transfer. The *phph*-based manipulation strategy offers great potential for obtaining alien transfers with minimum disturbance to the recipient genome. Pairing between wheat and alien chromosomes is a prerequisite for successful gene transfer. The *phph* stock showed extensive homoeologous pairing because of the lack of a dominant *Ph* gene. Such homoeologous pairing leads to the development of translocations, substitutions, and addition lines.

Regular meiotic behavior deciphers the transmission of the alien chromosomes to the progenies, and high fertility is beneficial for meeting the applied agricultural gains. Traditionally, alien introgressions in wheat have been characterized through meiotic metaphase I pairing. Germ plasm stability is of prime importance towards alleviating the constraints with biotic/abiotic screening for esoteric traits. Cytogenetics is valuable for a quick and accurate diagnosis of expected gene transfer and establishing stable gene introgressions. Cytogenetic manipulations can help in engineering desirable alien chromatin into wheat genome. Although high variability for biotic and abiotic stresses resides in the tertiary gene pool, very little has found its way to modern cultivars due to complex inheritance, unstable meiotic transmissions, and deleterious linked effects. Elucidating the compensating translocations, substitutions, and additions is facilitated by the newer technologies.

That most of the genetic stocks developed by the cytogeneticists over the past few decades are mostly maintained as oddities in germ plasm collections is unfortunate. These genetic stocks need to be characterized for response to biotic and abiotic stresses so that they can be used in applied breeding for stress tolerance.

Phenological characterization. Data on 11 morphological parameters was recorded for Chinese Spring wheat, the intergenric wheat/*Th. bessarabicum* hybrid, the seven addition lines, and the *phph* translocation stock. Three plants of each entry were used to record the data and the arithmetic mean was calculate (Table 46).

Days-to-heading. The amphiploid and *phph* translocation stock were very late heading compared to CS, the amphiploid, the *phph* translocation stock, and the seven addition lines, which were early heading. The addition lines had similar heading days.

Days-to-physiological maturity. The amphiploid had a rather early maturity compared to CS and the *phph* stock. Days-to-maturity in the addition lines was 104–116 days. Addition line $6E^b$ had early maturity and $7E^b$ was late.

Plant height. Plant height of the addition lines was 55–82 cm. Line $3E^b$ ws the tallest. Additions $3E^b$ and $4E^b$ were taller than the amphilploid and *phph* stock. The maximum plant height was in CS.

Tillers/plant. The number of productive tillers determines yield in terms of the number of spikes and grain produced. The amphiploid, *phph* stock, and $6E^b$ addition line showed poor growth. Tillering in the addition lines ranged from 7 to 14. The maximum number of tillers was in the $2E^b$ addition and the minimum in $3E^b$.

Spike length. Spike length contributes to yield determining the number of spikelets/spike and grain/plant. In CS, the amphiploid, and the *phph* stock, spike length ranged from 9 to 13 cm; in the addition lines, it ranged from 6 to 13 cm. The maximum spike length was in in addition line $3E^b$.

Grains/spike. Seed set in the addition lines was 17–41 grains/spike and 67 in CS. Addition $6E^b$ had poor seed set and poor growth in the screen house. The maximum seed set was in the $1E^b$ addition line. The minimum grains/spike was in addition $4E^b$ but it produced the maximum number of grains/spikelet.

Grains/plant. The *phph* stock, amphiploid, and the $6E^b$ addition line had poor reproductive growth. these lines had the least seed set. Grains/plant in the other addition lines ranged from 100 to 427. The maximum number of grains/plant was produced by $5E^b$ and the minimum by the $7E^b$ addition lines. A heavy pest and disease infestation on the $5E^b$ addition line did not preclude it from producing the maximum number of grains.

1,000-kernel weight. 1-000-kernel weight is an important yield parameter. Genotype were scored as either low-yielding or high-yielding, ranging from 17 to 34 g. The maximum grain weight was in addition line $7E^b$ and the least in the $2E^b$ addition line. Although the $7E^b$ addition produced the least number of grains, it had the maximum kernel weight, even greater than the Chinese Spring parent.

Grain color. The color of grain is an important parameter for characterizing and identifying a genotype and studying gene action. In these lines. grain color was brown, blackish-brown, or light brown.

Awn color. All the genotypes except Chinese Spring had a creamy white awn color.

Presence of awns. Chinese Spring is awnless. The $3E^b$ addition also was awnless. The amphiploid, its *phph* stock, and the $1E^b$, $2E^b$, $5E^b$, and $6E^b$ addition lines were awnletted. Additions $4E^b$ and $7E^b$ had long awns resembling normal wheat.

The vegetative morphology of the amphiploid resembled that of Chinese Spring, with spikes intermediate between the wheat parent and wheatgrass, a common observation for most intergeneric hybrids within the Triticeae and a valid morphological indicator of alien genetic expression in a wheat background. Seed set in the amphiploid and translocation stock was poor. A small number of seeds were produced but only sufficient for cytogenetic investigations in future. In the disomic addition lines, the phenotype of the wheat parent dominated. The addition lines showed good vegetative and reproductive growth, except for the $6E^b$ addition. This line produced an enormous amount of seed, which can be further utilized for cytogenetic manipulations and breeding. Cytological procedures helped in the purification of the genetic stocks.

Statistical analysis. Based on the statistical analysis, the genetic stocks derived from *Th. bessarabicum* showed considerable diversity (Table 47). Considerable variation was observed for days-to-heading and maturity, plant height, and number of grains/plant. These traits can be exploited further by breeders.

Table 47. Basic statistics for eight quantitative traits under study in *Thinopyrum bessarabicum*-derived addition lines.

Trait	Mean	Range	Standard deviation
Days-to-heading	94.70	78–136	±25.276
Days-to-physiological maturity	128.20	104–174	±28.448
Plant height (cm)	71.99	55–120	±18.999
Tillers/plant	8.45	2–14	±3.941
Spike length (cm)	10.78	6.9–13.2	±1.961
Grains/spike	32.50	17–67	±15.152
Grains/plant	228.30	32–427	±152.596
1,000-kernel weight (g)	23.65	17.31–33.38	±5.074

Currently, bread wheat improvement priorities worldwide are associated with the genetic security against the three rusts, Fusarium head blight and heat, drought, and salinity tolerance. Crop improvement is dependent on a continued supply of genetic variability. Alien grass species of tertiary gene pool can enrich

cultivated wheat. Considerable yield benefits can be gained from alien chromosome segments or through synthetic wheats using the genetic diversity residing in wild wheat relatives. *Thinopyrum* species have been extensively hybridized with wheat and have played an important role in wheat improvement. Many wheat–*Thinopyrum* amphiploids have been produced with varied chromosome constitutions and can be used as efficient bridges to transfer genes to wheat. Translocations between alien and wheat chromosomes are of worth because they carry a smaller number of alien genes into the crop. They may prove to be a potent source of variation and durable resistance under field conditions because of thier diverse origin. For this to happen, plant breeding, cytogenetics, and biotechnology should go hand-in-hand to accelerate germ plasm improvement programs.

Response of wheat germ plasm derived from the primary and tertiary Triticeae gene pools for Karnal bunt resistance.

Muhammad Ifnan Khan, Nasir Mahmood Minhas, Muhammad Zakria, Alvina Gul Kazi, Hadi Bux, Awais Rasheed, and Abdul Mujeeb-Kazi.

Wheat production is influenced by susceptibility to biotic and abiotic stresses. Karnal bunt (*Neovossia indica*) is a key biotic stress of significant concern to wheat production output; the pathogen is a serious quarantine matter. Fungicide can be used to control the disease, but this is highly uneconomical, hence, resistant cultivars are desirable. For resistance breeding, genetic diversity is required, and this diversity is present in all three gene pools of the wheat family. We exploited the primary and tertiary gene pools. The evaluation of synthetic hexaploids (SH) based on *Ae. tauschii* from the primary gene pool and on *S. cereale* from the tertiary gene pool.

Karnal bunt, or partial bunt, has been described as a smut disease of wheat. First reported in 1931 in experimental wheats at the Botanical Station at Karnal, India, for many years, it was known only in the plains of India. However, since 1974, Karnal bunt has been noted in many locations across northern India, and later Pakistan. Karnal bunt is known to occur in Mexico. Although it is reported from Iraq, this report has not been yet confirmed in the field. A similar situation occurred in Afghanistan and Lebanon, where the pathogen was found in wheat samples imported from the U.S.A. and India, respectively. Karnal bunt occurs naturally on bread wheat, durum wheat, and triticale. The Karnal bunt fungus, originally classified as *Tillitia indica*, was later placed in *Neovossia* on the basis of a long promycelium with a whorl of 32–128, nonfusing conidia at the apex. Karnal bunt is difficult to control because it is seed and soil borne. The focus of quarantine has been to restrict movement of the pathogen from country to country and within the countries where it has been detected. Chemical control of Karnal bunt is not feasible, thus, resistant cultivars are the best option. Derived SH wheat from durum/*Ae. tauschii* crosses plays a major role in the incorporation of Karnal bunt resistance in commercial cultivars. Karnal bunt can survive long periods of time outside its host.

Our focus was to (a) identify agronomically suitable, primary SH wheats based upon phenotype, (b) evaluate the response of advanced wheat germ plasm derived from crosses between breadwheat cultivars and Karnal bunt resistant, SH wheats for resistance, and (c) screen germ plasm of the tertiary gene pool for Karnal bunt resistance.

Forty-seven entries, including KB-12, 57, 171, 203, 245, 248, 268, 269, 272, 290, 301, 310, 369, 376, 380, 391, 405, 419, 520, 551, 640, 648, 706, 739, 789, 940, 1030, 1035, 1036, 1087, 1088, 1114, 1127, 1159, 1200, 1266, 1267, 1280, 1288, 1290, 1321, 1431, 1452, 1454, 1455, 1469, and 1475, were sown in four replications in a randomized complete block design. The infested grain ratio was calculated by the formula: % infestation = total number of infested grains / total number of grains x 100.

Phenotypic evaluation. Data were recorded for several characters (Table 48, p. 186).

Pubescence – pubescence grading was negative (–) or positive (+). Thirty-six genotypes were pubescent and 11 lines were not. The pubescent lines are resistant to insects/pests.

Plant height – Plant height in the genotypes under study was 88–97 cm.

Number of tillers/plant – ranged from 9.25 to 17.50 cm.

Number of grains/spike – ranged from 20.75 to 59.00 cm.

1,000-kernel weight – ranged from 39.25 to 57.50 g.

Spike length – ranged from 7 to 13 cm.

Days-to-flowering – was 89–105 days.

Table 48. Phenotypic characterization of 47 synthetic hexaploid wheat lines. For pubescence, + = present and – = absent; for waxiness, + = present and – = absent.

Entry	Pubescence	Waxiness	Plant height (cm)	Tillers/plant	Days–to–physical maturity	Spike length (cm)	Grains/spike	Days–to–flowering	Spikelets/spike	1,000–kernel weight (g)
KB–12	+	+	88	12	143	5.0	37	96	18	39
KB-57	+	–	87	18	129	11.0	57	96	18	58
KB-171	–	–	84	15	143	13.0	49	89	16	53
KB-203	+	+	87	12	127	9.0	25	95	18	37
KB-245	+	–	81	9	179	8.5	39	105	16	40
KB-428	+	+	85	12	132	10.0	34	99	18	45
KB-268	+	–	91	14	143	11.5	24	93	16	41
KB-269	–	+	82	12	136	8.0	49	100	18	49
KB-272	+	–	85	11	157	11.5	51	97	17	55
KB-290	+	+	81	10	162	10.0	24	98	15	44
KB-301	+	–	95	15	165	7.5	52	86	13	57
KB-310	–	–	96	13	179	8.0	47	102	13	52
KB-369	+	+	85	11	143	5.5	27	95	21	41
KB-376	+	–	94	10	151	12.0	32	89	15	38
KB-380	+	–	71	11	149	14.0	38	96	13	55
KB-391	+	+	89	12	143	10.0	27	94	13	56
KB-405	–	–	97	10	156	12.0	52	96	21	41
KB-419	+	+	85	11	179	12.0	29	100	17	41
KB-520	+	+	89	9	143	7.6	23	95	20	46
KB-551	+	–	85	10	172	8.3	34	95	19	45
KB-640	+	–	89	10	154	13.0	34	95	12	31
KB-648	–	+	84	14	149	9.0	47	92	19	47
KB-706	+	+	91	10	169	10.0	45	98	21	54
KB-739	–	–	85	12	127	11.5	43	92	17	58
KB-789	+	–	83	9	143	9.0	29	91	19	53
KB-940	+	+	85	11	127	9.0	38	97	16	47
KB-1030	+	–	94	10	158	11.0	36	84	18	40
KB-1035	+	+	89	15	143	9.0	52	98	20	55
KB-1036	–	–	89	10	159	11.0	51	94	19	55
KB-1087	+	–	71	10	168	11.0	30	95	17	49
KB-1088	–	+	89	10	162	13.0	33	96	18	45
KB-1114	+	–	97	12	143	9.0	29	95	19	44
KB-1127	+	–	85	10	179	11.0	38	92	15	47
KB-1159	+	+	89	7	163	11.0	26	100	20	42
KB-1200	–	+	85	12	169	13.0	49	95	19	52
KB-1266	+	–	89	14	127	8.0	59	97	17	48
KB-1267	+	–	84	14	179	7.0	51	105	18	57
KB-1280	+	–	84	10	179	10.0	34	98	16	45
KB-1288	+	+	89	9	161	8.0	39	93	15	48
KB-1290	–	+	85	12	173	6.8	45	100	20	45
KB-1321	+	–	89	9	143	8.5	42	97	19	56
KB-1431	+	+	87	9	158	12.0	21	98	17	48
KB-1452	+	–	95	13	149	8.0	21	86	18	48
KB-1454	+	–	94	10	152	12.0	33	102	19	48
KB-1455	–	–	82	12	143	8.0	24	97	15	45
KB-1469	+	–	94	12	140	11.0	22	98	14	41
KB-1475	+	+	85	16	139	10.0	46	92	16	48

Karnal bunt, disease-resistant genotypes. Of the 47 SH wheat genotypes, 15 were resistant to Karnal bunt (Table 49). These lines are recommended for further use in breeding programs in order to improve wheat yield and quality.

Table 49. Karnal bunt resistant genotypes selected for the breeding program of the Wheat Wide Crosses and Cytogenetics Program, National Agriculture Research Centre, Islamabad.

Line	Total grain	Infested grain	Karnal bunt score					
			0	1	2	3	4	5
KB-203	23	0	23	0	0	0	0	0
KB-245	22	0	22	0	0	0	0	0
KB-272	16	0	16	0	0	0	0	0
KB-290	10	0	10	0	0	0	0	0
KB-380	32	0	32	0	0	0	0	0
KB-391	25	0	25	0	0	0	0	0
KB-520	37	0	37	0	0	0	0	0
KB-1127	31	0	31	0	0	0	0	0
KB-1280	21	0	21	0	0	0	0	
KB-1321	29	0	29	0	0	0	0	0
KB-1452	26	0	26	0	0	0	0	0
KB-1454	38	0	38	0	0	0	0	0
KB-1455	12	0	12	0	0	0	0	0
KB-1469	21	0	21	0	0	0	0	0
KB-1475	31	0	31	0	0	0	0	0

Screening of wheat, a Thinopyrum bessarabicum amphiploid, and its disomic chromosome addition lines for salt tolerance.

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Salinity of arable land is a global problem that has restricted the productivity of 955 x 10^6 ha of land. Salinity has developed due to the accumulation of water soluble salts in the soil and its interactions with ground water to a level significantly affecting agricultural production, environmental health, and the economic welfare of many countries, especially those in the arid regions. Pakistan is located in an arid to semiarid climate zone, so high evapotranspiration is the basic cause of salt accumulation on the soil surface. The annual rainfall varies between 100–700 mm throughout the country. The evaporation rate exceeds the precipitation rate, rendering soil salinity and sodicity problems as common production constraints in arid and semiarid regions. In Pakistan, about 6.30 x 10^6 ha are salt affected, out of which 1.89 x 10^6 ha are saline, 1.85 x 10^6 ha are permeable saline-sodic, 1.02 x 10^6 ha impermeable saline-sodic, and 0.028 x 10^6 ha are sodic in nature. Of the 1.89 x 10^6 ha of saline patches, 0.45 x 10^6 ha are present in Punjab, 0.94 x 10^6 ha in Sindh, and 0.5 x 10^6 ha in KPK. The magnitude of the saline problem can be gauged from the fact that the area of productive land is being damaged at the rate of about 40,000 ha annually.

Thinopyrum bessarabicum (2n=2x=14, E^bE^b = JJ) is a wild, rhizomatous, maritime, sand couch grass distributed in Crimea recognized for its high tolerance to salinity and capable of withstanding up to 350 mol/mL^3 of NaCl. Understanding the organization of E^b genome and its phylogenetic relationship with other related genomes greatly facilitates the utilization of these grasses for the introgression of useful genes into wheat. *Th. bessarabicum* has been preferred for genetic transfers due to its diploid status, which theoretically permits swift genetic exchanges to occur as compared with an equally tolerant decaploid (2n=10x=70) *Th. ponticum*.

Alien chromosome addition lines are usually generated to detect agronomically important gene(s) of wild relatives expressed in a wheat background. Alien chromosome addition lines are useful for localizing genes for valuable traits on specific chromosomes, construction of DNA libraries for specific chromosomes following microdisjunction, and research on genome composition and chromosome structure.

Our objectives were the (a) *in vitro* screening for salt tolerance using K^+:Na^+ discrimination as an evaluation parameter under hydroponic testing conditions coupled with additional tests comprising of protein, proline, total chlorophyll content, sugars, super-oxide dismutase (SOD), and root and shoot fresh/dry weight, and (b) identification of the addition line/s that bestow salt tolerance.

The experimental lines were nine entries, including the wheat cultivar Chinese Spring, a CS/*Th. bessarabicum* amphiploid (2n=8x=56; $AABBDDE^bE^b$), and the seven, $1E^b$ to $7E^b$, disomic, chromosome addition lines with 44 chromosomes (2n=6x=42 + $1E^b1E^b$ to $7E^bE^b$). All addition lines were subjected to cytological validation for determining the presence of disomic addition status (42 + 1J1J–7J7J or $1E^b1E^b$ to $7E^bE^b$) through routine mitotic analysis and giemsa C-banding with a focus on detecting the added pair of *Th. bessarabicum* chromosomes. Standard wheat cultivars recognized as salt tolerant (Kharchia 65, Shorawaki, Lu26S, and S-24) and susceptible (PDW34 and PBW343) also were included in the experimental evaluation (Table 50, p. 187).

K^+:Na^+ discrimination. Salt-tolerant species have the ability to maintain low Na^+ and high K^+ concentration in leaves, therefore, a high K^+:Na^+ value indicates a high level of salt tolerance and a greater ability of a plant to exclude Na^+ and accumulate K^+ at high NaCl concentrations. The accumulation of more K^+ compared to Na^+ under saline conditions is a character that determines salinity tolerance at the seedling stage.

Table 50. Pedigree of the disomic, *Th. bessarabicum* addition lines (2n = 6x = 42 + 2 E^b) used to screen for salinity tolerance.

Pedigree	Addition chromosome	Status
CS/*Th. bessarabicum*//Gen81	1J	42 + 1J1J ($1E^b$)
CS/*Th. bessarabicum*//CS	2J	42 + 2J2J ($2E^b$)
CS/*Th. bessarabicum*//2*Gen81	3J	42 + 3J3J ($3E^b$)
CS/*Th. bessarabicum*//2*Gen81	4J	42 + 4J4J ($4E^b$)
CS/*Th. bessarabicum*//2*Gen81	5J	42 + 5J5J ($5E^b$)
CS/*Th. bessarabicum*//2*Gen81	6J	42 + 6J6J ($6E^b$)
CS/*Th. bessarabicum*//2*Gen81	7J	42 + 7J7J ($7E^b$)

The amphiploid, Chinese Spring, Kharchia-65, Shorawaki, and S-24 were found to be the most tolerant to salinity at 75 mol/m^3 with K^+:Na^+ values 6.00, 5.13, 4.81, 4.88, and 5.10, respectively (Table 51). Genotypes PBW-343 and PDW-34 were salt susceptible at 75 mol/m^3 with K^+:Na^+ values of 1.50 and 1.20, respectively. Among the addition lines, the K^+:Na^+ value ranged from 2.60–5.76. Addition lines 1J, 2J, 3J (translocated), and 6J were found to be semitolerant. Addition lines 3J, 4J, 5J, and 7J were the most salt tolerant at 75 mol/m^3 with K^+:Na^+ values of 5.21, 5.76, 4.98, and 4.78, respectively.

Agronomic characteristics under salt stress. Shoot length, root length, shoot and root fresh weight, and shoot and root dry weight were recorded on 35-day-old plants under 75 mol/m^3 NaCl salt stress (Table 52). The greatest shoot length of 38.0 cm was found in the cultivar S-24, and the greatest root length of 9.9 cm was found in Kharchia-65. The shortest shoot length of 30.0 cm was found in PDW-34, and the shortest root length of 4.5 cm was found in PBW-343. Both of these cultivars are susceptible checks and were found to be agronomically poor, with low biomass production. Overall, Chinese Spring, Kharchia-65, Shorawaki ,and S-24 showed better agronomic performance along with high K^+:Na^+ values, were regarded as tolerant genotypes (Table 52), and are of top priority for use in breeding for salt tolerance.

Addition line 4J showed good agronomic performance with high shoot (3.87 cm) and root (10.60 cm) lengths. Line 6J had the lowest shoot length (26 cm) and the lowest root length was 3.3 cm in addition 1J. Overall, addition lines 3J, 4J, 5J, and 7J were better than the others.

Table 51. Mean values for leaf fresh weight, leaf dry weight, and K^+:Na^+ for disomic *Thinopyrum bessarabicum* addition lines and standard wheat cultivars recognized as tolerant (Kharchia-65, Shorawaki, and S-24) and susceptible (PBW-343 and PDW-34) for salt tolerance.

Entry	Leaf fresh weight (gm)	Leaf dry weight (gm)	Na^+%	K^+%	K^+:Na^+
1J	0.106	0.0049	2.80	10.255	3.60
2J	0.920	0.0035	7.60	19.843	2.60
3J	0.159	0.0089	2.77	14.451	5.21
4J	0.132	0.0088	2.45	14.121	5.76
5J	0.108	0.0073	3.12	15.532	4.98
7J	0.167	0.0078	2.98	14.143	4.75
3J (translocation)	0.884	0.0056	6.30	17.566	2.78
6J	0.102	0.0086	5.90	17.465	2.96
Amphiploid	0.141	0.0094	1.80	10.772	6.00
PBW-343	0.031	0.0033	13.6	20.220	1.50
Chinese Spring	0.118	0.0088	2.73	14.000	5.13
PDW-34	0.084	0.0049	12.4	15.111	1.20
Kharchia-65	0.114	0.0075	3.21	15.440	4.81
Shorawaki	0.106	0.0073	2.99	14.581	4.88
S-24	0.109	0.0069	2.80	14.231	5.10

Table 52. Mean values for the agronomic parameters shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight for disomic *Thinopyrum bessarabicum* addition lines and standard wheat cultivars recognized as tolerant (Kharchia-65, Shorawaki, and S-24) and susceptible (PBW-343 and PDW-34) for salt tolerance.

Entry	Shoot length (cm)	Root length (cm)	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)
1J	33.7	3.30	0.3173	0.0384	0.0268	0.0048
2J	30.8	7.40	0.0400	0.0624	0.0301	0.0058
3J	32.8	6.80	0.4449	0.0718	0.0349	0.0060
4J	37.8	10.6	0.3963	0.0951	0.0391	0.0081
5J	34.3	4.00	0.4999	0.0578	0.0389	0.0035
7J	34.7	3.60	0.4018	0.0877	0.0365	0.0076
3J (translocation)	30.0	3.90	0.3564	0.0605	0.0325	0.0062
6J	26.0	5.20	0.3349	0.0572	0.0309	0.0060
PBW-343	30.2	4.50	0.3673	0.0670	0.0355	0.0065
Chinese Spring	35.4	7.90	0.4995	0.1090	0.0428	0.0101
PDW-34	28.0	4.80	0.2600	0.0387	0.0279	0.0044
Kharchia-65	33.0	9.90	0.4150	0.0792	0.0407	0.0123
Shorawaki	35.5	6.80	0.4760	0.0895	0.0410	0.0085
S-24	38.8	9.80	0.5340	0.1193	0.0556	0.0899

Addition lines with high K^+:Na^+ values also showed high shoot dry weight, indicating sn association between K+/Na+ value and plant performance under stress. Lines 3J, 4J, 5J, and 7J showed high values for both parameters, whereas 1J, 2J, 3J (Tr), and 6J had average performance. Chinese Spring, Kharchia-65, Shorawaki, and S-24 exhibited high values for both parameters, whereas genotypes PBW-343 and PDW-34 were the lowest for both. These data are consistent with germ plasm tolerance catergorization.

Chlorophyll content. We found that the chlorophyll content significantly decreased under salt stress. The genotypes with tolerance to salinity had a low chlorophyll content compared to susceptibile lines. The tolerant genotypes Chinese Spring, Kharchia-65, Shorawaki, and S-24 had total chlorophyll content values of 1.1, 1.2, 1.0, and 1.3 mg/g, respectively, with high K^+:Na^+ values. Addition lines 3J, 4J, 5J, and 7J showed total chlorophyll values of 1.1, 0.9, 1.2, and 1.1 mg/g, respectively, with higher K^+:Na^+ values and more biomass production.

Proline content. Plants accumulated proline in response to abiotic stresses thereby protecting the plant by reducing oxidative damage created due to osmotic stress. The genotypes had increased proline contents in stress conditions. The maximum increase in proline content was observed in Kharchia-65 (4.111 mg/g) followed by Chinese Spring (4.068 mg/g), Shorawaki (3.998 mg/g), and S-24 (3.010 mg/g). The susceptible genotypes had proline content values of 1.061 and 1.010 mg/g. In the addition lines, the maximum proline content was 5.131 mg/g in line 3J, followed by 4J (4.666 mg/g), 5J (3.918 mg/g), and 7J (3.777 mg/g). Addition lines 3J, 4J, 5J, and 7J also showed a higher K^+:Na^+ value.

Protein content. The amount of soluble protein content increased during stress. Genotypes Chinese Spring, Kharchia-65, Shorawaki, and S-24 showed protein content values in the range of 2.001, 1.118, 1.669, and 1.703 mg/g, respectively, whereas genotypes PBW-343 and PDW-34 showed values as low as 0.968 and 0.898 mg/g, respectively. In the tolerant addition lines, high proteing content values were in 3J (2.101 mg/g), 4J (1.998 mg/g), 5J (1.896 mg/g), and 7J (1.999 mg/g). whereas the susceptible addition lines 1J, 2J, 3J(Tr), and 6J had values of 0.911, 1.018, 1.211, and 1.023 mg/g, respectively. Because 3J, 4J, 5J, and 7J showed higher K^+:Na^+ values, they were the most tolerant.

Sugar content. Under stress conditions, tolerant genotypes with high K^+:Na^+ showed high sugar content values, including Chinese Spring (22.9 mg/g), Kharchia-65 (24.6 mg/g), Shorawaki (19.5 mg/g) and S-24, whereas the susceptible genotypes PBW-343 (11.8 mg/g) and PDW-34 (13.6 mg/g) were low. Addition lines with high sugar content values were 3J (17.4 mg/g), 4J (20.2 mg/g), 5J (24.7 mg/g), and 7J (27.3 mg/g), were found to be the most salt-tolerant with high K^+:Na^+ values.

Superoxide dismutase (SOD) content. The maximum superoxide dismutase content was found in Shorawaki at 30.000 units/g fresh weight (u/gfw). Other genotypes with high SOD content incuded Chinese Spring (28.611 u/gfw), Khar-

chia-65 (27.000 u/gfw), and S-24 (21.671 u/gfw). PDW-34 had an SOD content 13.556 u/gfw, whereas the least value was 10.444 u/gfw in PBW-343. In the addition lines, the maximum SOD value was 28.056 u/gfw in line 5J.

Conclusion. The Chinese Spring, Kharchia-65, Shorawaki, and S-24 genotypes were found to be most tolerant to salinity at 75 mol/m^3; PBW-343 and PDW-34 were susceptible. Among the addition lines, K^+:Na^+ values were 2.6–6.0 in 3J, 4J, 5J, and 7J, the most tolerant to salinity at 75 mol/m^3. The tolerance of conventional wheats Chinese Spring, Kharchia, Shorawaki, S-24, and the susceptibility of PBW-343 and PDW 34, supports earlier observations by de Leon et al. (2010). The role of chromosomes 3J, 4J, 5J, and 7J suggests multiple influences, which is consistent with reports of Dvorak et al. (1988) for 3J, 4J, and 7J. The inclusion of 5J in this group is surprising, but it agrees with Forster et al. (1988). Future progress appears to be with more stringent testing with more replications and, if the tolerance comes from multiple alien chromosomes, exploit the amphiploid in a shot-gun manner to effect homoeologous exchanges around the *ph1b* system (Mujeeb-Kazi 2006).

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Characterization of wheat genotypes for salinity tolerance based on physiological attributes.

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Salinity is one of the major environmental stresses that cause significant reduction in grain yield productivity worldwide, especially in irrigated lands (Zhang et al. 2010). Establishment of ion homeostasis is an essential requirement for plants to survive under salt stress conditions and ion transport and homeostasis are issues of special significance (Pardo et al. 2006). Salt effected soils can be brought under cultivation by producing germ plasm tolerant to salinity, which involves identifying genotypes or cultivars on the basis of physiological/biochemical traits that are tolerant to salinity or the use of new genetic resources to introduce new genes for salt tolerance in existing cultivars (Farshadfar et al. 2008). The trait of salinity tolerance is present in D-genome ancestor of wheat *Ae. tauschii* and transferred to synthetics when crossed with susceptible durum parents (Schachtman et al. 1992). Material (landraces) developed in Pakistan before the Green Revolution possess very useful genes for tolerance to abiotic stresses, such as to salt and drought that prevail in the arid and semiarid regions. Our aim is to exploit the variation for salt tolerance within wheat to produce new salt-tolerant wheat cultivars. Many mechanisms, including growth response, selective uptake and transport of Na^+, maintaining high K^+:Na^+ in their cytoplasm, chlorophyll, antioxidative enzymes, compatible solute productio,n and osmotic adjustment have been associated with genetic variation in salt tolerance.

Thirty-two wheat genotypes (advanced lines, landraces, synthetic hexaploids, and checks (LU 26S and Kharchia 65) were obtained from Wheat Wide Crosses, Wheat Program and National Gene Bank, Plant Genetics Resources Program, IABGR, National Agricultural Research Centre, Islamabad, Pakistan. Wheat plants were grown in Jiffy-7 peat pots. Ten-day-old seedlings were transferred to black painted boxes (3 dm^3), containing 3 L full-strength, Hoagland's culture solution (Hoagland and Arnon 1950) and grown in a growth chamber at Plant Physiology Program, CSI, NARC, Islamabad. Thirteen-day-old wheat plants were exposed to 100 mM NaCl. Six days after salinization, recently matured fresh leaves were collected and Na^+ and K^+ concentrations were measured using the method of Yeo and Flower (1983). Chlorophyll content was calculated according to Arnon (1949). Twenty-day-old plants were harvested and shoot and root biomass were recorded.

Based on physiological parameters such as K^+:Na^+ ratio and chlorophyll content, seven genotypes, V-05066, NR-372, PK-01, V07194, Elite-10, Elte-9, and Elite-8, were found to be the most salt tolerant; PR-102, PK-04, V-05082, Elite-4, and Elite-3 were semi-tolerant, and rest of the genotypes were sensitive (Fig. 31, p. 191). The K^+:Na^+ ratio in tolerant genotypes was 2.0–3.5, 1.6–2.2 in the semi-tolerant genotypes, and 0.4–1.5 in the sensitive genotypes. Chlorophyll content in tolerant genotypes was 12.5–14.5 mg/g dry wt, 10.5–12.4 mg/g dry wt in the semi-tolerant genotypes,

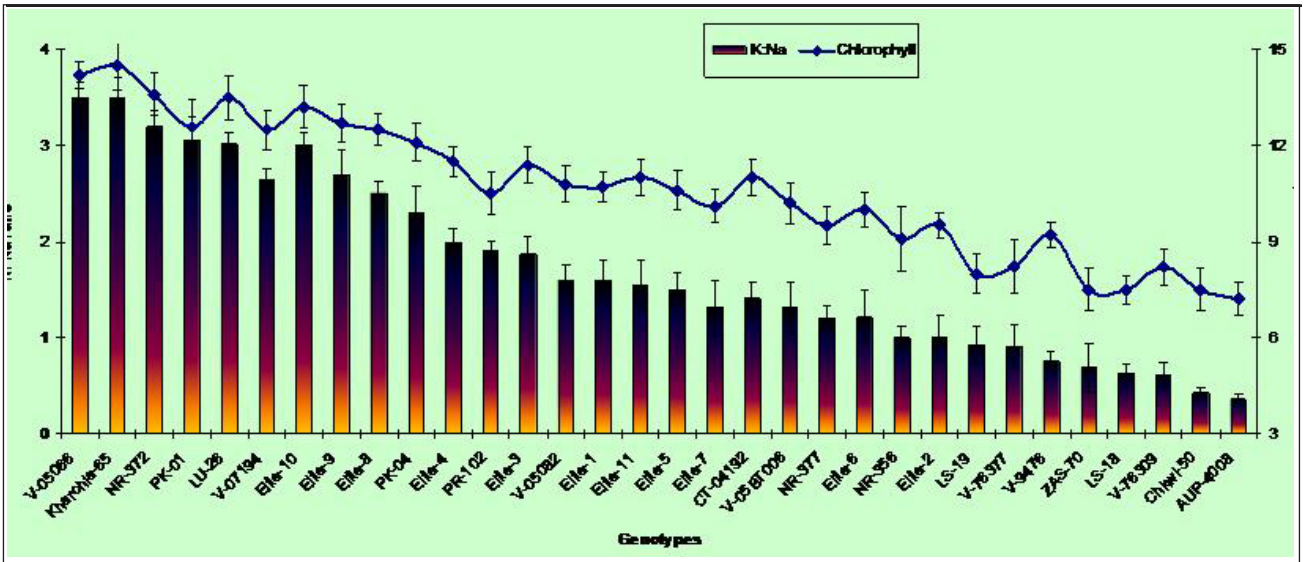

Fig. 31. The K^+:Na^+ ratio and chlorophyll content (mg/g dry weight) of the wheat genotypes is at 100 mM NaCl stress. Each bar represents the mean value of ten plants with standard error of means.

and 7.5–10.0 mg/g dry wt in the sensitive genotypes (Fig. 31). Shoot fresh and dry weight of the wheat genotypes were in the following order: tolerant genotypes > semi-tolerant > sensitive genotypes. The root dry weight of tolerant and semi tolerant genotypes were nearly equal, however the sensitive genotypes had lower root dry weight than tolerant and semi-tolerant genotypes (Table 53, p. 192).

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Genotypic differences in drought tolerance of wheat (Triticum aestivum L.) genotypes.

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Introduction. Drought is one of the most important factors, limiting plant growth and productivity more than any other environmental factor. In Pakistan about 15 x 10^3 ha of cultivated land is affected by this syndrome. Wheat is the most important grain crop grown, comprising about one-third of the total annual cereal production. In wheat, the reproductive stage is considered to be the most sensitive to drought. The best option to improve crop yield under moisture stress is to develop drought-tolerant crop cultivars. One important component is the evaluation of genetic variability in the cultivars to identify a tolerant cultivar that may sustain a reasonable yield under moisture stress. An increase in drought tolerance may be more successful if selection is based directly on the physiological characters conferring tolerance. Proline accumulation is considered to be associated with drought tolerance in wheat. Grain yield is a product of an organized interplay of several components that are highly susceptible to drought. This study assessed the drought tolerance of some commonly grown cultivars, advanced lines, and landraces collected from drought-prone areas.

Table 53. Shoot fresh weight, shoot dry weight, and root dry weight of wheat genotypes at 100 mM NaCl stress. The data represents the mean value of ten plants with standard error of means.

Genotype	Shoot fresh weight (g)	Shoot dry weight (g)	Rood dry weight (g)
V-05066	0.43±0.038	0.045±0.051	0.017±0.002
Kharchi-65	0.38±0.029	0.036±0.034	0.017±0.002
NR-372	0.40±0.040	0.042±0.049	0.016±0.001
PK-01	0.39±0.032	0.04±0.0470	0.022±0.002
LU-26	0.45±0.045	0.046±0.052	0.018±0.001
V-07194	0.37±0.034	0.037±0.039	0.015±0.002
Elite-10	0.35±0.045	0.032±0.021	0.018±0.003
Elite-9	0.34±0.060	0.033±0.036	0.016±0.004
Elite-8	0.31±0.050	0.029±0.032	0.015±0.003
PK-04	0.34±0.031	0.035±0.034	0.019±0.003
Elite-4	0.27±0.026	0.025±0.040	0.016±0.001
PR-102	0.38±0.029	0.039±0.044	0.015±0.004
Elite-3	0.24±0.022	0.022±0.026	0.015±0.002
V-05082	0.32±0.045	0.036±0.038	0.015±0.003
Elite-1	0.21±0.042	0.02±0.0250	0.014±0.001
Elite-11	0.22±0.037	0.019±0.022	0.013±0.003
Elite-5	0.23±0.025	0.02±0.0180	0.012±0.004
Elite-7	0.21±0.033	0.019±0.024	0.012±0.002
CT-04192	0.29±0.060	0.03±0.0280	0.016±0.001
V-05BT006	0.30±0.050	0.031±0.030	0.014±0.002
NR-377	0.31±0.026	0.033±0.035	0.015±0.001
Elite-6	0.20±0.020	0.018±0.017	0.011±0.001
NR-356	0.28±0.022	0.032±.0320	0.014±0.003
Elite-2	0.19±.0190	0.015±0.019	0.011±0.003
LS-19	0.17±0.037	0.015±0.033	0.010±0.004
V-76377	0.28±0.042	0.029±.0330	0.013±0.004
V-9476	0.31±0.037	0.032±.0370	0.012±0.002
ZAS-70	0.28±0.025	0.027±.0310	0.011±0.001
LS-18	0.15±0.025	0.013±0.027	0.009±0.002
V-76309	0.25±0.033	0.026±.0280	0.014±0.003
Chakwal-50	0.27±0.020	0.029±.0210	0.016±0.002
AUP-4008	0.25±.0190	0.024±.0250	0.012±0.001

Forty-five wheat genotypes, ZAS-70 NR-366, Inqilab-91, Zarlashta-99, NR-367, Wafaq-2001, Margalla-99, AUP-4008, NR-268, NR-375, ESH-9525, V-9476, Chakwal-97, NR-372, NR-377, Chakwal-50, NR-234, NR-368, NR-267, NARC-2009, QS-III, NR-360, HB-10, NR-358, NR-371, NR-356, GA-2002, V-05066, Sehar-2006, V-76309, V-10, Sariab-92, SD-4085/3, PR-102, EBWYT-514, V-07194, Punjab-96, V-76377, V-05082, V15, NR-356, DN-62, CT-04192, V-05BT006, and V-05BT006, were used in the study. Plants were grown in pots containing 10 kg sandy loam soil in a glasshouse at the National Agricultural Research Centre, Islamabad, during the winter/spring of 2005 and 2006 with average day/night temperatures 30±8ºC and 13±5ºC, respectively. A fertilizer mixture of 500 mg N, 300 mg P, 200 mg K, and 50 mg K per pot as urea, di-ammonium sulphate, potassium phosphate, and zinc sulphate was mixed in the soil before sowing. The pots were arranged in factorial, randomized, complete block design. Plants were subjected to three consecutive drought cycles at pre-anthesis (80 days after sowing) growth stages by withholding irrigation for 5–7 days, or until the signs of temporary wilting/leaf rolling started. After the drought stress treatment, fully emerged young leaves from control and stressed plants were sampled for quantification of proline and chlorophyll content. Control pots were irrigated as frequently as needed. After the drought treatment and sample collection, plants were regularly irrigated with water. Plants were harvested at physiological maturity and yield data recorded. Coleoptile emergence was recorded by germinating seeds in a 30% PEG (6000) for one week.

Results. Drought imposed at pre-anthesis significantly reduced grain yield (19–45%) in all the tested genotypes (Fig. 32). Relative reduction in grain yield due to drought was less in ZAS-70 (16%), NR-366 (19%), Inqalab-91 (19%), Zarlashta-99 (21%), NR-367(21%), Wafaq-2001(22%), Margalla-991(23%), AUP-4008 (26%), and NR-268 (26%). Under drought stress, a few additional genotypes, such Chakwal-97, NR-377, NR-360, and NR-356, also had high yields. However, based on some physiological traits such as coleoptile emergence, proline accumulation, chlorophyll content, and the yield component seeds/plant, the overall performance of wheat genotypes ZAS-70, NR-366, Inqalab-91, Zarlashta-99, NR-367, and Wafaq-2001, and the advance lines developed by NARC, NR-267 and NR-268, were found to be better than the other test cultivars. They produced a higher number of caryopses and a fewer number of sterile florets per spike under water stress conditions.

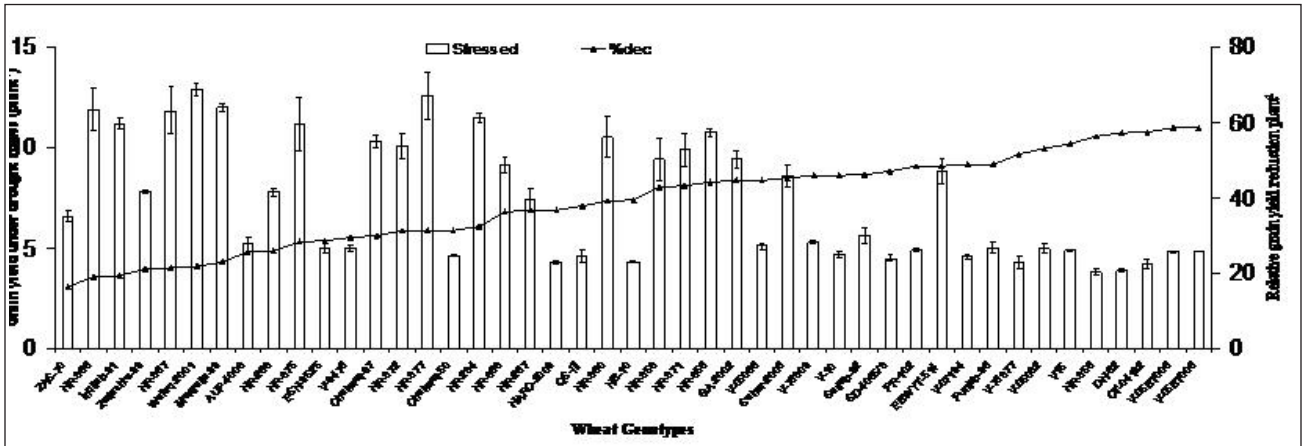

Fig. 32. The effect of drought stress on the relative grain yield reduction and grain yield (under drought stress) in wheat genotypes.