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RESEARCH ARTICLE

Polyethylene Glycol Mediated Osmotic Stress Impacts on Growth and Biochemical Aspects of Wheat (*Triticum aestivum* L.)

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Abstract

Seed germination and seedling growth establishment are the most critical growth stages, and drought stress imposed at these stages highly limits crop productivity. In this regard, a hydroponic water culture experiment was conducted with the aim to assess the potential of 20 wheat genotypes against drought stress at the seedling stage. Water deficit was induced through polyethylene glycol (PEG-6000), by maintaining two osmotic potentials in water culture medium, i.e. -0.7 MPa (medium water stress) and –1.0 MPa (high water stress). After seed germination, drought stress was applied for 8 days. Seedlings shoot and root length and biomasses were restricted with an increase in osmotic deficit. Photosynthetic pigments and nitrate reductase activity (NRA) of wheat seedlings were reduced, while proline, total soluble sugars, total phenolics, and mineral ions (K^+ and Ca^{2+}) were augmented with the rise in water deficiency in most of the genotypes. On the basis of growth and biochemical attributes, six genotypes (NIA-AA-01, NIA-AA-08, NIA-AA-09, NIA-AA-13, NIA-AA-12, and NIA-AA-14) were categorized as drought tolerant, and three as medium tolerant. These genotypes exhibited better growth by showing the least reduction in root and shoot length, and fresh and dry biomasses, as well as modulation in biochemical processes to survive under water deficit. All studied traits indicated tolerance potential of these genotypes against moderate and extreme drought stress, which could also give better growth in arid and semi-arid regions of the country that facing water scarcity.

Key words : Drought, photosynthesis, phenolics, polyethylene glycol, proline, soluble sugars, wheat seedlings

Introduction

Drought is one of the most devastating environmental stresses which reduces crop yield. Diverse climatic changes have affected the patterns of rainfall and inadequate water assets are depleting to fulfill water requirements of crops (Abedi and Pakniyat 2010; Al-Ghamdi 2009). Unbalanced and widespread agriculture is also exhausting water resources and disturbs the ground water table. All these factors are the leading causes of intensifying water scarcity.

Wheat is the staple food crop cultivated in many countries. It is one of the most cultivated cereal crops with a global annual production of 651.4 million metric tons (FAO 2012). Still, there is not enough food to fulfill the food requirements of an increasing human population. Hence, it is necessary to expand the land used for wheat cultivation. However, its production can be increased by overcoming the problem of drought by cultivating drought-resistant wheat varieties in drylands.

The effects of drought stress on plants depend upon severity and duration of water shortage as well as the phase of plant growth. Seed germination and seedling stand establishment are the most sensitive to drought (Noorka and Khaliq 2007). Drought conditions slow the process of seed emergence by lowering the water potential of seed. When the seedling stand establishes successfully and transforms from the seedling to plant stage, its growth becomes less susceptible to environmental fluctuations as the plant is able to evolve defense mechanisms to respond under multiple abiotic factors including soil moisture, light, temperature, and gravity (Farooq et al. 2009).

There are many morphological, phonological, and physiological characters representing the adaptive responses of plants under drought stress. Water is involved in the hydrolysis of lipids, proteins, and stored starch of seed endosperm, metabolite transport, and enzymatic reactions, etc. (Biaecka and Kepczynski 2010). In order to withstand water deficiency, the metabolic processes of plants are modulated through variations in the

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pattern of production and utilization of metabolites (Suseela et al. 2015). Recent advances in metabolomics highlighted that plants produce multiple compounds such as carbohydrates, amino-acids, phenolic acids, lipids, and amino complexes, which act as osmoregulators or antioxidant defense compounds and have a role in partial mitigation of abiotic stress (Rivas-Ubach et al. 2012). These molecules accumulate in the cytosol of the plant cell which increases its osmotic potential and helps in maintaining the turgor of cell as well as establishes the gradient for more uptake of water (Rhodes and Samaras 1994).

It becomes critical to select wheat genotypes with good drought tolerance capabilities under dry environmental conditions (Tuberosa and Salvi 2006). However, consistent and controlled drought conditions are difficult to maintain in the field as rainfall removes the water deficit conditions. Thus, *in vitro* screening method is proving effective in the selection of drought tolerant genotypes on the basis of drought-resistant traits. Many chemicals can be used for inducing *in vitro* drought stress. Polyethylene glycol (PEG) acts as osmotica to reduce water potential of water culture medium, thus creating water stress on plant tissues by the outward flow of water from plant tissues to concentrated solution of PEG (Meneses et al. 2011). PEG molecules are inert in nature, non-ionic, and induce uniform drought stress without entering the plant cells. Many early drought screening studies had also involved PEG-6000 solutions for induction of drought under controlled conditions (Jatoi et al. 2014; Nawaz et al. 2013; Singh et al. 2008).

The main motive of this study is to estimate drought tolerance potentials of newly developed wheat genotypes of the Nuclear Institute of Agriculture (NIA), Tandojam at the early seedling stage by estimating their growth and physico-chemical responses under controlled environmental conditions.

Materials and Methods

Water culture experiment was conducted in plastic pots (8.6 × 4.6 cm) under controlled laboratory conditions in the Plant Physiology main laboratory, Nuclear Institute of Agriculture Tandojam. Twenty advance wheat genotypes including two check varieties (Khirman and Chakwal-86) were collected from the plant breeding and genetics division, Nuclear Institute of Agriculture (NIA) Tandojam. The experimental plan constituted two drought treatments applied in factorial arranged completely randomized design (CRD) containing three replications. Seeds of all wheat genotypes were first germinated in Petriplates containing Whatman's filter paper no. 1 soaked with 1/4th Hoagland's solution. After 96 hours of sowing, when all seeds were germinated with reasonable lengths of plumule and radical, 30 seedlings from each genotype were transplanted on wire nets embedded in plastic pots containing water culture medium. Drought stress was imposed through PEG-6000 solutions in water culture medium by maintaining the osmotic potentials of -0.7 MPa (medium drought stress) and -1.0 MPa (high drought stress) (Michel and Kaufmann 1973), while 0 MPa Hoagland's solution (1/4th) was used as control treatment. Bowls were kept in growth incubator (Vindon, England) provided with 10 h day period with 25˚C temperature and 14 h night period with 20˚C night temperature. After 8 days of exposure to drought stress, seedlings were harvested and processed for the following growth and biochemical analyses.

Growth attributes

Five seedlings from each pot were harvested for recording shoot and root length using calibrated meter rod. Fresh weights of these seedlings were also measured instantly and dry weights were taken after oven drying for 72 h at 60°C.

Biochemical Aspects

Photosynthetic pigments

Chlorophyll pigment were quantified after extraction of freshly chopped leaves in 80% acetone solution, overnight according to the method of Lichtenthaler (1987). Chlorophyll a and b were measured by taking optical densities (OD) of filtered solutions at 663 and 645 nm, respectively, using the following equations:

$$Chl.a = 12.25A_{663} - 2.79A_{646}$$
$$Chl.b = 21.5A_{646} - 5.1A_{663}$$

NR Enzyme estimation

Nitrate reductase (NR) enzyme was assessed through the methodology of Sym (1984). Fresh seedling leaves (0.5g) were homogenized in 5 mL of 2 M phosphate buffer (pH 7.5) containing 0.02% KNO_3 at 32°C in the dark. Out of it, 1 mL solution was mixed with 0.5mL 1% sulphnailamide prepared in 3N HCl and 0.02% (1-Naphthyl)-ethylene diamine-dihydrochloride and incubated at 25°C for 20 min and absorbance was noted at 542nm.

Inorganic solutes estimation

Accumulation of potassium and calcium ions in wheat seedling was analyzed by extracting dried seedlings in 0.2 M acetic acid solution in water bath at 100°C for 1 h after Anderson and Ingram (1989). Plant samples were filtered; suitable dilutions were made with distilled water and concentration of ions was measured using flame photometer (Jenway, Model PEP7).

Estimation of organic osmolytes

Osmolytes including proline, total soluble sugars, and phenolic contents were determined by following the respective protocols of Bates et al. (1973), Riazi et al. (1985), and Waterhouse (2002).

For the estimation of proline, 0.5 g fresh leaves were ground in 10 mL 3% sulfosalicylic acid (w/v) and the extract was filtered. For the reaction, 2 mL of each plant extract, ninhydrin reagent and glacial acetic acid were heated at 100°C for 1 h in a water bath and cooled instantly. Then, 4 mL toluene was added in the above mixture and vortex for few seconds. As a result, two layers were developed, upper

pink-reddish colored layer was used to read OD at 520 nm, and proline was calculated from the standard curve.

For measurement of soluble sugars, crushed seedlings were dipped in 80% ethanol for 24 h and filtered the solution. For reaction, 0.1 mL of extract and 3 mL anthrone reagent were reacted at 98°C in a water bath for 10 min and cooled instantly. ODs of transparent green solutions were estimated at 630 nm.

Phenolic compounds were determined by grinding fresh plant tissues in 80% acetone. Then, 60 µL plant extract was reacted with 200µL Folin-Ciocalteu reagent and 3mL distilled water. After 5-8 min, 600 µL of 2 M Na_2CO_3 was added and heated at 40°C for 30 min in a water bath. Development of bluish coloration was the indication of phenolics and absorbance of samples was taken at 765 nm.

Criteria for scoring genotypes

On the basis of relative reduction in growth and biochemical aspects, wheat genotypes were characterized as drought tolerant, medium tolerant, and sensitive ones. The genotypes showing less than 30% relative reduction and higher mean values were categorized as drought tolerant, 30-60% reduction as medium tolerant, and more than 60% reduction were termed as sensitive ones.

Statistical Analyses

The experiment was executed in a factorial arranged complete randomized design with three replicates. All attributes were statistically analyzed using analysis of variance techniques to check the significant differences among wheat genotypes and drought treatments using Duncan's multiple range test and least significance difference (LSD) was computed at 5% probability level using Statistix 8.1 software.

Results

Growth

Seedling growth was more restricted with the increase in osmotic stress. Better shoot lengths were measured at -0.7 MPa as compared to -1.0 MPA PEG-induced drought stress. Maximum shoot length was maintained in NIA-AA-09 followed by NIA-AA-14 and NIA-AA-08 with 15.25, 13.69, and 30.56% relative reduction at -1.0 MPA osmotic stress, respectively (Fig. 1). However, seedling roots exhibited anomalous behavior at -0.7 MPa stress; as it was increased in some genotypes while decreased in others as compared to control while at -1.0 MPa osmotic potential, all genotypes showed reducing trend. Better root lengths were also recorded in NIA-AA-13 and NIA-AA-09 at -1.0 MPa osmotic stress (Fig. 2).

Plant biomass was also influenced by reduced water contents. Maximum shoot fresh weight was noted in genotype NIA-AA-09 at both medium and high osmotic stress. NIA-AA-08 and NIA-AA-13 also exhibited better fresh biomasses of shoots with minimum relative reductions under drought. Dry biomass of shoot varied among genotypes as it was increased in NIA-AA-09 and NIA-AA-08 while decreased in all other genotypes under both osmotic potential levels. Maximum mean values of all treatments were also observed in NIA-AA-09 and NIA-AA-08 for shoot fresh and dry biomasses (Table 1). Likewise, uppermost fresh biomasses of roots were measured in NIA-AA-01 followed by NIA-AA-09 with maximum mean values of all treatments. Root dry weights were increased in some genotypes while decreased in others under drought stress. NIA-AA-11 and NIA-AA-10 showed the highest root dry weights under mild drought stress while NIA-AA-08 produced maximum root dry weight

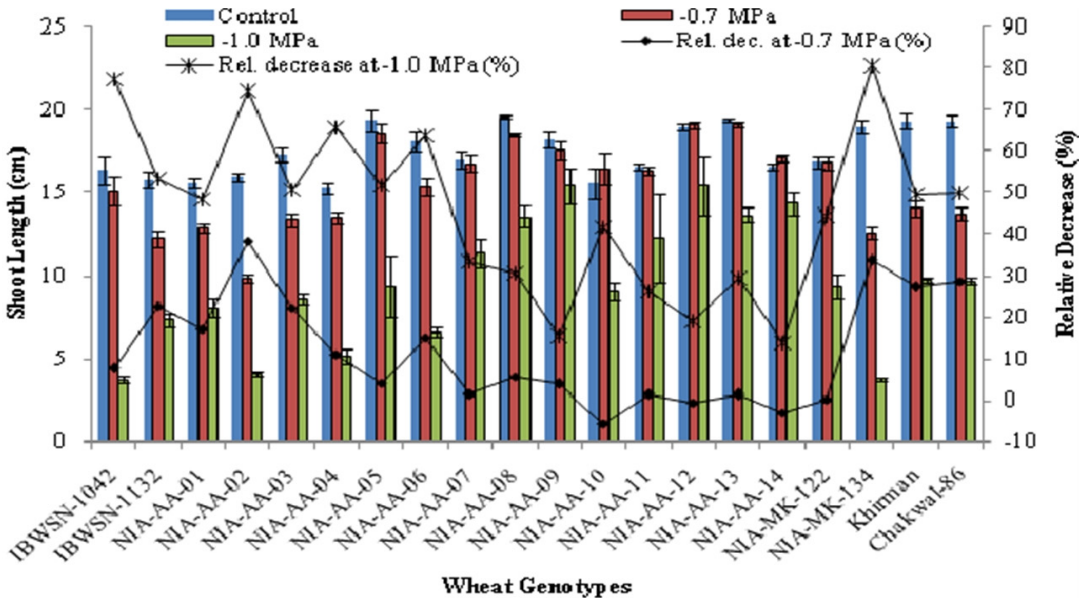

Fig. 1. Impact of PEG-induced osmotic stress on shoot length of various wheat genotypes.

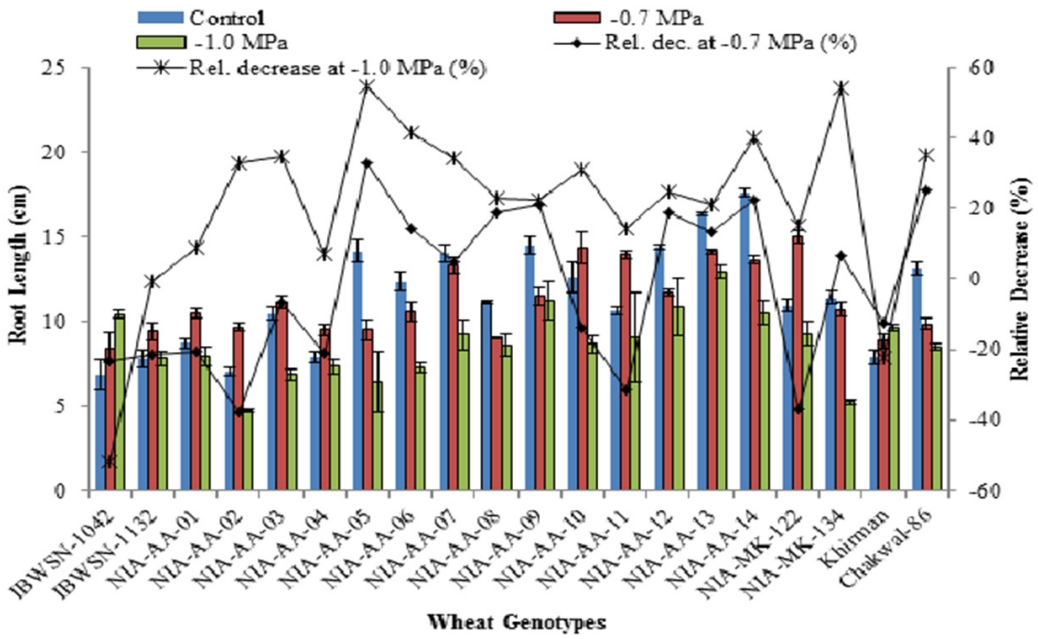

Fig. 2. Impact of PEG-induced osmotic stress on root length of various wheat genotypes.

Table 1. Shoot fresh and dry weights (mg) of wheat genotypes under PEG-induced osmotic stress.

Wheat genotypes	Fresh weight of shoot (mg)				Dry Weight of shoot (mg)			
	Control	-0.7 MPa	-1.0 MPa	Ranking	Control	-0.7 MPa	-1.0 MPa	Ranking
IBWSN-1042	110.73	76.44 (30.97)	41.23 (62.77)	17	18.37	13.93 (24.16)	5.62 (69.38)	15
IBWSN-1132	107.00	78.12 (26.99)	36.00 (66.35)	18	17.27	11.81 (31.58)	7.02 (59.32)	17
NIA-AA-01	128.08	101.56 (20.71)	83.42 (34.87)	11	19.55	18.51 (5.30)	18.25 (6.65)	3
NIA-AA-02	103.67	67.77 (34.63)	35.14 (66.10)	20	15.28	9.71 (36.47)	5.33 (65.10)	20
NIA-AA-03	118.40	71.78 (39.38)	30.80 (73.99)	19	16.70	11.41 (31.67)	6.51 (61.01)	19
NIA-AA-04	122.89	87.00 (29.20)	32.22 (73.78)	16	14.72	12.74 (13.43)	8.58 (41.74)	18
NIA-AA-05	147.78	109.89 (25.64)	43.44 (70.60)	12	18.36	17.82 (2.91)	11.60 (36.80)	5
NIA-AA-06	138.67	82.67 (40.38)	45.33 (67.31)	15	16.11	13.22 (17.93)	9.12 (43.38)	14
NIA-AA-07	127.44	102.67 (19.44)	56.44 (55.71)	14	15.71	16.98 (-8.06)	10.00 (36.35)	11
NIA-AA-08	181.07	156.20 (13.73)	83.93 (53.65)	2	18.23	17.75 (2.60)	21.66 (-18.81)	2
NIA-AA-09	174.73	159.20 (8.89)	114.00 (34.76)	1	18.31	18.38 (-0.36)	29.01 (-58.42)	1
NIA-AA-10	112.58	121.47 (6.01)	41.23 (68.10)	13	18.55	9.16 (50.63)	12.27 (33.85)	13
NIA-AA-11	166.27	124.05 (25.39)	74.55 (55.16)	7	18.55	8.71 (53.06)	14.17 (23.58)	12
NIA-AA-12	159.33	139.80 (12.26)	86.20 (45.90)	5	16.36	15.74 (3.79)	14.47 (11.53)	6
NIA-AA-13	182.40	137.33 (24.71)	92.07 (49.52)	3	19.81	17.06 (13.87)	13.53 (31.71)	4
NIA-AA-14	155.67	125.27 (19.53)	77.80 (50.02)	8	15.76	15.56 (1.27)	12.33 (21.74)	8
NIA-MK-122	150.23	137.34 (8.58)	42.24 (71.88)	9	18.15	8.37 (53.87)	10.69 (41.09)	16
NIA-MK-134	230.71	71.11 (69.18)	23.92 (89.63)	10	22.2	13.20 (40.54)	7.87 (64.56)	10
Khirman	198.35	99.43 (49.87)	70.60 (64.41)	6	19.93	14.73 (26.09)	9.20 (53.85)	7
Chakwal-86	209.19	105.17 (49.72)	86.40 (58.70)	4	20.93	13.07 (37.58)	9.47 (54.78)	9
LSD		22.034				2.627		
St. error		11.127				1.327		

Mean followed by various alphabets differed significantly at p=0.05; *Values in brackets ( ) represents relative reduction (%)

at high drought stress. NIA-MK-34 also retained better root fresh and dry biomasses under both osmotic stresses.

Photosynthetic pigments

Photosynthetic pigments (chl. a and b) were degraded under reduced moisture concentrations. Better chlorophyll contents were analyzed at -0.7 MPa osmotic potential with minimum reduction as compared to at -1.0 MPa stress. Maximum chl. “a” was analyzed in NIA-AA-11 and NIA-AA-14 under -0.7 MPa and -1.0 MPa osmotic stress, respectively. NIA-AA-07 and NIA-AA-09 also retained better chl. a pigments under stress. Similarly, utmost Chl. “b”

Table 2. Root fresh and dry weights (mg) of wheat genotypes under PEG-induced osmotic stress.

Wheat genotypes	Fresh weight of root (mg)				Dry Weight of root (mg)			
	Control	-0.7 MPa	-1.0 MPa	Ranking	Control	-0.7 MPa	-1.0 MPa	Ranking
IBWSN-1042	57.30	40.51 (29.31)	22.26 (61.15)	20	5.47	5.10 (6.76)	4.17 (23.71)	20
IBWSN-1132	61.18	39.81 (34.93)	26.54 (56.62)	18	6.04	5.77 (4.47)	5.20 (13.86)	18
NIA-AA-01	84.02	74.71 (11.08)	62.92 (25.12)	01	8.80	9.80 (-11.36)	8.08 (8.22)	07
NIA-AA-02	53.66	43.95 (18.10)	25.31 (52.83)	19	5.10	5.53 (-8.50)	4.87 (4.44)	19
NIA-AA-03	69.32	56.96 (17.84)	31.86 (54.04)	11	6.28	6.10 (2.81)	5.86 (6.59)	17
NIA-AA-04	68.89	52.67 (23.55)	31.00 (55.00)	13	6.80	8.62 (-26.80)	8.51 (-25.16)	12
NIA-AA-05	88.44	50.56 (42.84)	31.33 (64.57)	07	7.54	8.97 (-18.85)	8.63 (-14.43)	10
NIA-AA-06	63.00	39.44 (37.39)	29.78 (52.73)	16	6.67	7.10 (-6.50)	7.24 (-8.67)	15
NIA-AA-07	65.67	48.56 (26.06)	29.11 (55.67)	14	7.03	9.79 (-39.18)	7.37 (-4.74)	11
NIA-AA-08	82.73	53.67 (35.13)	44.00 (46.82)	05	6.94	8.61 (-24.11)	10.12 (-45.82)	09
NIA-AA-09	88.00	61.13 (30.53)	53.73 (38.94)	02	9.37	10.41 (-11.02)	9.69 (-3.41)	05
NIA-AA-10	63.57	39.34 (38.12)	26.89 (57.71)	17	9.08	16.75 (-84.43)	7.63 (16.01)	02
NIA-AA-11	77.94	42.31 (45.72)	41.56 (46.68)	10	10.01	17.30 (-72.88)	6.87 (31.38)	01
NIA-AA-12	61.53	51.60 (16.14)	44.00 (28.49)	12	6.35	7.88 (-24.03)	7.33 (-15.42)	14
NIA-AA-13	74.47	60.67 (18.53)	58.87 (20.95)	04	7.45	8.28 (-11.19)	7.11 (4.48)	13
NIA-AA-14	81.13	38.07 (53.08)	77.80 (4.11)	03	6.79	7.17 (-5.59)	6.57 (3.24)	16
NIA-MK-122	60.07	47.48 (20.95)	34.41 (42.71)	15	8.89	16.03 (-80.28)	6.23 (29.91)	03
NIA-MK-134	91.07	50.21 (44.87)	28.09 (69.16)	08	9.60	12.53 (-30.56)	8.87 (7.64)	04
Khirman	82.63	48.91 (40.81)	41.87 (49.33)	06	7.93	11.60 (-46.22)	8.87 (-11.76)	06
Chakwal-86	78.63	43.84 (44.24)	39.67 (49.55)	09	7.47	10.33 (-38.39)	8.60 (-15.18)	08
LSD		13.923				1.530		
St. error		7.0308				0.773		

Mean followed by various alphabets differed significantly at p=0.05; *Values in brackets ( ) represents relative reduction (%)

Table 3. Impact of PEG induced drought stress on photosynthetic pigments (chl. a and b).

Wheat genotypes	Chlorophyll a			Chlorophyll b			Total chlorophyll		
	Control	-0.7 MPa	-1.0 MPa	Control	-0.7 MPa	-1.0 MPa	Control	-0.7 MPa	-1.0 MPa
IBWSN-1042	0.141	0.127 (10.02)	0.046 (67.17)	0.113	0.105 (6.72)	0.022 (80.13)	0.253	0.232 (8.55)	0.069 (72.94)
IBWSN-1132	0.183	0.170 (7.33)	0.067 (63.39)	0.167	0.164 (1.63)	0.056 (66.68)	0.350	0.334 (4.61)	0.123 (64.96)
NIA-AA-01	0.173	0.086 (50.27)	0.082 (52.64)	0.169	0.080 (52.76)	0.076 (55.12)	0.341	0.166 (51.50)	0.157 (53.87)
NIA-AA-02	0.137	0.133 (2.89)	0.071 (48.15)	0.129	0.128 (0.57)	0.051 (60.42)	0.266	0.261 (1.77)	0.122 (54.10)
NIA-AA-03	0.102	0.100 (2.23)	0.057 (44.63)	0.093	0.092 (1.46)	0.051 (44.61)	0.195	0.191 (1.86)	0.108 (44.62)
NIA-AA-04	0.177	0.130 (26.27)	0.088 (50.28)	0.152	0.121 (20.44)	0.077 (49.52)	0.329	0.251 (23.57)	0.165 (49.93)
NIA-AA-05	0.169	0.154 (8.79)	0.115 (32.15)	0.160	0.141 (11.87)	0.087 (45.41)	0.329	0.295 (10.28)	0.202 (38.61)
NIA-AA-06	0.184	0.153 (17.10)	0.076 (58.97)	0.180	0.128 (29.05)	0.065 (64.16)	0.364	0.280 (23.0)	0.140 (61.54)
NIA-AA-07	0.265	0.196 (26.22)	0.154 (41.98)	0.259	0.187 (27.64)	0.141 (45.41)	0.524	0.383 (26.93)	0.295 (43.67)
NIA-AA-08	0.145	0.137 (5.63)	0.131 (9.82)	0.168	0.125 (25.44)	0.109 (34.79)	0.624	0.523 (16.26)	0.479 (23.21)
NIA-AA-09	0.244	0.133 (45.48)	0.174 (28.79)	0.230	0.120 (47.72)	0.163 (29.19)	0.948	0.507 (46.57)	0.674 (28.98)
NIA-AA-10	0.284	0.217 (23.77)	0.145 (48.93)	0.258	0.177 (31.17)	0.155 (39.94)	0.541	0.394 (27.29)	0.300 (44.66)
NIA-AA-11	0.281	0.266 (5.23)	0.094 (66.57)	0.277	0.226 (18.35)	0.097 (65.03)	0.558	0.492 (11.74)	0.191 (65.81)
NIA-AA-12	0.159	0.114 (28.65)	0.141 (11.17)	0.126	0.102 (18.87)	0.120 (4.37)	0.285	0.216 (24.34)	0.262 (8.17)
NIA-AA-13	0.158	0.135 (14.81)	0.131 (17.61)	0.141	0.120 (14.89)	0.139 (1.29)	0.299	0.255 (14.84)	0.295 (1.28)
NIA-AA-14	0.151	0.150 (1.03)	0.149 (1.53)	0.133	0.132 (0.39)	0.120 (9.14)	0.284	0.282 (0.73)	0.271 (4.55)
NIA-MK-122	0.297	0.230 (22.56)	0.180 (39.63)	0.261	0.215 (17.64)	0.141 (46.25)	0.559	0.445(20.26)	0.320 (42.72)
NIA-MK-134	0.191	0.150 (21.09)	0.056 (70.60)	0.187	0.152 (18.95)	0.043 (77.05)	0.380	0.304 (20.02)	0.099 (73.81)
Khirman	0.165	0.138 (16.46)	0.087 (47.07)	0.163	0.133 (18.20)	0.053 (67.29)	0.329	0.272 (17.33)	0.141 (57.17)
Chakwal-86	0.227	0.190 (16.59)	0.127 (44.09)	0.219	0.203 (7.01)	0.106 (51.43)	0.446	0.393 (11.89)	0.233 (47.69)
LSD		0.053			0.064			0.1519	
St. error		0.027			0.033			0.0767	

Mean followed by different alphabets were found significant at p≤0.05 according to Duncan's multiple range Test.

contents were determined in NIA-AA-11 and NIA-AA-09 at -0.7 MP aand -1.0 MPa PEG induced stress, respectively. Overall, highest total chlorophyll were observed in NIA-AA-08 and NIA-AA-09 with minimum relative reduction over control under both drought stresses (Table 3).

Table 4. Accumulation of mineral ions (K+ and Ca2+) under the influence of PEG induced drought stress.

Wheat genotypes	Potassium (K+) ions				Calcium (Ca2+) ions			
	Control	-0.7 MPa	-1.0 MPa	Ranking	Control	-0.7 MPa	-1.0 MPa	Ranking
IBWSN-1042	0.67	0.87 (29.50)	0.93 (38.51)	14	0.30	0.49 (64.58)	0.54 (78.89)	18
IBWSN-1132	0.89	0.90 (0.47)	1.18 (32.48)	10	0.61	0.62 (1.09)	0.79 (29.10)	11
NIA-AA-01	0.95	1.72 (81.50)	1.06 (11.81)	9	0.65	1.18 (80.08)	0.78 (19.67)	8
NIA-AA-02	0.68	0.74 (9.20)	1.13 (66.01)	13	0.46	0.49 (6.01)	1.00 (118.03)	12
NIA-AA-03	0.56	0.98 (75.37)	1.39 (149.25)	11	0.28	0.68 (142.51)	1.08 (289.22)	10
NIA-AA-04	0.59	0.52 (-11.35)	1.18 (101.42)	16	0.36	0.33 (-7.56)	0.84 (133.72)	15
NIA-AA-05	0.42	0.59 (39.60)	1.05 (148.51)	18	0.23	0.36 (55.36)	0.68 (191.07)	19
NIA-AA-06	0.56	0.49 (-12.69)	1.13 (102.24)	17	0.32	0.34 (6.58)	0.73 (128.95)	17
NIA-AA-07	0.53	0.48 (-10.24)	0.64 (20.47)	19	0.26	0.33 (25.88)	0.37 (42.17)	20
NIA-AA-08	1.93	2.71 (40.33)	3.82 (97.84)	1	1.03	1.15 (10.81)	1.65 (59.52)	1
NIA-AA-09	1.94	2.40 (23.67)	3.01 (55.51)	5	0.91	0.96 (5.88)	1.20 (32.35)	7
NIA-AA-10	0.69	0.68 (-0.49)	0.97 (40.78)	15	0.52	0.45 (-11.97)	0.60 (16.18)	14
NIA-AA-11	0.59	0.72 (21.47)	1.28 (117.23)	12	0.48	0.43 (-11.11)	0.76 (57.64)	13
NIA-AA-12	1.50	2.71 (80.58)	3.49 (132.19)	3	0.72	1.27 (75.98)	1.34 (85.22)	5
NIA-AA-13	1.80	2.11 (17.46)	3.70 (105.94)	4	0.85	0.99 (16.77)	1.48 (75.15)	6
NIA-AA-14	1.86	2.66 (42.49)	3.78 (102.86)	2	0.93	1.17 (25.18)	1.47 (57.50)	2
NIA-MK-122	0.52	0.56 (6.71)	0.96 (84.03)	18	0.47	0.40 (-14.95)	0.59 (26.69)	16
NIA-MK-134	1.31	1.41 (7.89)	2.22 (69.72)	8	0.80	0.65 (-18.50)	0.97 (20.37)	9
Khirman	2.02	2.05 (1.28)	1.07 (-47.31)	7	1.10	1.19 (8.26)	1.13 (2.06)	4
Chakwal-86	1.44	1.45 (0.75)	3.22 (123.14)	6	0.82	0.83 (2.04)	1.81 (121.77)	3
LSD		0.4603				0.2505		
St. error		0.2324				0.1265		

Values in brackets represents "relative increase/ decrease (-) as compared to control"

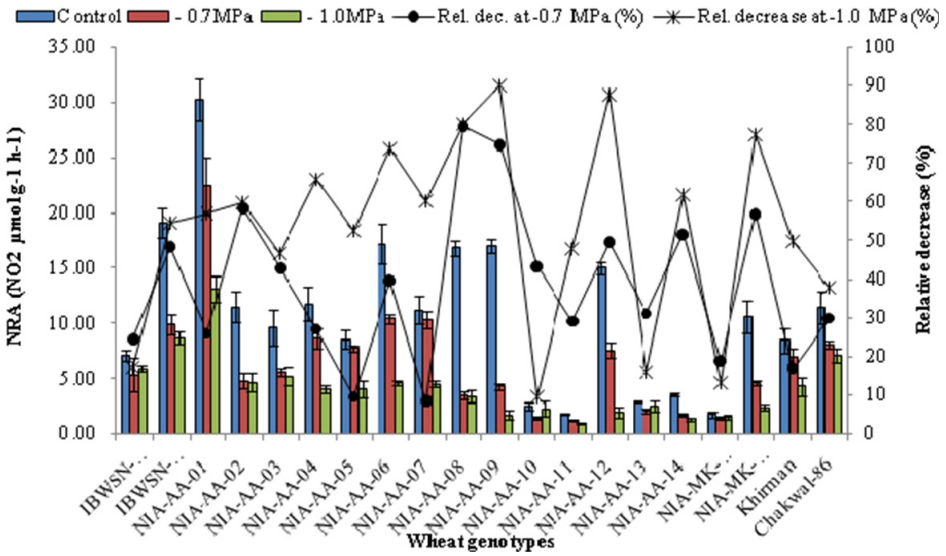

Fig. 3. Impact of PEG-induced osmotic stress on nitrate reductaseenzymatic activities of various wheat genotypes.

NR enzyme activity

Nitrate reductase activity was highly reduced due to increasing moisture deficit. However, NIA-AA-01 retained better NR-activity under both drought levels. NIA-AA-13also exhibited least reduction in NRA under both osmotic stresses (Fig. 3).

Accumulation of inorganic solutes

Wheat genotypes showed haphazard responses in terms of accumulation of K^+ and Ca^{2+} ions under PEG-induced drought stress. Accumulation of these solutes was increased in many genotypes while decreased in some genotypes under water scarce conditions. Maximum K^+ contents were measured in NIA-AA-14, NIA-AA-13, and NIA-AA-12 under both drought levels while highest Ca^{2+} ions were analyzed in NIA-AA-08

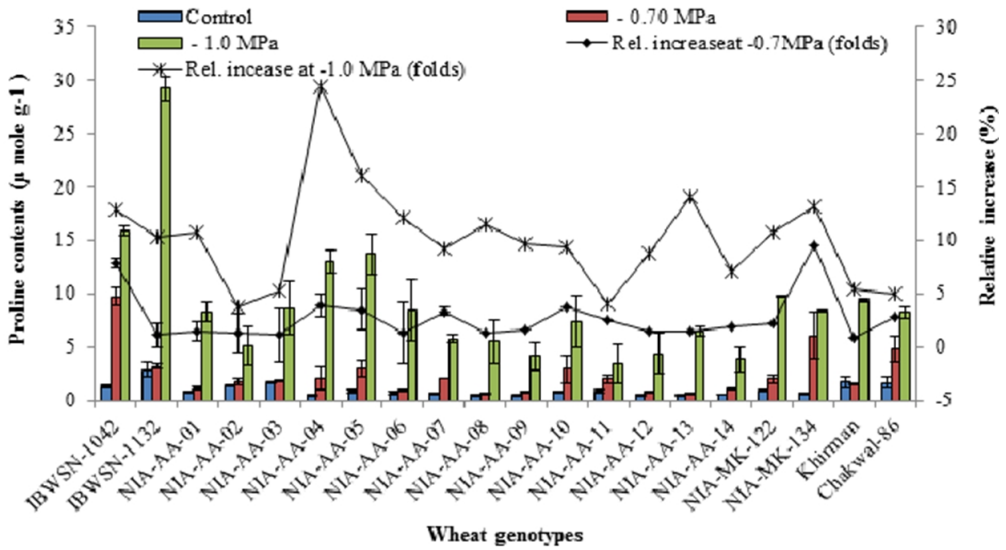

Fig. 4. Impact of PEG-induced osmotic stress on endogenous proline concentration.

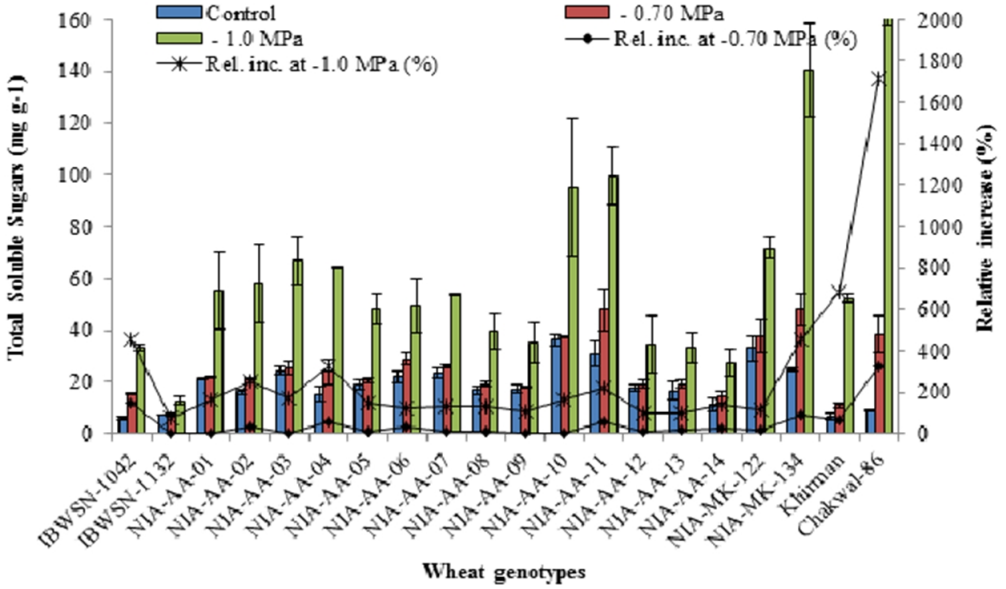

Fig. 5. Impact of PEG-induced osmotic stress on endogenous amelioration of total soluble sugars.

and NIA-AA-14. Nevertheless, NIA-AA-08 ranked at top among means of all treatments in terms of K^+ as well as Ca^{2+} ions accumulation.

Accumulation of organic osmolytes

Concentration of various osmolytes such as proline, TSS, and phenolic compounds were increased under the influence of drought stress. Maximum augmentation in endogenous proline was scrutinized in IBWSN-1132 and IBWSN-1042 under reducedo smotic potentials. However, NIA-AA-04 and NIA-AA-05 also accumulated better proline contents under -1.0 MPa osmotic stress (Fig. 4). In the same way, utmost TSS were assessed in wheat genotypes NIA-MK-134 and NIA-AA-11 under mild drought stress while NIA-MK-134 and Chakwal-86 produced the highest TSS under high osmotic stress (Fig. 5).

Concentration of total phenolic compound showed variation in different genotypes under drought stress. Nevertheless, greatest TPC were analyzed in genotype NIA-AA-11 followed by NIA-AA-10 under both levels of water shortage. Negative response was observed in genotypes IBWSN-1042 and IBWSN-1132 in this regard (Fig. 6).

Correlation analysis

Pearson correlation analysis was determined among growth and physiological parameters (Table 5). Chlorophyll contents showed positive and significant correlation with most of growth traits. Proline exhibited negative but significant

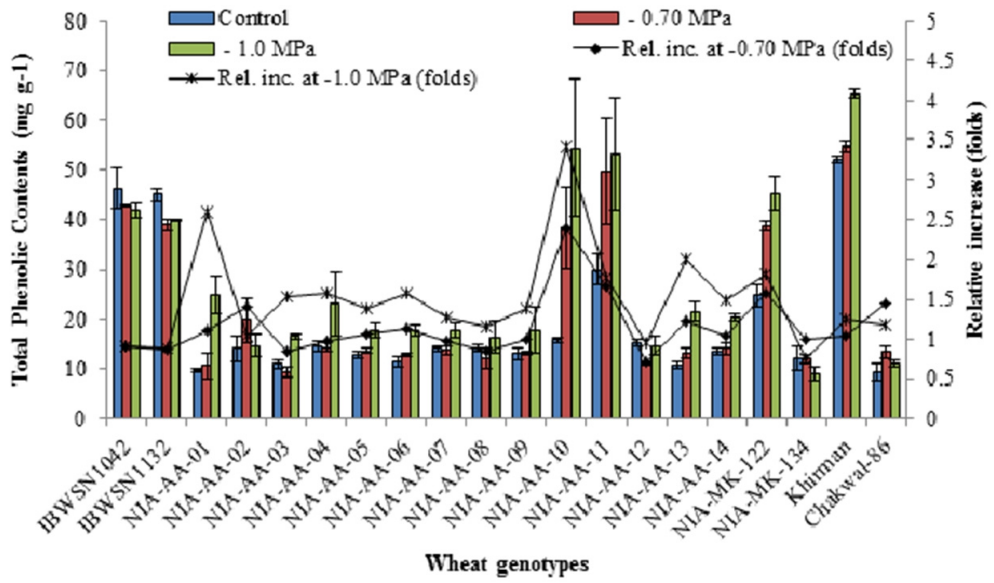

Fig. 6. Impact of PEG-induced osmotic stress on endogenous amelioration of total phenolics.

Table 5. Pearson correlation analysis among growth and biochemical analysis.

	Chl	NRA	Pro	TSS	TPC	K+	Ca2+
Shoot Length	0.5367*	-0.2830	-0.5224*	-0.1906	-0.1842	0.6532*	0.5041*
Root Length	0.4100	-0.4552*	-0.5250*	-0.0070	-0.1850	0.4624*	0.2698
Shoot FW	0.6296*	-0.1962	-0.5538*	0.1030	-0.1338	0.7828**	0.7245**
Shoot DW	0.6102*	0.2503	-0.3979	-0.1692	-0.2843	0.6307*	0.5517*
Root FW	0.2493	0.2269	-0.5538*	-0.0856	-0.3413	0.6301*	0.6392*
Root DW	0.5652*	-0.2502	-0.3681	0.6241*	0.1732	0.0350	0.0540

*= Significant at p= 0.05; **significant at p=0.001
Chl. = Chlorophyll; NRA nitrate reductase activity; Pro = proline; TSS total soluble sugars; K^+ potassium ions; Ca^{2+} calcium ions

association with shoot and root's lengths and fresh weights. However, significant and positive correlation existed among growth traits, and K^+ and Ca^{2+} ion accumulation.

Discussion

Water deficit negatively regulates wheat growth and development (Sikuku et al.2012). All wheat genotypes showed diverse responses in terms of their capacities to regulate growth and metabolic processes under severe drought. Drought tolerant plants maintained better seedling growth while sensitive ones were not able to maintain metabolic homeostasis resulting in stunt growth (Jogaiah et al. 2013). Seedling traits related to the effective seedling stand establishment including coleoptiles length and biomass can enhance dehydration tolerance by early ground cover which decreases the surface evaporation (Spielmeyer et al. 2007). Drought resistant genotypes showed less reduction in shoot and root length under reduced water stress (Moucheshi et al. 2012). Desiccation tolerant genotypes maintained their root growth (length and biomass) as an adaptive feature under drought which facilitate in effective water uptake from deep soils (Ehdaie et al. 2012; Rodrigues et al. 1995). In present study, some wheat genotypes exhibited increase in root length of seedlings under water deficit which was also supported by Shabbir et al. (2015). Several researchers had documented the reduction in root and shoot length in many crops under extreme water shortage (Baloch et al. 2012; Jaleel et al. 2008; Mujtaba et al. 2016).

Reduced water supply imposed negative impacts on photosynthesis. Chlorophyll pigments (a and b) were degraded under drought stress which resulted in low concentrations of total chlorophyll content in wheat seedlings. This decrease in chl. a and b pigments ultimately impaired the process of photosynthesis (Kalaji et al. 2011) and might be attributed to lower activity of RuBisCO (ribulose-1, 5 bisphosphate carboxylase/oxygenase) enzyme and reduced ATP formation (Dulai et al. 2006). These adverse effects of drought were found comparable to some previous studies (Chaves et al. 2009; Faisal et al. 2017; Pandey et al. 2012).

Water stress had exerted negative effects on activity of nitrate reductase enzyme. NR- activity is decreased in all

wheat genotypes under the exposure of drought stress, however, relatively less reduction occured in tolerant genotypes (Correia et al. 2005). Decrease in NR activity is associated with declined photosynthesis (Kaiser and Forster 1989).

Drought stress disturbs the ionic balance of plants (Hasanuzzaman et al. 2014). Uptake of K^+ and Ca^{2+} ions raised in tolerant genotypes under hypertonic medium. Potassium ions help in stromal alkalization, which stimulates non-stomatal photosynthesis in dehydrated plants (Berkowitz et al. 1983). K^+ in leaf also facilitates in maintaining turgor pressure. Significantly higher K^+-contents are a prerequisite for drought tolerance as it helps in enhancing dry matter accumulation as compared to low K^+ contents (Egilla et al. 2001). Both K^+ and Ca^{2+} ions being inorganic solutes, are also involved in osmotic adjustment under water-scarce conditions. Under stress conditions, cytoplasmic calcium concentration arises in plants which is involved in various stress signaling processes (Gupta and Kaur 2005; Klimecka and Muszynska 2007). Previous studies also highlighted that drought-tolerant genotypes retained higher K^+ contents (Ashraf et al. 2010).

Desiccation-tolerant plants have ability to maintain metabolic homeostasis by accumulation of various solutes, while sensitive ones are unable to do this and suffer from dehydration-induced injuries which ultimately lead towards the death of such plant species (Jogaiah et al. 2013). Concentration of various organic solutes such as proline, sugars, and phenolics were enhanced under low osmotic potentials. These osmotic contents are involved the defense of plant seedlings under increasing drought stress. Proline is one of the osmoprotectants which prevents denaturation of cellular membranes and subcellular constituents, and scavenges the active species of oxygen under water deficit (Hayat et al. 2012; Kavikishor and Sreenivasulu 2014). Tatar and Gevrek (2008) had also verified the increment in the quantity of proline under water stress.

The carbohydrate pools in plants undergo modifications depending upon duration and severity of drought stress, however, a reduction in starch contents along with the accumulation of soluble sugars are frequently noticed under mild water deficit (Basu et al. 1999; Vu et al. 1998; Yang et al. 2001). Kerepesi and Galiba (2000) reported the increase in the amount of soluble carbohydrates under drought conditions. Hence, sucrose accumulation might be the indication of drought tolerance.

Phenolics have a role in plant defense responses under abiotic stress. They have oxidation properties due to their ability to donate hydrogen and act as reducing agent as well as quenches singlet oxygen (Amarowicz et al. 2004). A strong association exists among drought tolerance and up-regulation of phenolic compounds. In the present research, phenolic contents were increased in tolerant genotypes while decreased in sensitive ones under both water deficit levels. Hassan et al. (2015) have also demonstrated amelioration in phenolic contents under drought stress.

Categorization of genotypes

On the basis of a relative reduction in growth and biochemical aspects, wheat genotypes were characterized as drought tolerant, medium tolerant, and sensitive ones. Among all, six genotypes such as NIA-AA-01, NIA-AA-08, NIA-AA-09, NIA-AA-13, NIA-AA-12, and NIA-AA-14 were classified as drought tolerant, three genotypes (NIA-AA-05, NIA-AA-10, and NIA-MK-134) were marked as medium tolerant, and all others came in the category of sensitive genotypes. These genotypes showed the least reduction in seedling growth, photosynthesis, and NRA, while effective enhancement in biochemical traits. These genotypes have the potential to give better yields in drought prone and rain fed areas.

References

Abedi T, Pakniyat H. 2010.Antioxidant enzyme changes in response to drought stress in drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). Czech J. Genet. Plant Breed. 46:27-34

Al-Ghamdi AA. 2009. Evaluation of oxidative stress tolerance in two wheat (*Triticum aestivum*) cultivars in response to drought. Int. J. Agric. Biol. 11: 7-12

Amarowicz R, Pegg RB, Rahimi-Maghaddam P, Barl B, Weil JA. 2004. Free-radical scavenging capacity and antioxidant activityof selected plant species from the Canadian prairies. Food Chem. 84: 551-562

Anderson JM, Ingram JSI. 1989. TSBF: A handbook of methods of analysis. CAB International, pp 39

Basu PS, Sharma A, Garg ID, Sukumaran NP. 1999. Tuber sink modifies photosynthetic response in potato tuber under water stress. Environ. Exp. Bot.42: 25-39

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water stress studies. Plant Soil 39: 205-20 7

Berkowitz GA, Chen C, Gibbs M. 1983. Stromal acidification mediates *in vivo* water stress inhibition of non-stomatal-controlled photosynthesis. Plant Physiol. 72: 1123-1126

Biaecka B, Kepczynski J. 2010. Germination, a-, b-amylase and total dehydrogenase activities of *Amaranthus caudatus* seeds under water stress in the presence of ethephon or gibberellin A3. Acta Biol. Cracov. Ser. Bot. 52:7-12

Chaves MM, Flexas J, Pinheiro C. 2009. Photosynthesis under drought and salt stress, regulation mechanisms from whole plant to cell. Ann. Bot-London 103: 551-560

Correia MJ, Fonseca F, Azedo-Silva J, Dias C, David MM, Barrote I, Osorio ML, Osorio J. 2005. Effects of water deficit on the activity of nitrate reductase and content of sugars, nitrate and free amino acids in the leaves and roots of sunflower and white lupin plants growing under two nutrient supply regimes. Physiol. Plant.124: 61-70

Dulai S, Molnár I, Prónay J, Csernak A, Tarnai R, Molnár-Láng M. 2006. Effects of drought on photosynthetic parameters and heat stability of PSII in wheat and in Aegilops species originating from dry habitats. Acta Biol. Szeged. 50: 11-17

Egilla JN, Davies FT, Drew MC. 2001. Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: Plant growth, leaf macro- and micronutrient content and root longevity. Plant Soil 229: 213-224

Ehdaie B, Layne AP, Waines JG. 2012. Root system plasticity to drought influences grain yield in bread wheat. Euphytica186: 219-232

Faisal S, Mujtaba SM, Khan MA, Mahboob W. 2017. Morpho-physiological assessment of wheat (*Triticum aestivum* L.) genotypes for drought stress tolerance at seedling stage. Pak. J. Bot. 49(2): 445-452

Farooq M, Wahid N, Kobayashi FD, Basra SMA. 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Develop. 29: 185-212

Gupta AK, Kaur N. 2005. Sugar signaling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. J. Biosci. 30(5): 761-76

Hasanuzzaman M, Alam MM, Nahar K, Jubayer-Al-Mahmud, Ahmad KU, Fujita M. 2014. Exogenous salicylic acid alleviates salt-stress oxidative damage in *Brassica napes* by enhancing the antioxidant defense and glyoxalase systems. Aust. J. Crop. Sci.8: 631- 648

Hassan MA, Fuertes MM, Sánchez FJR, Vicente O, Boscaiu M. 2015. Effects of salt and water stress on plant growth and on accumulation of osmolytes and antioxidant compounds in cherry tomato. Not. Bot. Horti. Agrobo. 43:1-11

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. 2012. Role of proline under changing environments: a review. Plant Signal. Behav.7:1456-1466

Jaleel CA, Gopi R, Sankar B, Gomathinayagam M, Panneerselvam R. 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. CR Biol. 331: 42-47

Jatoi SA, Latif MM, Arif M, Ahson M, Khan A, Siddiqui SU. 2014. Comparative assessment of wheat landraces against polyethylene glycol simulated drought stress. Sci. Tech. Develop. 33: 1-6

Jogaiah S, Govind SR, Tran LSP. 2013. Systems biology-based approaches toward understanding drought tolerance in food crops. Crit. Rev. Biotechnol. 33: 23-29

Kalaji MH, Bosa K, Kościelniak J, Hossain Z. 2011. Chlorophyll "*a*" fluorescence – A useful tool for the early detection of temperature stress in spring barley (*Hordeum vulgare* L.). OMICS: Afr. J. Integ. Biol. 15: 925-934

Kavikishor PB, Sreenivasulu N. 2014. Is proline accumulation *per se* correlated with stress tolerance or is proline homeostasis a more critical issue? Plant Cell Environ. 37: 300-311

Kerepesi I, Galiba G. 2000. Osmotic and salt stress induced alteration in soluble carbohydrate content in wheat seedlings. Crop Sci. 40: 482-487

Klimecka M, Muszynska G. 2007. Structure and functions of plant calcium-dependent protein kinases. Acta Biochim. Pol. 54(2): 219-233

Lichtenthaler HK. 1987. Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. In L Packer, R Douce, eds, Methods in Enzymology, Academic Press, pp 350-382

Meneses CHSG, Bruno RLA, Fernandes PD, Pereira WE, Lima LHGM, Lima MMA, Vidal MS. 2011. Germination of cotton cultivar seeds under water stress induced by polyethyleneglycol-6000. Sci. Agric.(Piracicaba, Braz.).68 (2): 131-138

Michel BE, Kaufmann MR. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 51: 914-916

Moucheshi A, Heidari B, Assad M. 2012. Alleviation of drought stress effects on wheat using arbuscular mycorrhizal symbiosis. Int. J. Agrisci. 2(1): 35-47

Mujtaba SM, Faisal S, Khan MA, Mumtaz S, Khanzada B. 2016. Physiological studies on six wheat (*Triticum aestivum* L.) genotypes for drought stress tolerance at seedling stage. Agric. Res. Technol. Open Access J. 1(2): 001-005

Nawaz S, Ahmed N, Iqbal A, Khaliq I. 2013. Optimization of regeneration protocols for wheat under drought and salt stress. Pak. J. Agri. Sci. 50: 663-670

Noorka IR, Khaliq I. 2007. An efficient technique for screening wheat (*Triticum aestivum* L.) germplasm for drought tolerance. Pak. J. Bot. 39(5): 1539-1546

Pandey HC, Baig MJ, Bhatt RK. 2012. Effect of moisture stress on chlorophyll accumulation and nitratereductase activity at vegetative and flowering stage in Avena species. Agri. Sci. Res. J. 2: 111-118

Riazi A, Matruda K, Aslam A. 1985. Water stress induces changes in concentration of proline and other solutes in growing regions. J. Exp. Bot. 36:1716-1725

Rivas-Ubach A, Sardans J, Perez-Trujillo M, Estiarte M, Penuelas J. 2012.Strong relationship between elemental stoichiometry and metabolome in plants. Proc. Natl. Acad. Sci. USA. 109:4181-4186

Rhodes D, Samaras Y. 1994. Genetic control of osmoregulation in plant. In K Strong, eds, Cellular and Molecular Physiology of Cell Volume Regulation. CRC Press, Boca Raton, pp 347-361

Rodrigues M, Pacheco C, Chaves M. 1995. Soil–plant water relations, root distribution and biomass partitioning in *Lupinus albus* L. under drought conditions. J. Exp. Bot. 46: 947-956

Shabbir RN, Ashraf MY, Waraich EA, Ahmad R, Shahbaz M. 2015.Combined effects of drought stress and NPK foliar spray on growth, physiological processes and nutrient uptake in wheat. Pak. J. Bot. 47(4): 1207-1216

Singh GP, Chaudhary HB, Rajbir Y, Tripathi S. 2008. Genetic analysis of moisture stress tolerance in segregating populations of bread wheat (*Triticum aestivum* L.). Ind. J. Agric. Sci. 78(10): 848-852

Sikuku PA,Onyango JC, Netondo GW. 2012. Yield components and gas exchange responses of Nerica rice varieties (*Oryza sativa* L.) to vegetative and reproductive stage water deficit. Global J. Sci. Front.Res. 12: 51-62

Spielmeyer W, Hyles J, Joaquim P, Azanza F, Bonnett D, Ellis M, Moore C, Richards RA. 2007. A QTL on chromosome 6A in bread wheat (*Triticum aestivum* L.) is associated with longer coleoptiles, greater seedling vigour and final plant height. Theor. Appl. Genet.115: 59-66

Suseela V,Tharayil N, Xing B, Dukes JS. 2015. Warming and drought differentially influence the production and resorption

of elemental and metabolic nitrogen pools in *Quercus rubra*. Glob. Change Biol. 21(11): 4177-4195

Sym GJ. 1984. Optimization of in-vivo assay conditions of nitrate reductase in Barley (*Hordeumvulgare* L.cv.ir). J. Sci. Food Agric. 35(7): 725-730

Tatar O, Gevrek MN. 2008. Influence of water stress on proline accumulation, lipid peroxidation and water content of wheat. Asian J. Plant Sci. 7(4): 409-412

Tuberosa R, Salvi S. 2006. Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci.11: 405-412

Vu JCV, Baker JT, Pennanen AH, Allen Jr H, Bowes G, Boote KJ. 1998. Elevated CO_2 and water deficit effects on photosynthesis, ribulose bisphosphate carboxylase–oxygenase, and carbohydrate metabolism in rice. Physiol. Plant. 103: 327-339

Waterhouse AL. 2002. Determination of total phenolics. Curr. Prot. Food Anal. Chem. 11(1): 1-8

Yang J, Zhang J, Wang Z, Zhu Q. 2001. Activities of starch and hydrolytic enzymes and sucrose-phosphate synthase in the stems of rice subjected to water stress during grain filling. J. Exp. Bot. 52: 2169-2179