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Germination response of three Argentinian *Prosopis* species to osmotic priming

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Abstract

Prosopis **is a widely distributed genus with 13 species endemic to Argentina. Several of these are harvested for multiple uses. The regeneration of this specie can only be achieved through the implantation of seedlings or direct sowing of seeds. High rate and percentages of germination are key factors affecting the success of reintroduction of these species. Among the treatments used to enhance germination and emergence rate the osmotic priming (imbibition of seeds in osmotic solutions) is one of the most successful. In the present study we investigate the effect of osmotic priming on seed germination rate and germination percentage for three species of *Prosopis (P. Argentina, P. chilensis and P. flexuosa)*. We subjected mechanically scarified seeds of each species to 16 treatments resulting from the combination of PEG solutions with four osmotic potentials (-0.8; -1.0; -1.2 and -1.4MPa) during four time periods (24, 48, 72 and 96h). Treatment seeds were contrasted against a control consisting of scarified seeds. The osmotic priming significantly increased germination rate and radicle length relative to control seeds for three species. The effectiveness of priming treatments in enhancing germination of native *Prosopis* species constitutes a significant achievement that may well increase the success of reforestation programs.**

Keywords: Argentina, germination rate, monte desert, peg.

INTRODUCTION

The genus *Prosopis* L. are main components of Argentinian arid and semi-arid ecosystems and contribute significantly by stabilizing primary production (FAO, 1997). Various studies have shown the importance of different *Prosopis* species in ecosystems because they generate specific conditions that favor associated animal populations and plant communities (Kingsolver et al., 1977; Mares et al., 1977). Their adaptive response to moisture stress and saline water, allow these species to produce high biomass in extremely arid environments (Villagra and Cavagnaro, 2006; Cavagnaro and Passera, 1993; Cony, 1993). Several species of the genus are intensively exploited for their multiple uses resulting in a marked degradation of the natural resources in arid and semi-arid habitats (Karlin and Diaz, 1984). They are as source of food, fibre, firewood, fodder, timber and they are use in agroforestry systems (Kaushik and Kumar, 2003).

Transplanting of seedlings is a common approach for regenerating the vegetative cover on degraded sites. For instance *Prosopis* seedlings produced in nurseries have been used in reforestation plans (FAO, 1997). However, the direct sowing of seeds, an alternative technique rarely adopted in forestry, can also result in successful recovery of sites where *Prosopis* has been overexploited. Direct sowing is easier, simpler, and cheaper than planting seedlings (Shen and Hess, 1983), especially in inaccessible sites where establishing a nursery to produce and maintain plants can be avoided.

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It also reduces costs of transport and labor during soil preparation and sowing. This approach has been reported for the rehabilitation of gallery forests (Barbosa et al., 1992) and drainage channels (Sun et al., 1995), the restoration of impoverished forests (Catalan et al., 1994), the enrichment of forests after logging (Guariguata and Pinard, 1998), and the restoration of degraded lands after mining (Parrotta and Knowles, 1999).

Seed germination and germination rate are strongly associated with the success of reforestation plans, especially if direct sowing is used. This is a key characteristic particularly in regions with adverse and unpredictable climate and saline soils, as the germination and seedling stages are the most critical periods in the life cycle of desert plants (Pratolongo et al., 2003; Sosebee and Wan, 1987; Rathcke and Lacey, 1985). Moreover, rapid emergence and growth are generally considered as highly advantageous since they favor resource appropriation by seedlings and therefore increase successful establishment probabilities (Black and Bewley, 2000; Mooney et al., 1977). A long period between the sowing and emergence predisposes the seedling to deterioration resulting in a reduced number of viable plants (Parera and Cantliffe, 1994). Among the treatments used to enhance germination and emergence velocity and synchronization, osmotic priming (imbibition of seeds in osmotic solutions) is without doubt one of the most successful (Sadeghi et al,. 2011; Govinden-Soulange and Levantard, 2008; Farooq et al., 2006; Murungo et al., 2003; Zheng et al., 1994; Parera et al., 1993; Liptay and Zarifa, 1993; Frett et al., 1991). In the present study we investigate the effect of osmotic priming on seed germination velocity, synchronization and percentage for three species of the genus *Prosopis*. By combining the advantages of mechanical scarification with osmotic treatments during pre-sowing we expect to enhance the three parameters previously mentioned. The results obtained from this study present useful basic knowledge that will be helpful in the production of seedlings in nurseries as well as under direct sowing of seeds.

MATERIALS AND METHODS

Species used

We selected *Prosopis chilensis* Mol. Stuntz emend. Burk, *Prosopis flexuosa* DC. and *Prosopis argentina* Burkart because of their relevance as source of wood, fodder and their use in agroforestry systems.

P. chilensis and *P. flexuosa* grow simpatrically in most of the Monte, with *P. flexuosa* being the most abundant. They are characteristically phreatic trees (Roig and Ruiz Leal, 1959) which allow them to cope with water deficit (Villagra and Cavagnaro, 2006; Cavagnaro and Passera, 1993). They also succeed in saline habitats (Cony, 1993). Their adaptive response to moisture stress and saline water, allow these species to produce high biomass in extremely arid environments. *P. argentina* on the other hand, is a shrub species that occupies sites in the non-saline soils of sand dunes (Villagra and Cavagnaro, 2006).

We collected seeds of the three species from several locations in San Juan, Argentina (31°32'03"S and 68°31'34"W). The seeds extracted manually from the pods with tweezers and then scarified approximately 25 % of their seed coat with sandpaper (Ortega et al., 2002; Catalán and Balzarini, 1992). We visually selected healthy seeds, and discarded the broken and insect-damaged ones. We stored the seeds at 20 ºC and 20 % of relative humidity prior to conducting the osmotic treatments.

Osmotic priming treatment

We immersed groups of 120 scarified seeds in 250 ml of aereated solutions of polyethylene glycol 6000 (PEG) during different periods of time. For each species, the 16 treatments conducted resulted from the combinations of two factors: osmotic potential (-0.8; -1.0; -1.2 and -1.4 MPa); and immersion period (24, 48, 72, 96 and 120 h). The treatments were contrasted with a control consisting of scarified seeds that were not immersed in any of the osmotic solutions. The immersion time of 120 h waste for evaluation because any seed showed radicular protrusion. We prepared 250 ml of the solutions at 25 ºC following the Vant`Hoff equation (Salisbury and Ross, 1994) and adjusted them using an osmometer (Wescor, USA). After completing the osmotic treatment we dehydrated the seeds at 25 ºC and 30 % RH to their original humidity (10 %) prior to germination.

Germination

We performed the germination assays using the wet towel method. We moistened two layers of paper towels with distilled water, placed 25 seeds on top of the wet towels and spaced them so that they did not touch each other. We rolled up the towel so that each seed stayed moistened on both sides, placed the rolls loosely in a plastic bag and positioned them upright. The rolls were kept in a growth chamber at 25 ºC. We evaluated seed germination at 16, 24, 48 and 72 hours after sowing by counting germinated seeds. Seeds were considered germinated when their radicle was at least 2 mm long. We calculated the emergence rate index (ERI) (Shmueli and Goldberg, 1971) for each species subjected to each treatment.

$$ERI = \sum_{n=n0}^{n=c-1} Xn \ (c - n) / N$$

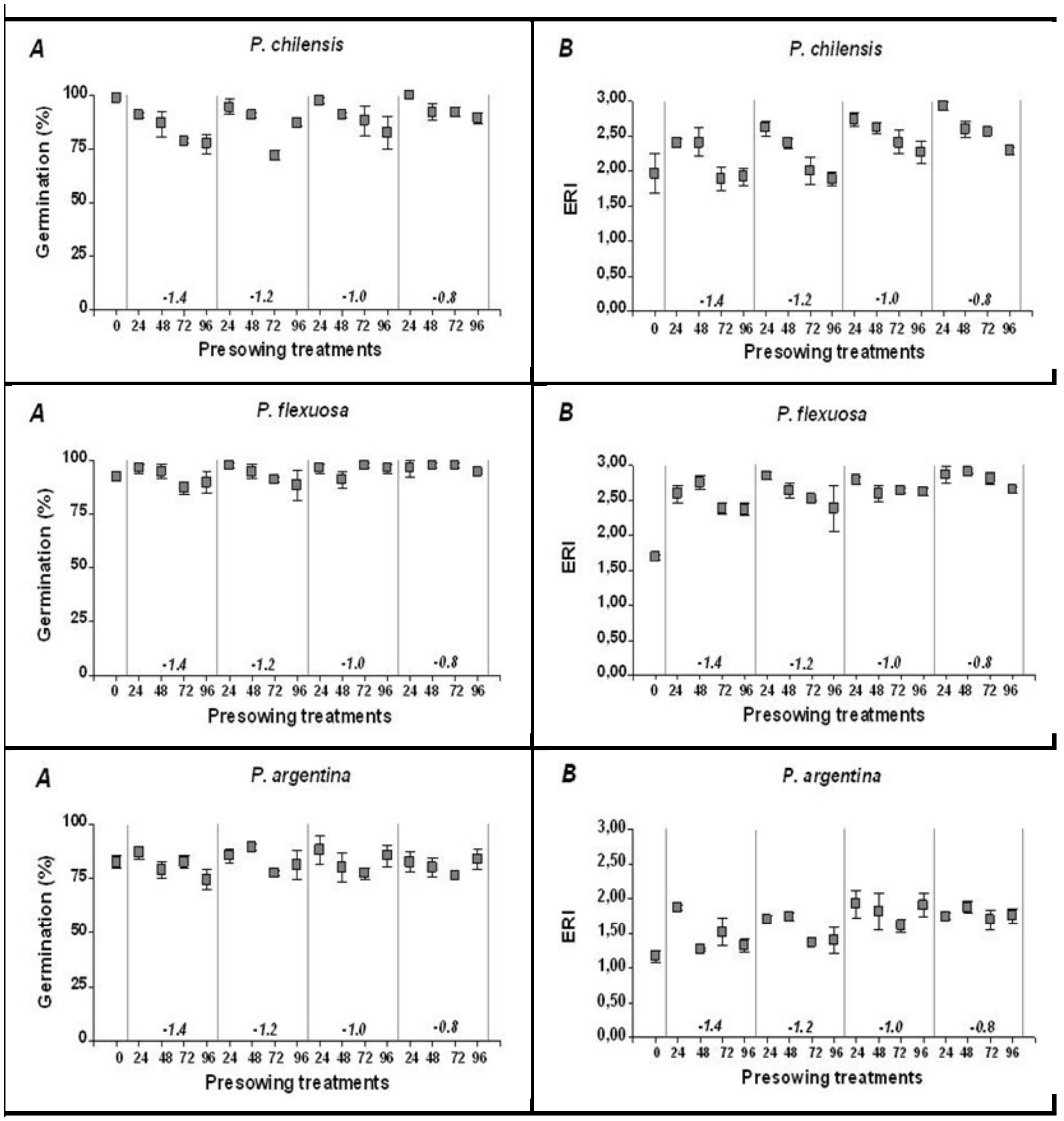

Figure 1. Box plot A) Germination percentages and B) emergence rate index (ERI) after osmotic pre-sowing treatments (0=control; 24, 48, 72 and 96 hours at -1.4, -1.2, -1.0 and –0.8 MPa) in *P. chilensis*, *P. flexuosa* and *P. argentina*. Vertical bars are s.e. of mean.

Where: X_n: number of germinated seed on the day n; *c:* number of days counted from sowing until the end of the emergence; *n:* day of the counting, expressed as number of days after sowing; n_0: day when the emergency started, expressed as number of days alter sowing; *N*: number of germinated seed.
After 72 hours we measured radicle length and determined dry mass of the radicle and hypocotyls separately.

Experimental design and statistical analysis

We used a completely randomized block design with three replications for the germination testing. We analyzed the data using ANOVA and transformed germination percentages using the arc-sine square root transformation to achieve normality. When significant differences were detected, we used an a posteriori Tukey test to discriminate means (Zar, 1984). Unless otherwise indicated, all treatments were determined to be significantly different al $P<0.05$. We used SAS program (SAS Institute, 1996) for the statistical analysis.

RESULTS

Germination percentages

At 72 hours after sowing, we found significant differences in germination percentages among the treatments in *P. chilensis* seeds; yet the increments with respect to the control seeds were negligible (Figure 1A). No significant differences were found in germination percentages for *P. flexuosa* or *P. argentina*. On the other hand, we observed for the three species, but in particular for *P. chilensis*, that for any given osmotic potential, the longer periods of seed immersion resulted in lower final germination percentages.

Germination rate

Combinations of immersion period and osmotic potential significantly increased germination rate between 35 to 60% respect to control seeds in *P. chilensis*, *P. flexuosa* and *P. argentina*. In the case of *P. flexuosa*, all treatments increased germination rate. In *P. chilensis* the only treatments that did not differ from the control seeds were the immersion of seeds in PEG (-1.2 MPa) for 24 and 96 hours and in PEG (-1.4 MPa) for 72 and 96 hours. The immersion of *P. argentina* seeds in PEG (-1.2 MPa) for 72 and 96 hours and in PEG (-1.4 MPa) during 48, 72, and 96 hours did not increase germination rate relative to control seeds.

Among the priming treatments, germination rate seemed to increase linearly as the immersion period and osmotic potential increased in all three *Prosopis* species (Figure 1B). The effect of the priming treatments on germination rate seemed to be strongest in *P. flexuosa*, followed by *P. chilensis* and then *P. argentina* (Figure 1B).

The increment in germination rate following the priming treatments also resulted in an increased speed of the germination process in all the species. In *P. chilensis, P. flexuosa* and *P. argentina* the effects of priming notably affected germination rate during the first 16 hours after the sowing. The treated seeds reached germination percentages of 50% to 80% depending on the solution used, while only 15% or less of the control seeds had germinated. Only after 72 hours did the control seeds reach germination percentages comparable to those of the priming treated seeds (Figure 2). The priming treatments conducted with lower osmotic potential (-0.8 and -1.0 MPa) and during shorter periods of time (24 or 48 hours) resulted in the highest synchronization of the seed germination process.

Radicle and hypocotyls

The priming treatments affected each of the species differently regarding the radicle length (Figure 3). In the case of *P. chilensis*, although the ANOVA resulted significant none of the treatments increased radicle length relative to the control. On the other hand, most of the treatments (except for PEG -1.0 MPa for 72h, -1.2 MPa for 96hs and -0.8 MPa during 72 and 96h) enhanced radicle length compared to the control in *P. flexuosa* seeds (Figure 3). In *P. argentina* the ANOVA resulted significant between tretments respect to control, the radicle length of seed treated at -1.4 MPa for 96 hs doubled the length of the control seeds (Figure 2).

The dry mass of radicle and hypocotyls in treated seeds were not significantly different from control seeds in *P. chilensis*. In *P. flexuosa* seeds the ANOVA for dry mass of radicle was significant, however, only a few treatments resulted in higher mass than control seedlings: PEG -0.8, -1.0, -1.2, and -1.4 MPa immersed during 24 hs (data not show). The dry mass of hypocotyls in treated seeds was not different from the control. In *P. argentina* there was no difference between treatments for dry mass of radicle or hypocotyls.

DISCUSSION

The germination and seedling stages are probably the most critical periods in the life cycle of desert plants, and a long period between the sowing and emergence may predispose the seedling to deterioration resulting in less viable plants (Parera and Cantliffe, 1994). Osmotic priming has been successful in enhancing germination and emergence rate and synchronization in cultivated plants (Murungo et al*.,* 2003; Parera et al., 1993; Frett et al., 1991). Frett et al. (1991) were able to increase emergence rate in treated tomatoes and asparagus seeds relative to control seeds. Liptay and Zarifa (1993) observed that seeds treated with PEG solutions resulted in a higher emergence rate as well as a higher amount of radicles protruding from the seed coat. Zheng et al. (1994) working on *Brassica napus* seeds were able to increase the emergence rate and final germination percentages with priming treatments. Our results suggest that priming treatments could be effective in a variety of other non-cultivated plants of potential ecological importance.

In general, for the three *Prosopis* species, scarified (control) seeds reached high germination percentages after 72 hours. This has been previously observed in other studies for several species of *Prosopis*, where high germination percentages are obtained after mechanically scarifying seeds (Vilela and Ravetta, 2001). In addition, our results indicate that longer periods of immersion in osmotic solution have negative effects on the final germination percentages of *Prosopis* seeds. This may be related to the advance of germination towards the radicle protrusion phase during the treatment (Bradford, 1995; Bewley, 2001). The longer the treatment the closer the seed gets to the phase of radicle protrusion, and then there is a higher risk of damaging the seed's embryo during the dehydration period that follows the

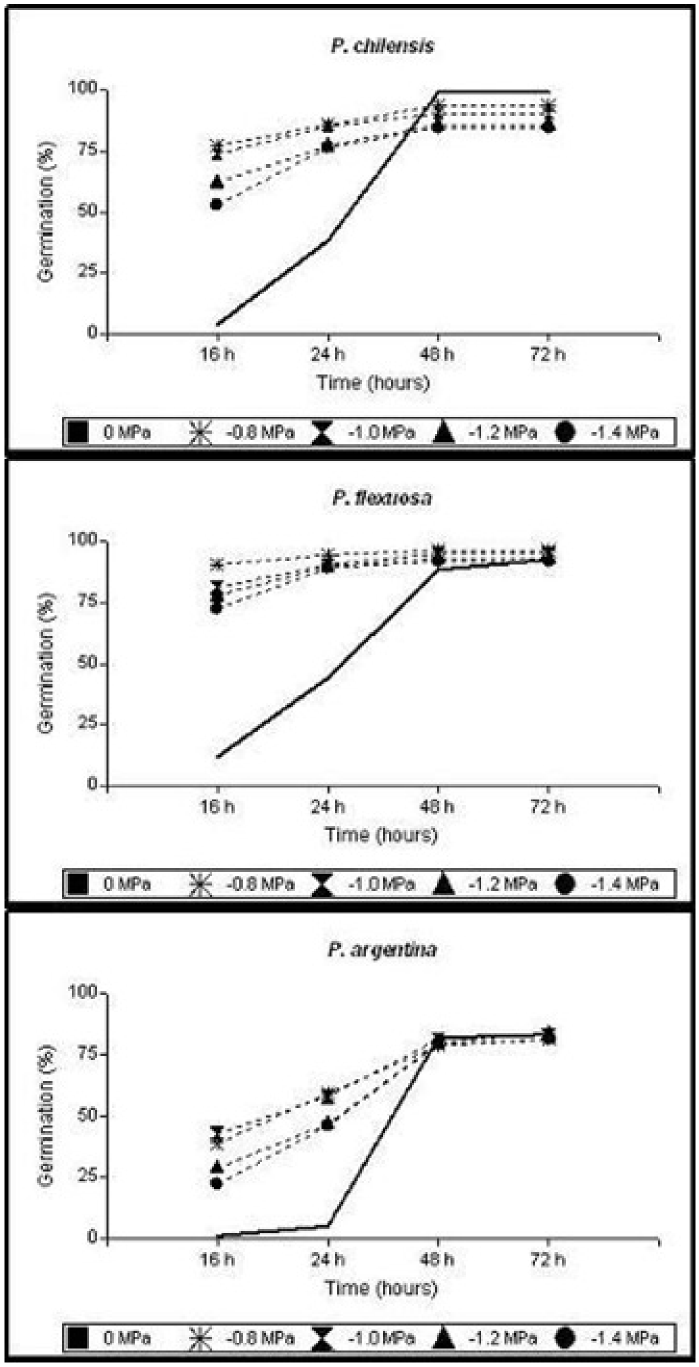

Figure 2. Germination percentages at 16, 24, 48 and 72 hours in osmotic pre-sowing treatments in different solutions (control; -0.8Mpa; -1.0MPa; -1.2MPa and -1,4MPa) in *P. chilensis*, *P. flexuosa* and *P. argentina*.

pre-sowing treatment.

For the three *Prosopis* species studied, seeds under osmotic priming greatly increased their germination rate relative to control seeds. The different parameters in a priming treatment interact resulting in different optimum combinations. For example, immersion time in the osmotic solution may show optimal results depending on the type of osmolite used, the osmotic potential of the solution, the temperature and the species being treated (Parera and Cantliffe, 1994).

The changes induced during priming that result in enhanced emergence rate are physiological as well as

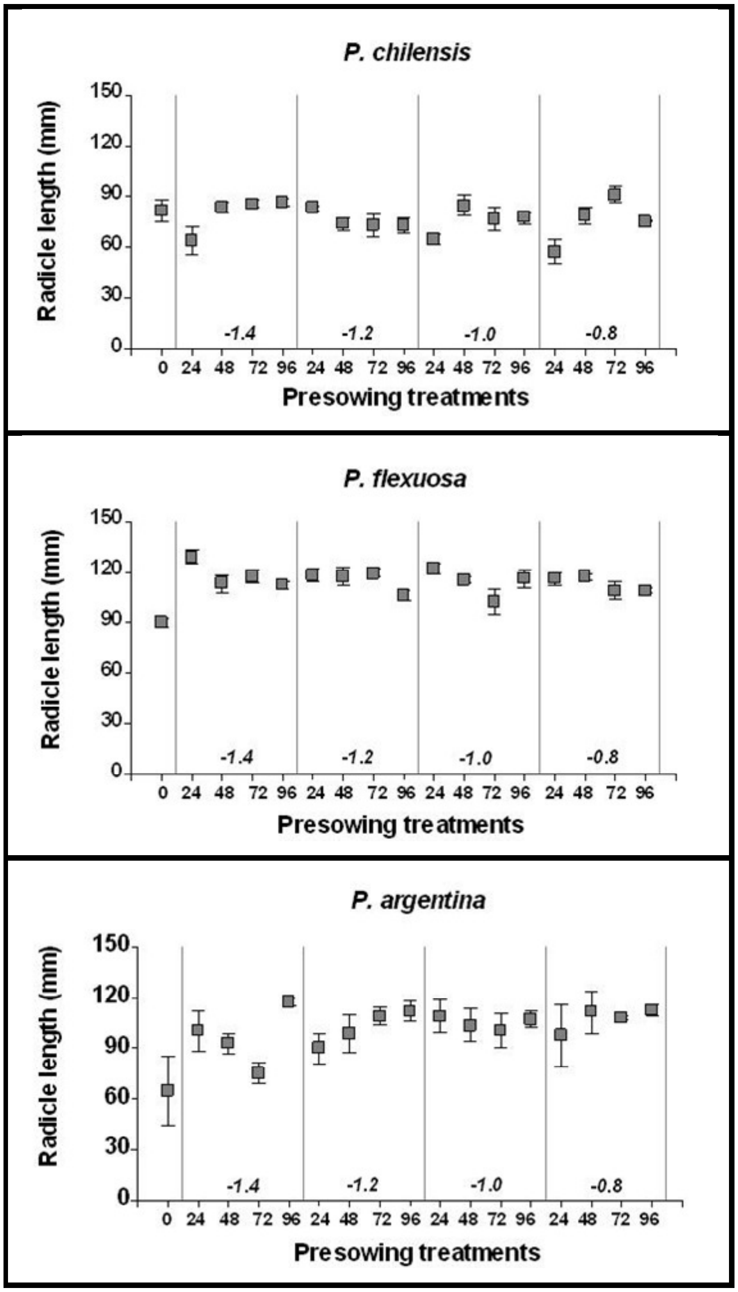

Figure 3. Radicle length (mm) of three *Prosopis* species seedling after osmotic pre-sowing treatments (0=control; 24, 48, 72 and 96 hours at -1.4, -1.2, -1.0 and –0.8 MPa) in *P. chilensis*, *P. flexuosa* and *P. argentina*. Vertical bars are s.e. of mean.

structural modifications. An adequate osmotic potential allows for sufficient seed hydration to activate the cellular metabolism but at the same time avoid radicle protrusion (Bradford, 1995). If the osmotic potentials of the solution are closer to zero, the germination advances towards the phase of growth and radicle emergence, making it impossible to dehydrate the seeds without the death of the embryo. The same pattern has also been observed for other species, for example Danneberger et al. (1992) found that in *Lolium perenne* seeds osmotic potentials of -1.1 MPa enhanced germination more than osmotic potentials of -0.5 MPa and -1.4 MPa.

Among the studied *Prosopis* species, the effect of the priming treatments on germination rate seemed to be strongest in *P. flexuosa*, followed by *P. chilensis* and then *P. argentina*. Probably for the last species other combinations of osmotic potential and immersion time should be tested to reach a higher emergence velocity. The priming treatments tested with *P. chilensis, P. flexuosa* and *P. argentina* seeds significantly increased the germination rate relative to control seeds. The treatments were particularly effective especially during the 24 hours after the sowing. This constitutes an advantage for both the production of seedling in nurseries and direct sowing of seeds, since the treatments are able to reduce to a great extent the time between sowing and emergence. The shortening of this period results in a reduction of the time the seeds are exposed to soil microorganisms that often are responsible for the deterioration of the seeds. However, the risk of a post-emergence drought destroying the entire seeded population is also greater if all the seedlings emerge at the same time. The use of PEG solutions of osmotic potentials between -0.8 MPa and -1.0 MPa ensures better results than using more concentrated solutions. Also, for the three species studied, between 24 and 48 hours constitute the optimal priming immersion periods.

The increase in radicle length after priming treatments that occurred in *P. flexuosa* and marginally *P. argentina* were somewhat expected, since a faster germination rate could be easily associated with a faster growth rate. Moreover, it constitutes a noteworthy result with direct implications on the seedlings production in nurseries or after direct sowing. It has been observed by other authors that the growth of several species of the genus *Prosopis* is extremely slow during the earlier stages of development (Felker, 1996; Catalan et al., 1994) and that, under natural conditions, seedlings show very low survival percentages (Scifres and Brock, 1970a, 1970b). In general, rapid emergence and growth are supposed to be highly advantageous since they favor resource appropriation by seedlings and therefore increase successful establishment probabilities (Mooney et al., 1977). These are key characteristics particularly for the desert where climate can be quite adverse and unpredictable and saline soils are abundant (Sosebee and Wan, 1987; Rathcke and Lacey, 1985). What is surprising from our results is that none of the priming treatments increased radicle length relative to control seeds in *P. chilensis.* This suggests that the effects of osmotic priming on radicle growth and seedling development and the mechanisms involved in the process need further study.

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