Osmotic Priming Hastens Germination and Improves Seedling Size of *Pinus brutia* var. *eldarica*

Shad K. Khalil, John G. Mexal, and Melchor Ortiz

Assistant professor, Department of Agronomy, Agriculture University, Peshawar, Pakistan; professor, Department of Agronomy and Horticulture, and professor emeritus, Department of Experimental Statistics, New Mexico State University, Las Cruces, New Mexico

Production of eldarica pine—Pinus brutia *var.* eldarica *seedlings for Christmas tree or ornamental use could be improved by more rapid germination. Seeds of this species exhibit shallow dormancy and generally do not require stratification. However, osmotic priming may speed emergence. Our objective was to examine the response of eldarica pine seeds to osmotic priming. Seeds were preconditioned at room temperature in an aerated solution of polyethylene glycol (PEG) 8000 for 2, 5, 7, 9, and 11 days, at one of the following concentrations-200, 300, and 400 g PEG /kg water. After treatment, seeds were sown in plastic trays and grown in a greenhouse for 12 weeks. PEG reduced germination 7 to 15 percentage points. However, speed of germination measured as days to 50% germination (T_{50}) was reduced 4 days by treatments of up to 9 days' duration. Because of the more rapid germination, shoot length and shoot dry weight measured 12 weeks after sowing were increased by priming. Concentrations of 200 or 300 g PEG/kg water for 9 days provided the best response. Although osmotic priming increased speed of germination and subsequent seedling size, other invigoration treatments such as stratification or controlled hydration may offer greater benefits in nursery production at lower cost.* Tree Planters' Notes 48(1/2): 24-27; 1997.

Rapid germination is desired in conifer seedling production because it increases seedling size and uniformity and improves yield. Rapid germination also can reduce to risk to soil-borne diseases during the germination process. Eldarica *pine—Pinus brutia* var. *eldarica—is* an important species for live Christmas trees, windbreaks, and ornamental use in the southwestern United States. Unfortunately, germination of eldarica pine is often variable in speed and capacity, and the cost of seed is high (>$0.02 for each pure live seed). Therefore, improvements in germination and establishment would help growers meet local seedling demands. *Pinus brutia* provenances vary in their response to stratification, with southern provenances germinating over a wide temperature range without stratification (Skordilis and Thanos 1995). Northern provenances have poor germination without stratification. Operational data from growers indicate that *Pinus brutia* var. *eldarica* behaves like the southern provenances in the aforementioned study.

Several methods have been used to precondition seeds to improve seedling establishment of vegetable and field crops. Osmotic priming is one technique offering promise for improvement in germination speed and completeness (Khan and others 1990). Priming is a controlled hydration technique that enables seed to absorb water and start pregerminative metabolic activities while maintaining seeds under mild water stress to prevent radicle emergence (Bradford 1986; Heydecker and Coolbear 1977). Water uptake is regulated with large-molecular-weight osmotic agents, such as polyethylene glycol (PEG), which are not absorbed as readily as salts.

Osmotic priming reduces days to 50% germination for shortleaf pine *(Pinus echinata* Mill.), Scots pine *(P. sylvestris* L.), and loblolly pine *(P. taeda* L.) (Hallgren 1987, 1989; Simak and others 1984). However, effects of PEG on germination percent are variable. Priming increased total germination of slash pine *(P. elliottii* Engelm.), Scots pine, and loblolly pine (Hallgren 1987; Haridi 1985; Simak and others 1984). Decreases in total germination have been reported for Norway spruce *(Picea abies* (L.) Karst.) (Simak 1985), white spruce *(P. glauca* (Moench) Voss) (Downie and others 1993), and slash pine (Hallgren 1989). This experiment measures the effect of polyethylene glycol (PEG 8000) concentrations and soaking durations on greenhouse germination and seedling growth of eldarica pine.

Materials and Methods

Seeds of eldarica *pine—Pinus brutia* var. *eldarica-* were soaked in PEG 8000 solutions (200, 300, and 400 g PEG/kg water) for 2, 5, 7, 9, or 11 days at room temperature. The osmotic potential of PEG 8000 solutions was —0.5 MPa for 200 g/kg water, —1.1 MPa for 300 g/kg, and —1.8 MPa for 400 g/kg (Michel 1983). Seeds were aerated during soaking in erlenmeyer flasks with an aquarium pump. Distilled water was added daily to maintain water level and water potential. After priming, the seeds were rinsed with tap water for about 2 minutes. The control treatment consisted of soaking seeds in distilled water for 8 hours. Unpublished work indicated that soaking seeds in aerated water for 5 or more days resulted in germination during the soaking treatment. Thus, a water duration treatment was dropped.

Seeds were sown on December 1, 1989, in a greenhouse in plastic trays measuring 52 (37 cm filled with peat moss. The seeds were covered with a thin layer of vermiculite. The temperature in the greenhouse was 25 °C (= 77 °F) with max/min variation of 5 °C (9 °F). Each treatment contained 60 seeds replicated 3 times. Emergence counts were made on alternate days until the 30th day. Emerging seedlings were tagged with colored rings to record time of emergence (Mexal and Fisher 1987). Shoot length and shoot dry weight were recorded by randomly selecting 3 seedlings from each treatment every 2 weeks from week 4 to week 12. At 12 weeks, the remaining seedlings were oven-dried at 70 °C (= 158 °F) for 3 days. The experiment was split block designed as a 3 x 6 factorial. Data were analyzed using analysis of variance technique and the least significant difference (LSD) test was applied when F-values were significant.

Results

Germination. Germination percentage was decreased by duration of priming (D) but not by the concentration of PEG (table 1). Furthermore, there was a significant interaction between PEG concentration and duration. The control (water soak for 8 hours) had the best germination percentage (94.7%), but the poorest speed of germination (figure 1). Germination

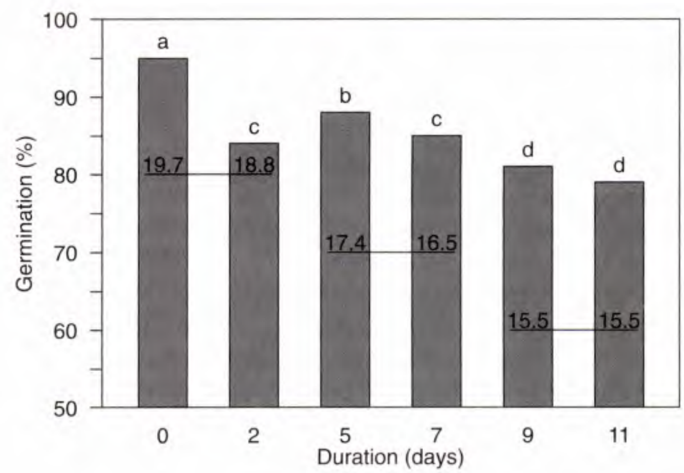

Figure 1—*Germination percent and days to 50% germination (numbers within bars) of eldarica pine seed following priming with PEG 8000 for various durations. Bars with the same letters, and numbers under the same line are not significantly different* (P = 0.05) *using least significant differences.*

decreased as seed treatment duration increased and poorest emergence was for seed soaked for 9 and 11 days. However, these 2 treatments had the fastest germination. The days to 50% germination (T_{50}) were about 4 days earlier for 9- and 11-day priming treatments compared to the control. Additionally, T_{50} was affected by PEG concentration. The lowest concentration (200 g/kg) resulted in faster emergence (T_{50} = 16.7 days) compared to the highest concentration (T_{50} = 17.9 days). The T_{50} for the intermediate concentration (T_{50} = 17.1 days) was not significantly different from the lowest concentration.

Seedling morphology. Duration of priming significantly affected shoot length and shoot dry weight, but

Table 1—*Analysis of variance of percentage germination, days to 50% germination (T_{50}), shoot length, and shoot and root dry weight of eldarica pine as affected by PEG 8000 concentration and seed priming duration.*

		Mean squares				
					Dry weight (g)	
Source	DF	Germination (%)	T_{50} (days)	Shoot length (cm)	Shoot	Root
Replication	2	12.62	12.66	0.6848	0.0037	0.0005
		—	—	—	—	—
Concentration	2	1.68	6.89	0.1631	0.0001	0.0001
		(.746)	(.003)	(.412)	(.726)	(.287)
Duration	5	103.81	27.38	0.6723	0.0010	0.0001
		(<.001)	(<.001)	(.004)	(<.001)	(.797)
C×D	10	25.62	10.00	0.2459	0.0004	0.0001
		(<.001)	(.060)	(.212)	(.138)	(.351)
Error	34	29.35	0.98	0.2868	0.0003	0.0001

Values in parenthesis are P values for mean squares values above it.

not root dry weight (table 1). Shoot length tended to increase with each increment of seed treatment duration (figure 2). The control treatment had the shortest seedlings, while the 9-day priming treatment had the tallest seedlings at the end of the study. Shoot weight tended to follow similar relationships. However, root weights were similar (figure 3), and the R/S ratio was not clearly related to treatment.

Improved growth of primed seed seemed attributable to the earlier emergence. Growth curves of all treatments fit the same linear relationship (figure 4). The only difference was that the 5- day priming treatment reached T_{50} = 2.3 days earlier than the control, and the 9-day priming treatment reached T_{50} = 4.2 days earlier. Other priming treatments were intermediate in response but not shown. The difference in time of emergence and growth over time seem to fit reasonably well on the regression line, which would explain the differences in morphology at the end of the growth period.

Furthermore, significant differences were lacking among the regression slopes of the different priming treatments. The response for shoot dry weight was similar (data not shown).

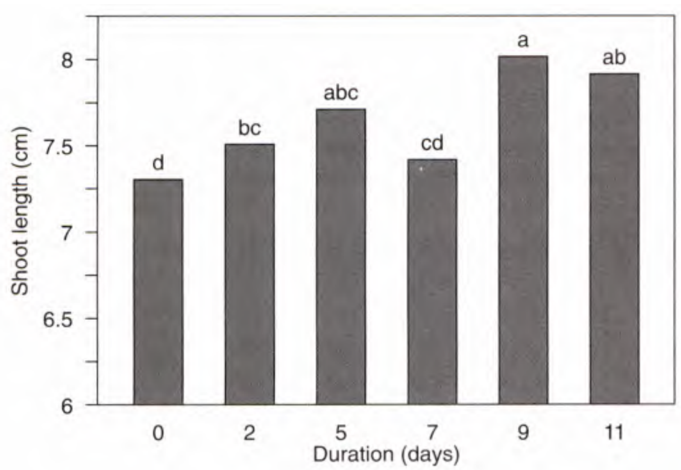

Figure 2—*Shoot length (cm) of eldarica pine seed following priming with PEG 8000 for various durations. Bars with the same letters are not significantly different* (P = 0.05) *using least significant differences.*

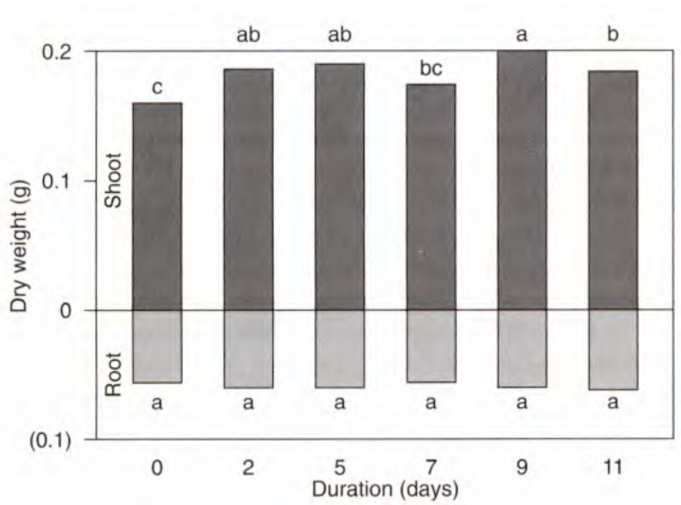

Figure 3— *Shoot and root dry weight (g) of eldarica pine seed following priming with PEG 8000 for various durations. Bars with the same letters are not significantly different* (P = 0.05) *using least significant differences.*

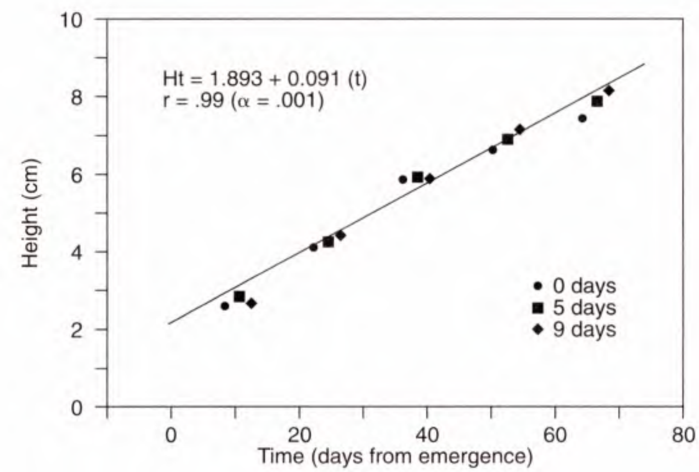

Figure 4—*Relationship between days from emergence and height (cm) for eldarica pine seed following priming with PEG 8000 for 0, 5, or 9 days.*

Discussion

Priming with PEG 8000 decreased total emergence of eldarica pine seed compared to seed soaked in distilled water. Similar responses of total germination were reported by Hallgren (1987, 1990) for slash and loblolly pine. Days to 50% germination (T_{50}) was reduced by priming. Maximum reduction in days to 50% emergence was observed for seed treatment duration of 9 days and a PEG concentration of 200 g/kg. Murray (1990), Lopes and Takaki (1988), Carpenter (1989), and Dearman and others (1986) also reported reduction in T_{50} when seeds were treated with PEG for different durations

Shoot length and shoot dry weight increased with each increment of seed treatment duration up to 9 days duration, but further increases in duration were of no additional benefit.

Osmotic priming hastens germination, resulting in increased seedling size of eldarica pine. However, priming also reduces overall germination. A reduction in germination is an undesirable feature that mitigates against recommending this as a promising treatment. It is possible that part of the improvement in germination speed results from the mortality of late germinants. These germinants likely would die anyway, as late emerging seedlings suffer increased mortality compared to early emerging neighbors (Mexal and Fisher 1987). In nurseries where prompt emergence is important to avoid washing from heavy rains, the tradeoff between prompt yet lower emergence may be worthwhile. Treating the seed for 9 days speeds the emergence in the nursery by about 4 days. This could reduce to risk from seedbed washing or seedling loss to soil-borne damping off, and increase the size of the crop. However, earlier sowing or possibly stratification could accomplish the same effect. This technique should be used only in emergency situations where inventory indicates a need for additional seedlings.

Address correspondence to: Dr. John Mexal, New Mexico State University, Dept. of Agronomy and Horticulture, Box 30003, Dept. 3Q, Las Cruces, NM 88003; e-mail: jmexal@nmsu.edu

Literature Cited

Bradford KJ. 1986. Manipulation of seed water relation via osmotic priming to improve germination under stress conditions. HortScience 21: 1105-1112.

Carpenter WJ. 1989. *Salvia splendens* seed pregermination and priming for rapid and uniform plant emergence. Journal of the American Society for Horticultural Science 114(2): 247-250.

Dearman J, Brocklehurst PA, Drew RL. 1986. Effect of osmotic priming and aging on onion seed germination. Annals of Applied Biology 108: 639-648.

Downie B, Bergsten U, Wang BSP, Bewley JD. 1993. Conifer seed germination is faster after membrane tube invigoration than after prechilling or osmotic priming. Seed Science Research 3: 259-270.

Hallgren SW. 1987. Priming treatments to improve pine seed vigor. In: Proceedings, Intermountain Forest Nursery Association Conference; 1987 August 10-14; Oklahoma City, OK. Tech. Rep. RM-151. Ogden, UT: USDA Forest Service, Intermountain Forest and Range Experiment Station: 33-35

Hallgren SW. 1989. Effects of osmotic priming using aerated solutions of polyethylene glycol on germination of pine seeds. Annales des Sciences Forestieres 46: 31-36.

Haridi MB. 1985. Effect of osmotic priming with polyethylene glycol on germination of *Pinus elliottii* seeds. Seed Science and Technology 13: 669-674.

Heydecker W, Coolbear P 1977. Seed treatment for improved performance-survey and attempt prognosis. Seed Science and Technology 5: 353-25.

Khan AA, Miura H, Prusinski J, Ilyas S. 1990. Matriconditioning of seeds to improve emergence. In: Proceedings, National Symposium on Stand Establishment for Horticultural Crops. 1990 April 4-6: 29-40.

Lopes VB, Takaki M. 1988. Seed germination of *Phaseolus vulgaris:* 2. Effect of water potential and photoperiod. Arquivos de Biologia e Technologia 31(2): 307-312.

Mexal JG, Fisher JT. 1987. Size hierarchy in conifer seedbeds: 1. Time of emergence. New Forests 1: 187-196.

Michel BE. 1983. Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiology 72: 66-70.

Murray AG. 1990. Priming sweet corn seed to improve emergence under cool conditions. HortScience 25(2): 231.

Simak, M. 1985. Seed germination after incubation at different moisture stress. In: Proceedings, IUFRO International Symposium on Seed Problems Under Stressful Conditions; 1986 June 3-8; Vienna and Gmunden, Austria. FBVA Berichte 12: 125-137.

Simak M, Sahlen K, Thorsen J. 1984. Germination of PEG treated Scots pine seeds under various stress conditions. In: Pert K, ed. Ecology and Management of Forest Biomass Production Systems. Swedish University Agricultural Science Report 15: 309-322.

Skordilis A, Thanos CA. 1995. Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis.* Seed Science Research 5: 151-160.