Pak. J. Bot., 45(4): 1173-1177, 2013.

EFFECTS OF VARIOUS TREATMENTS ON SEED GERMINATION AND GROWTH OF CAROB (*CERATONIA SILIQUA* L.)

ESMA GUNES[1], HAMIDE GUBBUK[1*], TOMAS AYALA-SILVA[2], SADIYE GOZLEKCI[1] AND SEZAI ERCISLI[3]

[1]*Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya 07058, Turkey*
[2]*National Germplasm Repository System, Subtropical Horticulture Research Station, Miami, FL, 33158, USA*
[3]*Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey*
[*]*Corresponding author's e-mail: gubbuk@akdeniz.edu.tr*

Abstract

In this study, *Ceratonia siliqua* L. carob seeds harvested from both wild and cultivated genotypes in Turkey were subjected to mechanical scarification, soaking in hot water and dipping in sulfuric acid. All treatments hastened seed germination and seedling growth of carob compared to control. The germination percentage of control seeds were similar for both wild and cultivated genotypes (13%) and it was increased up to 95% and 93% in wild and cultivated genotypes, respectively, following sulfuric acid treatment. Seed germination percentage of wild and cultivated carobs was similar in all treatments indicating that domestication does not appear to have influenced germination behavior in both genotype.

Introduction

Ceratonia siliqua L. (Fabacea) or carob is one of the important plants of the Mediterranean basin. Carob trees as wild or cultivated have a high aesthetic value and are also used as an ornamental plant in Mediterranean countries. They grow well on poor, sandy, calcareous and limestone soils unreliable (Batlle & Tous, 1997). It is a drought resistant fruit species and is particularly useful in areas where irrigation is impractical or rainfall is unreliable (Batlle & Tous, 1997; Janick & Paull, 2008; Shepperd, 2008). Consequently, it is a favorite fruit tree in arid and semiarid regions of the southern Mediterranean basin (Haq, 2008**).** Carob is an economically important multipurpose tree and can also be used for charcoal, wood industry and to prevent soil erosion (Barwick, 2004; Baskin & Baskin, 1998; Pérez-García, 2009).

Commercial propagation of carob is a major constraint to carob cultivation. The plant is commercially propagated by grafting and vegetative propagation by cuttings has not been achieved commercially. Grafting requires the generation of seedlings and carob seeds are very recalcitrant and have physical dormancy (Batlle & Tous, 1997; Mitrakos, 1987). Consequently seed germination is difficult without pretreatment (Martins-Louçao *et al*., 1996; Ortiz *et al*., 1995; Pérez-García, 2009; Piotto & Di Noi, 2003; Tsakaldimi & Ganatsas, 2001; Yoursheng & Sziklai, 1985). This type of dormancy is very common in many species of several plant families (Eşen *et al*., 2009; Rehman *et al*., 2011; Arefi *et al*., 2012; Nadeem *et al*., 2012), but found more frequently in seeds of the Leguminosae, Malvaceae, Geraniaceae, Chenopodiaceae, Convolvulaceae, Solanaceae and Liliaceae (Cicero *et al*., 1986; Kramer & Kozlowski, 1960; Popinigis, 1985).

Carob seeds have a very hard seed coat and do not imbibe water easily (Batlle & Tous, 1997; Carvalho & Nakagawa, 2000; Coit, 1951). Previously, carob seeds from different countries have demonstrated variable germination characteristics according to pretreatments and seed origin (Martins-Louçao *et al*., 1996; Pérez-García, 2009; Piotto & Di Noi, 2003; Tsakaldimi & Ganatsas, 2001). In general, each country that grows carob uses their own seed materials to obtain rootstocks for grafting (Martins-Louçao *et al*., 1996; Pérez-García, 2009; Piotto & Di Noi, 2003; Tsakaldimi & Ganatsas, 2001) because such seeds are thought to be better adapted to local habitat conditions. Thus, germination studies in carob are important in each country to obtain information on the effects of pretreatments, the optimal conditions for seed germination and the influence of seed provenance, all of which are crucial for carob produ

ction, conservation and restoration programs as well. Moreover, data from different carob bioclimatic regions within the Mediterranean basin are needed in order to create a protocol for carob seed germination that can be used by the international seed testing association.

Morphological studies on carob have demonstrated significant differences between wild, *in situ*-managed, and cultivated genotypes, suggesting that such differences are associated with selection and may have also occurred on seeds. Various studies conducted on carob seeds showed that seed morphology and yield of wild and cultivated genotypes were significantly different and these properties varied among regions (Di Lorenzo, 1991; Marakis *et al*., 1988; Tetik *et al*., 2011; Tous *et al*., 1995). The seed ratio has been reported between 5 to 27% among carob genotypes grown in the Mediterranean basin (Caja *et al*., 1988; Crescimanno *et al*., 1988; Marakis *et al*., 1988).

Previously, pretreatments on seed germination of carob have only been carried out with wild carob genotypes (Martins-Louçao *et al*., 1996; Pérez-García, 2009; Piotto & Di Noi, 2003; Tsakaldimi & Ganatsas, 2001) with no comparable work being conducted on cultivated genotypes. During domestication processes in plant species, artificial selection may determine changes in physiological aspects such as loss of seed dormancy, faster and synchronous germination, and variations in periods of fruit maturation (Frary & Doganlar, 2003; Hawkes, 1983).

The purpose of this study was to determine the effects of pretreatments on seeds of both cultivated and wild carob genotypes on imbibition, germination, and root and shoot development.

Materials and Methods

Pods containing mature seeds of carob were harvested in August 2009 from the cultivar 'Etli' and a wild genotype in Demre (Antalya province). Seeds were subjected to following treatments:

1. Mechanical scarification (MST); a small cut, 1-2 mm in length was made on the individual seed coat opposite the hilum without causing any damage to the cotyledons (Karaguzel *et al*., 2002).
2. Soaking in hot water (HWT); seeds were immersed in hot water at 100°C for 5 min (Awodola, 1994; Pérez-García, 2009), then removed, rinsed three times with cold tap water.
3. Dipping in sulfuric acid (SAT); seeds were immersed in 98% sulfuric acid for 20 min at 24°C, then thoroughly washed in running tap water and dried on paper towels.
4. Untreated seeds were used as control.

All seeds were tested for germination following published procedures (Karaguzel *et al*., 2002; Yoursheng & Sziklai, 1985) using three replicates (each with 9 seeds).

Seeds were placed on 2 layers of blotting paper moistened with 10 ml distilled water in 11 cm diameter Petri dishes incubated at 25±1°C for nine days. Additional water was added as needed. A seed was considered as germinated when the radicle was approximately 5 mm in length. The rate of imbibition (%), germination rate (%), germination index, mean germination time (days), germination energy (%), and the lengths of roots and shoots (cm) were assessed. The amount of imbibition and germination were measured and scored daily during the nine day treatment periods.

Imbibitions was calculated as (Baskin & Baskin, 2000): Wi (%) = (Wi-Wd)/Wd x100; where Wi and Wd are the weights of seeds after imbibing water and the initial seed weight, respectively.

Germination was defined by the presence of a radicle at least 2 mm long (Karaguzel *et al*., 2002; Mackay *et al*., 1995).

Germination index (GI) and mean germination time (MGT) were calculated according to the following formulas (Alvarado *et al*., 1987; Ruan *et al*., 2002).

$$GI = \Sigma\ (Gt/Tt)$$

where Gt is the number of seeds germinated on day t^{th} and Tt is the number of days up to and

$$MGT = \Sigma Ti\ Ni/\Sigma Ni$$

where Ni is the number of newly germinated seeds at time Ti.

Energy of germination was determined as the percentage of germinating seeds 9 days after treatment (Karaguzel *et al*., 2002).

Statistical analysis: The experiment had 3 replicates of each treatment arranged in a completely randomized block. Analysis of variance (ANOVA) was carried out with SAS version 8.1 (Anon., 2000). Significance between means was tested by LSD - a multiple range test ($p = 0.05$).

Results

The rate of imbibition between seeds of cultivated and wild genotypes is shown in Figs. 1 and 2. Fastest imbibition was recorded with the MST followed by SAT and then the HWT, all of which were markedly higher than the control (Figs. 1 and 2).

Seed germination percentage was significantly affected by pre-sowing treatment but there were no significant differences between genotypes (Table 1). The lowest germination rate was found in the control for both seed genotypes. The highest germination percentages were obtained in both the MST and SAT treatments. Nine days after the treatment, there was a significant positive correlation between imbibition and germination percentage in both the cultivated and the wild genotypes ($R^2 = 0.991$, $p<0.01$ and $R^2 = 0.911$, $p<0.01$, respectively).

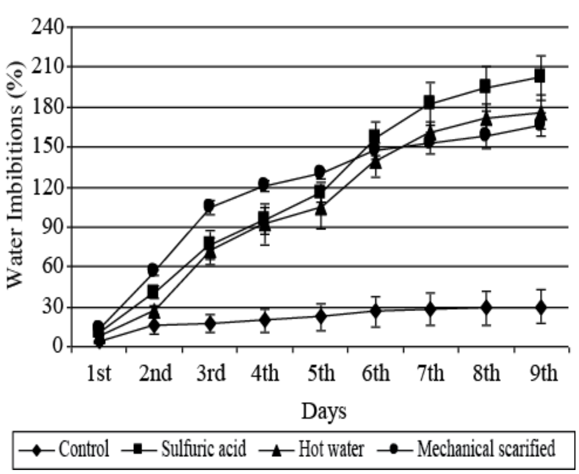

Fig. 1. The effect of various pre-sowing treatments on the percentage of water imbibition in seeds of the cultivated genotype.

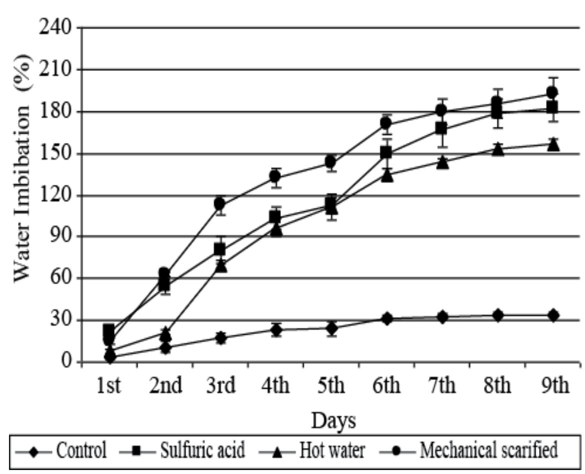

Fig. 2. The effect of various pre-sowing treatments on the percentage of water imbibition in seeds of wild genotype.

Table 1. The effect of various pre-sowing treatments on the rate of germination (%) in cultivated and wild carob seed genotypes.

Genotypes	Treatments				Mean
	Control	Sulfuric acid	Hot water	Scarification	
Cultivated	13.57 C*	92.60 A	77.80 B	98.77 A	70.68***
Wild	13.57 C	95.07 A	74.10 B	92.60 A	68.83
Treatments mean	13.57 c**	93.84 a	75.95 b	95.69 a	

*Type x treatment means with the same capital letters is not significantly different ($p<0.05$)
** Treatment means with the same letter are not significantly different ($p<0.05$)
***N.S.: Not significant

Table 2. The effect of various pre-sowing treatments on the average germination time in cultivated and wild carob seed genotypes (days).

Genotypes	Treatments				Mean
	Control	Sulfuric acid	Hot water	Scarification	
Cultivated	5.73 AB*	5.51 BC	5.33 C	4.20 E	5.19 a**
Wild	5.83 A	5.08 D	5.59 AB	3.74 F	5.06 b
Treatments mean	5.78 a**	5.30 b	5.46 b	3.97 c	

*Type x treatment means with the same capital letters is not significantly different ($p<0.05$)
** Treatment means with the same letter are not significantly different ($p<0.05$)

There were significant differences in the mean germination time of seeds for both cultivated and wild genotypes and among the pre-sowing treatments (Table 2). The average germination time was longest in the control and shortest in the MST for both seed genotypes (Table 2). Germination energy was affected statistically only among pre-sowing treatment and no significant differences between both cultivated and wild genotypes (Table 3). The lowest germination energy was in the control for seeds of both genotypes and the highest in the MST treatment. Similar results were obtained for the germination index (Table 4). The HWT increased the energy of germination and germination index compared to the control, but germination was not rapid and uniform.

The maximum root length was found in sulfuric acid treatment and followed by mechanical scarification of the seeds (Table 5). However the greatest shoot length was determined with mechanical scarification and the lowest in HWT treatment (Table 6).

Table 3. The effect of various pre-sowing treatments on seed germination energy (%) in cultivated and wild carob seed genotypes.

Genotypes	Treatments				Mean
	Control	Sulfuric acid	Hot water	Scarification	
Cultivated	13.56 F*	54.30 D	53.07 D	79.02 A	49.99***
Wild	13.60 F	67.89 C	38.24 E	70.34 B	47.52
Treatments average	13.58 d**	61.10 b	45.66 c	74.68 a	

*Type x treatment means with the same capital letters is not significantly different ($p<0.05$)
** Treatment means with the same letter are not significantly different ($p<0.05$)
***N.S.: Not significant

Table 4. The effect of various pre-sowing treatments on seed germination index in cultivated and wild carob seed genotypes.

Genotypes	Treatments				Mean
	Control	Sulfuric acid	Hot water	Scarification	
Cultivated	3.05 G*	13.86 D	12.07E	19.73 B	12.18***
Wild	3.03 G	16.46 C	9.04 F	21.37 A	12.48
Treatments mean	3.04 d**	15.16 b	10.56 c	20.55 a	

*Type x treatment means with the same capital letters is not significantly different ($p<0.05$)
** Treatment means with the same letter are not significantly different ($p<0.05$)
***N.S.: Not significant

Table 5. The effect of various pre-sowing treatments on root length (cm) in cultivated and wild carob seed genotypes.

Genotypes	Treatments				Mean
	Control	Sulfuric acid	Hot water	Scarification	
Cultivated	2.28 B*	2.74 AB	2.35 B	2.86 AB	2.56***
Wild	2.55 B	3.29 A	2.67 AB	2.81 AB	2.83
Treatments mean	2.42 b**	3.02 a	2.51 b	2.84 ab	

*Type x treatment means with the same capital letters is not significantly different (p<0.05)
** Treatment means with the same letter are not significantly different (p<0.05)
***N.S.: Not significant

Table 6. The effect of various pre-sowing treatments on shoot length (cm) in cultivated and wild carob seed genotypes.

Genotypes	Treatments				Mean
	Control	Sulfuric acid	Hot water	Scarification	
Cultivated	4.66 ABC*	5.04 AB	4.22 C	5.39 A	4.83***
Wild	4.67 ABC	4.83 ABC	4.33 BC	5.07 AB	4.73
Treatments mean	4.67 bc**	4.94 ab	4.28 c	5.23 a	

*Type x treatment means with the same capital letters is not significantly different (p<0.05)
** Treatment means with the same letter are not significantly different (p<0.05)
***N.S.: Not significant

Discussion

Physical dormancy is an adaptive trait, allowing seed germination over time and space, thus increasing the probability of enhancing the propagation of a species (Baskin & Baskin, 2000). Similar seed dormancy characteristics were reported in carob by Perez-Garcia (2009). Seed dormancy can be broken by mechanical scarification and certain pretreatments such as acid, hot water and dry heat treatments (El-Shatnawi *et al*., 2001; Karaguzel *et al*., 2002; Pérez-García, 2009; Piotto & Di Noi, 2003).

All pretreatments enhanced the seed coat permeability to varying degrees resulting in higher imbibitions in carob. Consequently, mechanical scarification and sulfuric acid treatment were found to be superior in terms of imbibition, germination percentage, average germination time, energy of germination and germination index. However, mechanical scarification is not as feasible as a practical treatment compared to the other pretreatments. Perez-Garcia (2009) used various treatments to enhance seed germination in carob and found the lowest germination rate in the control seeds (25%) and highest (99%) in scarified seeds. Similarly, Tsakaldimi & Ganatsas (2001) found that the highest germination rate (87%) was found on seeds dipped in concentrated sulfuric acid for 15 minutes but that the shortest average germination time (8 days) was observed with hot water soaking for 5 minutes. In this study, the highest germination percentage was obtained with mechanical scarification and also with sulfuric acid treatment, but the shortest mean germination time was found using scarification. Piotto & Di Noi (2003) have demonstrated that if the carob seeds had been either soaked in hot water, or dipped in concentrated sulfuric acid and then washed in water, or mechanically scarified, then there is no subsequent problem in terms of seed germination. Tsakaldimi & Ganatsas (2001) reported that the shortest root and shoot lengths were obtained from hot water treatment due to damage to the embryo.

Conclusions

In conclusion, mechanical scarification and sulfuric acid treatment were the most successful treatments for enhancing seed germination in carob. Results for the wild and cultivated genotypes were similar from all pretreatments suggesting that domestication of the specie does not appear to have influenced germination. In future work this hypothesis should be tested by using additional wild and cultivated genotypes. These results will have application by farmers, ornamental nurseries and forestry authorities in the Mediterranean basin where there is a high demand to establish carob orchards particularly in dry areas.

Acknowledgements

This paper was supported by the Scientific Research Projects Coordination Unit of Akdeniz University. The authors thank to Dr. Uri Lavi, Volcani Center (Israel), for their critical review of this manuscript.

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. *J. Amer. Soc. Hort. Sci.,* 112: 427-432.

Anonymous. 2000. *Statistical Analysis System*. SAS Institute. Procedures Guide: Version 8.0. Cary, North Carolina, USA.

Arefi, I.H., S.K. Nejad and M. Kafi. 2012. Roles of duration and and concentration of priming agents on dormancy breaking and germination of caper (*Capparis spinosa* L.) fort he protection of arid degraded areas. *Pak. J. Bot*., 44(2): 225-230.

Awodola, A.M. 1994. Aspects of germination in seeds of African locust bean tree *Parkia biglobosa* (Jacq) Don. *J. Trop. Forest Resour*. 10: 82-91.

Barwick, M. 2004. *Tropical and subtropical trees*. An Encyclopedia. Timber Press Portland, Oregon.

Baskin, C.C. and J.M. Baskin. 1998. *Seeds. Ecology, biogeography, and evolution of dormancy and germination*. San Diego: Academic Press, pp. 666.

Baskin, J.M. and C.C. Baskin. 2000. Evolutionary considerations of claims for physical dormancy-break by microbial action and abrasion by soil particles. *Seed Sci. Res*., 10: 409-413.

Batlle, I. and J. Tous. 1997. Carob tree. *Ceratonia siliqua* L. Promoting the conservation and use of under-utilised and neglected crops Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy. pp. 93.

Caja, G., E. Albanell and R. Casanova. 1988. Caracterización morfológica de frutos de algarrobo cultivados en España. In: P., Fito, A. Mulet(Eds.), *Proceedings of the II^nd International Carob Symposium Valencia*, *Spain*: 119-229.

Carvalho, N.M. and J. Nakagawa. 2000. Sementes: ciência, tecnologia e produção. 4: ed. Jaboticabal: FUNEP, In Portuguese, pp. 588.

Cicero, S.M., M. Filho, J. Silva and W.R. Org. 1986. Atualização em produção de sementes. Campinas: Fundação Cargill, In Portuguese, pp. 223.

Coit, J.E. 1951. Carob or St. John's Bread. *J. Econ. Bot*., 5: 82-96.

Crescimanno, F.G., A. De Michele, R. Di Lorenzo, G. Occorso and A. Raimondo. 1988. Aspetti morfologici e carpologici di cultivar di carrubo (*Ceratonia siliqua* L.). In: (Eds.): P. Fito and A. Mulet, *Proceedings of the II^nd International Carob Symposium Valencia, Spain*: 169-181.

Di Lorenzo, R. 1991. Carrubo. Frutticoltura speciale. Ed. REDA, Rome.

El-Shatnawi, M.K. and K.I. Ereifej. 2001. Chemical composition and livestock ingestion of carob (Ceratonia siliqua L.) seeds. J. Range Management., 54: 669-673.

Eşen, D., N. Güneş and O. Yildiz. 2009. Effects of citric acid presoaking and stratification on germination behavior of *Prunus avium* L. seed. *Pak. J. Botany*., 41 (5): 2529-2535.

Frary, A. and S. Doganlar. 2003. Comparative genetics of crop plant domestication and evolution, *Turk. J. Agri. Forest*., 27: 59-69.

Haq, N. 2008. Ceratonia siliqua L**.,** Carob (p. 387-391). In: *The Encyclopedia of Fruits and Nuts.* (Eds.): J. Janic and R.E. Paull, Cambridge, MA. CABI: pp. 954.

Hawkes, J.G. 1983. *The diversity of crop plants*, Harvard University Press, London.

Janick, J. and R.E. Paull. 2008. The encyclopedia of fruits and nuts. CAB International. Cambridge, MA: 387-396.

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: 40-47.

Kramer, P., and T.T. Kozlowski. 1960. *Physiology of trees*. McGraw-Hill Book Company. New York, pp. 642.

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.

Marakis, S., J. Kalaitzakis and K. Mitrakos. 1988. Criteria for recognizing carob tree varieties. In: (Eds.): P. Fito and A. Mulet, *Proceedings of the II^nd International Carob Symposium Valencia, Spain*: 558-566.

Martins-Louçao, M.A., P.J. Duarte and C. Cruz. 1996. Phenological and physiological studies during carob (*Ceratonia siliqua* L.) seed germination. *Seed Sci. Technol*., 24: 33-47.

Mitrakos, K. 1987. The botany of *Ceratonia*. In: (Eds.): P. Fito and A. Mulet, *Proceedings of the II^nd International Carob Symposium Generalitat Valenciana*, Conselleria d'Agricultura i Pesca, Sept/Oct-86, Valencia, Spain: 209-218.

Nadeem, M., F. Al-Qurainy, S. Khan, M. Tarroum and M. Ashraf. 2012. Effect of some chemical treatments on seed germination and dormancy breaking in an important medicinal plant *Ochradenus arabicus* Chaudhary, Hill C. & AG Mill. *Pak. J. Bot*., 44(3): 1037-1040.

Ortiz, P.L., M. Arista and S. Talavera. 1995. Germination ecology of *Ceratonia siliqua* L. (Cesalpinaceae), a Mediterranean tree. *Flora*, 190: 89-95.

Pérez-García, F. 2009. Germination characteristics and intrapopulation variation in carob (*Ceratonia siliqua* L.) seeds. *Spanish J. Agri. Res*., 7: 398-406.

Piotto, B. and A. Di Noi (Eds.). 2003. *Seed propagation of Mediterranean trees and shrubs*. Agenzia Nazionale per la Protezione dell'Ambiente (ANPA). Roma, Italy, pp. 116.

Popinigis, F. 1985. *Fisiologia da Semente*. 2. 2e. Brasilia, Agiplan, pp. 289.

Rehman, S., H.R. Choi, M. Jamil and S.J. Yun. 2011. Effect of GA and ABA on germination behavior of black raspberry (*Rubus coreanus* Mıquel) seeds. *Pak. J. Bot*., 43(6): 2811-2816.

Ruan, S., Q. Xue and K. Tylkowska. 2002. Effects of seed priming on emergence and health of rice (*Oryza sativa* L.) seeds*. Seed Sci. Technol.,* 30: 451-458.

Shepperd, W.D. 2008. *Ceratonia siliqua* L. carob. In: *The Woody Plant Seed Manual*. (Eds.): *The Woody Plant Seed Manual*. *Agric. Handbook* No. 727. (Eds.): Bonner, T. Franklin, Karrfalt, P. Robert. Washington, DC. U.S. Department of Agriculture, Forest Service. p. 371-373.

Tetik, N., I. Turhan, H.R. Oziyci, H. Gubbuk, M. Karhan and S. Ercisli. 2011. Morphological and chemical characterization of *Ceratonia siliqua* L. germplasm in Turkey. *Scientia Hort*., 129: 583-589.

Tous, J., I. Batlle and A. Romero. 1995. Prospección de variedades de algarrobo en Andalucía. Información Técnica *Económica Agraria,* 91: 164-174.

Tsakaldimi, M. and P. Ganatsas. 2001. Treatments improving seeds germination of two mediterranean sclerophyll species *Ceratonia siliqua* and *Pistacia lentiscus*. In: *Proceedings of the Third Balkan Scientific Conference on Study, Conservation and Utilization of Forest Resources*, Sofia, Bulgaria, Vol. II, ISBN 954-90896-3-0, 119-127.

Yoursheng, C. and O. Sziklai. 1985. Preliminary study on the germination of *Toora sinensis* (A. Juss). Roem*. Seed from eleven Chinese provenances*. *For. Ecol. Manage*., 10: 269-281.

(Received for publication 12 August 2011)