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EARLY GROWTH RESPONSE OF SIX WHEAT VARIETIES UNDER ARTIFICIAL OSMOTIC STRESS CONDITION

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An experiment was carried out under laboratory conditions where seeds of six wheat varieties (Damani, Hashim-8, Gomal-8, DN-73, Zam-04 and Dera-98) were raised in Petri dishes and were either treated with distilled water (control) or 15% polyethylene glycol (PEG) 6000 solution. Seeds were treated with 15% PEG solution to establish an artificial osmotic stress condition (water stress) and observe its effect on germination percentage, coleoptile length, shoot and root length, fresh weight of shoot and root. A significant difference ($P<0.05$) was recorded between varietal and treatment means regarding all traits. Variety Hashim-8 gave maximum germination percentage (93.33%) whereas maximum coleoptile (1.78cm) and shoot length (5.77cm) was observed in variety DN-73 which was statistically at par with variety Hashim-8. Similarly, root length (3.63g), fresh shoot (0.15g) and root weight (0.12g) was maximum in variety Dera-98 which was statistically at par with variety Hashim-8. A second experiment was carried out under glasshouse environment where plants were treated with non-stress (100% field capacity) and water stress (35% field capacity) treatments. Although total grain yield was significantly ($P<0.05$) reduced in all six wheat varieties when grown in water stress condition however Hashim-8 showed the lowest reduction (13%) while Zam-04 showed the highest (32%). The outcome of both experiments indicated that these varieties have great potential to incorporate with the existing commercial wheat varieties in order to obtain high yield in water stress regions.

Keywords: Wheat, water stress, drought, polyethylene glycol, PEG-6000.

INTRODUCTION

The stress factors especially drought, negatively affect plant growth and development and causes a sharp decrease of plants productivity (Pan *et al.*, 2002). It can have a substantial impact on the ecosystem and agriculture of the affected region. Droughts can persist for several years; even a short, intense drought can cause significant damage and harm the local economy. Drought study has been one of the main directions in global plant biology and biological breeding. Plant responses to drought is a complex physical-chemical process, in which many biological macro and micro molecules are involved, such as nucleic acids (DNA, RNA, microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements (Ingram and Bartels, 1996).

The effects of drought on yield of crops depend on severity and the stage of plant growth during which it occurs. Seed germination is the first stage of growth that is sensitive to water deficit. Therefore, seed germination, vigour and coleoptile length are rudiments for the success of stand establishment of crop plants. Under semiarid regions, low moisture is often a limiting factor during germination. The rate and degree of seedling establishment are extremely important factors to determine both yield and time of maturity (Rauf *et al.*, 2007). The importance of coleoptile length in achieving optimum height and establishment of a crop is already reported, particularly when seed is planted deep to reach moisture in dry soils (Dilday *et al.*, 1990). Thus there is a need to improve the genetic tolerance of crops at the seedling stages. Improvement in grain yield of wheat has traditionally relied on direct selection for this trait (Braun *et al.*, 1992). Development of stress tolerant varieties is always a main objective of many breeding programs, but success has been limited by adequate screening techniques, and the lack of genotypes that show clear differences in response to various environmental stresses. Therefore, wheat breeders are always looking for means and sources of genetic improvement for grain yield and other agronomic traits. Plant breeders are also adopting new technologies such as molecular markers to increase wheat grain yield under drought stress regions.

Polyethylene glycol (PEG), a drought induced chemical, is frequently used to screen out drought tolerant varieties at early stage of seedlings under laboratory conditions. Previous studies revealed 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 (Lagerwerff *et al.*, 1961; Money, 1989; Zhu *et al.*, 1997). PEG molecules with a Mr_6000 (PEG 6000) are inert, non-ionic and virtually impermeable chains that have frequently been used to induce water stress without causing physiological damage and maintain uniform water potential through out experiment periods (Lu and Neumann, 1998).

Molecules of PEG 6000 are too small to influence the osmotic potential but large enough not to be absorbed by plant and even not expected to penetrate intact plant tissues rapidly (Carpita *et al.,* 1979). Water is withdrawn from the cell because PEG does not enter into the apoplast therefore PEG solution mimics dry soil more closely than solutions of low Mr_Osmotica, which infiltrate the cell wall with solutes (Veslues *et al.,* 1998). It is envisaged from the above findings that PEG solution can be used in the laboratory for screening drought tolerance varieties. Therefore, present experiment was designed to treat seeds of six wheat varieties with PEG solution for drought tolerance screening of these varieties under laboratory condition. On the basis of this experiment further experiment was devised under water stress glasshouse environment to evaluate the surviving ability of these varieties under *in-vivo* conditions which would eventually validate the reliability of using PEG technique for the screening of drought resistant varieties.

MATERIALS AND METHODS

Laboratory experiment: Six bread wheat varieties such as Damani (a local variety), Hashim-8 (ICW91), Gomal-8 (CM85836), DN-73 (CMSS96T03253T), Zam-04 (CRG732) and Dera-98 (CM76688) were used in laboratory experiment at Ecology Lab., School of Biological Sciences, University of Reading to find out a reproducible, fast and easy technique for screening wheat varieties using polyethylene glycol (PEG) solution for drought tolerance. Thirty seeds of each wheat variety were treated with three treatments viz. T_1 - only distilled water was applied (control), T_2 - 15% PEG solution was applied and T_3 - 25% PEG solution was applied. Solutions of PEG 6000 were prepared according to weight by volume (Bayoumi *et al.,* 2008) i.e. to prepare 15% PEG solution (T_2), 150 g of PEG was dissolved in 200 ml of distilled water and total volume was raised up to one litre. Similarly, to prepare 25% PEG solution (T_3), 250 g of PEG was dissolved in 200 ml of distilled water and total volume was raised up to one litre. Seeds of each variety were placed on the moist Whatman germination papers in Petri dishes and 5 ml of PEG solution was applied on each day up to 15 days however distilled water was also sprinkled on these seed to provide appropriate moisture. However, seeds treated with 25% PEG did not germinate at all. The reason of this failure might be the high concentration of the chemical (PEG) which desiccated endosperm of the seeds rapidly, eventually they failed to grow. Data were recorded after two weeks to estimate germination percentage, shoot length, root length, fresh weight of shoot and fresh weight of root. Coleoptile length was measured as the length of protective sheath that covers the shoot during emergence. A spilt plot design was used for ANOVA using the Genstat version 11 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK, 2008). The same software was used to estimate simple correlation coefficient between different traits at seedling stage. The significance of correlation was tested against the value of t-tabulated.

Glasshouse experiment: Seeds of six bread wheat varieties (Damani, Hashim-8, Gomal-8, DN-73, Zam-04 and Dera-98) were sown in pots (4L) in a glasshouse under ambient environment. These pots were filled with the John Innes No. II growing media. At emergence, only three seedlings per pot were left growing while others were thinned out. Plants were exposed to two treatments; T_1 (control, 100% field capacity) and T_2 (35% field capacity). Pot weight plus dried soil was recorded as 2.84kg, afterward it was irrigated to make it at field capacity (FC) and its weight was increased to 4.07kg (moisture content was estimated as 1.23kg/pot). Pots in control treatment (T_1) were irrigated weekly to keep them at 100% FC during the whole growing period. Pots in T_2 treatment were allowed to deplete moisture content up to 35% of the FC and then these pots were re-irrigated up to 100% of the FC (Fig.1). This practice was continued until harvesting. There were four replications of each treatment. Total yield per plant was taken at harvest (137 days after sowing). A spilt plot design was used for ANOVA using the Genstat version 11 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK, 2008).

RESULTS

eeds of Zam-04 and Dera-98 germinated 90% under control and 15% PEG treatment (Table 1). However, seeds of Hashim-8 germinated 93% under PEG treatment and 84% in control. Similarly, 90% seeds of Damani variety germinated in PEG treatment as compared to 86% in control. Seed germination percentage of Gomal-8 was decreased in PEG treatment (80%) whereas it was 87% in control. About 19% decease in seed germination of DN-73 was observed between PEG (60%) and control (79%) treatments. Coleoptile length was significantly ($P<0.05$) reduced in PEG treatment (Table 1) as compared to control. The coleoptile S size of Damani was 4.63cm in control which was dramatically reduced in PEG treatment (1.39cm). Similarly, the coleoptile length in Hashim-8, Gomal-8, DN-73, Zam-04, and Dera-98 was measured as 2.97, 3.01, 3.00, 3.19 and 3.20cm respectively. However, it was decreased to 1.75, 1.66, 1.78, 1.52 and 1.73cm respectively in the above-mentioned varieties when treated with 15% PEG solution. Shoot length of all varieties decreased significantly ($P<0.05$) when treated with 15% PEG solution (Table 1). Damani variety produced maximum (24.8cm) shoot length when grown in control which was decreased to 5.23cm in 15% PEG treatment followed by Gomal-8 (16.3 to 5.50cm), Hashim-8 (15.8 to 5.67cm), DN-73 (15.3 to 5.77cm), Dera-98 (15.2 to 5.57cm) and Zam-04 (14.8 to 5.39cm). More or less similar trend was observed in root length parameter

Table 1. Response of six wheat varieties to control and PEG treatments regarding germination %age, coleoptile length, shoot length, root length, fresh shoot and root weight parameters

Treatments	Varieties	Germination (%)	Coleoptile length (cm)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)
Control	Damani	85.56±2.94	4.63±0.22	24.78±2.08	16.44±3.57	0.35±0.046	0.21±0.035
Control	Hashim-8	84.44±1.11	2.97±0.05	15.78±0.49	12.33±1.02	0.26±0.012	0.17±0.006
Control	Gomal-8	86.67±5.10	3.01±0.06	16.33±1.35	13.11±1.75	0.31±0.046	0.19±0.049
Control	DN-73	78.89±1.11	3.00±0.00	15.33±0.51	14.78±2.57	0.27±0.031	0.18±0.026
Control	Zam-04	90.00±5.78	3.19±0.13	14.78±1.50	7.67±1.93	0.24±0.025	0.16±0.017
Control	Dera-98	90.00±1.93	3.20±0.05	15.22±0.78	12.67±2.08	0.25±0.010	0.17±0.011
15% PEG	Damani	92.22±2.23	1.39±0.19	5.23±0.18	3.33±0.53	0.13±0.010	0.12±0.009
15% PEG	Hashim-8	93.33±3.34	1.75±0.06	5.67±0.06	3.61±0.56	0.14±0.003	0.12±0.001
15% PEG	Gomal-8	80.00±3.85	1.66±0.13	5.50±0.10	3.44±0.49	0.13±0.003	0.10±0.003
15% PEG	DN-73	60.00±6.95	1.78±0.24	5.77±0.34	3.02±0.33	0.14±0.008	0.10±0.002
15% PEG	Zam-04	90.00±5.10	1.52±0.30	5.39±0.27	3.02±0.67	0.13±0.009	0.10±0.003
15% PEG	Dera-98	90.00±1.93	1.73±0.16	5.57±0.18	3.63±0.42	0.15±0.006	0.12±0.005
SED	Treatments	2.10^{NS}	0.11^{*}	0.98^{*}	1.47^{*}	0.03^{*}	0.02^{**}
	Varieties	4.05^{**}	0.15^{**}	0.69^{**}	1.57^{NS}	0.02^{**}	0.02^{NS}
	Interaction	5.64^{*}	0.22^{**}	1.32^{**}	2.50^{NS}	0.04^{**}	0.03^{NS}

Values showing and stand for significant at 0.05 and 0.01 probability level, respectively, whereas ^{NS} represents a non-significant value. SED stands for standard error of difference between varietal means.

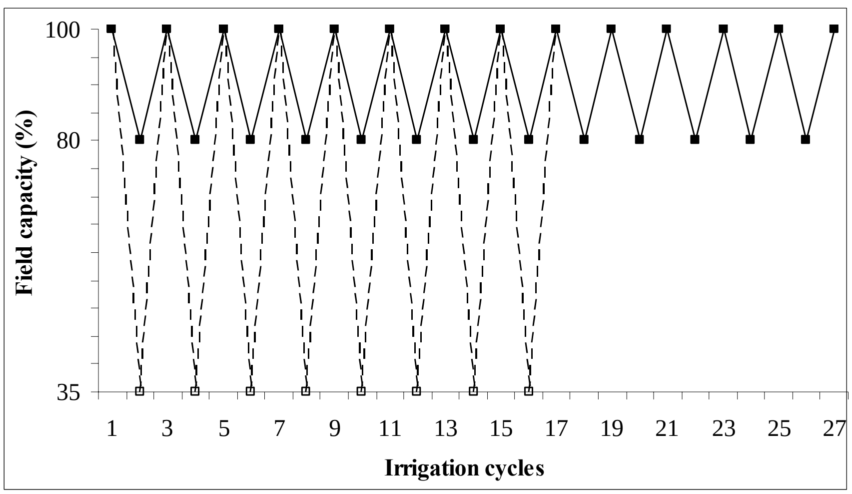

Figure 1. Schematic representation of irrigation cycles and their field capacity of T_1 (■ 100% FC) and T_2 (□ 35% FC)

(Table 1). Wheat variety Damani produced maximum root length (16.4cm) when grown in control and it was decreased to 3.33cm when grown in 15% PEG treatment. Similarly, root length of DN-73, Gomal-8, Dera-98, Hashim-8 and Zam-04 was measured as 14.8cm, 13.1cm, 12.7cm, 12.3cm, 7.67cm in control which was significantly (P<0.05) decreased to 3.02cm, 3.44cm, 3.63cm, 3.61cm and 3.02cm, respectively in PEG treatment. Table 1 showed that fresh shoot weight was highest (0.35g) in Damani variety followed by Gomal-8 (0.31g), DN-73 (0.27g), Hashim-8 (0.26g), Dera-98 (0.25g) and Zam-04 (0.24g) when grown in control. Fresh shoot weight was significantly (P<0.05) decreased when these varieties were treated with PEG solution i.e. 0.14g in Hashim-8 and DN-73; 0.13g in Damani, Gomal-8 and Zam-04; and 0.15g in Dera-98. Similarly, highest fresh root weight (Table 1) was recorded in Damani variety (0.21g) followed by Gomal-8 (0.19g), DN-73 (0.18g), Hashim-8 (0.17g), Dera-98 (0.17g) and Zam-04 (0.16g) when grown in control. However, these plants when treated with PEG solution significantly (P<0.05) reduced fresh root weight i.e. 0.12g in Hashim-8, Damani and Dera-98 and 0.10g in Gomal-8, Zam-04 and DN-73.

Correlation studies among different traits (Table 2) showed that germination %age showed positive but non-significant correlation with coleoptile length, shoot length, root length, fresh shoot weight and fresh root weight. Coleoptile length showed positive and highly significant (P<0.01) correlation with shoot length, root length, fresh shoot weight and fresh root weight. Similarly, shoot length showed positive and highly correlation with root length, fresh shoot weight and fresh root weight. Similar trend was observed in root length character which showed positive and highly significant correlation with fresh shoot weight and fresh root weight. Highly significant and positive correlation between fresh shoot weight and fresh root weight was determined.

As compared to varieties in control treatment (100% FC), water stress (35% FC) caused significant (P<0.05) percent reductions in total grain yield per plant (Fig.2). However, all varieties produced considerable total grain yield under stress environment. Total grain yield per plant was decreased up to 23% in Damani, 13% in Hashim-8, 28% in Gomal-8, 24% in DN-73, 32% in Zam-04 and 26% in Dera-98. Among all, variety Hashim-8 showed minimum reduction in total grain yield trait when subjected to stress condition and showed a promising potential variety for dryland farming areas.

DISCUSSION

As screening technique, the survival ability of the six wheat varieties to tolerate chemical desiccation by PEG during the growth of seedling showed a significant variation among wheat varieties. Varieties Damani, Hashim-8, Zam-04, Dera-98 and Gomal-8 germinated well when treated with 15% PEG solution however DN-73 showed less germination percentage. Other parameters related to plant growth such as coleoptile, shoot and root length also envisaged as prominent characteristics for drought resistant screening process of wheat varieties (Byrne *et al.,* 2007; Bayoumi *et al.*, 2008; Foito *et al.*, 2009). It has been emerged from the present investigation that drought resistant varieties such as Damani, Hashim-8, Zam-04 and Dera-98 were not only had maximum survival percentage but they also have adequate coleoptile and shoot and root length under water stress condition. This indicated that these six varieties were more

Table 2. Correlation coefficient among various traits of six wheat varieties

	Coleoptile length	Shoot length	Root length	Fresh shoot weight	Fresh root weight
Germination % age	0.001^{NS}	0.06^{NS}	0.04^{NS}	0.05^{NS}	0.17^{NS}
Coleoptile length		0.99^{**}	0.91^{**}	0.95^{**}	0.94^{**}
Shoot length			0.95^{**}	0.98^{**}	0.97^{**}
Root length				0.96^{**}	0.97^{**}
Fresh shoot weight					0.99^{**}

Values showing * and ** stand for significant at 0.05 and 0.01 probability level, respectively, whereas ^{NS} represents a non-significant value.

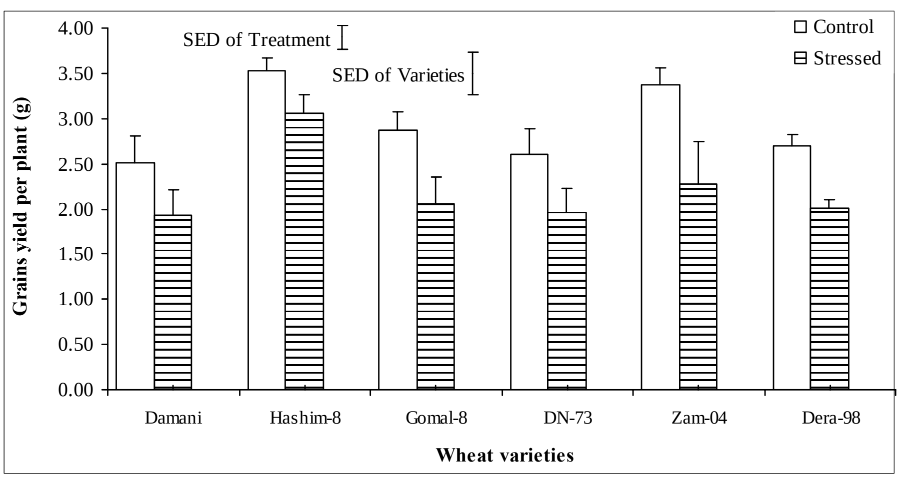

Figure 2. Effects of water stress on grains yield per plant (g) of six bread wheat varieties. White bars represent the non-stress control treatment (100% FC) whereas bars showing horizontal lines represent the water stress treatment (35% FC). SED stands for standard error of difference between treatment and varietal means at 5% probability level. Standard errors, where large than the points are shown on bars represent the variability within replicates

adoptive when grown under water stress environment than others in respect of plant growth (Leila, 2007). However, these traits were reduced in quantitative respect as compared to non-stress treatment. The reduction in the shoot length and root length may be due to an impediment of cell division and elongation leading to a kind of tuberization. This tuberization and the lignifications of the root system allow the water stress plant to enter a slow-down state, while waiting for the conditions to become favourable (Fraser *et al.*, 1990). Similar results have been reported in wheat varieties Sahel 1, Giza 168 and Rufom-5 when treated with 15% PEG which increased their root length to reach deeper water under stress condition. Similarly, shoot length and coleoptile length of the same varieties were also decreased under PEG treatments (Bayoumi *et al.*, 2008). Badiane *et al.* (2004) concluded that the expression of certain genes controlling root formation is stimulated by drought conditions which indicated a promising role of some dominant drought tolerance genes in wheat varieties which developed a decent root system under water deficit condition.

Plant of all six varieties reduced shoot weight and root weight when treated with 15% PEG solution was attributed that plants under water stress conditions used limited availability of food energy supplied by the seed in an effective way for their establishment and to start photosynthesis to provide energy for growth and development. Other workers such as Khan *et al.* (2002), Dhanda *et al.* (2004) and Rauf *et al.* (2007) also conducted similar studies and reported that germination and growth of wheat seedlings were affected significantly with change in water stress levels.

These promising characteristics observed in all six varieties in general confirmed the observations obtained from the glasshouse experiment, where all varieties thrive best against water stress condition. However, among these, Hashim-8 showed minimum reduction in total grain yield when grown under water stress condition and showed a promising potential variety for dryland farming areas as compared to others. These results are also in line with the findings of Blum and Pnuel (1990) who reported that grain yield of twelve spring wheat varieties was significantly decreased under drought condition. Similarly, Bayoumi *et al.* (2008) observed that water stress caused 43% reduction in grain yield of wheat varieties and subjecting the seeds of wheat varieties to artificial osmotic stress condition in the laboratory (treating with PEG solution) is an adequate tool for the presumption of their thriving against water stress field condition.

CONCLUSION

All six wheat varieties survived and established well when they were treated with PEG solution in the laboratory which indicated that these varieties can be raised in the water stress areas. Keeping in view this assumption, these varieties were exposed to water stress condition under glasshouse environment where only 35% FC was maintained. By combining the findings of both experiments it can be concluded that these varieties have full potential not only to subsist but to produce substantial yield under water scarce regions. Moreover, the drought tolerance traits of these varieties can be incorporated in to other high yielding varieties to get maximum plant population and yield. It has also emerged that the PEG technique would be suitable for screening drought tolerance in large populations prior to yield testing trials which could significantly reduce the over all cost and manpower. Using this method maximum germplasm can be tested for drought tolerance under limited space and resources.

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