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Effect of hydropriming on seed germination indices of sunflower (*Helianthus annuus* L.) under salt and drought conditions

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Abstract

A laboratory experiment was conducted to evaluate the effect of aerated hydropriming (24h) on two cultivar of sunflower (Urfloar and Blazar) seed germination under a range of drought stress and salt stress. Cultivar Urfloar had the more germination index (Gmax), germination rate (R50), days to 50% germination (D50), germination index (GI), root and shoot length and dry weight as compared with cultivar Blazar. Hydropriming for 24 h increased germination percentage, germination rate, germination index, root and shoot length, root and shoot weight of seed sunflower as compared with the control. However, as salinity and/or drought level increased, all of these parameters reduced under both conditions. Primed seeds produced higher germination rate and percentage, D50 and GI under all salinity and drought levels as compared with non-primed seeds. There was interaction between cultivar and priming on the germination rate and D50 as hydropriming was more effective in cultivar Urfloar. There was also interaction between priming and drought and salt stress on the Gmax, R50, D50 and GI. Primed seeds clearly produced higher Gmax, R50, D50 and GI than non-primed seeds under all drought and salinity levels. There was interaction among cultivar, drought and salt stress for all the parameters which were measured. Cultivar Urfloar clearly produced higher Gmax, R50, D50, GI, root and shoot length and their dry weight as compared with cultivar Blazar. The results suggested that hydropriming for 24 h was enhanced germination and seedling growth of sunflower under stress conditions. Therefore, this treatment may be used to improved seed performance of sunflower under normal and stress conditions.

Key words: germination index, germination percentage, germination rate, hydropriming, *Helianthus annuus.*
Abbreviations: Gmax: germination index, R50: germination rate, D50: days to 50% germination and GI: germination index.

Introduction

One major constraint to seed germination is soil salinity, a common problem in irrigated areas, with low rainfall (Kaya et al., 2003) Soil salinity may effect the germination of seed either by creating an osmotic potential external to the seed preventing water uptake, or through the toxic effects of Na and Cl ions on the germination seeds (Khajeh-Hosseini et al., 2003). Another major constraint to seed germination is water deficit, water deficit conditions have been reported to affect seedling growth adversely by altering carbohydrate metabolism and the transport of sucrose in chickpea seedling (Gupta et al., 1993) However, growth regulators like gibberellic acid and kinetin may partially reverse effects of water deficit stress during germination by inducing changes in the activities of enzymes of carbohydrate metabolism (Kaur et al., 2000). Although the priming of seeds had been reported to result in better seedling growth under water deficit stress conditions, little is known about the metabolic changes by seed priming (Kaur et al., 2002). Sunflower (*Helianthus annuus*) is one of the most important oil seed crops. The achene of sunflower is an important source of edible oil. However, sunflower germination is very susceptible in real conditions of field because of bad seed bed preparation and varying environment. Despite the introduction of improved germplasm, and this is largely conduted to hampered seed/seedling vigor under field condition. In this respect the use of seed with enhanced vigor can be a practicable strategy to obtain healthy seedling and a better crop stand under a range of environmental situation in real condition of fields. Several methods have been used to preconditioning seeds in an attempt to improve germination and seedling establishment of many vegetables and filed crops. These include alternate wetting and drying, pre-germination and controlled hydration by means of an osmotic such as polyethylene glycol. This method of controlled hydration is called priming or osmoconditioning (Khan et al., 1990). The general purpose of seed priming is to a point where germination processes are begun, but not completed. Treated seeds are usually re-dried to primary moisture before use, but they would exhibit rapid germination when re-imbibed under normal or stress conditions (Ashraf and Foolad 2005). Priming of seeds in osmoticums such as mannitol, polyethylene glycol and sodium chloride (osmo-priming) and in water (hydro-priming) has been reported to be an economical, simple and a safe technique for increasing the capacity of seeds to osmotic adjustment and enhancing seedling establishment and crop production under stressed conditions. Among above methods for seed priming soaking and misting seeds in water and redrying them before they complete germination (hydropriming) is the simplest approach to hydrating seeds (Mc Donald 2000). The adverse effects of drought and salinity stress can be alleviated by various measures, including seed priming (Ashraf and Foolad, 2005). Such controlled imbibitions of seed followed

by dehydration was shown to improve germination and early seedling growth under salt and drought stress, compared to seedling grown from untreated seed. Various pre-hydration or priming treatment have been employed to increase the speed and synchrony of seed germination (Bradford 1986). Priming could be due to faster emergence of root and shoots, lower incidence of resowing, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher grain yield under adverse conditions (Cayuela et al., 1996). Unlike other vigour enhancement treatment where hydration is stopped before germination is completed, pre-germination (which is very much similar to traditional soaking practice) is characterized by seed hydration to the point of radicle protrusion. The result is more uniform, faster germination and almost 100% seedling establishment (Mc Donald 2000). Harris et al. (2001) reported that the direct benefits of seed priming in all crops included faster emergence, better, more and uniform stands, less need to re-sow, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest and higher grain yield. In recent years, a lot of works has been done on the invigoration of seed to improve the germination rate and uniformity of growth and reduce the emergence time of many vegetables and some field crops (Basra et al., 2003). Additional information on effect of seed priming in advert condition could be beneficial both more yield production and completing data on this area so the aim of the study was to evaluate whether hydropriming results in enhancement of seed vigour in sunflower under a range of drought stress and salt stress due to PEG and NaCl, respectively.

Results

Effect of cultivar on germination indices

Cultivar Urfloar had the most germination index (Gmax), germination rate (R50), days to 50% germination (D50), germination index (GI), root and shoot length and their dry weight as compared with cultivar Blazar (Table1). Among the trials were measured, cultivar Urofelor had the most effective on the root length, as resulted increase about 419.60 % root length, in contrast had the lower effective on the shoot length (Table 1).

Effects of priming treatment on germination indices

Hydropriming for 24 hours enhanced Gmax, GI, shoot length and shoot weight as compared with control (Table2). Hydropriming was the most effective for Gmax, as hydropriming was increased Gmax about 33.39% as compared with control. Hydropriming for 24 h had the negative effect on the R50 and D50, as resulted decrease R50 and increase D50 as compared with control (Table 2).
Results showed that hydropriming lead to increase all of germination parameters in both of two cultivar sunflower. But cultivar Urfloar better responded to hydropriming as compared with cultivar Blazar. A signification two-way interaction (cultivar and priming) was found for R50 and D50. Highest and lowest of R50 was recorded with non-primed seeds of cultivar Urfloar and non-primed seeds of cultivar Blazar respectively (table4). But the highest and lowest of D50 was recorded with primed seeds of cultivar Urfloar and non-primed seeds of cultivar Blazar respectively (Table4).

Effect of drought stress on germination indices:

The two hybrid sunflower cultivars were tested for evaluation of PEG drought at germination stage. The parameters that were measured decreased with increasing drought levels (Table3). Gmax, R50, root and shoot length and shoot weight reduced with increasing in drought levels up to -9 bar, but increased thereafter, while GI and root weight reduced up to -12 bar (Table3). In drought condition cultivar Urfloar seemed to be better than the cultivar Blazar for parameters were measured. A signification two-way interaction (cultivar and drought stress) was found for Gmax, R50, D50, GI, root length, shoot length, root weight and shoot weight. Highest and lowest of these parameters were recorded with cultivar Urfloar under control conditions and cultivar Blazar under -6 bar PEG drought level, respectively. The cultivar Blazar seeds were not able to germinate at -9 and -12 bar PEG (Table 5). All of the parameters of germination were highest for primed seed as compared with non-primed seeds under all of the drought levels. A signification two-way interaction (priming and drought stress) was found for Gmax, R50, D50 and GI. Highest and lowest of these parameters except D50 were recorded with non-primed seeds under control conditions and primed seeds under -12bar PEG drought level, respectively. Highest and lowest of D50 recorded with non-primed seeds under control conditions and primed seeds under -6 bar PEG drought, respectively. The non-primed seeds were not able to germinate at -9 and -12 bar PEG (Table 6).

Effect of salt stress on germination indices

The two hybrid sunflower cultivars were tested for evaluation of NaCl salinity at germination stage. The parameters that were measured decreased with increasing NaCl levels (Table3). Cultivar Urfloar seemed better than the cultivar Blazar for parameters were measured. A signification two-way interaction (cultivar and salt stress) was found for Gmax, R50, D50, GI, root length, shoot length, root weight and shoot weight. Highest and lowest of these parameters except shoot weight were recorded with cultivar Urfloar under control conditions and cultivar Blazar under 23.5 dsm^{-1} salinity levels, respectively. Highest and lowest of shoot weight were recorded with cultivar Urfloar under 18.4dsm^{-1} and cultivar Blazar under 23.5 dsm^{-1} salinity levels, respectively (Table 5). A signification two-way interaction (priming and salt stress) was found for Gmax, R50, D50 and GI. Highest and lowest of these parameters except D50 were recorded with non-primed seeds under control conditions and non-primed seeds under 23.5 dsm^{-1} salinity levels, respectively. Highest and lowest of D50 were recorded with primed seeds under 12.7dsm^{-1} and non-primed seeds under control conditions (Table 6).

Discussion

It was revealed from this study that two cultivars of sunflower had various tolerances to salt and drought stress in germination stage. Cultivar Urfloar in comparison with the Blazar had more tolerance to drought and salt stress. It is

Table 1. Effect of cultivar on the germination characters of sunflower seeds.

Cultivar	Parameters							
	Shoot weight (mg)	Root weight (mg)	Shoot length (mm)	Root length (mm)	GI	D50(d^{-1})	R50(d^{-1})	Gmax (%)
Urfloar	27.97[a]	13.83[a]	13.32[a]	18.29[a]	364.57[a]	3.50[a]	8.40[a]	60.56[a*]
Blazer	15.40[b]	6.26[b]	8.77[b]	3.52[b]	151.56[b]	1.82[b]	3.37[b]	24.66[b]

*Mean within columns with the same letters is not significantly different at 5% level.
GI (Germination Index), D50 (Days to 50 % germination), R50 (Germination rate) and Gmax (Germination percentage)

Table2. Effect of priming on the germination characters of sunflower seeds.

Priming	Parameters							
	Shoot weight (mg)	Root weight (mg)	Shoot length (mm)	Root length (mm)	GI	D50 (d^{-1})	R50 (d^{-1})	Gmax (%)
Hydroprimin g	22.98[a]	10.81[a]	11.97[a]	12.61[a]	283.59[a]	3.36[a]	5.70[a]	48.33[a*]
Control	20.16[b]	9.15[a]	10.03[b]	10.90[a]	228.59[b]	1.94[b]	5.97[a]	36.23[b]

*Mean within columns with the same letters is not significantly different at 5% level.

Table3. Effect of osmotic potential on the germination characters of sunflower seeds.

Osmotic potential	Parameters							
	Shoot weight (mg)	Root weight (mg)	Shoot length (mm)	Root length (mm)	GI	D50(d^{-1})	R50(d^{-1})	Gmax (%)
-3 PEG	19.42[d]	11.77[c]	9.73[d]	19.73[b]	297.00[c]	3.07[b]	5.54[d]	51.38[c*]
-6 PEG	7.09[e]	3.85[ef]	2.91[e]	5.98[def]	59.00[e]	4.75[a]	0.76[f]	17.50[f]
-9 PEG	0.90[f]	2.16[ef]	0.53[e]	1.26[f]	5.67[f]	2.42[bc]	0.08[f]	2.77[g]
-12 PEG	1.55[f]	0.93[f]	0.70[e]	1.41[ef]	5.17[f]	1.81[c]	0.09[f]	3.05[g]
6.5 NaCl	42.71[a]	19.63[b]	21.71[b]	23.66[ab]	522.56[a]	2.49[bc]	13.08[b]	81.38[a]
12.7 NaCl	35.06[b]	11.70[c]	16.76[c]	12.90[c]	406.50[b]	2.76[bc]	9.24[c]	65.27[b]
18.4 NaCl	27.30[c]	8.71[cd]	11.21[d]	9.51[cd]	268.83[c]	2.57[bc]	5.25[d]	43.88[d]
23.5 NaCl	18.00[d]	5.45[de]	7.86[d]	6.51[de]	177.33[d]	2.08[bc]	3.10[e]	31.11[e]
Distilled water	42.09[a]	25.60[a]	27.38[a]	25.11[a]	268.83[a]	1.88[bc]	15.38[a]	84.16[a]

*Mean within columns with the same letters is not significantly different at 5% level.

evident from the results that priming had more effect on germination parameters of sunflower seeds. Nearly all the germination parameters which were measured, was more in hydroprimed seeds than non-primed seeds, suggesting that hydropriming of sunflower seeds may be an effective method to improve seed vigour and establishment in salt soil and dry area. Hydropriming clearly improved germination parameters under salt and drought stress conditions. The results are in line with the findings of Thornton and Powell (1992) in Brassica and Srinivasan et al. (1999) in Mustard. In many crops, pre-soaking causes improvement in germination and seedling establishment (Ashraf and Foolad, 2005). In seed of some plant species, trypsin-like proteolytic enzymes, which are produced during seed development, are important during seed development, are important during germination. The activity such enzymes, however, is often prevented by trypsin inhibitors, which may be present in the seed and play regulatory roles in protein mobilization during germination (Bewley and Black, 1994). Priming, however, may reduce the inhibitory activities of such enzymes and promote germination. For example in sorghum, soaking seed in distilled water or salt solution reducer inhibitory activities of trypsin and chymotrypsin, although the effect of the latter treatment was greater (Mulimani and Vadiraj, 1994). According to McDonald (2000), experiment the major pre-germination steps such as DNA and RNA synthesis are accomplished in the seed during the priming states consequently the seeds are physiologically close to germination and have fewer steps to complete than unprimed seeds. Hydropriming treatment increased seedling dry weight in both drought and salt stress condition across genotypes.

Thus, increase in seedling dry weight may be one of reason responsible for positive effect of hydropriming on seed performance under adverse environmental conditions. Similar to these results, Sadeghian and Yavari (2004) reported that seedling growth severely diminished with drought stress and genetic differences were found in sugar beet. Kaur et al., (2002) reported that hydropriming showed three to four more growth with respect to root and shoot length under comparison with seedling obtained from non-primed seeds. Basra et al., (2003) found that wheat seeds responded to different presowing seed treatments with hydropriming showing the maximum invigoration followed by hydropriming 48 h. These findings support the other work where improved germination rate and percentage were observed following hydropriming for 48 h in wheat (Basra et al., 2003). Hydropriming increased Gmax, R50, D50, GI, root and shoot length, root and shoot weight under normal and drought stress conditions. Drought was reduced these parameters. These results confirm the findings of EL-Midaoui et al., (2003) in sunflower, Demir and Van De venter (1999) in watermelon and Murillo- Amador et al., (2002) in cowpea. Demir and Van De venter (1999) reported that drought and salinity may influence germination by decreasing the water uptake. Drought and salinity stress have adverse effects on germination while the effects of drought stress were more severe than salinity stress. Accordance to our results Janmohammadi et al. (2008) reported that both salinity and drought stress affected germination adversely while the effects of drought stress were more severe than salinity stress. Also they reported that compared to the control osmo and hydropriming showed enhanced

Table 4. Interaction effect cultivar and priming on the R50 (d^{-1}) and D50 (d^{-1}).

Priming	Cultivar	Parameters	
		D50	R50
Hydropriming	Urfloar	4.66^{a*}	7.42^{b*}
	Blazer	2.18^{b}	3.63^{c}
Control	Urfloar	2.39^{b}	9.53^{a}
	Blazer	1.48^{b}	2.42^{d}

*Mean within columns with the same letters is not significantly different at 5% level.

Table5. Interaction effect cultivar and osmotic potential on the Gmax (%), R50 (d^{-1}), D50 (d^{-1}), GI, root length (mm), shoot length (mm), root weight (mg) and shoot weight (mg).

Cultivar	Parameters								
	Osmotic potential	shoot weight	root weight	shoot length	Root length	GI	D50	R50	Gmax
Urfloar	-3 PEG	31.01^{d}	19.56^{c}	14.30^{d}	36.13^{a}	533.00^{b}	3.36^{bcd}	10.05^{d}	99.22^{a*}
	-6 PEG	12.56^{e}	7.35^{fgh}	5.20^{e}	11.33^{bcd}	111.00^{f}	7.42^{a}	1.43^{h}	33.33^{d}
	-9 PEG	1.80^{fg}	4.33^{ghi}	1.06^{ef}	2.53^{e}	11.33^{gh}	4.85^{b}	0.17^{hi}	5.55^{ef}
	-12 PEG	3.11^{fg}	1.86^{i}	1.40^{ef}	2.83^{e}	10.33^{gh}	3.62^{bc}	0.18^{hi}	6.11^{ef}
	6.5 NaCl	42.35^{ab}	26.20^{ab}	21.46^{bc}	32.70^{a}	638.00^{a}	1.79^{cdef}	17.58^{a}	95.00^{a}
	12.7 NaCl	40.45^{abc}	14.46^{d}	17.76^{cd}	19.23^{b}	602.33^{a}	2.11^{cde}	14.47^{b}	92.22^{a}
	18.4 NaCl	47.30^{a}	13.66^{de}	18.76^{cd}	16.36^{b}	481.00^{b}	2.79^{cd}	9.47^{de}	78.33^{b}
	23.5 NaCl	33.79^{cd}	10.51^{def}	14.96^{d}	12.66^{bc}	339.00^{d}	3.56^{bc}	5.97^{f}	59.44^{c}
	Distilled water	39.40^{bc}	28.06^{a}	25.03^{ab}	33.26^{a}	599.00^{a}	1.65^{def}	18.50^{a}	87.77^{ab}
Blazer	-3 PEG	7.83^{ef}	3.98^{hi}	5.16^{e}	3.33^{de}	61.00^{gf}	2.77^{cd}	1.03^{hi}	10.55^{e}
	-6 PEG	1.16^{fg}	0.36^{i}	0.63^{ef}	0.63^{e}	7.00^{gh}	2.08^{cde}	0.08^{hi}	1.66^{ef}
	-9 PEG	0.00^{g}	0.00^{i}	0.00^{e}	0.00^{e}	0.00^{h}	0.00^{f}	0.00^{i}	0.00^{f}
	-12 PEG	0.00^{g}	0.00^{i}	0.00^{e}	0.00^{e}	0.00^{h}	0.00^{f}	0.00^{i}	0.00^{f}
	6.5 NaCl	43.08^{ab}	13.06^{de}	21.96^{bc}	14.63^{bc}	407.00^{c}	3.18^{bcd}	8.59^{e}	67.77^{c}
	12.7 NaCl	29.68^{d}	8.56^{efg}	15.76^{d}	6.56^{cde}	210.67^{e}	3.42^{bcd}	4.02^{g}	38.33^{d}
	18.4 NaCl	7.31^{efg}	3.76^{hi}	3.66^{ef}	2.66^{e}	56.67^{fgh}	2.35^{cde}	1.01^{hi}	9.44^{ef}
	23.5 NaCl	2.21^{fg}	0.40^{i}	0.76^{ef}	0.36^{e}	15.67^{gh}	0.60^{ef}	0.23^{hi}	2.77^{ef}
	Distilled water	44.78^{ab}	23.15^{a}	30.13^{a}	16.96^{b}	526.67^{b}	2.11^{cde}	12.26^{c}	80.55^{b}

*Mean within columns or rows with the same letters are not significantly different at 5% level

Table6. Interaction effect priming and osmotic potential on the Gmax (%),R50 (d^{-1}), D50 (d^{-1}) and GI.

Cultivar	Osmotic potential	Parameters			
Urfloar		GI	D50	R50	Gmax
	-3 PEG	286.00^{efg}	3.24^{bcd}	4.04^{e}	53.33^{ep*}
	-6 PEG	85.33^{hi}	5.81^{a}	1.08^{fg}	25.00^{h}
	-9 PEG	11.33^{j}	4.85^{ab}	0.17^{g}	5.55^{j}
	-12 PEG	10.33^{j}	3.62^{bc}	0.18^{g}	6.11^{j}
	6.5 NaCl	603.00^{a}	2.30^{cd}	13.20^{b}	43.89^{a}
	12.7 NaCl	482.67^{c}	3.11^{bcd}	9.75^{c}	76.11^{cd}
	18.4 NaCl	313.67^{de}	2.87^{cd}	6.05^{d}	50.55^{ef}
	23.5 NaCl	247.33^{fg}	2.36^{cd}	4.13^{e}	43.33^{fg}
Blazer	Distilled water	532.67^{b}	2.05^{cd}	12.30^{b}	81.11^{bc}
	-3 PEG	308.00^{def}	2.89^{cd}	6.68^{d}	49.44^{ef}
	-6 PEG	32.67^{ij}	3.70^{bc}	0.43^{g}	10.00^{ij}
	-9 PEG	0.00^{j}	0.00^{e}	0.00^{g}	0.00^{j}
	-12 PEG	0.00^{j}	0.00^{e}	0.00^{g}	0.00^{j}
	6.5 NaCl	442.00^{c}	2.67^{cd}	12.97^{b}	68.98^{d}
	12.7 NaCl	350.33^{d}	2.42^{cd}	8.74^{c}	54.44^{e}
	18.4 NaCl	224.00^{g}	2.26^{cd}	4.44^{e}	37.22^{g}
	23.5 NaCl	107.33^{h}	1.80^{d}	2.07^{f}	18.88^{hi}
	Distilled water	593.00^{ab}	1.70^{de}	18.45^{a}	87.22^{ab}

*Mean within columns or rows with the same letters are not significantly different at 5% level.

performance under stress conditions. Hydropriming technique compared with osmopriming clearly improved seed germination and seedling early growth under both stress and non-stress conditions. Hydroprimed seeds could achieve earlier and more uniform germination, or by higher GI and heavier seedlings. Basra et al. (2006) also reported that hydroprimed seeds of sunflower and wheat could germinate faster and produced longer seedling under salinity stress, compared with untreated seeds. Salt stress was decreased Gmax, R50, D50, GI, root length, shoot length, root weight and shoot weight growth in both primed and non-primed seeds. These results confirm the findings of Hassanpouraghdam et al., (2009) in rapeseed. Salt deposit in the root growing medium is the mean reason for physiological drought and subsequently reduced cell division and enlargement in the root growing region and ultimately reduced root growth (Godfery et al., 2004). Meanwhile these parameters in primed seeds at all salinity levels was higher than of non-primed seeds. Primed seeds had better efficiency for water absorption from growing media, and it is obvious that metabolic activities in seed during germination process commence much earlier than radicle and plumule appearance, i. e. emergence (Ascherman-Koch et al., 1992). Beneficial effects of hydropriming under normal and stress conditions could be due to earlier metabolic activities, faster and imbibitions, lesser mechanical restriction of seed coat as a result of softening of seed coat (Mc Donald, 2000). This research has shown that seed priming improved the sunflower germination traits in the laboratory. Treatment with hydropriming to cause better germination than control in salinity and drought stress. Hydropriming treatment may therefore be used to improved seed performance of sunflower. This treatment, using water alone, is a simple, cheap and environmentally friendly technique that does not need expensive chemical and sophisticated equipment.

Material and methods

This experiment was carried out at the seed technology laboratory of college of agriculture Isfahan university of Technology in 2010. Seed moisture content was determined by grinding the seeds and then drying at 130 ºC for 4 h (ISTA, 2003) and was found to be 4.4% on a fresh weight basis. The seeds were stored in a cold room maintained at 4 ºC and 30% RH.

Experimental design

The experimental design was three factors factorial (2×2×9) arranged in a completely randomized design; with three replication and 30 seeds in per replicate. The first factor was sunflower cultivars (Urfloar and Blazar), the second, seed treatments (control and hydropriming), and the third was osmotic potential levels (0, -.3, -.6, -.9 and -1.2 Mpa of PEG and 6.5, 12.7, 18.4 and 23.5 $ds.m^{-1}$ NaCl). These two cultivars of sunflower are the ones most commonly grown in Iran.

Seed treatments

Seeds of sunflower were divided into 2 sub-samples. One sub-sample was kept as the control (unprimed) and the other sub-sample was subjected to hydropriming for 24 hours. The ratio of seed weight to solution volume was 1:5 (g/ ml) (Basra et al., 2004). Seeds were dried back in to their original moisture content (4.4%) after treatment at room temperature. The PEG was prepared according to the formula of Michel and Kaufmann (1973).

Germination tests

Three replications of 30 seeds placed on filter paper that wetted with 10 ml of respective test solutions and germinated in a germinator at 25ºC and 16 hour light for 12 days. The filter papers were replaced every 2 days to prevent accumulation of salts (Rehman et al., 1996). Germination was considered to have occurred when the radicles were 2 mm long. Germination percentage was recorded every 24 h for 12 days. Germination rate (Coolbear et al., 1984), dry weight of root and shoot, root and shoot lenght, vigor 1 and vigor 2 (Abdual-baki and Anderson, 1973) were measured after the 12^{th} day.

Statistical analysis

Data were subjected to normal distribution tests and analysis of variance and least significant difference (LSD) for comparison of means were performed, using SAS (ver. 9.1) software.

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