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MORPHO-PHYSIOLOGICAL ASSESSMENT OF WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES FOR DROUGHT STRESS TOLERANCE AT SEEDLING STAGE

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Abstract

As water deficit is the major constrained for agriculture crop production, germination potentials of twenty six wheat genotypes were assessed under various drought stress levels while six genotypes were evaluated for drought tolerance potential through growth and physiological studies at early seedling stage. The experimental layout was comprised of three drought treatments in completely randomized pattern with three replicates under controlled conditions. Drought stress was induced through polyethylene glycol-6000 solutions by maintaining three osmotic potentials (-0.5MPa, -0.75MPa and -1.0MPa) in water culture medium while 1/4th Hoagland's solution with zero osmotic potential was applied as control. Germination rate, seedling's growth and photosynthesis were declined with increased levels of water deficiency. Seedling length, fresh and dry biomasses of root and shoot, and photosynthetic pigments executed more reduction at higher water deficit conditions. However, genotype TD-1 followed by ESW-9525 and IBWSN-1010 showed better performance with minimum reduction in seedling's length and biomasses at -0.75MPa and -1.0MPa osmotic stress. TD-1 exhibited least reduction (15.26%) in chlorophyll pigments and enhanced accumulation of K^+ ions at highest osmotic stress level. Maximum K^+/Ca^{2+} ratio was determined in ESW-9525 and TD-1 which is the tolerance trait. Hence, TD-1 and ESW-9525 have more drought stress tolerance capacity as compare to other genotypes.

Key words: Drought; Germination; Growth; Polyethylene-glycol; Photosynthesis; Potassium ions; Wheat

Introduction

Drought is a natural catastrophe which intensifies the water deficiency and imparts adverse impacts on the economy of a country. It is a global issue, which has constrained the quality and productivity of agricultural crops. About 86% of the cultivated land of the world is under the rainfed regions (Kumar, 2005). According to an estimate, 50% population of the world will be living with complete water shortage areas until the year 2025 (Anon., 2007). Severity of drought cannot be predicted accurately because it relies on many factors including temperature, average rainfall, humidity, vapor pressure, transpiration rate and field capacity of the cultivated land soil.

Wheat is one of the most cultivated crops worldwide which covers about 1/3rd food requirement of the whole world. There is an increase in demand for wheat production due to exponentially increasing human population. According to an estimate, wheat requirement will increase up to 750-million tons by the year 2025 (Mujeeb-Kazi & Rajaram, 2002). Therefore, it is need of time to increase the production of wheat through enhancement in their genetic potential by understanding the physiological mechanisms (Shahryari *et al*., 2008). Due to extreme climatic conditions, current wheat production rate is not adequate for meeting the rising food demands due to limited water resources and low ground water table (Moaveni, 2011). Drought imparts serious threat towards the global wheat production. It causes up to 70% loss in grain yield (Nouri-Ganbalani *et al*., 2009). Water deficiency is more critical for seed germination as minimum available water decreases water potential of seeds which completely inhibits or slows down the process of germination. Drought affects crop plants more severely at early developmental stages where they have developed very least resistance against water scarcity. It inhibits or delays seed germination and crop stand establishment, reduces leaf area and plant height ultimately leading towards reduction in grain yield (Farooq *et al*., 2009).

Apart from genetic basis, many physiological responses are modulated under drought stress. Photosynthesis is reduced due to degradation of chlorophyll under water scarcity. Osmotic adjustment is another tolerance mechanism which involves accumulation of secondary and tertiary organic metabolites (sugars, amino acids etc.) as well as mineral ions for protection of sub-cellular organelles (Iannucci *et al*., 2002; Dijksterhuis & De Vries, 2006). Low soil water potential results in alteration in uptake of minerals from soil which affects the leaf expansion. About 67-82% K^+ uptake was reduced under moderate and extreme water scarcity, which ultimately affects the grain yield (Baque *et al.*, 2006).

Many compounds can be used for inducing *In vitro* drought stress in plants grown in water culture by modulating the osmotic potential of solutions. Polyethylene glycol (PEG) molecules are inert and non-ionic in nature which are impermeable to cellular membranes, due to which it can induce symmetrical osmotic stress without any physiological damage to plant cells (Zhu, 2006). PEG causes reduction in water potential of plant cell leading towards *in vitro* drought stress (Govindaraj *et al*., 2010).

In order to utilize the uncultivated lands of arid and semi-arid areas, there is a need to sort out drought resistant wheat genotypes which have potential to produce good yield under water limited conditions. There are many factors including rainfalls which cause difficulty in maintaining constant drought conditions in field. Thus, genotypes are screened under controlled conditions through PEG-induced drought stress on the basis of growth and physiological traits.

Materials and Methods

Germination test: The germination potential of twenty six upcoming wheat genotypes of Plant Breeding and Genetics Division, Nuclear Institute of Agriculture (NIA) Tandojam, Sindh, Pakistan, were assessed under various levels of water stress. Twenty seeds of each genotype were sown on filter paper in Petri-plates (following ISTA standard conditions for testing of seed) under three water stress treatments. Water deficit conditions were created by using PEG-6000 solution by maintaining the osmotic potentials of -0.5MPa, -0.75MPa and -1.0MPa while 1/4th Hoagland's solution with zero osmotic potential was used as control. Germinated seed were counted daily and germination was noted up to 192 h after seed sowing. Germination percentage and germination stress tolerance index (GSI) were computed according to Bouslama & Schapaugh (1984).

Seedling experiment: Six wheat genotypes including two check varieties, Khirman and Chakwal-86, were evaluated for drought tolerance potential at seedling stage. The experiment was conducted in plastic bowls under control conditions at Plant Physiology laboratory, Nuclear Institute of Agriculture, Tandojam, Pakistan. The layout of the trial was in complete randomized design (CRD) with four drought treatments (0, -0.5, -0.75, -1.0MPa PEG solutions) and three replicates. Drought stress was induced by polyethylene glycol (PEG-6000) with 12.24% (-0.5MPa), 18.4% (-0.75MPa) and 24.5% (-1.0MPa) solutions prepared in 1/4th Hoagland's solution. The conditions maintained in growth incubator (Vindon, England) included 14 h day and 10 h night periods, with 25°C day and 20°C night temperatures. The seeds of wheat genotypes IBWSN-1010, IBWSN-1025, TD-1, ESW-9525, Khirman and Chakwal-86 were obtained from plant breeding and genetics division, Nuclear Institute of Agriculture (NIA) Tandojam, Sindh, Pakistan. Healthy seeds were sterilized in 3% sodium hypochlorite solution for 15 min. and thoroughly washed with distilled water. Thirty seeds were sown in each bowl. Bowls were air tighten and placed in dark for 48 h for germination of seeds. After the emergence of plumule and radical, bowls were provided with light in growth chamber (Vindon, England) and germination was noted up to 96 h. Germination percentage and germination index was computed. Two weeks old seedlings were harvested and subjected to the following growth and physiological analysis:

Seedling growth: Seedling growth was evaluated through the measurement of root and shoots lengths; and fresh and dry biomasses. Seedling vigour index (SVI) was also computed.

Photosynthetic pigments: Chlorophyll (chl. a and b) and carotenoids were determined from fresh leaves by extracting in 80% following the method of Lichtenthaler (1987).

Determination of ionic constituents: Mineral ions including potassium and calcium were analyzed through flame photometer (Jenway, Model PFP 7) following the methodology of Ansari & Flowers's, (1986). According to it, 0.1g dried plant material was extracted in 0.1 M acetic acid at 95ºC for an hour, the extract was filtered and suitable dilutions were made accordingly.

Statistical analysis: The data of all parameters was statistically analyzed through analysis of variance techniques to check the significant differences among wheat genotypes at 0.05 probability level and correlation among growth and physiological parameters was computed by using MSTAT-C software (Steel *et al*., 1997).

Results

Drought stress had severely affected the rate of germination. Most of genotypes executed good germination percentage with minimum percent reduction over control at lowest drought stress of -0.5MPa while germination percentage decreases with the increment of drought stress. At medium water scarcity (-0.75MPa), best results were sown by Benazir and MASR-64 followed by Sarsabz variety with least % reduction in germination over control. Likewise, Benazir and Sarsabz genotypes gave maxiumum germination percentages and germination stress tolerance indices (GSI) at -1.0 MPa osmotic stress. Wheat lines IBWSN 1149 followed by DH-9/1 showed poor germination potentials with highest % reduction over control and lowest GSI at highest level of drought stress (Table 1).

Growth and physiology of early wheat seedlings was negatively influenced under low osmotic potentials. Significant reduction in shoot and root length, fresh and dry weight at higher concentrations (-0.5, -0.75 and -1.0MPa) of PEG was observed as compared to control. However, TD-1 executed highest shoot length at -1.0MPa PEG induced osmotic stress with 24.86% reduction over control. Genotypes IBWSN-1010, IBWSN-1025 and ESW-9525 showed good shoot length up till -0.75MPa stress while more reduction occurred at -1.0MPa osmotic stress (Fig. 1). Increase in root length was observed at -0.5MPa osmotic stress while reduction in root occurred at -0.75MPa and -1.0MPa osmotic stress. However, TD-1 showed more root length with least reduction as compared to other genotypes at highest level of moisture stress. Similar trend of root behavior was also noted in check varieties (Fig. 2).

Fresh and dry biomasses of wheat seedlings showed variation at various levels of osmotic stress. Reducing trend was observed with the increase in drought stress. Highest fresh and dry biomasses were calculated in genotype IBWSN-1010 at -0.5MPa and -0.75MPa osmotic stresses while more reduction occurred at -1.0MPa drought stress. At highest level of water deficit condition, TD-1 genotype exhibited better seedling fresh and dry biomasses with least reduction over control (Figs. 3, 4, 5 & 6). Variations were also noted in case of TD-1 showed more root dry mass at -0.75MPa stress.

Table 1. Germination percentage and % reduction over control under PEG induced osmotic stress.

Wheat genotypes	Control (0 MPa)	-0.5 MPa			-0.75 MPa			-1.0 MPa		
		G (%)	Rel. dec. (%)	GSI	G (%)	Rel. dec. (%)	GSI	G (%)	Rel. dec. (%)	GSI
IBWSN-1010	100 a	100 a	0 d	100 bcd	95 ab	5 gh	95.5 a-d	68.3 bcd	41.7 fgh	52.8 fgh
IBWSN-1025	100 a	98.3 a	1.67 d	104.0 a	96.7 ab	3.3 gh	105.7 a	43.3efg	56.7 cde	43.2 hij
IBWSN-1042	100 a	100 a	0 d	98.2b-e	78.3 def	21.7 cde	82.5b-g	36.7gh	63.3 bc	33.6 jk
IBWSN-1132	100 a	100 a	0 d	99.3b-e	91.7 abc	8.3 fgh	78.3-k	61.7bcd	38.3 fgh	22 kl
IBWSN-1144	100 a	100 a	0 d	100bcd	98.3 a	1.7 gh	84b-i	56.7cde	43.3 efg	18.5 l
IBWSN-1148	96.7 bc	86.7d	10.35 b	86.6 f	85 cd	12.1 efg	79.7 f-k	33.3 gh	65.5 bc	10.8 lmn
IBWSN-1149	100 a	100 a	0 d	98.7 b-e	78.3 de	21.7 cde	75.6 fgh	0 i	100 a	0 n
IBWSN-1150	98.3 ab	98.3 a	0 d	98.5 b-e	81.7 cde	17 def	70.1 c-g	26.7 h	72.9 b	23 kl
IBWSN-1156	100 a	96.7 ab	3.33 cd	96 cde	91.7 abc	8.3 fgh	91.7 a-g	32.5 gh	67.5 bc	15.3 lm
IBWSN-1157	100 a	100 a	0 d	98 b-e	81.6 cde	18.3 def	61.7 lm	5 i	95.0 a	5.0 lmn
DH-9/1	100 a	100 a	0 d	100 bcd	90 a-d	10 e-h	89.3 b-h	6.7 i	93.3 a	4.7 lmn
DH-9/6	100 a	98.3 a	1.67 d	97.7 b-e	95 ab	5 gh	93.7 a-f	60 bcd	40 fgh	40.7 hij
DH-12/7	100 a	98.3 a	1.67 d	97 cde	95.3 ab	4.7 gh	94.8 a-e	70 bc	30 gh	59.8 ef
DH-12/31	98.3 ab	98.3 a	0 d	98.6 b-e	95ab	3.4 gh	96 a-d	53.3 def	45.76 def	46.3 ghi
MASR-08	98.3 ab	98.3 a	0 d	102.5ab	95 ab	3.39 gh	94.5 a-f	38.3 fgh	61 bcd	33.1 jk
MASR-22	100 a	100 a	0 d	100 bcd	98.33 a	1.7gh	97.2 a-d	66.7bcd	33.3 fgh	56.5 efg
MASR-64	100 a	100 a	0 d	99.3 b-e	100 a	0 h	99.3 ab	65 bcd	35 egh	52.2 fgh
ESW-9525	100 a	92 bcd	8.3bc	95.3 de	68.3 fgh	31.7 abc	72.7 i-l	40fgh	60 bcd	33.8 jk
MSH-14	95 c	90cd	1.754 d	94.7 e	61.7 gh	35.1 ab	56.7 m	41.7e-h	56.1 cde	43.9 hij
NIA Amber	98.3 ab	95 abc	3.39 cd	98.5 b-e	58.3 h	40.7 a	67.9 j-m	38.3 fgh	61bcd	39.2 ij
NIA Saarang	98.3 ab	96.7 ab	1.70 d	97.3 cde	96.7 ab	1.7 gh	70.5 i-m	71.7bc	28.3gh	66.6 de
Sarsabz	98.3 ab	96.7 ab	1.70 d	98.3 b-e	96.7ab	1.7 gh	98.3 abc	93.3a	5.1 i	88.8 ab
Benazir	100 a	100 a	0 d	100 bcd	100 a	0 h	100 ab	96.7a	3.3 i	80.5 bc
TD-1	100 a	71.67 e	28.33 a	64.0 g	71.7 efg	28.3 bcd	66.7 klm	73.3bc	26.7 h	73.1 cd
Khirman	100 a	100 a	0 d	102.5 ab	96.7 ab	3.3 gh	99.2 ab	93.3a	6.7 i	94.4 a
Chakwal-86	100 a	100 a	0 d	100 bcd	100 a	0 h	100 ab	98.3a	1.7 i	94.8 a
LSD	2.2205	6.3462	6.1855		11.686	11.815		15.599	15.693	
St. Error	0.7817	2.2342	3.0796		4.1140	5.8822		5.4916	7.8133	

Mean ± standard error along with various letters is significantly different at a level of p≤0.05, rel. dec.= relative decrease, GSI= germination stress tolerance index

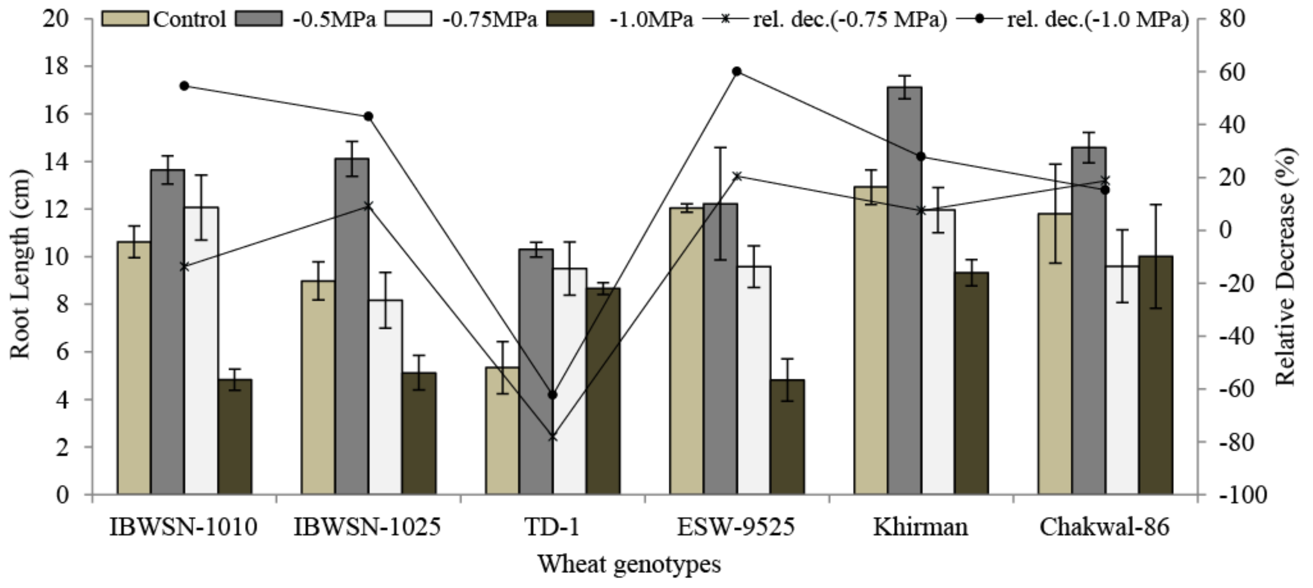

Fig. 1. Effect of drought stress on shoot length in different wheat genotypes

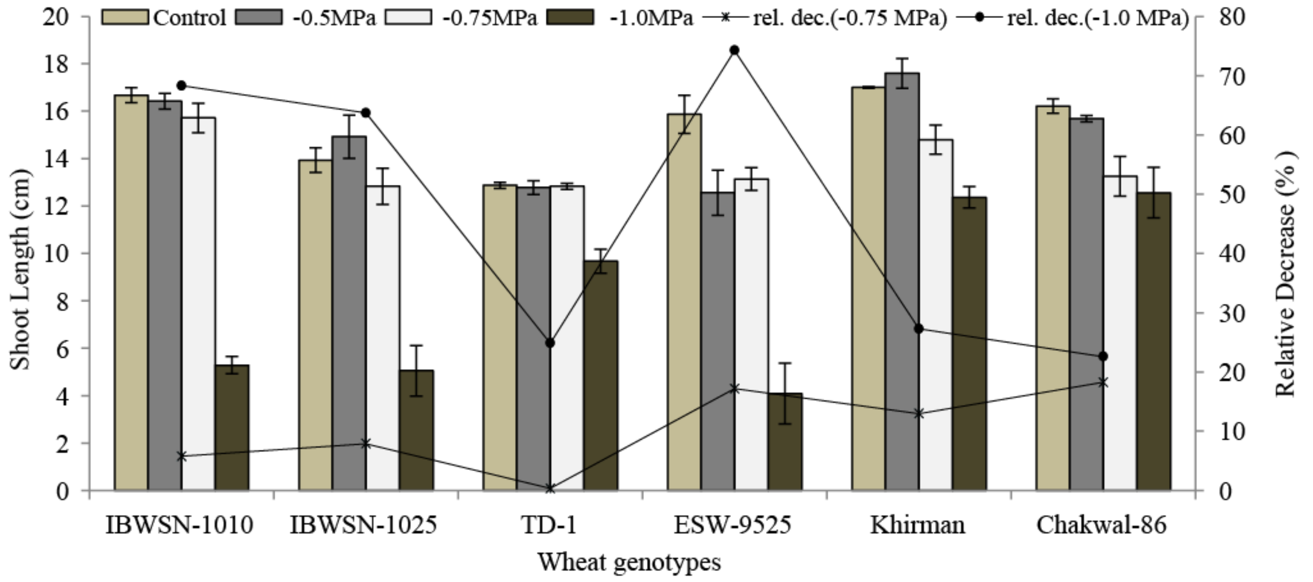

Fig. 2. Effect of drought stress on root length of different wheat **g**enotypes

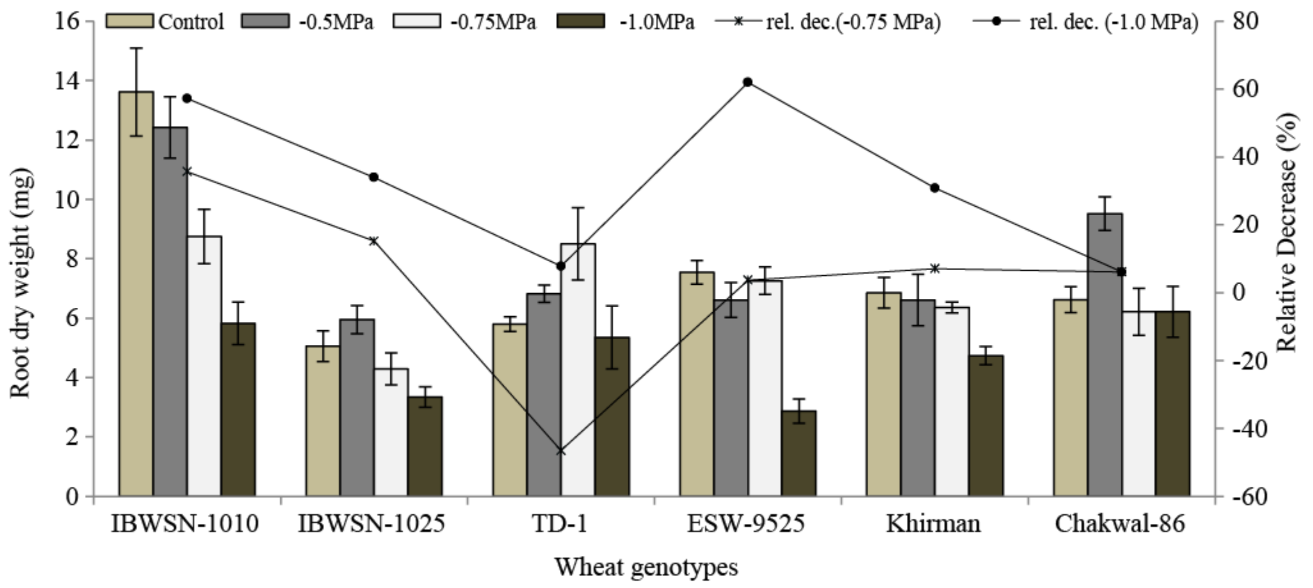

Fig. 3. Effect of drought stress on shoot fresh weight of wheat genotypes

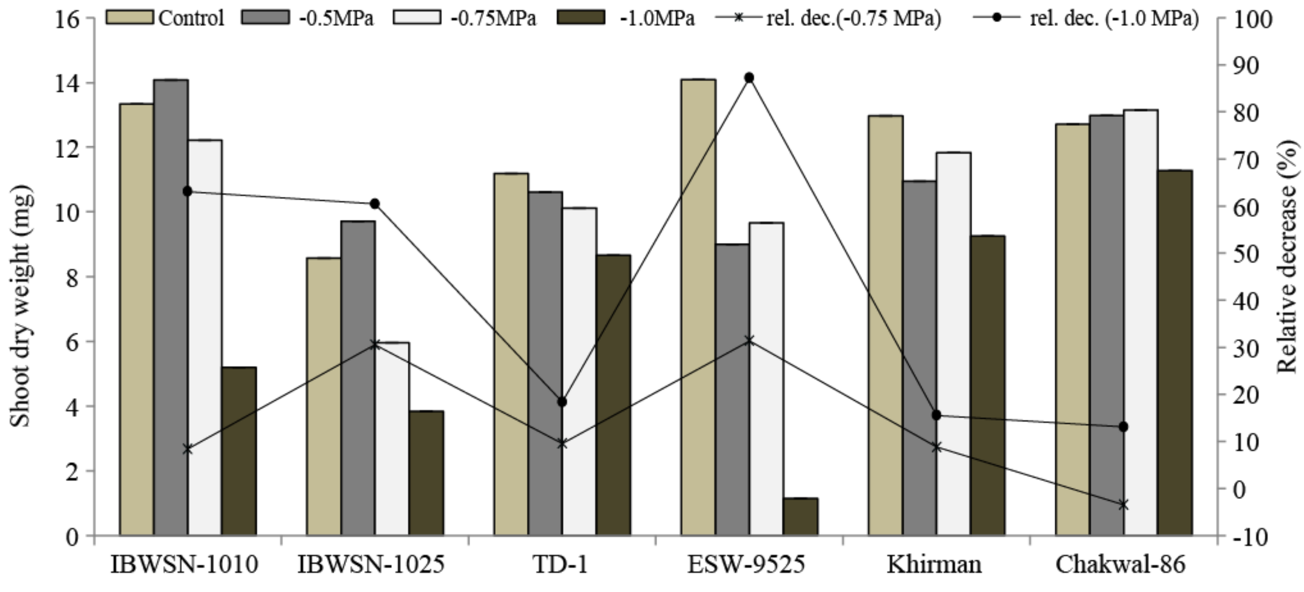

Fig. 4. Effect of drought stress on shoot dry biomass in different wheat genotypes

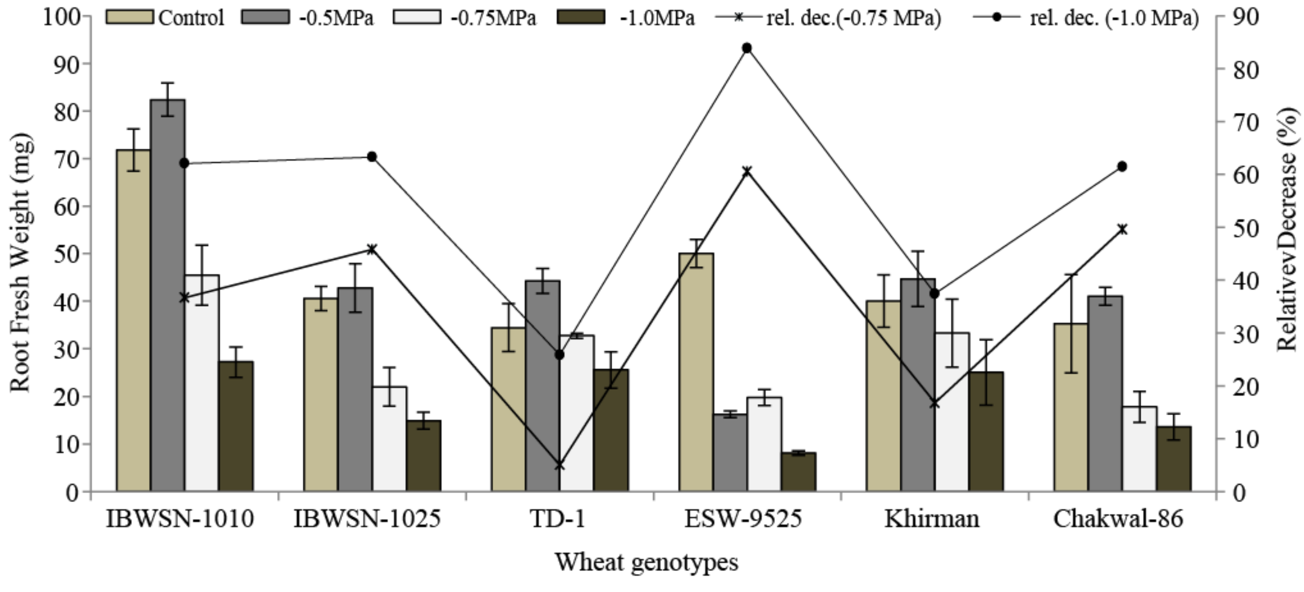

Fig. 5. Effect of drought stress on root fresh biomass in different wheat genotypes

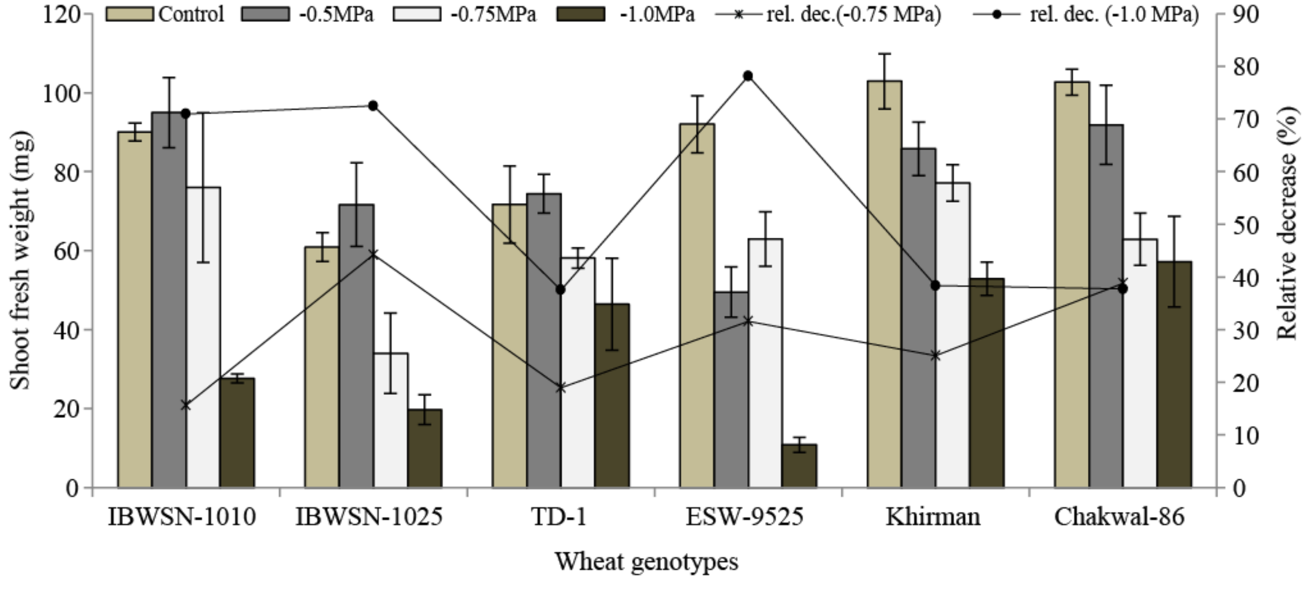

Fig. 6. Effect of drought stress on root dry weight of different wheat genotypes

Table 2. Effect of PEG induced drought stress on photosynthetic pigments (chl. a + b) in wheat genotypes

Wheat genotypes	Control	-0.5 MPa stress		-0.75 MPa stress		-1.0 MPa stress	
		Total Chl. (mg g^{-1})	Relative decrease (%)	Total Chl. (mg g^{-1})	Relative decrease (%)	Total Chl. (mg g^{-1})	Relative decrease (%)
IBWSN-1010	0.272 d	0.258 de	5.24	0.246 b	9.81	0.121 cd	55.46
IBWSN-1025	0.289 d	0.193 f	33.12	0.254 b	12.22	0.173 c	40.14
TD-1	0.308 d	0.272 d	11.89	0.274 b	11.30	0.261 b	15.26
ESW-9525	0.413 c	0.398 c	3.60	0.370 b	10.58	0.240 b	41.83
Khirman	0.747 a	0.722 a	3.33	0.622 a	16.69	0.424 a	43.16
Chakwal-86	0.577 b	0.541 b	6.11	0.370 b	35.90	0.402 a	30.30
LSD (≤0.05)	*0.0678*	*0.0449*		*0.1397*		*0.0626*	
St. Error	*0.0323*	*0.0214*		*0.0665*		*0.0298*	

Mean along with various letters in column are significantly different at $p<0.05$

Table 3. Effect of of PEG induced drought stress on ionic contents in wheat genotypes

Wheat genotypes	Shoot potassium ions (%)			K^+/Ca^{2+} Ratio	Root potassium ions (%)			K^+/Ca^{2+} Ratio
	Control	-0.75MPa	-1.0MPa		Control	-0.75MPa	-1.0MPa	
IBWSN-1010	0.84 bc	1.22 b	0.21 c	0.38 c	0.36 b	0.40 d	0.44 b	0.76 ab
IBWSN-1025	0.75 c	0.73 b	0.61 bc	0.79 bc	0.35 b	0.61 cd	0.42 b	0.69 b
TD-1	1.03 ab	1.18 b	1.30 ab	1.05 b	0.53 a	0.89 ab	0.54 b	0.89 ab
ESW-9525	0.93 abc	1.86 a	0.38 c	1.19 b	0.46 ab	0.76 bc	0.45 b	1.17 ab
Khirman	1.05 a	1.87 a	1.66 a	1.95 a	0.44 ab	0.70 bc	0.91 a	1.38 a
Chakwal-86	0.86 abc	1.89 a	1.35 a	1.72 a	0.45 ab	1.11 a	1.12 a	0.84 ab
LSD (≤0.05)	*0.1967*	*0.5762*	*0.7393*	*0.4965*	*0.1532*	*0.2571*	*0.2782*	*0.6481*
St. Error	*0.0883*	*0.2586*	*0.3318*	*0.2228*	*0.0688*	*0.1154*	*0.1249*	*0.2909*

Mean along with various letters in column are significantly different at $p\leq0.05$

Chlorophyll contents of all wheat genotypes were affected by drought. Photosynthetic pigments (Chl. a and b, carotenoid contents) were reduced with increased in water scarcity. Most of wheat genotypes exhibited better chlorophyll contents at -0.75MPa osmotic stress while TD-1 and ESW-9525 maintained maximum photosynthetic pigments even at -1.0MPa stress. Both these genotypes were statistically at par in terms of photosynthesis (Table 2).

Uptake of mineral ions was also disturbed under drought stress. There was found no significant difference in ionic concentration of -0.5MPa drought treatment and control. However, more accumulation of potassium ions in roots and shoots was analyzed at -0.75MPa and -1.0MPa osmotic stress in check varieties. Maximum shoot and root K^+ ions were assessed in TD-1 at highest level of drought stress. While highest K^+/Ca^{2+} ratio was computed in check varieties followed by ESW-9525 and TD-1 at -1.0 MPa osmotic stress (Table 3).

Discussion

Various levels of water deficit had inhibitory effects on plant seedling's growth and functionality. Seed germination of most of wheat genotypes was suppressed or delayed with the increase in water deficit levels because of lack of required level of moisture for initiation of germination. All wheat genotypes behaved diversely in terms of seedling's growth and development for tolerating chemical dehydration induced by PEG solutions. Plant growth related parameters such as root and shoot length, seedlings fresh weight etc. are characterized as major parameters for screening of efficient wheat varieties under low water contents (Foito *et al*., 2009). Growth of wheat seedlings was inhibited at all levels of water stress resulting in reduction of biomass. Germination percentage of all wheat genotypes was declined under osmotic stress. Decrease in shoot and root length, and biomass had been observed in all genotypes at water deficit conditions. It may be the result of diminish relative turgidity and dehydration of protoplasm which is correlated with turgor loss and reduced cell expansion and impediment of mitosis. Khodarahmpour (2011) documented the deduction in germination percentage, shoot and root length through PEG induced osmotic stress. Ahmad *et al*. (2013) also assessed decrease in seedling's growth including seedling length and biomasses with increase in osmotic stress.

Table 4. Pearson's correlation among various growth and physiological parameters under drought stress.

	SL	RL	SFW	SDW	RFW	RDW	TC	SK
RL	0.782**							
SFW	0.8558**	0.6724**						
SDW	0.6667**	0.3573*	0.7813**					
RFW	0.6091**	0.5242	0.6369	0.4633				
RDW	0.5585**	0.4464	0.6025**	0.5237**	0.7621			
TC	0.5509**	0.5304	0.5691**	0.3462	0.0523**	0.0570**		
S K	0.4236**	0.4691**	0.3031	0.3363*	0.0612	0.2057	0.3122*	
RK	-0.0328	-1.013	-0.1268	0.0239	-0.3287*	-0.1385	0.0769	0.4343**

SL= Shoot length, RL= Root length, SFW = Shoot fresh weight, SDW = Shoot dry weight, RFW = Root fresh weight, RDW = Root dry weight, TC = Total chlorophyll, SK = Shoot potassium, RK = Root potassium

* Significant at p = 0.05, ** Significant at p = 0.001

Photosynthesis, a key process of plant cells, is regulated under low osmotic potential of water culture medium. More the chlorophyll pigments more will be the photosynthesis. Chlorophyll contents executed more reduction in all wheat genotypes with the increment in levels of water stress because thylakoid membranes disintegrate upon dehydration of cells (Zeng *et al*., 2016). Previous studies also highlighted the reduction in chlorophyll under water deficit conditions (Kidokoro *et al.*, 2009) while tolerant genotypes maintained better amount of these pigments under drought (Seher *et al*., 2015).

The under tested wheat genotypes also varied in response to solute accumulation and mineral uptake in water stress. More the K^+/Ca^{2+} ratio; more will be the dearth tolerance potential. In this regards, ESW-9525 executed highest K^+ contents as well as root and shoot K^+/Ca^{2+} ratio along with both check varieties at highest drought stress treatment. Asghari *et al*. (2001) also documented elevated K^+/Ca^{2+} ratio in tolerant cultivars of wheat under drought stress.

Pearson correlation among growth and physiological traits was also calculated (Table 4). Strong, positive and significant coefficients of correlation were marked among all seedling traits except root potassium under control and drought stress. Darvishzadeh *et al*. (2010) stated that correlations are also the good criteria for selecting drought tolerant genotypes.

These wheat genotypes exhibited tolerance potential against extreme water deficit under controlled conditions at the critical developmental stage of plant life cycle, i.e. seeding stage. Hence, it is recommended that these genotypes which performed better in water stress condition may increase production of arid and rainfed lands.

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