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Full Length Research Paper

Effect of osmopriming on antioxidant activity of Bersim clover (*Trifolium alexandrinum* L.)

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Rapid and uniform seed germination and stand establishment are critical factors of crop production under any condition. Seed priming has been known as one of the most effective seed enhancement techniques to improve germination under unfavorable conditions. Antioxidants are critical factors for vigorous seed germination. Seeds with higher antioxidant profiles are more tolerant to unsuitable conditions than other seeds. A laboratory study was conducted to evaluate the influence of the seed priming technique on seed performance during germination and on antioxidant activity of Bersim clover seeds, an economically important forage crop. Seeds were osmoprimed using solutions of polyethylene glycol-6000 (PEG-6000) giving osmotic potentials of -0.8, -1,-1.2,-1.4 and -1.6, MPa for two priming periods (8 and 16 h). Then best priming treatments were further assessed by measurements of antioxidant enzyme activities. The general finding is that seed priming improved final germination percentage, germination rate, seedling length and vigor indexes. Maximum invigoration was observed in seeds osmoprimed at -0.8 MPa for 16 h. Antioxidant enzyme activities (superoxide dismutase, catalase, and peroxidase) in treated seeds of Bersim clover were significantly increased compared to those in control untreated seeds.

Key words: seed, priming, germination, vigor and antioxidant.

INTRODUCTION

Bersim clover (*Trifolium alexandrinum* L.) is highly used as a forage crop, being popular for farmers because of its fast growth, high production mow and fresh forage production with good quality and quantity (Shrestha et al., 1996; Musavi Aghdam, 1985; Khoshgoftar, 1992). Research results indicate that the yield of Bersim clover depends on sowing date, climate conditions, soil fertility, shrub height, the number of harvests and variety. For example, in Iran the following yields of fresh forage have been recorded: 55 to 70 t/ha in the Mazandaran province, 20 to 30 t/ha in the Gilan province and 100 t/ha in the Khuzestan province (Zarrineh et al., 1985; Khoshgoftar, 1992). Considering the important role of this crop in the production of dairy and protein products, an important objective of Iranian Agriculture authorities is to increase the performance of forage crops and to develop novel production strategies (1st national forage crops congress of Iran, 2005).

Seed germination and seedling establishment play important role in controlling crop yields. Consequently, technologies improving seed performance, such as priming, can be very useful to increase the yield of crops (Heydecker et al., 1973; Bradford, 1986; Harris et al., 1999; Kersulec et al., 1997). Among seed priming invigoration treatments, seed osmopriming, also known as osmo-conditioning, is a pre-sowing treatment of seeds in an osmotic solution (made from polyethylene glycol (PEG), sorbitol, or mannitol in water) that allows seeds to imbibe water to proceed to the first stage of germination permitting reactivation of primary metabolism but prevents radicle protrusion through the seed coat (Ashraf and

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Abbreviations: PEG, polyethylene glycol; **SOD,** superoxide dismutase; **CAT,** catalase; **POX,** peroxidase.

Foolad, 2005). Since seeds remain desiccation tolerant up to radicle protrusion, then the treated seeds can be dried back to original moisture contents for storage purposes (Heydecker et al., 1973). It is assumed that this technique improves seed germination and seedling vigor by conferring an advance in germination timing of the treated seeds over untreated seeds. This technique has been widely used in vegetable seeds to increase germination rate, total germination and seedling uniformity, especially under unfavorable environmental conditions (Bradford et al., 1990; Ozbingol et al., 1998; Moosavi et al., 2009; Nascimento and Arago, 2004; Korkmaz, 2006). Indeed, when primed seeds are sowed in the field, they usually display fast and uniform germination (Bourgne et al., 2000). It is reported that for sunflower, seeds priming can increase germination and seedling growth under stressful conditions (Demir et al., 2006). Because of its fast growth and the number of possible harvests, one of the current plans of the Agriculture Ministry of Iran is to increase the production of Bersim clover (www.dfid.psp.org.). To this end, the aim of the present study was to increase germination percentage and seed vigor in this species by osmopriming with PEG, and to define optimal combinations of PEG concentration and treatment duration.

MATERIALS AND METHODS

This study was carried out in the Department of Agronomy, Faculty of Agriculture, University of Tehran, Iran. Bersim clover from Shahid Beheshti culture and industry of Dezful., which is commonly grown in Iran, was used as seed material. Germination and early seedling growth were daily investigated for seven days and seeds with 2-mm long radicles were considered as a germinated seeds ISTA (1997).

Seed priming

Preliminary experiments showed that osmopriming can improve Bersim clover germination performance (Rouhi et al., 2010). Effects of osmopriming on germination and vigour traits of Bersim clover (*Trifolium alexandrinum* L.). Not. Sci. Biol. 2: 59-63). In the present work, experiments were conducted to investigate if seed priming has a positive effect on seed germination of Bersim clover seeds and to decipher if priming exerts an effect on seed enzyme activities. Mature dry Bersim clover seeds (5.5% seed moisture content) were immersed in various solutions of polyethylene glycol 6000 giving osmotic potentials of −0.8, −1, −1.2, −1.4, and −1.6 MPa) according to Michel and Kaufmann (1973) at 20°C for 8 and 16 h under dark conditions. Then seeds were rinsed three times with distilled water. The treated seeds were surface-dried and dried back to their original moisture content at room temperature (about 21°C, 45% relative humidity), as determined by measuring changes in seed weight.

Germination tests

Three replicates of 50 seeds were subjected to standard germination test according to ISTA (1997). Seeds were placed between double layers of filter paper in 90-mm diameter Petri dishes. Water (5 ml) was added to each Petri dish. Petri dishes were sealed with plastic bags to avoid moisture loss. Seeds were allowed to germinate at 20 ± 1°C in dark conditions for 7 days (ISTA, 1997). Germination was considered to have occurred when the radicles were 2 mm long. Germination percentage was recorded every 12 h for 7 days. Germination rate (GR) was calculated (ISTA, 1997). Seedling length was measured after the 7th day of imbibition.

Antioxidant enzyme assay

Measurements of antioxidant enzyme activities were performed with control and osmoprimed seeds. Three replicates of seed samples (each with 3 sub-samples of 15 seeds) were hand-ground at 4°C in a mortar and pestle with 4 ml of 0.1 M potassium phosphate buffer (pH 7.0), followed by centrifugation at 20,000 × g for 20 min. The supernatants were used for the determination of enzyme activities. Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by the decrease in absorbance due to NADH oxidation (Stewart and Bewley, 1980). Peroxidase (POX, EC 1.11.1.7) activity was determined according to the procedure of Johnson and Cunningham (1972)http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TC3-4JCSMCX-1&_user=1400009&_coverDate=04%2F10%2F2006&_alid=539323364&_rdoc=1&_fmt=full&_orig=search&_cdi=5159&_sort=d&_docanchor=&view=c&_ct=5&_acct=C000052577&_version=1&_urlVersion=0&_userid=1400009&md5=a9135077ded5f142667f59077c27a21d. Catalase (CAT; EC 1.11.1.6) activity was assayed following the changes in absorbance at 250 nm (Kato and Shimizu, 1987). The SOD activity was expressed as the unit of enzyme activity needed to inhibit the reaction by half, per seed and per min. The activities of POX and CAT were expressed per mg protein, and one unit represented 1 μmol of substrate undergoing reaction per mg protein per min.

Statistical analysis

The statistical analysis was two factors factorial (6 × 2) arranged in a randomized completely block design, with three replications and 50 seeds per replicate. The first factor was osmotic potential level (−0.8, −1.0, −1.2, −1.4, −1.6 and 0 MPa) in PEG solutions and the second was the duration of seed priming, namely 8 and 16 h. Data for germination and abnormal germination percentage were subjected to arcsine transformation before analysis of variance was made using MSTAT-C program (Michigan State University). Mean separation was performed by Fisher's least significant difference (LSD) test if F-test was significant at ($P < 0.05$).

RESULTS AND DISCUSSION

Data variance analysis show that the duration of treatment significantly affected final germination percentage, germination rate. The osmotic potential of the osmopriming solution influenced germination performance in Bromus seeds. From these results, we investigate and discuss the impact of the duration of treatment and of the value of the osmotic potential of the solutions used for osmopriming the seeds.

Final germination percentage (FGP) and germination rate (GR)

The most effective treatment was observed by

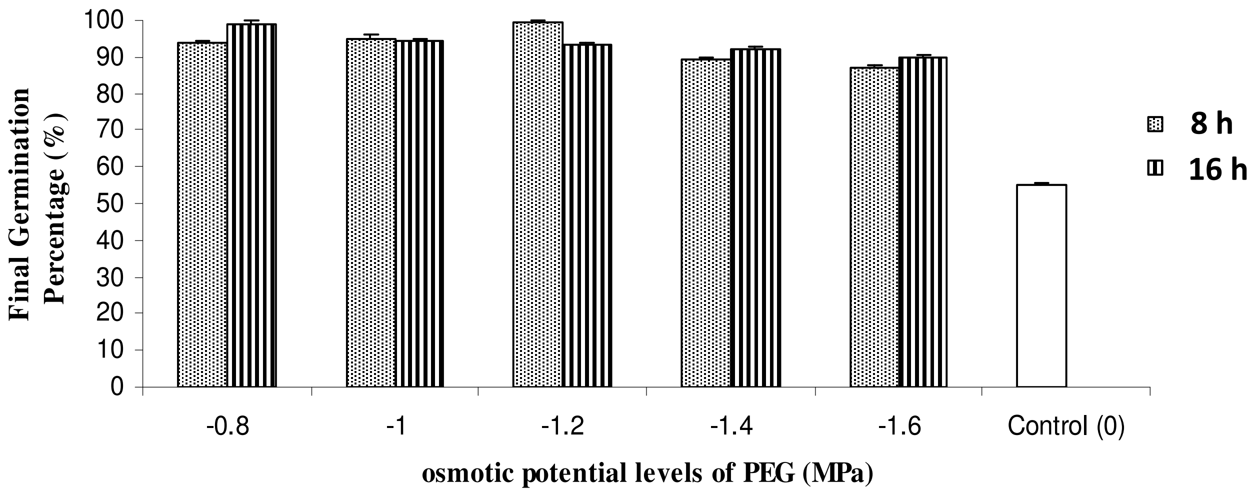

Figure 1. Influence of osmotic potential levels of osmopriming treatments on the final germination percentage (FGP) in Bersim Clover seeds under 8 and 16 h durations +S.E (Error bars indicate standard deviation from the mean). Seed priming with -0.8 MPa PEG; -1 MPa PEG; -1.2 MPa PEG; -1.4 MPa PEG; -1.6 MPa PEG; seed priming with polyethylene glycol and 0 MPa as a control group.

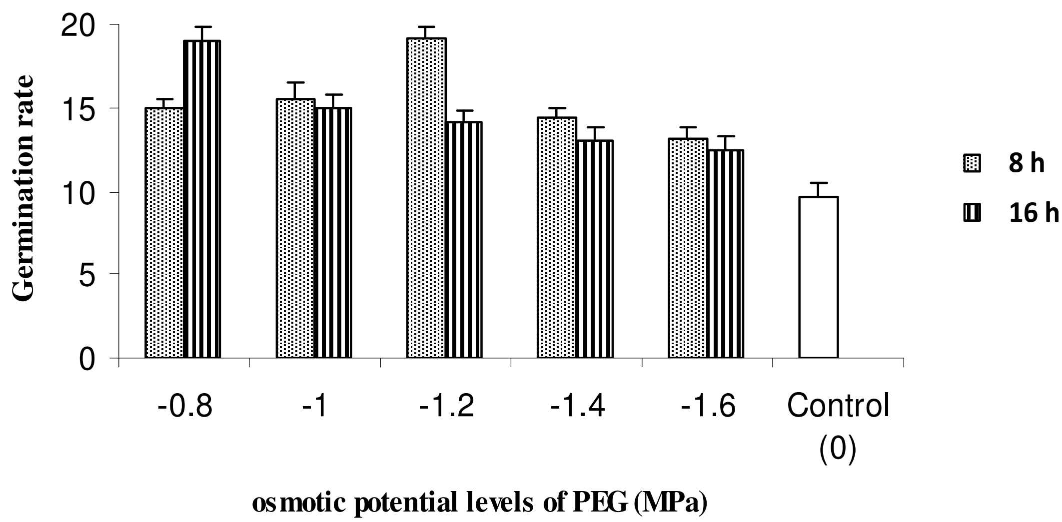

Figure 2. Influence of osmotic potential levels of osmopriming treatments on the germination rate in Bersim Clover seeds under 8 and 16 h durations +S.E. Seed priming with -0.8 MPa PEG; -1 MPa PEG; -1.2 MPa PEG; -1.4 MPa PEG; -1.6 MPa PEG; seed priming with polyethylene glycol and 0 MPa as a control group.

osmopriming seeds for 8 h at an osmotic potential of -1.2 MPa. Here, FGP and GR values were higher in comparison with other conditions and control untreated seeds (Figures 1 and 2). In these conditions, seed priming increased final germination percentage by about 30% compared to control values. It was reported that osmopriming of watermelon seeds caused a decrease in mean germination time and an increase of germination percentage (Demir and Van de Vanter, 1999). Also in agreement with our data, several studies reported a positive effect of priming on seed germination percentage (Rouhi et al., 2010; Misra and Dwivedi, 1980; Bradford et al., 1990; Job et al., 1997; Ozbingol et al., 1998; Chiu et al., 2002).

Subedi and Ma (2005) noted that one of the most important conditions for crop potential performance is fast and uniform germination, which is a resultant of seed priming in the farm. Furthermore, the positive effect of seed priming on crop performance was demonstrated under real field conditions, and the interest of this technique was highlighted notably for on-farm priming (Harris et al., 2001; Giri and Schilinger, 2003; Finch-Savage et al., 2004).

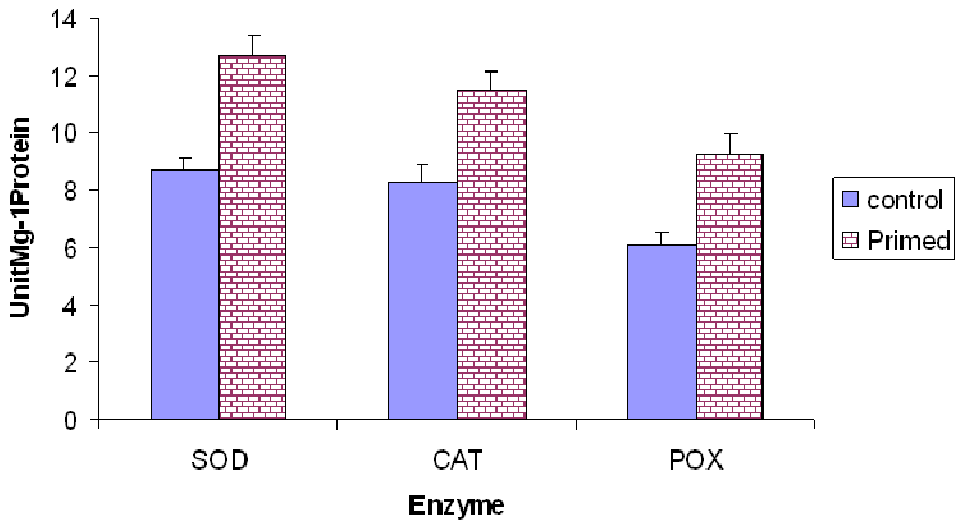

Figure 3. Effect of seed priming on different antioxidant enzymes (catalase, superoxide dismutase and peroxidase) of Bersim clover seeds.

Antioxidant enzyme activities

The present study shows that seed priming increased antioxidant enzyme activities during the seed germination process. Thus, SOD activity significantly increased in primed seeds (Figure 3). Catalase and peroxidase also increased in primed seeds. During seed priming, POX activity was the most highly influenced antioxidant activity (Figure 3). Moosavi et al. (2009) reported that seed priming highly increased POX and polyphenol oxidase (PPO) activities in Amaranth genotypes. Oxidative stress blocks growth and development by decreasing cell division, therefore protection from oxidative stress is critical for seed germination (Bailly et al., 2008). From intracellular signaling networks to cell death, the dual role of reactive oxygen species is seen in seed physiology (Comptes Rendus Biologies 331: 806-814). Recent studies have documented the presence of several antioxidative enzymes in dry cereal grains, and a considerable rise in their activities after the start of seed imbibition (Reichheld et al., 1999; Chang et al., 2000; Demeke et al., 2001; Morohashi, 2002, Moosavi et al., 2009). Higher germination percentage and seedling length of osmoprimed seeds could result from an increase in the antioxidant profile of the treated seeds. Plant peroxidases (EC 1.11.1.7) are hemeproteins that play an important role in catalysis of H_2O_2 dependent oxidation of a wide variety of reducing substrates (Job et al., 1978; Teraoka et al., 1983). Higher POX activities could increase cell division, and therefore such enzymes could play a key role in seedling growth. Also, increasing SOD and CAT activities could increase seed tolerance to environmental stressful conditions. It is noted that the SOD content of the Bersim clover seeds was higher than those for the other investigated antioxidant activities, both in primed and control seeds, a finding that therefore highlights the potential role of this enzyme in seed germination performance.

Conclusions

This study shows that osmopriming treatments conducted at -0.8 M Pa during 16 h or at -1.2 MPa during 8 h were associated with the best performance of seeds and seedlings compared to other conditions presently investigated. However, a detailed comparison of these two optimal treatments suggests that the longest priming duration (16 h) at -0.8 MPa is superior, i) because seed water uptake water may occur more gradually in these conditions, and ii) because the lower PEG amount used in this condition makes the osmopriming treatment less expensive. Our results are in good agreement with those of Akinola et al. (2000), showing that a higher duration of seed priming results in higher cumulative germination in wild sunflower and also with the results of Caseiro et al. (2004), showing that conducting onion seed is more effective when seeds are hydrated for 96 h compared to 48 h. In agreement with Khajeh-Hosseini et al. (2003), the present study revealed that PEG had no toxic effect since all seeds germinated and produced vigorous seedlings. Indeed, Mehra et al. (2003) and Michel (1983) showed that PEG molecules do not penetrate into the seed. It is generally admitted that to maintain high quality of primed seeds for extended storage periods; treated seeds should be stored at low temperature and low moisture content. It is also suggested that increase in antioxidant enzymes might result in increasing seedling tolerance to unfavorable environmental conditions (for example, water stress, and salt stress). Our results establish that the most effective invigoration treatment for Bersim clover seeds is soaking in -0.8 MPa PEG solution for 16 h. However, if users are willing to conduct Bersim clover seed priming more rapidly, we show here that an alternative protocol is to conduct the priming treatment at -1.2 MPa for 8 h. Finally, it is recommended that the present results have be confirmed in farm conditions.

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