Pak. J. Bot., 36(1): 65-74, 2004.

INFLUENCE OF SEED COAT TREATMENTS ON GERMINATION AND EARLY SEEDLING GROWTH OF *LUPINUS VARIUS* L.

OSMAN KARAGUZEL, SADIK CAKMAKCI*, VELI ORTACESME AND BILAL AYDINOGLU*

Department of Landscape Architecture,
Faculty of Agriculture, Akdeniz University, Antalya 07070, Turkey.

Abstract

The seed coat treatments (mechanical scarification, boiling seeds for 4, 6, 8 and 10 minutes and scarifying in concentrated sulfuric acid for 4, 8, 12, 16, 20 and 24 h) were tested to overcome the seed coat impermeability and improve germination and early seedling growth characteristics of *Lupinus varius*. Mechanical scarification provided rapid and highest imbibitions, germination percentage and seedling establishment, and also the highest values of early seedling growth characteristics. Embryos of *L. varius* could survive after boiling the seeds for 4, 6, 8 and 10 minutes and final germination percentages were 68.3%, 80.0%, 71.7% and 63.3%, respectively. Boiling treatments also improved some other germination and early seedling growth characteristics compared with untreated seeds, but it was not completely able to overcome the seed coat impermeability in any durations of boiling time. Concentrated sulfuric acid treatments increased imbibitions and germination percentages and improved early seedling growth characteristics as the duration of scarification time increased. However, shoot height and root length and their dry weights decreased, and also abnormal geotropic growth occurred in radicles when seeds were scarified in sulfuric acid for 20 and 24 h. Considering similarities in most of germination and seedling growth characteristics, scarifying seeds in sulfuric acid for 12 or 16 hours was found as alternative seed coat treatments to mechanical scarification. Results indicate that the seed coat dormancy in *L. varius* is harder to overcome than those of other *Lupinus* species and seed coats are not only impermeable but also might be heat resistant.

Introduction

Lupinus varius (Leguminosae), short to medium softly hairy annual, is native to Mediterranean basin and to some regions of Anatolia, which have exact or partly Mediterranean climatic characteristics (Chamberlain, 1965). The plant has attractive leaf and flower shape and color, flowering and branching characteristics (Blamey & Gray-Wilson, 1998; Burnie, 2000). Therefore it has been considered that *Lupinus varius* has potential for use as a bedding plant or a new cut flower crop in respect to sustainable landscaping approaches and needs for new cut flower crops.

Seeds of *L. varius* have impermeable seed coat dormancy that presumably is a part of adaptation mechanism for species survival under Mediterranean rainfall regime (Karaguzel *et al*., 2002). Under cultural conditions this dormancy results in the lack of uniform germination, and seedling and subsequent plant growth, which restrict the use of *L. varius* as a bedding plant or cut flower crop. Studies on *Lupinus* species showed that some of the other species, genotypes and cultivars have similar seed coat dormancy and mechanical or sulfuric acid scarification is needed to obtain uniform and rapid germination, and hot water or boiling treatments can improve germination (Valenti *et al*., 1989; Davis *et al*., 1991; Mackay *et al*., 1995; Mackay *et al*, 1996; Centenera *et al*., 1999).

*Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya 07070, Turkey.

Recent studies on germination of a native *L. varius* population from South Anatolia (Gazipasa-Antalya) indicated that boiling seeds for two minutes and scarifying in concentrated sulfuric acid for two hours did not provide adequate germination percentages (38.9% and 31.1%, respectively) and mechanical scarification was the most effective treatments to obtain uniform and rapid germination and seedling establishment, whereas germination percentages in untreated seeds were 6.7% and 15.3% (Karaguzel *et al*., 2002). However, mechanical scarification treatments have not been found practical for large seed lots and it is still needed to investigate more effective seed coat treatments on the germination of this native population of *L. varius*. The aim of this study was to determine the effects of seed coat treatments on germination and early seedling growth characteristics of *L. varius* native to South Anatolia.

Materials and Methods

General procedures

Seeds of *Lupinus varius* L., used in this study were collected from plants grown in field plots at the Research and Application Station of Faculty of Agriculture, Akdeniz University at Antalya (Turkey) in July 2001. They were dusted with 3α, 4, 7, 7α-tetrahydro-2[(trichlor methyl) thio]-1*H*-isoindole-1, 3(2*H*)-dione (captan) and stored at room temperature and relative humidity until the experiments commenced.
Seed coat treatments were:

1. Mechanical scarification: The scissors were used to cut a score (1-2 mm in diameter) on the seed coat at the opposite site of hilum without causing any damage on cotyledons;

2. Boiling: 2-liter water heater in which water temperature could be kept at 100±2°C by setting up the thermostat at 100°C was used. Each time 20 dry seeds in a small cellular cotton bag were soaked in the hot water (100±2°C) for adequate time that was planned for the experiments as a level of boiling treatment. At the end of the time, seeds were taken out of the hot water and were rinsed with tap water four times to be cooled;

3. Acid scarification: Seeds were placed in concentrated (36 *N*) sulfuric acid (20 seeds per 30 ml) for adequate time for each level of acid scarification treatments at 24°C and then rinsed with distilled water several times.

In boiling and acid scarification treatments, after rinsing, the seeds were left to dry out on the blotter paper on benches for 24 h at laboratory temperature before placing them in Petri dishes or sowing in seed trays. Untreated (control) seeds were only counted for each replicate of experiments before sowing without any pre-sowing treatment.

In the experiments, 11-cm Petri dishes containing three layers of blotter papers were used and blotter papers were moistened with 10 ml distilled water before placing the seeds. After the first watering, distilled water was used exclusively for moistening blotter paper and it was applied as needed. In the experiment carried out in greenhouse, plastic seed trays with 54 holes were used as the seed pad after filling with the mixture of peat and perlite (2:1 by volume). The seeds were sown in germination medium with one seed for each hole in 15 mm depth and watering was made by hand using tap water as needed.

Specific experimental procedures

Expt. I: This experiment was conducted to determine the effects of certain level of different seed coat treatments on imbibitions and germination percentages and to investigate relationship between imbibitions and germination percentages during 120 h testing period. Untreated (control) seeds, seeds scarified mechanically, seeds boiled for 6 minutes and seeds scarified in concentrated sulfuric acid for 12 h were placed in Petri dishes and then Petri dishes were placed in an incubator in dark at 25±1°C. There were three replicates of each treatment arranged in a single completely randomized block and each Petri dish containing 20 seeds was one replicate. Imbibitions and germination were measured and scored at 24 h intervals during 120 h testing period. The imbibitions were calculated using the following formula:

$$Wi = (Wi-Wd)/Wd$$

where Wi and Wd were weights of imbibed and dry seeds, respectively (Baskin *et al*., 1998). Germination was defined by the presence of a radicle at least 2 mm long (Mackay *et al*., 1995).

Expt. II: In this experiment, the effects of different level of duration time of boiling and sulfuric acid scarification treatments on some germination and seedling growth characteristics were tested in comparison to mechanically scarification and untreated seeds. Therefore, untreated (control) seeds, the seeds scarified mechanically, seeds boiled for 4, 6, 8, and 10 min. and seeds scarified in concentrated sulfuric acid for 4, 8, 12, 16, 20 and 24 h were placed in Petri dishes. After that Petri dishes were placed in the growth chamber under light (40.8 μmols m^{-2} s^{-1} PAR) provided with cool-white fluorescent lamps for 12 h daily at 24±2.4°C. Five days after sowing, covers of Petri dishes were opened to provide upright growth of seedlings. The experiment was designed in a single completely randomized block for each treatment with three replications and each Petri dish containing 20 seeds was one replicate. During the experiment, germination was recorded at 24 h intervals. Shoot height and root length were measured and samples were prepared for dry weights at the end of 12 days testing period. Germination index and mean germination time were calculated using the following formula:

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

where Gt is the number of seeds germinated on day t and Tt is the number of days. Mean germination time (MGT) = $\sum Ti\ Ni/\sum Ni$, where Ni is the number of newly germinated seeds at time Ti (Alvarado *et al*., 1987; Ruan *et al*., 2002). Energy of germination and germination percentage were recorded the 6^{th} and 12^{th} day, respectively. Energy of germination was the percentage of germinating seeds 6 days after sowing relative to the number of seeds tested (Ruan *et al*., 2002). The shoots and roots of seedling were separated by cutting tissues with razor blade on connecting lines that could be easily seen with different color of stem and root tissues. Then the distances between cutting points and tip of longest leaves and longest roots were measured as shoot height and root lengths, respectively. Shoot (including cotyledons) and root samples were oven dried at 70°C for five days before weighing.

Expt. III: After consulting the data obtained from *Experiment II*, the effects of selected seed coat treatments on seedling establishment, emergence and growth characteristics in seed trays were tested. Untreated (control) seeds, the seeds scarified mechanically, seeds

boiled for 4 and 6 min. and seeds scarified in concentrated sulfuric acid for 4, 8, 12 and 16 h were sown in plastic seed trays as described above and trays were placed in greenhouse on 15 April 2002. Air temperature was recorded using thermograph for 15 days (until 1 May 2002) and mean minimum and maximum temperatures were 17.8±3.4 and 27.6±4.2°C, respectively. A single completely randomized block design for each treatment with three replications was used, and 20 seeds assigned per replicate. Seedling vigor index was calculated using the number of seedlings that had emerged by 5, 7, 9, 11 and 13 days after sowing as follows:

Seedling vigor index = number of seedlings emerged/number of days of first count + number of seedlings emerged/number of days of second count +…+ number of seedlings emerged/number of days of the last count.

Speed of emergence was calculated according to Ruan *et al*., (2002) by using following equation:

Speed of emergence = number of seedling emerged 5 days after sowing/ number of seedling emerged 13 days after sowing X 100.

Seedling establishment was the percentage of seedlings established 15 days after sowing relative to number of seeds sown. Shoot height and root length and also shoot and root dry weights were determined as the growth characteristics of seedlings at the end of 15 days period. Leaf score was defined as the development stage of cotyledons and leaves (Ruan *et al*., 2002) and scaled as 0 = no emergence, 1 = cotyledons emerged on the germination medium surface, 2 = first leaf developed, 3 = second leaf developed, 4 = third leaf developed, 5 = fourth leaf developed. Shoot and root measurements were made as described in the second experiment.

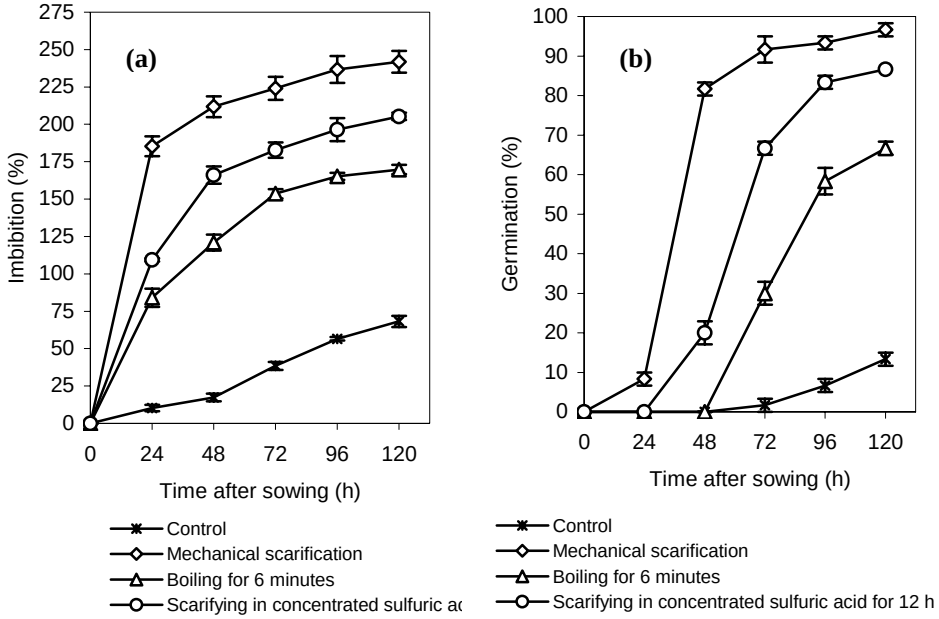

Fig. 1. Effect of seed coat treatments on imbibitions (a) and germination percentage (b) of *Lupinus varius* seeds. Each value is the mean of three replicate samples and vertical bars indicate SE when lager than the symbol.

Statistical analysis

Data obtained from the *Experiment I* are shown in graphs with error bars ($_{SE}$). All data from *Experiment II* and *Experiment III* were analysed using analyses of variance (ANOVA) and Duncan's multiple range test was used to compare means. Before using ANOVA, percentages were transformed according to y = arcsin[sqr(x/100)].

Results

Imbibitions and germination (Experiment I)

Results indicated that seed coat treatments significantly affected imbibitions and germination percentages of *Lupinus varius* seeds (Fig. 1a, 1b). The most rapid increase in imbibitions was recorded in mechanically scarified seeds 24 h after sowing and this treatment was followed by scarifying the seeds in sulfuric acid for 12 h and boiling for 6 min., respectively. Differences in imbibitions continued with gradually limited increases in rates until the end of 120 h testing period (Fig. 1a). Despite this, increase in weights of untreated (control) seeds was limited to 24 h after sowing and lowest at the end of testing period.

First germination was observed in mechanically scarified seeds after 24 h. The time required for first germinations was >24 h in seeds scarified in sulfuric acid for 12 h; >48 h in seeds boiled for 6 min., and about 72 h in untreated (control) seeds (Fig. 1b). While highest germination speed in mechanically scarified seeds occurred during 24 and 48 h after sowing, the seeds scarified in sulfuric acid for 12 h and boiled for 6 min. germinated with higher increases in the percentage during 48 and 72 h and 48 and 96 h after sowing, respectively. Germination increase in untreated (control) seeds were almost constant during the testing period and final germination percentage was only 13.3%. At the end of 120 h testing period, the highest germination percentage (96.7%) was recorded in mechanically scarified seeds. Scarifying in sulfuric acid for 12 h and boiling for 6 min., showed 86.7 and 66.7% final germination, respectively (Fig 1b). At the end of 120 h testing period, there was significant linear regression (R^2 = 0.991) between imbibitions and germination percentages (Fig. 2). Regression equation (y = 0.495x-18.882) in Figure 2 shows that higher imbibitions rates result in higher germination percentages.

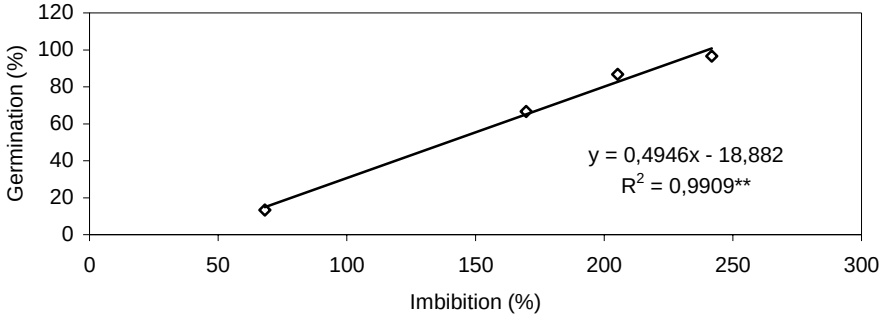

Fig. 2. Relationship between imbibitions and germination percentages in *L. varius* seeds. Germination percentage was plotted as a function of imbibitions at the end of 120 h testing period. A positive significant correlation (**:P<0.01) was observed.

Germination and seedling growth responses in Petri dishes (Experiment II)

Seed coat treatments significantly ($P \leq 0.001$) affected germination and seedling growth characteristics of *L. varius*. At the end of 12 days testing period, the highest final germination percentage of 100.0% were recorded in mechanically scarified seeds and seeds scarified in sulfuric acid for 24 h. The treatments including scarifying the seeds in sulfuric acid for 20, 16, 12 and 8 h followed these treatments without significant differences (Table 1). In boiling treatments, germination percentage increased by 80% when seeds boiled for 6 min. However, final germination percentages decreased in seeds boiled for 8 and 10 min., compared to boiling for 6 min., treatment and lowest germination percentage (30%) was obtained in untreated (control) seeds.

The results related to energy of germination and germination index showed similar differences to final germination percentages (Table 1). Therefore, the highest energy of germination and germination index were counted in mechanical scarification treatment and scarification of seeds in sulfuric acid for 24, 20, 16 and 12 h, respectively. Boiling treatments increased the energy of germination and germination index compared to control, but increases were not found adequate for rapid and uniform germination. The lowest energy of germination and germination index were recorded in untreated (control) seeds. As a main criterion for rapid germination, the shortest mean germination time was counted in mechanically scarified seeds and seeds scarified in sulfuric acid for 16 h. Boiling treatments did not significantly shorten the mean germination time compared with untreated (control) seeds (Table 1).

The highest shoot height and root length, and shoot and root dry weight were measured and weighed in mechanically scarified seeds, seeds scarified in sulfuric acid for 16, 12 and 8 h and seeds boiled for 6 min. without significant differences, respectively (Table 1). The lowest values were counted in seeds boiled for 8 and 10 min., and seeds scarified in sulfuric acid for 20 and 24 h and also untreated (control) seeds. In addition, abnormal (spirally) geotropic growth in radicles was observed in the seeds scarified in sulfuric acid for 20 and 24 h. Also, germination percentages of enlarged seeds were lower in the seeds boiled for 8 and 10 min., although enlarged seeds could germinate about 100% in other treatments within a short period.

Seedling establishment and growth responses in seed trays (Experiment III)

Analyses of data related to *Experiment III* showed that selected seed coat treatments had significant ($P \leq 0.001$) effect on seedling establishment and early growth characteristics of *Lupinus varius* in seed trays under greenhouse condition (Table 2). All seed coat treatments increased seedling vigor index, speed of emergence, seedling establishment and leaf score compared with untreated (control) seeds. However, the highest seedling vigor index, speed of emergence, seedling establishment and leaf score values were counted in mechanically scarified seeds and seeds scarified in sulfuric acid for 12 and 16 h (Table 2). Shoot height and dry weight in treated seed were higher than those of untreated (control) seeds without significant ($P \geq 0.05$) differences between seed coat treatments. Root length was highest in mechanically scarified seeds and seeds scarified sulfuric acid for 12 h. There were no significant differences among other treatments. While the lowest root dry weight was recorded in untreated (control) seeds, root dry weight in mechanically scarified seeds and seeds scarified in sulfuric acid for 12 h were higher than those of other seed coat treatments.

Table 1. Effect of seed coat treatments on seed germination and seedling growth of *Lupinus varius* in Petri dishes under light (40.8 µMol·S^{-1}·m^{-2} PAR) at 24±2.4°C in growth chamber.

Treatment	Germination (%)	Energy of germination (%)	Mean germination time (days)	Germination index	Shoot height (cm)	Shoot dry weight (mg/plant)	Root length (cm)	Root dry weight (mg/plant)
Control	30.0 e	20.0 e	4.7 ab	6.80 g	4.57 cd	164.6 cde	6.41 de	22.1 bc
Mechanical scarification	100.0 a	100.0 a	2.4 e	38.85 a	5.93 a	208.5 a	7.57 a	26.7 a
Boiling								
4 min	68.3 cd	56.7 d	5.0 a	16.20 f	4.73 abcd	171.5 bcd	6.53 cde	19.0 cdef
6 min	80.0 bc	70.0 bc	3.9 abc	22.61 de	5.22 abc	177.3 bc	7.42 ab	25.7 ab
8 min	71.7 cd	58.3 cd	4.3 ab	18.40 ef	4.60 cd	168.3 cd	6.33 de	21.4 cd
10 min	63.3 d	56.7 d	4.3 ab	16.32 f	2.88 e	145.3 e	5.94 e	20.2 cde
Scarifying in concentrated sulfuric acid								
4 h	76.7 c	71.7 b	3.6 bc	24.45 cd	4.68 bcd	175.2 bc	6.67 bcd	18.5 def
8 h	93.3 ab	80.0 b	4.1 ab	29.11 bc	5.08 abcd	174.9 bc	7.21 abc	19.7 cde
12 h	96.7 a	96.7 a	3.0 cd	32.73 ab	5.37 abc	183.1 abc	7.37 ab	22.5 abc
16 h	96.7 a	96.7 a	2.4 e	34.69 ab	5.81 ab	194.0 ab	7.50 a	25.7 ab
20 h	98.3 a	98.3 a	3.0 cd	33.40 ab	4.68 bcd	165.4 cde	6.21 de	17.7 ef
24 h	100.0 a	100.0 a	3.0 cd	33.07 ab	4.04 d	152.0 de	4.45 f	16.3 f
Significance								
Treatment:	***	***	***	***	***	***	***	***

***-P< 0.001.

Testing period was 12 days. Within columns, the same letter following two mean values indicates that the difference between the two treatments is not significant at p=0.001 level, as determined by Duncan's multiple range tests.

Table 2. Effect of seed coat treatments on seedling establishment and growth of *Lupinus varius* in seed trays under greenhouse conditions.

Treatment	Seeding establishment (%)	Seedling vigor index	Leaf score	Speed of emergence (%)	Shoot height (cm)	Shoot dry weight (mg/plant)	Root length (cm)	Root dry weight (mg/plant)
Control	28.3 e	1.31 e	0.67 d	13.33 c	8.80 b	151.2 c	12.80 c	32.1 d
Mechanical scarification	100.0 a	11.53 a	3.73 a	83.33 a	13.56 a	233.0 a	19.78 a	55.1 a
Boiling								
4 min	70.0 d	6.68 d	1.97 c	50.18 b	11.55 a	198.5 ab	15.64 abc	43.6 abc
6 min	78.3 cd	8.17 bc	2.68 b	66.10 ab	11.90 a	204.9 ab	15.08 bc	42.0 bc
Scarifying in concentrated sulfuric acid								
4 h	76.7 cd	6.81 cd	2.63 b	63.06 ab	11.38 ab	195.5 b	12.66 c	35.3 cd
8 h	83.3 bc	8.62 b	2.83 b	63.97 ab	11.75 a	201.7 ab	16.09 abc	44.9 abc
12 h	93.3 ab	10.79 a	3.50 a	82.16 a	13.39 a	230.1 ab	18.07 ab	50.4 ab
16 h	100.0 a	11.12 a	3.62 a	81.32 a	13.01 a	223.5 ab	15.11 abc	42.2 bc
Significance								
Treatment:	***	***	***	***	***	***	***	***

***- P<0.001.

Testing period was 15 days. Within columns, the same letter following two mean values indicates that the difference between the two treatments is not significant at p=0.001 level, as determined by Duncan's multiple range tests.

Discussion

Results indicated that seeds of *L. varius* population native to South Anatolia had impermeable seed coat dormancy, which was harder to overcome than those of other *Lupinus* species. Breaking down impermeability of seed coat by means of scarification methods or certain level of seed coat treatments resulted in considerable increase in imbibitions and germination percentages (by 100%) in relatively short time. Similar seed coat dormancy is reported in *Lupinus hawardii*, *Lupinus angustifolius*, *Lupinus texensis*, *Lupinus perennis*, *Lupinus hispanicus* and many legumes (Davis *et al.*, 1991; Mackay *et al.*, 1995; Mackay *et al.*, 1996; Baskin *et al.*, 1998; Centenera *et al.*, 1999).

Mechanical scarification provided rapid and highest imbibitions, germination percentage, seedling establishment and also the highest values of seedling growth characteristics such as shoot height and root length, shoot and root dry weight and leaf score (Fig. 1, Table 1 & 2). These results are similar to those reported for *Lupinus texensis* (Davis *et al*., 1991), *Lupinus hawardii* (Mackay *et al.*, 1995), *Lupinus perennis* (Mackay *et al*., 1996), *Lupinus hispanicus* (Centenera *et al.*, 1999) and *Lupinus varius* (Karaguzel *et al.*, 2002).

Boiling the seeds for 4, 6, 8 and 10 min., improved some germination, seedling establishment and growth characteristics considered in the study compared with untreated (control) seeds, but they did not completely overcome the seed coat impermeability in any duration of boiling time (Fig. 1, Table 1 & 2). Placing of *Lupinus texensis* seeds in hot (85°C) water and then cooling for 24 h promoted emergence compared to untreated controls, but was less effective than other methods (Davis *et al.*, 1991). Soaking the seeds of *Lupinus havardii* in water for 24 h failed to promote seed germination regardless of water temperature (Mackay *et al*., 1995). Boiling *Lupinus hispanicus* seeds for 240 seconds was not found as a useful treatment compared with mechanical scarification and immersion in sulfuric acid (Centenera *et al*., 1999). Baskin *et al.* (1998) boiled *Senna marilandica* and *Senna obtusifolia* seeds from 0 to 240 seconds and found that boiling for ≥20 seconds was detrimental to seeds of *Senna obtusifolia* and only 6% of *Senna marilandica* seeds boiled for 240 seconds germinated. Soaking in hot water and boiling are mentioned as useful techniques for many members of Leguminosae, but the durations of boiling or soaking times are critical (Macdonald, 1999). In this study, embryos of *Lupinus varius* seeds boiled for 6 min., could survive and germinated by 80.0%, and also germination percentages in seeds boiled for 8 and 10 min., were 71.7% and 63.3%, respectively. These results allude to the fact that the seed coat of *L. varius* seeds is not only impermeable but also might be heat resistant.

Scarifying the seeds in concentrated sulfuric acid increased imbibitions, and improved germination, seedling establishment and growth characteristics of *L. varius* as the duration of scarification time increased up to 16 h at 24°C (Fig. 1, Table 1 & Table 2). Some seedling growth characteristics such as shoot height and dry weight and root length and dry weight decreased, and also abnormal (spirally) geotropic growth occurred in radicles when seeds scarified in sulfuric acid for 20 and 24 h (Table 1). Results related to efficiency of acid scarification on germination of other lupines are similar with differences in the duration of scarification times required for optimal germination. The length of the scarification time needed for optimum germination is 30 to 60 min. for *Lupinus texensis* (Davis *et al*., 1991), 90 to 120 min. for *Lupinus havardii* (Mackay *et al*., 1995), 45 min. for *Lupinus perennis* (Mackay *et al*., 1996) and 60 min. for *Lupinus*

hispanicus hispanicus and *Lupinus hispanicus bicolor* (Centenera *et al*., 1999). Scarifying the seeds of *Lupinus varius* in sulfuric acid for 120 min. resulted in 31.1% of germination (Karaguzel *et al*., 2002). It would suggest that the duration of scarification time needed for optimum germination in seeds of *Lupinus varius* native to South Anatolia are longer (12 to 16 h) than those of other *Lupinus* species.

Acknowledgements

This study was supported by the Administration Unit of Scientific Research Projects of Akdeniz University.

References

Alvarado, A.D., K.J. Bradford and J.D. Hewitt. 1987. Osmotic priming of tomato seeds. Effects on germination, field emergence, seedling growth and fruit yield. *Journal of American Society of Horticultural Science*, 112: 427-432.

Baskin, J.M., N. Xiaoying and C.C. Baskin. 1998. A comparative study of seed dormancy and germination in an annual and a perennial species of *Senna* (Fabaceae). *Seed Science Research*, 8: 501-512.

Blamey, M. and C. Grey-Wilson. 1998. *Mediterranean Wild Flowers*. HarperCollins, London, p. 85.

Burnie, D. 2000. *Wild flowers of the Mediterranean*. Dorling Kindersley, London, London, p.90.

Centenera, E., C. Cuadra, C. de la, de la Cuadra and G.D. Hill. 1999. *Control of seed viability in Lupinus hispanicus*. Towards to 21st Century. Proceedings of the 8th International Lupin Conference, Asilomar, California, USA, May 11-16 1996, 416-419.

Chamberlain, D.F. 1965. *Lupinus*. In: *Flora of Turkey and The East Aegean Islands*. Vol 3, (Ed.): P.H. Davis. Edinburgh University, Edinburgh, pp. 38-40.

Davis, T.D., S.W. George, A. Upadhyaya and J. Parsons. 1991. Improvement of seedling emergence of *Lupinus taxensis* Hook. following seed scarification treatments. *Journal of Environmental Horticulture*, 9(1): 17-21.

Karaguzel, O., I. Baktir, S. Cakmakci, V. Ortacesme, B. Aydınoglu and M. Atik. 2002. Effects of scarification methods, temperature and sowing date on some germination characteristics of *Lupinus varius* L. *2nd National Congress on Ornamental Plants*, October 22-24, Citrus and Greenhouse Research Institute, Antalya, Turkey, p. 40-47.

Mackay, W.A., T.D. Davis and D. Sankhla. 1995. Influence of scarification and temperature treatments on seed germination of *Lupinus havardii*. *Seed Science & Technology*, 23: 815-821.

Mackay, W.A., T.D. Davis, D. Sankhla and D.E. Reinmenschneider. 1996. Factors influencing seed germination of *Lupinus prennis*. *Journal of Environmental Horticulture*, 14(4): 167-169.

Ruan, S., Q. Xue and K. Tylkowska. 2002. The influence of priming on germination of rice (*Oryzo sativa* L.) seeds and seedling emergence and performance in flooded soil. *Seed Science & Technology*, 30: 61-67.

Valenti, G.S., L. Melone, M. Ferro and A. Bozzini. 1989. Comparative studies on testa structure of 'hard-seeded' and 'soft-seeded' varieties of *Lupinus angustifolius* L. (*Leguminosae*) and on mechanism of water entry. *Seed Science & Technology*, 17: 563-581.

(Receiced for publication 17 May 2003)