Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (4) October-December, pp.455-464/Zare
Research Article

A STUDY OF DIFFERENT TREATMENT EFFECT ON SEED GERMINATION CHARACTERISTICS AND SEEDLING SURVIVAL MONTPELLIER MAPLE (*ACER MENSPESSOLANUM* SUBSP. *TURCOMANICUM* RECH. F.)

***Alireza Zare**
Department of Agriculture and Natural Resources Research Center of Khorasan Razavi
**Author for Correspondence*

ABSTRACT
Montpellier maple (*Acer monspessulanum subsp. turcomanicum* Rech. f.) is a valuable species in the mountain forests of Hezar Masjed in Northern Khorasan and Khorasan Razavi province that is expanded in the northeastern highlands of mountains of thousands of Hezar Masjed and Kope Dagh. This species because of the drought resistance is suitable for reforestation in semi-arid climate. Its seed is collected from highlands of the southern slopes of the Kopet Dagh and pretreatment test of dormancy breaking which includes moist chilling (in four levels 0,3, 4 and 5 months) and scarification (Sulphuric acid and hydrochloric acid) and applying gibberellic acid hormone is done (at two levels of 250 and 500 ppm concentration). Indicators including seed viability, germination rate, mean of germination time, duration and completion of the germination and establishment percentage of seedlings were recorded and analyzed after six months in the greenhouse environment.This research is applied in a completely randomized design with four replications and data were analyzed by using LSD test method and SAS software. The results showed that seed germination percentage has significant difference among treatments at level ($P < 0.001$). The highest percentage of germination referred to the 500 ppm and 250 ppm gibberellic acid treatments with five months moist chilling which were 38 and 34 %, respectively. The evaluation of the survival percentage of seedlings after 6 months in the greenhouse environment showed that the highest survival rate relates to five and four months moist chilling treatment which are 56 and 53 %.

Keywords: *Moist Chilling, Germination Percentage, Gibberellic Acid, Scarification, Turkmen Acer Cineracens*

INTRODUCTION
Forested mountains of arid and semi-arid have important environmental role including the production of fresh water, soil stabilization, climate tension adjustment, oxygen production and ultimately are responsible for creating a stable ecosystem. The lower mezzanine of these forests is composed of pasture plants. Natural regeneration of plants, especially trees and shrubs do not occur due to the loss of topsoil. According to the destructive environmental impact of deforestation and pasture, one day the need for reforestation is felt and conducting extensive afforestation with native species will be on the agenda of the government and people of Iran, so the answer to the sexual regeneration and seedling production of this species is one of the important functions of Forest Research Branch, Agricultural and Natural Resources research Centers. Acer cineracens Turkmen maple Acer monspessulanum L. subsp. turcomanicum Rech. f. is one of the four subspecies of Acer cineracens existing in Iran (Mozaffarian, 2004). The habitat of this species is in the North East of Iran and south of Turkmenistan. The proliferation of this species is important because it is the key elements of the mountainous forests of the semi-arid areas. The mentioned species is more drought tolerant than other maple species and normally grows in areas with rainfall of 400 mm (Tissier *et al.*, 2004). However, due to soil erosion the lack of regeneration of this species in field surveys is completely evident in recent years. Therefore, the recognition of practical and suitable methods is important for seed proliferation in the nursery and transferring the seedlings to the field of natural resources. Seed dormancy is a process that delays germination in the nature until the suitable conditions to be provided for seed germination and plant establishment. In most tree species of temperate regions dormancy period is associated with chilling requirement of seed. This process is a physiological

mechanism that leads to the growth arrest and until the chilling requirement is not provided, the developing fetus will not start (Lang, 1985). Additionally, plant species that grow in harsh conditions have the complex seed dormancy organization; this phenomenon increases their ability to cope with the live, non-live and adverse environmental tensions and guarantees plant reproduction (Tajbakhsh, 1996). Two factors alone or in combination are effective in providing seed dormancy. A mechanical and external factor such as impermeability of seed hulls to water and oxygen and the mechanical strength of seed hulls and the other refers to the internal factors including chemical and biological effects like the existence of growth-inhibiting substances in the environment, growth recession in the fetus and immaturity of the fetus (Latifi, 2001). Seeds of some maple species, such as A. campestre, A. monspessulanum A. pseudoplatanus, A. platanoides, A. glabrum have dormancy (Thomas *et al.*, 1973; Piotto and Di-Noi, 2003), but in some other species such as A. rubrum and A. saccharinum pre-treatment is not required for seed germination. The first material of sexual breeding in plants is seed but seed dormancy problem is a major obstacle in the path of the plants' proliferation especially forest species,
but by applying some pretreatments such as scarification of seed hulls with sulfuric acid (Song et al., 1990), changes in temperature (Pawłowski, 2007, 2009), moist chilling (Rouhi *et al.*, 2010; Pinfield *et al.*, 1990; Aygun, 2011; Shock *et al.*, 2009), soaking in gibberellic acid (Prased *et al.*, 1996; Kabar, 1997, Dobrescu and Catrina, 1984), the mentioned problem is solved in artificial conditions. In a report in addition to focusing on the internal factors of seed dormancy in some maple species, it is emphasized to spend three to five ° C cooling for 56 to 84 days to meet: Acer cineracens maple seed dormancy and germination reaching to 40% (Piotto and Di-Noi, 2003). Mustafa (2006) has reported that for the physiological dormancy removal of maple seed, Acer trautvetteri Medv, spending a 90-day cooling period is necessary. A part of Korko Maple seed's dormancy, Acer campestre L, relates to the impermeability of seed layer, so, 6 to 7 months of cold dormancy is broken by spending a month with heat (12 ° C) and germination occurs (Bewley and Black, 1982; Blomme, 1972). In another report, the duration of cold at a temperature of 3 ° C for 10 weeks resulted in Acer platanoides L. seed germination to the rate of 54% (Draghici and Abrudan, 2011).
Furthermore, Jensen (2001) demonstrated that 36% humidity and a temperature of 0 to 1 ° C is effective to break seed dormancy of Maple Acer platanoides L. and five to seven degrees Celsius rise in temperature delayed the seed dormancy breaking, but the temperature increases to 15 to 18 ° C led to the secondary seed dormancy. The investigations conducted on Iranian Acer cineracens by Nasiri (2008) showed that the increase of moist chilling from 3 to 6 months increased the seed germination from 19 to 33%. In addition, the combined effect of heating and chilling compared to the chilling in the same period was more effective. Temperature changes, especially moist chilling had more impact on internal hormonal balance of the seed and increase fetal sensitivity to growth hormones and thereby removes seed dormancy (Kucera *et al.*, 2005). Applying the hormonal substances break seed dormancy, in a way that gibberellic acid unlike abscisic acid plays an important role in resolving seed dormancy and germination (Xia *et al.*, 2000; Ogawa *et al.*, 2003). This acid plays this role in two ways: a) Increased growth potential of fetal and b) Overcoming the inhibitory mechanism of seed coating layers and breaking the tissue surrounding the root (Ogawa *et al.*, 2003). In addition to the utilization of chemical seed germination stimulant, the seed dormancy period can be reduced by decreasing the amount of chemical inhibitor; in this method, by removing the seed hulls due to the reduction of the abscisic acid rate the seed germination of Acer velutinum Boiss increases (Farhadi *et al.*, 2006).

MATERIALS AND METHODS

The seeds were collected in the fall of 2012 from the mountainous region of northern Tandooreh National Park at an elevation of 1300 meters above sea level in the geographic coordinates of 47' and 32″ and 37˚ north latitude and 20' and 37" and 58˚ eastern longitude (Figure 1).
After transferring to the physiology Laboratory of Department of Forest and Range of Agriculture and Natural Resources Research Center of Khorasan Razavi and isolating ballet of fruit, seeds were obtained free from physical contamination. Seed germination treatments were included following issues:

A) Scarification with hydrochloric acid and sulfuric acid each with concentration of 32 and 98% respectively, by seed immersion in acid solution at two temporal level of zero (control) or 10 minutes before chilling treatment,
B) Soaking seeds in gibberellic acid hormone at two concentration of 250 and 500 million in episode for 24 hours before chilling treatment,
C) Chilling at four levels of zero, three, four and five months
The seeds were disinfected for 15 min with 1% hypochlorite (20% volume commercial bleach containing a drop of liquid soap) and then washed several times with distilled water (Nassiri *et al.*, 2003). Then, the germination test treatments were applied on these seeds as follows.
The disinfected wet sand was used as the treatment substrate due to the long period of chilling in this experiment and because of fungus possibility in a Petri dish environment (Nassiri, 2008, Lee *et al.*, 2006). Sand after washing with municipal water was dried in the open air for 2 hours and was placed at 120 ° C in a furnace. Sterilized sands were placed in plastic containers with suitable ventilation and their moisture was increased to the 50% saturation moisture by using distilled water and was used as seeds' substrate in chilling treatments. During the test conducting, if the surfaces of sand to be dry a little water was sprinkled on it. After termination of the chilling period, seeds come out of bed and after disinfection for conducting germination tests seeds were placed in Petri dishes on a wet filter paper. The humidity inside the container was maintained in standard mode. The mentioned containers were placed in germinator at 15 ° C and every day was monitored and in addition to providing moisture optimally and recording the seeds' germination then they were excluded from Petri dishes and were planted in substrate containing coco peat and perlite in 80 to 20 ratio (Figure 2). Then were transferred to the greenhouse and after a 6-month the survival status of seedlings was investigated.

Figure 1: Rootstock of prepared seed for determining seed viability test

Germination percentage (Formula 1), germination rate (Formula 2) and germination index (Formula 3) which are as a measure of germination time were calculated by mathematical equations (Bewley, Black, 1994) as follows:

Formula 1 $$\frac{N}{S} * 100$$

N is the number of seeds germinated, S is the number of seeds tested.

Formula 2 $$\sum_{1}^{j} \frac{n_i}{D_i}$$

n_i is the number of germinated seeds in the counting days and D is the number of days after starting the test

$$\frac{\sum T_i N_i}{N}$$

Formula 3

T_i counting time (days) after planting, N_i the number of germinated seeds per counting (day) and N is the number of green seeds

Starting and termination time of seed germination were recorded in each treatment and after calculation the period lasted for germination of all green seeds were determined. The test was conducted in the frame of completely randomized block design includes 20 treatments and each treatment includes four replications and each replication contains 50 seeds. It should be mentioned that the results of the treatments that had no positive effect on germination were excluded from statistical analysis to obtain a better result from the statistical analysis. The statistical data were analyzed by using SAS software and graphs were plotted by using Excel software.

Figure 2: Green Acer cineracens of Turkmen in the mixed bed of cocopeat + perlite

RESULTS AND DISCUSSION

Results

The results showed that treatments with sulfuric acid scarification alone or in combination with chilling had no effect on seed dormancy breaking of Turkmen Acer cineracens. As shown in Table 1, the treatments conducted on germination, germination rate, germination time, start time, end germination and seedling establishment percent show a significant difference. The variance analysis of tested treatments' effect showed that the difference is significant at probability level of 0.1%.

Table 1: ANOVA analysis and the significant effect of treatment on the mean of index of germination and seedling establishment

Sum of squares						Source of Variations
Seeding establishment percentage	**Ending of germination**	**Starting of germination**	**Germination time**	**Germination speed**	**Germination %**	
311**	53***	66***	62***	9***	305***	Treatment
100	3.6	1.7	1.6	0.24	5.7	Error
25	9	12	10	19	12	Total
51	81	92	92	92	94	Coefficient of variation
						r^2

****, **, *, and ns stands for level of significance at 0.1%, 1%, 5% and no significant, respectively*

Gibberellic acid treatments + chilling had the most effect on the germination percentage and germination rate. So that, the highest germination percent of 38.5 % relates to the treatment of four months chilling of seed that soaked for 24 hours in 500 M.d.q gibberellic acid solutions and the lowest germination rate 10.5% relates to the two months chilling (Figure 3). In contrast to the results of other experiments in this research, seedling establishment percentage determination test which was out of the laboratory environment and inside the greenhouse had high variation coefficient and less correlation coefficient and by increasing chilling duration from three to five months the germination rate increased and duration of germination period was reduced in all treatments (Table 1, Figure 4 and 5). As can be seen in Table 2, the application of gibberellic acid at two levels of 250 and 500 M.d.q. had significant impact on increasing germination percentage particularly in chilling treatment of five months, so that their germination percentage was respectively, 34 and 38.5%. Moreover, the use of this hormone in chilling treatment of five months shows the greatest influence on the germination rate of 6.2 and 5.2 respectively for the two levels of 250 and 500 M.d.q. Gibberellic acids.

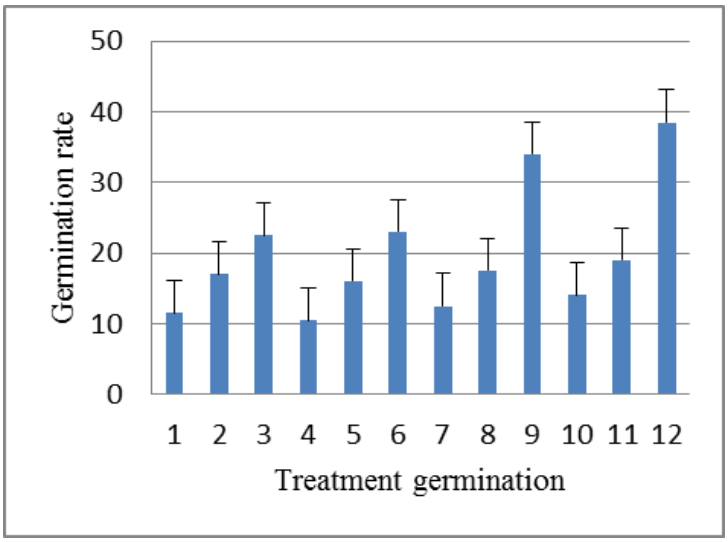

Figure 3: The effect of different treatments on seed germination percentage of Turkmen Acer cineracens

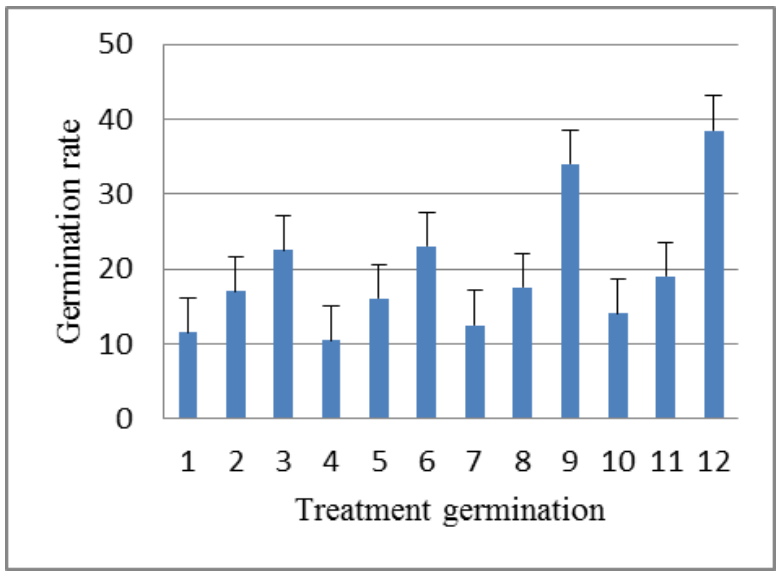

Figure 4: The effect of germination treatment on seed germination rate

Table 2: The mean comparison of the germination percentage, growth rate, germination index and the establishment percentage of Turkmen Acer cineracens in condition of different treatments

Establishment percentage	The time of eding garminatio n	The time of starting gaminatio n	Garminatio n time	Garminatio n speed	Germinatio n percentage	Kind of effective treatment on breaking sleep	Treatmen t
35/0bc	26/0a	16/5ab	17/6a	1/0f	11/5fg	Sc (HCl Scarification) . Then 3 M.c (Month cold).	1
41/0abc	27/0a	14/5bc	16/3a	2/1de	17/0cde	Sc. Then 4 M.c.	2
44/9abc	24/5a	9/5ef	12/1b	4/2b	22/5b	Sc. Then 5 M.c.	3
47/5abc	26/0a	18/0a	18/0a	0/8f	10/5fg	3 M.c.	4
53/5ab	20/5bc	13/0cd	12/6b	1/8def	16/0cdef	4 M.c.	5
56/1a	17/5c	8/0fg	8/3cd	3/1bc	23/0b	5 M.c.	6
31/6c	21/0b	11/0de	12/8b	1/1ef	12/5efg	250 PPM G.a (GA3 scarification). Then 3 M.c.	7
33/7c	18/0bc	8/5fg	8/8cd	2/4cd	17/5cd	250 PPM G.a. Then 4 M.c.	8
37/2abc	18/5bc	7/0gh	8/3cd	5/2a	34/0a	250 PPM G.a. Then 5 M.c.	9
28/4c	17/5c	9/0efg	8/2cd	1/2ef	14/0defg	500 PPM G.a. Then 3M.c.	10
32/0c	16/5bc	7/0gh	9/4c	2/6cd	19/0bc	500 PPM G.a. Then 4M.c.	11
31/2c	17/5c	5/0h	6/5d	6/2a	38/5a	500 PPM G.a. Then 5M.c.	12

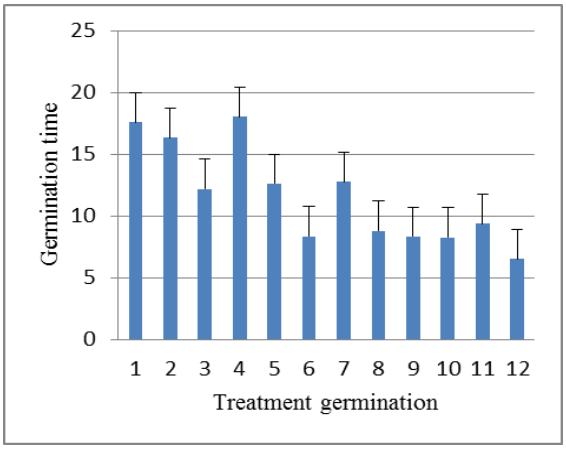

Figure 5: The effect of pretreatment on seed germination period of Turkmen Acer cineracens

Unlike the positive effect of gibberellic acid hormone on germination percentage, rate and reducing its duration, the use of this hormone had no positive effect on seedling establishment but also showed the lowest percentage of seedling establishment, unlike the most effect related to the chilling for five months (Figure 6 see Table 2). Comparing the number of seedlings established after six months in the greenhouse condition compared to the number of initial treated seed shown that treatments 6, 9 and 12, respectively, with 13, 12.5 and 12 percent had the best results and shows significant difference with other treatments (Figure 7).

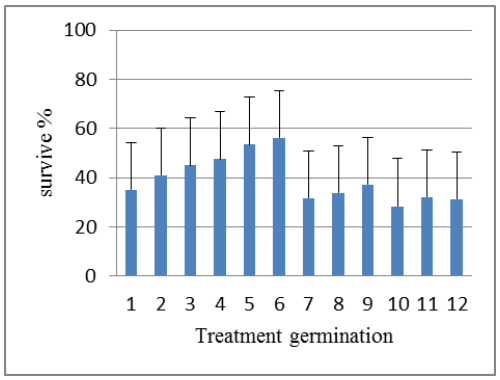

Figure 6: The effect of germination pretreatment of Turkmen Acer cineracens seed on seedling establishment

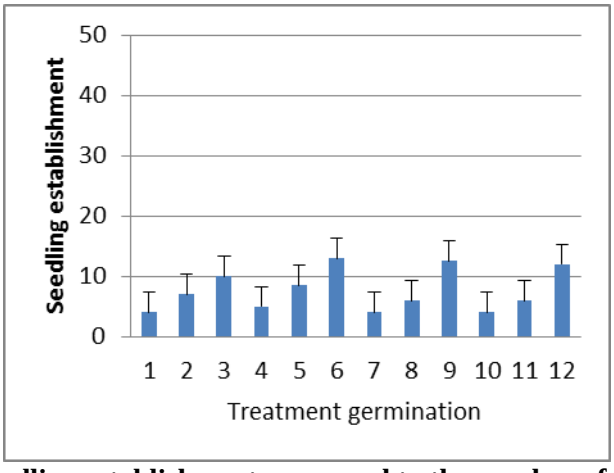

Figure 7: Seedling establishment compared to the number of treated seed

Discussion

The application of sulfuric acid in seed dormancy breaking treatments had different effect so that using sulfuric acid in some plants such as Cotoneaster nummularia Fisch, Albizia julibrissin Durazz had significant effect on germination percentage increasing (Nasir and Isvand, 2001), while in relation with some other species such as Ceratonia siliqua L., Ribes cereum Dougl had no significant effect on seed dormancy breaking (Nassiri and Isvand, 2001; Rosner *et al.*, 2003). In this experiment, as Nasiri (2008) also reported that, using sulfuric acid alone or combined with cold could break the seed dormancy of Maple: Turkmen Acer cineracens. On the other hand, the application of gibberellic acid given that leads to the loss of abscisic acid hormone could increase seed germination percentage (Gebre and Karam, 2004; Chvancern *et al.*, 2004; Prased *et al.*, 1996; Salisbury and Ross, 1992; Nasiri, 2008). However, the application of gibberellic acid without the effect of chilling leads to the seed germination of Maple: Acer cineracens that a similar result was reported by Dobrescu and Catrina (1984). Hormones such as gibberellic acid alter the pattern and structure of protein, which is helpful to break seed dormancy (Pawłowski 2007, 2009). Results of the seedling establishment test showed that chilling in addition to preparing germination stimuli and removing physiological barriers will increase resistance of seedling and contributes to its establishment and next growth; the action that gibberellin alone cannot do it. Therefore, gibberellin can be used as stimulating factor to aid in germination (Nassiri and Isvand, 2001). Moreover, cooling can increase the viability and germination speed (Bewley and Black, 1982). Note that in this study, the highest germination percentage was related to chilling treatment for five months, the physiological dormancy of fetus can be considered as reason (Phartyal *et al.*, 2003). On the other hand, given that the purpose of seed germination is healthy and strong seedling production, the application of chilling is the best way to germinate the Maple: Acer cineracens of Turkmen.

REFERENCES

Aygun C, Olgum M, Sever AL, Kara I, Erdogdu I and Atalay AK (2011). Evaluation of germinabilities of different shrubs by some methods, *Biological Diversity and Conservation* **4**(3) 52-57.

Bewley JD and Black M (1994). *Seeds – Physiology of Development and Germination* (Plenum Press) New York 445.

Bewley JD and Black M (1982). *Physiology and Biochemistry of Seed in Relation to Germination* (Springer –Verlag) New-York 375.

Blomme R and Degeyter L (1979). Problems in the Germination of *Acer campestre* (field maple), *Verbondsnieuws voor de Belgische Sierteelt* **23**(21) 849-852.

Chvancern D, Bochv W, Wangian C, Jingl J and Hvan J (2004). Effects of chemical and physical factors to improve the germination rate of *Echinocea angustifolia* L. seeds, *Colloids and Surfaces B: Biointerfaces* **37** 101-105.

Dobrescu Z and Catrina I (1984). The Nuclear Radiation Effects on Forest Seeds during Dormant State, *Buletin Informativ al Filialei Cluj-Napoca a ASAS* **22** 255-266.

Draghici C and Abrudan IV (2011). The effect of different stratification methods on the germination of *Acer platanoides* and *Acer campestre* seeds, *Bulletin of the Trancilvania Universiry of Brasov*, Series II: Forestry, Wood Industry, *Agricultural Food Engineering* **53**(1) 29-34.

Farhadi M, Sharifani M, Heydari HA and Kouharkhi AR (2006). The effect of seed hull and moist chilling on seed germination of Acer velutinum Boiss, *Agricultural Sciences and Natural Resources* **52**(2) 44-49.

Gebre GH and Karam NS (2004). Germination of Cercis siliquastrum seeds in response to gibberellic acid and stratification, *Seed Science and Technology* **32** 255-260.

Jensen M (2001). Temperature Relations of Germination in *Acer platanoides* L. Seeds, *Scandinavian Journal of Forest Research* **16**(5) 404-414.

Kabar K (1997). Comparison of reversal abscisic acid induced inhibition of seed germination and seedling growth of some *Greminae* and *Liliaceae* members by kinetin and giberellic acid, *Turkish Journal of Botany* **21** 203-210.

Kucera B, Cohn MA and Leubner-Metzger G (2005). Plant hormone interaction during seed dormancy release and germination, *Seed Science Research* **15** 281-307.
Lang GA (1985). Dormancy: A new universal terminology, *Hortcultural Science* **21** 704.
Latifi N (2001). Techniques of Seed Science and Technology, Agricultural Sciences and Natural Resources University of Gorgan, Gorgan 310.
Lee YH, Hong SH, Kang BH, Lee JK, Slim SL and Kim W (2006). Effect of stratification on the dormancy of Chinese milkvetch seeds (*Astragalus sinicus* L.). *Korean Journal Breed* **38**(4) 250-254.
Mozafariyan VA (2004). *Iranian Trees and Shrubs* (contemporary culture Publications) Tehran 991.
Nasiri M (2008). Determination of the optimal treatment to break dormancy and increase seed germination of Acer monospessulanum L, *Genetic Investigation and Modifying Pastures and Forests Plants* **16** 94-105.
Nasiri M, Babakhanlou P and Madah Arefi H (2003). The first report of dormancy breaking and seed germination of Diplotaenia damavandica Mozaffarian, Hedge and Lamond, *Genetic and Iranian Pasture and Forest Plants Modification* **11**(2) 256-275.
Nasiri M and Isvand HR (2001). Investigating the effect of sulfuric acid on the dormancy breaking and seeds germination of Albizia julibrissin Durazz and Ceratonia siliqua L., *Genetic and Iranian Pasture and Forest Plants Modification* **8**(1) 95-113.
Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y and Yamaguchi S (2003). Gibberellin biosynthesis and response during Arabidopsis seed germination, *The Plant Cell* **15** 1591-1604.
Pawłowski TA (2007). Proteomics of European beech (Fagus sylvatica L.) seed dormancy breaking: Influence of abscisic and gibberellic acids, *Proteomics* **7** 2246–2257.
Pawłowski TA (2009). Proteome analysis of Norway maple (Acer platanoides L.) seeds dormancy breaking and germination: influence of abscisic and gibberellic acids, *Biomed Central Plant Biology* **9**(48)1-13.
Phartyal SS, Thapliyal RC, Nayal JS and Joshi G (2003). Seed dormancy in Himalayan Maple (*Acer caesium*), II: Bioassay of inhibitors, *Seed Science and Technology* **31** 13-20.
Pinfield NJ, Stutchbury PA, Bazaid SA and Gwarazimb VEE (1990). Abscisic acid and the regulation of embryo dormancy in the genus Acer, *Tree Physiology* **6** 79-85.
Piotto B and Di Noi A (2003). Seed Propagation of Mediterranean, Trees and Shrubs. Agency for the Protection of the Environment and the Technical Services, APAT, Rome 125.
Prased JS, Kumar R, Mishra M, Kumer R, Singh AK and Prased US (1996). Characteristics of litchi seed germination, *Horticulture Science* **31** 1187-1189.
Rosner LS, Harrington JT, Dreesen DR and Murray L (2003). Sulfuric Acid Scarification of Wax Currant Seeds from New Mexico, *Native Plants* 65-71.
Rouhi HR, Tavakkol A and Shakosami K (2010). Seed treatments to overcome dormancy of waterlily tulip (*Tulipa kaufmanniana* Regel), *Australian Journal of Crop Science* **4**(9) 718-721.
Salisbury FB and Ross CW (1992). *Plant Physiology* (Wadsworth publishing company) Belmont 682.
Shock C, Feibert E and Saunders L (2009). Emergence of native plant seeds in response to seed pelleting, planting depth, scarification, and soil anti-crusting treatment, Malheur Experiment Station Oregon State University 5.
Song EC, Park KY and Cho YY (1990). Effects of temperature polyethylene glycol and sulfuric acid treatment on germination of Chinese milkvetch, *Korean Journal of Crop Science* **35** 248-253.
Tajbakhsh M (1996). *Seed Recognition - certification and Its Control* (Ehrar Tabriz Publications) 182.
Thomas H, Webb DP and Wareing PF (1973). Seed dormancy in Acer: maturation in relation to dormancy in *Acer pseudoplatanus*, *Journal of Experimental Botany* **24** 958-967.
Tissier J, Lambs L, Peltier JP and Marigo G (2004). Relationships between hydraulic traits and habitat preference for six *Acer* species occurring in the French Alps, *Annals of Forest Science* **61** 81–86.
Xia JH and Kermode AR (2000). Dormancy of yellow cedar (*Chamaecyparis nootkatensis* [D. Don] Spach) seeds is effectively terminated by treatment with 1-propanol or nitrate in combination with warm water soak, gibberellin and moist chilling. *Seed Science and Technology* **28** 227-240.

Yilmaz M (2007). Depth of dormancy and desiccation tolerance in *Acer traurvetteri* Medv. seeds, *Turkish Journal Agriculture and Forest* 201-205.