Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 6 (2): 288-293, 2007
ISSN 1682-3974

Endangered Status and Propagation of an Endemic Plant Species, *Thermopsis turcica* (Fabaceae)

Suleyman Cenkci, Mustafa Kargioğlu, Sergun Dayan and Muhsin Konuk
Department of Biology, Afyon Kocatepe University, ANS Campus, Afyonkarahisar, 03200 Turkey

Abstract: The objective of this study were to investigate present endangered status and propagation of *Thermopsis turcica*, which is a very unusual plant species for Turkey. *T. turcica* grows in a very narrow area located between the Eber and Akşehir Lakes. The field studies indicated that holding unhealthy seeds due to pest infestation and clearing new agricultural fields are two major threatened factors for *T. turcica*. In sterile conditions, concentrated sulfuric, hydrochloric and nitric acid pre-treatments for 0, 30, 60, 90 and 120 min used to germinate *T. turcica* seeds which were only obtained from small Eber populations. 99% seed germination within a few days was achieved from 120 min. sulfuric acid pretreatment; whereas the hydrochloric and nitric acid treatments did not have effect on the germination (<20%) of *T. turcica* seeds. In non-sterile conditions, however, a maximum 61% germination was obtained from 120 min sulfuric acid pretreated seeds. Although 20% non-sterile seedlings were survived, 86% *in vitro* seedlings acclimatized to green house conditions were alive after 14 weeks. This propagation technique, *in vitro* germination-acclimatizing to the soil, could be used to propagate endangered plant species suffering from germination and seedling development.

Key words: *Thermopsis turcica*, endemic, propagation, conservation

INTRODUCTION

There are 25 species of the genus of *Thermopsis* R. Br. belonging to the Thermopsideae tribe of Leguminosae (Fabaceae) family and they are found mainly spread in the mountainous and humid regions of Central Asia and North America (Dement and Mabry, 1975; Davis *et al*., 1988; Wojciefowski, 2003). The only endemic representative of this genus in Turkey is *Thermopsis turcica* Kit Tan, Vural and Küçüködük (Kit *et al*., 1983; Davis *et al*., 1988). *T. turcica* grows over a narrow area located between the southern part of Eber Lake and the south-western part of Akşehir Lake (Tan *et al*., 2003) and the species was classified as critically endangered CR in the Red Data Book of Turkish Plants (Ekim *et al*., 2000). Some morphological and anatomical specifications of *T. turcica* evident in the Flora of Turkey and East Aegean Islands (Davis *et al*., 1988) have been described as follows; densely white-villous perennial herb, with long rhizome; 35-80 cm stems erect; 3 carpellate ovaries containing 10 ovules on adaxial suture; 2-3 seeded legumes which remain closed during maturity, the seeds being 4-5×3 mm in dimension, smooth and of a pale purplish color.

Most legume seeds have a hard seed coat inhibiting water and/or oxygen intake and radicula emergency (Salisbury and Ross, 1992). Some of the methods used successfully to weaken the hard seed shells of wild plants to promote germination are mechanical (e.g., sanding and drilling holes), chemical (e.g., soaking in acid) and physical (e.g., boiling and freezing) scarifications (Rehman and Park, 2000; Kaye and Kuykendal, 2001a, b; Sy *et al*., 2001; Olvera-Carrillo *et al*., 2003). The chemical scarification of the legume seeds using concentrated acids is practical and effective germination method (Tetsuya and Takahashi, 2003); but the exposing time should be precisely adjusted to have maximum germination without damaging seed germination capacity (Sy *et al*., 2001).

As an alternative to protect endangered plant species with *in situ* methods, *in vitro* germination procedures are often used to obtain sterile seedlings preferably used as explant source for micropropagation of endangered plant species with low seed production, germination problems or problematic seedling development (Pence, 1999; Cerabolini *et al*., 2004). The objective of this study was twofold; firstly we aimed to determine the present endangered status of *T. turcica* species and secondly, we intent to find out the best germination and seedling development procedures using sterile and non-sterile techniques to propagate this critically endangered plant species.

Corresponding Author: Suleyman Cenkci, Department of Biology, Afyon Kocatepe University, ANS Campus, Afyonkarahisar, 03200 Turkey Tel: +90 272 228 13 11 Fax: +90 272 228 12 35

MATERIALS AND METHODS

Seed collection: The seeds of *T. turcica* were obtained from a small plant population of less than 50 units located in the marsh area (~960 m altitude) south of Eber Lake in late August of 2004 and 2005 (Fig. 1). Relatively small amounts of seeds were collected (less than 40%) to avoid major damage to this small population. After shelling the seeds from their legumes, they were kept in glass jars in a dark room at room temperature.

Sterile seed germination: Untreated *T. turcica* seeds were cleaned under running water and sterilized in sodium hypochlorite solution (5%) containing a few drops of Tween-20 surfactant for 20 min. Then, the seeds were dipped into 70% ethanol for 90 sec, rinsed thrice with sterile distilled water and transferred onto an agar medium. The other seeds were immersed into concentrated sulfuric, hydrochloric or nitric acid for different periods (0, 15, 30, 60, 90 and 120 min). The scarified seeds were only rinsed three times with sterile distilled water before being transferred onto the agar medium.

Solid agar composition included sucrose (20 g L^{-1}), basal MS salts and vitamins (4.3 g L^{-1}) (Murashige and Skoog, 1962) and agar (7 g L^{-1}). The medium pH was adjusted to 5.7±0.1 and then autoclaved at 120°C and 2 atm for 15 min. In each treatment, only 15 seeds due to limited number available were placed into a germination box (11×11×9 cm) containing 200 mL of basal MS medium and four replicates were used for each treatment. The seeds were kept in the germination cabinet for a period of 4 weeks under Grolux^{Tm} fluorescent lamp light with 16/8 h light/dark cycle at 25±1°C. To minimize the effects within the cabinet, the places of the germination boxes were randomly changed on a daily basis. The seeds were accepted as germinated when the emerging radicula was approximately 2 mm in length (Rehman and Park, 2000). The germination data was recorded on a daily basis for 28 days.

Non-sterile seed germination: The non-sterile germination experiments were conducted during 20 Feb-31 May 2006. After 0, 15, 30, 60, 90 and 120 min exposing to only concentrated sulfuric acid, *T. turcica* seeds were washed a few times with dH_2O. Pretreated and untreated seeds were than soaked in water for 24 h. Subsequently, the seeds were sown into soil, sand and perlite mixture (1:1:1) 1.5 cm in depth as described for *Thermopsis lupinoides* seeds (Tetsuya and Takahashi, 2003). Fifteen seeds were sown to 50×10×20 cm plastic pots for each treatment and 4 replicates were used for each experiment. All the non sterile germination experiments were conducted in the greenhouse conditions and the seed pots were watered (containing 1/8 MS salt and vitamins) twice a week for the first month and later once a week. The seeds were accepted as germinated by the cotyledonal emergency. The germination data was recorded on daily basis for 100 days.

Seedling developments: The sterile germination boxes were opened for five days at the 30th day of germination experiment and randomly selected 50 sterile seedlings directly transformed to soil, perlite and sand mixture (1:1:1) in greenhouse conditions. The seedling survival data of sterile and non-sterile originated plantlets was recorded on daily bases for 100 days.

Data analyses: Final germination percentage was calculated when no further germination had taken place for several days. To determine the proportion in degrees and germination percentages of arcs transformed in germination, they were subjected to analysis by *Duncan* test using SPSS 10 (for Windows). The formula given below was used to calculate mean germination time (MGT) as described by Chuanren *et al.* (2004);

$$MGT = \Sigma (nd)/N$$

where n is the number of seeds germinated between scoring days; d is incubation period days and N is the total number of seeds germinated in treatment.

RESULTS

Field studies: *T. turcica* spreads over a 2×20 km^2 (~4 000 ha) area on the banks of Eber and Akşehir Lakes (Fig. 1). The field work carried out in this restricted area revealed that *T. turcica* has 5 separated populations (Table 1). Four of these populations, Akbaba 1 & 2 and Kavakli 1 & 2, are close to each other and consist of a high number of various plants, however the Eber population is made up of less than 50 plants, consisting of two clone groups with 14 and 35 plants, situated at least 15 km away from the other populations.

According to our individual observations, most land demands have taken place in the closed populations (Akbaba 1 & 2, Kavakli 1 & 2) where *T. turcica* is the most widespread, so young and clonally propagated *T. turcica* seedlings have covered the ploughed fields.

Table 1: The sizes, locations and altitudes of *T. turcica* populations

Populations	Population size	Locations	Altitude
A1, Akbaba 1	<1000	38°27' N, 31°21' E	952
A2, Akbaba 2	Intense	38°27' N, 31°20' E	963
K1, Kavakli 1	<5 000	38°29' N, 31°19' E	967
K2, Kavakli 2	Intense	38°29' N, 31°18' E	966
E1, Eber	<50	38°37' N, 31°11' E	968

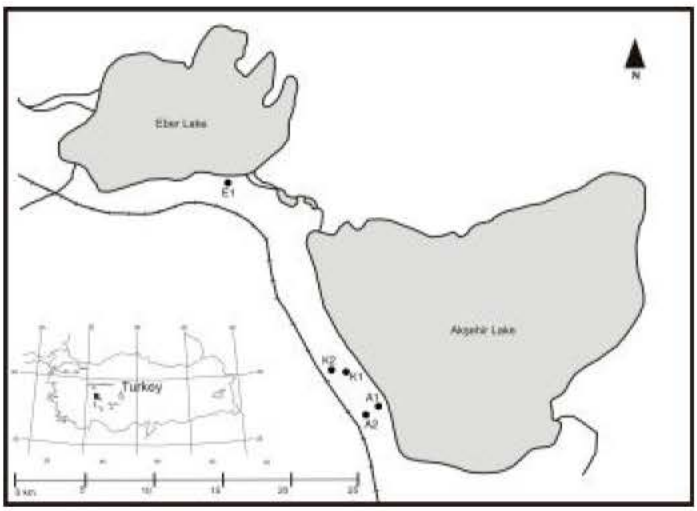

Fig. 1: Location of the study plant species *Thermopsis turcica*

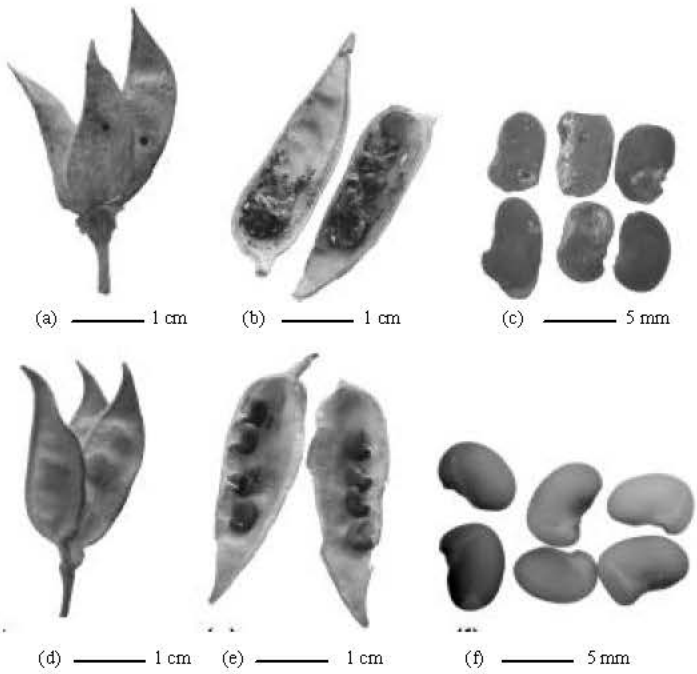

Fig. 2: The comparison of healthy and unhealthy *T. turcica* samples collected from Kavaklı 2 population (a, b, c) and Eber population (d, e, f), respectively; (a) a three carpelled healer legume, (b) a view of the inside of a carpel containing granulated and damaged seeds, (c) unhealthy seeds, (d) a well developed three carpelled legume, (e) clear view of the inside of a carpel and (f) healthy seeds

Also we have recognized that *T. turcica* carpels remain on the plant at least two years without seed dispersing. Furthermore, hardly any healthy seeds were observed among the existing one or two year old legumes in the most intensely spread populations. Surprisingly, each *T. turcica* legume obtained from these populations had a drilled hole in its hull (Fig. 2a) and was filled with granulated or damaged seeds (Fig. 2b) black or pale purple in color (Fig. 2c). On the contrary, all the legumes obtained from the small Eber population contained healthy seeds and some well developed legumes held up to 9 seeds which were brown in color (Fig. 2d, e and f).

Table 2: The effects of acidic scarification on germination and mean germination time (MGT) of *T. turcica* seeds

Exposure duration (min)	Sulfuric acid*		Hydrochloric acid		Nitric acid		Sulfuric acid **	
	Germination (%)	MGT (days)	G (%)	MGT (days)	Germination (%)	MGT (days)	Germination (%)	MGT (days)
0	6.66±2.72a***	7.0±0.70^{a}	6.66±2.72^{a}	7.0±0,70^{a}	6.66±2.72^{a}	7.0±0.70^{a}	0a	0a
15	23.33±0.00^{b}	4.4±1.95^{b}	5.0±1.66^{a}	6.6±0.19^{a}	8.3±1.66^{a}	6.7±0.17^{ab}	0a	0a
30	83.8±1.92^{c}	4.0±0.03^{b}	6.6±2.72^{a}	6.72±0.13^{a}	8.3±4.19^{a}	6.8±0.08^{ab}	11.66±3.19ab	26.67±2.07ab
60	83.8±1.95^{c}	3.69±0.07^{b}	6.66±2.72^{a}	6.65±0.17^{a}	8.33±4.19^{a}	6.75±0.09^{ab}	36.66±3.27b	26.85±3.84ab
90	94.99±1.66^{d}	3.59±0.11^{b}	8.33±1.66^{a}	6.52±0.24^{a}	10.03±3.33^{a}	6.42±0.16^{ab}	46.65±9.02c	28.8±6.91ab
120	98.33±1.66^{d}	3.83±0.46^{b}	8.35±1.66^{a}	6.5±0.20^{a}	20.0±2.72^{b}	5.8±0.18^{b}	61.66±7.30d	13.3±0.74b

*Germination tests were undertaken on M and S agar beads at 25°C and at 16/8 h photoperiod or **in soil under greenhouse conditions. *** Values in the same line with the same letter (s) do not differ significantly (Duncan test with p<5%)

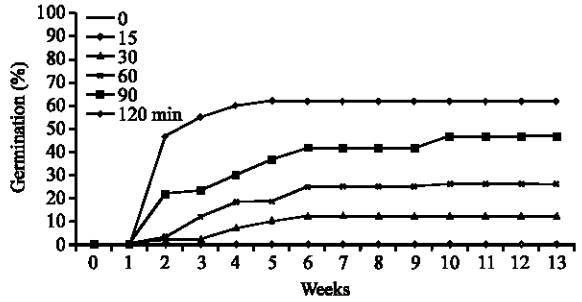

Fig. 3: Seed germination of *T. turcica* seeds exposed to various sulfuric acid pretreatments in non-sterile conditions

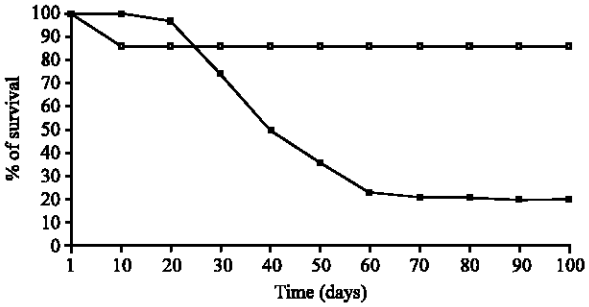

Fig. 4: Survival percentages of *T. turcica* seedlings obtained from; (▫) sterile conditions and (▪) non-sterile conditions

Sterile seed germination: Since none of the seeds of other population were germinated with any treatment used in this study, sterile and non-sterile germination trials have been carried out only on the limited amount of healthy seeds obtained from the Eber population. The germination rates of untreated seeds did not exceed 10% on MS agar (Table 2). *T. turcica* seeds treated with hydrochloric or nitric acid for different time periods showed similar germination results with untreated seeds with the exception of nitric acid application for 120 min where 20% germination occurred. On the other hand, only 15 min sulfuric acid min pretreatment of the seeds was resulted in 23% germination (Table 2).

The highest germination values (>94%) were obtained with 90/120 min sulfuric acid pretreatment of *T. turcica* seeds in sterile conditions. The Mean Germination Time (MGT) of the intact and hydrochloric acid pretreated seeds was approximately 7 days and nitric acid slightly decreased the MGT to 5.8 days (Table 2). On the other hand, 60-120 min sulfuric acid scarification sharply reduced the average germination time to almost half as compared to the intact seeds.

Non-sterile seed germination: Unlike the germination results of sterile conditions, none of the untreated and 15 min sulfuric acid treated *T. turcica* seeds germinated in greenhouse within 14 weeks (Fig. 3). The increased acidic scarification time was induced the germinations and the maximum germination (61.66%) was obtained from 120 min pretreated group.

We have calculated the GMT values as almost 27 days for 30, 60 and 90 min pretreated groups; in fact some germination were recorded at the 84th day of the experiment initiation in 90 min pretreated group. However, the average germination time was 13 days for 120 min pretreated group.

Seedling survivals: Totally, 86 of 360 seeds were germinated and 80% of the plantlets were dead in non-sterile conditions after 100 days (Fig. 4). Most of the seedling mortalities were observed between 20-60 days of the experiment. On the other hand, only 7 sterile seedlings were lost during the acclimatization process, so 86% of acclimatized *T. turcica* seedlings were surviving after 14 weeks.

DISCUSSION

Based on our field observations, *T. turcica* has some ecological advantages such as not grazing by domestic animals, not used for medical purposes and vegetating via their rhizomes. On the other hand, there are some important factors placing this endangered plant species into a more critical position. Because of the favorable

climate and land structure and also due to the fact that Eber and Akşehir Lakes have receded over 5 km during the last decades (Tan *et al*., 2003), efforts to clear more land for agricultural purposes in the natural habitat of this plant species has become a preliminary concern of the local farmers and this situation manifests itself with radical measures taken by the local people. Kit *et al.* (1983) and Davis *et al.* (1988) previously described the habitat of the *T. turcica* as marsh, pasture and muddy field land but presently *T. turcica* populations, except Eber, are completely in the agricultural fields. Therefore, newly vegetated *T. turcica* seedlings are mainly reproduced vegetating via their rhizomes on ploughed fields, because Akbaba 1 & 2 and Kavakli 1 & 2 populations are also suffering from insect attacks which inhibits the natural seed dispersion. In contrast, all *T. turcica* legumes of the small Eber population contained healthy seeds. This might be due to the fact that they are at least 15 km away from the other populations and naturally protected against insect attacks.

A sharp reduction in the number of the present populations and propagation via rhizomes possibly causes reduction in genetic variation among the *T. turcica* populations. Ellstrand and Elam (1993) argued that genetic drift, inbreeding and gene flow were likely to put rare plants and small populations at genetic risks. Barrett and Khon (1991) claimed that the loss of variation is thought to reduce the ability of populations to adapt to changing environments and increase their susceptibility to pest and disease pressures which might be a good description for the present endangered status of *T. turcica* populations.

The germination results indicated that *T. turcica* seeds have a strong physical dormancy caused by the hard seed coat which prevents seed germination without any pretreatment as reported for other legume species (Rehman and Park, 2000; Cerabolini *et al*., 2004; Kaye and Kuykendall, 2001b; Aparicio and Guisande, 1997). In the present study, 120 min sulfuric acid scarification is enough to obtain 98% germination in a few days in sterile conditions. Moreover, germination in sterile conditions and subsequently acclimatizing the sterile seedlings to the non-sterile conditions was found as effective and practical method for the propagation of this rare plant species as compared to the propagation in only greenhouse conditions. The reason of low germination and seedling survival in green house conditions remains to be investigated whether they are due to lower embryo fitness (Jarne and Charlesworth, 1993) or to eco-physiological or nutritional causes (Aparicio and Guisande, 1996). It was reported that some rare plant species seeds strictly need high nutrients and/or phytohormones to germinate, for instance, Cerabolini *et al.* (2004) showed the germination of *Physoplexis comosa* seeds only in sterile conditions. Fay (1992) and Pence (1999) argued that appropriate techniques must be selected based on the requirements of each species, particularly rare and/or endemic species. Therefore, high nutrient requirement might be an important factor for germination and early seedling development as removing the hard seed coat to have successful propagation strategy for *T. turcica*. It may be possible to reintroduce populations of *T. turcica* through direct seeding of habitats and trough transplanting sterile origin seedlings. In conclusion, the present study demonstrates that *ex situ* propagation of *T. turcica* is possible from seeds.

ACKNOWLEDGMENT

The authors wish to thank Afyon Kocatepe University Research Fund (Project No: 042.FENED.04) for supporting this study financially.

REFERENCES

Aparicio, A. and R. Guisande, 1997. Replenishment of the endangered *Echinospartum algibicum* (Genisteae, Fabaceae) from the soil seed bank. Biol. Conserv., 81: 267-263.

Barrett, S.C.H. and J.R. Kohn, 1991. Genetics and Evolutionary Consequences of Small Population Size in Plants: Implications for Conservation. In: Genetics and Conservation of Rare Plants. Falk, D.A. (Ed.), Oxford University Press, New York, pp: 3-31.

Cerabolini, B., R. Andreis, R.M. Ceriani, S. Pierce and B. Raimondi, 2004. Seed germination and conservation of endangered species from the Italian Alps: *Physoplexis comosa* and *Primula glaucescens*. Biol. Conserv., 117: 351-356.

Chuanren, D., W. Bochu, L. Wanqian, C. Jing, L. Jie and Z. Huan, 2004. Effect of chemical and physical factors to improve the germination rate of *Echinacea angustifolia* seeds. Colloids and Surfaces B: Biointerfaces, 37: 101-105.

Davis, P.H., R.R. Mill and T. Kit, 1988. Flora of Turkey and the East Aegean Islands. Edinburgh, 10: 111-112.

Dement, W.A. and T.J. Mabry, 1975. Biological implication of flavonoid chemistry in *Baptisia* and *Thermopsis*. Biochem. Sys. Ecol., 3: 91-94.

Ekim, T., M. Koyuncu, M. Vural, H. Duman, Z. Aytaç and N. Adigüzel, 2000. Red Data Book of Turkish Plants (*Pteridophyta* and *Spermatophyta*), 1st Edn., TDKA and Van Centennial University Press, Ankara.

Ellstrand, N.C. and D.R. Elam, 1993. Population genetic consequences of small population size: Implications for plant conservation. Ann. Rev. Ecol. Sys., 24: 217-242.

Fay, M.F., 1992. Conservation of rare and endangered plants using *in vitro* methods. *In vitro* Cellular Develo. Biol. Plant, 28: 1-4.

Jarne, P.H. and D. Charlesworth, 1993. The evolution of selfing rate in functionally hermaphrodite plant and animals. Ann. Rev. Ecol. Sys., 24: 441-466.

Kaye, T.N. and K. Kuykendall, 2001a. Germination and Propagation Techniques for Restoring Rare Pacific Northwest Prairie Plants. In: Conservation of Washington's Native Plants and Ecosystems. Reichard, S.H., P.W. Dunwiddie, J.G. Gamon, A.R. Kruckeberg and D.L. Salstrom (Eds.), Washington Native Plant Society, Seattle, pp: 213-224.

Kaye, T.N. and K. Kuykendall, 2001b. Effects of scarification and cold stratification on seed germination of *Lupinus sulphureus* ssp. *kincaidii*. Seed Sci. Technol., 29: 663-668.

Kit, T., M. Vural and M. Küçüködük, 1983. An unusual new *Thermopsis* from Turkey. Notes RBG Edinb, 40: 515-518.

Murashige, T. and F.A. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant, 15: 473-479.

Olvera-Carrillo, Y., J. Márquez-Guzmán, V.L. Barradas, M.E. Sánchez-Coronado and A. Orozco-Segovia, 2003. Germination of the hard seed coated *Opuntia temontesa* S.D., a cacti from the Mexico valley. J. Arid Environ., 55: 29-42.

Pence, V.C., 1999. The Application of Biotechnology for the Conservation of Endangered Plant Species. In: Plant Conservation Biotechnology. Benson, E.E. (Ed.), London, pp: 227-242.

Rehman, S. and I. Park, 2000. Effect of scarification, GA and chilling on the germination of goldenrain-tree (*Koelreuteria paniculata* Laxm.) seeds. Sci. Hortic., 85L: 319-324.

Salisbury, F.B. and C.W. Ross, 1992. Plant Physiology. Wadsworth Publishing, California, pp: 451-497.

Sy, A., M. Grouzis and P. Danthu, 2001. Seed germination of seven Sahelian legume species. J. Arid Environ., 49: 875-882.

Tan, A., H. Duman, İ.F. Niksarli, A. İnal and A. Karagöz, 2003. Ecosystem conservation and management for threatened species projects: Description of target species and their habitats. TACN (Turkish Association for the Conservation of Nature), Ankara.

Tetsuya, K. and K. Takahashi, 2003. Breaking of physical dormancy and germination ecology for seeds of *Thermopsis lupinoides* Link. J. Jpn. Soc. Regevet. Technol., 30: 163-168.

Wojciefowski, M.F., 2003. Reconstructing the Phylogeny of Legumes (*Leguminosea*): An Early 21st Century Perspective. In: Advances in Legume Systematics. Klitgaard, B.B. and A. Bruneau (Eds.), Part 10, Higher Level Systematics, Royal Batanic Gardens, Kew, pp: 5-35.