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IN SITU ASSESSMENT OF MORPHO-PHYSIOLOGICAL RESPONSE OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES TO DROUGHT

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Abstract

In situ studies were conducted to assess the morpho-physiological responses of wheat genotypes to PEG-induced water stress. Wheat genotypes were raised in hydroponic cultures where plants were nourished with ½ strength Hoagland solution. Plants were exposed to 00, 10, 20, 30 and 40% PEG-6000 at 4-leaf stage. PEG was applied in split doses @ 10% with an interval of 15 days. Significant differences ($p \leq 0.05$) were recorded for all the parameters studied due to genotypes and PEG concentrations. Wheat genotypes showed negative but variable response to PEG concentrations for shoot length, root length, root/ shoot ratio and root mass whereas PEG imposed stress had positive impact on proline content and abscisic acid (ABA). Genotype Khattakwal attained maximum shoot length in PEG induced stress. Maximum root/shoot ratio and root mass was recorded in Ghaznavi-98 while Tatara and Khattakwal attained maximum relative water content. Endogenous proline and ABA content increased up to 10 fold in response to 40% PEG. Maximum proline was accumulated by Khattakwal whereas maximum ABA by ICP-3.

Introduction

Research on plant response to water stress is becoming increasingly important as most climate-change scenarios suggest an increase in aridity in many areas of the world (Petit *et al*., 1999). Drought is one of the most important abiotic stress which affect crop growth and yield (Lutts *et al.,* 2004; Mahajan & Tuteja, 2005; Chaves *et al*., 2003). With increasing aridity and growing population, water will become an even scarcer commodity in the near future and its continuous shortfall will be disastrous for agriculture (Passioura, 2002). A better understanding of the effects of drought on plants is vital for improved management practices and breeding efforts in agriculture and for producing the fate of natural vegetation under climate change.

It is important to gain an understanding of how the crop reacts to drought for developing a breeding program for the improvement of drought resistance in a crop. Once the overall drought reaction has been established, the next step would be to identify mechanisms of drought escape, avoidance and tolerance, which contribute to performance under drought conditions. In turn, different genotypes could be studied in order to determine their genetic variability, heritability and performance under drought stress. Exposure of plants to water-limiting environment causes a set of metabolic and physiological changes, which can lead to plant survival or alternatively too severe damage and plant death. The one or other the possibility depends basically on the intensity and duration of the constraint. Rapid and severe variations in the environment, which represent the driving, force away from cellular homeostasis, often cause irreversible tissue injuries. Disruption in

plant organs and tissues in response to extreme water stress has been observed in several plants (Stewart, 1989). On the other hand, gradual adaptation to increasing intensity of water stress, a condition closer to that which occurs naturally, allows adjustments of the damaged organs compatible with the imposed stress. A step-wise adaptation allows biochemical and structural modifications, which is prerequisite of the acquired tolerance (Leon *et al*., 1994). Screening could be performed successfully both under field and controlled conditions. Fluctuation in the environment including unpredictable rains etc, could restricts the usage of the field experimentation. Many studies have been conducted covering the subjects from whole plant strategies to control water status under drought to the physiological and biochemical processes (Cornic & Massacci, 1996). Polyethylene (PEG) induced water stress has successfully used to screen drought tolerance in many plant species (Gopal & Iwama 2007; Bayoumi *et al.*, 2008; Ahmad *et al.*, 2009). Several physiological and biochemical changes when plants are exposed to water deficit stress have been extensively studied (Passioura, 2007). Abscisic acid (ABA) plays an important role under drought stress. Accumulated evidence has shown that root-originated xylem sap ABA can move to crop reproductive structures and accumulate there to a high level under drought conditions in wheat crop (Lie *et al.*, 2003). Biochemical (proline) changes plants growing under water stress conditions have been broadly investigated in many crop species (Hu *et al.*, 2007; Teixeira & Pereira, 2007; Cha-um *et al.*, 2009). Nevertheless, there is still insufficient data on the molecular and physiological mechanisms underlying plant responses to drought and other stresses. Keeping in view the importance of wheat and its lower yield in drought proven area, the present study was conducted to screen wheat genotypes for drought tolerance.

Materials and Methods

The experiment was conducted on 8 locally developed wheat cultivars viz., Ghaznavi-98, Fakhr-e-Sarhad, Inqilab-91, Tatara, Takbeer, Margalla-99, Pirsabak-85 and SARC-3, one line from ICARDA viz., ICP-3 and a local landrace (Khattakwal). Five hundred clean seeds of each genotype were surface sterilized with 0.1% H_2O_2 for 20 minutes, rinsed thoroughly with de-ionized water, and then soaked overnight in sterile water at room temperature. Seeds were then germinated in large trays in sterilized inert quartz sand. Seedlings of 5 cm (at 2 leaf stage) were then transferred to plastic trays containing 40 L ½ strength Hoagland nutrient solution (Hoagland & Arnon, 1950) with continuous aeration. Plants were fixed with the help of cotton/polyester foams plugs in holes (made with the help of cork borer) in thermo-pore plates at 5 cm apart as plant-to-plant and row-to-row distances. Holes were made in such a way that finally there were 20 columns and 10 rows. Each genotype was randomly grown in two columns containing 20 plants of each genotype in each treatment. Five PEG concentrations (0, 10, 20, 30 and 40%) induced water stress was studied. Nutrient solution was replaced after every three days. The experiment was replicated four times and repeated five times. At 4-leaf stage, uniform sized plants were retained and others were discarded. Fifteen days after 4-leaf stage 1st data (as reading 1) on various parameters was recorded on 5 plants of each genotype in each treatment then water stress was induced by the addition of various concentrations of PEG-6000 in the medium. PEG was applied in five split doses of 10% increment and data was recorded after each addition (denoted as reading 1 to 5). Osmotic potential of PEG was determined according to the methods of Lee Stadelmann & Stadelmann (1989). Medium was aerated continuously to avoid anoxia. Data was recorded on shoot length, root length, root shoot ratio, relative water content (RWC; Smart, 1974), proline (Bates *et al*., 1973) and ABA (Parry & Horgon, 1991) content.

Statistical analysis

All data are presented as mean values of four replicates. Data were analyzed statistically for analysis of variance (ANOVA) following the method described by Gomez & Gomaz (1984). MSTATC computer software was used to carry out statistical analysis (Bricker, 1991). The significance of differences among means was compared by using Least Significant Difference (LSD) test (Steel &Torrie, 1997).

Results

Plant growth: Shoot length showed significant ($p \leq 0.05$) differences due to genotypes, PEG concentrations and readings and interaction between PEG concentrations X readings whereas all other interactions were non-significant ($p \geq 0.05$; Table 1). Shoot length ranged from 23.4 cm (Takbeer) to 30.3 cm (Khattakwal, Table 2). PEG induced stress significantly ($p \leq 0.05$) decreased shoot length. Maximum shoot length of 37.0 cm was recorded in controls and decreased consistently with the increase in the PEG concentration. Shoot growth was very rapid from 1st reading to 5th reading (15.3 to 45.1 cm, respectively, Fig. 1) In case of interaction between PEG concentrations and readings shoot length ranged from 15.1 to 66.7 cm (Fig. 1). On 1st reading all the shoots were of almost the same length in all treatments. From 2nd onward readings, shoot length increased from the previous readings in all treatments but the rate of increase was slower in PEG stressed conditions in comparison to the shoot length of the plants grown in PEG free medium.

Significant ($p \leq 0.05$) differences were noted for root length due to PEG concentrations, readings and interaction between PEG concentrations X readings while all other interactions were non-significant ($p \geq 0.05$). Root length ranged from 9.8 cm (Takbeer) to 10.1 cm (ICP-3). Variability in root length existed in wheat genotypes but these differences were marginal ($p \geq 0.05$). PEG induced stress caused a negative effect on root length and hence slowed down the root growth when compared with control plants. Root length consistently increased in subsequent reading and ranged from 5.1 to 20.0 cm from 1st reading to 5th reading. Interaction between PEG treatments X readings revealed that root length ranged from 5.1 to 20.0 cm (Fig. 2). Root length increased in all treatments during the experimental period. Maximum length was recorded in PEG free medium where root length increased from 5.1 cm to 20.0 cm.

The effect of genotypes, PEG concentrations, readings and all interactions were significant at $p \leq 0.05$ on root-shoot ratio (Table 1). Different genotypes showed variable response for root shoot ratio (Table 4), however, root-shoot ratio ranged from 0.334 (ICP-3) to 0.380% (Ghaznavi-98). PEG induced stress imposed a negative effect on root shoot ratio. Root shoot ratio decreased consistently with the increase in PEG dosages when compared with control. Root-shoot ratio ranged from 0.336% to 0.408% (1st to 5th reading, Fig. 3). Root shoot ratio increased from 2nd reading onward but this increase was inconsistent. Root mass was significantly ($p \leq 0.05$) differed for PEG treatments, readings and interaction between PEG concentrations X readings (Table 1). Root mass was in the range of 15.52 cm^3 Ghaznavi-98 to 16.18 cm^3 in Inqilab-91 (Table 5). Root mass consistently dropped with the increase of PEG concentration in the medium. Maximum root mass was produced by the plants grown in PEG free medium (control), whereas minimum root mass was produced by plants survived in 40% PEG stress. Root mass increased consistently but at variable rate from 1st reading to 5th reading (Table 9). In all PEG treatments, root mass increased from 1st reading to 5th reading but the rate of increase varied in different PEG concentrations (Fig. 4).

Table 1 . Analysis of variance for different parameters of wheat genotypes.

Source	DF	RWC	SL	RL	Ratio	RM	Pro	ABA
A-Genotypes	9	*	*	NS	*	NS	*	*
B-PEG conc.	4	*	*	*	*	*	*	*
AB	36	NS	NS	NS	*	NS	*	*
C-Readings	4	NS	*	*	*	*	*	*
AC	36	NS	*	NS	*	NS	*	*
BC	16	NS	*	*	*	*	*	*
ABC	144	*	NS	NS	*	NS	*	NS
Error	1000							
Total	1249							
LSD			18.00	1.041	0.055	0.784	1.748	0.392

DF= Degree of freedom; RWC= Relative water content; SL= Shoot length; RL= Root length;
Ratio= Root shoot ratio; RM= Root mass
Pro= Proline content, ABA= Abscisic acid
*= Significant at $p \leq 0.05$
NS= Non-significant

Table 2. Mean values of shoot length (cm) of wheat genotypes grown in five PEG conc.

Genotypes	PEG concentration %					Mean
	00	10	20	30	40	
Ghaznavi-98	35.6	27.7	23.3	23.3	22.0	26.4
Fakhr-e-Sarhad	35.8	25.9	22.6	20.8	20.1	25.0
Inqilab-91	35.2	23.8	21.1	20.2	18.6	23.8
Tatara	34.9	28.2	23.6	23.0	22.7	26.6
Takbeer	34.8	24.8	21.4	19.2	16.7	23.4
Margalla-99	35.0	23.9	20.6	20.2	18.4	23.7
Pirsabak-85	35.0	27.5	23.5	23.0	22.8	26.5
ICP-3	34.7	31.3	29.1	28.1	27.7	30.2
SARC-3	34.6	31.5	28.9	28.3	27.9	30.2
Khattakwal	34.1	31.4	29.4	28.5	28.1	30.3
Means	37.0	28.8	25.1	24.9	24.2	

$LSD_{(5\%)}$ for genotypes = 1.038; for PEG Conc. = 0.846; for genotype X PEG = 1.392

Table 3. Mean root length (cm) of wheat genotypes at five PEG conc.

Genotypes	PEG concentration %					Means
	00	10	20	30	40	
Ghaznavi-98	13.6	10.4	9.4	8.5	8.2	10.0
Fakhr-e-Sarhad	13.4	10.4	9.5	8.5	8.3	10.0
Inqilab-91	12.9	10.3	9.4	8.5	8.3	9.9
Tatara	13.3	10.4	9.4	8.6	8.3	10.0
Takbeer	13.2	9.7	9.4	8.6	8.3	9.8
Margalla-99	13.3	10.3	9.3	8.4	8.3	9.9
Pirsabak-85	13.3	10.5	9.0	8.8	8.1	10.0
ICP-3	13.0	10.4	9.5	8.6	8.1	9.9
SARC-3	13.5	10.5	9.5	8.5	8.5	10.1
Khattakwal	13.1	10.4	9.5	8.7	8.2	10.0
Means	13.3	10.3	9.4	8.6	8.3	

$LSD_{(5\%)}$ for genotypes = 0.273; for PEG Conc. = 0.476; for genotype X PEG Conc. = 0.805

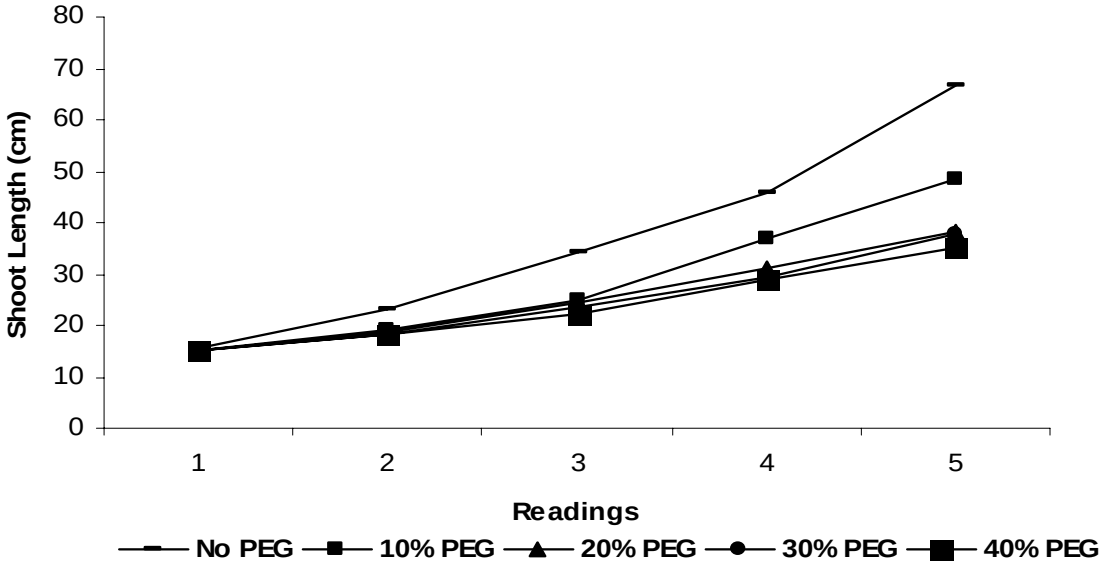

Fig. 1. Effects of PEG induced stress on shoot length of wheat genotypes on various readings.

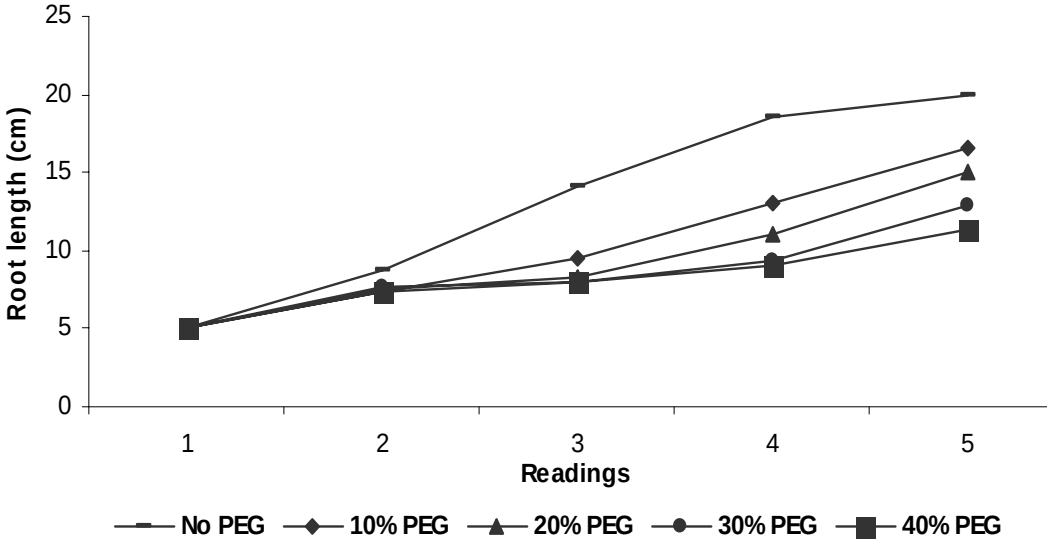

Fig. 2. Effects of PEG induced stress on root length of wheat genotypes on various readings.

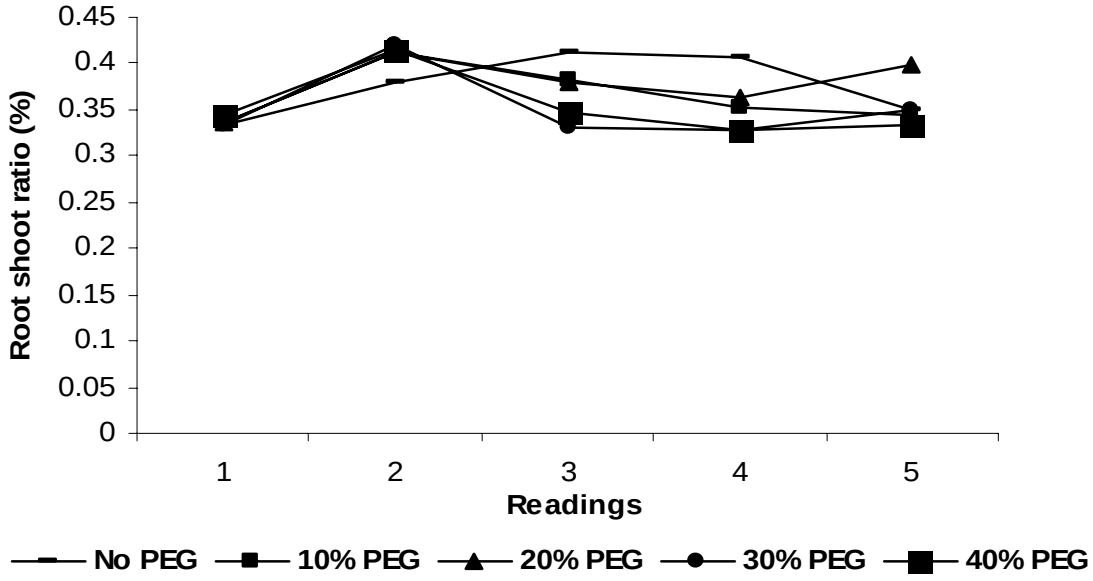

Fig. 3. Effects of PEG induced stress on root shoot ratio of wheat genotypes on various readings.

Leaf relative water content (RWC) showed significant differences ($p \leq 0.05$) due to genotypes, PEG, reading and interaction between genotypes X PEG concentrations and PEG concentrations X readings. Leaf RWC for PEG concentrations ranged from 28.76% (in 40% PEG) to 34.98% (in PEG free medium) indicating a consistent reduction in RWC in response to PEG concentrations. Similarly, RWC declined consistently with the passage of time and least values were observed on last reading (Fig. 5). RWC for interaction between genotype X PEG concentration ranged from 17.76 to 43.24, however, RWC consistently decreased in response to PEG dosages in comparison to PEG free medium in all genotypes (Table 6). RWC for interaction of PEG concentrations X readings was in the range of 35.18 to 35.97 on 1st reading to 33.78 on 5th reading.

Shoot proline content: Significant differences ($p \leq 0.05$) were noted for all main effects and their interactions (Table 1). Proline content was in the range of 4.689 and 7.976 µg g^{-1} fresh weights (Table 7). The highest proline accumulation was recorded in Khattakwal while least in Ghaznavi-98. On the basis of proline accumulation, the ten wheat genotypes used in the present experiments could be classified into three different groups; low (Ghaznavi-98 and Pirsabak-85), medium (Fakhr-e-Sarhad, Inqilab-91, Tatara, Takbeer and Margalla-99) and high (Khattakwal, ICP-3 and SARC-3) proline accumulating genotypes. Proline accumulation in different PEG-6000 induced stress ranged from 1.156 to 11.358 µg g^{-1} fresh weight (Table 7). Proline accumulation consistently increased with the increase in PEG concentration in the medium. Exposure of plants to 40% PEG in the medium resulted in 800% increase in proline accumulation when compared with control plants (Fig. 6).

Shoot abscisic acid: Shoot abscisic acid (ABA) showed significant ($P \leq 0.05$) differences due to genotypes, PEG, reading and their interactions. Shoot ABA ranged between 0.098 ng g^{-1} fresh weight (Fakhr-e-Sarhad) to 0.123 ng g^{-1} fresh weights (Khattakwal; Table 8). Abscisic acid varied from 0.015 ng g^{-1} fresh weight in PEG free medium to 0.216 ng g^{-1} fresh weight in 40% PEG medium (Table 8). A gradual increase in endogenous ABA content was observed with each increment of PEG in the medium. ABA accumulation at different time intervals ranged from 0.003 to 0.110 ng g^{-1} fresh weights (Fig. 7). Lowest ABA was measured from the plants on 1st reading whereas highest on 5th reading.

Discussion

The present study investigate the effect of PEG-induced water stress on plant growth and development of different wheat genotypes. The growth related parameters of all the 10 tested genotypes were negatively affected by different PEG concentrations whereas proline and ABA accumulations were significantly enhanced in these genotypes. However, the various genotypes under study behaved differently towards the drastic effects of PEG-induced stress.

Significantly reduced shoot and root lengths were observed in PEG-induced stress since PEG may interfere with cell division and growth. The findings of the present study are similar to some earlier studies where water stress reduced shoot and root fresh and dry weights, shoot length, total leaf area per plant, grain yield and gas exchange characteristics and increased shoot proline contents in wheat genotypes (Kamran *et al*., 2009). Similarly, imposition of water stress reduced shoot and root fresh and dry weights and chlorophyll contents of maize cultivars (Ali *et al*., 2007).

Table 4. Mean values of root: shoot ratio (%) of wheat genotypes grown in five PEG conc.

Genotypes	PEG concentration %					Means
	00	10	20	30	40	
Ghaznavi-98	0.380	0.375	0.374	0.370	0.365	0.373
Fakhr-e-Sarhad	0.371	0.364	0.362	0.356	0.351	0.361
Inqilab-91	0.362	0.397	0.416	0.426	0.438	0.408
Tatara	0.373	0.371	0.367	0.364	0.359	0.367
Takbeer	0.381	0.413	0.431	0.443	0.472	0.428
Margalla-99	0.371	0.397	0.403	0.413	0.446	0.406
Pirsabak-85	0.378	0.365	0.363	0.360	0.354	0.364
ICP-3	0.373	0.335	0.333	0.312	0.303	0.331
SARC-3	0.414	0.342	0.334	0.317	0.307	0.343
Khattakwal	0.380	0.335	0.329	0.323	0.304	0.334
Means	0.378	0.371	0.369	0.368	0.365	

$LSD_{(5\%)}$ for genotypes= 0.036; for PEG Conc. = 0.034; for genotype X PEG Conc. = 0.108

Table 5. Mean values of root mass (cm^3) of wheat genotypes grown in five PEG conc.

Genotypes	PEG concentrations %					Means
	00	10	20	30	40	
Ghaznavi-98	30.81	14.97	11.32	10.52	9.97	15.52
Fakhr-e-Sarhad	31.17	14.80	11.66	11.13	10.32	15.82
Inqilab-91	31.91	13.76	11.32	10.60	13.31	16.18
Tatara	31.72	14.91	11.59	10.80	10.29	15.86
Takbeer	31.29	14.79	11.52	10.90	10.31	15.76
Margalla-99	31.19	14.85	11.73	10.85	10.24	15.77
Pirsabak-85	31.77	14.50	11.31	10.67	10.07	15.66
ICP-3	31.76	14.90	11.58	10.88	10.39	15.90
SARC-3	31.50	14.86	11.60	10.66	10.35	15.79
Khattakwal	32.19	15.00	11.70	11.03	10.47	16.08
Means	31.53	14.73	11.53	10.80	10.57	

$LSD_{(5\%)}$ for genotypes = 0.52; for PEG Conc. = 0.88; for genotype X PEG Conc. = 1.26

Table 6. Mean values of relative water contents (%) of wheat genotypes grown PEG conc.

Genotypes	PEG concentration %					Means
	00	10	20	30	40	
Ghaznavi-98	43.16	37.10	33.42	33.05	31.86	35.72
Fakhr-e-Sarhad	33.59	31.85	30.66	28.87	28.44	30.68
Inqilab-91	25.54	21.84	19.71	18.97	17.76	20.76
Bakhtawar-92	41.31	35.30	34.39	36.84	34.38	36.44
Takbeer	29.48	25.27	24.90	24.66	22.40	25.34
Margalla-99	32.16	35.03	30.96	30.13	28.99	31.45
Pirsabak-85	26.73	21.68	20.98	19.74	17.45	21.32
Tatara	43.24	38.68	35.31	37.39	42.37	39.40
ICP-3	33.59	30.36	28.27	27.95	27.19	29.47
Khattakwal	41.03	38.22	35.93	38.50	36.75	38.09
Means	34.98	31.53	29.45	29.61	28.76	

$LSD_{(5\%)}$ for genotypes= 2.421; for PEG conc.= 1.312 for genotypes X PEG 9conc. = 4.521

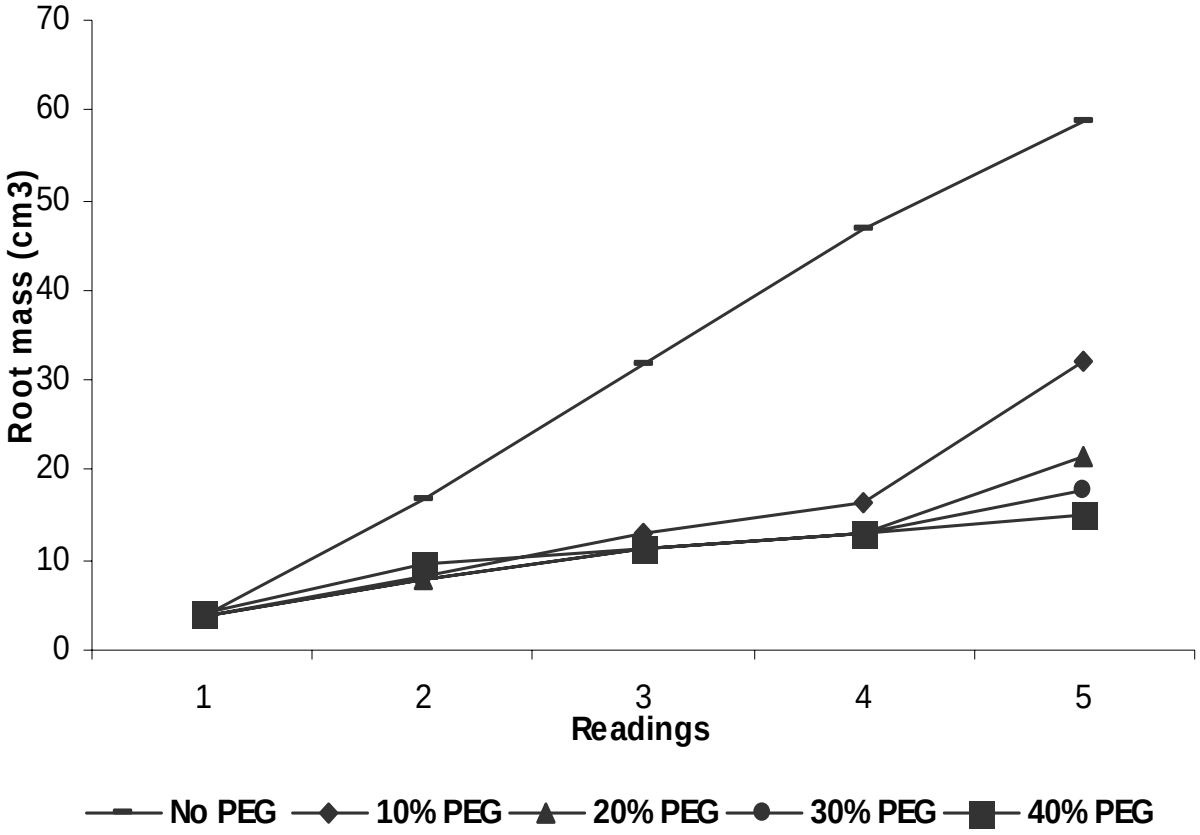

Fig. 4. Effects of PEG induced stress on root mass of wheat genotypes on various readings.

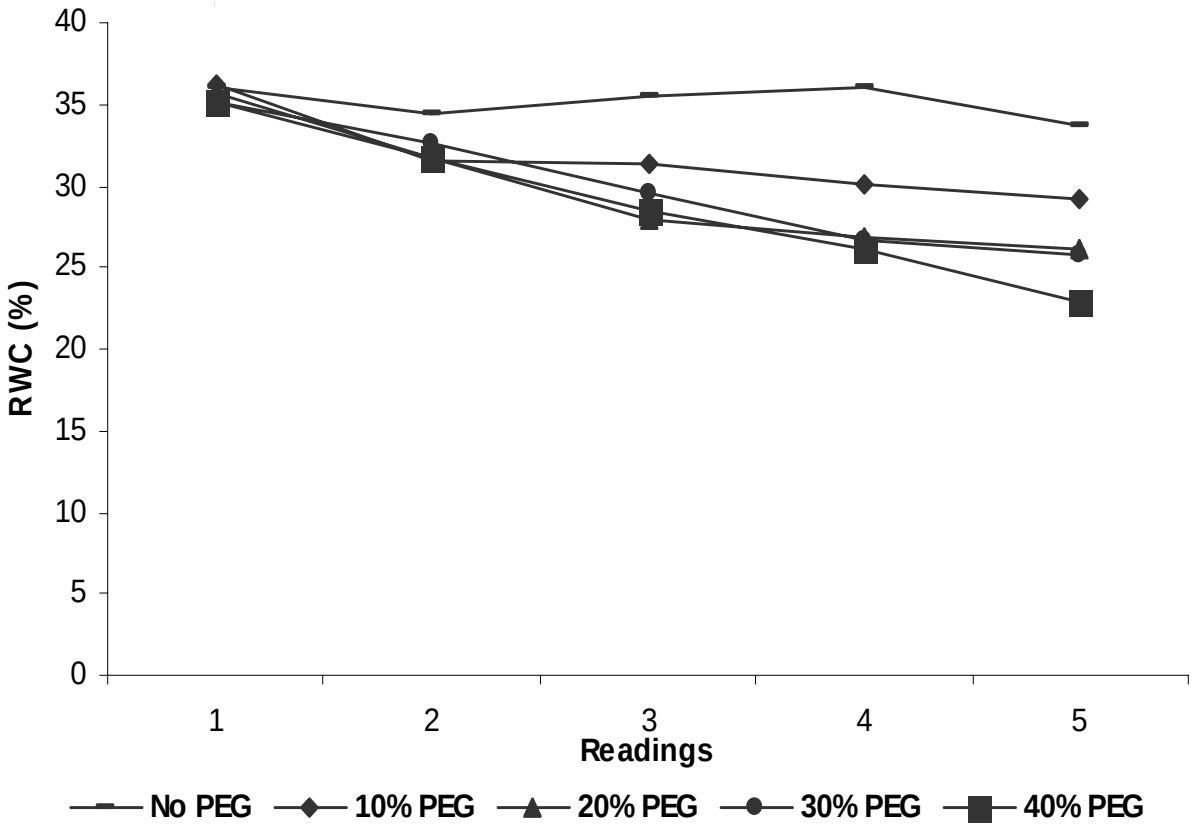

Fig. 5. Effects of PEG induced stress on relative water content (RWC) of wheat genotypes on various readings.

Table 7. Mean values of proline content (µg g^{-1} fresh weight) of wheat genotypes grown in five PEG conc.

Genotypes	PEG concentration %					Means
	00	10	20	30	40	
Ghaznavi-98	1.114	2.005	3.709	8.487	9.990	5.061
Fakhr-e-Sarhad	1.159	2.052	3.753	8.738	9.462	5.033
Inqilab-91	1.151	2.031	3.604	7.334	9.325	4.689
Tatara	1.170	2.105	3.714	8.512	10.488	5.198
Takbeer	1.115	2.083	3.855	8.386	10.125	5.113
Margalla-99	1.152	2.027	3.959	8.779	10.203	5.224
Pirsabak-85	1.198	2.111	3.946	7.591	9.023	4.774
ICP-3	1.139	2.401	5.597	12.951	14.531	7.324
SARC-3	1.176	2.534	5.963	13.166	15.175	7.603
Khattakwal	1.191	2.611	6.049	14.772	15.258	7.976
Means	1.157	2.196	4.415	9.872	11.358	

$LSD_{(5\%)}$ for genotypes= 0.663; for PEG Conc. = 0.469; for genotype X PEG Conc. = 1.484

Table 8. Mean values of abscisic acid (ng g^{-1} fresh weight) of wheat genotypes grown in five PEG conc.

Genotypes	PEG concentrations %					Means
	00	10	20	30	40	
Ghaznavi-98	0.013	0.057	0.087	0.121	0.198	0.095
Fakhr-e-Sarhad	0.014	0.057	0.085	0.110	0.191	0.091
Inqilab-91	0.016	0.061	0.086	0.118	0.188	0.094
Tatara	0.014	0.056	0.086	0.122	0.212	0.098
Takbeer	0.015	0.057	0.087	0.123	0.204	0.097
Margalla-99	0.014	0.059	0.094	0.119	0.193	0.096
Pirsabak-85	0.016	0.061	0.089	0.120	0.228	0.103
ICP-3	0.015	0.074	0.097	0.141	0.252	0.116
SARC-3	0.016	0.070	0.103	0.136	0.243	0.114
Khattakwal	0.016	0.073	0.107	0.140	0.247	0.112
Means	0.015	0.063	0.092	0.125	0.216	

$LSD_{(5\%)}$ for genotypes= 0.0024; for PEG Conc. = 0.0017; for genotypes X PEG Conc. = 0.0055

The extent and pattern of root development are closely related to the ability of the plant to absorb water. Optimization of rooting behavior would thus seem to be an obvious way of increasing water use-efficiency. Passioura (2002) has suggested that restricted seminal root system would reduce water use before anthesis, leaving more water available during the grain-filling period, with consequent increased grain yield. Price *et al*., (1997) reported that root growth is an important component of the adaptation of rice to drought-prone environments. Nagarajan & Rane (2000) recorded decreased root length and root and shoot weight in response to water stress.

A most common observation concerning roots under drought stress is the increase in root/shoot dry matter weight ratio. Increase in ratio results from the relatively greater decrease in shoot growth than in root growth under drought stress. However, in some rare cases root weight increased in absolute terms under drought stress (Malik *et al*., 1979). The increase in dry matter root/shoot ratio often implies the development of a larger ratio of root length density to leaf area, which translates into a better capacity for sustaining plant water status under a given evapo-transpirational demand (Blum & Arkin 1984).

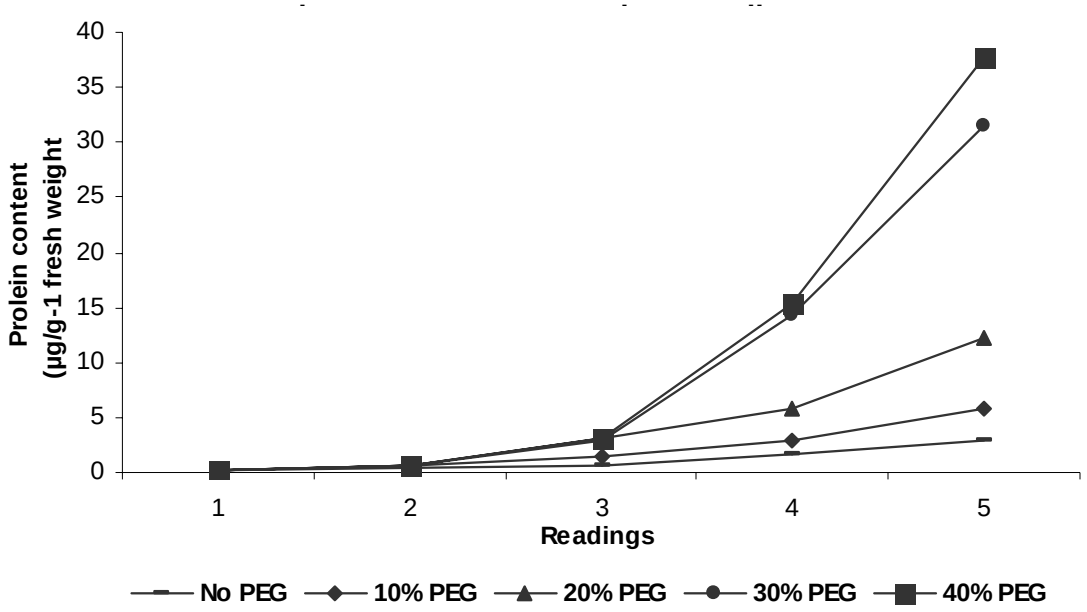

Fig. 6. Effects of PEG induced stress on prolein content of wheat genotypes on various readings.

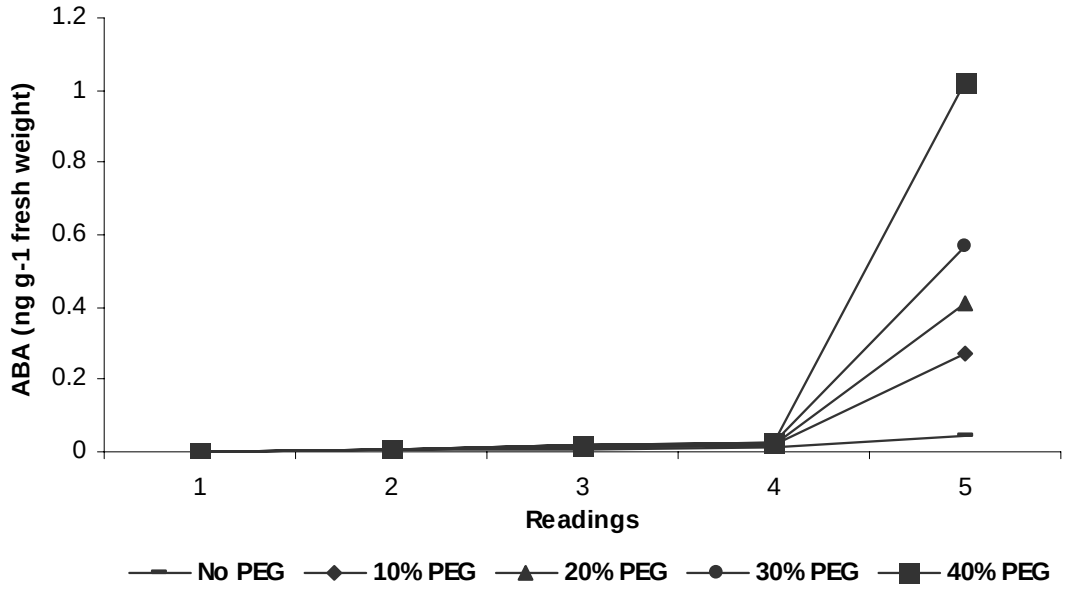

Fig. 7. Effects of PEG induced stress on ABA of wheat genotypes on various readings.

Relative water content (RWC) is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. Consistent decrease in RWC in response to PEG-induced water stress have been reported in wheat (Bajji *et al*., 2001, Zhang & Li, 2000; Swati *et al*., 2000), in *Brassica* (Zakirullah *et al*., 2000) and in rice (Hsu *et al*., 2003a). Strauss & Agenbag (2000) have suggested that leaf relative water content and proline content could be useful parameters for determining water stress in plants.

It is now well documented that drought stressed plants exhibit various physiological, biochemical and molecular changes to thrive under water limited conditions (Arora *et al*., 2002). Under various environmental stresses including drought, increased accumulation of proline and ABA is a characteristic feature of most plants (Lie *et al*., 2003; Hsu *et al*., 2003a; Hsu *et al*., 2003b; Teixeira & Pereira, 2007; Cha-um *et al*., 2009).

PEG-treatment also increased abscisic acid (ABA) content and decreased ethylene production (Hsu *et al*., 2003 a and b). The accumulation of proline is generally correlated with stress tolerance as tolerant species accumulate more proline as compared to sensitive ones (Zhang & Li, 2000; Nayyar & Walia, 2003). Proline is one of the most studied compatible solutes of imino group. In plants, proline is synthesized in the cytosol and mitochondria from glutamate via Δ1-pyrroline-5-carboxylate (P5C) by two successive reduction catalyzed by P5C synthetase (P5CS) and P5CR respectively (Hare *et al*., 1999). Genes encoding these enzymes have been cloned in several plant species and the expression of P5C was shown to be up-regulated by water and osmotic stress involving both ABA-dependent and ABA-independent signaling cascade. Further studies indicated that P5C might also serve as a regulator of cellular stress responses (Deuschle *et al*., 2001). It is now well established that accumulation of proline in plants provides energy for their growth and stress tolerance. Proline also plays an important role in protection of membrane organelles, proteins and enzymes under stress (Ashraf & Foolad, 2007; Hoque *et al*., 2007). It may also act as a regulatory or signaling molecule to activate a variety of responses (Maggio *et al*., 2002).

Conclusion

The results of these experiments suggested that screening procedures such as the one used in the present study are relatively simple, reproducible and less labor-intensive than growing plants to maturity in the field. Genotypic variability exists among the wheat genotypes for PEG-induced water stress. Landrace Khattakwal was found more tolerant whereas SARC-3 and ICP-3 also showed some tolerance to PEG-induced water stress. The amount of proline and ABA accumulation consistently increased in response to PEG increments in the medium; therefore, these parameters could be used as physiological indices for drought tolerance.

References

Ahmad, S., R. Ahmad, M.Y. Ashraf, M. Ashraf &E.A. Waraich. 2009. Sunflower (*Halianthus annuus* L.) to drought stress at germination and seedling stages. *Pak. J Bot.,* 41: 647-654.

Ali, Q., M. Ashraf and H.R. Athar. 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. *Pak. J. Bot*., 39: 1133-1144.

Arora, A., R.K. Sairam and G.C. Srivastava. 2002. Oxidative stress and antioxidative systems in plants. *Curr. Sci*., 82: 1227-1238.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. *Environ. Exp. Bot*., 59: 206-216.

Bajji, M., S. Lutts and J.M. Kinet. 2001. Water deficit effects on solute contribution to osmotic adjustment as a function of leaf aging in three durum wheat (*Triticum durum* Desf.) cultivars performing differently in arid conditions. *Plant Sci*., 160(4): 669-681.

Bates, L.S., R.P. Weldren and I.D. Teare. 1973. Rapid determination of free proline for water-stressed studies. *Plant Soil*., 39: 205-207.

Bayoumi, T.Y., M.H. Eid & E.M. Metwali. 2008. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. *Afri. J Biotechnol.* 7: 2341-2352.

Blum, A. and G.F. Arkin. 1984. Sorghum root growth and water-use as affected by water supply and growth duration. *Field Crop Res*., 9: 131-142.

Bricker, B. 1991. MSTATC: A micro-computer program for the design, management and analysis of agronomic research experimentation. Crop and Sci. Department MSU East Lansing Mi 48824 USA.

Cha-um, S. and C. Kirdmance. 2009. Proline accumulation, photosynthetic abilities and growth characteristics of sugar cane (*Saccharum offinarum* L.) plantlets in response to iso-osmotic salt and water deficit. *Agric. Sci. China*, 8: 51-58.

Chaves, M.M., Maroco J.P. and J.S. Pereira. 2003. Understanding plant responses to drought- from genes to the whole plant. *Functional Plant Biol*., 30: 239-264.

Cornic, G. and A. Massacci. 1996. Leaf photosynthesis under drought stress. In: *Photosysntyhesis and the environment*. (Eds.): N. Baker. Kluwer Academic Publishers. New York. pp. 347-366.

Deuschle, K., D. Funck, I.L. Hellmann and W. Frommer. 2001. A nuclear gene encoding mitochondrial sigma-proline-5-carboxylate dehydrogenase and its potential role in protection from proline toxicity. *The Plant J.*, 27: 345-356.

Gomez, K.A. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd Ed. John Willey & Sons, Inc. NewYork. 641.

Gopal, J. and K. Iwama. 2007. In vitro screening of potato against water stress mediated through sorbotl and polyethylene glycol. *Plant Cell Rep*. 26: 693-700.

Hare, P., W. Cress and J. VanStaden. 1999. Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *J. Expt. Bot*., 50: 413-434.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. *California Agric. Exp. Stn. Circ*., 347.

Hoque, M.A., E. Okuma, M.N.A. Banu, Y. Nakamura, Y. Shimoishi and N. Murata. 2007. Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *J. Plant Physiol.,* 164: 553-561.

Hsu, S.Y., Y.T. Hsu and C.H. Kao. 2003a. The effect of polyethylene glycol on proline accumulation in rice leaves. *Biol. Plant*., 46: 73-78.

Hsu, S.Y., Y.T. Hsu and C.H. Kao. 2003b. Ammonium ion, ethylene, and abscisic acid in polyethylene glycol-treated rice leaves. *Biol. Plant*., 46: 239-242

Kamran, M., M. Shahbaz, M. Ashraf and N.A. Akram. 2009. Alleviation of drought-induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as pre-sowing seed treatment. *Pak. J. Bot*., 41(2): 621-632.

Lee-Stadelmann, O. and E.J. Stadelmann. 1989. Plasmolysis and deplasmolysis. *Methods Enzymol.,* 174: 225-247.

Leon, A., A. Costa, M. Tucci and S. Grillo. 1994. Adaptaion verses shock response to polyethylene glycol-induced low water potential in cultured potato cells. *Physiol. Plant.*, 92: 21-30.

Lie, F., M.N. Anderson and C.P. Jensen. 2003. Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structure in soybean. *Funct Plant Biol.,* 30: 271-280.

Lutts, S., M. Almansouri and J.M. Kinet. 2004. Salinity and water stress have contrasting
effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. *Plant Sci.,* 167: 9-18.

Maggio, A., S. Miyazaki, P. Veronese, T. Fujita, J.I. Ibeas, B. Damsz, M.L. Narasimhan, P.M. Hasegawa, R.J. Joly and R.A. Bressan. 2002. Does proline accumulation play an active role in stress-induced growth reduction? *Plant J*., 31: 699-7 12.

Mahajan, S. and N. tuteja. 2005. Cold, salinity and drought stresses: An overview. *Arch. Biochem. Biophys.,* 444: 139-158.

Malik, R.S., J.S. Dhankar and N.C. Turner. 1979. Influence of soil water deficits on root growth and cotton seedlings. *Plant and Soil,* 53: 109-112.

Nagarajan, S. and J. Rane. 2000. Relationship of seedling traits with drought tolerance in spring wheat cultivars. *Indian J. Plant Physiol*., 5: 264-270.

Nayyar, H. and D.P. Walia. 2003. Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and absisic acid. *Biol. Plant*., 46: 275-279.

Parry, A.D. and A.D. Horgan. 1991. Abscisic acid, physiology and biochemistry. (Ed.): W.J. Davies and H.G. Jones. *Environmental Biology Series*. Bios Scientific Publishers Limited. Pp 5-22.

Passioura, J.B. 2002. Environmental plant biology and crop improvement. *Functional Plant Biol*., 29: 537-546.

Passioura, J.B. 2007. The drought environment: physical, biological and agricultural prespective. *J. Exp. Bot*., 58: 113-117.

Petit, J.R., J. Jouzel, D. Raynaud, N.I. Barkov and M. Stievenard. 1999. Climatic and atmospheric history of the past 420,000 years from the Vostoc ice core Antartica. *Nature*, 399: 429-436.

Price, A.H., A.D. Tomos and D.S. Virk. 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.). I: A hydroponic screen. *Theor. & Appl. Genet*., 95: 132-142.

Smart, R.E. 1974. Rapid estimate of relative water content. *Plant Physiol*., 53: 258-260.

Steel, R.G.D. and J.H. Torrie. 1997. Principles and procedures of statistics: A Biometrical Approach. McGraw Hill, New York USA.

Stewart, C.R. 1989. Desiccation injury, anhydrobiosis and survival. In: *Plants under stress*. (Eds.): T.J. HG Jones, T.J. Flowers and MB Jones. pp. 115-130. Cambridge Univ. Press, Cambridge, UK.

Strauss, J.A. and G.A. Agenbag. 2000. The use of physiological parameters to identify drought tolerance in spring wheat cultivars. *Plant and Soil*, 17: 20-29.

Swati, Z.A., M. Imtiaz, S. Ali and Raziuddin. 2000. Effect of moisture stress on leaf water potential and relative water content in wheat (*Triticum aestivum* L.). *Pak. J. Biol. Sci.*, 3: 87-90.

Teixeira, J. and S. Pereira. 2007. High salinity and drought act on an organ-dependant manner on potato glutamine synthetase expression and accumulation. *Environ.. Exp. Bot.,* 60: 121-126.

Zakirullah, Z.A. Swati, A. Ahmad and Raziuddin. 2000. Morpho-physiological response of selected *Brassica* lines to moisture stress. *Pak. J. Biol. Sci*., 3: 130-132.

Zhang, Y.Z. and T. Li. 2000. Changes of proline levels and abscisic acid content in tolerant/sensitive cultivars of soybean under osmotic conditions. *Soybean Genetics Newslett*., 27: 5 -11.

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