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Screening of wheat *(Triticum aestivum* L.), genotypes under osmotic stress at germination and seedling stage

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Abstract
Drought stress drastically decreases the yield of various crops including wheat. Germination stage is the most critical stage that is badly affected by drought stress. To screen out the wheat genotypes for osmotic stress, stress was induce by PEG-6000 to identify drought tolerant wheat genotypes after passing through screening criteria at seedling stage in wheat by using Petri dish. Twenty seven wheat genotypes were studied for seed germination percentage, germination rate index, root/shoot fresh and dry weight and root/shoot length. Relative net decrease was (64.9%). In germination rate index (GRI), (61.52%) germination, (91.64%), shoot length (SL), (91.13%) root length, (67.06%) shoot dry weight and (87.90%) in root dry weight was recorded under drought treatment after comparison all genotypes with control condition. In present in vitro experimental studies there was no germination in osmotic induce PEG (6000) treatment for genotypes (9409) PTSS08GHB00011S-0SH-099SHB-099SHB-52Y-0Y, (9414), PTSS08GHB00011S-0SH-099SHB-099SHB-89Y-0Y, (9418) SKOKOLL/WESTONIA and (9422) PUB94.15.12/WBLL1 respectively, therefore showed 100% decrease value in germination and badly affected by osmotic stress.

Keywords: Wheat (*Triticum aestivum* L.), osmotic stress, germination, seedling stage

Introduction

Wheat (*Triticum aestivum* L.) is one of the most essential cereal crops of the globe, due to its extensive utilization and nutritive significance. Therefore it is staple food for more than one third of the world's population (Asif *et al.*, 2005) [9]. About two million peoples obtain food from wheat crop and almost 55% of the carbohydrates and 20% of the food calories consumed worldwide (Breiman and Graur, 1995) [12]. The forecasted global insist for wheat in year 2025 may increase up to 750 million tons (Mujeeb-Kazi and Rajaram, 2002) [31]. Due to unfavorable effect of abiotic stresses e.g. cold salinity drought and high temperature the production of wheat is reducing slowly and gradually. But as compare to any other type of stress drought are the most destructive stress since it be inclined to harm wheat development, growth and deliberate the optimum production (Farooq *et al.*, 2011a; Ahmadizadeh *et al.* 2013) [20, 3]. A variety of factors affects the yield of a crop like seed germination, seedling vigor, growth rate, mean emergence time and desiccation tolerance (Crosbie *et al.*, 1980; Noorka *et al.* 2007) [16, 33]. But seed germination among them is the most critical stages of plant which are poorly disturbed by low soil moisture content. In seedling stage scarcity of water is the most aggressive problem in wheat production that can inhabit seed germination. However the plants which could tolerate shortage of water emerge as secure plants (Jajarmi, 2009). The prime causes of low germination in various crop seeds is the soil moisture scarcity (Mohammad *et al.*, 2002) [30]. Therefore seed germination is the first stage of plant growth which is highly sensitive to water deficit condition (Ali *et al.* 2007). In shortage in soil moisture germination rate and percentage are reduced (Delachiave and de Pinho, 2003) [17]. In arid and semiarid climates of Pakistan, the main limiting growth factor at critical growth stages of crops was water shortage. However, establishment seedling under this stressful environment is a superior marker for determining crop growth and maturity (Rauf *et al.*, 2012) [36]. Exposures to drought stress posses' severe challenges for the continued existence of plants, because it results in impaired germination and seedling growth (Ashraf *et al.*,, 2006). Various researchers had reviewed that drought stress was the inauspicious effects on germination and seedling development in many crops such as wheat (Dhanda *et al.*, 2004; Almaghrabi, 2012) [18, 8], barley, corn and canola (Gharoobi *et al.*, 2012) [23]. Study by using poly ethylene glycol (PEG 6000) in the medium at the early stage is very significant for resistance of water shortage is most frequently practiced (Rauf *et al.*, 2006) [35]. PEG 6000 are inert, non-ionic molecule, nearly produced impermeable chains and have commonly been used to induce water shortage without causing any major physiological harm to crop plants (Carpita *et al.*, 1979) [13]. Therefore Poly ethylene glycol (PEG 6000) can be utilized to alter the osmotic potential of nutrient solution culture and can induce plant water deficit in a comparatively controlled method, proper to experimental protocols (Lagerwerff *et al.* 1961) [29]. By utilizing different concentrations of PEG 6000 have showed significant differences for different seedling traits for earlier studies focusing on identification of the drought tolerant wheat genotypes (Rauf *et al.* 2006; Singh *et al.* 2008) [35].

The seedling characters when mutual together could distinguish between drought tolerant and susceptible genotypes (Noorka and Khaliq, 2007) [33]. The purpose of our research work to study the effect of osmotic stress on wheat genotypes at seedling stages by using poly ethylene glycol stimulated drought stress. It was also preferred to explain tolerance mechanisms in wheat crop in osmotic stress environment of water stress to inhibit seed germination and to identify possible adaptive mechanisms.

Materials and methods

Plant materials

The research material comprises of 27 wheat genotypes. Among them 26 genotypes were CIMMYT international, 2013, while one are local check NR2009.

Experimental design

In vitro experiment was conducted to calculate the germination characters of selected wheat genotypes in both drought and control condition at Wheat Wide Crosses and Cytogenetic lab, National Agriculture Research Centre (NARC) Islamabad Pakistan during 20th January 2017. The experiment was designed in Randomized Complete Design (RCD) having three replications. Ten similar sized and healthy seeds of each genotype were placed in Petri dishes having 13.5cm diameter on double layer of moist germination papers (filter paper). At every Petri dish 5ml 20% PEG-6000 solution were drop on the paper to provide suitable moisture stress for drought wheat plant, whereas 5ml distal water was drop for control condition in every Petri dish to moisture the germination paper. All the Petri dishes were kept in the germinator room at room temperature. The seeds were moisturized when necessary.

Measurement and determination

After 48 hours, germination was recorded, whereas root and shoot length was measured through scale after 10 days of germination. Mean data were determined for germination percentage, germination rate index, root length/shoot length and shoot/ root dry weight. Seed germination Percentage (%)

Seed germination

After 10 days of germination seed germination percentage ratio was determined by using the behind formula:

$$\text{Seed Germination (\%)} = \frac{\text{Germinated Seeds}}{\text{No of total seeds}} \times 100$$

Root and shoot length

The root and shoot length was measured in centimeter after 10 days of sowing at the time of experiment termination by using scales.

Fresh and dry weight of shoot and root

Roots and shoots were separated from each other and weighed in grams (g) with an electronic digital balance. Roots and shoots were than dried in hot air oven at 80^{0}C for 72 hours and then dry weight was measured by using electronic digital balance.

Results and discussion

Germination rate index

The current experiment was the first time to conducted on the stress condition in KP, Pakistan, is a major limiting factor in wheat crop production in arid and semiarid regions of the world. It inhibits plant growth and development severely and ultimately causes decline in yield. The response of PEG induced drought was evident in this study where germination/seedling traits were declined drastically. In the current studied screening of 27 wheat genotypes under chemical analysis induce by PEG-6000 at early seedling stage was conducted in vitro condition. Analysis of data showed that the effect of osmotic stress induced by PEG on germination rate index is decline as compare to irrigated environment presented in (Fig 1). Under osmotic stress treatment net decrease (64.9%) was recorded in germination rate index (GRI) when compare average of all genotype with control set. In present studied attributes regarding germination rate index (GRI) there was no germination in osmotic induce PEGS (6000) treatment for genotypes (9409) PTSS08GHB00011S-0SH-099SHB-099SHB-52Y-0Y,(9414),PTSS08GHB00011S-0SH-099SHB-099SHB-89Y-0Y, (9418) SKOKOLL/ WESTONIA and (9422) PUB94.15.12/ WBLL1 respectively, therefore showed 100% decrease value in germination rate index. Similarly the germination rate index in genotypes (9412) PTSS08GHB00011S-0SH-099SHB-099SHB-66Y-0Y was high in osmotic stress environment as compare to control level. The highest 88.00% decrease value was recorded for genotype (9421) SKOLL/WBLL1, followed by genotype (9404) PTSS08GHB00011S-0SH-099SHB-099SHB-72Y-0Y, 79.17% and genotype (9408) PTSS08GHB00011S-0SH-099SHB-099SHB-32Y-0Y 76.92%, while minimum 6.67% decrease value was recorded for genotype (9427) BORLAUG100F2014 respectively.

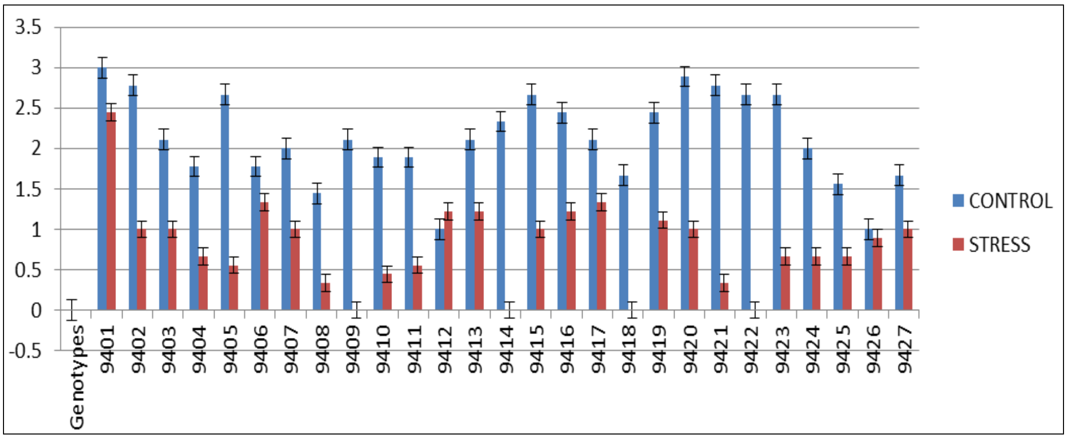

Fig 1: Effect of osmotic stress PEG-6000 on germination rate index

Seed germination percentage (%)

In the current research work, performance of twenty seven wheat genotypes under both chemical dehydration, induced by PEG (6000) and well irrigation during early seedling stage was evaluated under in-vitro conditions. Analysis of data showed that the effect of osmotic stress induced by PEG on seed germination percentage (%) was high as presented in (Fig 2). At irrigated level maximum seed germination percentage was recorded and decreased in the osmotic stress level was enlarged by using PEG-6000 in all wheat genotypes. Under the control level, maximum seed germination were recorded in local check NR2009 (9401) and SER1/BAV92//PUB94.15.1.12/WBLL1 (9420) (90% and 86.7%) whereas minimum seed germination was noticed in genotypes FRANCOLIN#1 (4926) (30%) shown in (Fig 2). Similarly under osmotic stress condition maximum seed germination percentage was recorded for genotype local check NAR2009 (73.3%), SKOKOLL/WESTONIA (40%), while minimum value was recorded for genotype PTSS08GHB00011S-0SH-099SHB-099SHB-32Y-0Y (6.7%) respectively. In these twenty seven genotypes seed germination percentage was high in genotype (4912) in osmotic stress condition as compare to control condition, while genotypes (9409) PTSS08GHB00011S-0SH-099SHB-099SHB-52Y-0Y, (9414), PTSS08GHB00011S-0SH-099SHB-099SHB-89Y-0Y, (9418) SKOKOLL/WESTONIA and (9422) PUB94.15.12/WBLL1 were badly affected by osmotic induce stress treatment and have no germination as shown in (Fig 2). Dodd and Donavon (1999) [19] reported that seed germination and seedling growth are decrease under osmotic stress conditions. Because osmotic stress induce by PEG-6000 reduces water potential gradient between seeds and their surrounding environment hence. Therefore Dodd and Donavon (1999) [19] recorded that osmotic stress can be causes reduction in seed germination. Investigation of genetic difference among the genotypes that could be significant to build up new genotypes that can be fitted in arid and semiarid areas was suggested by Alaei *et al.*, (2010) [6] and Jaijarmi (2009) [25].

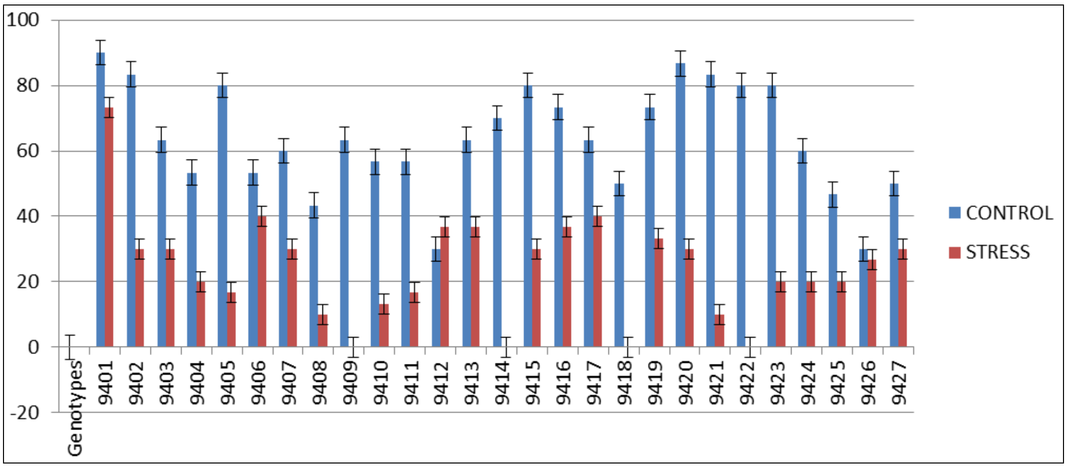

Fig 2: Effect of PEG-6000 on germination percentage of selected wheat genotype

Shoot and root length

In the current studied wheat genotypes were tested under irrigated and osmotic induce PEG-6000 treatment. Significant differences were found in shoot and root length at both treatments. In control environment the highest value in stem length was recorded for (9419) BCN/WBL1//ROLF07 (9.49cm) follow by local check (9.40cm), (9415) SKOKOLL/WESTONIA (9.22cm) and minimum value for genotype BAJ#1 (5.41cm), while osmotic stress condition the highest value for (9401) local check (3.52cm), (1.74cm) for genotype (9402) PTSS08GHB00011S-0SH-099SHB-099SHB-28Y-0Y and lowest value (0.17cm) for genotypes (9410) PTSS08GHB00011S-0SH-099SHB-099SHB-76Y-0Y.

Similarly under control environment the highest value in root length was recorded for (9401) local check (12.77cm) and (9405) PTSS08GHB00011S-0SH-099SHB-099SHB-143Y-0Y (11.55cm), while minimum (4.40cm) for genotype (9425) BAJ#1 was displayed. In osmotic induce treatment the highest value was recorded for genotype local check (3.95cm) and (9402) PTSS08GHB00011S-0SH-099SHB-099SHB-28Y-0Y (1.82cm), while minimum value (0.17cm) for genotypes (9410) PTSS08GHB00011S-0SH-099SHB-099SHB-76Y-0Y shown in (Tables 1,2,3; fig.3). Relative decrease (91.64%) in shoot length (SL) and (91.13%) in root length was recorded under drought treatment when compare all genotypes with control condition. The highest 96.92% decrease value was recorded for genotype (9421) SKOLL/WBLL1 followed by genotype (9411) PTSS08GHB00011S-0SH-099SHB-099SHB-23Y-0Y (95.53%) and genotype (9408) PTSS08GHB00011S-0SH-099SHB-099SHB-32Y-0Y (95.11%), while minimum 80.79 decrease value was recorded for genotype (9416) SKOKOLL/WESTONIA respectively. Similarly in root length the highest 94.54% decrease value was recorded for genotype (9421) SKOLL/WBLL1 followed by genotype (9405) PTSS08GHB00011S-0SH-099SHB-099SHB-143Y-0Y (94.02%), while minimum 81.35% decrease value was recorded for genotype (9412) PTSS08GHB00011S-0SH-099SHB-099SHB-66Y-0Y respectively. Therefore shoot length of all genotypes are reduced due to PEG-6000 20% treatment. Same result was also reported by Fraser *et al.*, (1990) [22] that under water stress environment root and shoot length was decreased as a result of an inhibition of cell division and elongation. Under drought stress the declining trend in shoot and root growth was also recorded by Kamran *et al.*, (2009) [27] and Chachar *et al.*, (2014a 2014b) [14]. Root length was less affected by drought stress as contrasted to other seedling traits. Alike findings were reported by Thornley (1998). Inadequate supply of water and nutrients in water deficient environment often results growth inhibition of shoot than roots. Some hormonal messages are also induced in roots when they encountered drought stress (Sharp and Davis, 1985; Misra, 1990, 1994; Noorka *et al.* 2009) [34].

These results are in corroboration with the findings of Akram *et al.*, (1998) [5] and Baalbaki *et al.*, (1999). These results are similar to the findings of Rauf *et al.*, (2006) [35] and Singh *et al.*, (1994). 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 environment. 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 (Foito *et al.* 2009) [21] 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.*, 2011) [28] 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 by using 15% and 25% PEG-6000 solution.

A great variation in wheat genotypes has already been observed by Ashraf *et al.*, (1996) in response to PEG-induced water stress. In current experiment, PEG induced water stress increased the time to germination and decreased the final germination percentage. The increase in PEG concentrations caused a decrease in the uptake of water by seeds, which caused a decline in germination percentage (Kaydan & Yagmur, 2008). The germination stress tolerance index (GSI) can be used as an effective criterion for genotype screening against various drought stress regimes (Fernandez, 1992).

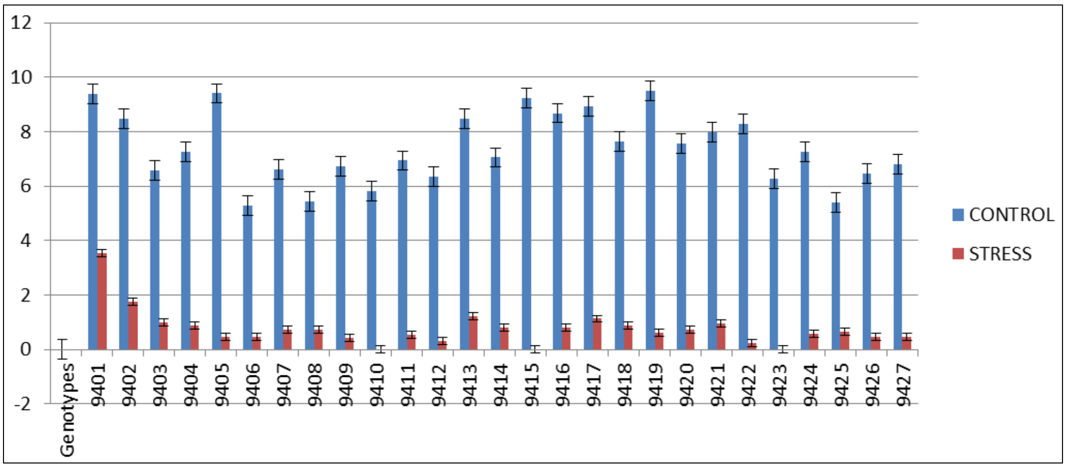

Fig 3: Effect of PEG-6000 on stem length of selected wheat genotypes

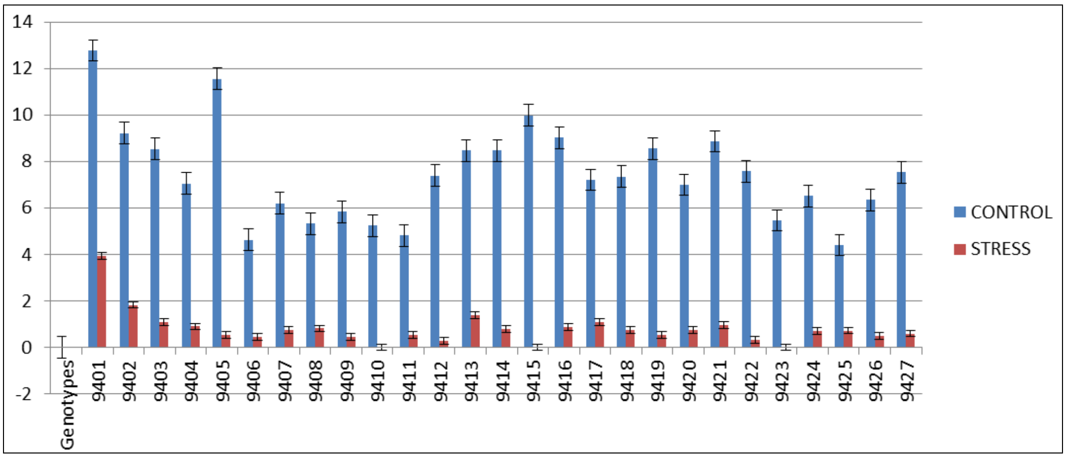

Fig 4: Effect of PEG-6000 on root length of selected wheat genotypes

Shoot and root fresh and dry weight

In the current studied attributes significant differences were recorded in both osmotic induce PEG-6000 treatment and irrigated condition regarding shoot/ root fresh and dry weight. In control level the highest value was recorded for genotype PTSS08GHB00011S-0SH-099SHB-099SHB-136Y-0Y (0.66g), PTSS08GHB00011S-0SH-099SHB-099SHB-32Y-0Y (0.54g) and (0.53g) for genotype PTSS08GHB00011S-0SH-099SHB-099SHB-89Y-0Y, while minimum value for genotypes (9426)FRANCOLIN#1 and (9412) PTSS08GHB00011S-0SH-099SHB-099SHB-66Y-0Y (0.34g) whereas in osmotic induce treatment the highest value was observed for genotypes SER1/BAV92//PUB94.15.1.12/WBLL1 (0.14g) and (0.12g) for genotypes PTSS08GHB00011S-0SH-099SHB-099SHB-32Y-0Y respectively. Analysis of data revealed that maximum reduction was recorded for genotypes (9409) PTSS08GHB00011S-0SH-099SHB-099SHB-52Y-0Y, (9410) PTSS08GHB00011S-0SH-099SHB-099SHB-76Y-0Y and (9427) BORLAUG100F2014 in shoot fresh weight (11.07%), (6.07%), (5.97%), shoot dry weight (5.03%), (3.01%), and (2.88%), while minimum reduction was recorded for genotypes (9426) Francolin#1 in shoot fresh weight (2.06%) and shoot dry weight for genotype (9404) PTSS08GHB00011S-0SH-099SHB-099SHB-72Y-0Y (1.68%) respectively Table 3; Fig 4.

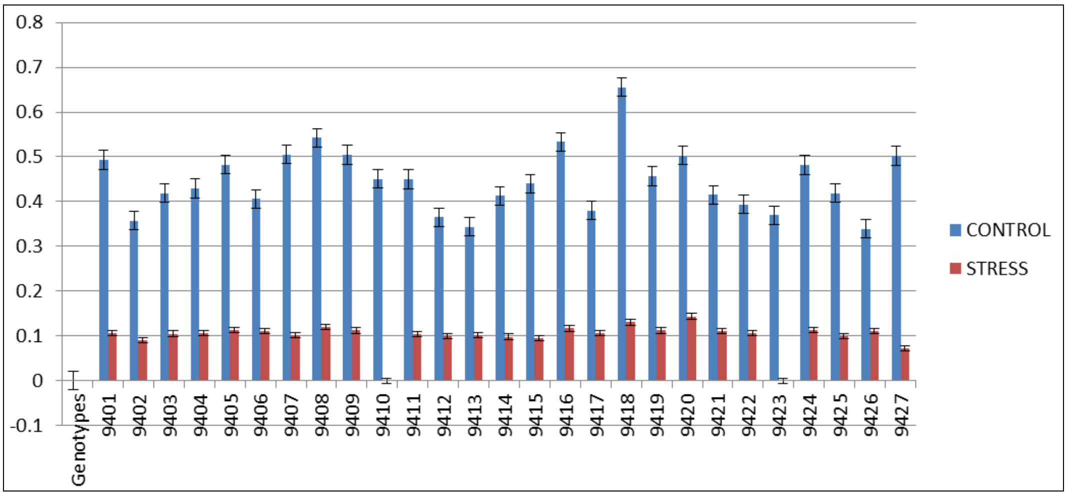

Fig 5: Effect of PEG-6000 on fresh weight of stem of selected wheat genotypes

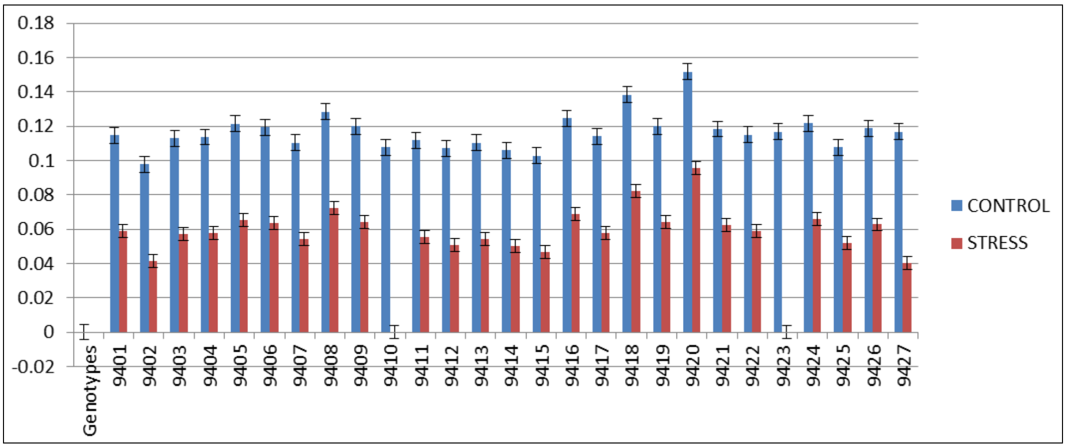

Fig 6: Effect of PEG-6000 on dry weight of stem of selected wheat genotypes

In root fresh weight under control environment the highest value was recorded for genotypes SKOKOLL/WESTONIA (0.56g), PTSS08GHB00011S-0SH-099SHB-099SHB-32Y-0Y (0.45g) and PTSS08GHB00011S-0SH-099SHB-099SHB-52Y-0Y (043g) whereas minimum decrease value was noted for genotype PTSS08GHB00011S-0SH-099SHB-099SHB-66Y-0Y (0.27g). Although in osmotic stress treatment the highest value for genotype SER1/BAV92//PUB94.15.1.12/WBLL1 (14g) and (0.11g) for genotype PTSS08GHB00011S-0SH-099SHB-099SHB-72Y-0Y, PTSS08GHB00011S-0SH-099SHB-099SHB-32Y-0Y, PTSS08GHB00011S-0SH-099SHB-099SHB-89Y-0Y and SOKOLL respectively, whereas minimum value for genotype BORLAUG100F2014 (0.07g) respectively. Similarly in root fresh weight (9.43%), (6.38%), (5.24%), root dry weight (2.86%), (1.69%) and (1.61%) decrease value was recorded for genotypes (9410) PTSS08GHB00011S-0SH-099SHB-099SHB-76Y-0Y, (9409) PTSS08GHB00011S-0SH-099SHB-099SHB-52Y-0Y and (9427) BORLAUG100F2014 respectively. There was decrease in shoot fresh, dry weight, root fresh and root dry weight in osmotic induce PEG-6000 treatment. Minimum reduction was recorded for genotypes 9412 (2.98%) in root fresh weight and root dry weight for genotype 9415 (0.93%) respectively (Table 1,2,3; Fig 5). This decrease value was high in osmotic induce PEG-6000 treatment as compare to control condition. Many scientists also noted decline in root and shoot dry weight (Kamran *et al.* 2009; Ahmad *et al.* 2013; Chachar *et al.*, 2014a, 2014b) [27, 14], who observes that drought stress had a major effect on shoot and root dry weight. Because the decrease in shoot-root dry and fresh weight was credited due to inferior number and development of smaller leaves with increased Poly ethylene glycol-6000 level in growing medium. Various scientists observed that water stress resistance is measured by tiny decline of shoot growth in drought stress condition (Ming *et al.* 2012; Mouchesh *et al.*, 2012 and Saghafikhadem 2012; Sassi *et al.* 2012). For the selection of drought resistance genotypes root morphology and biomass are very significant characters (Steven *et al.*, 2016). Decline in shoot and root dry weight was also recorded by other scientists (Kamran *et al.* 2009; Ahmad *et al.* 2013; Izabela *et al.*, 2013) [27] who noticed that shortage of water had a major effect on shoot and root dry matter production.

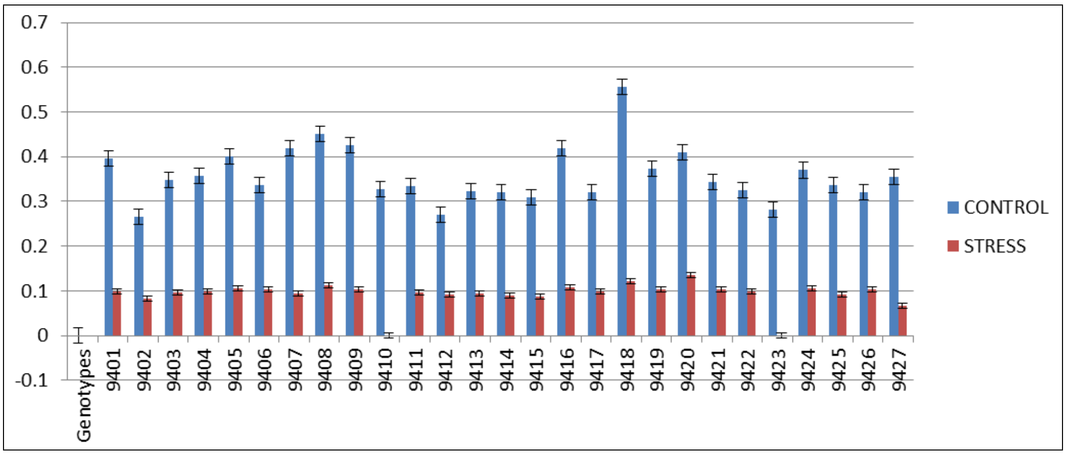

Fig 7: Effect of PEG-6000 on fresh weight of root of selected wheat genotypes

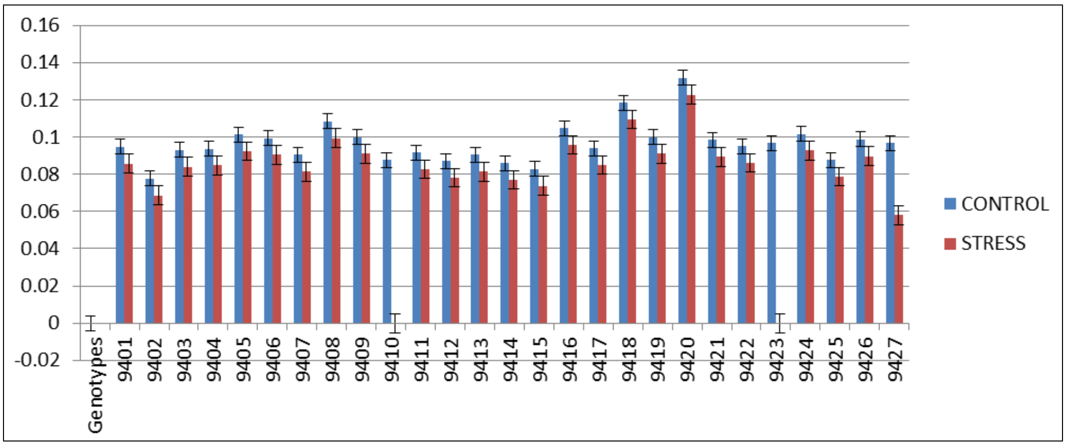

Fig 8: Effect of PEG-6000 on dry weight of root of selected wheat genotypes

Table 1: Pearson coefficient correlation (r) for invitro studied attributes

Variables	Treat	Germination%	STL	GRI	RDW	RFW	RTL	SDW
GRI	Control	1.0***						
	Stress	1.2***						
RL	Control	0.49***	0.48***					
	Stress	0.57***	0.56***					
RFW	Control	0.0426	0.0414	0.0436				
	Stress	0.0695	0.0694	0.224*				
RDW	Control	0.1746	0.1737	-0.0693	0.65***			
	Stress	0.0609	0.0607	0.2104	0.99***			
STL	Control	0.49***	0.48***	0.73***	0.0108	-0.0166		
	Stress	0.55***	0.54***	0.99***	0.247*	0.234*		
SFW	Control	0.0638	0.0625	0.0675	0.87***	0.58***	0.0029	
	Stress	0.0739	0.0738	0.231*	0.999***	0.296***	0.253*	
SDW	Control	0.1746	0.1737	-0.069	0.65***	1.0***	-0.0166	0.58***
	Stress	0.0342	0.034	0.1651	0.97***	0.98***	0.1893	0.96***

Table 2: Mean square of ANOVA analysis for the germinated wheat

	G%age	GRI	Root length	Root FW	Root DW	Stem length	tem FW	Stem DW
Genotypes	895.0***	0.995***	9.67***	0.0096***	0.0016***	4.23***	0.0114***	0.0014***
Treat	61.85***	68.76***	1772.37***	0.8352***	0.0093***	1767.74***	07917***	0.1457***
Treat + Genotypes	709.2***	0.7871***	4.46***	0.0043***	0.0007***	2.17***	0.0068***	0.0003***
Error	67.8	0.0752	0.11	0.0009	0.00009	0.3	0.0012	0.00009
Total	160	60	60	60	160	160	160	160

Conclusion

In our in vitro experiment osmotic induces stress PEG-6000 badly affected the seed germination, shoot length and root length. A significant difference was recorded in both osmotic stress and control level. All the twenty seven wheat genotypes exhibited tolerance against osmotic stress under in vitro conditions. Hence, it is recommended that these genotypes which performed better in water stress environment could increase production of arid lands.

Table 3: Comparison of mean between osmotic stress and well irrigated genotypes of wheat

Genotypes	Germination index		stem length		Root length		Stem fresh weight		Stem dry weight		Root fresh weight		Root dry weight		Germination %ge	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
1	3	2.45	9.4	3.53	12.77	3.95	0.49	0.11	0.115	0.059	0.4	0.1	0.095	0.086	90	73.3
2	2.78	1	8.48	1.74	9.22	1.82	0.36	0.09	0.098	0.042	0.27	0.08	0.078	0.069	83.3	30
3	2.11	0.89	6.58	1.01	8.54	1.07	0.42	0.1	0.113	0.05	0.35	0.09	0.093	0.077	63.3	26.7
4	1.78	0.67	7.27	0.74	7.05	0.78	0.43	0.11	0.114	0.065	0.36	0.11	0.094	0.092	53.3	20
5	2.66	0.78	9.41	0.42	11.55	0.44	0.48	0.11	0.121	0.061	0.4	0.1	0.101	0.088	80	23.3
6	1.78	1.22	5.28	0.62	4.62	0.6	0.41	0.11	0.119	0.062	0.34	0.1	0.099	0.089	53.3	36.7
7	2.00	0.78	6.62	0.75	6.2	0.79	0.51	0.11	0.11	0.062	0.42	0.1	0.09	0.089	60	23.3
8	1.44	0.22	5.44	0.58	5.33	0.63	0.54	0.12	0.128	0.072	0.45	0.11	0.108	0.099	43.3	6.7
9	2.11	0.00	6.73	0.00	5.83	0.00	0.5	0.00	0.12	0.00	0.43	0.00	0.1	0.00	63.3	0.00
10	1.89	0.33	5.82	0.17	5.24	0.17	0.45	0.04	0.108	0.021	0.33	0.03	0.088	0.03	56.7	10
11	1.89	0.89	6.94	0.43	4.82	0.45	0.45	0.1	0.112	0.056	0.33	0.1	0.092	0.083	56.7	26.7
12	1.00	1.22	6.33	0.62	7.38	0.6	0.37	0.1	0.107	0.048	0.27	0.09	0.087	0.075	30	36.7
12	2.11	0.78	8.47	1.11	8.47	1.28	0.34	0.1	0.11	0.054	0.32	0.09	0.09	0.081	63.3	23.3
13	2.33	0.00	7.05	0.00	8.47	0.00	0.41	0.00	0.106	0.00	0.32	0.00	0.086	0.00	70	0.00
15	2.66	1.00	9.22	0.27	9.97	0.29	0.44	0.1	0.103	0.055	0.31	0.09	0.083	0.082	80	30
16	2.44	1.33	8.68	0.96	9.02	0.96	0.53	0.12	0.125	0.067	0.42	0.11	0.105	0.094	73.3	40
17	2.11	1.33	8.94	1.13	7.2	1.09	0.38	0.11	0.114	0.058	0.32	0.1	0.094	0.085	63.3	40
18	1.66	0.00	7.65	0.00	7.35	0.00	0.66	0.00	0.138	0.00	0.56	0.00	0.118	0.00	50	0.00
19	2.45	1.11	9.49	0.61	8.55	0.53	0.46	0.11	0.12	0.064	0.37	0.1	0.1	0.091	73.3	33.3
20	2.89	1.00	7.56	0.73	6.99	0.73	0.5	0.14	0.152	0.096	0.41	0.14	0.132	0.123	86.7	30
21	2.78	0.33	8	0.95	8.86	0.95	0.41	0.11	0.118	0.062	0.34	0.1	0.098	0.089	83.3	10
22	2.67	0.00	8.29	0.00	7.57	0.00	0.39	0.00	0.115	0.00	0.32	0.00	0.095	0.00	80	0.00
23	2.67	0.67	6.28	0.98	5.46	0.00	0.37	10	0.117	0.059	0.28	0.09	0.097	0.034	80	20
24	2	0.67	7.27	0.58	0.0	0.69	0.48	0.11	0.122	0.066	0.37	0.11	0.102	0.093	60	20
25	1.56	0.67	5.41	0.67	4.4	0.72	0.42	0.1	0.108	0.052	0.34	0.09	0.088	0.079	46.7	20
26	1.00	0.89	6.47	0.46	6.35	0.5	0.34	0.11	0.119	0.063	0.32	0.1	0.099	0.09	30	26.7
27	1.67	1.00	6.82	0.45	7.53	0.59	0.5	0.07	0.117	0.04	0.35	0.07	0.097	0.058	50	30

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