African Journal of Biotechnology Vol. 7 (13), pp. 2156-2162, 4 July, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments

Mehmet Yağmur* and Diğdem Kaydan

Yüzüncü Yıl University, Faculty of Agriculture, Department of Field Crops, VAN, Turkey.

Accepted 27 March, 2008

Effects of seed priming treatments with 0.5% KH_2PO_4 (w/v) solution and water were determined on germination and seedling characters of hexaploid triticale (Triticosecale Witm., cv. Presto) in different osmotic potential of NaCl and PEG solutions. Drought and salt osmotic stress conditions were separately created by using PEG 6000 and NaCl, respectively, at different osmotic potentials (-0.45, -0.77, -1.03 and -1.44 MPa and control). At the equivalent osmotic potential, the effects of PEG 6000 were more harmful than NaCl on germination and seedling stage. Germination percentage and seedling growth and also relative water content (RWC, %) decreased with the decrease in osmotic potential of PEG 6000 and NaCl. But root-to-shoot length ratios increased with the effects of osmotic stress of PEG 6000 and NaCl. Despite the negative effects of two stress conditions, the two priming treatments were effective in improving germination percentage and seedling growth in Presto. But seed primed treatment was effective at the lowest osmotic potentials; therefore, seedling growth survived at the highest concentrations. Consequently, the effect of hydropriming is very pronounced particularly in improving germination and seedling growth in low stress.

Key words: Triticosecale Witm., priming, NaCl, PEG 6000, KH_2PO_4.

INTRODUCTION

Breeders and farmers aim to get higher seedling establishment in crops, but some biotic and abiotic stresses reduce seedling establishment in field conditions. Recently, salt and drought stress are perhaps the two most important abiotic stresses that limit plant growth and development. These abiotic stresses occur in field condition due to lack of some environmental components. The uncertainty of rainfall is immediately after plant emergence, leading to early season drought in rainfed farming systems (El Hafid et al., 1998). Another most negative effect on seed germination is soil salinity (Yağmur et al., 2007). Generally, salt stress causes both osmotic stress and ionic stress (Ueda et al., 2003). There are many strategies to overcome the negative effects of drought and salinity. A good strategy is the selection of cultivars and species for salinity and drought conditions (Ashraf et al., 1992). But an alternative strategy for the possibilities to overcome salt and drought stresses is by seed treatments with hydropriming or other treatments.

Seed priming techniques such as hydropriming, hardening, osmoconditioning, osmohardening, and hormonal priming have been used to accelerate emergence of roots and shoots, more vigorous plants, and better drought tolerance in many field crops like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur et al., 2002), sunflower (Kaya et al., 2006) and cotton (Casenave and Toselli, 2007).

Seed priming with potassium hydrophosphate (KH_2PO_4) had shown good potential to enhance germination, emergence and plant growth of wheat (Das and Choudhury, 1996; Korkmaz and Pill, 2003; Ghana and Schillinger, 2003). Çakmak (2005) reported that the improvement of K-nutritional status of plants might be of great importance for the survival of crop plants under environmental stress conditions, such as drought, chilling, and high light intensity.

*Corresponding author. E-mail: myagmur@yyu.edu.tr.

Hydropriming method has also been used successfully in wheat (Harris et al., 2001), in sunflower (Kaya et al., 2006), chickpea (Kaur et al., 2002) and cotton (Casenave and Toselli, 2007). Moreover hydropriming increased germination and seedling growth under salt and drought stresses (Kaur et al., 2002; Kaya et al., 2006; Casenave and Toselli, 2007). Although priming improves the rate and uniformity of seedling emergence and growth particularly under stress conditions (Parera and Cantliffe, 1991), the effectiveness of different priming agents varies under different stresses and different crop species (Iqbal and Ashraf, 2005).

NaCl and Polyethylene glycol (PEG) compounds have been used to simulate osmotic stress effects in petri dish (*in vitro*) for plants to maintain uniform water potential throughout the experimental period (Kulkarni and Deshpande, 2007).

Generally, triticales are grown in marginal fields with higher stress conditions in Turkey. Therefore, it is aimed that higher seedling growth and optimum number of seedling per area in triticale under stress conditions can be induced. Therefore, the objective of the present study was to assess the various treatments (hydropriming and KH_2PO_4) as a pre-sowing seed treatment which could ameliorate the effect of different osmotic potential of salt and drought stress on the germination and the seedling growth of triticale (Presto cultivar).

MATERIAL AND METHODS

The experiment was conducted in the laboratory of the Department of Field Crops and Department of Plant Protection, Faculty of Agriculture, University of Yüzüncü Yıl in 2007.

Seed material

New seed materials of triticale (Presto cultivar) that are commonly cultivated in Turkey were used in the present study. Hand selected seed were initially treated with a 1.0% solution of sodium hypochlorite for 3 min for surface sterilization (McGee, 1988). Residual chlorine was eliminated by thorough washing of seeds with distilled water.

Priming techniques

Two priming media were used such as water and (w/v) 0.5% KH_2PO_4 solutions. Two priming media were prepared in distilled water. Seed was fully immersed in priming media at a temperature of 24°C for durations of 12 h in germination chamber (Ghana and Schillinger. 2003). Seed were removed from priming media at the same time, then rinsed thoroughly with distilled water and lightly dried using blotting paper. The treated seeds were dried back to their original moisture content. Control treatment consists of untreated seed.

Osmotic stress of PEG and NaCl

Four drought stresses with different osmotic potentials of -0.45, -0.77, -1.03 and -1.44 MPa were arranged as described by Michel and Kaufmann (1973). Salt concentrations that had the same osmotic potentials of -0.45, -0.77, -1.03 and -1.44 MPa were adjusted using NaCl. The osmotic potential of the control solution was -0.20 MPa. The osmotic potentials of the two osmotic agents (PEG 6000 and NaCl) were read with a Wescor Vapour Pressure Osmometer- 5520).

Seed germination test and seedling growth

The treated and untreated seeds were then transferred to Petri dishes (20 seeds per petri dish with four replications) containing two (Whatman No 2) filter paper moistened with 10 ml of half-strength control solution or the same solution added with NaCl and PEG-6000. In order to avoid water losses, edges of Petri dishes were tightly sealed with an impermeable colourless parafilm. Petri dishes placed in a germination chamber to germinate in the dark condition for 72 h at 21 ± 2°C. Then the Petri dishes were transferred to germination chamber at 21 ± 2°C under a 12 h daylight photoperiod. The Petri dishes were controlled in one day intervals for solutions content. Germination percentages were obtained on the 8^{th} days using radicle extrusion (2 mm long) (ISTA, 1996).

The relative water content was estimated according to Turner (1981) and was evaluated from the equation:

$$RWC = (FW - DW)/(TW - DW)X100$$

where *FW* is the fresh weight of the shoots, *TW* is the weight at full turgid, measured after floating the shoots for 24 h in distilled water in the light at room temperature and *DW* is the weight estimated after drying the shoots at 70°C or until a constant weight is achieved. Root and shoot length (mm), and seedling fresh and dry weights (mg $plant^{-1}$) were measured on the 8^{th} day (Bray, 1963). Root to shoot length ratio was estimated by dividing root length to shoot length. Dry weights were measured after drying samples at 70°C in an oven until a constant weight is achieved.

The experimental design was arranged in a completely randomized design with 3 factorials with 4 replications. The first factor was osmotic agents, the second was solution levels, and the third was the primings. The data were statistically analyzed by MSTATc computer programme.

RESULTS AND DISCUSSION

Germination

In the present study, a significant three way interaction (osmotic agents osmotic potentials and primings) was found ($P < 0.01$) for all investigated characters. The seed germination percentage decreased with decreasing of osmotic potential of PEG and NaCl. Germination percentage of Presto was significantly reduced in response to the highest dose of NaCl (-1.44 MPa); low germination percentage was recorded. But, there was a little reduction in germination in osmotic potentials of NaCl above -0.77 MPa compared to control solution. This result correlated with the findings of Almansouri et al. (2001) who reported that moderate stress intensities only delayed germination, whereas the highest concentration of NaCl and PEG reduced germination percentages. In response to the highest dose of PEG 6000 (-1.44 MPa), no seed germination was obtained in untreated treatments (Figure 1B). The explanation of the reduction in germination percentage by effects of PEG 6000 and NaCl may be that the decrease in water potential gradient between seeds and their surrounding media adversely affects seed germination (Dodd and Donovan, 1999). Thus, the mobilization of stored reserves and synthesis of proteins in germinating embryos (Ramagopal, 1990) are not able to begin (Bouaziz and Hicks, 1990) subsequent growth processes. Moreover, the decrease in germination percentage was higher in PEG concentration than in NaCl. In other words, the negative effect of PEG was higher than NaCl on germination percentage. This is related to the accumulation of Na^+ by the imbibing seed embryo functions to promote a water potential gradient between the embryo and substrate, and maintain water uptake during seed germination under low osmotic potential of NaCl (Dod and Donovan, 1999).

Seed primed with KH_2PO_4 and water increased germination percentage under salt and water stress compared to untreated seeds. Although no seed germination was obtained at the highest dose of PEG 6000 (-1.44 MPa) in untreated seeds, KH_2PO_4 and hydropriming enhanced germination, as well as germination recorded at the same osmotic potentials of PEG. In addition, KH_2PO_4 and hydropriming increased germination percentage in lower osmotic stress of PEG and NaCl compared to untreated seeds. Seed primed with KH_2PO_4 was more effective in NaCl stress for germination percentage; whereas, hydropriming was effective in PEG stress (Figure 1A, B).

Seed primed with KH_2PO_4 and water improved germination percentage compared to untreated seed treatments. Similarly Korkmaz and Pill (2003) reported that priming with KH_2PO_4 improved the germination synchrony of low vigour cultivar in lettuce. Ghana and Schillinger (2003) observed that seed primed with KH_2PO_4 and water treatments enhanced germination in wheat under normal condition compared to untreated seed. This may be related to rapid water uptake in priming techniques such as KH_2PO_4 and hydropriming treatments in comparisons untreated seed. Therefore higher water uptake ability in seed primed with KH_2PO_4 and water resulted in higher germination percentage in osmotic stress of PEG and NaCl. Kaya et al. (2006) reported that primed seeds had more rapid water uptake abilities than untreated seeds in sunflower.

Seedling growth

PEG and NaCl increased root length at low concentrations (-0.45 MPa), but this increase in root length stopped at osmotic potential of -0.77 MPa in NaCl. In other words root length decreased sharply at osmotic potential of -0.77. Radhouane (2007) proposed that varieties having longer root length under drought condi-tion reflects an adaptive response involving an increase in root length to reach deeper water in the soil. Although root length was affected due to both kinds of osmotic stresses, significant and higher inhibition due to PEG was very evident at higher concentration of PEG. At –1.03 MPa of osmotic potential of PEG, root growth stopped after emergence of primary root from the seed in untreated seeds. But KH_2PO_4 and hydropriming treatments diminished both PEG and NaCl negative effects on root growth. Although root growth stopped at -1.03 MPa of PEG and also at 1.44 MPa of NaCl in untreated seeds, higher root growth was observed at -1.03 MPa of PEG and NaCl in primed seed with KH_2PO_4 and hydropriming treatments (Table 1). Moreover, Kaya et al. (2006) have previously reported that seed primings enhanced root length at osmotic stress of water and NaCl compared to untreated seeds in sunflower.

It is important that drought resistance in variety is characterized by small reduction of shoot growth in drought stressed conditions. In the present study, greater reduction in shoot length was obtained under same osmotic potential of PEG in comparison with NaCl. Also shoot growth did not progress at –0.77 MPa of PEG and at –1.03 MPa of NaCl in untreated seed of Presto (Table 1). However, KH_2PO_4 and hydropriming enhanced shoot growth at –0.77 MPa of PEG and also at –1.03 MPa of NaCl in Presto (Table 1). The effecting of primed seed was higher at higher concentrations of NaCl and PEG.

Root to shoot length ratio increased with the increasing of PEG and NaCl concentrations (Table 2). The results showed more inhibition of shoot growth than root growth under both PEG and NaCl. But greater increase in root to shoot length ratio was obtained in the increasing of PEG concentration in comparison to NaCl (Table 2). The selection in variety for root length and root-to-shoot length ratio under osmotic stress could be instrumental in predicting the drought tolerance of genotypes (Dhanda et al., 2004). Increase of root to shoot length ratio under stress condition depends on low water uptakes and results in reduction of shoot growth more than root growth. In drought condition, cultivars with strong root growth are particularly important to avoid drought (Dhanda et al., 1995). Okçu et al. (2005) observed that water stresses depressed the shoot growth of the cultivars rather than their root growth in pea. In the present study, root growth was similarly under both NaCl and PEG, but shoot growth was more depressed in PEG than in NaCl. Our findings confirm the observation of Hsiao and Xu (2000) and Munns and Sharp (1993) who reported that shoot growth was often more reduced than root growth by salinity, a phenomenon common with dry soil. Thornley (1998) observed that root biomass of plants in drying soil may increase relative to that in well-watered conditions. There were no significant differences in root to shoot length between primed seed and untreated

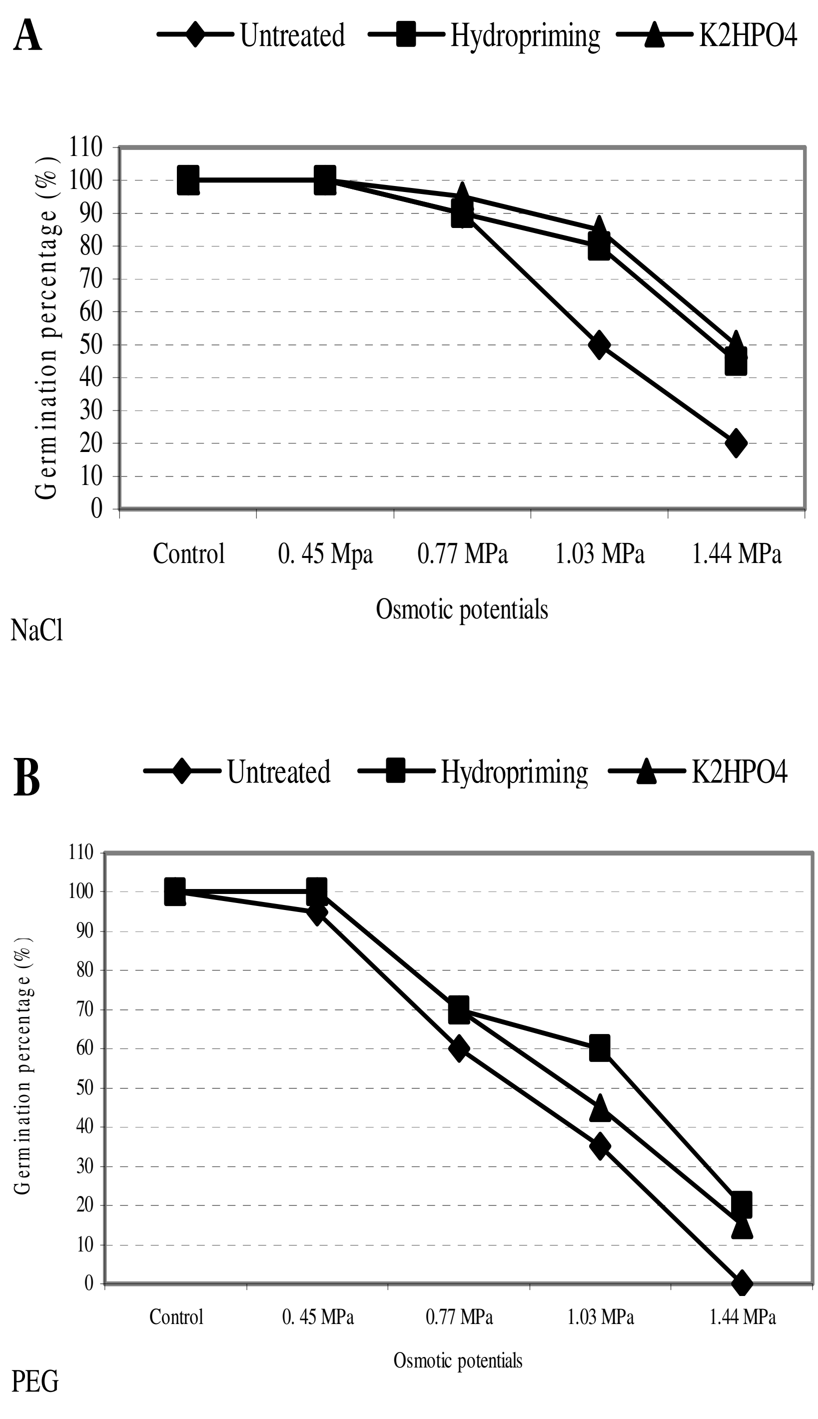

Figure 1. Effects of priming with water and KH_2PO_4 on germination percentage (%) of Presto seeds under osmotic stresses of NaCl (A) and PEG (B).

seeds in control solution. KH_2PO_4 and hydropriming lowered the negative effects of NaCl on root and shoot. But the increase in shoot growth with primed seed was higher compared to root growth. In PEG stress, root to shoot length ratio increased with increase in concentration with hydroprimed seeds compared to control solution. These results showed that hyroprimed seeds enhanced root length in comparisons with shoot growth.

According to results of relative water content (RWC) in Table 2, osmotic stress of PEG and NaCl significantly reduced RWC of shoot compared to control. RWC gradually declined with increase in concentration of PEG

Table 1. The effects of hydropriming and KH_2PO_4 on root length (mm) and shoot length (mm) of Presto under osmotic stresses of PEG and NaCl.

Priming treatment	Osmotic agent	Root length (mm)					Shoot length (mm)				
		Osmotic potentials (-MPa)									
		Control	0.45	0.77	1.03	1.44	Control	0.45	0.77	1.03	1.44
Untreated	NaCl	64.8	105.0	56.8	17.3	-	100.8	72.3	23.5	-	-
	PEG		90.0	30.7	-	-		31.3	-	-	-
Hydropriming	NaCl	49.8	69.3	69.0	24.3	-	106.8	73.5	21.5	9.3	-
	PEG		100.8	73.0	28.3	-		25.8	20.0	-	-
KH_2PO_4	NaCl	58.8	99.5	73.5	24.5	-	107.0	72.8	34.8	13	-
	PEG		70.3	47.5	26.0	-		23.5	15.0	-	-
		LSD ($P<0.05$) =12.6					LSD ($P<0.05$) =5.53				

Table 2. The effects of Hydropriming and KH_2PO_4 on root to shoot length ratios (%) and RWC (%) of Presto under osmotic stresses of PEG and NaCl.

Priming treatment	Osmotic agent	Root to shoot lenght ratios					RWC (%)				
		Osmotic potentials (-MPa)									
		Control	0.45	0.77	1.03	1.44	Control	0.45	0.77	1.03	1.44
Untreated	NaCl	0.64	1.44	2.51	-	-	88.8	80.3	60.0	-	-
	PEG		2.88	-	-	-		67.0	-	-	-
Hydropriming	NaCl	0.47	0.94	1.81	1.85	-	94.5	80.5	62.0	53.8	-
	PEG		3.99	3.65	-	-		70.0	44.3	-	-
KH_2PO_4	NaCl	0.55	1.35	2.11	2.31	-	86.2	75.0	62.0	60.8	-
	PEG		2.99	3.07	-	-		58.5	55.0	-	-
		LSD ($P<0.05$) =0.44					LSD ($P<0.05$) =6.31				

and NaCl. Greater reduction in RWC was obtained in PEG stress compared to NaCl stress. El Tayeb (2006) reported that drought caused a decrease in RWC in Vicia faba cultivars. Clarke and McCaig (1982) found out that some drought tolerant cultivars can retain more shoot water content than do drought-susceptible cultivars. Decrease in RWC with the negative effect of PEG is related to low water uptake by germinating seed compared to NaCl. But the two priming techniques used in this study enhanced relative water content due to higher water uptake. KH_2PO_4 and hydropriming increased relative water content of shoot compared to untreated seeds. But the effect of hydropriming was very pronounced particularly in improving relative water content in control and low concentration of PEG and NaCl (-0.45 MPa). But at higher stress condition, KH_2PO_4 and hydropriming enhanced RWC similarly.

As a consequence of very poor germination at the lowest of osmotic potential of PEG and NaCl, Presto could not produce any fresh or dry matter (Tables 3 and 4). NaCl increased fresh weight of root at low concentrations (-0.45 MPa). But fresh weight of root decreased sharply at osmotic potential of -0.77 MPa compared to control. In contrast, PEG decreased fresh weight of root with increase in PEG concentration. Although root length increased due to low concentration of PEG (-0.45 MPa), fresh weight of root declined at same osmotic potential of PEG. This result showed that PEG increased root length at low concentration, but fresh weight of root was low. Priming with KH_2PO_4 and water increased fresh root weight compared to untreated seed under different osmotic potential of PEG and NaCl conditions.

Greater reduction in dry root weight due to PEG and NaCl was obtained in the present study (Table 3). KH_2PO_4 and hydropriming were effective in improving dry root weight. The results showed that KH_2PO_4 and hydropriming stimulated root growth at the osmotic potential of -1.03 MPa of PEG.

Increasing concentrations of PEG and NaCl decreased fresh weight of shoot compared to control, with the highest negative effect at -1.03 MPa of NaCl and –0.77 MPa of PEG in untreated seed (Table 3). Priming with KH_2PO_4 and water increased fresh shoot weight compared to untreated seed.

Similarly, PEG and NaCl caused great reduction in dry shoot weight (Table 4). The decrease in dry shoot weight was significantly higher in PEG than in NaCl stress. Although no shoot growth was recorded at the -1.03 MPa of NaCl and at –0.77 MPa of PEG 6000 in untreated seed, shoot growth was recorded at -1.03 MPa of NaCl and at

Table 3. The effects of hydropriming and KH_2PO_4 on fresh root weight (mg plant^{-1}) and fresh shoot weight (mg plant^{-1}) of Presto under osmotic stresses of PEG and NaCl.

Priming treatment	Osmotic agent	Fresh root weight (mg plant^{-1})					Fresh shoot weight (mg plant^{-1})				
		Osmotic potentials (-MPa)									
		Control	0.45	0.77	1.03	1.44	Control	0.45	0.77	1.03	1.44
Untreated	NaCl	51.3	73.8	41.5	28.5	-	66.7	55.6	22.5	-	-
	PEG		28.5	22.5	-	-		21.3	-	-	-
Hydropriming	NaCl	84.3	62.0	42.5	30.3	-	83.3	54.6	26.5	20.0	-
	PEG		33.3	31.5	19.8	-		20.5	15.1	-	-
KH_2PO_4	NaCl	60.3	80.3	68.0	30.5	-	82.8	61.0	36.2	12.5	-
	PEG		30.8	15.0	17.8	-		22.8	12.5	-	-
		LSD (P<0.05) =11.0					LSD (P<0.05) =3.6				

Table 4. The effects of hydropriming and KH_2PO_4 on dry root weight (mg plant^{-1}) and dry shoot weight (mg plant^{-1}) of Presto under osmotic stresses of PEG and NaCl.

Priming treatment	Osmotic agent	Dry root weight (mg plant^{-1})					Dry shoot weight (mg plant^{-1})				
		Osmotic potentials (-MPa)									
		Control	0.45	0.77	1.03	1.44	Control	0.45	0.77	1.03	1.44
Untreated	NaCl	5.8	6.3	5.3	2.3	-	6.5	6.5	3.0	-	-
	PEG		6.5	4.5	-	-		4.0	0	-	-
Hydropriming	NaCl	7.5	6.3	5.5	4.3	-	7.6	6.5	3.0	2.0	-
	PEG		6.0	6.8	3.8	-		3.0	3.3	-	-
KH_2PO_4	NaCl	5.8	8.3	7.3	4.5	-	7.4	7.5	5.6	2.0	-
	PEG		4.5	5.0	3.4	-		4.4	3.3	-	-
		LSD (P<0.05) =1.42					LSD (P<0.05) =0.96				

–0.77 MPa of PEG 6000 with KH_2PO_4 and hydropriming (Table 4). Although both KH_2PO_4 and hydropriming were effective in improving dry shoot weight of triticale under different osmotic potential of PEG and NaCl, hydropriming stimulated more shoot growth in control compared to KH_2PO_4. In contrast, KH_2PO_4 stimulated more shoot growth in PEG and NaCl osmotic potential at -0.45 MPa and –0.77 Mpa, respectively.

The decrease in osmotic potential of NaCl and PEG 6000 reduced seedling growth such as root and shoot length, dry and fresh weight of root and shoot in Presto. The reduction in water uptake by germinating seed in stress condition resulted in decreases of seedling growth (Alam, 2001). Similar results were reported by other scientists showing reduction in seedling growth and different response of cultivars to drought in wheat (Almansouri et al., 2001) and pea (Okçu et al., 2005). Moreover, Radhouane (2007) observed that decreases in the external osmotic potential induced decreased shoot growth. Moreover, Soltani et al. (2006) found that reduction in seedling dry weight in response to drought and salinity in wheat cultivars is a consequence of decrease in mobilized seed reserve due to low water uptake by the germinating seeds.

The present study indicated that osmotic stress of PEG and NaCl had greater inhibitory effects on seedling growth compared to seed germination. The root and shoot growth stopped after emergence of radicle and plumula from the seed at the lowest osmotic potential of NaCl (–1.44 MPa) and at the -1.03 MPa of osmotic potential of PEG. Our results were correlated with the findings of Okçu et al. (2005).

Conclusion

In many crops pre-soaking or priming causes improvement in germination and seedling establishment (Harris et al., 2001). Increases in the seedling growth correlated with higher water uptake by primed seed resulted in higher seedling growth. Kaur et al. (2002) reported that hydropriming showed three to four fold more growth with respect to root and shoot length in comparison with seedlings obtained from non-primed seeds in drought condition.

Consequently, primed seed with KH_2PO_4 and water increased germination percentage and seedling growth that promoted optimum seedling in per unit area and strong seedlings. Generally in the present study hydropriming and KH_2PO_4 had similar effects on germination and seedling growth. Therefore hydropriming may be selected to improve seedling growth in field condition because it is cheaper. Moreover these priming techniques or other treatments should be studied in triticale cultivars under field condition to get more favourable results for the effects of priming on seedling growth and yield parameters.

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