Assessment of wheat cultivars for drought tolerance via osmotic stress imposed at early seedling growth stages

ASSESSMENT OF WHEAT CULTIVARS FOR DROUGHT TOLERANCE VIA OSMOTIC STRESS IMPOSED AT EARLY SEEDLING GROWTH STAGES

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ABSTRACT

A study was conducted in the Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Pakistan during the year 2009. Sixteen spring wheat cultivars (*Triticum aestivum* L.) were screened under osmotic stress with three treatments i.e. control-no PEG (polyethylene glycol), 15 percent and 25 percent PEG-6000 solution. The analysis of variance indicated significant differences among treatments for all seedling traits except seed germination percentage. Varieties also differed significantly in germination percentage, coleoptile length, shoot root length, shoot weight, root/shoot ratio and seed vigour index. However, shoot and root weights were non-significant. Significant interactions revealed that cultivars responded variably to osmotic stress treatments; hence provided better opportunity to select drought tolerant cultivars at seedling growth stages. The relative decrease over averages due to osmotic stress was 0.8 percent in seed germination, 53 percent in coleoptile length 62.9 percent in shoot length, 74.4 percent in root length, 50.6 percent in shoot weight, 45.1 percent in root weight, 30.2 percent in root/shoot ratio and 68.5 percent in seed vigour index. However, relative decrease of individual variety for various seedling traits could be more meaningful which indicated that cultivar TD-1 showed no reduction in coleoptile length, while minimum decline was noted in Anmol. For shoot length, cultivar Sarsabz expressed minimum reduction followed by Anmol. However, cultivars Anmol, Moomal, Inqalab-91, and Pavan gave almost equally lower reductions for root length suggesting their higher stress tolerance. In other words, cultivars Anmol, Moomal, Inqalab-91, Sarsabz, TD-1, ZA-77 and Pavan had relatively longer coleoptiles, shoots and roots, and were regarded as drought tolerant. Correlation coefficients among seedlings traits were significant and positive for all traits except germination percentage which had no significant correlation with any of other trait. The results indicated that increase in one trait may cause simultaneous increase in other traits; hence selection for any of these seedling attributes will lead to develop drought tolerant wheat cultivars.

KEYWORDS: ***Triticum aestivum;*** **cultivars; seedlings; osmotic pressure; stress; Pakistan.**

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INTRODUCTION

Development of stress tolerant varieties has been a major objective of many breeding programmes. However, success has been limited by inadequate screening techniques, and lack of genotypes that illustrate apparent differences in response to well-defined environmental stresses. Yield has been the foremost criteria for such programmes and is very complex trait in terms of number of genes controlling it. This trait is also largely influenced by the environmental factors that cause selection for such traits less effective. Another problem is that due to major environmental factors, the heritability of yield and its components is also very low (7). Drought related traits can, however, effectively be measured in off-season or in controlled laboratory conditions in early generations, which could be a cost effective and still potenial approach.

Seedling emergence is one of the most sensitive growth stages that are susceptible to water-deficit. Therefore, seed germination, seedling vigour and coleoptiles's length are prerequisites for successful stand establishment of crop plants. Rauf *et al*. (10) observed that in semiarid regions, low moisture is limiting factor during germination, hence, the rate and degree of seedling establishment are extremely important in determining both yield and time of maturity The availability of soil moisture has a major effect on germination and subsequent emergence. Besides reduction in total germination, relatively low soil moisture causes delay in seed emergence also. Germination percentage and seedling growth have been reported to decrease at low moisture levels as noted by Ashraf and Abu-Shakra (1). Dilday *et al*. (5) have demonstrated the importance of coleoptile length (protective sheath that covers the shoot during emergence) in achieving optimum stand establishment particularly when seed is planted deep to reach moisture in dry soils. It appears that there is dire need to improve the genetic tolerance of crops at seedling stages so as to save the resources in field trials and rather do the extensive screening at early stages of crop growth and development.

Selection for drought tolerance at early stage of seedlings is most commonly carried out by using chemical desiccators like polyethylene glycol (PEG-6000) medium. Lagerwerff *et al.* (6) indicated that PEG can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water-deficit in a relatively controlled manner yet appropriate to experimental protocols (8, 13). Rauf *et al*. (10) evaluated 16 spring wheat genotypes at four levels of PEG-6000 and noted significant reduction in germination percentage, germination rate index, shoot length, root length, fresh and dry weights of shoot, fresh and dry weights of root, and root/shoot ratio in all treatments of PEG except control, yet the greatest decrease in these traits occurred with 33 percent PEG concentration. Sayar *et al*. (12) observed that PEG at 25 percent concentration decreased the germination by 9 percent in sensitive varieties while at 30 percent PEG, only few seeds from drought tolerant varieties germinated. Bayoumi *et al*. (2) evaluated nine *Triticum aestivum* L. cultivars by using PEG with 0, 15 and 25 percent. PEG considerably reduced the shoot, root biomass and coleoptile length in susceptible genotypes, while less reduction was noted in drought tolerant genotypes.

Morgan *et al*. (9) stated that drought avoidance due to a profound root system enhances the ability of a plant to capture water from deeper layers of soils and is a fundamental adaptation mechanism to drought. So root system characteristics are of fundamental importance for soil exploration and below-ground resource acquisition, and are strongly related to plant adaptation to sub-optimal conditions such as drought stress. In a comprehensive review of traits likely to improve yield in water-limited environments, Ludlow and Muchow (7) placed the rooting depth and density higher in their list of priorities of drought-adaptive attributes to be exploited in crop improvement programmes. Previous studies also suggest that root-to-shoot ratio increases under water-stress conditions to facilitate water absorption which is related to abscisic acid (ABA) content of roots and shoots.

The present study was aimed to find-out a rapid and straightforward technique for screening wheat genotypes for drought tolerance at early seedling growth stages by using PEG-6000 and also quantify the associations between drought related seedling traits.

MATERIALS AND METHODS

This study was conducted in a growth room of the Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Pakistan during 2009. The experiment was laid-out in split-plots arranged in randomized complete blocks with three replications where osmotic stresses were considered as main plots and varieties as sub-plots. Sixteen spring wheat genotypes viz. Anmol, Inqalab-91, Moomal, TJ-83, Sarsabz, Khirman, SKD-1, TD-1, Kiran, Abadgar, Marvi, Mehran, Bhitai, ZA-77, Pavan and Imdad were studied to see the effect of osmotic stress imposed by using polyethylene glycol (PEG) 6000. Solutions were prepared according to weight by volume i.e. T_1 (control with distilled water), T_2 (15% PEG solution) and T_3 (25% PEG solution). It means while preparing 15 percent PEG, 150g of PEG was dissolved with a stirrer in 1000ml of distilled water. Similarly for making 25 percent PEG, 250g of PEG was dissolved in 1000ml of distilled water. The temperature in growth room was kept at 25 ± 2^{0}C with 60 percent relative humidity. Thirty seeds were placed in Whatman No.1 filter paper in Petri dishes measuring 90mm. After placing the seeds in Petri dishes, measured volume of 10ml PEG solution or distilled water was taken by Eppendorf pipette and poured into Petri dishes. Thereafter, every day, 5ml of distilled water was added in 15 and 25 percent PEG treatments whereas same amount of distilled water was added to control Petri dishes so as to keep them moist and maintain the evaporation losses. The seeds were considered germinated when radicle (coleorhizae) measured at least 5mm while the germination percentage and other measurements were recorded after 10 days of treatment. None of the seeds germinated in 25 percent PEG solutions, hence this treatment was discarded from the experiment. The data were recorded on 15 seedlings of each variety. The observations were taken on germination percentage, coleoptile length (cm), shoot length (cm), root length (cm), fresh weight of shoot (g), fresh weight of root (g), root/shoot ratio and seed vigour index (calculated by multiplying the sum of root and shoot lengths with germination percentage).

The data collected were statistically analyzed by using Genstat 11th Edition software to workout the significant differences among genotypes as affected by PEG osmotic stress. Least significant difference test was applied at 5 percent probability level to compare the mean differences. Pearson's simple correlation coefficient between different traits at seedling stage was also computed with Genstat 11th Edition.

RESULTS AND DISCUSSION

Analysis of variance revealed significant treatment effect on all traits except germination and root/shoot ratio which were declared non-significant (Table 1). With regard to varietal differences and treatment x varietal interaction,

Table 1. Mean squares of various spring wheat seedling traits affected by PEG-6000 treatments.

Traits	Treatment (T)	Error (a)	Variety (V)	V x T	Error (b)
	1df	2df	15df	15df	60df
Germination%	14.00ns	81.37	60.92**	48.82**	22.65
Coleoptile length	59.588**	0.0669	0.1767**	0.1532**	0.0557
Shoot length	1960.234**	2.255	3.255**	3.165**	0.799
Root length	2111.876**	21.207	13.814**	15.469**	4.283
Shoot weight	0.390**	0.00129	0.0006NS	0.0005NS	0.0005
Root weight	0.13261**	0.0006	0.0028NS	0.0035NS	0.0031
Root/shoot ratio	2.0723**	0.2051	0.1078**	0.0427NS	0.0098
Seed vigour index	73250739.0**	190754.0	116685.0NS	155015.0NS	104552.0

**Significant at 1% probability level, NS = Non-significant.

significant differences existed for germination percentage, coleoptile length, shoot length, root length, while root/shoot ratio was significant for varieties but not for interaction. Dhanda *et al*. (4), Rauf *et al*. (10), and Bamouni *et al*. (2) also noted significant effect of PEG treatment on wheat seedling traits, significant varietal and differential response of varieties to PEG treatment.

Varying response of genotypes to PEG treatment is very important for plant breeders as drought tolerant genotypes could be screened and tagged at seedling stage before extensive and expensive field tests are conducted. The expression of mean performance of all seedling traits was higher in control than in osmotic stress (15% PEG) treatment (Table 2 Fig. A-G). Similarly, ranges in mean values of control treatment were greater and more variable than PEG induced stress conditions. This is probably due to significant depression in traits due to PEG-desiccation. The relative decrease (RD) over averages was 0.8 percent in seed germination 53 percent in coleoptile length, 62 percent in shoot length, 74.4 percent in, root length, 50.6 percent in shoot weight, 45.1 percent in root weight, 30.2 percent in root/shoot ratio and 68.5 percent in seed vigour index (Table 2). The results further showed that maximum decrease occurred in root length and minimum in germination percentage. However, data of individual variety in osmotic stress, indicated no decrease in seed germination in more than half of varieties (Table 3). Therefore, seed germination cannot be used as a criterion for osmotic stress tolerance because it could not obviously discriminate among the varieties. This has also been shown by non-significant mean square differences among the varieties (Table 1). These results are in consonance with those of Sayar *et al*. (12) who concluded that seed germination under stress conditions could not distinguish among varieties. So it can be regarded as an inappropriate test for drought tolerance screening. Similar to present results, Roza *et al.* (11) noted significant decrease in radicle and plumule length and dry matter. Longer coleoptile length helps seedlings to emerge from the soil even at low soil moistures levels, hence can play an important role in the establishment of plant stand. Variety TD-1 did not show any decrease under osmotic stress being less affected, nonetheless minimum decrease (38.1%) was also observed in Anmol but maximum in Marvi (66.5%). Shoot length showed substantial vulnerability to osmotic stress (Fig.B), because all varieties exhibited considerable decrease in shoot length due to PEG desiccation. However, minimum decrease (57.5%) was noted in Sarsabz being drought tolerant and maximum decrease (68.4%) was observed in Bhitai showing higher susceptibility to osmotic stress. The development of root system in water deficit conditions appears to be a very viable criterion to select for water stress because it takes the moisture from lower layers of soil and also

Table 2. Average values of various spring wheat seedling traits under Polyethylene glycol-6000 treatments.

Variety	SG (%)	CL. (cm)	SL (cm)	RL (cm)	SW (g)	RW (g)	R/S ratio	SVI
	T_1 = control no PEG application							
Anmol	96.7	2.58	13.04	9.49	0.251	0.159	0.721	2178
Inqalab-91	91.1	3.01	14.00	10.23	0.246	0.135	0.719	2215
Moomal	93.3	2.89	12.82	9.53	0.218	0.130	0.723	2068
TJ-83	94.4	3.06	14.23	10.98	0.239	0.144	0.755	2378
Sarsabz	100.0	3.12	13.93	12.12	0.253	0.158	0.874	2606
Khirman	96.7	3.04	14.83	14.70	0.231	0.171	0.987	2845
SKD-1	87.8	2.82	13.74	15.20	0.277	0.194	1.109	2546
TD-1	96.7	2.56	13.10	14.94	0.264	0.218	1.144	2711
Kiran	94.4	2.90	14.88	13.17	0.276	0.134	0.885	2650
Abadgar	95.6	3.22	15.34	17.00	0.271	0.183	1.107	3090
Marvi	95.6	3.40	15.26	13.44	0.245	0.168	0.869	2726
Mehran	96.7	2.88	15.48	14.83	0.232	0.165	0.956	2925
Bhitai	94.4	3.39	17.11	12.72	0.262	0.194	0.742	2816
ZA-77	93.3	3.14	15.00	10.84	0.273	0.171	0.709	2416
Pavan	94.4	2.91	14.33	10.28	0.239	0.152	0.719	2322
Imdad-05	93.3	2.78	12.89	12.28	0.271	0.174	0.934	2325
Range	91.1-100	2.9-3.4	12.9-17.1	9.5-17.0	0.22-.77	0.13-.73	0.71-1.14	2068-3090
Average	94.7	2.98	14.38	12.61	0.253	0.166	0.872	2551
LSD var. (5%)	5.5	0.27	1.32	3.19	NS	NS	0.188	NS
	T_2 = 15% PEG-6000							
Anmol	90.0	1.60	5.50	3.41	0.134	0.080	0.619	805
Inqalab-91	95.6	1.48	5.43	3.72	0.124	0.084	0.685	884
Moomal	87.8	1.48	5.40	3.39	0.126	0.087	0.627	767
TJ-83	98.9	1.44	5.33	3.10	0.121	0.083	0.581	834
Sarsabz	98.9	1.66	5.92	3.21	0.129	0.079	0.561	903
Khirman	93.3	1.20	5.03	3.02	0.122	0.082	0.600	754
SKD-1	91.1	1.36	5.29	3.78	0.122	0.084	0.715	821
TD-1	86.7	2.56	5.21	3.24	0.121	0.085	0.622	727
Kiran	95.6	1.14	5.00	3.01	0.118	0.074	0.604	763
Abadgar	97.8	1.36	5.29	2.89	0.130	0.091	0.545	801
Marvi	96.7	1.14	5.00	3.19	0.123	0.085	0.643	791
Mehran	97.8	1.27	5.26	3.10	0.127	0.073	0.587	818
Bhitai	80.0	1.47	5.40	2.84	0.123	0.077	0.537	660
ZA-77	96.7	2.02	6.11	3.20	0.134	0.087	0.529	900
Pavan	97.8	1.32	5.18	3.84	0.123	0.036	0.742	883
Imdad	97.8	1.19	5.06	2.77	0.128	0.074	0.545	764
Range	80-89.9	1.4-2.6	5-6.1	2.8-3.7	0.12-0.13	0.04-0.09	0.53-0.74	727-903
Average	93.9	1.40	5.34	3.23	0.125	0.091	0.690	804
RD (%)	0.8	53.0	62.9	74.4	50.6	45.1	30.2	68.5
LSD V. (5%)	5.5	0.27	1.32	3.19	NS	NS	0.188	NS
LSD T (5%)	NS	0.23	1.32	4.05	NS	NS	NS	383
LSD VxT(5%)	8.5	0.39	1.19	4.81	NS	NS	0.428	NS

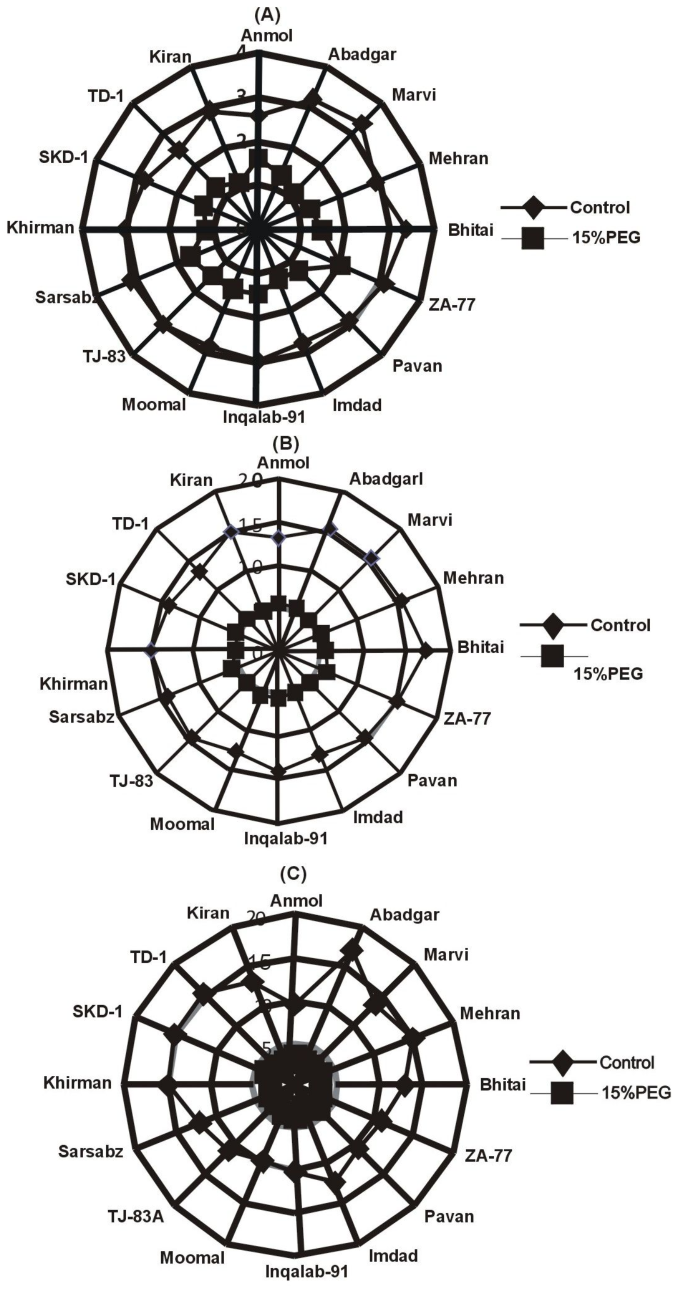

Fig. A-C Graphical presentation of coleoptile length (A), shoot length (B) and root length (C) of wheat seedlings grown under PEG induced osmotic stres conditions.

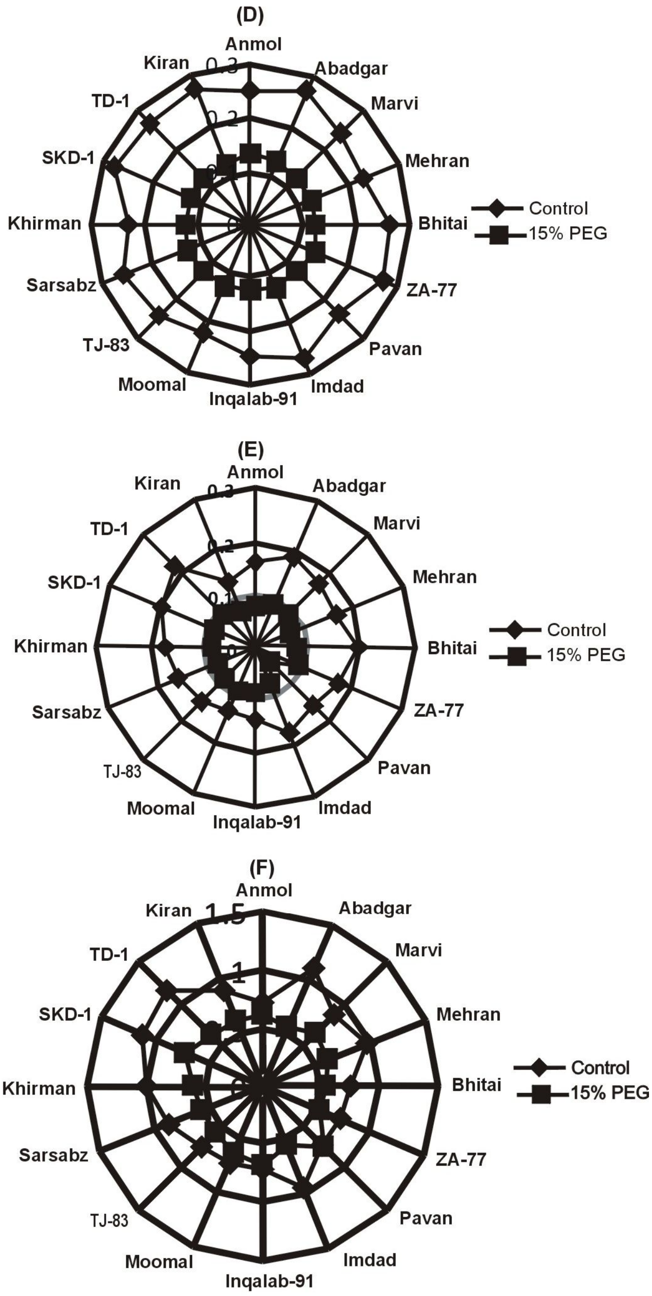

Fig. D-F Graphical presentaiton of shoot weight (D), root weight (E) and root/shoot ratio (F) of wheat seedligns grown under PEG induced osmotic stress conditions.

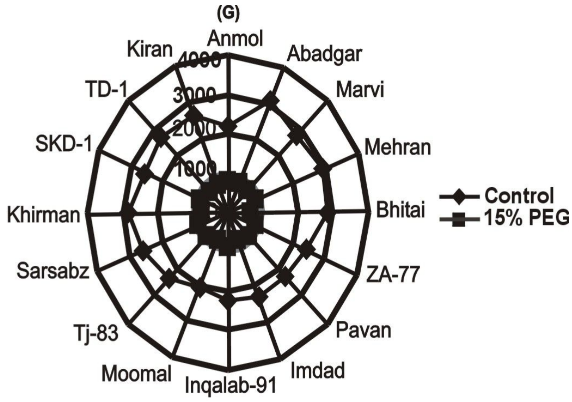

Fig. G Graphical presentation of seed vigour index under PEG induced osmotic stress

Table 3. **Relative decrease (%) in individual variety with 15% PEG treatment (T_2) against control (T_1) for various wheat seedlings traits.**

Varieties	Treat-ment	SG (%)	CL (cm)	SL (cm)	RL (cm)	SW (g)	RW (g)	R/S ratio	SVI
Anmol	T_1	96.7	2.58	13.04	9.49	0.251	0.158	0.721	2178
	T_2	90.0	1.60	5.50	3.41	0.134	0.080	0.619	805
	RD (%)	6.9	38.1	57.8	64.1	46.6	49.4	14.1	63.0
Inqalab-91	T_1	91.1	3.01	14.00	10.23	0.246	0.135	0.719	2215
	T_2	95.6	1.48	5.43	3.72	0.124	0.084	0.685	884
	RD (%)	ND	50.8	61.2	63.6	49.6	37.8	4.73	60.1
Moomal	T_1	93.3	2.89	12.82	9.53	0.218	0.130	0.723	2068
	T_2	87.8	1.48	5.40	3.39	0.126	0.087	0.627	767
	RD (%)	5.9	48.8	57.9	64.4	42.2	33.1	13.3	62.9
TJ-83	T_1	94.4	3.06	14.23	10.98	0.239	0.143	0.755	2378
	T_2	98.9	1.44	5.33	3.10	0.121	0.083	0.581	834
	RD (%)	ND	52.9	62.5	71.8	49.4	42.1	23.0	64.9
Sarsabz	T_1	100.0	3.12	13.93	12.12	0.253	0.158	0.874	2606
	T_2	98.9	1.66	5.92	3.21	0.129	0.079	0.561	903
	RD (%)	1.1	46.8	57.5	73.5	49.0	50.0	39.9	65.3
Khirman	T_1	96.7	3.04	14.83	14.70	0.231	0.171	0.987	2845
	T_2	93.3	1.20	5.03	3.02	0.122	0.082	0.600	754
	RD (%)	3.5	60.5	66.1	79.5	47.2	52.0	39.2	73.8
SKD-1	T_1	87.8	2.82	13.74	15.20	0.277	0.194	1.109	2546
	T_2	91.1	1.36	5.29	3.78	0.122	0.084	0.715	821
	RD(%)	ND	51.8	61.5	75.1	56.1	56.7	35.5	67.8
TD-1	T_1	96.7	2.56	13.10	14.94	0.264	0.218	1.144	2711
	T_2	86.7	2.56	5.21	3.24	0.121	0.085	0.622	727
	RD (%)	10.3	ND	60.3	78.3	54.2	61.0	45.6	73.2
Kiran-95	T_1	94.4	2.90	14.88	13.17	0.276	0.134	0.885	2650
	T_2	95.6	1.14	5.00	3.01	0.118	0.074	0.604	763
	RD (%)	ND	60.7	66.4	77.1	57.2	44.8	31.8	71.2
Abadgar	T_1	95.6	3.22	15.34	17.00	0.271	0.183	1.107	3090
	T_2	97.8	1.36	5.29	2.89	0.130	0.091	0.545	801
	RD (%)	ND	57.8	65.5	83.0	52.0	66.7	50.8	74.1

Table 3 contd….

Marvi	T_1	95.6	3.40	15.26	13.44	0.245	0.168	0.869	2726
	T_2	96.7	1.14	5.00	3.19	0.123	0.085	0.643	791
	RD (%)	ND	66.5	67.2	76.3	49.8	49.4	26.0	71.1
Mehran	T_1	96.7	2.88	15.48	14.83	0.232	0.165	0.956	2925
	T_2	97.8	1.27	5.26	3.10	0.127	0.073	0.587	818
	RD (%)	ND	55.9	66.0	79.1	45.3	55.8	38.6	72.0
Bhitai	T_1	94.4	3.39	17.11	12.72	0.262	0.194	0.742	2816
	T_2	80.0	1.47	5.40	2.84	0.123	0.077	0.537	660
	RD (%)	15.3	56.6	68.4	77.7	53.1	60.3	27.6	76.6
ZA-77	T_1	93.3	3.14	15.00	10.84	0.273	0.171	0.709	2416
	T_2	96.7	2.02	6.11	3.20	0.134	0.087	0.529	900
	RD (%)	ND	35.7	59.3	70.5	50.9	49.1	25.4	62.7
Pavan	T_1	94.4	2.91	14.33	10.28	0.239	0.152	0.719	2322
	T_2	97.8	1.32	5.18	3.84	0.123	0.036	0.742	883
	RD (%)	ND	54.6	63.9	62.6	48.5	76.3	ND	62.1
Imdad	T_1	93.3	2.78	12.89	12.28	0.271	0.174	0.934	2325
	T_2	97.8	1.19	5.06	2.77	0.128	0.074	0.545	764
	RD (%)	ND	57.2	60.7	77.4	52.8	57.5	41.6	67.1

ND= No decrease, SG= Seed germination, CL = Coleoptile length, SL= Shoot length, RL= Root length, SW= Shoot weight, RW = Root weight, R/S = Root-shoot ratio, SVI = Shoot vigour index.

obviously suffers more than any other seedling trait. Dhanda *et al*. (3) reported that continued growth of roots in dry soil is particularly important to avoid drought. As compared to shoot, root length demonstrated a high percentage decrease due to osmotic stress. The variety Pavan presented minimum reduction in root length (62.6%) while maximum reduction was registered by Abadgar (83.0%). In case of shoot weight Moomal showed minimum reduction (42.2%) against maximum in Kiran-95 (57.23). About root weight, Moomal proved as more tolerant while Pavan was less. Regards to root/shoot ratio, no decrease occurred in Pavan whereas the highest reduction (50.8%) was noted in Abadgar being most vulnerable to osmotic stress. Considerable declines were noted for seed vigour index in all varieties ranging from 60.1 (Inqalab-91) to 76.6 percent (Bhitai). Similar to present findings, Bayoumi *et al*. (2) and Rauf *et al*. (10) also noted significant reductions in all seedling traits by osmotic stress, yet some of the varieties gave little decline, and were regarded as drought tolerant.

Correlations among seedling traits

Correlations are important statistical parameters for selection and crop improvement. The correlation values (Table 4) indicated that seed germination percentage was not affected by PEG and also did not show correlation with any other seedling trait. Coleoptile length expressed significantly positive correlation with shoot length (r=0.95), root length (r=0.81), shoot weight (r=0.90), root weight (r=0.57), root/shoot ratio (r=0.45) and seed vigour index (r=0.90). These results indicated that increase in

Table 4. Correlation coefficients (r) among various seedling traits of spring wheat genotypes treated with polyethylene glycol (PEG-6000).

Seedling traits	Coleoptile length	Shoot length	Root length	Shoot weight	Root weight	Root/ shoot ratio	Seed vigour index
Germination percentage	0.05ns	0.06NS	-0.006	0.04NS	0.04NS	-0.09NS	0.10NS
Coleoptile length	1	0.95**	0.81**	0.90**	0.57**	0.45**	0.90**
Shoot length		1	0.89**	0.95**	0.59**	0.55**	0.97**
Root length			1	0.89**	0.64**	0.84**	0.97**
Shoot weight				1	0.62**	0.59**	0.94**
Root weight					1	0.51**	0.63**
Root/shoot ratio						1	0.71**

****Significant at 1% probability level, NS = Non-significant**

coleoptile length caused a simultaneous increase in other traits. Similarly, shoot length showed significant and positive correlations with root length (r=0.89), shoot weight (r=0.95), root weight (r=0.59), root/shoot ratio (r=0.55) and seed vigour index (r=0.97). Likewise root length, shoot weight, root weight and seed vigour index manifested significant and positive correlations with other seedling traits further suggesting that increase in any one seedling attribute correspondingly increased the other traits. It means if one reliable attribute is picked in osmotic stress and used as a selection criterion that will lead to improve other seedling traits for drought conditions and better varieties could be selected to cope with stress environments. Several other workers (2, 4, 10) also noted positive correlations among wheat seedling traits under osmotic or water stress conditions.

CONCLUSION

In this study osmotic stress caused significant effect on all the seedling traits. However, the effect on individual variety was more meaningful which indicated that cultivar TD-1 showed no reduction in coleoptile length while minimum reduction was recorded in Anmol. Similarly, Sarsabz and Anmol expressed minimum reductions in shoot and root length suggesting their higher stress tolerance, so these seedling traits may be used as good indicators for drought tolerance. Correlations among seedling traits were significant and positive except germination percentage which suggested that increase in one seedling trait could cause simultaneous increase in another trait; hence may lead to screen and develop drought tolerant wheat cultivars.

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