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STUDY OF THE EFFECT OF PEG-6000 IMPOSED DROUGHT STRESS ON WHEAT *(Triticum aestivum L.)* CULTIVARS USING RELATIVE WATER CONTENT (RWC) AND PROLINE CONTENT ANALYSIS

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Drought drastically affects plant growth and development, and thus reduces crop productivity. Drought stress tolerance in wheat is a prime factor for stabilization of crop performance in the drought-prone environments. It is not a simple response but mostly conditioned by many components' responses, which interacts and may differ for crops, concerning types, intensity and duration of water deficiency. Moreover, most agronomical characters inherit differently in normal and stress conditions and are known to be affected by environmental factors. Therefore, selection based on the phenotype is difficult for such traits. Wheat (*Triticum Aestivum L.*) occupies 220 million hectares i.e., 17% of the total cultivated land in the world and supports nearly 35% of the world's population. The main objective of this study was to observe the response of 40 wheat cultivars to drought tolerance by evaluation through polyethylene glycol (PEG) mediated moisture stress under laboratory conditions. Seedling traits studied in two different concentrations of PEG (20% & 30%). The result showed that with increasing PEG concentration the seedling traits decreased and at 30% of PEG, no germination observed. Physiological parameter such as relative water content (RWC) was very responsive to drought stress and has been shown associated well with drought tolerance. Under osmotic stress, maximum relative water contents observed in *Inqilab-91*, *Chakwal-50* and *LLR-17*. Furthermore, under stress condition amount of proline content increased significantly in all wheat cultivars in comparison to control. Based on root/shoot/coleoptile length, dry root/shoot weight and proline contents *Chakwal-97*, *Inqilab-91*, *Pak-81*, *Faisalabad-08* and *Fareed-06* found drought-tolerant cultivars.

Keywords: Drought tolerance, Wheat cultivars, Polyethylene glycol (PEG), Relative Water Content (RWC), Proline content.

INTRODUCTION

Wheat (*Triticum aestivum L.*) is the world's most widely acclimatized and consumed crop. It is also a vital and major cereal crop of Pakistan grown under irrigated and rainfed environments (Nasim *et al.,* 2017). It is expected that the world would require one billion metric tons of wheat by the year 2020. Wheat production and productivity are challenged by periodic drought and heat stresses related to climate change (Alexandratos and Bruinsma, 2012; Knox *et al.,* 2012; Daryanto *et al.,* 2016). Therefore, to meet this challenge of increasing food demand of growing populations more yield would be required through the integration of plant breeding, various accommodating disciplines and sustainable farming systems (Mujeeb-Kazi and Rajaram, 2002; Ray *et al.,* 2013).

Wheat being the ancient crop of Asia is the most leading crop of many countries and occupies a central position in agriculture and economy. It occupies 220 million hectares i.e., 17% of the total cultivated land in the world and supports nearly 35% of the world's population. One of the biggest adaptability of wheat is that it can grow in a variety of environments, ranging from fully irrigated to high rainfall and drought regions, but it also faces a wide range of biotic and abiotic stresses. Therefore, wheat crop needs more focus on its improvement in each area for getting more produce and to fulfil the consumers' demand (Dreisigacker *et al.,* 2004).

Drought drastically affects plant growth and development and thus reduces crop productivity. The effect of drought in plants response by stomata closure and reduction of water content thus leading to turgor loss, ultimately the plant dies, due to disturbance in metabolism (Nowsherwan *et al.,* 2017). Several researchers had documented the reduction in root and shoot length in many crops under extreme water shortage (Mujtaba *et al.,* 2016). The effects of drought on the yield of crops depend on the severity and the stage of plant growth during which it occurs. Seed germination is the first stage of growth that is very much sensitive to water deficit. Therefore, seed germination, vigor and coleoptile length are the basics for the success of stand establishment of crop plants. Under semi-arid region, low moisture is often a limiting factor during germination. The rate and degree of the seedling establishment are extremely important factors to determine both yield and time of maturity (Rauf *et al.,* 2008). In the last few decades, great efforts have been made by breeders to improve plant tolerance to drought stress (Galovic *et al.,* 2005). These efforts have been focused mostly on exploiting high yield potential and cultivar selection for morphological, physiological and agronomic traits indicative of drought tolerance under field conditions (Dhanda *et al.,* 2004).

Drought stress tolerance in wheat is a prime factor for stabilization of crop performance in the drought-prone environments. Soil moisture stress results mainly due to occasional rains and non-accessibility of irrigation amenities in rainfed areas. This is the main reason of lesser yields of the wheat crop when compared to yields of irrigated areas (Tokatlidis, 2014). Therefore, drought is one of the main environmental constraint in agriculture, which occurs in many parts of the world every year and often have devastating effects on crop productivity. Therefore, improved tolerance to drought has been a goal in crop improvement programs since the dawn of agriculture (Ludlow and Muchow, 1990). Drought tolerance is not a simple response but mostly conditioned by many of the components' responses. Which interacts and may differ for different crops, with types, intensity and duration of water deficit. Moreover, most agronomical characters inherit differently in normal and stress conditions and are known to be affected by environmental factors (Hittalmani *et al.,* 2003).

Several physiological traits that are associated with drought tolerance have been identified in wheat and thus different varieties have been identified as drought-tolerant based on yield under drought condition (Ahmed *et al.,* 2000). Conventional breeding approaches are mostly used for the characterization of breeding materials under irrigated and drought field conditions. Along with these in *vivo* studies, several in *vitro* studies have also been done using drought inducing chemical polyethylene glycol (PEG) for identifying the traits contributing to drought tolerance and thus different varieties have been identified showing tolerance to drought (Abdel and Naggar, 2007).

Different chemicals can be used for inducing in *vitro* drought stress. Polyethylene glycol (PEG) molecules are inert, non-ionic, and induce uniform drought stress without entering the plant cells. It is usually produced in a range of molecular weight (4000 – 8000). High molecular weight PEG 6000 is mostly used for desiccation (Premachandra and Shimada, 1987). At a given concentration of PEG 6000 osmotic potential linearly increases with temperature. PEG 6000 performs and works well with plants than the PEG of low molecular weight (Michel and Kaufmann, 1973). Many early drought screening studies had also involved PEG-6000 solutions for induction of drought under controlled conditions (Nawaz *et al.,* 2013: Jatoi *et al.,* 2014). Polyethylene glycol (PEG) acts as osmotic to decrease water potential of water culture medium, thus creating water stress on plant tissues by the outward flow of water from plant tissues to a concentrated solution of Polyethylene glycol (PEG) (Meneses *et al.,* 2011). Therefore, this study was carried out to identify new sources of drought tolerance and most diverse cultivars for utilization in breeding programs that would support to develop drought-tolerant varieties. The specific objective of this research work was to study and identify the drought-tolerant wheat cultivars based on seedling traits.

MATERIALS AND METHODS

***Plant Material*:** Forty wheat cultivars were collected from different R&D institutes working on wheat. These cultivars evaluated in *in vitro* for drought tolerance.

In Vitro Studies: The moisture stress created to various polyethylene glycol (PEG) levels. The applied treatments were

- Control (0% PEG)
- Treatment 1 (20% PEG)
- Treatment 2 (30% PEG)

The seed of all accession was initially treated with 1.5% sodium hyper chlorite for 15 minutes and then residual chlorine was eliminated by a thorough washing of seed with distilled water. Four seeds of each accession placed on the moist filter papers in a petri dish. 2ml of distilled water added in each petri dish after every one day under control treatment. At the same time, 2ml of PEG solution added in each petri dish under osmotic stress conditions. All the Petri dishes placed in a growth chamber for 10 days at an average

temperature of 22°C and 50% relative humidity. The data of root length, shoot length, coleoptile length, fresh and dry root and shoot weight, and seed vigor index of each accession recorded.

Relative Water Content (RWC): Leaf samples placed in vials and weighed to determine leaf sample weight (W) and the samples hydrated to full turgidity for 4h under normal room light and temperature. Then samples rehydrated by floating on deionized water in a close petri dish. After 4h, the samples were taken out from the water and weighed instantly to obtain fully turgid weight (TW). After weighing, samples oven-dried at 80°C for 24h and then weighed again (after being cooled in an incubator) to determine the dry weight (DW). The relative water content of leaf samples calculated by using formula (1) as under:

$$RWC\ (\%age) = [(W - DW) / (TW-DW)] \times 100 \quad \ldots \quad (1)$$

Where W represents sample fresh weight, TW represents sample turgid weight and DW sample dry weight (Weatherley and Slatyer, 1957).

Proline Content: Proline content determined by the method: 5ml of 3% sulfosalicylic acid added in test tubes and then fresh samples of weighed leaves added into these (Bates *et al.*, 1973). Thereafter, ground these samples and left for a while to set. Then 2ml of supernatant taken from it in separate test tubes, and 2ml glacial acetic acid and ninhydrin reagent added. Samples boiled for 1h in a water bath at 100°C and then 4ml toluene added in it after cooling the samples. After shaking, the upper layers of toluene transferred into another set of test tubes. Then absorbance observed at 520nm wavelength on UV Spectrophotometer Biochem. Proline contents calculated by (2):

$$\text{Proline } (\mu g/g) = (\text{Absorbance of Sample} * \text{K Value} * \text{Dilution Factor}) / [\text{Weight of Sample (g)}] \quad \ldots \quad (2)$$

Where K represents concentration/absorbance.

Statistical Analysis: For statistical analysis of all the data recorded in *in vitro* condition procedures followed which were outlined by Steel and Torrie (1986).

RESULTS AND DISCUSSION

Forty wheat cultivars evaluated in *in vitro* for drought tolerance (Table 1).

***In Vitro* Studies:** Under *In Vitro* conditions, data of root length, shoot length, coleoptile length, fresh root weight, dry root weight, fresh shoot weight, dry shoot weight and seedling vigor index were recorded.

***Root length (cm)*:** Analysis of variance for root length (Table 2) revealed significant differences in stress levels, Cultivars and Cultivar * stress level interaction. Two treatments of PEG 6000 (20% and 30%) were used along with control treatment. Under control conditions, root length ranged from 9.88 – 17.26cm with a mean of 13.57cm. At 20% PEG, it ranged from 4.22 – 9.18cm with a mean of 6.70cm.

Maximum root length observed in Inqilab-91 (17.26cm), Chakwal-97 (16.96cm) and Faisalabad-08 (16.45cm) under control conditions while minimum root length found in AS-02 (9.88cm), WC-16 (9.96cm) and WC-15 (10.20cm) as shown in Fig. 1.

Under 20% PEG, maximum root length observed in Chakwal-97 (9.20cm), Inqilab-91 (9.08cm) and Faisalabad-08 (9.03cm) while minimum length observed in Bahawalpur-97 (4.22cm), AS-02 (4.38cm) and WC-16 (4.49cm).

Table 1. Wheat Cultivars and Parameters.

S/No.	*Cultivars*							*S/No.*	*Parameters*
1	AS-2002	11	LLR-17	21	Miraj-2000	31	WC-11	1	Root Length (cm)
2	Bahawalpur-97	12	LLR-18	22	Pak-81	32	WC-12	2	Shoot Length (cm)
3	Chakwal-50	13	LLR-19	23	Punjnad-2001	33	WC-13	3	Coleoptile Length (cm)
4	Chakwal-97	14	LLR-20	24	SA-42	34	WC-14	4	Fresh Root Weight (g)
5	Faisalabad-2008	15	LLR-29	25	Saher-06	35	WC-15	5	Dry Root Weight (g)
6	Faisalabad-85	16	LLR-30	26	SH-2002	36	WC-16	6	Fresh Shoot Weight (g)
7	Fareed-06	17	LLR-31	27	Shafaq-06	37	WC-17	7	Dry Shoot Weight (g)
8	Inqilab-2000	18	LLR-32	28	Shahkar-95	38	WC-18	8	Seedling Vigor Index
9	Inqilab-91	19	LLR-34	29	Ufaq-2002	39	WC-19	9	Relative Water Content (%age)
10	LLR-16	20	LLR-35	30	WC-10	40	WC-20	10	Proline Content (µg)

Table 2. Analysis of Variance for Root Length under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	2035.080	2035.080	11800.645	0.000
Cultivar	39	720.068	18.463	107.061	0.000
Level*C'type	39	79.186	2.030	11.774	0.000
Error	160	27.593	0.172		
Total	239	2861.927		**CV**	4.19%

Table 3. Analysis of Variance for Shoot Length under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	2206.629	2206.629	18321.396	0.000
Cultivar	39	214.694	5.505	45.707	0.000
Level*C'type	39	50.667	1.299	10.787	0.000
Error	160	19.270	0.120		
Total	239	2491.261		**CV**	5.60%

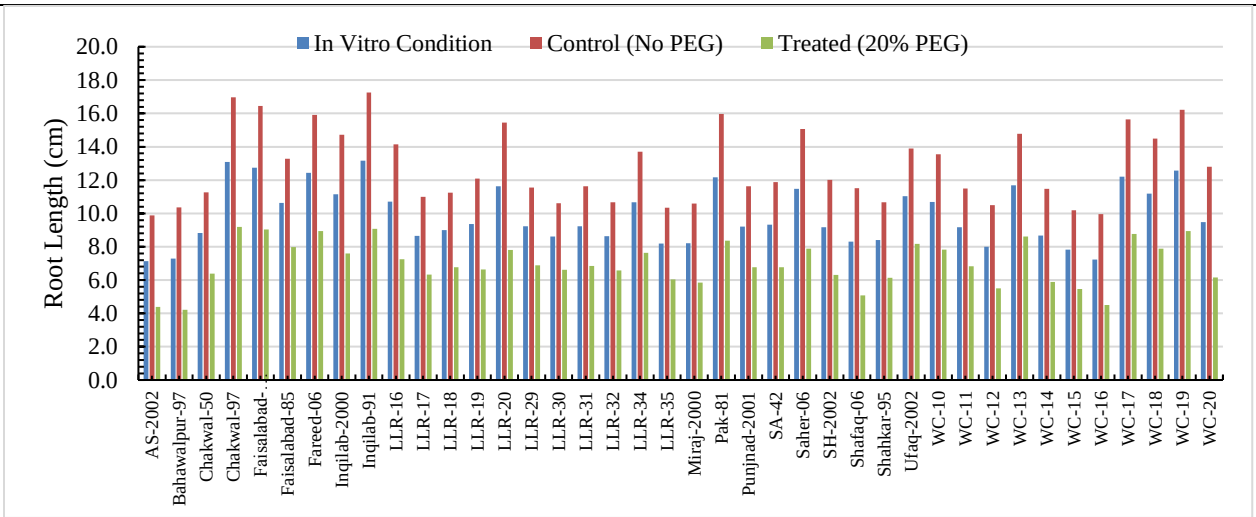

Figure 1. Root Length under *In Vitro*, Control and PEG Treated Conditions.

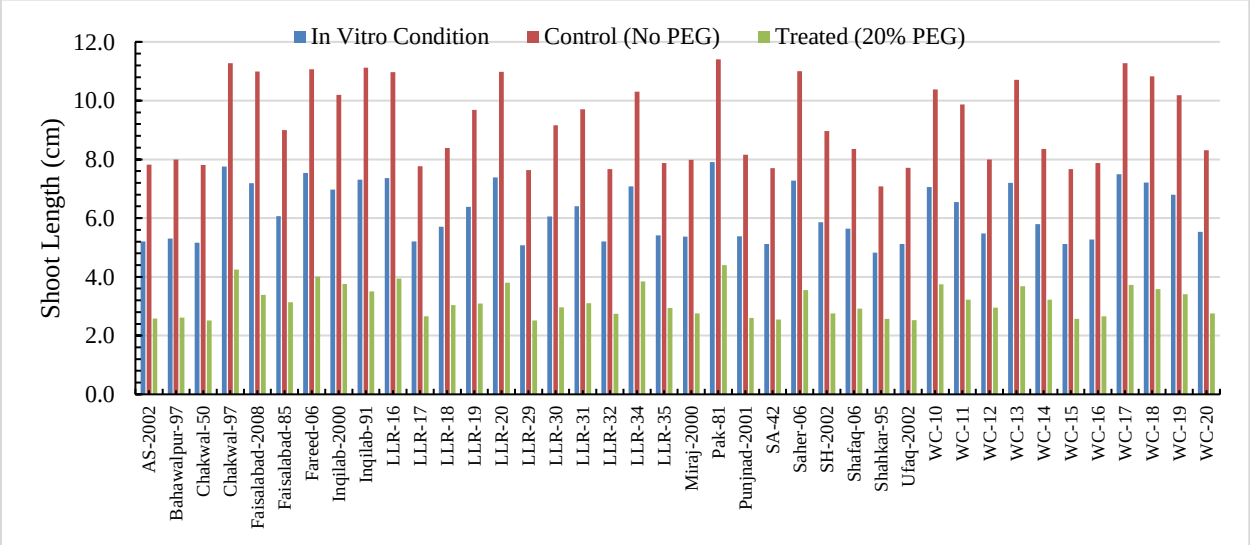

Figure 2. Shoot Length under *In Vitro*, Control and PEG Treated Conditions.

At 30% PEG, no growth observed in all cultivars. In many studies, it was observed that root length decreases with increase in osmotic stress in wheat (Kamran *et al.*, 2009) and increase in the PEG concentration reduced in the root length (Yagmur and Kaydan, 2008).

Shoot Length (cm): It observed that shoot length decreased with the increasing concentration of PEG. Significant differences observed for shoot length among stress level, Cultivar and stress level * Cultivar interaction (Table 3). Minimum shoot length observed in LLR-29 (2.52cm), Chakwal-50 (2.52cm) and Ufaq-02 (2.53cm) while maximum shoot length observed in Pak-81 (4.40cm), Chakwal-97 (4.25cm) and Fareed-06 (4.01cm) at 20% PEG. Under control conditions maximum shoot length observed in Pak-81 (11.41cm), Chakwal-97 (11.27cm), WC-17 (11.27cm) and

Table 4. Analysis of Variance for Coleoptile Length under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	180.579	180.579	694534.000	0.000
Cultivar	39	23.211	0.595	2289.080	0.000
Level*C'type	39	16.382	0.420	1615.580	0.000
Error	160	0.042	0.000		
Total	239	220.214		**CV**	0.99%

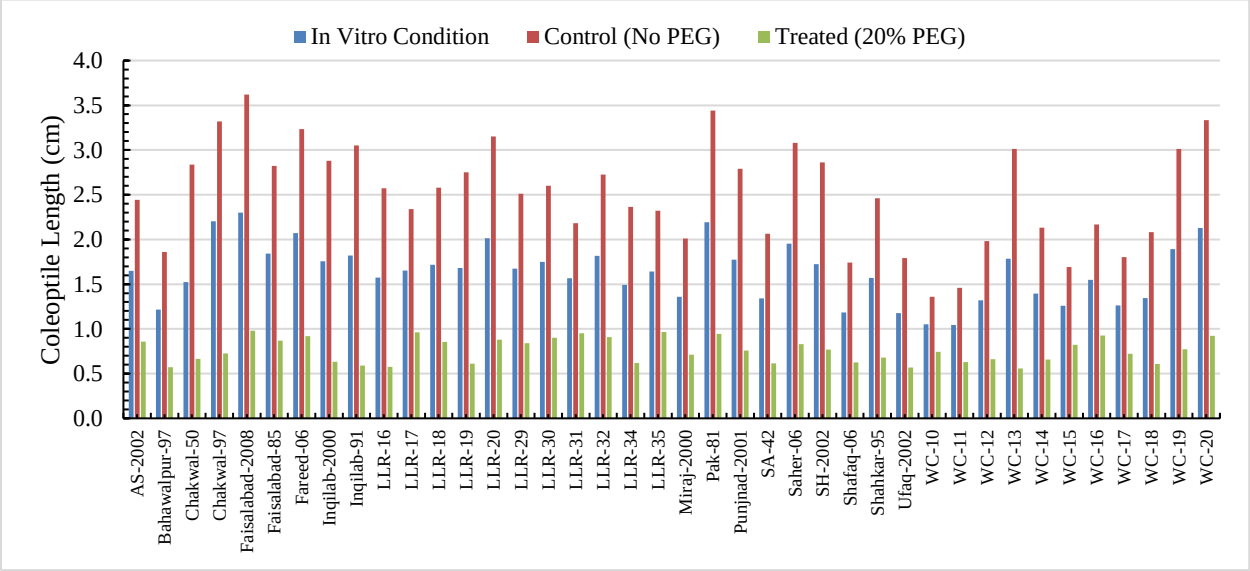

Figure 3. Coleoptile Length under *In Vitro*, Control and PEG Treated Conditions

Inqilab-91 (11.12cm) whereas Shahkar-95 (7.08cm), LLR-29 (7.64cm) and LLR-32 (7.67cm) showed minimum shoot length (Fig. 2).

Under control conditions, shoot length ranged from 7.08 – 11.41cm with a mean of 9.25cm and in 20% PEG, it ranged from 2.52 – 4.40cm with a mean of 6.92cm. Nagarajan and Rane (2000) recorded decreased shoot length and root and shoot weight in response to water stress.

Coleoptile Length (cm): In the present study significant differences observed in coleoptile length for stress levels, Cultivars and Cultivars * stress level interaction (Table 4). Minimum coleoptile length under 20% PEG found in WC-13 (0.557cm), Ufaq-2002 (0.567cm) and Bahawalpur-97 (0.570) whereas maximum coleoptile length detected in Faisalabad-2008 (0.980cm), LLR-35 (0.967) and LLR-17 (0.963cm).

Under control conditions, coleoptile length ranged from 1.36 – 3.62cm with mean of 2.49cm and in 20% PEG, it ranged from 0.58 – 0.98cm with mean of 0.77cm (Fig. 3).

Maximum coleoptile length observed in Faisalabad-2008 (3.620cm), Pak-81 (3.440cm) and WC-20 (3.333cm) under control conditions while minimum coleoptile length found in WC-10 (1.360cm), WC-11 (1.460cm) and WC-15 (1.690cm). It revealed that on the application of osmotic stress the coleoptile length reduced (Bayoumi *et al.*, 2008). It also exposed from studies that a different osmotic pressure for different wheat varieties, gave significant differences in coleoptile length (Shahryari *et al.*, 2008), similarly, the cultivars in the present study differed significantly from each other in coleoptile length.

Fresh Root Weight (g): The statistical analysis for fresh root weight showed significant differences for stress levels, Cultivars and Cultivars * stress level interaction (Table 5). In certain replications, fresh root weight means value under control condition ranged from 0.132 – 0.267g with an average of 0.198g and at 20% PEG fresh root weight ranged from 0.086 – 0.169g with an average of 0.126g.

Under control condition, maximum fresh root weight recorded in Inqilab-91 (0.267g) followed by Faisalabad-2008 (0.265g) and Pak-81 (0.245g). Minimum fresh root noted in SA-42 (0.130g), Miraj-2000 (0.133g) and WC-12 (0.136g) as shown in Fig. 4.

At 20% of PEG, minimum fresh root weight observed in Miraj-2000 (0.086g), WC-15 (0.087g) and LLR-17 (0.089g) whereas maximum fresh root weight observed in Faisalabad-2008 (0.169g), Fareed-06 (0.167g) and Chakwal-97 (0.165g). Fresh root weight decreased with the increasing level of polyethylene glycol (PEG) and intense weight observed in control conditions. Similar results of fresh root weight were observed by Rauf *et al.* (2008), the variable response of

Table 5. Analysis of Variance for Fresh Root Weight under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	0.173	0.173	363352.000	0.000
Cultivar	39	0.184	0.005	9941.250	0.000
Level*C'type	39	0.027	0.001	1475.230	0.000
Error	160	0.000	0.000		
Total	239	0.384		**CV** 0.47%	

Table 6. Analysis of Variance for Dry Root Weight under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	0.047	0.047	73265.500	0.000
Cultivar	39	0.068	0.002	2709.510	0.000
Level*C'type	39	0.008	0.000	330.980	0.000
Error	160	0.000	0.000		
Total	239	0.123		**CV** 0.73%	

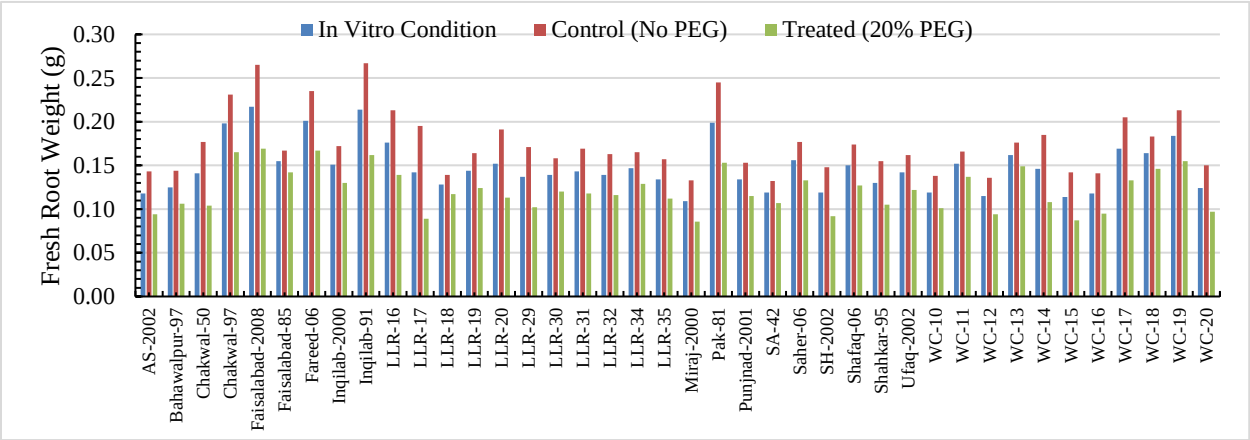

Figure 4. Fresh Root Weight under *In Vitro*, Control and PEG Treated Conditions.

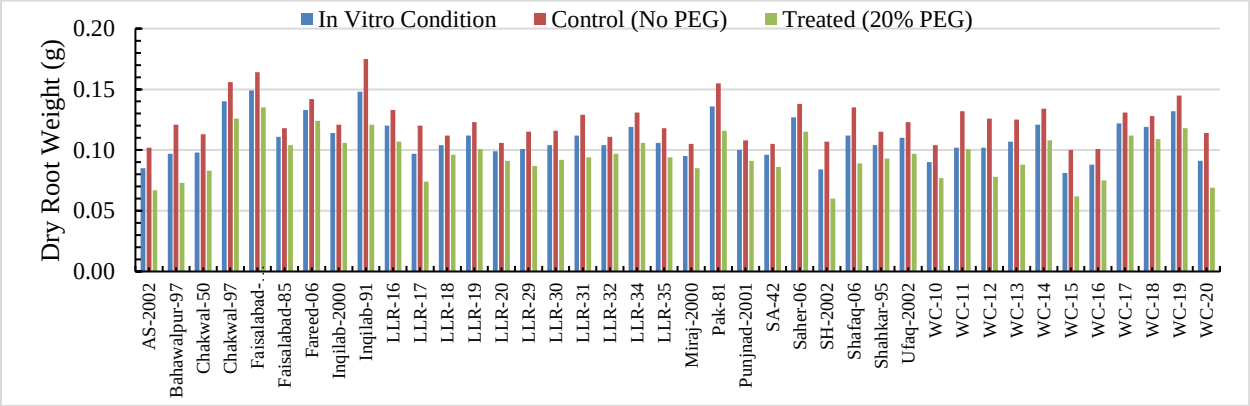

Figure 5. Dry Root Weight under *In Vitro*, Control and PEG Treated.

cultivars towards fresh root weight which coordinated with our results.

Dry Root Weight (g): The analysis of variance showed that differences among stress level as well as cultivars regarded dry root weight were highly significant (Table 6). In case of control, maximum value of dry root weight observed in Inqilab-91 (0.175g), Faisalabad-2008 (0.164g) and Pak-81 (0.155g). While minimum value of dry root weight under control condition recorded in WC-11 (0.103g), AS-2002 (0.103g), WC-10 (0.104g) and Miraj-2000 (0.105g).

At 20% PEG, maximum value of dry root weight observed in Faisalabad-2008 (0.135g), Chakwal-97 (0.127g) and Fareed-

Table 7. Analysis of Variance for Fresh Shoot Weight under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	0.996	0.996	18077.388	0.000
Cultivar	39	0.267	0.007	124.277	0.000
Level*C'type	39	0.150	0.004	69.615	0.000
Error	160	0.009	0.000		
Total	239	1.422		**CV** 3.97%	

Table 8. Analysis of Variance for Dry Shoot Weight under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	0.212	0.212	53206.500	0.000
Cultivar	39	0.234	0.006	1504.320	0.000
Level*C'type	39	0.103	0.003	663.210	0.000
Error	160	0.001	0.000		
Total	239	0.549		**CV** 1.52%	

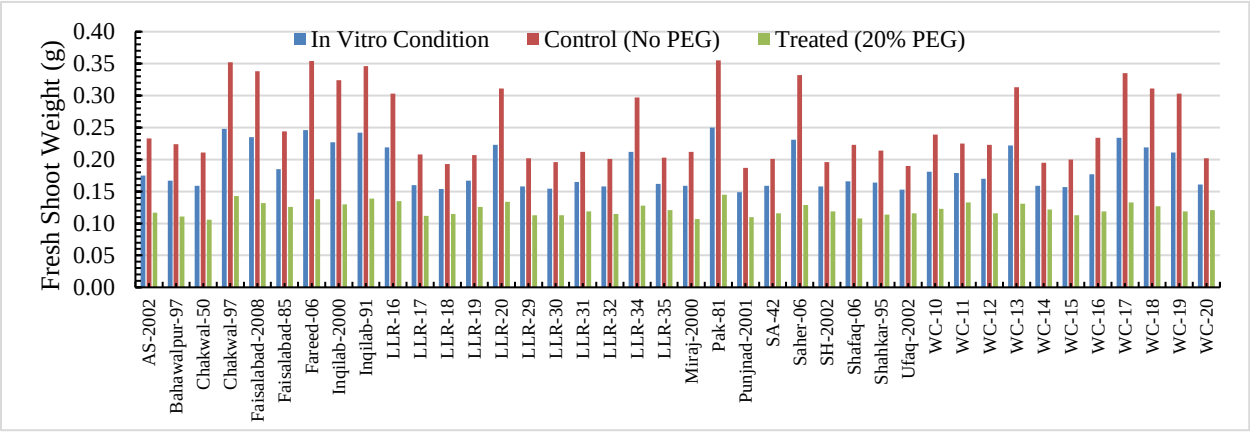

Figure 6. Fresh Shoot Weight under *In Vitro*, Control and PEG Treated Conditions.

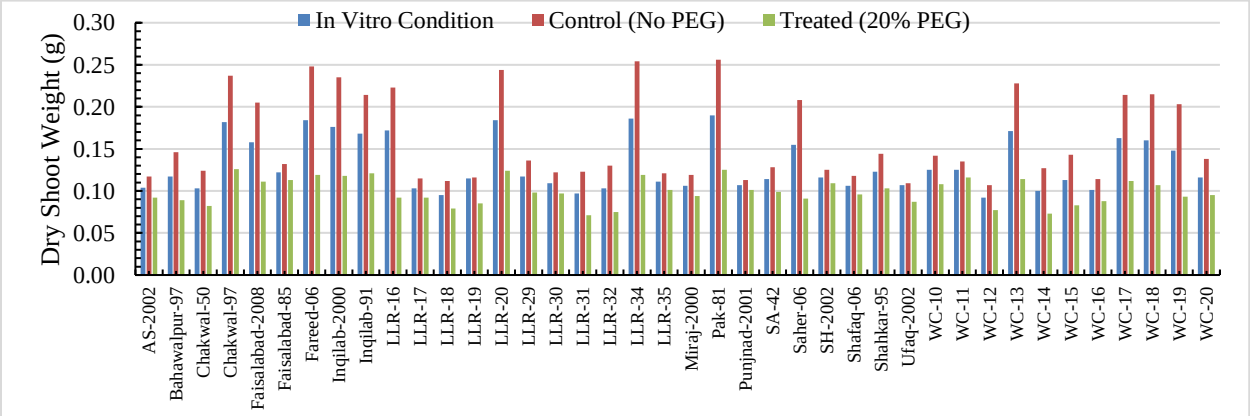

Figure 7. Dry Shoot Weight under *In Vitro*, Control and PEG Treated Conditions.

06 (0.125g). Whereas minimum dry root weight recorded in SH-2002 (0.060g), WC-15 (0.062g) and AS-2002 (0.067g). Under control conditions, dry root weight ranged from 0.103 – 0.175g with a mean of 0.139g and at 20% PEG, it ranged from 0.060 – 0.135g with a mean of 0.097g. The result indicates that by increasing osmotic stress level the dry root weight decreases as compared to normal condition (Fig. 5). Achakzai (2009) reported that concerning various levels of induced water stress, dry root weight significantly decreased which supported our findings.

Fresh Shoot Weight (g): Substantial variations noted for fresh shoot weight among osmotic stress level, Cultivars and

Table 9. Analysis of Variance for Seed Vigor Index under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	580269.923	580269.923	14011.281	0.000
Cultivar	39	123380.370	3163.599	76.389	0.000
Level*C'type	39	57710.005	1479.744	35.730	0.000
Error	160	6626.317	41.414		
Total	239	767986.614		**CV**	8.96%

Table 10. Analysis of Variance for Relative Water Contents under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	35817.312	35817.312	1789.826	0.000
Cultivar	39	4510.216	115.647	5.779	0.000
Level*C'type	39	2043.228	52.390	2.618	0.000
Error	160	3201.859	20.012		
Total	239	45572.616		**CV**	6.39%

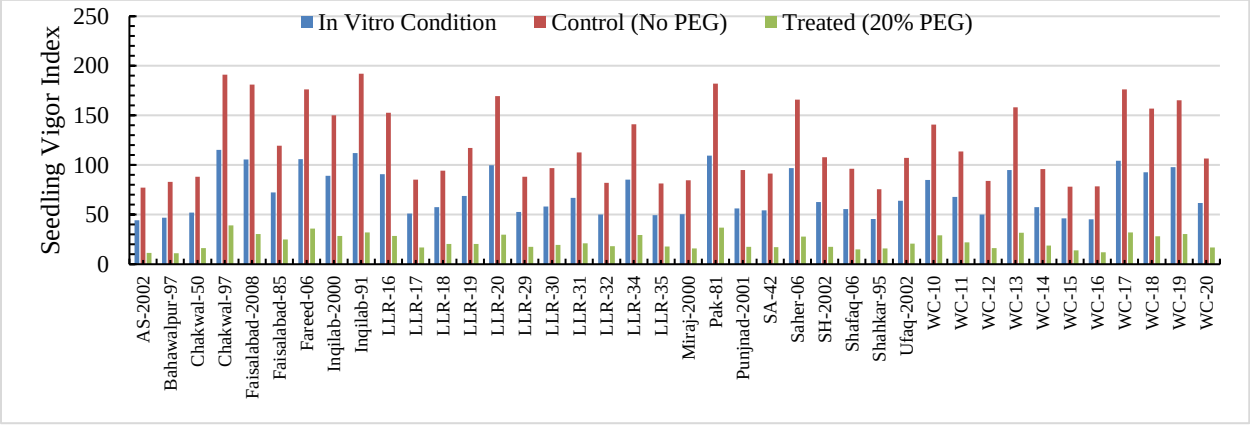

Figure 8. Seed Vigor Index under *In Vitro*, Control and PEG Treated Conditions.

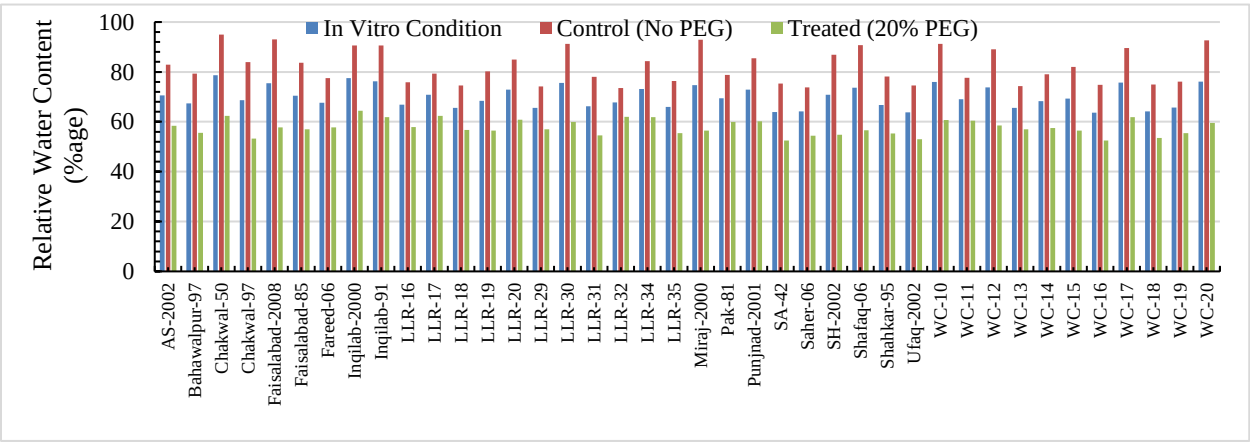

Figure 9. Relative Water Contents under *In Vitro*, Control and PEG Treated Conditions.

Cultivars * stress level interaction (Table 7). The results showed that the maximum shoot weight observed in Pak-81 (0.355g), Fareed-06 (0.251g) and Chakwal-97 (0.352g). Whereas, minimum fresh shoot weight observed in Punjnad (0.187g), Ufaq (0.190g) and LLR-18 (0.193g) under normal conditions as shown in Fig. 6.

Similarly, at 20% PEG, maximum weight showed in Pak-81 (0.145g), Chakwal-97 (0.143g) and Inqilab-91 (0.139g) while minimum weight observed in Chakwal-50 (0.106g), Mirag-2000 (0.107g) and Shafaq-06 (0.108g). Ahmad *et al.* (2007) reported reduced dry shoot weight under water stress

conditions for different cultivars which were similar to our results.

Dry Shoot Weight (g): Analysis of variance of dry shoot weight showed difference among cultivars and osmotic stress levels (Table 8). Under controlled conditions, our results showed that maximum dry shoot weight found in Pak-81 (0.256g) followed by LLR-34 (0.0254g) and Fareed-06 (0.249g).

Whereas, minimum dry shoot weight observed in WC-20 (0.107g), Ufaq-2002 (0.109g) and LLR-18 (0.112g). Whereas, other cultivars showed a similar response to fresh shoot weight. At 20% PEG application maximum dry shoot weight recorded in Chakwal-97 (0.127g), Pak-81 (0.125g) and LLR-20 (0.124g). While LLR-31 (0.071g), WC-14 (0.073g) and LLR-32 (0.075) showed minimum dry shoot weight at 20% PEG. There were significant variations for fresh shoot weight under increasing osmotic stress levels. The results showed that intense dry shoot weight found under control (distilled water) and with increasing concentrations there was a decrease in dry shoot weight (Fig. 7). Such results in wheat cultivars based on the genotypic response in respect to dry shoot weight to various water stress treatments as reported by Mahmood *et al.* (2004).

Seedling Vigor Index: The analysis of variance showed that differences among stress level as well as cultivars concerning seedling vigor index were highly significant (Table 9). Maximum seedling vigor index was found in Chakwal-97 (39.12) followed by Pak-81 (36.82) and Fareed-06 (35.83) at 20% of PEG while minimum seedling vigor index observed in Bahawalpur-97 (11.03), AS-2002 (11.38) and WC-16 (12.00) as shown in Fig. 8.

Highest seedling vigor index observed in Inqilab-91 (191.99), Chakwal-97 (191.20) and Pak-81 (182.18) whereas the lowest value of minimum seedling vigor index shown by Shahkar-95 (75.56) followed by AS-2002 (77.34) and WC-15 (78.25) under control conditions.

Similar findings revealed that the reduction of genetic variance and heritability under stress is partly a direct result of large environmental variance within the stress environment and partly a result of the suppression of genetic variability under such conditions (Ludlow and Muchow, 1990).

Relative Water Content (%age RWC): Physiological parameter such as relative water content (RWC) is very responsive to drought stress and has been associated well with drought tolerance. The analysis of variance for relative water contents showed significant differences among stress levels, Cultivars and Cultivars * stress level interaction (Table 10).

The highest value of relative water content found in Chakwal-50 (94.88), Faisalabad-2008 (92.99) and Miraj-2000 (92.90) under normal condition whereas lowest water contents observed in LLR-32 (73.51), Saher-06 (73.81) and LLR-29 (74.13) as indicated in Fig. 9.

Under osmotic stress level minimum, relative water contents observed in WC-16 (52.45), SA-42 (52.50) and Ufaq (52.95) while maximum relative water contents shown in Inqilab-91 (64.40), Chakwal-50 (62.35) and LLR-17 (62.34) under osmotic stress condition. Relative water contents differences according to genotype in wheat cultivars under water stress were also observed by Schonfeld *et al.* (1988), that suggest

Table 11. Analysis of Variance for Proline Content under Control and Drought.

Source	DF	SS	MV	FV	P
Stress level	1	36440000.000	36440000.000	9181226.000	0.000
Cultivar	39	6537416.000	167626.000	42231.700	0.000
Level*C'type	39	5934219.000	152159.000	38335.100	0.000
Error	160	635.071	3.969		
Total	239	48190000.000		**CV**	0.44%

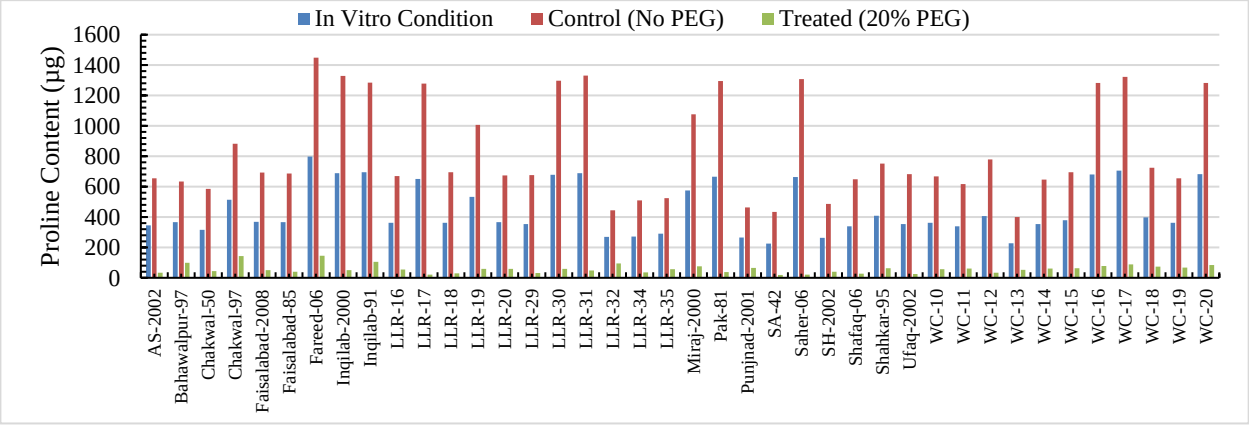

Figure 10. Proline Content under *In Vitro*, Control and PEG Treated Conditions

the use of relative water contents as a selection criterion for drought resistance in wheat (Schonfeld *et al.,* 1988). The plants having more relative water contents are more adaptive in water shortage environment (Gupta *et al.,* 2012).

Proline Content (µg): High proline accumulation observed under drought stress condition whereas under normal condition proline accumulation was low. The analysis of variance indicated significant differences for stress levels, Cultivars and Cultivars * stress level interaction under drought and normal (control) conditions (Table 11).

Under control conditions, proline content ranged from 399.90 – 1449.40µg with mean of 894.65µg and in 20% of PEG, it ranged from 17.90 – 146.10µg with mean 82.00µg. In Fareed-06 (1449.40µg), LLR-31 (1330.10µg) and Inqilab-2000 (1328.40µg) recorded high proline content while WC-13 (399.90µg), SA-42 (433.70µg) and LLR-32 (443.40µg) showed low proline content at 20% of PEG. Similarly, under control conditions SA-42 (17.90µg), Saher-06 (20.70µg) and LLR-17 (21.70µg) showed low proline content whereas Fareed-06 (146.10µg), Chakwal-97 (142.50µg) and Inqilab-91 (104.80µg) indicated high proline content (Fig. 10).

It is now well known that drought-stressed plants exhibit various physiological and biochemical changes to thrive in limited water (drought) conditions (Arora *et al.,* 2002). Under various environmental stresses including drought, increased accumulation of proline and abscisic acid (ABA) is a characteristic feature of most plants (Hsu *et al.,* 2003). PEG treatment also increased abscisic acid (ABA) content and decreased ethylene production (Hsu *et al.,* 2003). The accumulation of proline is generally correlated with stress tolerance as tolerant species accumulate more proline as compared to sensitiveness (Nayyar and Walia, 2003).

Conclusion: In this study, sufficient variations observed in the selected cultivars for drought tolerance. At 30% of PEG, no germination observed in all wheat cultivars. The results showed that with increasing PEG concentration the seedling traits decreased. Relative water content (RWC) was very responsive to drought stress and had been shown associated well with drought tolerance. Under osmotic stress, maximum relative water contents observed in *Inqilab-91*, *Chakwal-50* and *LLR-17*. Furthermore, in laboratory condition found that under stress condition amount of proline content increased significantly in all wheat cultivars in comparison to control. Based on root/shoot/coleoptile length, dry root/shoot weight and proline contents *Chakwal-97*, *Inqilab-91*, *Pak-81*, *Faisalabad-08* and *Fareed-06* found drought-tolerant cultivars.

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