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Investigation the effect of osmopriming and hydropriming on germination behaviour of alfalfa (*Medicago sativa*) and maize (*Zea mays*)

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Abstract

The present study was performed to investigate the effect of hydropriming and osmopriming on the germination of alfalfa (*Medicago sativa*) and maize (*Zea mays*) seeds in 2013 at the College of Agriculture and Natural Resources of Islamic Azad University of Karaj. In this study, factorial experiment was used in a completely randomized design with four replications. The osmopriming treatments were studied in five concentration level of PEG solution (0%, 25%, 50%, 75%, 100%) with potentials of 0.021, -0.045, -0.071, -0.097, -0.024, -0.051, -0.078, -0.102, -0.022, -0.048, -0.074 and -0.099 MPa, while hydropriming treatments were studied with distilled water. The results showed that there was a higher germination percentage in PEG solutions (osmopriming) under various concentrations in comparison with priming with distilled water (hydropriming). Therefore the highest germination percentage of seeds was observed in priming with polyethylene glycol (PEG) and the least one in hydropriming. The results revealed that priming treatments had significant influence on all traits. The higher germination percentage for osmoprimed seeds goes back to the controlled delivery of water to seeds while in hydroprimed ones, the seed coat bursts due to the non-controlled absorption of water by the seed, its inside metabolic substances leak out and the fungi and microorganisms are activated. Our findings showed that the higher germination percentage in the osmopriming treatments with PEG indicate the osmotic effects of this solution on the germination of the seeds.

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Introduction

Osmotic seed preparation defines as controlled dehydration of seeds in an osmotic solution. This dehydration enables metabolic activities prior to the germination, while it is insufficient for the emergence of radicle out of seed coat (Bradford, 1986). This is a common treatment method before planting seeds which, especially under environmentally undesirable conditions, increases the rate, percentage and uniformity of germination and emergence of seeds or seedlings (Demir and Mavi, 2004). Its overall mechanism is described as that during the preparation of osmotic seeds, there would be initiated a transfer of storage substances, activation and synthesis of several enzymes, synthesis of DNA and RNA, ATP production and an improvement in their cytoplasmic membrane (Bray, 1995). According to Fu *et al.* (1988), after treatment we would observe rapid growth of the fetus by the removal of barriers to germination. Some of the biochemical and physiological changes which occur in seeds during osmotic preparation or upon its completion are as macromolecules synthesis, the activity of several enzymes, increased vigor and dormancy breaking. In order to osmotically prepare seeds among different species, varieties and even seed masses, there exists different needs in terms of the composition and osmotic potential of solution, the temperature and duration of treatment, and that the response of seeds to the osmotic preparation largely depends on the osmotic potential of the related solution. In addition the duration of treatments is of importance and has been reported for some plants (Bradford, 1986). There are numerous priming methods including hydropriming (water absorption), halopriming (absorption in inorganic salts solutions), thermopriming (seed treatment under high or low temperature), osmopriming (absorption in various organic osmotic solutions), solid matrix priming (seed treatment with solid matrices) and biopriming (hydration by utilizing biological compositions). To create artificial environments to control water potential, usually solids with high molecular mass are used which do not affect tissue uptake and that are not being absorbed into the plant. Among priming methods, osmopriming is of considerable importance within which water is provided to seed under significant control by using substances such as polyethylene glycol (PEG), potassium nitrate (KNO_3), etc. Osmopriming points to water absorption by seed in sugar, polyethylene glycol (PEG), glycerol, sorbitol or mannitol solution which makes the seed hydrated prior to planting. The low water pressure in treatment solution provides a limited possibility for hydration so that metabolic activities are started prior to the germination but inhibits the germination (Sivritepe and Dourado, 1995). Pretreatment of seeds with salts solutions or different osmotic potentials (osmopriming or halopriming) is an easy, low cost and low risk method which is considered as a common strategy for increasing the rate and uniformity of germination, emergence of seeds and qualitative and quantitative improvement of crop under undesirably environmental conditions and could increase tolerance of plants against salinity stress (Strogonov, 1964; Rehman *et al.,* 1995; Cayuela *et al.,* 1996; Guzmán and Olave, 2004; Iqbal *et al.,* 2006). The priming of pepper seeds with polyethylene glycol 6000 under 1 MPa potential at a temperature of 20 °C increased the overall germination percentage and the priming increased the uniformity and rate of germination and also the emergence of seeds (Demir *et al.,* 1999). Osmotic potential of polyethylene glycol solution increases the rate, uniformity and percentage of germination of various primed seeds compared with unprimed ones and seed priming in osmotic solution increases the water amount being absorbed by the seed and eventually boosts the rate of germination of the seeds and the growth of the radicle and shoot (Michel and Kaufmann, 1973). Based on the studies conducted on onion and tomato seeds under priming conditions, it was determined that priming onion seeds with PEG at the osmotic potential of -1.6 MPa for 14 days has a significant effect on seed germination and that for tomato seeds, 18 days of soaking in a solution with an osmotic potential of -10 MPa is suitable. The reason was attributed to the increase and uniformity of rate and percentage of germination in primed seeds (Haigh *et al.,* 1986). Passam and Kakouritis (1994) reported

that the treatment of cucumber seeds with 0.7 gr mannitol in dark at 25 °C for 3 days increased the germination rate, radicle development, seedling emergence and the development of the first leaf but didn't have any significant effect on the growth rate of the second leaf or the photosynthetic activity of the first or the second leaf. Priming seeds using PEG 6000 at a potential of -15 Bar at 16 °C for 4 days yields an increase of germination and that osmotic potential is an influential factor on priming (Madakazole and Smith, 2000). Furthermore hydropriming improves seedling establishment and vigor of crop plants which in turn yields accelerated growth, flowering, maturity and yield. Hydropriming raises the tolerance of plants against dryness and decreases the damages caused by pests due to the accelerated emergence of seedlings out of soil (Harris *et al.,* 1999). In hydropriming method, seeds are treated with pure water and without any chemical substance which means a simple and inexpensive method. In this method water absorption is controlled by the time seeds are in contact with water. Also radicle emergence is prevented by decreasing this time and treating at low temperatures (Fujikura *et al.,* 1993). Hydropriming causes improved establishment of seedling and boosts the vigor of corn, rice and chickpea seeds which in turn accelerates growth, flowering, maturity and yield. In addition this method heightens the tolerance of plants against dryness and lowers their vulnerability against pests and diseases (Harris *et al.,* 2000). Various reports indicated that hydropriming causes an increase of percentage, rate and uniformity of germination and emergence of seeds (Afzal *et al.,* 2002).

It was reported that hydropriming increases the percentage and rate of the germination of sunflower seedlings and their dry weight under dry conditions and also decreases their abnormal seedlings (Demir Kaya *et al.,* 2006). Under this treatment, metabolic activities of germinations are stimulated and reaches each other at appoint and this created balance improves the rate of germination, uniformity of the emergence of bushes, germination under diverse environments, vigor improvement and accelerated seedling growth (Artola *et al.,* 2003). But there exists conflicting results. Tylkowska and Van Denbulk (2001) reported that hydropriming significantly decreased the germination percentage of two varieties of carrot. The cause was attributed to the leakage of metabolic substances from the seeds and the expansion of the activities of micro-organisms and fungi, or the imposition of premature aging on the living parts of the carrot seeds and the negative effect created by this treatment on the seed mass.

This study was designed and implemented in 2013 to investigate the germination behavior of alfalfa and maize seeds under osmopriming and hydropriming treatments and to find effective alternatives to increase percentage and rate of germination of these seeds.

Materials and Methods

Experimental design

The seeds of crop plants being used in this study were alfalfa (*Medicago sativa*) and maize (*Zea mays*) seeds provided from Seed and Plant Improvement Institute of Karaj (SPII). In this regard an experiment based on factorial and in a completely random design was conducted with 4 replications. The factors included the levels of PEG (0, 0.021, -0.045, -0.071, -0.097, -0.024, -0.051, -0.078, -0.102, -0.022, -0.048, -0.074 and -0.099 MPa) which zero was the control treatment, under the concentrations of 0, 25, 50, 75 and 100%. To create the osmotic potential levels, PEG 6000 was used. The solutions were prepared according to the below equation and by solving appropriate amounts of PEG 6000 in distilled water (Michel and Kaufmann, 1973).

$$S = -(1.18\times10^{-2})C - (1.8\times10^{-4})C^2 + (2.67\times10^{-4})CT + (8.39\times10^{-7})C^2T$$

Seed preparation and priming

In order to apply the osmopriming, alfalfa and maize seeds were put into the PEG solutions with different concentrations for 24 hours at 20±2 °C. After this stage the seeds were washed with distilled water three times and were dried in dark at 23±2 °C (Noumani *et al.,* 2012). After being dried, we placed 25 seeds in

each petri dish between two filter papers and then 7 mL of distilled water was added to each unit. The petri dishes were put into the growth chamber at alternating temperature of 18/25 °C day/night.

Measurements

The germinated seeds were counted on a daily basis and at specific times and the traits related to germination and seedling growth were calculated for 14 days. The percentage of germination was calculated based on below formula (Chung *et al.,* 2001).

$$GP = (\frac{N_i}{S}) \times 100$$

In this equation, GP holds for germination percentage, Ni for the number of the seeds germinated in day i, and S is the total number of the seeds being cultivated. Finally after conducting a preliminary analysis on the data and their dispersion, SAS 9.1 statistical software was used to analyze the data. To compare the means, Duncan's multiple range tests were utilized and Excel software was of use in drawing diagrams.

Results and discussion

Analysis of variance

In Tables 1 and 2, you could see the effects of polyethylene glycol (PEG) on the germination percentage, radicle and shoot lengths, and wet and dry weights of the seeds of alfalfa and maize. The variance analysis showed that the germination percentage, radicle and shoot length, and wet and dry weight of the seedlings were statistically under the influence of PEG and different concentrations. The effect of these two was at the level of 1% (Table 1).

Table 1. Analysis of variance for maize characteristics.

S.O.V.	d.f.	Mean Square (M.S.				
		Germination Percentage	Shoot Length	Root Length	Seedling Wet Weight	Seedling Dry Weight
PEG	2	33.06	197.16**	176.1**	0.0021**	0.0000219**
Concentration	4	2358.93**	2477.40 **	258.4**	0.0275**	0.0002753**
Interaction	8	33.73	37.18**	29.73**	0.0004**	0.0000041**
Error	45	30.66	6.17	6.17	0.0030	0.0000006
C.V.	-	8.75	5.69	6.27	7.86	6.59

* and **: significant differences at 0.05 and 0.01 probability level respectively.

Table 2. Means Comparison for maize characteristics.

Treatment / Traits		Germination Percentage (%)	Shoot Length (mm)	Root Length (mm)	Seedling Wet Weight (g)	Seedling Dry Weight (g)
Concentration (%)	PEG (MPa)					
100	-0.097	71^{a}	23.09^{e}	17.09^{e}	0.076^{e}	0.0056^{e}
	-0.102	71^{a}	25.27^{e}	19.27^{e}	0.084^{e}	0.0064^{e}
	-0.099	74^{a}	22.64^{e}	16.64^{e}	0.075^{e}	0.0055^{e}
75	-0.071	70^{ab}	37.60^{d}	31.60^{d}	0.125^{d}	0.0105^{d}
	-0.078	69^{ab}	335.96^{d}	29.96^{d}	0.119^{d}	0.0099^{d}
	-0.074	69^{ab}	377.04^{d}	31.04^{d}	0.0.123^{d}	0.0103^{d}
50	-0.045	69^{ab}	38.94^{d}	32.94^{d}	0.129^{d}	0.0109^{d}
	-0.051	69^{ab}	47.26^{c}	41.26^{c}	0.157^{c}	0.0137^{c}
	-0.048	68^{ab}	45.52^{c}	39.52^{c}	0.151^{c}	0.0131^{c}
25	-0.021	72^{a}	45.79^{c}	39.79^{c}	0.152^{c}	0.0132^{c}
	-0.024	71^{a}	56.68^{b}	50.68^{b}	0.188^{b}	0.0168^{b}
	-0.022	61^{b}	55.47^{b}	49.47^{b}	0.184^{b}	0.0164^{b}
0	0	39^{c}	54.77^{b}	48.77^{b}	0.182^{b}	0.0162^{b}
	0	39^{c}	64.55^{a}	58.55^{a}	0.215^{a}	0.0195^{a}
	0	37^{c}	63.56^{a}	57.56^{a}	0.211^{a}	0.0191^{a}

Means within a column followed by the same letters are not significantly different at the %1 level according to Duncan's multiple range test.

Table 3. Analysis of variance for alfalfa characteristics.

S.O.V.	d.f.	Mean Square (M.S.)				
		Germination Percentage	Shoot Length	Root Length	Seedling Wet Weight	Seedling Dry Weight
PEG	2	13.95	7.32	7.32	0.0000081	0.00000001
Concentration	4	996.98**	2728.74**	2728**	0.030318**	0.0000302**
Interaction	8	26.78	4.25	4.25	0.0000048	0.00000003
Error	45	36.10	8.71	8.71	0.0000097	0.00000001
C.V.	-	9.30	5.45	6.40	5.45	6.39

* and **: significant differences at 0.05 and 0.01 probability level respectively.

Table 4. Means Comparison for alfalfa characteristics.

Traits / Treatment		Germination Percentage (%)	Shoot Length (mm)	Root Length (mm)	Seedling Wet Weight (g)	Seedling Dry Weight (g)
Concentration (%)	PEG (MPa)					
100	-0.097	73^{a}	34.52^{e}	26.52^{e}	0.0115^{e}	0.00088^{e}
	-0.102	69^{a}	33.48^{e}	25.48^{e}	0.0111^{e}	0.00085^{e}
	-0.099	73^{a}	37.78^{e}	29.78^{e}	0.0125^{e}	0.0009^{e}
75	-0.071	64^{a}	44.22^{d}	36.22^{d}	0.0147^{d}	0.0012^{d}
	-0.078	65^{a}	44.14^{d}	36.14^{d}	0.0147^{d}	0.0012^{d}
	-0.074	65^{a}	44.42^{d}	36.42^{d}	0.0148^{d}	0.0012^{d}
50	-0.045	72^{a}	54.92^{c}	46.92^{c}	0.0183^{c}	0.0015^{c}
	-0.051	72^{a}	53.72^{c}	45.72^{c}	0.0179^{c}	0.0015^{c}
	-0.048	67^{a}	54.44^{c}	46.44^{c}	0.0181^{c}	0.0015^{c}
25	-0.021	68.25^{a}	63.72^{b}	55.72^{b}	0.0212^{b}	0.0018^{b}
	-0.024	68^{a}	63.08^{b}	55.08^{b}	0.0210^{b}	0.0018^{b}
	-0.022	65^{a}	62.77^{b}	54.77^{b}	0.0209^{b}	0.0018^{b}
0	0	50^{b}	73.02^{a}	65.02^{a}	0.0243^{a}	0.0021^{a}
	0	45^{b}	73.16^{a}	65.16^{a}	0.0243^{a}	0.0021^{a}
	0	52^{b}	74.22^{a}	66.22^{a}	0.0247^{a}	0.0022^{a}

Means within a column followed by the same letters are not significantly different at the %1 level according to Duncan's multiple range test.

Effect of priming on germination characteristics

The maximum germination percentage was obtained at the concentration of 100% with osmotic potential of -0.99 MPa which was equal to 74% and the least one was attributed to the control treatment as 37%. By increasing the concentration, we observed a reduction in the length of shoot, radicle, wet and dry weight of the seedlings but an increase for the controlled treatments. Specifically saying, the minimum of the mean values for the length of shoot, radicle, wet and dry weight of the seedlings were 22.64, 16.64, 0.075 and 0.0055 and the maximum ones were 64.55, 58.55, 0.215 and 0.0195, respectively. Baker *et al.* (1980) studied the germination and emergence of foxtail millet seedlings under osmotic conditions and observed the highest germination in potential of -12 Bar. They claimed that priming increases water absorption, the percentage and rate of germination, and the growth of radicle and shoot. Michel and Kaufmann (1973) investigated the osmotic potential of PEG solution for various seeds and were able to increase the percentage, rate and uniformity of the germination in primed seeds compared with unprimed ones. They said that priming of seeds in osmotic solution increases water absorption by seed and in turn boost the rate of germination of seed, and the growth of radicle and shoot. In Table 3 and 4, you could see the effect of PEG on the germination percentage, the radicle length, the shoot length, the dry weight and the wet

weight of alfalfa and maize seeds. The variance analysis indicated that the germination percentage, the radicle length, the shoot length, the dry weight and the wet weight of the seedlings were statistically affected by the concentration of PEG at a level of 1% (Table 3). The highest germination percentage was obtained at the concentration of 100% which was equal to 73% and the least one was observed for the control treatment (concentration of 0%) as 45%. The concentration and germination percentage were in direct relationship with each other but increasing the concentration yielded decrease of the length of radicle, shoot, wet and dry weight of the seedlings so that these traits were respectively equal to 33.48, 25.48, 0.0111 and 0.0009 in the highest concentration (100%0 and equal to 74.22, 66.22, 0.0247 and 0.002 in the lowest concentration (control). Madakazole and Smith (2000) showed that priming seeds with PEG 6000 in potential of -15 Bar at 16 °C for 4 days increases germination and declared the effectiveness of osmotic potential on priming.

Conclusion

The results of our study revealed that polyethylene glycol (PEG) has a stimulating effect on the germination of seeds and the growth of alfalfa and maize seedlings, although this effect was of varying degree in the different seeds studied. The findings indicated that the influence of PEG on germination percentage and seedling growth is related to osmotic effects. The higher germination percentage in osmoprimed seeds attributes to the controlled delivery of water into seed while in hydroprimed seeds, due to the uncontrolled absorption of water by seed, seed coat burst, the metabolic substances inside it leaked out and the fungi and microorganisms became activated.

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