



EFFECT OF SEED OSMOPRIMING ON SEED GERMINATION BEHAVIOR AND VIGOR OF SOYBEAN (*Glycine max* L.)

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ABSTRACT

In order to evaluate the effect of seed Osmopriming by using PEG6000 priming media on germination behavior and seed vigor of soybean (cultivar 033) an experiment was conducted at Seed analysis laboratory of agricultural and natural resources center of Sari, Iran. Seeds were primed with six levels of Poly ethylene glycol (PEG6000) as priming media (distilled water as control, -0.4, -0.8, -1.2, -1.6 and -2 MPa) for 6, 12, 24 and 48 hours at 25°C. Experimental units were arranged factorial in a completely randomized design with three replications. Dry soybean seeds considered as a control treatment (non primed). Results of variance analysis made clear that different osmotic potential and priming duration had significant effect on germination percentage, mean germination time, germination index, and the time to get 50% germination, seed vigor and electrical conductivity of seeds. Also -1.2 MPa osmotic potential increased germination percentages, germination index and seed vigor meanwhile decreased mean germination time, the time to get 50% germination and electrical conductivity of seeds. Also it was observed that 12 h priming duration had most effect on studied traits as -1.2 MPa osmotic potential treatment. Generally primed seeds showed better condition than control treatment in aspect of studied criteria.

Keywords: Soybean, seed priming, priming duration, germination, osmotic potential.

INTRODUCTION

Oil seeds are very sensitive to the harsh environmental conditions. It is hypothesized that their oil content readily oxidize, which deteriorate the seed health in storage (Kausar *et al.*, 2009).

One of the most important aspects for oil seed production is rapid emergence and good seedling establishment in field. In the other hand germination and emergence are important issues in plant production and they have significant effect on the next stages of plant growth in field. Rapid and uniform field emergence is essential to achieve high yield with having good quality and quantity in annual crops (Yari *et al.*, 2010).

Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, particularly seeds of vegetables and small seeded grasses (Dell Aquila and Tritto, 1991; Donaldson *et al.*, 2001). In fact, this technique is a treatment that applied before germination in a specific environment that seeds are partially hydrated to a point where germination processes begin but radical emergence does not occur (Dell Aquila and Tritto, 1991; Giri and Schilinger, 2003; Kaur, 2002). In the other hand on seed priming the amount of water absorption is controlled so as necessary metabolic activities occurred for germination but radical emergence is prohibited. This technique used for improvement of germination speed, germination vigor, seedling establishment and yield (Talebian *et al.*, 2008; Bodsworth and Bewley, 1981). Improvement in priming is affected by some factors such as plant species, water potential form priming factor, priming duration, temperature, vigor and seed primed storage condition (Mubshar *et al.*, 2006). There is different treatment for seed priming which Polyethylene glycol (PEG) is one of the most prevalent treatment. This treatment dose not have any physiological

responses to seed and in order to having more molecular weight can not pass the cell wall and so it be used for water potential adjustment in germination experiments (Seyed Sharifi and Seyed Sharifi, 2008; Emmerich and Hardgree, 1990).

There are more reports about seed priming effects on different plants. It was reported that soybean seed priming made better seedling emergence and yield improvement (Arif *et al.*, 2008). Moradi Dezfuli *et al.*, (2008) revealed hydro primed seeds for 36 h had lowest values (T50 and MGT), followed by 24 h and 48 h seed treatments, whereas the lowest germination rate that was indicated by higher T50 and MGT was observed in osmoprimed seeds which were subjected to PEG-6000 solution and also Maximum final germination was seen in seed hydro primed for 36 h, which was statistically similar to seeds of cv. MO17 which was hydro primed for 48 h and seeds of cv. B73 which was hydro primed for 24 h. Ghiyasy *et al.*, (2008) declared osmopriming of maize (*Zea mays* L.) seeds with polyethylene glycol 8000 (PEG 8000) at -0.5 MPa osmotic potential had improved emergence, grain and biological yields compared with other treatments.

It was reported seed priming had significant effect on increment of germination percent; germination speed and seedling dry weight of sunflower vice versa of producing abnormal seedling decrement in drought condition (Demir Kaya *et al.*, 2006).

The objective of this study was to evaluate the effect of seed osmopriming with different concentration and duration on soybean germination behavior of cv. 033.

MATERIALS AND METHODS

In order to evaluate the effect of seed priming technique by using Poly ethylene glycol (PEG 6000)



priming media on germination behavior and seed vigor of soybean (cv. 033) an experiment was conducted at Seed analysis laboratory of agricultural and natural resources center of Sari, Iran. Seeds were primed with six levels of PEG 6000 as priming media (distilled water as control, -0.4, -0.8, -1.2, -1.6 and -2 MPa) for 6, 12, 24 and 48 hours at 25°C.

Experimental units were arranged factorial in a completely randomized design with three replications. Dry soybean seeds considered as a control treatment (non primed).

Solution osmotic potential was calculated as described in Michel (1973) (equation 1).

$$\text{Water potential (bar index)} = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T \quad (1)$$

Where C is Poly ethylene glycol concentration, T is temperature (centigrade).

After treatment, seeds were given three surface washings with distilled water and re-dried to original

weight with forced air under shade at $27 \pm 3^\circ\text{C}$ ((Mubshar *et al.*, 2006).

Fifty seeds from each of the treatments were placed in 90-mm-diameter Petri dishes on whatman No.2 filter paper moistened with 10 ml of distilled water. Seed was kept at room temperature (25°C) under normal light. Germination progress was measured at 12 h intervals and continued until fixed state. The number of germinated seeds was recorded 8 days after planting as final germination percent (FGP) (ISTA, 1993 and ISTA, 1999).

Mean germination time (MGT) was calculated according to the equation 2 (Moradi Dezfuli *et al.*, 2008).

$$\text{MGT} = \frac{\sum Dn}{\sum n} \quad (2)$$

Where n is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination.

The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formula:

$$\text{GI} = \frac{\text{No. of germinated seed}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seed}}{\text{Days of final count}} \quad (3)$$

The time to 50% germination (T50) was calculated according to the following formula of Coolbear *et al.* (1984) modified by Farooq *et al.* (2005):

$$\text{T50} = t_i + \frac{\{(N/2) - n_i\} (t_i - t_j)}{n_i - n_j} \quad (4)$$

Where N is the final number of germination and n_i , n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Energy of emergence (EG) was recorded on the 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Ruan *et al.*, 2002). The vigor index was calculated according to equation 5 (Orchard, T. 1977).

$$\text{Seedling vigor index (SVI)} = [\text{seedling length (cm)} \times \text{germination percentage}] \quad (5)$$

Conductivity test is used to quantify the leakage of electrolytes from the seed coat with respect to age, storage life and other factors i.e. temperature, humidity, soil and water stress. A sample of 50 seed was taken from each treatment, placed in a 250ml flask with 200ml of distilled water. The flasks were stirred to remove air bubbles and floating seed, covered with aluminum foil and were kept at 20°C for 24 h. after soaking, seeds were gently swirled and the conductivity of the soaked water was measured with a dip type cell (Cell Constant of 1.0) conductivity meter. Conductivity was expressed on a weight basis in deci-Siemens m-1(dSm-1) g-1 of seed (ISTA, 1993).

Experimental data were analyzed using SAS (Statistical software, SAS institute, 2002) and treatment means were compared using Duncan's multiple range tests at 5% level of probability.

RESULTS AND DISCUSSIONS

According to the results, all studied traits were affected by the experimental factors and there was completely significant difference between control (non primed seeds) and primed seeds (Table-1). Final germination percentage (FGP) was affected by PEG concentration as well it was increased (91%) by decreasing of PEG osmotic potential from 0 to -1.2 MPa and then it was declined by more reduction of PEG osmotic potential from -1.6 to -2 MPa. Also FGP was increased (90.22%) by increasing of hydro priming duration from 6 to 12 h but it was decreased by increasing of hydro priming duration from 18 to 24 h. Mean comparison by Duncan multiple range test displayed significant difference between control and primed seeds as well more FGP was attained in seed primed than control in all osmotic potential (Table-2).

Basra *et al.* (2003) and Salinas (1996) reported improvement in germination percent, emergence and seedling stand by using seed priming techniques. In fact priming induces a range of biochemical changes in the seed that required initiating the germination process i.e., breaking of dormancy, hydrolysis or metabolism of inhibitors, imbibitions and enzymes activation (Ajouri *et al.*, 2004). Some previous researcher indicated that some or all process that precede the germination are triggered by priming and persist following the re-desiccation of the seed (Asgedom and Becker, 2001). Thus upon sowing,



primed seed can rapidly imbibe and revive the seed metabolism, resulting in higher germination percentage and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995).

The highest germination index was attained from -1.2 osmotic potential and 12 h seed priming duration treatments (21.15 and 20.15, respectively). Meanwhile germination index decreased by osmotic potential reduction and increment of seed hydro priming duration. Furthermore there was significant difference between control and priming treatments so as germination index from primed seed was more than control (Tables 1 and 2). Huns and sung (1997) reported that seed priming resulted anti-oxidant increment as glutathione and ascorbate in seed. These enzymes make more germination speed via

reduction of lipid proxidation activity. It has been declared that priming had been resulted in more germination speed especially in drought stress, saline stress and low temperatures in sorghum, sunflower and melon (Sivritepe, *et al.*, 2003; Demir Kaya *et al.*, 2006; Foti *et al.*, 2002). Variance analysis and mean comparison results displayed that mean germination time and the time to get 50% germination were affected by different osmotic potentials and seed hydro priming duration. The least MGT and T50 was obtained from -1.2 osmotic potential (2.7 and 1.7 respectively) and 12 h hydro priming duration (2.89 and 2.13) treatments. Generally less MGT and T50 was attained from seed priming treatment than control (Tables 1 and 2).

Table-1. variance analysis of studied traits in soybean (cv 033).

SOV	Mean square								
	df	Final germination	Seedling weight	Electrical conductivity	Time to50 germination	Germination index	Mean germination time	Germination energy	Seedling vigor index
Treatment	24	21.86**	1588.4**	61.16**	0.659**	23.9**	3.02**	30.85**	1722.57**
Control VS. treatment	(1)	66.21**	2750.3**	144.27**	1.228**	40.19**	7.52**	61.97**	3093.98*
Osmotic potential	5	54.95**	4560.7**	245.54**	2.76**	61.72**	7.22**	43.35**	4866.31**
Duration	3	57.71**	3853.3**	28.11**	0.239**	69.21**	8.77**	59.351**	4293.72**
Duration × osmotic potential	15	0.706 ^{ns}	67.24 ^{ns}	0.768 ^{ns}	0.0049*	1.15 ^{ns}	0.178 ^{ns}	0.38 ^{ns}	69.01 ^{ns}
Error	26	2.11	741.59	0.43	0.00059	2.59	0.302	2.25	177.62
Total	74								

*and ** significant at 5% and 1%, respectively

The probable reason for early emergence of the primed seed maybe due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Arif, 2005). Like germination percentage, prime seeds had lower Mean Emergence Time (MET) compared with unprimed seeds. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Hassanpouraghdam *et al.*, 2009; Sivritepe *et al.*, 2003). The highest seedling dry weight and energy of germination (EG) was attained from -1.2 osmotic potential (1.669 and 1.76, respectively) and 12 h seed priming duration treatments (1.55 and 1.75, respectively). Also SVI in primed seeds was more than control treatment and its highest amount was achieved from -1.2 osmotic potential (152.22) and 12 h seed priming duration treatments (140.41) (Table-2). Priming presumably allowed some repairs of damaged to membrane caused by deterioration (Ruan *et al.*, 2002). It has been reported that primed seeds showed better

germination pattern and higher vigor level than non-primed (Ruan *et al.*, 2002). Nascimento and West (1998) indicated minimizing of seed coat adherence during emergence of muskmelon seeds. The improvement in germination and vigor of normal/low-vigor seed might be due to reserve mobilization of food material, activation and re-synthesis of some enzymes DNA and RNA synthesis start during osmotic priming. Rapid embryo growth resulted when the obstacle to germination was removed (Basra *et al.*, 2003). Variance analysis results showed that there was significant difference between priming and control treatment from the aspect of electrical conductivity (EC) so as the highest EC was related to control treatment. Furthermore the lowest EC was attained from -1.2 osmotic potential and 12 h seed priming duration treatments (38.01 and 43.72 ds cm⁻¹ gr⁻¹, respectively) among primed treatments. Priming can repair some damages that have been arisen from seed erosion and improve seed quality (Arif *et al.*, 2008). Sung *et al.* (1999) displayed reduced seed secretion and subsequently decrement of EC via priming which were in agreement with Xiang *et al.* (1995) findings(3).

**Table-2.** Means comparison of studied traits in soybean by Duncan multiple range test (DMRT).

	Final germination	Seedling dry weight	Electrical conductivity	Time to 50% germination	Germination index	Mean germination time	Germination energy	Seedling vigor index
Duration (hour)								
6	86.77 c	1.246 c	44.57 b	2.23 c	16.30 c	4.24 a	71.61 c	108.53 c
12	90.22 a	1.55 a	43.72 c	2.13 d	20.15 a	2.89 c	75.10 a	140.41 a
18	88 b	1.375 b	46.26 a	2.30 b	17.54 b	3.74 b	72.83 b	121.47 b
24	86.1 6c	1.237 c	46.18 a	2.40 a	15.75 c	4.47 a	71 c	107.08 c
Control (dry seed)	83	1.043	45.189	2.92	13.70	5.45	68	86.6
Osmotic potential								
0	84.75 e	1.096 d	49.5 b	2.75 b	14.51 d	4.95 a	67.83 d	93.08 d
-0.4	86.58 d	1.272 c	44.96 c	2.37 c	16.38 c	47.2 b	71.58 c	110.63 c
-0.8	88.16 bc	1.352 bc	44.56 c	1.99 d	17.54 bc	3.73 cd	73.16 b	119.59 bc
-1.2	91 a	1.669 a	38.01 e	1.70 f	21.15 a	2.70 e	76 a	152.22 a
-1.6	89 b	1.455 b	43.5 d	1.91 e	18.56 b	3.34 d	74.33 a	129.82 b
-2	87.25 cd	1.267 c	50.53 a	2.88 a	16.46 c	4.04 bc	72.41 bc	110.89 c
Control (dry seed)	83	1.043	45.189	2.92	13.70	5.45	68	86.6

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