

Osmopriming Alleviates Drought Stress in Soybean (*Glycine max* L.) Seeds During Germination and Early Growth Stages

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Abstract

Germination and seedling establishment are critical stages in the life cycle of plants especially under stress conditions. The objective of this study was to evaluate the effect of osmopriming on germination and seedling growth of Soybean (*Glycine max* L.) seeds under drought stress. Seeds were primed in aerated solutions of PEG 6000, KNO₃ and KH₂PO₄ have -1.2 MPa osmotic potential. Drought levels were 0 (control), -3, -6, -9 bar. Stress condition was created by using PEG6000. Final germination percentage, time to get 50% germination (T₅₀), seedling vigor index (SVI), germination index (GI), reduction percentage of germination (RPG), seedling dry weight and length were measured. The results indicated that inhibition of germination and seedling growth due to drought stress should be overcome by using osmopriming treatments in soybean. Among the materials used for osmopriming PEG 6000 has the greatest impact on mitigating the effects of drought stress on germination and early growth stages.

Keywords: Germination, osmopriming, PEG 6000, drought, soybean.

INTRODUCTION

Soybean (*Glycine max* L.) is one of the most important oil and protein crop throughout the world. Its oil is the largest component of the world's edible oils. The world production of edible oils consists of 30% soybean. Poor germination and low seed viability are among the serious problems in the production of soybean (Arif et al., 2010).

Plant growth and productivity affected by nature's wrath in the form of various abiotic stress factors. Plants are frequently exposed to the plethora of stress conditions such as salt, drought, Oxidative stress and others. All these stress factors are a means for plants and prevent them from reaching their full genetic potential and limit their crop productivity worldwide (Mahajan and Tuteja, 2005).

Lack of adequate soil moisture in the seedbed is a major obstacle to the establishment of the crop, because inadequate soil moisture can reduce germination, slow down seedling growth and decrease yield (Saglam et al., 2010).

There are many strategies to overcome the negative effects of drought. A good strategy is the selection of cultivars and species for drought condition (Pavlousek,

2011). However, an alternative strategy for the possibilities to overcome drought stress is by seed pre-sowing treatments (Ghiyasi et al., 2008). Seed priming was defined as pre-sowing treatments in water or in an osmotic solution that allows the seed to imbibe water to proceed to the first stage of germination, but prevents radical protrusion through the seed coat (Yari et al., 2012). Seed priming techniques such as hydropriming, hardening, osmopriming, osmohardening, hormonal priming and hydropriming have been used to accelerate emergence more vigorous plants and better drought tolerance in many field crop like wheat (Iqbal and Ashraf, 2007), chickpea (Kaur et al., 2002), sunflower (Kaya et al., 2006), cotton (Casenave and Toselli, 2007) triticale (Yagmur and Kaydan, 2008).

Primed seeds usually to exhibit an increased germination rate, greater germination uniformity and greater total germination percentage. Increased germination rate and uniformity have been attributed to metabolic repair during imbibitions build up of germination enhancing metabolites (Abbasdokht, 2012).

The objective of this study was to evaluate the effect of seed priming on soybean germination and vigor under drought stress.

MATERIAL AND METHODS

This study was carried out at the Department of Agronomy, Faculty of Agriculture, Urmia University, West Azerbaijan, Iran. Seeds of soybean cv. Williams were used. Seed moisture content was determined by high-temperature oven method at $130 \pm 2^\circ \text{C}$ for 4 hours (ISTA, 2003). The mean moisture content of seed sample was about 10.30%.

The experimental design was a two factor factorial arranged in a completely randomized design (CRD) with three replications. The first factor was osmopriming treatments consisted of soaking in -1.2 MPa solutions of KNO_3 , KH_2PO_4 and polyethylene glycol (PEG) 6000, and the second factor was drought levels (0, -0.3, -0.6 and -0.9 bar). Drought stress was simulated by high osmotic substance PEG of molecular weight (MW) 6000. Non-primed seeds were used as control. Before applying experimental treatments the seeds were sterilized by using 30% hypochlorite for five minutes and then washed three times with distilled water (Unair et al., 2012). The seeds were soaked in a series of all the osmopriming treatments for eight hours. After priming, seeds were given three surface washings with distilled water and re-dried, near to original weight under shade (Unair et al., 2011).

The standard germination test was performed by placing 100 seeds between two Whatman no. 1, filter paper in 120 mm Petri dishes (three Petri dishes were used in each replication). Petri dishes containing primed and control seeds were irrigated with solutions of drought stress levels. During the test filter papers in the Petri dishes were kept water saturated state. All Petri dishes moved to germinator with 25°C , temperature at dark condition (ISTA, 2003). Germinated seeds in each treatment were recorded every 24 hours for seven days. Seeds were considered as germinated when the radical length reached 2 mm long.

The time to get 50% germination (T_{50}) was calculated according to the following formulae of Coobear et al. (1984) modified by Farooq et al. (2005) as below:

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

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 $n_i < N/2 < n_j$.

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981) as under:

$$MGT = \frac{\sum Dn}{\sum n}$$

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.

Germination index (GI) was calculated as described in the Association of Official Seed Analysts (1983) as the following formulae:

$$\text{Germination index} = (Gt/Tt)$$

Where Gt is the number of seeds germinated on day t and Tt is the number of weekly germinated seeds at time Tt .

Seedling vigor index (SVI) was calculated following modified formula of Abdul-Baki and Anderson (1973):

$$SVI = [\text{seedling length (cm)} \times \text{germination percentage}]$$

The reduction percentage of germination (RPG) was calculated as quoted by Madidi et al. (2004):

$$RPG = (1 - N_x / N_c) \times 100$$

" N_x " is the number of germinated seedlings under drought treatments and " N_c " is the number of germinated seedlings under control.

For seedling growth test, 30 germinated seeds in each treatment were selected randomly and were transferred to plastic boxes ($20 \times 12 \times 10$) and were cultured on filter paper for ten days in three replications. Selected seedlings were weighted and then mean value was calculated to obtain seedling fresh weight (Jamal et al., 2011). Then these seedlings were dried in an oven at 80°C for 48 hours and again weighed after complete drying for recording seedling dry weight. Seedling length was measured with a ruler and accuracy of measurement was 1 mm. Data of germination and seedling growth tests were subjected to data transformation (arcsine) before the statistical analyses in order to unify the variance of data. Analysis of variance (ANOVA) was used to compare treatment means. Differences between means were determined by Duncan's multiple range tests (DMRT) at probability level 1%. A computer software SAS was used to carry out the statistical analysis. Drawings were made using Excel software.

RESULTS AND DISCUSSIONS

The effect of osmopriming and drought on all studies treated were significant ($p < 0.01$). In addition to, the interaction among these factors was also significant ($p < 0.01$). In osmopriming and control treatments with increasing stress intensity final germination percentage, MGT, T_{50} , seedling dry weight, seedling length and SVI were affected by drought stress. However the negative effects of drought stress on treated seeds were significantly lower than control. (Fig 1 – Fig 7). RGP in drought levels (-3, -6 and -12 bar) of control treatment were 11.32, 21.37 and 33.69 respectively (Fig 5). Although in osmopriming treatments with increasing drought stress RGP was increased, but reduced in germination percentage was significantly lower than the control ($p < 0.01$). RGP in PEG 6000, KNO_3 and KH_2PO_4 treatments in the drought levels (-3, -6 and -12 bar) were 5.57, 12.2, 21.47, 7.94, 15.35, 24.64, 5.61, 13.72 and 23.38 respectively (Fig 5). Despite germination and vigor of primed seeds with different materials under drought stress condition were better than control, but the results of the mean comparison indicated that the effects of various materials used for osmopriming in order to alleviation of this stress on germination of soybean seeds were statistically different ($p < 0.01$) (Fig 1 – Fig 7). In this regard, PEG 6000 has the greatest impact on improving germination and seedling growth under drought stress condition.

The results of this study showed that drought stress affected germination and seedling growth of soybean seed adversely and osmopriming treatments indicated that enhanced performance under stress condition compared to control.

McDold (2000), stated that PEG 6000 large molecular size is not allowed to enter the cell embryo during the operation of osmopriming, while KNO_3 and KH_2PO_4

can enter the seed embryo cells. It can be associated with some toxic effects (Noorbakhshin et al., 2011). Basra et al., (2006) reported that delayed and weak germination in seeds rice subjected to osmoconditioning for 24 and 48 h in KNO₃ was probably due to toxicity. KNO₃ toxicity results in injury to cellular organelles and membranes of wheat (Ghiyasi et al., 2008). The positive effect of inorganic salts such as KNO₃ and KH₂PO₄ in some primed seeds of different crops related to the presence of the selectively permeable tissue layer surrounding the embryo, which allows the uptake of water but prevents the diffusion of solutes into the seeds (Tajbakhsh and Ghiyasi, 2009).

Osmopriming of seed prolongs phase II of germination process, where considerable metabolic activities take place that prepare germination seeds for radical emergence, such as DNA repair, DNA replication, β -tubulin accumulation and mobilization of seed storage (Chen et al., 2010). Thus primed seeds with a prolonged phase I are likely more

prepared for germination and early growth than unprimed seeds. In addition to, osmopriming can contribute to improve germination rate and seedling emergence of seeds by increasing the expression of aquaporins, enhancement of ATP_{ase} activity, RNA and acid phosphatase synthesis (Gao et al., 1999).

CONCLUSIONS

The results of this study demonstrate that osmopriming with PEG6000, KNO₃ and KH₂PO₄ is effective for improvement of germination and seedling growth of soybean during germination and early growth stages especially under drought stress. Among these materials, primed seeds with PEG6000 recorded highest germination and vigor parameters under drought stress condition.

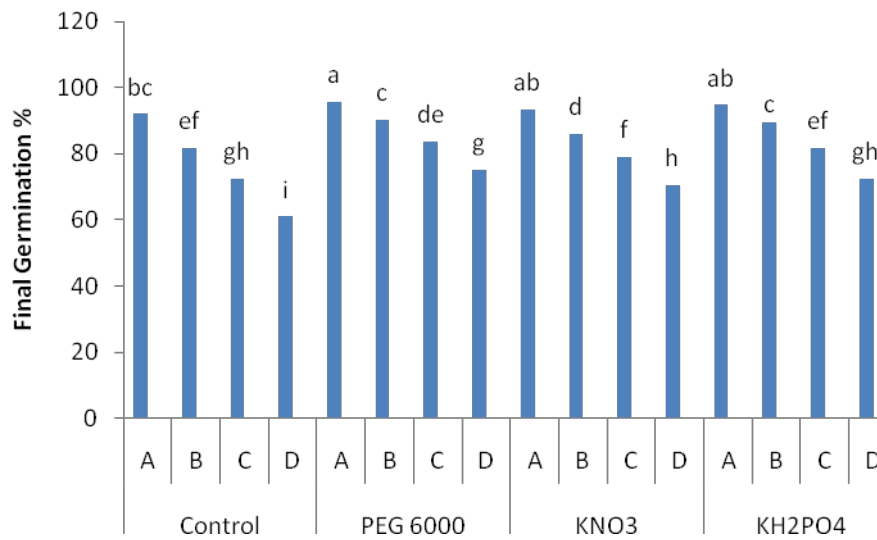


Fig 1. Effect of osmopriming treatments on final germination percentage of soybean seeds under different drought level. Different letters indicating significant differences at $p \leq 0.01$. A= 0 bar, B= -0.3 bar, C= -0.6 bar, D= -0.9 bar.

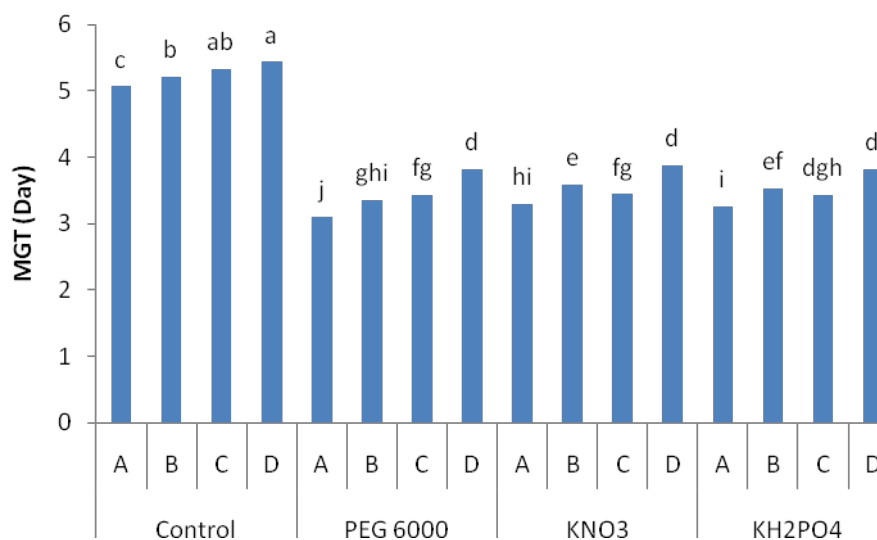


Fig 2. Effect of osmopriming treatments MGT of soybean seeds under different drought level. Different letters indicating significant differences at $p \leq 0.01$. A= 0 bar, B= -0.3 bar, C= -0.6 bar, D= -0.9 bar.

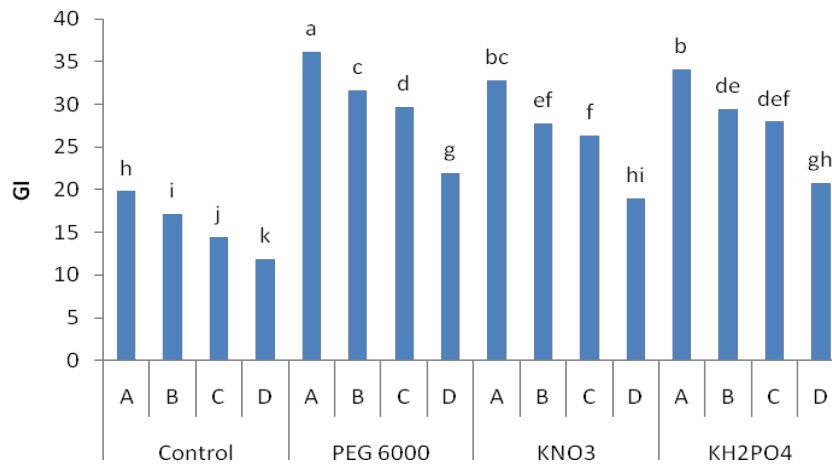


Fig 3. Effect of osmopriming treatments GI of soybean seeds under different drought level. Different letters indicating significant differences at $p \leq 0.01$. A= 0 bar, B= -0.3 bar, C= -0.6 bar , D= -0.9 bar.

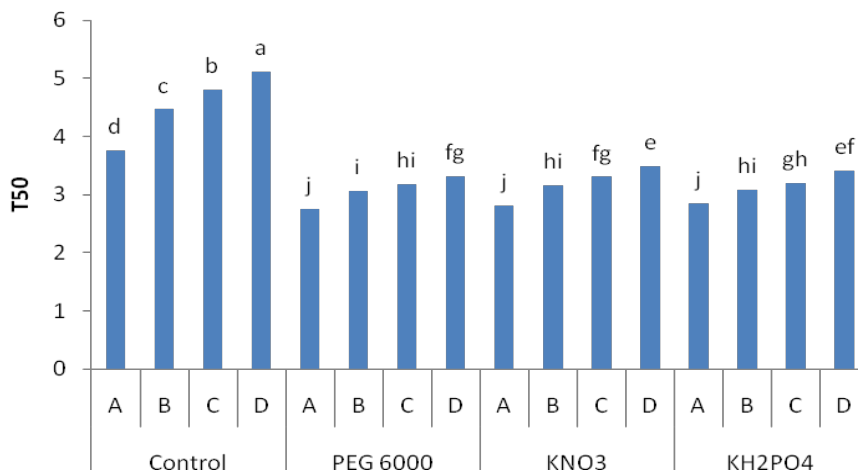


Fig 4. Effect of osmopriming treatments T50 of soybean seeds under different drought level. Different letters indicating significant differences at $p \leq 0.01$. A= 0 bar, B= -0.3 bar, C= -0.6 bar , D= -0.9 bar.

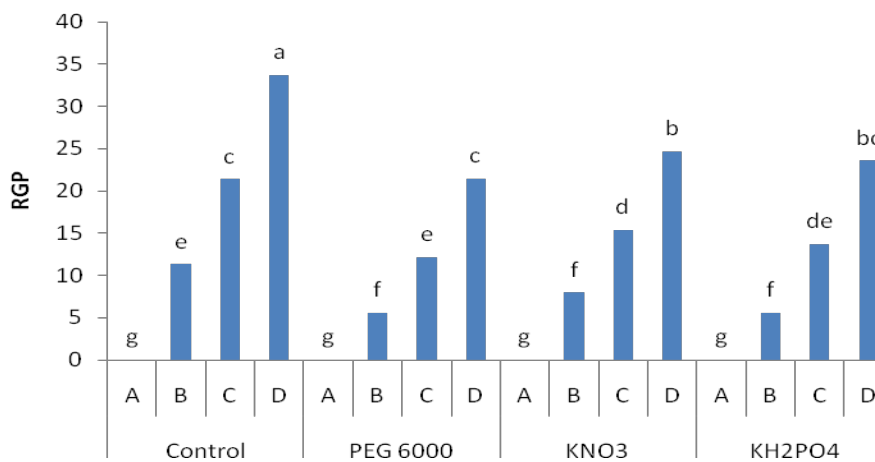


Fig 5. Effect of osmopriming treatments RGP of soybean seeds under different drought level. Different letters indicating significant differences at $p \leq 0.01$. A= 0 bar, B= -0.3 bar, C= -0.6 bar , D= -0.9 bar.

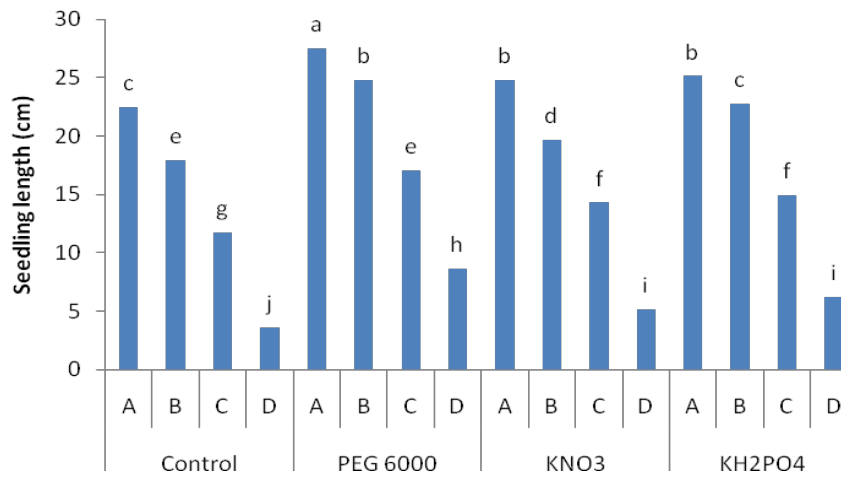


Fig 6. Effect of osmopriming treatments seedling length of soybean seeds under different drought level. Different letters indicating significant differences at $p \leq 0.01$. A= 0 bar, B= -0.3 bar, C= -0.6 bar , D= -0.9 bar.

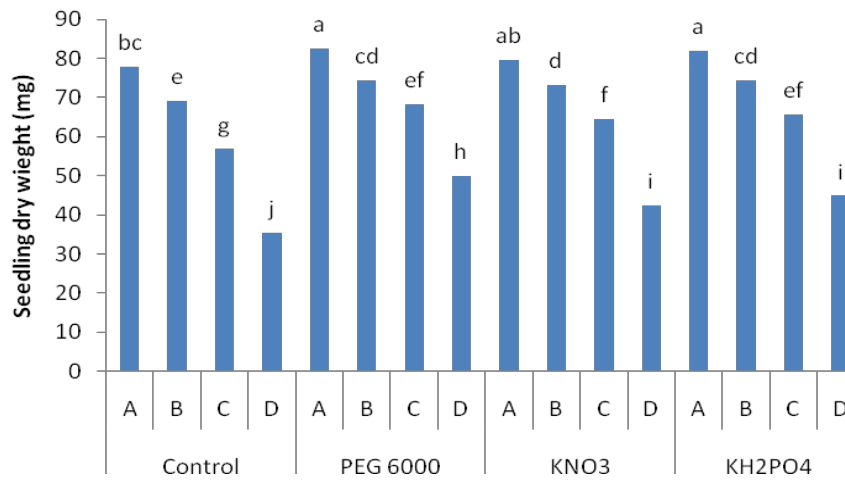


Fig 7. Effect of osmopriming treatments seedling dry wight of soybean seeds under different drought level. Different letters indicating significant differences at $p \leq 0.01$. A= 0 bar, B= -0.3 bar, C= -0.6 bar , D= -0.9 bar.

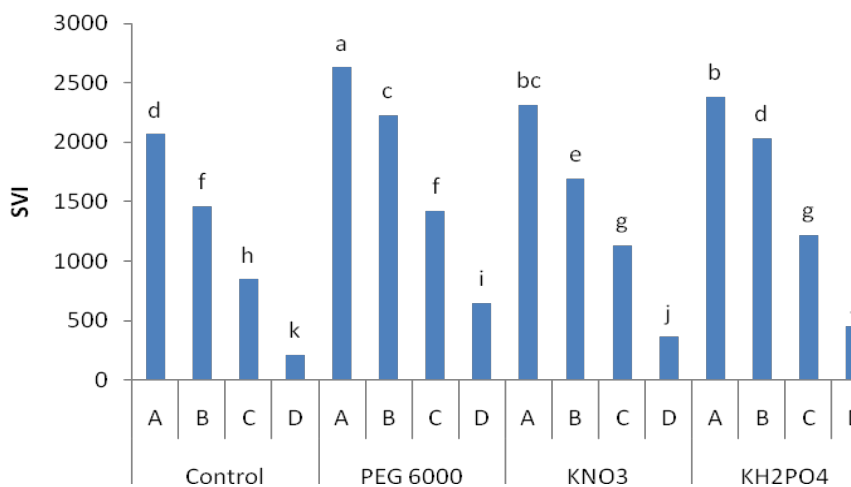


Fig 8. Effect of osmopriming treatments SVI of soybean seeds under different drought level. Different letters indicating significant differences at $p \leq 0.01$. A= 0 bar, B= -0.3 bar, C= -0.6 bar , D= -0.9 bar.

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