



Improvement of Osmotic Stress Tolerance in Wheat by Seed Priming

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Abstract

The present work was conducted to find out the role of seed priming on wheat germination. The study was carried out in the Central Laboratory of Centre for plant Sciences & Biodiversity (CPS&B), University of Swat, Pakistan. Three wheat genotypes (HU-12, HU-17, and HU-20) and two check cultivars (Inqilab-91 and Chakwal-50) were studied. Seven priming media were employed which included Urea 1% and 0.5%; Distilled water (dH₂O), diammonium phosphate (DAP) 0.5% and 1%; single super phosphate (SSP) 1% and 0.5% with priming duration 18 and 36 h. The priming media (PM), genotypes (gen) and priming duration (PD) significantly affected germination percentage, germination rate index, root diameter, root length and shoot length. Seeds primed with dH₂O for 18 h performed well for all the studied attributes followed by Urea 0.5%, Urea 1% and DAP 0.5%. Similarly, the wheat genotypes HU-20 and HU-12 performed well for all the studied attributes followed by HU-17, Inqilab-91 and Chakwal-50 respectively. Further, PD of 18 h was observed to be more appropriate when compared to 36 h priming duration. The genotype HU12, HU17 and Inq91 performed better regarding root fresh weight in both controlled and stress conditions. The genotype HU12, HU17 and HU20 performed well for chlorophyll a, chlorophyll b, total chlorophyll and proline content in both controlled and stress conditions when compared to check cultivars. Further, a less relative decrease in chlorophyll content and an increase in proline content indicated that priming with urea 0.5% for 18 h duration has enhanced the osmotic tolerance of the studied wheat genotypes.

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Introduction

Seed germination, seedling emergence and crop establishment are important aspects of agricultural and horticultural production and are important components of seed and seedling vigor. These factors are related to the early growth of the crop and may be related to resistance to early-season stresses and final yield (Kakhki *et al.*, 2008). Plant growth depends upon early seedling vigor and can potentially assist the crop to reach a high potential growth rate and also reduce its yield variability. Priming is a method to improve the rate and uniformity of germination and the physiological advancement of the seed lot.

It has been observed that seed priming can improve allometric traits, grain yield and harvest index (Farooq *et al.*, 2008). Seed priming significantly increased the number of leaves, fresh and dry weight per plant of wheat seedlings and wheat crop completed its growth phases earlier, took less time than the non-primed seeds to reach tillering, heading and flowering. According to (Ashraf and Foolad, 2005) many seed soaking methods are proposed, involving hydro-priming (priming with H₂O), halo-priming (priming in inorganic salt), osmopriming (priming in various solutions of organic osmotica), thermo-priming (soaking of seeds either low or high temperature), solid matrix treatment (priming of seed by solid matrix) and bio-priming (addition of water by applying natal compounds).

Wheat (*Triticum aestivum* L.) is a member of the grass family (*Poaceae*) and the tribe *Triticeae*. Wheat is the most important food of approximately 36% of the world population. Wheat crop produced about 55% carbohydrates and 20% of the food calories that are consumed throughout the world (Eivazi, 2012). Wheat yield can be enhanced through higher productivity and not by increasing the area.

In Pakistan, wheat is cultivated in almost every part of the country on the largest land. Most of the wheat seed used for wheat cultivation is non-certified and stored by farmers from their previous crop resulting in reduced yields (Khalil *et al.*, 2010).

On-farm seed soaking includes priming of seed in H₂O, surface drying and planted on the same day. The time which is required for germination is decreased by priming treatment and may also permit escaping of the seedling from deteriorating the soil physical nature (Farooq *et al.*, 2010; Hussian *et al.*, 2013) observed that emergence, stand establishment, production of straw and grains and crops index is enhanced by soaking of seeds. They also reported that primed crops produced larger seeds as compared with non-primed crops. Seed soaking with different salts of calcium, potassium, or priming with water is better to improve the vigor of seed and also enhanced stand establishment Eivazi, 2012 reported that seed priming in 2.5% potassium chloride (KCl) for 12 hours can enhance wheat grain yield up to 15%. Paul and Choudhury (1991) reported that priming of seeds with 0.5 to 1% treatment with potassium chloride or K₂SO₄ improved grain production, plant height, and yield components of wheat crops.

Nonetheless, the practicabilities of these priming techniques need further exploration. The current research work was therefore conducted to find out the effects of wheat osmopriming on germination behavior and osmotic stress tolerance of wheat genotypes.

Material and methods

Plant Material

The research work was conducted in the central laboratory of Centre for Plant Sciences & Biodiversity (CPS&B), University of Swat, Pakistan. The experimental material comprised of synthetic derived advanced wheat lines including HU12, HU17, HU20, and check cultivars Inqilab91 and Chakwal50.

The derived synthetics were developed earlier for drought tolerance by crossing primary synthetics from crosses of durum wheat (*T. turgidum*)/*Ae. tauschii* with susceptible bread wheat cultivars. The material originated from CIMMYT through the National Agriculture Research Center (NARC), Islamabad, Pakistan. The pedigree of the experimental material is given in Table 1.

Seed priming experiment

Clean and healthy seeds of each genotype were used for priming with 7 different priming media (PM) including diammonium phosphate (DAP) 0.5 and 1%; urea (N_2H_4CO) 0.5 and 1%; distilled water (DW); single super phosphate (SSP) 0.5 and 1%. Solution of DAP 1% (50g/L), DAP 0.5% (25g/L), SSP (1%) 114.5 g/L, SSP (0.5%) 57.25g/L, Urea (1%) 10g/L, Urea (0.5%) 5g/L was prepared in dH_2O and seeds were primed at 25 °C. Distilled water (DW) was used for hydro priming. Healthy seeds of each wheat genotype were primed separately in each solution for 18 and 36 h priming duration (PD) at room temperature in test tubes. From priming media, all the seeds were removed after 18 and 36 h and then rinsed by dH_2O and by using tissue paper the seeds were dried. The 18-h treatments were immersed 18 h after the first batch so that from priming media all the seeds were removed simultaneously. For each treatment 36 seeds were kept on 90 mm diameter Petri dishes. The Whatman filter papers were moistened with 10 mL of DW. The Petri dishes were kept at optimum room temperature. Seed germination was recorded daily with radical protrusion of 2 mm scored as germination. Final germination percentage (FGP; which is the percentage of the number of germinated seeds to total seeds sown multiply by 100) of seeds were obtained when the experiment was completed. Total germination (TG) was measured when the experiment was terminated. Germination Index (GI) was measured as described in the (AOSA, 1983) according to the following formulae:

$$GI = \frac{\sum T_i N_i}{S}$$

Where the number of days after planting is represented by T_i , the number of germinated seeds on day i by N_i , and the total number of sown seeds by S . The germination experiment was continued till 14 d so that each genotype achieved maximum root and shoot length. The root length (RL) and root diameter (RDia) were measured by using Smart Root software and the shoot length (SL) was measured by using the scale. Images were taken daily from 3 to 18 days after sowing (DAS) by using a sonny 8 megapixels camera.

Features of a root system at specific time points were determined with ImageJ (<http://rsbweb.nih.gov/ij/>), using SmartRoot plugin (Adu *et al.*, 2014); (Lobet *et al.*, 2011) (<http://www.uclouvain.be/en-smartroot>). Image analysis was carried out on grayscale images obtained from the red channel of the color images. Median and Gaussian filters were applied to eliminate noise. Variation in pixel strength over longer distances was removed by subtracting the mean background pixel intensity of neighboring pixels from the pixel intensity of the original image.

Wheat seeds primed with 0.5% urea were further studied under osmotic stress conditions. For this purpose, each genotype was surface sterilized by placing seeds in mercuric chloride (1 %) solution for 5 min and rinsed thoroughly with deionized water. Seeds were germinated in Petri plates on wet filter paper using a thermostat in the dark for 14 days at an irradiance of $130 \text{ lmol m}^{-2} \text{ s}^{-1}$, 22–25 °C air temperature and 12-h photoperiod. Osmotic stress was induced with the gradual addition of polyethylene glycol (PEG 6000) and simultaneously measured osmotic potential via a vapor pressure osmometer (Vapro5520, Wescor, Inc.) until 20 % PEG (-0.85 Mpa). A control set was maintained in distilled water only for comparison. Data were recorded for root fresh weight (RFW), root dry weight (RDW), chlorophyll and proline contents. (Hiscox and Israelstam, 1979) and (Arnon, 1949) were followed for the extraction of chlorophyll using dimethyl sulphoxide (DMSO) and chlorophyll determination at wavelengths 645 and 663 nm on a spectrophotometer (BMS-1602) respectively. For the determination of proline content, the method of (Bates *et al.*, 1973) was followed using a standard proline curve.

The experimental design employed was a three-factor factorial completely randomized design (CRD) with 70 treatment combinations replicated three times. The studied attributes were subjected to analysis of variance (ANOVA) by using computer software STATISTIX 8.1 (Steel, 1997) Critical differences (CD 0.05 and 0.01) were employed to evaluate variations among treatment means of the variables.

Results

Results regarding root length showed that the mean maximum RL was recorded in HU-20 and HU-17 (8.04 and 6.32 cm respectively), while the mean minimum RL (2.89 cm) was recorded in Inqilab-91. The maximum SL was recorded in HU-20 and HU-17

(10.59 and 7.81 cm, respectively) while minimum SL (4.92cm) was recorded in Inqilab-91. The maximum RDia was recorded in HU-20 and HU-17 (0.36 and 0.23 mm, respectively) while the mean minimum RDia (0.05 mm) was recorded in Inqilab-91 as shown in Fig. 1.

Table 1. List and pedigree of wheat genotypes used.

Acc. No.	Pedigree of Conventional Bread Wheat (CBW) and Check Cultivars (CCT)
HU-12	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (882)/6/ATTLA/10/
HU-17	DOY1/AE.SQUARROSA(333)/3/PRL/VEE#6//CHOIX/4/HAHN/PRL//CLMS/3/HAHN/PRL
HU-20	CROC_1/AE.SQUARROSSA (205)//BORL95/3/KENNEDY
Inqilab 91	WL-711/CROWS
Chakwal 50	ATTLA/3/HUITLE/CARCOMUN(CARC)//CHEN/(CHTO)CHORLITO/4/ATTLA[3811]

(Source: Wheat Wide Crosses and Cytogenetics, National Agriculture Research Centre, Islamabad, Pakistan.

Priming media significantly affected the RL and RDia in the present study. Maximum mean RL was recorded in dH₂O, Urea 0.5% and DAP 0.5% (7.15, 7.12 and 7.12 cm, respectively) while the minimum mean RL (0.92 cm) was recorded in SSP 0.5%. The maximum SL was recorded in dH₂O and DAP 1%

(11.63 and 10.13 cm, respectively) while minimum SL (4.25 cm) was recorded in SSP 1% treatment. The minimum RDia has recorded in SSP 1% and 0.5% priming media while the maximum RDia (2.32 and 2.01 mm) was recorded in DAP 0.5% and Urea 1% priming treatment as shown in Fig. 2.

Table 2. Summary statistics of the studied wheat lines.

	RDia (mm)	RL (cm)	SL (cm)	GP
Mean	1.7	5.5	7.4	54.5
Variance	0.9	21.7	35.4	340.7
SE Mean	0.1	0.3	0.4	1.3
C.V.	49.9	8.4	8.0	13.9
Minimum	0.0	0.0	0.0	20.0
Maximum	10.	15.1	21.0	100.0

CV, coefficient of variation; RDia, root diameter; RL, root length; SL, shoot length; GP, germination percentage.

Priming duration significantly affected RL and Dia. The maximum mean RL (5.87 cm) was recorded in 18 h PD as compared to 36 h. The maximum SL (8.11cm) was recorded for seed soaked for 36 h PD. The maximum RDia (0.27 mm) was recorded in 18 h PD as compared to 36 h PD (Fig.3).

Summary statistics of the studied wheat lines under different PM showed that maximum RDia of 9.9 cm was recorded with CV 49.9. The recorded maximum RL was 15.1 cm with variance 21.7 and CV 8.4. Similarly, the recorded maximum SL was 21.0 cm

with variance 35.4 and CV 8.0 (Table 2). Mean GP was observed by 54.5% with CV 13.9. Maximum GP (100%) was recorded when seeds were treated with dH₂O and Urea (1 and 0.5%) solution. Analysis of variance (ANOVA) showed that PM, PD and Gen have positive effects on the studied character. But no prominent effect was recorded for RDia. The interaction of the studied factors was also highly different except for RDia. The Genotypes and PM interaction also suggested that the effects of PM and PD on GP, RDia, RL and SL may be duration and genotypes dependent (Table 3).

Table 3. Mean squares for the studied wheat lines.

SOV	DF	RL	SL	RDia	GP
PD	1	4.82 ^{NS}	42.23 ^{NS}	1.09 ^{NS}	518.57 ^{NS}
PM	6	170.19 ^{***}	306.68 ^{***}	1.34 ^{NS}	2354.29 [*]
Gen	4	200.23 ^{***}	203.61 ^{***}	0.70 ^{NS}	217.86 ^{***}
PD*PM	6	29.88 ^{***}	43.45 ^{**}	1.48 ^{NS}	406.35 ^{NS}
PD*Gen	4	27.33 ^{**}	67.28 ^{***}	0.88 ^{NS}	84.05 ^{***}
PM*Gen	24	37.56 ^{***}	58.88 ^{***}	0.65 ^{NS}	813.41 ^{**}
PD*PM*Gen	24	13.97 ^{**}	22.07 [*]	0.57 ^{NS}	652.38 ^{***}
Error	141	6.17	12.44	0.75 ^{NS}	126.67

Where, *, ** and *** significant at the 0.05, 0.01 and 0.001 probability level.

NS, non-significant; PD, priming duration; PM, priming media; Gen, genotypes; RL, root length; SL, shoot length; RDia, root diameter.

Based on critical differences, it is evident from Table 4, that the genotype HU-20 performed well for RDia, RL and SL when compared to other genotypes along with check cultivars. Similarly, among different priming media, Urea 0.5% induced the best positive effects on all the studied attributes including GP.

Regarding PD, it was also observed that 18 h PD has more promising and positive effects on the studied attributes because 36 h PD exhibited no prominent effect. The results of the present study also showed that interaction of PM and Gen have a prominent effect on GP.

Table 4. Comparison of means among the genotypes, priming duration and priming media for the studied attributes.

Genotypes	RDia (mm)	RL (cm)	SL (cm)	GP
HU12	0.61 ^{NS}	5.95 [*]	10.39 [*]	52.1 ^{NS}
HU17	2.32 ^{NS}	6.32 [*]	7.81 [*]	53.1 [*]
HU20	3.57 [*]	8.08 ^{**}	10.39 ^{**}	53.8 ^{**}
Inq-91	0.46 ^{NS}	2.86 ^{NS}	4.84 ^{NS}	55.7 ^{NS}
Chakwal-50	1.69 ^{NS}	3.45 ^{NS}	5.64 ^{NS}	57.8 ^{NS}
Priming Media				
DAP 1%	0.28 ^{NS}	4.17 ^{NS}	5.42 ^{NS}	54.0 ^{NS}
DAP 0.5%	2.32 ^{NS}	7.11 [*]	9.07 ^{NS}	59.6 [*]
Urea 1%	2.02 ^{NS}	6.73 ^{NS}	9.40 [*]	59.7 ^{NS}
Urea 0.5%	6.11 [*]	7.12 [*]	10.03 ^{**}	60.7 ^{**}
DW	0.73 [*]	7.55 ^{**}	10.07 ^{**}	63.0 ^{**}
SSP 1%	0.31 ^{NS}	2.49 ^{NS}	3.43 ^{NS}	44.7 ^{NS}
SSP 0.5%	0.34 ^{NS}	2.16 ^{NS}	3.11 ^{NS}	40.0 ^{NS}
Priming Duration				
36 h	1.03 ^{NS}	5.16 ^{NS}	6.73 ^{NS}	56.1 ^{NS}
18 h	2.45 ^{NS}	5.50 ^{**}	7.70 [*]	52.9 [*]
Gen CD 0.05	1.20	5.58	7.46	25.35
Gen CD 0.01	1.72	8.00	10.69	36.34
PD CD 0.05	1.20	6.12	7.82	22.09
PD CD 0.01	1.71	8.78	11.22	32.96
PM CD 0.05	1.18	5.42	6.83	25.23
PM CD 0.01	1.70	7.77	9.79	36.17

CD_{0.05} and CD_{0.01} are critical differences at $P < 0.05$ and 0.01 respectively.

For trait codes, see table 3.

The result of the Osmopriming showed that the genotype HU12 and HU20 performed well for RFW in controlled and stress conditions. For RDW, it was observed that all the varieties responded quite fairly with various treatment levels, the differences among treatments and varieties were highly significant. The synthetic wheat showed variation in studied attributes and the maximum RFW, RDW, Chl a, Chl b and TChl was recorded for HU-20 followed by HU-17,

HU-12, Chk50 and Inq91, respectively. The genotype HU12, HU17 and inq91 performed well for chl a in both controlled and stress conditions, while HU20 proved better for chl b content both in controlled and stress conditions. The genotype HU12, HU17, HU20 and Inq91 perform well in stress and controlled conditions for total chlorophyll content. Similarly, in stress conditions HU17, HU20 and Inq91 exhibited high proline contents.

Table 5. Root Fresh Weight (RFW), Root Dry Weight (RDW) chlorophyll a (Chla), chlorophyll b (Chlb) total chlorophyll (TChl) and proline content (PRC).

Gen.	RFW (g)		RDW (g)		Chla (mg/g)		Chlb (mg/g)		TChl (mg/g)		PRC ($\mu\text{mol/g}$)	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
HU12	0.330**	0.220**	0.168 ^{NS}	0.123**	1.20**	0.92**	0.85*	0.61*	2.05**	1.54*	2.04 ^{NS}	5.07*
HU17	0.230 ^{NS}	0.163 ^{NS}	0.152 ^{NS}	0.085 ^{NS}	1.17*	0.79*	0.86*	0.63*	2.03**	1.42*	2.86*	6.63*
HU20	0.327**	0.213**	0.162 ^{NS}	0.107**	1.16*	0.97 ^{NS}	0.87**	0.67**	2.03**	1.64*	2.44 ^{NS}	6.25**
Inq91	0.187**	0.113*	0.089**	0.056**	0.97**	0.68*	0.71*	0.46*	1.68**	1.14*	1.50 ^{NS}	3.03**
Chk50	0.180**	0.133 ^{NS}	0.086**	0.066 ^{NS}	1.04 ^{NS}	0.83 ^{NS}	0.72 ^{NS}	0.58 ^{NS}	1.76 ^{NS}	1.41 ^{NS}	2.63*	4.71 ^{NS}
CD 0.05	0.042	0.033	0.034	0.017	0.064	0.086	0.059	0.074	0.116	0.155	0.575	0.831
CD 0.01	0.060	0.047	0.048	0.024	0.092	0.124	0.084	0.106	0.166	0.222	0.825	1.191

CD_{0.05} and CD_{0.01} are critical differences at $P < 0.05$ and 0.01 respectively.

Discussion

The result of the present study regarding root length and shoot length support the previous observations of enhanced nitrogen uptake for wheat seeds primed with dH₂O for 12h. Singh *et al.*, 2017 have also reported that seeds soaked in 2.5% potassium

chloride (KCl) for 12 h before planting exhibited enhanced production of grain up to 15%. It was also recorded that seed soaked with 0.5 to 1% KCl or K₂SO₄ solutions significantly improved root length, shoot length, yield and its components in wheat genotypes (Paul and Choudhury, 1991).

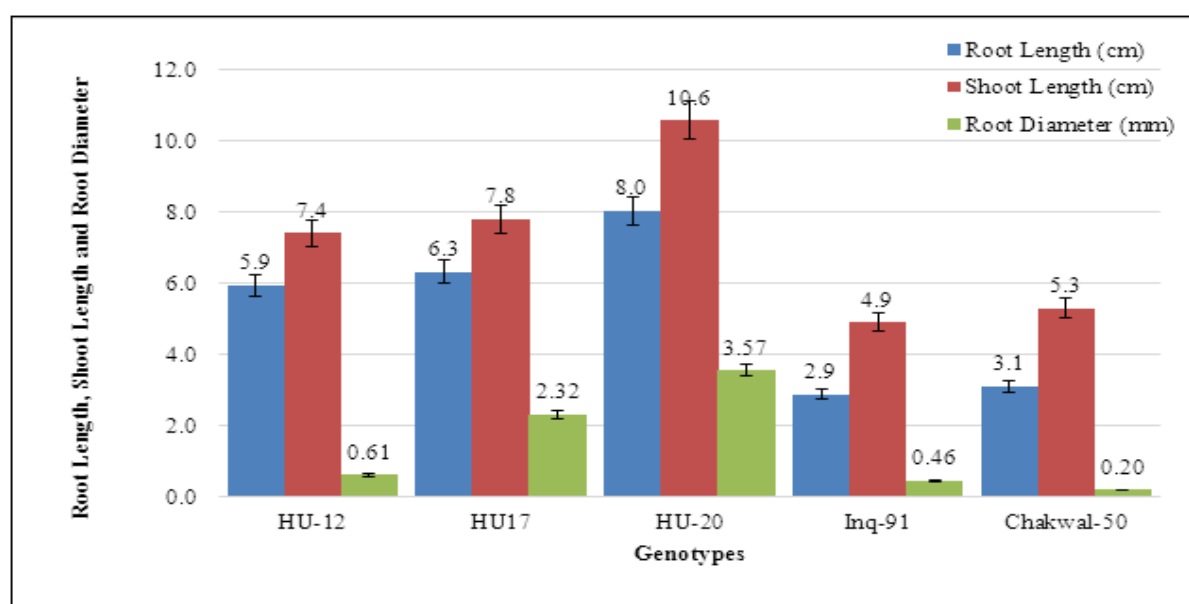


Fig. 1. Mean length and diameter of the studied wheat lines

Our finding is in contradiction with previous findings in which early germination and high yield were reported in seeds primed with phosphorus than unprimed and water primed seed. This may be due to the genotypic variability of the studied germplasm. The same could also be attributed to the priming

concentration of phosphorus which was different from which we employed in our experiment. Seed priming with both SSP and DAP solutions performed almost equal and better. In the present study, the SSP priming showed that the phosphorus has a negative role in early germination and upon RL and SL.

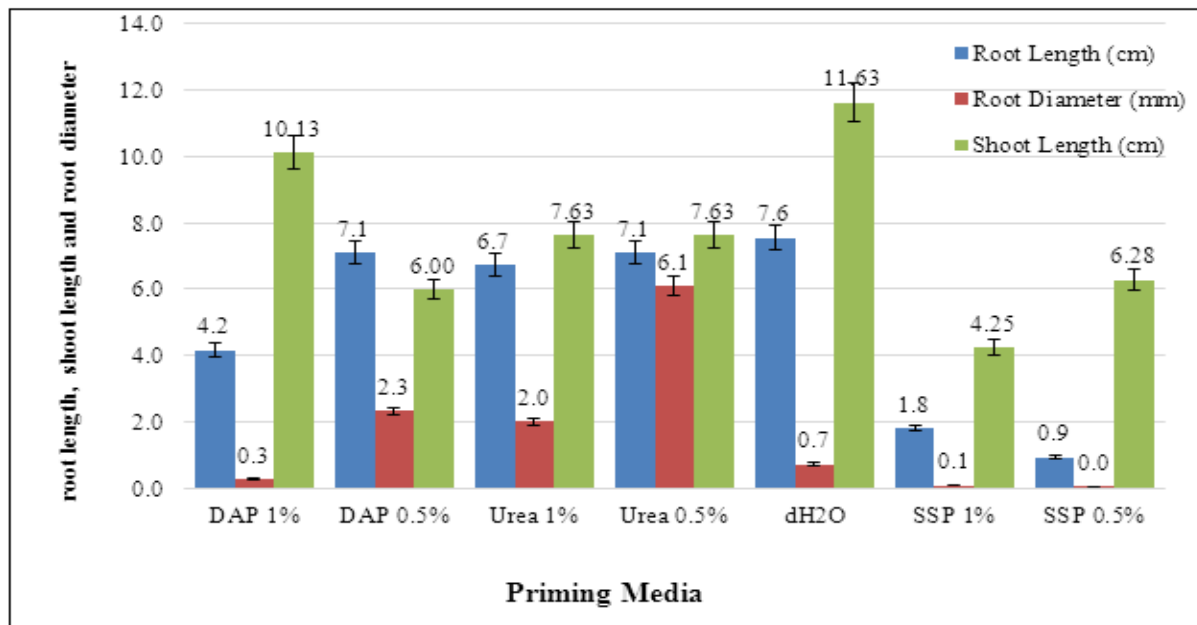


Fig. 2. Mean length and diameter of the studied wheat lines under different priming media.

The 18 h PD was observed to have a key role in the imbibition of seed and enhancement of seedling vigor. However, the effect of the priming duration is difficult to interpret because duration would be the main factor affecting rate and end-point of imbibition when priming in water, but would have less effect when the osmotic solution is used, as the rate and final level of imbibition are being controlled more closely by the potential difference. Our findings agree with Lemrasky and Hosseini, 2012 who reported that different priming treatments have positive effects on various characters like root and stem length, germination percent (GP) and rate of germination. RL is considered to be one of the most important traits in a stressful environment and genetic variations under osmotic stress conditions for RL have previously been studied in wheat (Shafi and FU, 2010).

Our findings are in general agreement with those reported by (Giri and Schillinger, 2003) They observed that wheat seeds soaked in H₂O for 12 h

duration were proving better than other priming media. High maize GP was recorded when seeds were treated with water for 36 h. Other research has also shown that soaking wheat genotypes in an osmotic solution of H₂O have enhanced germination percentage, seedling emergence and increase vigorous root length. The higher GP in treated seeds is due to the breaking of dormancy as fresh seeds were used during the investigation. In our study, the Urea showed highly significant results to studied attributes of wheat which do not agree with the finding of Eivazi, 2012 suggested that urea pretreatment had a negative effect on seedling related traits compared to control Seed priming for more than 18 h period in any priming media may lead to reduce the rate of germination. The results regarding root fresh weight are in agreement with those of (Jiang *et al.* 2016) who reported reduced root fresh weight under osmotic stress conditions. The decrease in root dry weight was also reported by other researchers and they believed that the treatment level had a significant effect on

root dry weight.

Seed priming with different chemicals, ions, organic compounds, hormones and antioxidants has been reported to have profound effects on seed

germination, growth and yield (Ashraf and Foolad, 2005). Osmotic stress is the major consequence of drought stresses that eventually reduce the water status and in turn, change the whole biochemistry of plant cells.

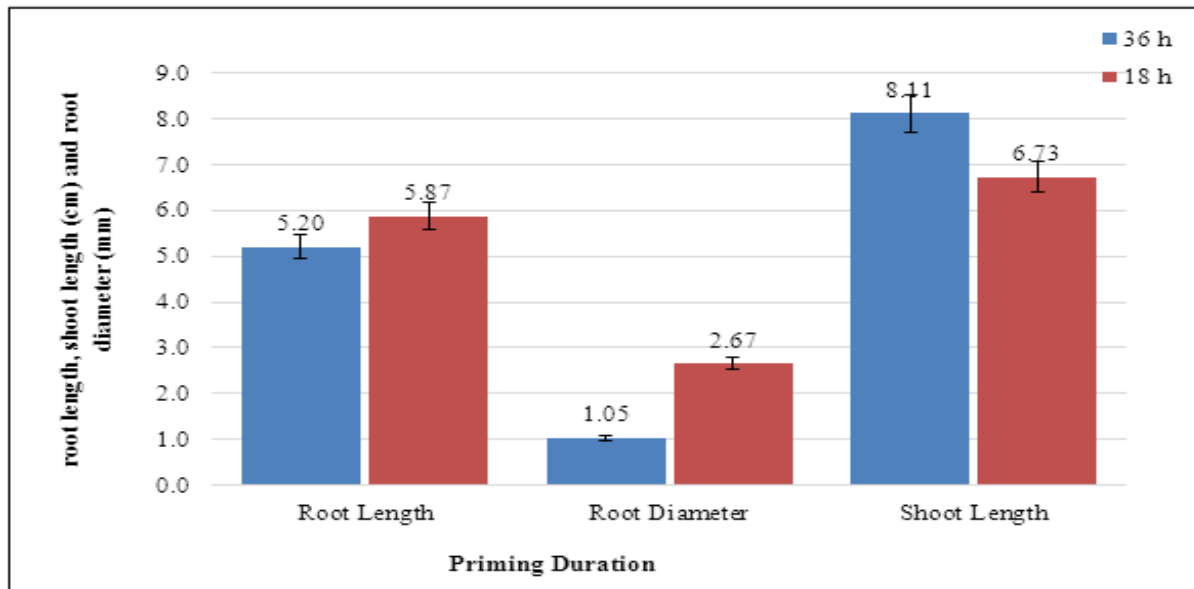


Fig. 3. Mean length and diameter of the studied wheat lines under different priming duration.

It is well established that drought stress affects water status, osmotic potential; seedling morphological attributes, and alters chlorophyll, protein and proline contents. In our study, leaf chlorophyll declined in the stress treatment, but the decrease was variable in different genotypes suggesting that different species have different threshold levels under different water regimes. A similar trend in drought stress conditions has also been reported by Tambussi *et al.*, 2000. Plant capacity to absorb water depends largely on the pattern and extent of root development; therefore, its optimization is considered as a useful way of enhancing water use efficiency (Abdel Latef and Tran, 2016).

Photosynthetic efficiency can be changed due to changes in photosynthetic pigments, such as chlorophylls 'a' and 'b' with the lowered capability to harvest light; hence chlorophyll content is commonly employed for characterizing the general photosynthetic status of a plant (Shikhov *et al.*, 2016) varied decreasing trend was observed in Chla, Chlb and Tchl content which may be ascribed to different

tolerance levels of the studied wheat genotypes. In general, the decrease was mild which may be due to increased osmoprotectant. Reports of such mild decreases or little altered chlorophyll contents have been reported by (Izanloo *et al.*, 2008). Our findings are in agreement with those of (Nyachiro *et al.*, 2001) and (Sayar *et al.*, 2008).

In wheat and other plants, different workers have reported an increase in proline concentration under osmotic stress conditions and are, therefore, recommended as a selection parameter for tolerance to drought stress (Shafi and FU, 2010); (ZGALLAĬ *et al.*, 2005). Our findings suggest that higher proline content improves the plant's ability to tolerate osmotic stress. Further, the relative increase rather than absolute value could be the appropriate indicator of stress tolerance. Wheat genotypes used in current research work differed in their morpho-physiological and biochemical attributes. Tolerant genotypes exhibited the ability to ameliorate the harmful effects of PEG-induced osmotic stress through better osmotic adjustment achieved through substantial

proline content, relatively stable root length with maximum water extraction capacity.

Conclusion

It was concluded from conducted research work that seed priming with dH₂O and Urea 0.5% and 1% solution for 18 h duration enhanced the rate of germination, RL, RDia and SL in wheat. The genotypes HU-12, HU-17 and HU-20 which are synthetic derived advanced wheat proved to be the promising genotypes in terms of stress tolerance by outperforming check cultivars. We can confer that the introgression of favorable alleles for stress tolerance has taken place from the diploid wild species *Aegilops tauschii*.

Conflicts of interest

The authors declare no conflict of interest.

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