

## Physiological Studies on Six Wheat (*Triticum Aestivum* L.) Genotypes for Drought Stress Tolerance at Seedling Stage

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**Submission:** January 01, 2016; **Published:** January 25, 2016

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### Abstract

Screening of drought tolerant varieties of wheat are important for ameliorating productivity of water scarce areas. Six wheat genotypes including two local checks were evaluated for drought tolerance against different osmotic stress levels induced by Polyethylene Glycol (PEG)-6000. Experiment was set in completely randomized design (CRD) with three replicates and four treatments [0, -0.5 MPa, -0.75 MPa and -1.0 MPa PEG concentrations]. Wheat genotypes Khirman and Chakwal-86 were employed as check varieties. Four test genotypes evaluated were SD-621, SD-222, NIA Saarang and 22-03. Data were recorded on various seedling parameters such as germination percentage, germination index, shoot length, root length, fresh and dry weight of shoot and root, chlorophyll contents (a and b) and ionic contents (K<sup>+</sup> and Ca<sup>2+</sup>, and K<sup>+</sup>/Ca<sup>2+</sup> ratio). No seedling survived at -1.0 MPa osmotic stresses. Best results were illustrated by wheat genotypes SD-222 and SD-621 with 98.89% and 96.7% germination at -0.5MPa and -0.75MPa osmotic stress, respectively. NIA Saarang and genotype 22-03 also exhibited better results at moisture stress. Reduction in chlorophyll and ionic contents was occurred with rise in water deficit in all genotypes. These findings appreciate the future use of these genotypes for obtaining high yield in water sparse areas.

**Keywords:** Chlorophyll; Drought; Germination; Polyethylene glycol; Seedling; Wheat

**Abbreviations:** G: Germination; GI: Germination Index; Wt.: Weight; PEG: Polyethylene Glycol; CRD: Completely Randomized Design; LSD: Least Significance Difference

### Introduction

Drought is a worldwide problem and present climatic changes have even made it worse in many parts of the world. Exposing plants to water stress adversely affect plant growth and productivity. Osmotic stress is produced when inadequate moisture is present for plant's growth and development [1]. Drought is one of the major environmental issues affecting the wheat production. Pakistan is facing extreme drought conditions from last three decades especially in Sindh and Balochistan.

Wheat is an important cereal food and feeds one third population of the world fulfilling 50% of their protein requirements [2]. It is the staple food of Pakistan. Seed germination is the vital stage in plant's life cycle and it is regulated by genetic and environmental factors as well as seed treatment methods. The time and degree of seedling establishment are also highly important factors in determining the yield [3].

In case of wheat, the main threatening issue is the deficit water at seedling stage, mid season stress and terminal stress.

Various factors affect the yield of a crop like seed germination, seedling vigor, mean emergence time, growth rate and desiccation tolerance [4]. Germination and seedling establishment are rigorously declined by drought [5]. Thus, development of drought tolerant wheat cultivars is encouraged to gain high yield under moisture stress. Growth involves cell division, cell elongation and differentiation, and is regulated by genetic, ecological, physiological and morphological phenomenon. Cell growth is the most drought-sensitive physiological processes due to the turgor pressure reduction in cell [6].

Osmotic adjustment is the process of maintaining water relations in osmotic stress. Under water deficit, accumulation of solutes occurs which results in lowering of the osmotic potential of the cell, which pulls water molecules in the cell and helps in maintaining turgor. PEG is used for artificial induction of osmotic stress. [3,7]. Poly ethylene glycol (PEG) molecules are non-ionic, inert and have impermeable chains that induce water stress with no physiological damage and maintain the same water potential throughout the trial [8]. As PEG molecules do

not enter apoplast, water is pulled out from the plant cell, thus create water deficiency. The endeavor of present research was to assess the tolerance capacity of four wheat varieties against drought stress based on seedling traits.

### Materials and Methods

The experiment was conducted in plastic bowls to assess the effects of different levels of drought stress at seedling stage in four wheat varieties at Plant Physiology laboratory, Nuclear Institute of Agriculture, Tandojam, Pakistan. The layout of the experiment was in completely randomized design (CRD) with four treatments (0, -0.5, -0.75, -1.0MPa PEG solution) and three replicates. Day and night lengths were 14/10 h, with 25°C and 20°C temperatures, respectively. Wheat genotypes, Khirman (C<sub>1</sub>) and Chakwal-86 (C<sub>2</sub>) were used as check varieties. Four test varieties included SD-62, SD-222, NIA-Saarang and 22-03. Healthy seeds were first sort out, sterilized in 3% solution of sodium hypochlorite for about 10 minutes and thoroughly washed with distilled water. 30 seeds of each variety were placed in each bowl containing PEG solution and Hoagland’s solution. Bowls were covered and placed in dark for 48 hours for germination. After germination had started, bowls were transferred in light in growth chamber (Vindon, England) and germination was noted up to 96 hours. Germination percentage and germination index was computed. Two weeks old seedlings were subjected to the following measurements.

### Growth analysis

Seedling growth was assessed by taking into account following characters, like root and shoots length (cm) of 10 randomly selected seedlings while 20 randomly selected seedlings were subjected to fresh and dry weight (g) of root and shoot.

### Biochemical analysis

Chlorophyll a and b were determined following the method of Lichtenthaler [9] based on fresh weight by using the following formulae.

$$\text{Chlorophyll } a = (12.70 \times O.D \ 663) - (2.69 \times O.D \ 645) \times Wt. \times \frac{V}{1000}$$

$$\text{Chlorophyll } b = (22.90 \times O.D \ 645) - (4.68 \times O.D \ 663) \times Wt. \times \frac{V}{1000}$$

### Determination of ionic constituents

Potassium and calcium concentrations, in roots and shoots, were analyzed by Ansari and Flowers’s [10] methodology. According to it, 0.1g dried plant material was digested with 0.1M acetic acid (CH<sub>3</sub>COOH) in water bath for 1 hour at 95°C, the extract was filtered and suitable dilution was made. K<sup>+</sup> and Ca<sup>2+</sup> contents were determined by flame photometer (Jenway, Model PFP7).

### Statistical analysis

All parameters were taken thrice and data was statistically analyzed using analysis of variance techniques to check the significant differences among wheat genotypes using least significance difference (LSD) test at 0.05 probability levels [11].

### Results

Early wheat seedling’s growth and physiology was adversely affected by the increase in level of drought stress. Significant reduction in shoot and root length, fresh and dry weight at higher concentrations of PEG was observed as compare to control. Deceleration in percentage germination and germination index (GI) was also occurred with alleviated levels of water stress. The variations among genotypes were also momentous for all studied traits. All varieties expressed better results in control treatment especially SD-222 and SD-621 along with local checks.

All genotypes exhibited 100% germination in control. In -0.5MPa PEG (T<sub>2</sub>) treatment, maximum germination was executed by SD-222 (98.89%) followed by genotype 22-03 (91.1%) whereas, NIA Saarang showed minimum germination (54.45%). Likewise, SD-222 and SD-621 had highest % germination (96.7%) and genotype 22-03 exhibited lowest germination (68.87%) under -0.75MPa PEG (T<sub>3</sub>) solution. While no seedling survived at -1.0 MPa PEG stress.

Significant reduction in seedling length was observed in drought stress in all genotypes. SD-621 had maximum shoot length (13.38cm) followed by SD-222 (13.33cm) with 21.62% and 20.80% reduction as compare to control at -0.5MPa PEG stress. Maximum reduction (30.04%) was given by genotype 22-03 with 10.97cm shoot length. At alleviated osmotic stress (-0.75MPa PEG), SD-621 showed maximum shoot length (11.42cm) (Figure 1). Highest root length was expressed by genotype SD-222, i.e. 19.84cm which gradually decreased to 12.12cm and 7.20cm in T<sub>2</sub> and T<sub>3</sub>, respectively (Figure 2).

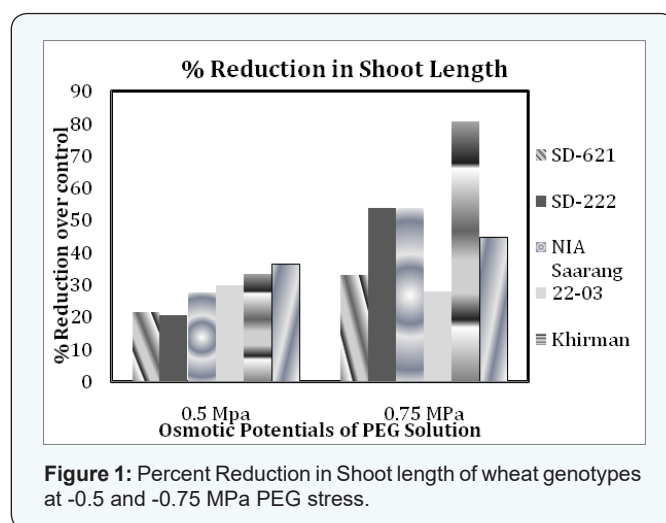


Figure 1: Percent Reduction in Shoot length of wheat genotypes at -0.5 and -0.75 MPa PEG stress.

Genotype 22-03 executed maximum shoot and root fresh weight with minimum % reduction over control, i.e. 0.079g (28.83% reduction over control) and 0.036g (16.28% reduction) respectively at -0.5MPa PEG stress which had decreased up to

0.073g (34.23% reduction) and 0.025g (41.86% reduction) under -0.75MPa PEG stress. SD-222 also showed good results in case of seedling fresh and dry weight (Figure 3, 4).

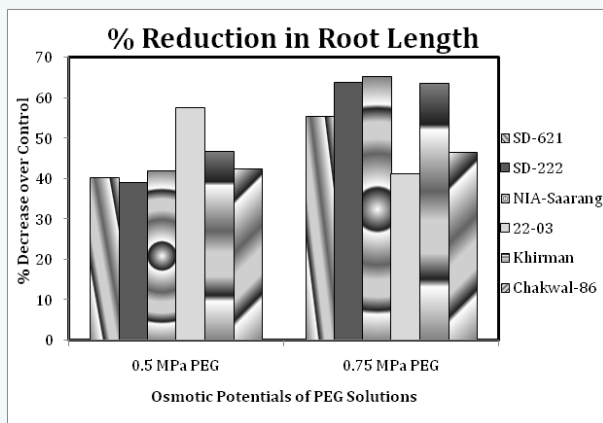


Figure 2: Percent Reduction in root length of wheat genotypes at -0.5 and -0.75 MPa PEG stress.

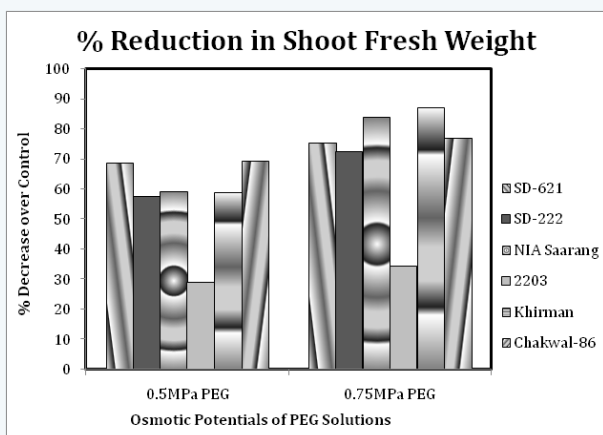


Figure 3: Percent Reduction in shoot fresh weight of wheat genotypes at -0.5 and -0.75 MPa PEG stress.

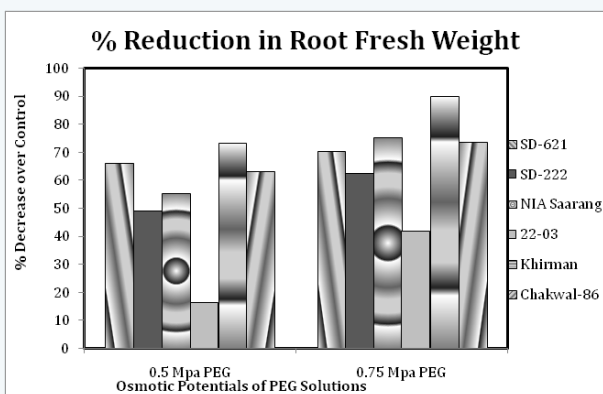


Figure 4: Percent Reduction in root fresh weight of wheat genotypes at -0.5 and -0.75 MPa PEG stress.

Chlorophyll contents of all wheat genotypes were affected by drought. Results of total chlorophyll contents expressed the presence of more chl. b as compare to chl. a in all treatments (Table 1). Highest chlorophyll contents were observed in SD-621

and NIA Saarang which were reduced slightly in comparison with chlorophyll contents of other genotypes with alleviated levels of PEG concentrations (Table 2). Low chlorophyll contents were present in wheat genotype 22-03.

**Table 1:** Growth responses of wheat genotypes under control and PEG treatments. Mean followed by same letters are non-significantly different at a level of  $P < 0.05$

Treatments	Varieties	% G	GI	Shoot Length (cm)	Root Length (cm)	Shoot Fresh wt. per plant (g)	Shoot dry wt. (g)	Root Fresh wt. (g)	Root dry wt. (g)
T <sub>1</sub> (Control)	SD-621	100a	7.50a	17.07ab	17.07b	0.118ab	0.015ab	0.071a	0.0073ab
	SD-222	100a	7.50a	16.83ab	19.84a	0.120a	0.014bc	0.061bc	0.0057bc
	NIA-Saarang	100a	7.50a	17.53a	17.59b	0.105bc	0.014bc	0.065ab	0.0063abc
	22-03	100a	7.50a	15.68b	18.17b	0.111abc	0.011cdef	0.043d	0.007ab
	Khirman	100a	7.50a	16.19ab	20.44a	0.109abc	0.012bcd	0.071a	0.0077ab
	Chakwal-86	100a	7.42a	16.50ab	17.97b	0.104c	0.012bcd	0.057c	0.0053bc
T <sub>2</sub> (-0.5MPa)	V <sub>1</sub> SD-621	80.0abc	6.04ab	13.38c	10.17de	0.037fg	0.018a	0.024fgh	0.0067abc
	V <sub>2</sub> SD-222	98.89 a	7.30a	13.33c	12.12c	0.051e	0.009efgh	0.031ef	0.0063abc
	NIA-Saarang	54.45bcd	5.73ab	12.67cd	10.18de	0.043ef	0.009fgh	0.029efg	0.005bc
	22-03	91.1ab	6.42ab	10.97e	11.53cd	0.079d	0.009efgh	0.036de	0.0064abc
	Khirman	73.33abc	5.50ab	10.77e	10.85cde	0.045ef	0.0098defg	0.019hij	0.0057bc
	Chakwal-86	86.67ab	6.42ab	10.47ef	10.32de	0.032ft	0.008ghi	0.021hij	0.0073ab
T <sub>3</sub> (-0.75 MPa)	V <sub>1</sub> SD-621	96.67 a	7.33a	11.42de	7.60f	0.029fg	0.005ij	0.021ghij	0.0057bc
	V <sub>2</sub> SD-222	96.7a	7.08a	7.75g	7.20fg	0.033fg	0.006hij	0.023ghi	0.0077ab
	NIA-Saarang	78.9abc	5.73ab	8.12g	6.08g	0.017hi	0.006hij	0.016ij	0.0037c
	22-03	68.87abc	5.75ab	11.27de	10.68cde	0.073d	0.014bc	0.025fgh	0.006bc
	Khirman	31.12 d	5.7ab	3.13h	7.43fg	0.014i	0.004j	0.007k	0.0047bc
	Chakwal-86	47.80cd	3.67b	9.07fg	9.61e	0.024ghi	0.012cde	0.015j	0.0093a
	LSD	37.438	3.0546	1.609	1.5114	0.0141	3.179	7.644	0.0027
	S.E	18.422	1.5031	0.792	0.7437	0.0385	1.564	3.762	2.679

NIA Saarang had highest shoot  $K^+/Ca^{2+}$  ratio (0.61) equalent to check variety Khirman (0.62) under normal conditions followed by SD-621(0.57) and SD-222 (0.49). Under drought, shoot  $K^+/Ca^{2+}$  ratio boost up in genotype 22-03 from 0.47 (control) up to 0.72 under -0.75 PEG stress. Similar ascending trend was observed in genotypes SD-621 and SD-222. Root  $K^+/Ca^{2+}$  ratio was not significantly affected by osmotic stress. Maximum  $K^+/Ca^{2+}$  ratio was marked in roots of genotype SD-222 (1.15) at -0.5MPa water deficit conditions (Table 2).

### Discussion

The present study showed that osmotic stress induced by PEG-6000 had inhibitory effects on plant seedling's growth and physiology. All wheat varieties showed diversity in their ability to tolerate chemical dehydration induced by PEG during the seedling's growth and development. 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 varieties [12]. Limited water conditions inhibited plant growth resulting in decline in biomass. The decrease in the 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 cell division. All the under tested varieties fall in the category of drought sensitive genotypes as their % reduction is greater than 30%. Khakwani et al. [7] also documented the deduction in shoot and root length and fresh seedling weight while studying early growth responses in wheat varieties by inducing osmotic stress using 15% and 25% PEG-6000 solutions.

**Table 2:** Total Chlorophyll and ionic contents of wheat genotypes (K<sup>+</sup>, Ca<sup>2+</sup> K<sup>+</sup>/Ca<sup>2+</sup> ratio).

Treatments	Varieties	Total chlorophyll contents (mgg <sup>-1</sup> )		% K <sup>+</sup> in shoot	% Ca <sup>2+</sup> in Shoot	Shoot k <sup>+</sup> / Ca <sup>2+</sup>	% K <sup>+</sup> in Root	% Ca <sup>2+</sup> in Root	Root K <sup>+</sup> / Ca <sup>2+</sup> ratio
		Chl. A	Chl. b						
T <sub>1</sub> (Control)	SD-621	0.178ab	0.300a	0.78b	1.36fg	0.57abcde	0.150abcde	0.185b	0.811cdef
	SD-222	0.124cd	0.193bc	1.01b	2.06abcd	0.49de	0.157abcd	0.142b	1.109abc
	NIA-Saarang	0.194a	0.301a	1.18b	1.94abcde	0.61abcd	0.135bcde	0.125b	1.081abcd
	22-03	0.019f	0.030d	1.04b	2.21ab	0.47de	0.103e	0.112b	0.932abcdef
	Khirman	0.016f	0.025d	1.03b	1.65cdefg	0.62abcd	0.113de	0.108b	1.051abcde
	Chakwal-86	0.011f	0.016d	0.95b	1.34g	0.72ab	0.140bcde	0.115b	1.231a
T <sub>2</sub> (-0.5MPa)	SD-621	0.136bcd	0.220bc	1.38b	2.14abc	0.64abcd	0.153abcde	0.202b	0.758cdef
	SD-222	0.156abc	0.252ab	1.09b	1.88bcdef	0.58abcde	0.175ab	0.153b	1.152ab
	NIA-Saarang	0.089de	0.149c	0.97b	1.87bcdefg	0.52cde	0.126cde	0.143b	0.878bcdef
	22-03	0.021f	0.033d	1.28b	1.97abcde	0.65abcd	0.132bcde	0.137b	0.961abcdef
	Khirman	0.014f	0.022d	1.01b	1.83bcdefg	0.55bcde	0.160abcd	0.160b	1.011abcde
	Chakwal-86	0.018f	0.025d	4.11a	1.50efg	0.70abc	0.137bcde	0.183b	0.736ef
T <sub>3</sub> (-0.75MPa)	SD-621	0.122cd	0.204bc	1.12b	1.82bcdefg	0.56abcde	0.178ab	0.220b	0.811cdef
	SD-222	0.045ef	0.068d	1.51b	2.45a	0.63abcd	0.175ab	0.223b	0.784cdef
	NIA-Saarang	0.115cd	0.182bc	1.03b	1.67cdefg	0.61abcd	0.148abcd	0.198b	0.761de
	22-03	0.017f	0.025d	1.12b	1.55defg	0.722ab	0.115de	0.130b	0.904bcdef
	Khirman	0.009f	0.016d	0.78b	2.06abcd	0.40e	0.195a	0.184b	1.090abc
	Chakwal-86	0.021f	0.029d	1.05b	1.38fg	0.7554a	0.167abc	0.780a	0.662f
	LSD	0.0470	0.0748	2.2066	0.5343	0.1993	0.0491	0.4174	0.3268
	S.E	0.0231	0.0368	1.0858	0.2629	0.0980	0.0242	0.2054	0.1608

Mean followed by same letters are non-significantly different at a level of P < 0.05.

Drought stress produces changes in photosynthetic constituents [13]. Reduction of chlorophyll was the result of disappearance of thylakoid structures and chloroplast disintegration [14]. SD-621 and NIA-Saarang exhibited better chlorophyll contents under moisture stress which showed their tolerance against drought. These results are supported by Khayatnezhad et al. [15] according to which chlorophyll contents of tolerant cultivars increased under drought stress.

The under tested wheat genotypes also varied in response to solute accumulation and mineral uptake in water stress. Wheat genotype 22-03 better maintained its turgor by accumulation of higher inorganic ions as compare to other genotypes under scarce water conditions. All other genotypes also had better K<sup>+</sup> and Ca<sup>2+</sup> contents in their root and shoot under stress condition.

**Conclusion**

All the tested wheat genotypes exhibited tolerance against osmotic stress under laboratory conditions. Hence, it is recommended that these genotypes which performed better in water stress condition could increase production of arid lands.

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