

EVALUATION OF DROUGHT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS AT EARLY SEEDLING STAGE USING POLYETHYLENE GLYCOL INDUCED OSMOTIC STRESS.

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ABSTRACT

Drought is a grave concern due to changing climate phenomenon which drastically affects wheat production throughout the world. Screening drought tolerant varieties of wheat are important for ameliorating productivity of water scarce areas. An experiment was conducted at physiology division Nuclear Institute of Agriculture Tandojam (Latitude: 25.433 Longitude: 68.533) Sindh, Pakistan during 2016-2017. Treatment included a factorial combination using a completely randomized design (CRD) with three replications. Six wheat cultivars (cv. cultivars IBWSN-1010, IBWSN-1025, TD-1, ESW-9525, Khirman and Chakwal-86) were investigated for their response at seedling stage under different water stress treatments (T-1 0, T-2 -0.5, T-3 -0.75 and T-4 -1.0 MPa) in controlled conditions. The results of experiments with reference to cultivars revealed that cultivar Khirman & IBWSN-1010 showed maximum shoot length (17.33 and 16.68 cm), while the cultivar Khirman and TD-1 showed maximum root length (10.02 and 8.67 cm), shoot fresh weight (34.46 g 10⁻¹shoots), root fresh wt. (71.76 g 10⁻¹shoots), shoot dry wt. (13.55 g 10⁻¹shoots), root dry wt. (13.62 g 10⁻¹roots), while the cultivar IBWSN-1010 observed more chlorophyll contents (0.27 mg g⁻¹ fresh wt) and ionic contents (K⁺ and Ca²⁺, and K⁺/Ca²⁺ ratio) (1.05, 1.03 and 0.93 %). Among all the wheat cultivars tested Khirman and IBWSN-1010 are tolerant cultivar and have the potential to perform better under drought conditions, whereas IBWSN-1025 and Chakwal-86 were moderately tolerant under water stress conditions. Moreover, the cultivars i.e. TD-1 and ESW-9525 are the sensitive cultivars under drought environment. It may be concluded from present in-vitro studies that osmotic stress significantly reduced the shoot/root length fresh and dry weight in all six wheat cultivars. The maximum reduction (P≤0.05) was found at higher osmotic stress induced by PEG-6000 (-1.0 MPa).

Keywords: Drought, Seedling, Germination, Polyethylene glycol, Chlorophyll, Wheat.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is a staple food for more than 35% of the world population and it is also the first grain crops in most of developing countries (Metwali *et al.*, 2011). Abiotic stress, especially drought stress is a worldwide problem, seriously constraining global crop production (Pan *et al.*, 2002), commonly reduces average yield for many crop plants by more than 50% (Wang *et al.*, 2003; Bayoumi *et al.*, 2008). The high yield of plant in sufficient irrigated conditions is not necessarily related to high yield under drought stress (Jajarmi *et al.*, 2009). Understanding of plant responses to drought is a fundamental part of developing stress-tolerant varieties

(Oneto *et al.*, 2016). Extreme water shortage causes considerable physiological, metabolic and morphological changes in plant, and ultimately reduces crop yield and quality (Maqsood *et al.*, 2012). In most of developing countries, wheat is mainly grown on rainfed lands without supplementary (590 mm) irrigation. About 37% of land area in these countries consists of semiarid environments in which available moisture constitutes a primary constraint to wheat production (Qayyum *et al.*, 2011). Seed germination and seedling growth characters are extremely important factors in determining yield. Scientists (Dhanda *et al.*, 2004; Rauf *et al.*, 2007; Noorka and Khaliq, 2007) have indicated that seed vigor index and shoot length are among the most sensitive to drought stress, followed by root length and coleoptiles length.

According to the wheat scientists, seedling growth is influenced under drought stress, but the influence changes from variety to variety. The selection of wheat variety with the best performance under water stress environments could increase the production of rainfed areas. Scientists (Noorka *et al.*, 2013; Ahmad *et al.*, 2014; Ahmed *et al.*, 2017). When plant faces or observe drought condition the amino acids are helpful to complete their life cycle (Abro *et al.*, 2019). Many studies suggested the seed treatments with poly ethylene glycol (PEG6000) that are helpful for alleviating the negative effect of drought stress on plant (Almansouri *et al.*, 2001; Okcu *et al.*, 2005; Kaya *et al.*, 2006 and Iqbal *et al.*, 2007). Selection of drought tolerance at early seedling stage is frequently accomplished using simulated drought induced by chemicals like poly ethylene glycol (PEG6000). Poly ethylene glycol can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water deficit in relatively controlled manner. Researchers (Manoj and Uday 2007; Foito *et al.*, 2009 and Khodarahmpour, 2011) reported that Poly ethylene glycol molecules are inert, non-ionic, and virtually impermeable to cell membranes and can induce uniform water stress without causing direct physiological damage. PEG as a factor causing drought stress by reducing water potential, results in reducing growth of seedling. This effect has been observed to a great extent more in the shoot than primary roots, and it was also suggested that PEG prevent water absorption by seeds, through penetrable ions inside cell by reducing potential results in water absorption at early germination stage (Khajeh *et al.*, 2000; Zhu *et al.*, 2002). The objective of this study was to screen out wheat (*Triticum aestivum* L.) cultivars under water stress condition at seedling stage using polyethylene glycol PEG 6000 with different concentrations.

MATERIALS AND METHODS

A laboratory experiment was conducted at physiology division Nuclear Institute of Agriculture Tandojam (Latitude: 25.433 Longitude: 68.533) Sindh Pakistan during 2016-2017. The purpose was to observe effects of water stress, using polyethylene glycol, on seedling growth parameters in wheat; the treatment included a factorial combination using a completely randomized design (CRD) with three replications. In the present study seeds of six cultivars from wheat (IBWSN-1010, IBWSN-1025, TD-1, ESW-9525, Khirman and Chakwal-86). Grains of six cultivars were subjected to four stress level of PEG6000 (0.0, -0.5, -0.75, -1.0 Mega Pascal MPa). PEG6000 was prepared by dissolving the required amount of PEG in distilled water at 30°C. Wheat grains were disinfected with 10% sodium hypochlorite solution for 30 seconds. After the treatment the grains were washed two times with distilled water. Twenty

grains from each cultivar were sown in glass bowls (15 and 10 cm intensive) in respective treatment from PEG6000. The glass bowls were placed in an incubator under a photoperiod ($4.9 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) for 20 days. Grains were considered germinated when they exhibited radicle extension of $> 3 \text{ mm}$. Every 24 hours after soaking, germinated grains were counted daily the experiment to determine following seedling parameters. Shoot length (cm) Root length (cm) Shoot fresh weight (g) Root fresh weight (g) Shoot dry weight (g) Root dry weight (g) and the Ionic contents (Ca and K) total Chlorophyll content (mg g^{-1} fresh wt), Shoot potassium K^+ (%), Root potassium content (%), Shoot calcium Ca (%), Root calcium (%), etc., were also observed from shoots and roots of wheat cultivars with help of flame photometer method (Amrutkar *et al.*, 2013).

Statistical analysis: The data recorded were subjected to analysis of variance to discriminate the superiority of treatment means and LSD test were applied following method of Gomez and Gomez (1984) to compare the means.

RESULTS

In our research trial the ability of the six wheat cultivars under water stressed, induced by PEG (6000) during early seedling stage was assessed under in-vitro environment. Statistical analysis data revealed that different six wheat cultivars depicted performance differently under the different PEG treatments. There was a significant two-way interaction (drought level and cultivars) ($P \leq 0.01$) for all seedling parameters. Data pertaining the effect of PEG induced stress on shoot and root length (cm), shoot/root fresh and dry weight, total chlorophyll content (mg g^{-1} fresh weight), shoot potassium K^+ , root potassium K^+ content (%), shoot calcium Ca^{2+} (%) and root calcium (%) is shown in table (1a, 1b, and 2a,2b).

Shoot and root length (cm): Under control conditions, the maximum shoot length was recorded in Khirman followed by, IBWSN-1010 (17.33 and 16.68 cm) as depicted in table.1. Whereas, the maximum root length was recorded in Khirman and ESW-9525 (12.92 and 14.80 cm), moreover, minimum root length was recorded in TD-1 (5.34 cm). Increasing concentration of osmotic stress reduced shoot and root length of all the wheat cultivars. Maximum reduction was recorded at the highest water stress level (i.e.-1.0 MPa), cultivar Khirman and Chakwal-86 also showed maximum shoot length (12.56 and 12.36 cm), followed by TD-1 (9.68 cm), IBWSN-1010 (5.29 cm), and IBWSN-1025 (5.06 cm), while minimum shoot length was observed in cultivars ESW-9525 (4.09 cm), respectively. Under highest water stress conditions the cultivar Khirman Chakwal-86 and TD-1 showed the maximum root length

(i.e. 10.02, 9.33 and 8.67 cm) followed by cultivars IBWSN-1025 (5.13 cm), IBWSN-1010 (4.83cm), while minimum root length i.e. (4.82 cm) was observed in cultivars ESW-9525, respectively. The plant growth related parameters such as root and shoot length, seedlings fresh weight etc., are visualized as major characteristics for screening of drought resistant wheat cultivars (Foito *et al.*, 2009). It is also evident that PEG induced water stress has drastic effect on shoot length of various cultivars. Because artificially osmotic stress cell division necessary for shoot elongation. Similar results

are found by the (Almaghrabi *et al.*, 2012) where he found that PEG has effect on shoot length of different wheat cultivars in his research experiment. It may be interpreted that the increasing concentrations of PEG has declining effect on the root length, possibly because of the inhibition of the cell division and elongation. (Chachar *et al.*, 2014) reported that the reduction in the root length under drought stress may due to an impediment of cell division and elongation leading to tender tuberization.

Table.1 a. Mean squares from analysis of variance for various morphological traits of wheat cultivar s at seedling stage under osmotic stress (PEG 6000).

Source of variation	DF	Morphological characters					
		Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
Replications	2	0.11	2.47	66.19	129.81	2.15	1.34
Treatments	3	201.97**	128.22**	8187.65**	3041.24**	135.78**	38.14**
Cultivar	5	32.86**	31.49**	2298.43**	1661.08**	58.23**	40.70**
G x T	15	12.24**	10.93**	494.15**	268.92**	20.68**	6.76**
Error	40	1.45	3.99	125.3	52.79	4.74	1.49

**= significant at P<0.01probability level, *= Significant at P<0.05probability level and ns. = Non-significant

Table.2a. Mean squares from analysis of variance for various physiological traits of wheat cultivar s at seedling stage under osmotic stress (PEG 6000).

Source of variation	DF	Physiological characters				
		Total chlorophyll	Shoot K ⁺	Root K ⁺	Shoot Ca ⁺	Root Ca ⁺
Replications	2	0.00144	0.01088	0.00111	0.01942	0.0017
Treatments	3	0.0894**	2.1955**	0.32288**	0.56984**	0.07825*
Cultivar s	5	0.30617*	0.74908*	0.14684*	0.65473**	0.01648**
G x T	15	0.00982**	0.39261**	0.0604**	0.07713**	0.02033**
Error	40	0.00354*	0.09586**	0.01358**	0.01489**	0.00325*

**= significant at P<0.01probability level, *= Significant at P<0.05probability level and ns. = Non-significant

Shoot/root fresh and dry weight (g10⁻¹roots): The shoot and root fresh weight values were decreased with increasing water stress in all wheat cultivars (table.1) Maximum shoot fresh weight was observed in Khirman (102.86g 10⁻¹shoots). Whereas, the minimum shoot fresh weight values were observed in IBWSN-1025(69.33 g 10⁻¹shoots), moreover, the cultivar IBWSN-1010 revealed more root fresh weight (71.76 g 10⁻¹shoots) and the minimum root fresh weight observed by cultivar TD-1 (34.46 g 10⁻¹shoots) under controlled conditions. The results for shoot and root dry weight is presented in table 1b. Results revealed that significant decrease with increasing water stress. At high water stress condition (-1.0 MPa) there was comparatively higher reduction in plant biomass with increasing water stress of the growing media. The cultivars Chakwal-86 Khirman and TD-1 showed maximum shoot fresh weight (57.22, 52.87 and 54.63 g10⁻¹shoots), followed by IBWSN-1010 (27.59 g 10⁻¹shoots) and IBWSN-1025 (19.73 g 10⁻¹shoots). While minimum shoot fresh weight (10.82 g 10⁻¹shoots) was observed in cultivar ESW-9525, respectively.

Root fresh weight: There was decrease in root fresh weight with the increasing in water stress in all wheat cultivars. The decrease was more in -1.0 MPa as compared to control. Mean values for root fresh weights in three treatments were recorded as 45.35, 39.2, 28.50 and 19.06 g10⁻¹roots under control, -0.5, -0.75 and -1.0 MPa, respectively. The cultivar IBWSN-1010 showed maximum root fresh weight (71.76 g 10⁻¹root), followed by Khirman (50.06), IBWSN-1025 (40.55), Chakwal-86 (40.00), and ESW-9525 (35.29 g10⁻¹shoot) was recorded, whereas minimum root fresh weight (34.46 g10⁻¹roots) was observed in cultivars TD-1, respectively. Root fresh weight at the highest -1.0 MPa water stress was observed as maximum in cultivar IBWSN-1010 (i.e. 27.22 g10⁻¹roots), followed by TD-1 (25.56), Khirman and Chakwal-86 (25.03 and 13.60 g10⁻¹roots). While, the cultivars IBWSN-1025 and ESW-9525 showed minimum (14.89 and 8.07 g10⁻¹roots) values for root fresh weight at highest osmotic stress, respectively. Our results are in agreement with the results found by (Wei *et al.*, 2013) where he found decreasing trend in shoot and root

fresh/dry weight. However, it was reported by various researchers that both parameters of shoot/root fresh and dry weight significantly increased shoot and root masses in both cultivars under well watered condition.

Shoot dry weight (g 10⁻¹shoots): Mean values for shoot dry weight in different treatments were recorded as 12.65, 11.09, 10.29 and 6.61 g 10⁻¹shoots in different four treatments as T1 control (0), T2 -0.5, T3 -0.75 and T4 -1.0 MPa, respectively. Under control condition, shoot dry weight of cultivar ESW-9525 was maximum i.e. (14.09 g 10⁻¹shoots), followed by TD-1 (13.99 g 10⁻¹shoots) and IBWSN-1010 (13.55 g 10⁻¹shoots). While, minimum shoot dry weight was observed in cultivars Khirman, Chakwal-86 and IBWSN-1025 (12.97, 12.71 and 8.58 g 10⁻¹shoots), respectively. The maximum shoot dry weight was recorded in cultivar Khirman (11.28 g 10⁻¹shoots), followed by Chakwal-86 and TD-1 as (9.25 and 8.32 g 10⁻¹shoots), at -1.0 MPa. The other cultivar were also showing better SDW, was IBWSN-1010 and IBWSN-1025 (5.20 and 3.84 g 10⁻¹shoots) respectively. Minimum values for SDW (1.75 g 10⁻¹shoots) were recorded in cultivar ESW-9525, respectively.

Root dry weight (g 10⁻¹roots): Results revealed that significant decrease with increasing water stress. High

water stress condition (-1.0MPa) there was comparatively higher reduction in root dry weight with increasing water stress of the growing media. Mean values for root dry weight in different treatments were observed such as (7.58, 7.99, 6.90 and 4.72 g 10⁻¹roots) in four different treatments of PEG viz T1 control (0), -T2 0.5, T3 -0.75 and T4 -1.0 MPa, respectively. Under control condition, root dry weight of cultivar IBWSN-1010 was maximum i.e. (13.62 g 10⁻¹roots), followed by ESW-9525 (7.54 g 10⁻¹roots), Khirman (6.85 g 10⁻¹roots) and Chakwal-86 (6.62 g 10⁻¹roots), whereas TD-1 (5.80 g 10⁻¹roots), whereas the minimum root dry weight were recorded in cultivar IBWSN-1025 (5.06 g 10⁻¹roots), respectively. Under highest osmotic stress (-1.0 MPa) condition, root dry weight of cultivar Khirman was maximum i.e. (6.22 g 10⁻¹roots), followed by IBWSN-1010, TD-1 and Chakwal-86 (5.82, 5.35 and 4.74 g 10⁻¹roots), while minimum root dry weight was observed in cultivar IBWSN-1025 and ESW-9525 (3.34 and 2.87 g 10⁻¹roots), respectively. The decreasing trend in root and shoot dry weight was also reported by other researchers (Kamran *et al.*, 2009; Ahmad *et al.*, 2013; Marcińska *et al.*, 2013) who found that water stress had a significant effect on root and shoot dry matter production.

Table.1 b. Mean value for various morphological traits of wheat cultivars at seedling stage under osmotic stress (PEG 6000).

Effect of peg on different drought levels on growth parameters of six wheat cultivars									
Morphological parameters									
Parameter	PEG level	Cultivars						MEAN	LSD
		IBWS-1010	IBWS-1025	TD-1	ESW-9525	Khirman	Chakwal-86		
Shoot length	Control	16.68	13.93	12.88	15.86	17.01	16.22	15.43	0.99
	0.5MPA	16.43	14.93	12.78	12.56	17.60	15.69	15.00	
	0.75MPA	15.72	12.83	12.33	13.14	14.80	13.26	13.68	
	1.0MPA	5.29	5.06	9.68	4.09	12.36	12.56	8.17	
	Mean	13.53	11.69	11.92	11.41	15.44	14.43		
Root length	Control	10.62	8.98	5.34	12.04	12.92	11.81	10.29	1.65
	0.5MPA	13.64	14.11	10.30	12.22	17.12	14.58	13.66	
	0.75MPA	12.07	8.17	9.50	9.59	11.96	9.60	10.15	
	1.0MPA	4.83	5.13	8.67	4.82	9.33	10.02	7.13	
	Mean	10.29	9.10	8.45	9.67	12.83	11.50		
Shoot fresh wt	Control	90.04	69.33	72.01	92.01	102.86	102.67	88.15	9.24
	0.5MPA	86.64	62.46	53.53	49.48	85.77	91.86	71.62	
	0.75MPA	79.35	31.75	58.11	62.97	77.08	62.84	62.02	
	1.0MPA	27.59	19.73	54.63	10.82	52.87	57.22	37.14	
	Mean	70.91	45.82	59.57	53.82	79.65	78.65		
Root fresh wt	Control	71.76	40.55	34.46	50.06	40.00	35.29	45.35	5.99
	0.5MPA	82.36	42.76	44.27	16.18	44.69	41.01	45.21	
	0.75MPA	45.43	21.98	32.73	19.76	33.30	17.79	28.50	
	1.0MPA	27.22	14.89	25.56	8.07	25.03	13.60	19.06	
	Mean	56.69	30.04	34.25	23.52	35.75	26.92		
Shoot dry wt	Control	13.55	8.58	19.99	14.09	12.97	12.71	13.65	1.80
	0.5MPA	13.36	9.71	8.52	9.00	10.95	12.98	10.75	
	0.75MPA	12.37	5.62	10.12	9.67	11.83	13.16	10.46	
	1.0MPA	5.20	3.84	10.32	1.75	9.25	11.28	6.94	
	Mean	11.12	6.94	12.24	8.63	11.25	12.53		

	Control	13.62	5.06	5.80	7.54	6.85	6.62	7.58	
	0.5MPa	12.42	5.95	6.83	6.61	6.61	9.52	7.99	
Root dry wt	0.75MPa	8.76	4.29	8.50	7.26	6.36	6.22	6.90	1.01
	1.0MPa	5.82	3.34	5.35	2.87	4.74	6.22	4.72	
	Mean	10.16	4.66	6.62	6.07	6.14	7.15		

Total chlorophyll content mg g⁻¹ fresh wt: Decreasing trend was recorded with increase of water stress, as depicted in table 2b. mean value for total chlorophyll content were recorded in different four treatment control (0), -T2 0.5, T3 -0.75 and T4 -1.0 MPa of PEG such as 0.43, 0.40, 0.35 and 0.27. as we observed the behaviors of different wheat cultivars on water stress, we found more total chlorophyll content in wheat cultivar Khirman, Chakwal-86 and ESW-9525 (0.75, 0.58 and 0.41 mg g⁻¹ fresh wt), followed by TD-1 and IBWSN-1025 (0.31 and 0.29 mg g⁻¹ fresh wt). While the maximum value found in IBWSN-1010 (0.27 mg g⁻¹ fresh wt) in control condition, respectively, at the highest water stress condition -1.0 MPa some of wheat cultivars performed well and others did not tolerate the stress. The higher reduction of total chlorophyll content in flag leaf was recorded in -1.0 MPa were found in the cultivar IBWSN-1010 (0.12 mg g⁻¹ fresh wt.) while the cultivar Khirman (0.42 mg g⁻¹ fresh wt) produced maximum total chlorophyll content at high water stress level. Our results revealed that a decreasing trend was observed with increasing water stress. Similarly, (Li *et al.*, 2006) observed that the values of chlorophyll content in drought tolerance cultivars of barley were significantly higher than those in drought sensitive cultivars under drought stress. It was also reported that the improvement of cultivar yield under drought stress has resulted from a more extended grain filling duration, a higher chlorophyll content, a more sustained turgor, or a combination of them (Paknejad *et al.*, 2007). Drought stress produces changes in photosynthetic constituents (Anjum *et al.*, 2003). Reduction of chlorophyll was the result of disappearance of thylakoid structures and chloroplast disintegration. (Cornoy *et al.*, 1988) Khirman and Chakwal-86 exhibited better chlorophyll contents under moisture stress which showed their tolerance against drought. These results are supported by (Khayatnezhad *et al.*, 2011), according to which chlorophyll contents of tolerant cultivars increased under drought stress.

Shoot/root potassium content K⁺ (%): To evaluate the ability of the six wheat cultivars and to distinguish the potassium and calcium concentrations, in roots and shoots, mean value for shoot K⁺ were recorded in different four treatment control (0), -T2 0.5, T3 -0.75 and T4 -1.0 MPa of PEG such as 0.91, 1.56, 1.45 and 0.91. The cultivars Khirman, TD-1 and ESW9525 revealed highest shoot K⁺ (1.05, 1.03 and 0.93 %) under normal conditions followed by Chakwal-86, IBWSN-1010 and IBWSN-1025 (0.86, 0.84 and 0.75 %), respectively. Increasing the water stress -1.0 MPa, shoot K⁺ ratio boost

up in cultivar Khirman (1.66), Chakwal-86 (1.35) and TD-1 (1.30) followed by, IBWSN-1025 (0.61) and ESW-9525 (0.38). Whereas the minimum ratio of shoot K⁺ was marked in cultivar IBWSN-1010 (0.21), respectively. Mean value for root K⁺ revealed under different treatments i.e., control (0), -T2 0.5, T3 -0.75 and T4 -1.0 MPa 0.43, 0.51, 0.74 and 0.54. Under control conditions, the cultivar ESW-9525 marked as maximum root K⁺ as (0.46), moreover, minimum root K⁺ was observed in IBWSN-1025 (0.35). Similarly, under higher osmotic stress level (-1.0MPa) the cultivar Khirman accumulated the maximum root K⁺ (0.91), while minimum accumulations of root K⁺ were marked by cultivar IBWSN-1025 (0.42) respectively. This might be explained that higher K⁺ concentration in plant growing medium offered more opportunity for roots absorbing K⁺ cellular membrane recovery enhanced K⁺ conservation in plant tissues. The results are supported by the research carried out by (Wei *et al.*, 2013) in his study, that adequate external K⁺ significantly increased K⁺ contents in both shoot and root of PEG6000-stressed.

Shoot/root calcium content Ca⁺ (%): The results of shoot and root calcium Ca⁺, mean value for shoot Ca⁺ were recorded as T1 control (0), -T2 0.5, T3 -0.75 and T4 -1.0 MPa of PEG such as 0.87, 1.11, 1.02 and 0.70. The cultivar TD-1 had marked highest shoot Ca⁺ (1.21%), whereas Khirman accumulate less Ca⁺ (0.62), respectively. whereas under high osmotic stress -1.0 MPa, shoot Ca⁺ ratio had accumulated more in cultivar TD-1 (1.13), while the ratio of Ca⁺ observed less in cultivar ESW-9525 (0.33) different wheat cultivars accumulated calcium at their root and shoot as well, cross talk about the root calcium, the mean value for root Ca⁺ revealed under different four treatments of PEG i.e., 0.52, 0.57, 0.68 and 0.58. root Ca⁺ was accumulate more by the cultivar IBWSN-1010 (0.53), while the less root Ca⁺ had marked by the cultivar IBWSN-1025 (0.49) under controlled conditions. Similarly under higher osmotic stress level (-1.0MPa) the cultivar Khirman accumulated the maximum root Ca⁺ (0.66), while minimum accumulations of root Ca⁺ were marked by cultivar ESW-9525 (0.40), respectively. The study depicted that accumulation of root/ for shoot calcium there was varietal difference. Same results were found by (Mujtaba *et al.*, 2016) in his study, he mentioned that PEG 6000 ratio was not significantly affected on Root K⁺/Ca²⁺ by osmotic stress. It was observed by (Mujtaba *et al.*, 2016) in his study, where he reported that Root K⁺/Ca²⁺ ratio was not significantly affected by osmotic stress.

Table 2b. Mean value for various physiological traits of wheat cultivars at seedling stage under osmotic stress (PEG 6000).

Parameter	PEG level	Physiological parameters						MEAN	LSD 0.05
		Cultivars							
		IBWS-1010	IBWS-1025	TD-1	ESW-9525	Khirman	Chakwal-86		
Total chlorophyll content	CONTROL	0.272	0.289	0.3087	0.4132	0.7467	0.5767	0.434383	14.2
	0.5MPA	0.2577	0.1937	0.2713	0.3987	0.722	0.5417	0.397517	
	0.75MPA	0.244	0.1937	0.2737	0.3697	0.622	0.3697	0.345467	
	1.0MPA	0.121	0.1733	0.2613	0.2403	0.4243	0.4023	0.270417	
	Mean	0.223675	0.212425	0.2788	0.355475	0.62875	0.4726		
Shoot K ⁺ %	CONTROL	0.8417	0.7542	1.025	0.9333	1.0542	0.8583	0.911117	18.51
	0.5MPA	1.4125	1.5833	1.7083	1.8208	1.625	1.2667	1.569433	
	0.75MPA	1.2158	0.7333	1.1808	1.8583	1.8667	1.8917	1.457767	
	1.0MPA	0.2083	0.6083	1.2958	0.3792	1.6583	1.35	0.91665	
	Mean	0.919575	0.919775	1.3025	1.2479	1.55105	1.341675		
Root K ⁺ %	CONTROL	0.3583	0.35	0.5333	0.4625	0.4375	0.45	0.431933	20.86
	0.5MPA	0.4125	0.4167	0.7042	0.525	0.475	0.5333	0.511117	
	0.75MPA	0.4	0.6125	0.8958	0.7625	0.7	1.1083	0.746517	
	1.0MPA	0.4417	0.4208	0.5375	0.4542	0.9125	0.5	0.54445	
	Mean	0.403125	0.45	0.6677	0.55105	0.63125	0.6479		
Shoot ca ⁺ %	CONTROL	1.0458	0.9958	1.2083	0.6667	0.6167	0.6833	0.869433	13.19
	0.5MPA	1.3042	1.5292	1.4417	0.7158	0.8875	0.775	1.1089	
	0.75MPA	1.1458	1.3083	1.0792	0.9133	0.8542	0.8183	1.01985	
	1.0MPA	0.5708	0.7708	1.125	0.325	0.7833	0.6458	0.70345	
	Mean	1.01665	1.151025	1.2136	0.6552	0.785425	0.7306		
Root ca ⁺ %	CONTROL	0.5292	0.4917	0.5458	0.5042	0.5375	0.5167	0.52085	9.71
	0.5MPA	0.5208	0.5792	0.6542	0.6083	0.5208	0.5542	0.572917	
	0.75MPA	0.5	0.6167	0.6833	0.7375	0.6792	0.8542	0.678483	
	1.0MPA	0.5833	0.6208	0.6083	0.3958	0.6625	0.5917	0.577067	
	Mean	0.533325	0.5771	0.6229	0.56145	0.6	0.6292		

Conclusion: In water deficits environments crops have developed biochemical and physiological approaches to tolerate. It is concluded from present research study that osmotic stress significantly reduced the shoot/root length fresh and dry weight all the tested wheat cultivars exhibited tolerance against osmotic stress under laboratory conditions. Among all the wheat cultivars tested Khirman, Chakwal-86 and TD-1 are the tolerant cultivars had the potential to perform better under drought conditions, whereas IBWSN-1010 and IBWSN1025 was moderate tolerant under water stress conditions. Furthermore the cultivars i.e. ESW-9525 is sensitive cultivars under drought environment. Hence, it is recommended that these cultivars which performed better in water stress condition could increase production of arid lands.

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