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ORIGINAL ARTICLE

Do Primed Seeds After Drought Stress Have Higher Germination Recovery Efficiency Compared To Unprimed Seeds?

Akbarpour Bahreh M.*, Sharif Zadeh F., Archin Sh.

Department of Agronomy & Plant Breeding, Faculty of Agricultural Science & Engineering, College of Agriculture & Natural Resources, University of Tehran, Karaj 4111, Iran

*E-Mail: ***ma_akbarpoor20@ut.ac.ir***

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From the ecological and economic point of view *Agropyron elongatum*, among perennial grasses, has a special place. This study was conducted to determine the most appropriate seed priming treatment and whether after a temporary stress removal, primed seeds have a higher recovery efficiency compared with unprimed seeds? In this research, seed was treated with different osmo and hydro priming and evaluated their effect by conducting germination test under drought stress (-1.2 and -1.4 MPa PEG) and recovery test. Hydro-primed seeds at 10 °C at all times (12, 24, 36 and 48 h) during priming; most indices of germination significantly improved compared to unprimed seeds. Also, seeds treated with osmopriming at both temperatures (10 & 15 °C) and all times of priming compared to unprimed seeds in the stress level of -1.2 MPa, the germination characteristics were improved. However, by increasing the potential of stress, few priming treatments have been able to maintain their superiority. It appears that priming can partially be effective on stress resistance and if the stress threshold is slightly higher than expected (this threshold for the Agropyron seeds in this study was -1.2 MPa) cannot have a noticeable effect on resistance to drought stress and can even be harmful too. All treatments which were placed in stressful situations and then moved to fresh water, showed a variety of recovery responses. As we viewed, primed seeds with a solution of -0.6 MPa urea for 12 h at 15 °C and followed by PEG solutions (-1.2 and -1.4 MPa) for 5 days and subsequently moved to fresh water conditions, had higher performance compared to unprimed seeds ($P<0.05$). In many of priming treatments by increasing the potential of drought stress; on recovery of germination percentage, final germination and normal seedling percentage were added. It seems that high concentrations induce a state of quiescence, and this may suggest an important adaptation for growth in arid and semi-arid environments.

Key words: Agropyron elongatum, drought stress, germination, priming, recovery

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Key words: Agropyron elongatum, drought stress, germination, priming, recovery

Efficient germination and rapid and uniform seedling emergence, assure successful establishment of plants in arid and semi-arid regions considering low rainfall and irregular

precipitation patterns (Chen, 2011). Major part of the arid and semi-arid rangelands and low-yielding lands arid have extensive forage production potential. Therefore, appropriate revival and corrective operations in these regions considering the existing need, requires special attention. One of the efficient approaches to achieve this important, is the sowing of pasture - forage plant seeds. Tall wheatgrass (*Agropyron elangatum*) is a cool-season grass. Will grow in areas with 12.5–20 cm annual rainfall. Native to the eastern Mediterranean region, from southern Europe to Asia Minor and Crimea, where it occurs in saline meadows and along seashores. This plant is very dense, strong with sheaf form, serotinous, long growth period and green during the summer and sometimes fall. Due to cultivation possibility in lands that is not appropriate for other species of this genus, this plant has utmost importance of using in rangeland modification programs (Fuller *et al*., 1982; Heath, 1975; Duke, 1983). Tall wheatgrass is used extensively for seeding alkaline sites in the northern Plains and Intermountain regions of the United States, Canada and in some areas of northern Europe and Central Asia. (Buxton *et al*., 1996; Duke, 1983). One of the affecting factors in the success sowing program is using of high quality and appropriate seed (Wilson *et al*., 2000). Seed poor vigor and environmental unfavorable conditions are some of the main factors in slow and non-uniform germination (Wang, 2005; Chen, 2011). The use of treatments such as priming in which we can enhance seed physiological quality, could be effective in enhancing germination prior to planting, especially, in stressful environmental conditions, such as drought stress, through increasing seed vigor (Farooq *et al*., 2006). Seed priming is a controlled imbibed treatment where metabolic activities take place pre-germination, but prevent from radicle emergence (Bradford, 1986). In this technique, different methods are used to control seed imbibition, which includes methods such as osmopriming and hydropriming. Osmopriming is special kind of preparation before seeds planting that seeds are laying in or put in to the solutions containing chemicals with low osmotic potential such as polyethylene glycol (PEG), mannitol and chemical fertilizers (such as urea) (Ashraf & Foolad, 2005a). In hydropriming, seeds are treated with pure water without any chemical treatments (Ashraf & Foolad, 2005; Farooq *et al*., 2006). Over the past two decades, seed priming is considered as a common method in order to increase speed and uniformity of germination in the field, seedling better establishment and faster passage of environmental unfavorable conditions during germination and emergence, and so on, in many important crops such as wheat (*Triticum aestivum*), maize (*Zea mays*), pepper (*Capsicum annuum*), soybean (*Glycine max*) and so on, have been used in both optimal and unfavorable environmental conditions. (Iqbal & Ashraf, 2007; Farooq *et al*., 2008; Korkmaz & Korkmaz, 2009; Zhuo *et al*., 2009) that in some have been successful and some have not. However, in this regard, few studies have been performed on *Agropyron*.The objectives of this study were to determine the most appropriate seed priming treatment for *A. elongatum* and whether priming can enhance *Agropyron* seeds germination traits in drought stress conditions and can cause tolerance to more stress? And whether after a temporary stress removal, primed seeds have a higher recovery efficiency compared with unprimed seeds or not?

MATERIALS AND METHODS

Plant material

Tall wheatgrass seeds used in this study were prepared from Bahar Researching Station of Hamedan, Iran (34˚ 48' N– 48° 31' E; AAP 315 mm) and were kept at 3 °C until beginning of the experiment. Seed moisture content was measured by the oven method (130 ° C for 1 h) (ISTA, 1996), before testing, which was equivalent to 7.8%. A germination test was carried out to assess the initial viability at 25 °C that showed 98% germination, the seedlings were also normal.

Priming Treatments

Seed pre-treatments were hydropriming (seeds being exposed to distilled water) and osmopriming. Urea solutions were used for osmopriming. Solution concentrations, including -0.4 and -0.6 MPa were prepared using Van't Hoff's formula (Wain-Tassi *et al.,* 2012). The seed subsamples were randomly selected and were placed in glass petri dishes with 15 cm diameter containing mentioned solutions. Then the petri dishes were randomly incubated at temperatures 10 and 15 °C for periods of 12, 24, 36 and 48 hour(h) in darkness. Solution volume in each petri was chosen based on the grounds that the seeds should not be completely submerged in the solution, i.e., on one side is almost in contact with air. At 15 °C primed seeds for 36 and 48 h entered into a germination stage. Therefore, in order to avoid interfering with the priming effect on germination results, were removed these treatments. After priming, seeds were washed several times with sterile distilled water, and then seeds surface moisture was removed with blotting paper. Afterwards were placed at the same temperature that were primed to reduce the moisture content to reach the surface of unprimed seeds.

PEG Concentrations

For drought stress and recovery tests were used two concentrations of -1.2 and -1.4 MPa PEG solutions. These potentials with polyethylene glycol (PEG) 6000 were prepared according to the method of Michel and Kaufmann (Michel & Kaufman, 1973).

Drought Stress Test

Samples of 300 seeds (three replicates of 100 seeds each) were surface sterilized with vitawax fungicide for two minutes, subsequently washed with sterile distilled water before being used in the germination experiments to avoid fungus attack (Ruan *et al*., 2002). Then seeds surface moisture was removed with blotting paper. Afterwards were placed in petri dishes with 9 cm diameter containing 7 ml of PEG solutions (50 seeds each). To avoid interfering solutions osmotic potential with filter paper matric potential, was not used the filter paper. Subsequently petri dishes were sealed in a plastic bag to prevent evaporation and were placed in the darkness for 21 days at a temperature 25˚ C (±1) (ISTA, 1996) for evaluating germination behavior. Daily were performed counts. A seed was considered to have germinated if a radical was 2 mm. At the end of the experiment, were measured the final germination percentage (FGP), germination index (GI), root length (RL), seedling vigor index (SVI) and seedling dry weight (SDW). The GI was calculated as GI=Σ (Gt/t) where Gt is the number of germinated seeds on day t (Zhang *et al*., 2012). The SVI was calculated as SVI= seedling Lengh × FGP. In order to measure the *RL*, 10 seedlings were randomly selected from each Petri and was determined their length. To determine the dry weight of seedlings, seedlings were placed at 70 °C for 72 h and were weighed dry weight with an

accuracy of 0.0001 by digital scale.

Recovery Test

In this experiment, the primed and unprimed seeds were placed in PEG solutions similar drought stress test (Section 2-4). With this difference that after 5 days of germination (the first day of counting of *A. elongatum* (ISTA, 1996)), the percentage of germinated seeds was recorded and then all seeds (whether germinated or not) were rinsed with distilled water to wash PEG from the surface. Thereafter treated seeds were placed in new petri-dishes with moistened filter paper and distilled water, and incubated under the same conditions (25 °C) for an additional 21 days to study the recovery of germination. In this experiment, each treatment was 150 seeds (three replicates of 50 seeds each). None of the seedlings were removed until the end of the experiment. Total germination percentage, recovery percentage and recovered normal seedling percentage was calculated at the end of the experiment. The germination recovery percentage was calculated using the following formula: [(A-B) /(C-B)] × 100 where A is the number of seeds germinated in PEG solutions plus those that recovered to germinate in the deionized water, B is the number of seeds germinated in PEG solutions and C is the total number of seeds tested (Dechang *et al*., 2012). The final germination percentage was recorded as (A/C) * 100.

Statistical Analysis

Considering that the primed seeds at 15°C for 36 and 48 h were removed due to germination during priming, it was not possible to evaluate the interactions between temperature and duration of priming for all treatments. In addition, we aimed to compare the priming treatments with a control. Therefore, priming treatments as a factor with 18 levels (each priming treatment as a level) and drought stress was considered at two levels as the second factor. Analysis of variance (ANOVA; $P < 0.05$) for comparing treatment effects in both the factorial experiment was conducted in a completely randomized design (CRD**)**. Arcsine data transformation was performed on percentage data before analysis of variance to ensure homogeneity of variance (non-transformed data appear in all tables). All data were analyzed via SAS v.9.1. If the ANOVA showed significant effects, Duncan's multiple range test ($P <0.05$) was used to determine differences among treatments.

RESULTS

Drought Stress Test

Final Germination Percentage: Germination was significantly affected by drought stress in primed and unprimed seeds (F-Value = 2.21; P = 0.008). The final germination percentage (FGP) difference was clear between primed and unprimed seeds ($P< 0.05$). All priming treatments at -1.2 MPa stress level had significantly higher germination than unprimed seeds (Table 1). By increasing the stress level in all priming treatments and unprimed seeds, germination reduced significantly. However, this difference in germination was not statistically significant, because the unprimed seeds in both the levels of stress had lower germination. At -1.2 MPa, the best priming treatment (hydropriming at 10 °C for 12 h) had 56% germination which was 24 percent more than unprimed seeds ($P< 0.05$). But in -1.4 MPa both had 28% germination. At -1.4 MPa the highest FGP allocated to osmopriming with -0.4 MPa urea at 15 °C for 24 h (39%) and the lowest to urea -0.4 MPa in both at 10 and 15 °C (20%) for 12 h that were also less than the unprimed seeds ($P< 0.05$). Although it was not possible for time and

temperature to be considered as separate factors; however, the effects of interaction of temperature and priming duration were palpable. At -1.2 MPa the germination of hydroprimed seeds was at 10 °C significantly greater than 15 °C for 12 h (P< 0.05). In this type of priming with increasing temperature from 10 to 15 °C for 24 h germination declined, although not significant. With an increasing hydropriming duration from 12 to 24 hours, from 24 to 36 h and from 36 to 48 h, respectively, germination significantly decreased, unchanged and reduced insignificantly. In osmopriming with urea -0.4 MPa at all durations and urea -0.6 MPa for 12 h was not observed significantly different between two priming temperatures on FGP. However, in urea -0.6 MPa for 24 h, the germination of primed seeds at 10 °C was significantly higher than 15 °C (P < 0.05). At the potential of drought stress, FGP of primed seeds with urea -0.4 MPa for 24 h was more than 12 hours (P < 0.05). In addition, primed seeds with urea -0.6 MPa at 10 °C for 24 and 48 h in comparison with 15 °C, and also, other durations of priming had higher FGP (P < 0.05) (Table 1).

Germination Index: Germination index (GI) which represents germination rate, similar FGP with increasing level of drought stress in most priming treatments and was reduced to half (F-Value = 3; P = 0.0004). At all -1.2 MPa drought stress and at -1.4 MPa most of the priming treatments were greater germination index than the unprimed seeds. The highest GI was related to the urea -0.4 MPa for 36 and 48 h and urea -0.6 MPa for 48 h (GI=15) which were twice times the unprimed seeds (GI=7) in -1.2 MPa drought stress (P<0.05). The lowest GI belonged to the urea -0.4 MPa at 15 °C for 12 h in -1.4 MPa drought stress. Unprimed seeds with both of the potential of -1.2 and -1.4 MPa drought stress, had germination index (GI = 7) and (GI = 3.6) respectively (P<0.05). Highest GI in hydropriming belonged to duration of 48 h and in osmopriming urea -0.4 MPa for 36 and 48 h and -0.6 MPa for 48 h (Table 2).

Root Length: In both levels of drought stress, root length (RL) was reduced to a minimum in all priming treatments and the unprimed seeds (F-Value = 2.96; P = 0.0005). The effect of levels of drought stress on RL between priming treatments with control were hardly significant. The highest and lowest RL was assigned to hydropriming for 48 and 12 h at 10 °C in -1.2 MPa and -1.4 MPa drought stress respectively (P < 0.05) (Table 3).

Seedling Vigor Index: The interaction between priming treatment and drought stress on seedling vigor index (SVI) similar GI, FGP and RL was significant (F-Value = 21.92; P < 0.0001). With increasing levels of drought stress, SVI in unprimed seeds was reduced to half respectively (-1.2 MPa: SVI = 151, -1.4 MPa: SVI = 76) (P<0.05). In some priming treatments, with increasing drought stress the SVI to less than half and in some also dropped to half or even more than half. At -1.2 MPa drought stress in almost all priming treatments SVI were significantly higher than the unprimed seeds (P< 0.05). In this potential, maximum SVI obtained for osmopriming with -0.6 MPa urea for 48 h (SVI = 352). At -1.4 MPa, the highest and lowest SVI were assigned to -0.4 MPa urea at 15 °C for 24 h (SVI= 197) (which was two and a half times more than the control) and 12 h respectively (P< 0.05). The SVI greatest difference observed between control and priming treatments in -1.2 MPa drought stress (P< 0.05 With increasing drought stress level all priming treatments were unable to demonstrate its superiority compared to unprimed seeds (Table 4).

Seedling Dry Weight: It was not unexpected that with increasing the stress level, seedling dry

weight (SDW) was reduced (*F-Value = 1021.61; P < 0.0001*). However, in both stress levels, the most of priming treatments were higher SDW than the control. At the -1.2 and -1.4 MPa, the highest SDW was assigned to osmopriming -0.4 MPa urea at 15 °C for 24 h, which was twice more than the control (*P< 0.05*). And the lowest similar to other germination traits was related to priming with -0.4 MPa urea in both priming temperatures in -1.4 MPa drought stress (Table 5).

Recovery Test

The interaction between priming and drought stress were significant for all traits (recovery of germination percentage: *F-Value = 8.63; P < 0.0001,* final germination percentage: *F-Value = 7.91; P < 0.0001* and normal seedling percentage: *F-Value = 9.95; P < 0.0001*).

Recovery of Germination Percentage: The highest percentage of germination recovery (RGP) was assigned to osmopriming urea -0.6 MPa for 12 h at 15 °C (*RGP* = 100%) and the lowest for osmopriming urea -0.4 MPa for 36 (*RGP* = 16%) both in -1.4 MPa drought stress (*P< 0.05*). In most priming treatments with increasing potential of drought stress, The RGP showed an increasing trend. But the treatments that are bold in the table (Table 6) which the control is also included, RGP decreased with increasing drought stress. At in -1.2 MPa drought stress, primed seeds to hydropriming method for 24 h at 15 °C; had the RGP (91%). However, the difference with control (RGP = 85%) was not significant. This means that none of priming treatments on the stress level could not with more RGP to demonstrate their superiority compared to the control. But instead, both osmopriming urea -0.4 and -0.6 MPa; at the period of times of 12 and 24 h priming; in both temperatures 10 and 15°C; higher RGP were allotted to them (*P< 0.05*) (Table 6).

Final Germination Percentage: Fully percentage (100%) of the final germination percentage (FGP) followed by recovery was obtained for osmopriming urea -0.6 MPa at 15°C for 12 h in drought stress of -1.4 MPa (P< 0.05). The lowest FGP was observed in hydropriming for 24 h at 10 °C in the stress level of -1.2 MPa. By increasing the potential of stress, the FGP was reduced from 90 to 75 % in unprimed seeds (P< 0.05). The treatments that are bold in the table, FGP decreased with increasing levels of stress, though, this decrease was not significant in most of them. In other priming treatments, with increasing level of stress; FGP increased. At -1.2 MPa, most of priming treatments was lower FGP than the control that in some significant and some were non-significant; but at -1.4 MPa was inverted (Table 7).

Normal Seedling Percentage: In the majority of priming treatments with increasing levels of stress, normal seedling percentage (NSP) followed by recovery increased; but the downward trend was observed in the control and other priming treatments. The highest NSP (99%) was assigned to the priming treatment with the highest FGP (urea -0.6 MPa at 15 ° C for 12 h in -1.4 MPa stress level) (*P<0.05*). At -1.4 MPa drought stress, the lowest percentage of normal seedling was devoted to osmopriming urea -0.4 MPa for 36 h (*NSP* = 56%) Which was also lower than the control (*NSP* = 62%), however, were not statistically significant (Table 8).

TABLE 1. Comparison of final germination percentage (FGP) primed and unprimed seeds under different levels of drought stress. Drought stress was imposed by PEG 6000 solutions of two osmotic potentials: -1.2 and -1.4 MPa. *Agropyron elongatum* seeds were primed with zero (hydropriming), -0.4 and -0.6 MPa Urea solutions at 10 and 15 °C for 12, 24, 36 and 48 hours. PT: Priming temprature, PD: Priming duration.

		Hydro-Priming		Osmo-Priming (-0.4 MPa)		Osmo-Priming(-0.6 MPa)	
		Drought Stress					
PT(C°)	PD(h)	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa
10	12	56 (1.2) a	28 (1) l-m	43 (5.5) d-g	20 (2.5) o	44 (6.6) d-g	22 (4) no
	24	51 (1.5) bc	26 (2.1) mn	53 (4.6) a-b	30 (0) l-m	53 (1.2) a-b	23 (2.6) n
	36	50 (2.6) b-d	30 (1.2) l-m	51 (2) bc	35 (2.3) hj	44 (2.5) d-g	33 (0.6) jk
	48	44 (7.1) d-g	30 (2.6) l-m	48 (4.5) c-d	32 (2.1) j-l	52 (4) b	32 (5.3) j-l
15	12	36 (3.4) hj	25 (2.6) mn	47 (4) c-e	20 (0.6) o	46 (5.5) c-f	23 (4.7) n
	24	46 (6.1) c-f	25 (2.6) mn	52 (1.5) b	39 (8.2) gh	46 (6.2) c-f	26 (0.6) mn
Control (unprimed)		32 (2.5) j-l	28 (1.5) l-m				

Means (± SD) without same lowercases indicate statistically significant differences for final germination percentage (P

TABLE 2. Comparison of germination index (GI) primed and unprimed seeds under different levels of drought stress. Drought stress was imposed by PEG 6000 solutions of two osmotic potentials: -1.2 and -1.4 MPa. *A. elongatum* seeds were primed with zero (hydropriming), -0.4 and -0.6 MPa Urea solutions at 10 and 15 °C for 12, 24, 36 and 48 hours. PT: Priming Temprature, PD: Priming Duration.

		Hydro-Priming		Osmo-Priming (-0.4 MPa)		Osmo-Priming(-0.6 MPa)	
		Drought Stress					
PT(C°)	PD(h)	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa
10	12	12.7 (1.2) bc	5 (0) l-n	9.4 (0.6) e-h	4 (1) no	9.3 (1.2) e-h	4.4 (1.5) mn
	24	11.3 (1.5) c-e	5.3 (0.6) k-n	10.3 (0.6) d-g	5.3 (0.6) k-n	10 (1) d-g	4.4 (0.6) mn
	36	10.7 (1.2) c-f	5.3 (0.6) k-n	15 (1.7) a	6.4 (1.5) k-m	11.8 (0.6) cd	7.3 (0.6)h-
	48	14 (1) ab	7.3 (0.6) h-k	15 (1) a	9.3 (0.6) e-h	15.4 (1.2) a	7.7 (1.2) h-j
15	12	8.3 (1.5) g-j	4 (0) no	11.4 (1.2) c-e	2 (0)o	8.7 (0.6) f-i	3.4 (1.5) no
	24	8.7 (2.3) g-j	4.7 (1.5) mn	11.7 (2.1) cd	7 (1) i-l	11.7 (1.2) cd	5 (0) l-n
Control (unprimed)		7 (1) i-l	3.6 (2.1) no				

Means (± SD) without same lowercases indicate statistically significant differences for germination index (P <0.05).

TABLE 3. Comparison of Root length (cm) primed and unprimed seeds under different levels of drought stress. Drought stress was imposed by PEG 6000 solutions of two osmotic potentials: -1.2 and -1.4 MPa. *A. elongatum* seeds were primed with zero (hydropriming), -0.4 and -0.6 MPa Urea solutions at 10 and 15 °C for 12, 24, 36 and 48 hours. PT: Priming temprature, PD: Priming duration.

		Hydro-Priming		Osmo-Priming (-0.4 MPa)		Osmo-Priming(-0.6 MPa)	
		Drought Stress					
PT(C°)	PD(h)	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa
10	12	2.8 (0.3) d-j	1.6 (0.6) k	2.5 (0.5) f-j	2 (0.1) i-k	2.5 (0.4) g-j	2.3 (0.6) h-
	24	3.1 (0.6) b-h	2 (0) j-k	2.7 (0.5) d-j	2.7 (0.6) d-j	2.8 (0.4) d-i	2 (0.1) i-k
	36	3.6 (0.4) a-c	2.3 (0.6) h-k	3.4 (0.5) a-e	2 (0.1) i-k	2.9 (0.5) c-h	2.3 (0.6) h-
	48	4 (0.2) a	2 (0.1) i-k	3.4 (0.3) a-d	2.7 (0.6) d-j	3.8 (0.4) ab	2.8 (0.5) d-j
15	12	2.8 (0.4) d-h	2 (0.5) i-k	3.1 (0.3) b-h	1.7 (0.6) k	3.3 (0.3) a-f	2 (0.1) i-k
	24	3.3 (0.4) b-g	2.3 (0.6) h-k	2.6 (0.4) e-j	3 (0.1) b-h	3.1 (0.1) b-h	2 (0.1) i-k
Control (unprimed)		2.3 (0.1)h-k	2 (0.1) i-k				

Means (± SD) without same lowercases indicate statistically significant differences for Root length (P <0.05).

TABLE 4. Comparison of seedling vigour index (SVI) primed and unprimed seeds under different levels of drought stress. Drought stress was imposed by PEG 6000 solutions of two osmotic potentials: -1.2 and -1.4 MPa. *A. elongatum* seeds were primed with zero (hydropriming), -0.4 and -0.6 MPa Urea solutions at 10 and 15 °C for 12, 24, 36 and 48 hours. PT: Priming temprature, PD: Priming duration.

		Hydro-Priming		Osmo-Priming (-0.4 MPa)		Osmo-Priming(-0.6 MPa)	
		Drought Stress					
PT(C°)	PD(h)	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa
10	12	275 (20.4) bc	79 (6.1) mn	179 (18.2) hi	63 (5.7) n	212 (21.7) fg	79 (9.2) mn
	24	241 (27.7) de	95 (8.1) lm	249 (4.6) de	107 (2.3) l	234 (20.7) ef	65 (11.3) n
	36	236 (16.7) ef	99 (7.5) lm	333 (21.2) a	119 (0.6) l	212 (19.5) fg	107 (7.1) l
	48	296 (18.2) b	111 (1.1) l	265 (22.4) cd	143 (7) k	352 (12.7) a	176 (3.2) hi
15	12	172 (11.6) ij	73 (8.1) mn	283 (31.6) bc	38 (3.8) o	208 (18) g	64 (6) n
	24	198 (6.5) gh	77 (4) mn	239 (16) e	197 (20.8) g-i	330 (12.1) a	73 (1.7) mn
Control (unprimed)		151 (14.7) jk	76 (6.5) mn				

Means (± SD) without same lowercases indicate statistically significant differences for seedling vigour index (P <0.05).

TABLE 5. Comparison of seedling dry weight(mgr) primed and unprimed seeds under different levels of drought stress. Drought stress was imposed by PEG 6000 solutions of two osmotic potentials: -1.2 and -1.4 MPa. *A. elongatum* seeds were primed with zero (hydropriming), -0.4 and -0.6 MPa Urea solutions at 10 and 15 °C for 12, 24, 36 and 48 hours. PT: Priming temprature, PD: Priming duration.

		Hydro-Priming		Osmo-Priming (-0.4 MPa)		Osmo-Priming(-0.6 MPa)	
		Drought Stress					
PT(C°)	PD(h)	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa
10	12	1.8 (0.1) a	0.8 (0.01) i-k	0.9 (0.1) ij	0.4 (0.1) pq	1.1 (0.1) fg	0.6 (0.03) m-o
	24	0.9 (0.03) hi	0.6 (0.1) no	1.2 (0.05) d-f	0.7 (0.03) k-n	1.1 (0.04) fg	0.9 (0.1)ij
	36	1 (0.1) gh	0.9 (0.1) ij	1.3 (0.1) c	0.6 (0.1) l-n	1.5 (0.04) b	0.7 (0.1) l-n
	48	1.3 (0.01) cd	0.7 (0.03) k-m	0.9 (0.05) h-j	0.7 (0.02) k-m	1.3 (0) c	1.1 (0.1) ef
15	12	0.7 (0.1) k-m	0.4 (0.1) pq	1.2 (0.1) d-f	0.3 (0.03) q	0.9 (0.1) hi	0.8 (0.1) j-l
	24	1 (0.01) gh	0.7 (0.05) k-m	1.3 (0.1) c-e	1.2 (0.03) d-f	1.3 (0.1) cd	0.6 (0.1) mn
Control (unprimed		0.8 (0.1) i-k	0.5 (0.03) op				

Means (± SD) without same lowercases indicate statistically significant differences for seedling dry weight (P <0.05).

TABLE 6. Comparison of recovery of germination percentage primed and unprimed seeds under different levels of drought stress. After 5 days of exposure in PEG 6000 solutions; seeds were rinsed with distilled water and then were placed in new petri dishes with two pieces of germination paper moistened with distilled water, and incubated under the same conditions (25ºC) for additional 21 days to study the recovery of germination. Priming treatments and PEG solutions similar to the first experiment. PT: Priming Temprature, PD: Priming Duration.

		Hydro-Priming		Osmo-Priming (-0.4 MPa)		Osmo-Priming(-0.6 MPa)	
		Drought Stress					
PT(C°)	PD(h)	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa
10	12	64 (4.3) h-m	87 (1.9) b-e	54 (3.7) k-n	95 (9.4) b	79 (1.7) d-h	92 (1.6) bc
	24	42 (6.3) n-o	75 (9.1) e-i	66 (12.4) g-k	68 (12.8) f-k	76 (3.1) e-i	93 (11.3) bc
	36	45 (3.2) m-o	68 (3.9) f-k	**35 (1.2) o** →	**16 (6.9) p**	67 (5.3) g-k	67 (2.6) g-k
	48	**74 (9.8) e-j** →	**66 (5.2) h-l**	**70 (1.8) f-k** →	**62 (3.9) h-m**	**62 (3) h-m** →	**47 (1.6) l-o**
15	12	73 (2.4) e-k	88 (0) b-e	83 (6.8) c-g	93 (0.3) bc	90 (13.6) bc	100 (0) a
	24	**91 (4.2) b-d** →	**58 (3.2) i-n**	84 (6.3) c-f	92 (3.5) bc	**66 (1.9) h-l** →	**56 (4) j-n**
Control (unprimed)		**85 (1.5) c-f** →	**74 (7.1) e-j**				

Means (± SD) without same lowercases indicate statistically significant differences for germination percentage (P <0.05).

TABLE 7. Comparison of final germination percentage primed and unprimed seeds under different levels of drought stress. After 5 days of exposure in PEG 6000 solutions ; seeds were rinsed with distilled water and then were placed in new petri dishes with two pieces of germination paper moistened with distilled water, and incubated under the same conditions (25ºC) for additional 21 days to study the recovery of germination. Priming treatments and PEG solutions similar to the first experiment. PT: Priming Temprature, PD: Priming Duration.

		Hydro-Priming		Osmo-Priming (-0.4 MPa)		Osmo-Priming(-0.6 MPa)	
		Drought Stress					
PT(C°)	PD(h)	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa
10	12	73 (3.2) g-l	88 (1.7) c-f	62 (1.2) j-m	96 (8.4) b	83 (0.8) c-g	93 (1.3) b-d
	24	53 (5.2) m	79 (8) e-h	**78 (10) e-i** →	**73 (11) f-l**	83 (2.3) c-g	95 (9.3) b
	36	61 (2.9) k-m	73 (3.2) g-l	**76 (0) f-k** →	**61 (20) l-m**	**82 (4.5) d-g** →	**79 (2.1) e-i**
	48	**85 (7.5) c-g** →	**80 (4.3) e-h**	**86 (1.6) c-g** →	**76 (4) f-k**	**85 (4) c-g** →	**77 (4.8) e-k**
15	12	78 (1.4) e-j	90 (0) b-e	87 (6.1) c-f	94 (0) bc	90 (10) b-d	100 (0) a
	24	**93 (3.4) b-d** →	**66 (2.4) h-m**	89 (4.7) b-e	93 (3.4) b-d	**75 (2) f-l** →	**63 (2.9) i-m**
Control (unprimed)		**90 (1.9) b-e** →	**75 (7.5) f-k**				

Means (± SD) without same lowercases indicate statistically significant differences for germination percentage (P <0.05).

TABLE 8. Comparison of normal seedling germination primed and unprimed seeds under different levels of drought stress. After 5 days of exposure in PEG 6000 solutions ; seeds were rinsed with distilled water and then were placed in new petri dishes with two pieces of germination paper moistened with distilled water, and incubated under the same conditions (25ºC) for additional 21 days to study the recovery of germination. Priming treatments and PEG solutions similar to the first experiment. PT: Priming temprature, PD: Priming duration.

		Drought Stress					
PT(C°)	PD(h)	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa	-1.2MPa	-1.4MPa
10	12	63 (2.9) k-o	82 (3) d-j	54 (1.1) no	93 (11) b	77 (2) e-k	85 (0.9) c-g
	24	50 (3.4) o	75 (4.7) e-l	70 (6.3) i-m	60 (8.3) l-o	80 (4.6) e-j	92 (1.4) b-d
	36	56 (2.3) m-o	70 (2.5) i-m	69 (0.7) j-n	56 (4.6) m-o	78 (4.1) e-k	73 (1.9) f-l
	48	83 (4.8) c-i	72 (5.1) g-l	79 (3.5) e-j	71 (5.7) h-m	78 (2.7) e-k	73 (3.2) f-l
15	12	71 (1.9) h-m	86 (0) c-f	85 (7.1) c-f	93 (1.3) bc	88 (11) b-d	99 (1.4) a
	24	92 (2.1) b-d	61 (2.9) l-o	84 (4.7) c-h	87 (2.6) b-e	68 (4.3) j-n	61 (2.4) l-o
Control (unprimed)		86 (1.6) c-f	62 (4.7) l-o				

Means (± SD) without same lowercases indicate statistically significant differences for germination percentage (P <0.05).

DISCUSSION

Seed germination is a fundamental process in the plant life cycle and start of living in natural and agricultural ecosystems and the basis of agricultural

crop production (Weitbrecht *et al*., 2011). Seed germination in arid and semi-arid regions depends greatly on environmental conditions after rainfall (Laity, 2008) and rapid germination is considered as an important factor. Thus, the use of high quality seeds; is considered one of the pillars of the success in these areas. Seed priming is applied as a method to improve the physiological quality of seeds for a long time. This technique can also be involved in improving the system's response to stress, for example, the osmopriming may play a role in stress-induced protein aggregation, such as the late embryogenesis abundant proteins (LEA), heat-shock proteins (HSPs) (Gallardo *et al*., 2001; Soeda *et al*., 2004; Cortez-Baheza *et al*., 2008; Catusse *et al*., 2011; Chen, 2011). Indeed, many of the activities associated with initiation of germination, such as energy metabolism, fluidity of the basic food supply, development of the embryo, endosperm weakening (Chen, 2011), accelerated glucose metabolism (Sun *et al*., 2010) and strengthen the antioxidant system (Gallardo *et al*., 2001; Chen, 2011; Chen & Arora, 2011) occur during priming. Hydro and osmopriming methods, comprise methods that are common for many years and has been widely considered by many researchers. The investigation reviews (Ashraf & Foolad, 2005; Farooq *et al*., 2006; Sun *et al*., 2010; Rouhi *et al*., 2011; Moradi *et al*., 2012) show that, hydropriming can be effective in increasing resistance to drought stress at germination stage. Ahead study also reflects this result. As mentioned in the results section, this type of priming at 10 °C at all times during priming; most indices of germination significantly improved compared to unprimed seeds. Srivastava *et al*.(2010) with studying of the Indian mustard seed (*Brassica juncea* L.), expressed hydropriming treatment effectively give discounts effect of drought and salinity stress compared to osmopriming and need to increase the antioxidant metabolism and accumulation of osmolyte decreased, which may positively change the epigenetic level in the early stages of seed treatment "stress imprint" is attributed which probably plays an important role in enabling the modulation of gene expression. Gallardo *et al*. (2001) also confessed to effect of hydropriming in increasing the level for kind of catalase (type 1 protein) and type 11 and 12 proteins in Arabidopsis. There are also several reports of osmotic priming, including the urea osmopriming, which apart from osmotic effects, is or has the nutritional positive effects on germination. As a result, it can be effective in improving seed germination under stress conditions (Al-Mudarsi & Jutzi, 1999; Mauromicale & Cavallaro, 1996). Moradi *et al*. (2012) reported in their study, priming with urea compared to priming with PEG was more effective in enhancing drought tolerance. In the present study also seeds treated with urea osmopriming at both temperatures and all times of priming compared to unprimed seeds in the stress level of -1.2 MPa, the germination percentage, germination index and seedling vigor index were improved. Several metabolic pathways and cellular events have been investigated in order to understand the physiology of osmopriming and its association with stress tolerance including cell division and expansion, induction of stress-responsive proteins (HSPs and LEA), activity of ATPase, plasma membrane fluidity, changes in antioxidant systems and changes in the proteome and transcriptome (Chen & Aora, 2011). Chen and Aora (2011) also the effect of osmopriming in the enhancement of stress tolerance of germinating seeds of *Spinacia oleracea* have been attributed to increase the potential of

germination of primed seeds mediated by antioxidant systems. They stated that antioxidant activities of germinating seeds were strongly associated with physiological status and radicle length. Gallardo *et al*. (2001) were also considered proteins of types 9 and 10 in *Arabidopsis* primed seeds specific to osmopriming, but in proteins of types of 7 and 8 accumulation pattern were similar in osmo and hydropriming. Sun *et al*.(2010) also reported that, after osmo and hydropriming Malondialdehyde (MDA) levels in rice seeds reduced under stress, additionally, glucose metabolism was accelerated, proline levels increased and superoxide dismutase (SOD) and catalase (CAT) activity improved. However, in our study, by increasing the potential of drought stress, few priming treatments (urea osmopriming for 24 and 36 h in 15 and 10 °C, respectively) have been able to maintain their superiority. Although we did not assess our treatment from physiological aspects. It appears that priming techniques can partially be effective on stress resistance and if the stress threshold is slightly higher than expected (this threshold for the *Agropyron* seeds in this study was -1.2 MPa) cannot have a noticeable effect on resistance to drought stress and can even be harmful too. It seems that in addition to priming conditions, kind of stress, intensity and duration of stress; the effectiveness of priming treatments be affected. In most of studies, drought stress at germination stage, the effects of a constant stress have been considered during germination, while in most of the seed beds; these stresses are fluctuating and the ability of the seedling to overcome stress may be important (Whalley *et al*., 2001). Intensity and duration of stress can be influenced subsequent growth of the seedlings. As in this study, all treatments which were placed in stressful situations and then moved to fresh water, showed a variety of recovery responses. The percentage of germination and normal seedling percentage were not exception in this case. Despite the drought stress was temporary, but the power of germination in most of the priming treatments and control was reduced after transferring to the fresh water and germination percentages of recovered seeds were lower than seeds that had germinated in fresh water without early exposure to stress conditions. As we viewed, primed seeds with a solution of -0.6 MPa urea for 12 h at 15 °C and followed by PEG solutions (-1.2 and -1.4 MPa) for 5 days and subsequently moved to fresh water conditions, primed seeds had higher performance compared to unprimed seeds ($P<0.05$). In many of priming treatments by increasing the potential of drought stress; on RGP, FGP and NSP were added. However, in the control and some priming treatments reduced, which in both hydro and osmopriming for 48 hours was obvious example. Whalley *et al*. (2001) also in their study on onion (*Allium cepa* L.) found that seeds exposed to higher water potential to lower potential had higher recovery percentage. Vulnerability to high concentrations of PEG followed by recovery less than lower concentrations. It is generally understood that seeds gradually lose their desiccation tolerance (Whalley *et al* 2001), that probably part of during priming and the remaining in the process of germination and radicle emergence loses. It seems that high concentrations induce a state of quiescence, and this may suggest an important adaptation for growth in arid and semi-arid environments. This is confirmed by the observations that primed seeds with higher osmotic potential and short-term priming by placing in severe drought stress conditions, keep better their desiccation tolerance and remain in a state of

quiescence, after removing the stress will be higher germination.

CONCLUSION

In arid and semi-arid areas, regions, erratic rainfalls and their intervals are variable. Therefore, in the rangelands modification programs in these regions; using a combination of primed seeds with priming different materials and unprimed seeds to create a collection such as a natural seed bank. Thus, in each period of rainfall; a series of the seeds germinate and the plant survival is preserved during the year. But one of the challenges that exist in priming techniques on how to choose the material, temperature and duration of priming proportional with the situation of the plant of case study and its standardization and another understanding of the molecular mechanisms of these experiments.

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