OSMOTIC PRIMING AND STRATIFICATION ENHANCE EMERGENCE AND SEEDLING SIZE OF *PINUS BRUTIA* VAR ELDARICA

Shad K. Khalil[1], John G. Mexal[2], Abdur Rehman, A. Z. Khan and Iftikhar Hussain Khalil[3]

Abstract

Pinus brutia var. eldarica is gaining popularity because it provides high quality Christmas tree under a wide range of growing conditions in addition to its use in pulp and other wood products. Rapid and complete germination is one of the problems associated with this species. Osmoconditioning has been used for the speedy germination of most plant species. Eldarica pine seeds were osmoconditioned with the objective to evaluate its response to priming. Seeds were preconditioned in an aerated solution of polyethylene glycol (PEG) 8000 and water for 1, 4, 8, and 12 days. The osmotic potential of PEG solution was –0.5 and –1.8 MPa. In two other treatments, the seeds were aerated in distilled water for 1 day and refrigerated having 100% moisture content (MC) or reducing MC to 85% and then refrigerated for the above duration. Seeds were sown in the greenhouse in Ray-Leach tubes at Fabian Garcia Center, New Mexico State University, USA. Seed soaked in distilled water for more than 4 days failed to germinate. Seed refrigerated after 1 day soaking had increased total emergence, reduced days to 50% emergence (T50). However, none of the treatments produced significantly heavier seedlings. Early emerged seedlings resulted in heavier shoots and roots than late emerged seedlings.

Key word: PEG 8000, concentration, duration, stratification, biomass, eldarica pine.

Introduction

Rapid and uniform germination not only increases seedling size and yield of conifers but also minimizes the chances of seed borne pathogens during germination. Although eldarica pine is an important Christmas tree, and an ornamental plant in southwestern United States, yet its germination is very slow and non-uniform. Speedy and increased germination and establishment of seedlings would provide sufficient plants to meet local demands for seedlings. Pine seeds need stratification to induce germination; however, stratification requirement is species dependent. Stratified seed showed 96% germination in loblolly pine compared to 53% germination of non-stratified seed (Hallgren 1987). Seed priming has been used successfully for improvement of germination of most of field crops and vegetables (Khan *et al*., 2005, Khalil *et al*., 2003, Musa *et al*., 2001, Harris *et al*., 2001 a&b, Khan *et al.,* 1990). In this method the quiescent seed is exposed to external water potential sufficiently low to prevent radical protrusion and yet stimulate physiological and biochemical activities (Khan 1992). Various osmotic agents are used to precondition the seeds but PEG 8000 having higher molecular weight could safely be used as the seed does not readily absorb it and the chances of seed damage during priming are minimized. (Khalid *et al*., 2001).

Seed treatment with PEG enhanced speed of emergence of various pine species such as loblolly (*Pinus taeda* L.) and short leaf pine (*Pinus echinata* Mill.) (Hallgren 1990),

1 Department of Agronomy, Agriculture University, Peshawar, Pakistan
2 Department of Agronomy and Horticulture New Mexico State University Las Cruces, New Mexico, USA
3 Department of Plant Breeding and Genetic, Agriculture University, Peshawar, Pakistan

slash pine (*Pinus elliottii* Englem) and Scots pine (*Pinus sylvestris* L.) (Simak *et al*.,1984). The effect of PEG on total germination was different. Hallgren (1987), and Simak *et al*. (1984) reported an increase in the total germination. However, Hallgren (1990), Downie *et al*. (1993) and Simak (1985) reported decrease in total emergence of PEG treated seed. Primed seed produced heavier seedlings (Khalil *et al*., 1997).

The objective of this research was to evaluate the effect of seed priming and stratification on germination and seedling growth of eldarica pine various polyethylene glycol (PEG 8000) concentrations and seed treatment durations.

Materials and Methods

Seeds of *Pinus brutia* var. eldarica were primed for 1, 4, 8 and 12 days at room temperature soaked in aerated solution of PEG 8000 having 200, and 400g PEG kg^{-1} water and distilled water. In two separate treatments the seeds were soaked in distilled water for 1 day and were refrigerated for 1, 4, 8 and 12 days immediately after soaking, while in last treatment the seeds were weighed after 1 day soaking, spread in the sun, reweighed till seed moisture content was reduced to 85% and then refrigerated for 1, 4, 8 and 12 days. The osmotic potential of the PEG treated seed was –0.5 and –1.18 MPa (Michel 1983). The seeds were aerated in erlenmeyer flasks using an aquarium pump. Distilled water was added every day so that moisture content of the solution would not drop from a constant level. After priming, the seeds were rinsed with tap water for about 2 minutes. Seed moisture content was determined by weighing 10 seeds individually from each treatment immediately after seed coat removal and reweighed after drying at 70°C for 3 days.

The seeds were sown in greenhouse, Fabian Garcia Center, New Mexico State University. The seeds were planted in ray-leach tubes measuring inches 6.5 x 1.3 inches filled with peatmoss covered with a thin layer of vermiculite at a temperature of 26°C±4°C. The design used was randomized complete block with split block having 5 × 4 factorial treatments comprised 60 seeds replicated 3 times. Germination counts were made on alternate days until the 30th day, thereafter no germination occurred. Days to 50% emergence (T50) were counted from sowing till 50% of the seedlings emerged in each treatment. All the germinants from each treatment were summed to record total germination data. Emerging seedlings were tagged with colored rings to record time of emergence (Mexal and Fisher 1987). Shoot length, shoot and root dry weights were recorded by randomly selecting 3 seedlings from each treatment starting from every 2 weeks from 4 weeks to 12 weeks. Seedlings were dried at 70°C for 3 days at 12 weeks to record shoot and roots weight. Data were analyzed using analysis of variance technique and LSD Test was applied when F-values were significant (Snedecor and Cochran 1989).

Results

Moisture Content

PEG concentration and priming duration significantly affected moisture content (Table 1). Seed soaked in distilled water absorbed more water (31.1%) than all other concentrations, followed by 200g PEG, which absorbed 26.9% moisture, while control (dry seed) contains only 5.9% moisture (Table 2). Moisture content of the seed increased with each increment of duration and maximum moisture (30.9%) was observed for 12 days duration. Seed soaked either in distilled water or 200 g PEGkg^{-1} water for 12 days absorbed maximum moisture. Radical protrusion was observed in seed soaked in distilled water for 12 days, but not in PEG treated seed.

Emergence

Concentration (C), Duration (T) and C×T interaction significantly affected emergence (Table 1). Generally stratified seed produced more emergence than PEG treated seed or seed soaked in distilled water and maximum germination was observed in seed soaked in distilled water for 1 day and stratified at 100% moisture content resulting in 94.1% emergence compared to 48.8% emergence noted for seed soaked in distilled water. (Fig.1).

Emergence decreased with each increment of seed treatment duration and minimum emergence (63.8%) was observed for 12 days duration (Fig. 2). Emergence curves fit inverse relationship (Fig. 3)

Days to 50% Emergence (T50)

Duration (T) and concentration (C) significantly affected T50 emergence (Table 1). T50 decreased with stratification and PEG concentration except 400g PEG where soaking duration did not enhance T50. No T50 was observed when seed soaked in water for 8 or 12 days Fig. 4). The best treatment was 200 g PEG soaked for 4 days. Seed treatment duration enhanced T50 by 0.5, 1, and 0.2 days for 4, 8, and 12 day's duration (Fig. 5). T50 curves fit the same inverse relationship (6).

Seedling morphology

Shoot Length

Concentration (C), emergence date (D), C×T, C×D, T×D and C×T×D significantly affected shoot length (Table 3). Seed soaked in distilled water produced tallest seedlings while 200 g PEG resulted in the shortest seedlings (Fig. 4). Seed treated for 1 day produced taller seedlings than 8 or 12 days treatment (Fig. 5).

Seedling emerged on day 9 resulted in tallest seedlings. Seedling height decreased with delay in emergence and shortest seedlings were recorded when emerged on day 29.

Stratified seed with 100% m.c. emerged on day produced tallest seedlings, while 200g PEG treated seed produced shortest seedlings (Table 4).

Shoot Dry Weight

Emergence date (D) significantly affected shoot dry weight (Table 3). Early emerged seedling produced heavier seedlings than late emerged seedlings (Fig. 7). Delay in emergence showed inverse relationship with shoot dry weight (Fig. 8).

Root Dry Weight

Concentration (C), and emergence date (D) significantly affected root dry weight (Table 3). Seed soaked in distilled water resulted in heavier roots, followed by seed treated with 400 g PEG, while stratified seed having 100% mc or seed treated with 200 g PEG resulted in lighter roots (Fig. 9). Early emerged seedlings produced heavier roots than late emerged seedlings and lighter roots were recorded from seedlings emerged on day 29 (Fig. 10). Root growth curves fit the same inverse relationship (Fig. 11).

Discussion

Stratification and seed treatment with PEG 8000 both increased total emergence of eldarica pine compared to seed soaked in distilled water. Emergence was maximum for the stratified seed compared to seed primed with PEG 8000. Increase in emergence of stratified seed of loblolly and slash pine was also reported by Hallgren (1987). Priming increased total emergence compared to non-primed seed. Similar results were reported by Simak *et al*. (1984) and Carpenter (1989) who reported increased total emergence of Scots pine and Salvia splendens. Increase in total germination of soybean was also reported by Khalil *et al*. (2001).

Seed treated with PEG for 4 days had the lowest T50. Seed soaked in water for more than 4 days failed to germinate. Seed treatment longer than 4 days may have deleterious effect on enzymatic system responsible for germination and thus the total germination might have been reduced. Reduction in T50 was also reported by Khalil *et al*. (1997), Murray (1990), Lopes and Takak (1988), Carpenter (1989) and Dearman *et al*. (1986). Faster emergence from primed seed of field crops has reported by Harris *et al*. (1999), Harris *et al*. (2000), Harris *et al*. (2001a&b) and Musa *et al*. (2001). Enhancement of T50 is species dependent and may not be true for all species. Carpenter (1990) and Bussel and Gray (1976) observed no enhancement in days to T50 when seeds of dusty miller and tomato were treated with PEG.

Seed treated with PEG for 1 or 4 days produced tallest seedlings. This increase in shoot length was due to the beneficial effect of PEG treated seed emerged earlier and resulted in taller seedlings than control. Khalil *et al*. (1997) also reported increase in shoot length. Seedlings that emerged earlier produced heavier seedlings than late emerged seedlings showing that increase in shoot weight is related with time of emergence. These results are in line with Khalil *et al*. (1997) who reported heavier seedlings from early-emerged seedlings than late emerged seedlings.

Osmotic priming and stratification increased total germination and enhanced T50, resulting in heavier seedling. Stratification seems to be the best treatment as it resulted in more and quick emergence and also out yielded the PEG treated seed.

Table 1. Analysis of variance table of %emergence and days to 50% (T50) emergence of eldarica pine as affected by PEG 8000 concentration and seed treatment duration. Values in parenthesis are P values for mean squares values above it.

Source	D.F.	Mean squares		
		Moisture	Emergence (%)	T50
Rep (R)	2	-	11.93	2.19
Con (C)	4	83.00	4500.42	46.81
-		(.001)	(.000)	(.000)
Duration T	3	148.28	482.09	15.24
		(.003)	(.000)	(.011)
C x T 1	2/10	-	670.95	19.57
-			(.000)	(.000)
Error	38/12*/31**	13.91	74.83	3.47

* D.F for moisture content
** D.F. for T50

Table 2. Moisture content (%) of eldarica pine as affected by PEG 8000 concentration and seed treatment duration (dry seed = 5.9%)

Treatment	Moisture content (%) Duration (days)				
	1 Mean		4	8	12
Water	24.4	31.5	31.5	37.0	31.1a*
200 g PEG	13.3	25.4	30.7	38.3	26.9 ab
300 g PEG	12.2	19.1	25.4	29.1	21.4 bc
100% m.c.	23.8	26.5	26.8	27.9	26.2 ab
85% m.c.	15.8	21.7	20.0	22.3	19.9c
	17.9C*	24.8b	26.9ab	30.9a	

* Means in a row or column with the same letters are not significantly different using LSD test at p = 0.05.

Table 3. Analysis of variance table of shoot length, shoot and root dry weight of eldarica pine as affected by emergence date PEG 8000 concentration and seed treatment duration. Values in parenthesis are P values for mean squares above it.

Source	D.F.	Shoot length	Shoot D. wt.	Root
Rep (R) 2	2	0.13	.0004	.0003
		-	-	-
Con (C) 4	4	2.26	.0015	.0017
		(.021)	(.171)	(.000)
Tart (T) 3	3	1.42	.0007	.0012
		(.123)	(.548)	(.000)
C x T 12	12	1.87	.0020	.0004
		(.010)	(.0020	.0004
Error (a) 38/36	38/36	0.69	.0014	.0004
		-	-	-
Dates (D)5	5	8.91	.0477	.0148
		(.003)	(.000)	(.000)
Error (b) 10	10	1.15	.0007	.0002
-		-	-	-
C x D 20	20	.94	.0012	.0003
		(.036)	(.212)	(.057)
T x D 15	15	.95	.0010	.0001
		(.046)	(.420)	(.708)
CxTxD	41/43*	.87	.0009	.0002
		(.025)	(.623)	(.262)
Error(c)	89/82*	.53	.0009	.0002

* D.F. for root D. wt.

Table 4. Shoot length (cm) of eldarica pine as affected by PEG 8000 concentration, seed treatment duration and emergence date

Concentration g kg^{-1} water	Day to Emergence						
	9	13	17	21	25	29	Mean
0	9.9ab	9.9ab	9.4bc	9.0cdef	8.8ef	10.0a	9.5
200	9.1cdef	9.3bcd	9.3bcd	8.6def	8.5ef	7.0g	8.6
400	9.6bc	9.8b	9.7b	8.8def	8.8def	8.4f	9.2
100% m.c.	10.6a	9.6bc	9.6bc	8.5ef	9.2cde	74g	9.2
85% m.c.	9.8b	9.6bc	9.8b	8.8def	8.9cef	8.5ef	9.2
Mean	9.8	9.6	9.6	8.7	8.8	8.3	-

Table 5. Shoot length (cm) of eldarica pine as affected by PEG 8000 concentration, seed treatment duration and emergence date

Duration (days)	Days to Emergence						
	9	13	17	21	25	29	Mean
1	10.0ab	10.0ab	9.8bc	9.0fg	9.0fg	8,2l	9.3
4	10.4a	9.6bcd	9.5cde	8.7gh	8.7gh	9.1efg	9.3
8	9.7bcd	9.7bcd	9.6bcd	8.5hi	9.1efg	7.6j	9.0
12	8.7cdef	9.4cdef	9.3def	8.4hi	8.3hi	8.7gh	8.8

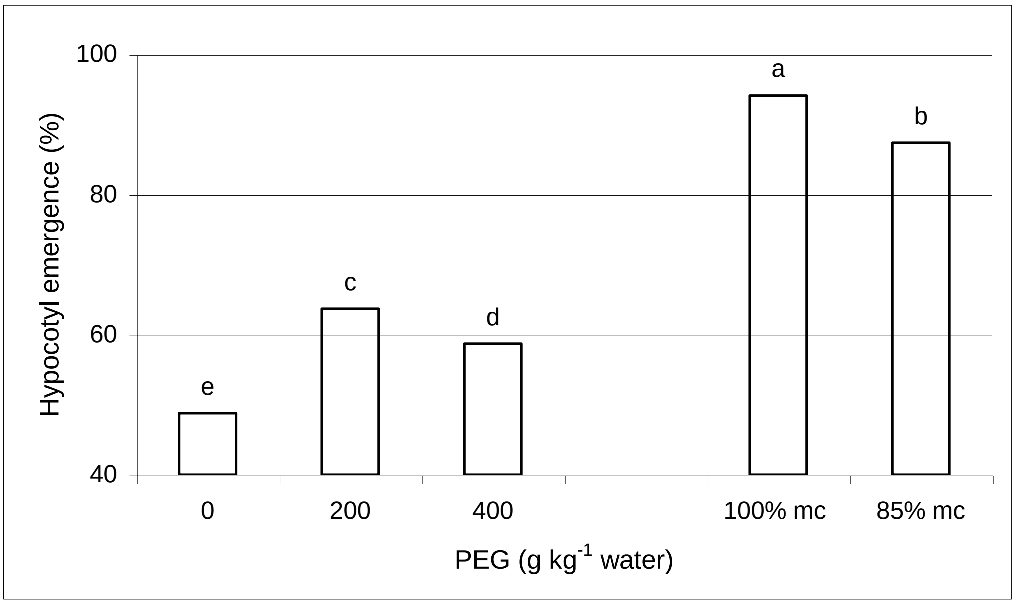

Fig 1. Hypocotyl emergence of eldaria pine as affected by PEG 8000 concentration and stratification

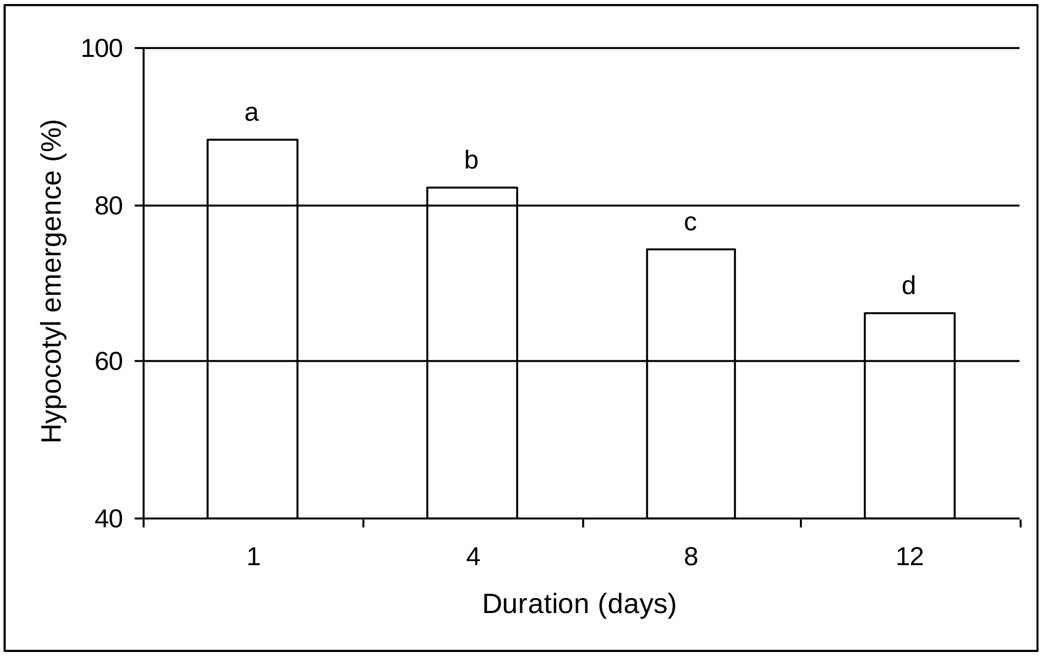

Fig. 2. Hypocotyl emergence of eldarica pine as affected by seed treatment Duration

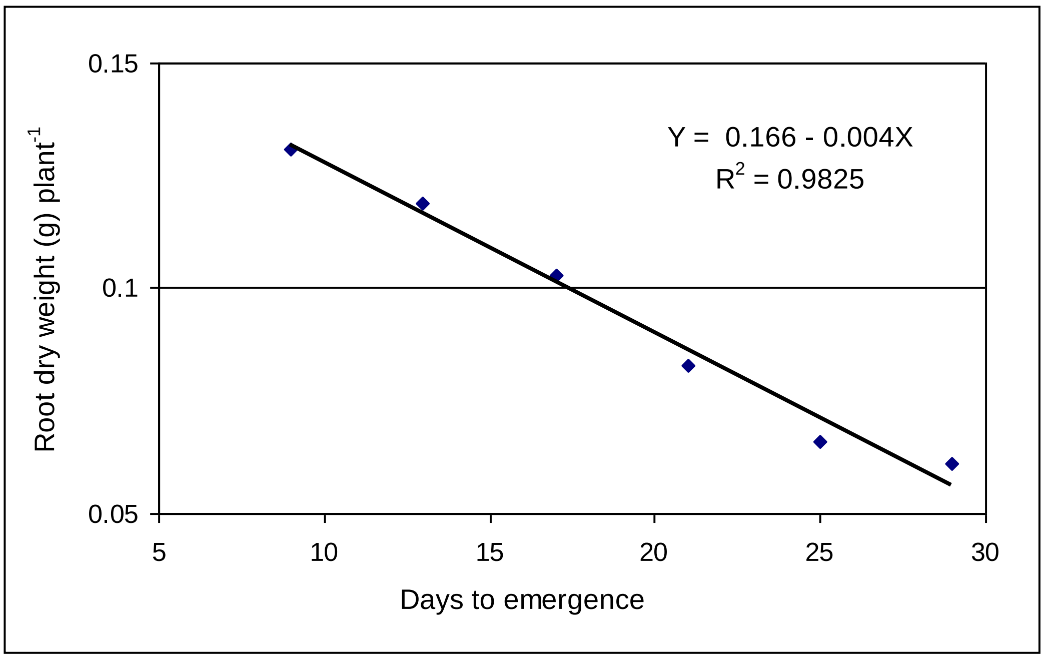

Fig. 3. Days to 50% emergence (T50) of eldarica pine as affected by seed treatment duration

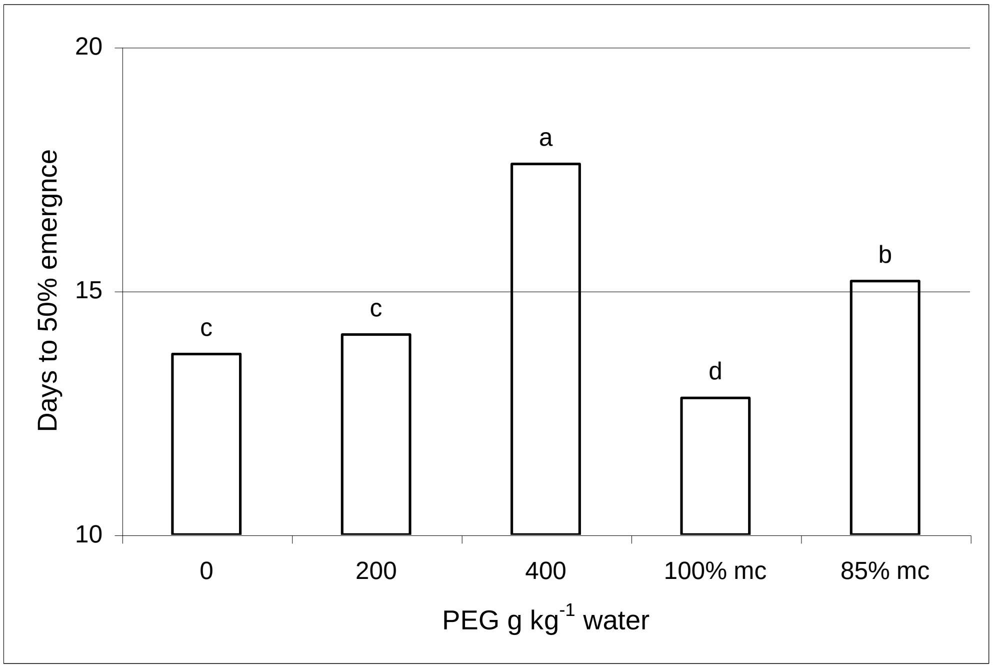

Fig. 4. Days to 50% emergence (T50) of eldarica pine as affected PEG 8000 concentration and refrigeration

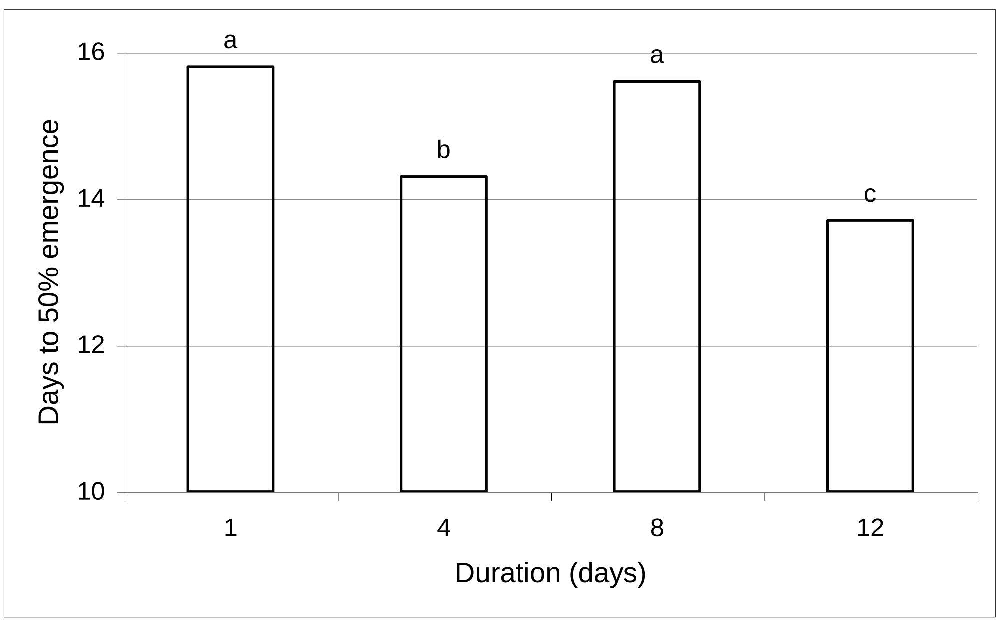

Fig. 5. Days to 50% emergence of eldarica pine as affected by seed treatment duration

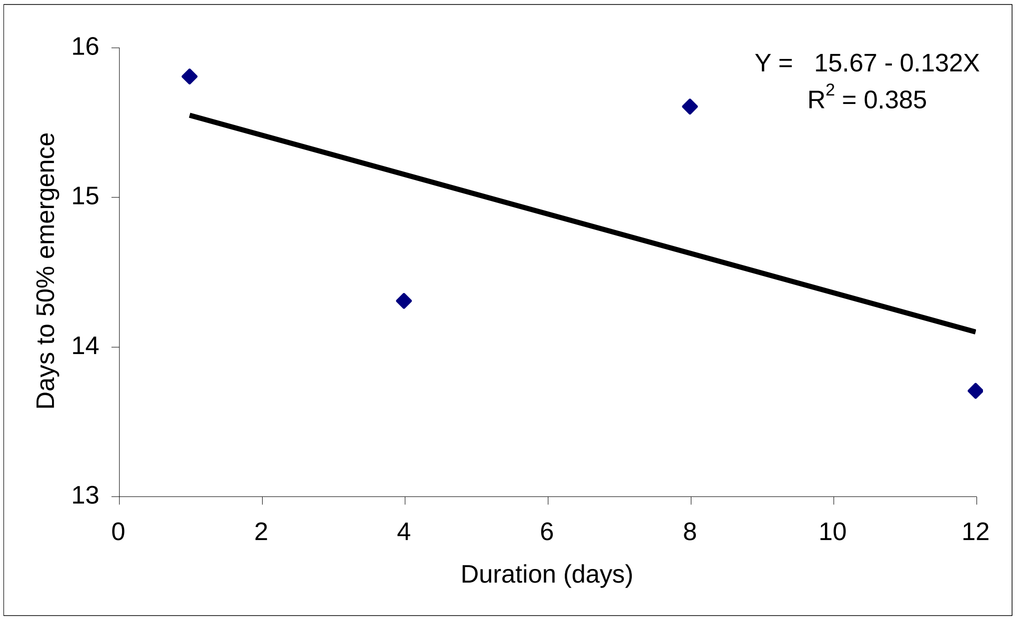

Fig. 6. Relationship between seed treatment duration and days to 50% emergence of eldarica pine.

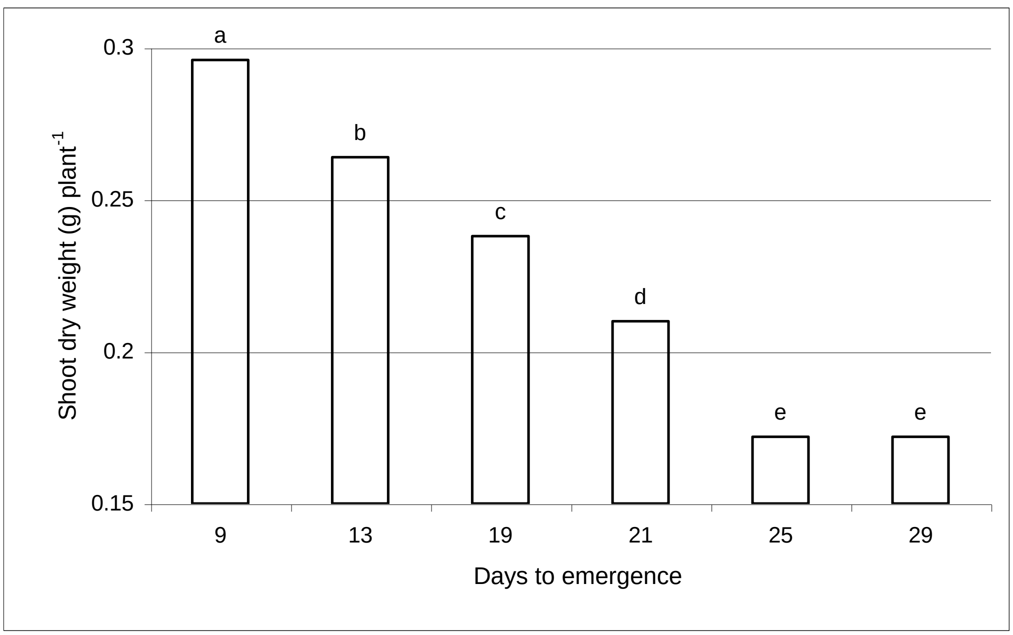

Fig. 7. Shoot weight (g) of eldarica pine as affected by emergence date

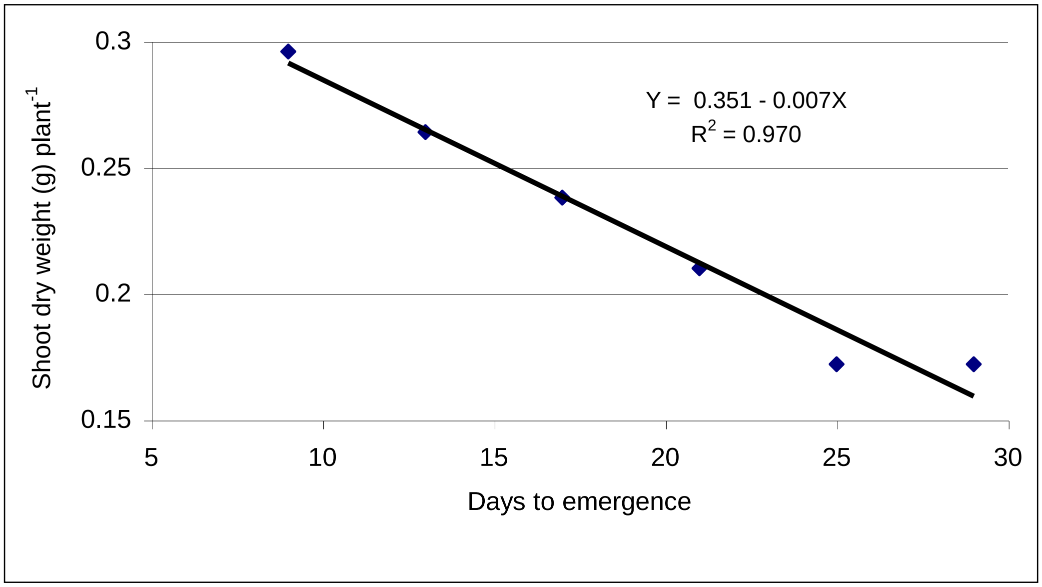

Fig. 8. Relationship between shoot dry weight and emergence date of eldarica pine.

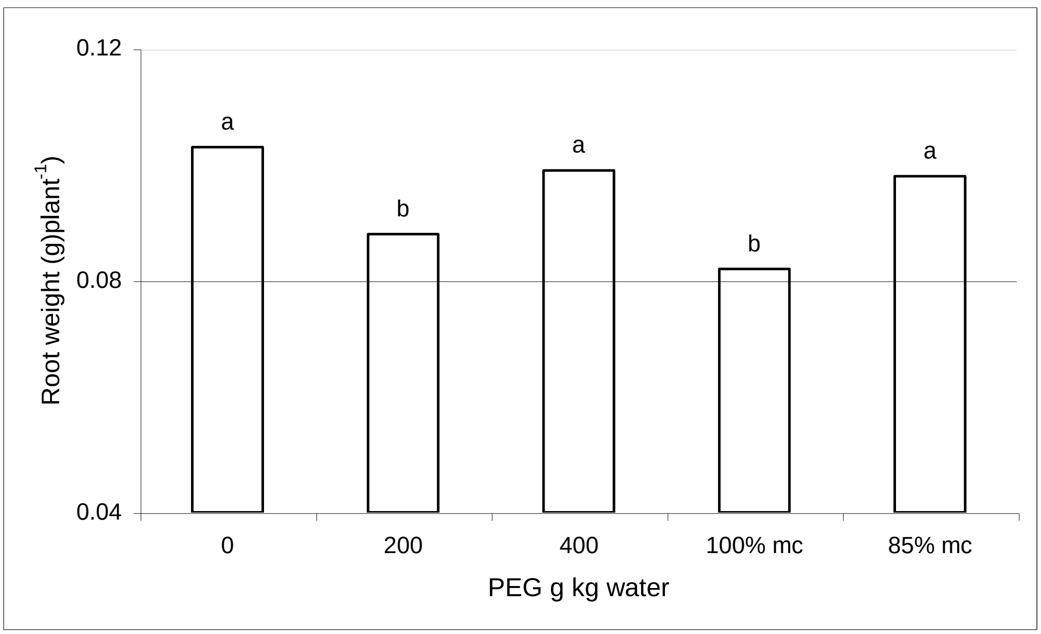

Fig. 9. Root dry weight (g) Plant^{-1} of eldarica pine as affected by PEG 8000 concentration and stratification

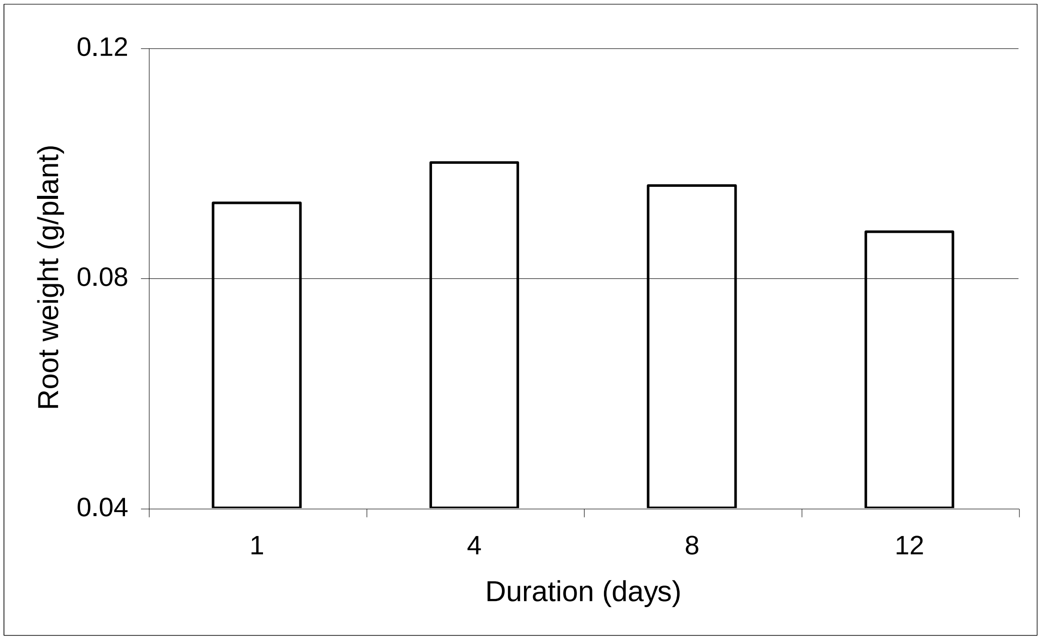

Fig. 10. Root weight of eldarica pine as affected by seed treatment duration

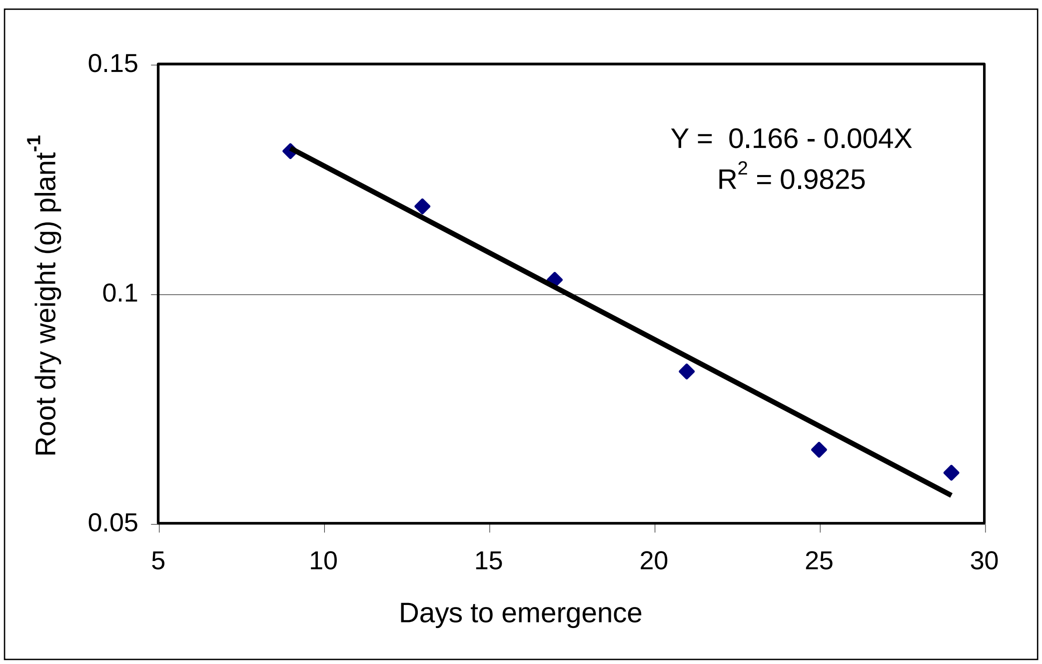

Fig.11. Relationship between root dry weight and date of emergence of eldarica pine.

References

Bussell, W. T. and D. Gray, 1976. Effects of pre-sowing seed treatments and temperatures on tomato seed germination and seedling emergence. Scientia Hort. 5(2):101-109.

Carpenter, W. J., 1989. Salvia splendens seeds pre-germination and priming for rapid and uniform plant emergence. J. Amer. Soc. Hort. Sci. 114(2): 247-250.

Carpenter, W. J., 1990. Priming dusty miller seeds: Role of aeration, temperature and relative humidity. Hort. Science 25(3): 299-302.

Dearman, J., P. A. Brocklehurst and R. L. Drew, 1986. Effect of osmotic priming and aging on onion seed germination. Ann. Appl. Biol. 108:639-648.

Downie, B., U. Bergsten, B. S. P. Wang and J. D. Bewley, 1993. Conifer seed germination in faster after membrane tube invigoration than prechilling or osmotic priming. Seed Science Research. 3: 259-270.

Hallgren, S. W., 1990. Osmotic priming southern pine seed. pp 59. In: Proceedings of 11th North Amer. Forest Biol. Workshop. Athens, GA, June 13-15.

Hallgren, S. W., 1987. Priming treatments to improve pine seed vigor. pp 33-35. In: Proc. Intermountain Forest Nursery Assoc., Okla., August 10-14.

Harris, D., A. Joshi, P. A. Khan, P. Gothkar and P. S. Sodhi, 1999. On-farm seed priming in semi-arid agriculture: development and evaluation in maize, rice and chickpea in India using participatory methods. Experimental Agriculture 35:15-29.

Harris, D. and R. S. A. Joshi, 2000. On-farm seed priming: to improve crop establishment and yield in direct seedbed rice. International workshop on dry seeded rice technology, January 25-28, Bangkok, IRRI.

Harris, D., A.K. Pathan, P. Gothkar, A. Joshi, W. Chivasa and P. Nyamudeza, 2001a. On-farm seed priming: using participatory methods to revive and refine a key technology. Agriculture Systems. 69:151-164.

Harris, B. S. Raghuwanshi, J. S. Gangwar, S. C. Singh, K. D. Joshi, A. Rashid and P. A. Hollington, 2001b. Participatory evaluation by farmers of on-farm seed priming in wheat in India, Nepal and Pakistan. Experimental Agriculture 37:403-415.

Khalil, S. K., J. G. Mexal and M. Ortiz, 1997. Osmotic priming hasten germination and improve seedling size of *Pinus brutia* var. eldarica. Tree Planters' Notes. On-48:24-27.

Khalil, S. K., A. Rehman, I. H. Khalil and S. Rehman, 2003. Osmoconditioning seed of mungbean varieties improve germination. Pak. J. Seed Tech. 1:13-22.

Khan, A. Khalil, S. K., S. Khan and A. Afzal, 2005. Priming affect crop stand of mungbean. Sarhad J. Agric. 21:535-538.

Khan, A. A., H. Miura, J. Prusinki and S. Ilyas, 1990. Matriconditioning of seeds to improve emergence. In: National symposium on stand establishment for horticultural crops. PP:29-40. Minnesota, April 4-6.

Khan, A. A., 1992. Preplant physiological seed conditioning. Hort. Rev. 13:131-181.

Lopes, V. B. and M. Takaki, 1988. Seed germination in Phaseolus vulgaris L. II. Effect of water potential and photoperiod. Arquivos de Biologiae Technologia 31(2):307-312.

Mexal, J. G. and J. T. Fisher, 1987. Size hierarchy in conifer seedbeds: 1. Time of emergence. New Forests 1:187-196.

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

Murray, G. A., 1990. Priming sweet corn seed to improve emergence under cool conditions. Hort. Science, 25:231.

Musa, A. M., D. Harris, C. Johansen and J. Kumar, 2001. Short duration chickpea to replace fallow after aman rice: the role of on-farm seed priming in the High Barind Tract of Bangladesh. Experimental Agriculture 37(4):430-435.

Simak, M., 1985. Seed germination after incubation at different moisture stress. In: Proceedings, IUFRO international symposium on seed problems under stressful conditions; June 3-8: Viennna and Gmunden, Austria. FBVA Berichte 12:125-137.

Simak, M., K. Sahlen and J. Thorsen, 1984. Germination of PEG treated Scots pine seeds under various stress conditions. In: Pert, K. (ed). Ecology and Management of forest biomass production systems. Dept. Ecol. and Envirn. Res., Swed. Univ. Agric. Sci. Rep., 15:309-322.

Snedecor, G. W. and W. G. Cochran, 1989. Statistical methods. Eight edition Iowa State University Press/AMES.