Volume 7, №4, 2007 ISSN 1311-9109

INTERNATIONAL
PLANT PROPAGATORS
SOCIETY
Region of Great Britain & Ireland

PROPAGATION OF ORNAMENTAL PLANTS

December

www.journal-pop.org

SEJANI
PUBLISHER
Sofia, Bulgaria

Propagation of Ornamental Plants
Vol. 7, № 4, 2007: 175-179

SEED GERMINATION OF THREE PROVENANCES OF *ARBUTUS ANDRACHNE* L. IN RESPONSE TO DIFFERENT PRETREATMENTS, TEMPERATURE AND LIGHT

Fahrettin Tilki* and Sinan Guner

Faculty of Forestry, Artvin Coruh University, Artvin-08000, Turkey,
*Fax: + 90-466-2126951, *E-mail: fahrettintilki@yahoo.com

Abstract

Seeds of *Arbutus andrachne* possess a physiological dormancy that prevents synchronized and rapid germination. The seeds were subjected to different pretreatments involving hot water, concentrated sulphuric acid, gibberellic acid and cold-moist stratification to break the dormancy. To determine the effects of temperature and light on seed germination they were treated with gibberellic acid and incubated at 20 and 24°C in light or dark. Germination ability of seeds from different sources was also compared to determine the effect of source environment. Compared with the control, sulphuric acid treatments were not effective in enhancing germination of *A. andrachne* seeds and hot water scarification was also not successful. When averaged over all provenances, cold moist-stratification at 4°C for 8 to 12 weeks or treatment of seeds with 200-800 mg l^{-1} gibberellic acid (GA_3) greatly increased germination percentage at 24°C in dark. Also, there was a remarkable effect of provenance on the germination percentage. Seeds treated with 400 mg l^{-1} GA_3 did not exhibit an obligate light requirement during germination, but germination rate was higher under light condition although temperature (20 or 24°C) did not affect germination performance.

Key words: eastern strawberry tree, GA_3, scarification, seed dormancy, stratification

INTRODUCTION

Arbutus andrachne L. (Eastern strawberry tree) is a bush or small tree reaching up to 5-6 m. This deciduous tree is a basic component of the maquis vegetation of coastal areas of the East Mediterranean (Kayacik 1982), and occurs also in Coruh river basin in the northeastern part of Turkey, Artvin. Since most stands of this species around Coruh river basin in Artvin, Turkey will be covered with the water of the dams, which are being constructed in this basin, its local gene pool is endangered. *A. andrachne* has potential use as a landscape and garden plant due to its ornamental features, which include twisted stems and branches, ornamental flowers, decorative fruits and a distinctive peeling reddish-brown bark (Karam and Al-Salem 2001).

Regeneration from seeds is the most often used and the cheapest method of propagation of many ornamental and forestry tree species. However, several germination inhibitors are present in the seed coats or embryos of dormant seeds of many species (Bewley and Black 1994, Bonner et al. 1994). The degree of dormancy may be expected to show some variation related to climate of origin, and seed dormancy can vary considerably among different clones, seed lots and among seeds within a seed lot (Edwards 1980, Leadem 1996). Stratification, scarification, gibberellins and other stimulators have a promotive effect on the germination of many species of angiosperms and gymnosperms (Bradbeer 1988, Bewley and Black 1994, Soyler and Khawar 2006, Tilki and Dirik 2007). Nevertheless, the effectiveness of these methods could vary from one species to another, pointing out the need for formulating species-specific treatments.

Arbutus species are considered to have dormant seeds, and therefore requiring special treatment before the germination (Roy 1974, Hartmann et al. 1990, Karam and Al-Salem 2001, Tilki 2004). Little work has been done on seed germination of *A. andrachne,* and studies yielded different results. Kose (1998) found that stratification for 2 months or treatment of seeds with 400-1000 mg l^{-1} were successful in breaking dormancy and stratification for 75 days reduced germination significantly when *A. andrachne* seeds were germinated at 20°C with 8 h light. According to Karam and Al-Salem (2001), stratification for 3 months or treatment with 250 or 500 mg l^{-1} GA_3 reduces seed

Received: June 26, 2007 *Accepted: September 21, 2007*

dormancy in *A. andrachne,* and 750 or 1000 mg l^{-1} GA_3 reduces germination percentage significantly at 20°C in dark. Thus, the aims of our research were to examine the effects of scarification, stratification, light and temperature on the germination of three provenances of *A. andrachne* seeds.

MATERIALS AND METHODS

Seed collection

Mature fruits of *A. andrachne* from Adana (S Turkey), Izmir (W Turkey), and Artvin (N Turkey) were collected from its natural habitat of three different provenances in October. Collected fruits were packed in plastic bags and transported to the laboratory where the study was undertaken. Fruits were soaked in water for two days before the seeds were extracted manually. They were washed and allowed to dry on filter paper at room temperature (20°C) for one day and stored at +4°C until use.

Sulphuric acid treatment

Seeds were treated with concentrated sulphuric acid (H_2SO_4, 96%) for 0, 5, 10 and 20 min at room temperature (20°C) and were stirred periodically. After acid scarification, seeds were thoroughly rinsed with distilled water for about 15 min and dried on paper toweling before placement in Petri dishes.

Hot water treatment

To test the effects of hot water on breaking coat-imposed dormancy, water was first heated up to 40, 60 and 80°C, and seeds in cloth bags were soaked in hot water for 0, 2, 4 and 6 min. The seeds were then dried on paper toweling and placed in Petri dishes.

Gibberellic acid treatment

In order to test the effect of gibberellic acid (GA_3) on seed germination, the seeds were immersed in five solutions of GA_3 (200, 400, 600, 800 and 1000 mg l^{-1}) at room temperature for 24 h.

Stratification treatments

Seeds were soaked in water for 24 h at 20°C and stratified in moist sand for 0, 4, 6, 8, 10 and 12 weeks at a constant temperature of 4 ± 1°C. After each stratification duration, the seeds were removed from the sand and placed into Petri dishes for germination.

Light and temperature treatments

Seeds treated with 400 mg l^{-1} GA_3 were germinated at 20 and 24°C in dark or 8-h light photoperiod provided by cool-white fluorescent lamps (20 µmol m^{-2} s^{-1}, appr.) to find the effects of light and temperature on seed germination percentage and germination rate.

Seed germination tests

The seeds were germinated in Petri dishes, 12 cm in diameter, on moist filter paper (with distilled water), and placed in germination chambers at a constant 24°C in dark (with the exception of light and temperature experiment). Seeds were monitored every day and moistened with distilled water if necessary. Germination counts were recorded every day for 40 days and seeds with 5 mm radicle were considered germinated (Karam and Al-Salem 2001). Germination percentage (GP%) was calculated each day and as the final value after 40 days. Mean germination time (MGT, day) was computed as follows: $MGT = \Sigma n_i d_i / n$ where n is the total number of germinated seeds during 40-day germination test, "n_i" is the number of germinated seeds on day "d_i" and "i" is the days during germination period (between 0 and 40 days) (Yousheng and Sziklai 1985).

Statistical analysis

All treatments were conducted in a completely randomized design using fifty seeds each in four replicates for all treatments. Data on percent germination were arcsin transformed to stabilize any heterogeneous variance (Zar 1984) and mean values were subjected to analysis of variance (ANOVA). Whenever significant differences were identified, Duncan's New Multiple Range Test was performed for the comparison of population and treatment means ($p \leq 0.05$). Results from the scarification, gibberellic acid, stratification and temperature/light treatments were analyzed separately.

RESULTS AND DISCUSSION

For different pretreatments, variability in germination response was significant. Also germination response varied significantly across the treatments (Tables 1-4).

Germination response for different scarification treatments revealed a significant difference (Table 1). In general scarification was not very successful in enhancing seed germination, which did not exceed 42.3% after water treatment at 40°C for 2 min., when all 3 provenances were averaged. The highest germination response was obtained in seeds treated with hot water, with maximum responses of a soaking duration of 2 or 4 min at 40°C in all provenances. With 60 and 80°C soaking treatments, germination decreased with soaking time, presumably reflecting the high temperature damage to the embryo with increasing soaking time. Soaking at 80°C for 2-6 min and acid scarification for 5-20 min were not as effective as the other scarification treatments. Hot water and concentrated sulphuric acid have been widely used to improve germination of several hard-seeded species (Msanga and Maghembe 1986, Tigabu and Oden 2001). In the present study, *A. andrachne* seeds showed poor response to sulphuric

Table 1. Effects of hot water and sulphuric acid scarification on seed germination (%) of *Arbutus andrachne*.

Treatment		Population			Treatment mean
		Artvin	Izmir	Adana	
Control		0	2	3	1.7 F
Hot water 40°C	2 min	43	39	45	42.3 A
	4 min	38	42	40	40.0 A
	6 min	35	29	34	33.7 B
Hot water 60°C	2 min	37	34	35	35.3 B
	4 min	31	30	35	32.0 B
	6 min	18	22	28	23.0 C
Hot water 80°C	2 min	9	10	15	11.3 D
	4 min	4	5	10	6.7 E
	6 min	3	2	5	3.3 F
Acid scarification	5 min	2	3	3	2.7 F
	10 min	4	8	7	6.3 E
	20 min	3	6	2	3.7 F
Population Mean		17.5 a	17.9 a	20.5 a	

Values in row followed by the same letter are not significantly different at $p \leq 0.05$.
Values in column followed by the same capital letter are not significantly different at $p \leq 0.05$.

acid treatments and hot water treatments, especially at 60 and 80°C. The decline in germination with further increase in temperature of water beyond 40°C might be due to the sensitivity of the seeds to higher temperature, which might have caused damage to the embryo. Gosling et al. (1995) and Tigabu and Oden (2001) stated that the poor performance of seeds after hot water exposure could be related to the thickness of the seed coat or soaking injury.

Germination response to GA_3 application was significantly higher than the control (Table 2). All GA_3 treatments improved germination by far better than scarification treatments. The highest mean germination (88%), averaged over three provenances, was achieved for seeds soaked in 400 mg l^{-1} GA_3. However, this was not a significantly higher germination response over 200, 600 and 800 mg l^{-1} GA_3 treatments. Mean germination percentage decreased as concentration was increased above 800 mg l^{-1}. Comparisons between provenances, across all GA_3 treatments, indicate a strong variability. On average for all GA_3 treatments, germination percentage was the lowest in Artvin provenance.

Gibberellins have a promotive effect on the germination of many species of angiosperms and gymnosperms (Groot and Karssen 1987, Bewley and Black 1994, Tigabu and Oden 2001). Karam and Al-Salem (2001) reported that the highest germination in one provenance of *A. andrachne* was obtained in seeds moistened with 10 ml of GA_3, 250 or 500 mg l^{-1} during germination at 24°C in dark (GP > 80%), and germination percentage decreased as concentration was increased above 500 mg l^{-1}, while Kose (1998) stated that *A. andrachne* seeds treated with 0.4, 0.6, 0.8 or 1.0 g l^{-1} for 24 h promoted over 90% germination under 20°C with 8 h light. In the present study, 200-800 mg l^{-1} GA_3

Table 2. Effect of different concentrations of GA_3 on seed germination (%) of *Arbutus andrachne*.

Treatment	Population			Treatment Mean
	Artvin	Izmir	Adana	
Control	1	4	3	2.7 A
200 mg l^{-1}	77	84	90	83.7 C
400 mg l^{-1}	82	93	89	88.0 C
600 mg l^{-1}	80	88	93	87.0 C
800 mg l^{-1}	76	85	88	83.0 C
1000 mg l^{-1}	64	72	83	73.0 B
Population Mean	63.3 a	71.0 b	74.3 b	

Values in row followed by the same letter are not significantly different at $p \leq 0.05$.
Values in column followed by the same capital letter are not significantly different at $p \leq 0.05$.

Table 3. Effects of cold stratification on seed germination (%) of *Arbutus andrachne*.

Provenance	Stratification duration (weeks)						Population mean
	0	4	6	8	10	12	
Artvin	2 a	39 b	49 c	69 d	84 e	87 e	55.0 A
Izmir	2 a	44 b	50 b	72 c	88 d	92 d	58.0 AB
Adana	4 a	40 b	59 c	91 d	95 d	93 d	63.7 B
Treatment Mean	2.7 a	41.0 b	52.7 c	77.3 d	89.0 e	90.7 e	

Values in the same row followed by the same lowercase letter are not significantly different at $p \leq 0.05$.
Values in column followed by the same uppercase letter are not significantly different at $p \leq 0.05$.

improved germination in *A. andrachne* seeds at 24°C in dark, and germination performance decreased when GA_3 concentration was above 800 mg l^{-1}.

Stratification greatly influenced germination percentage, and stratification requirements varied according to provenance (Table 3). On average for all provenances, germination percentages were significantly affected by 4-week cold-moist stratification. Furthermore, an increase in duration of stratification increased germination percentage, and stratification of seeds for 10 or 12 weeks resulted in the highest germination percentage (89.0 and 90.7%, respectively). This definite response to stratification is typical of dormant seed sources. Stratification treatments on *A. andrachne* revealed a significant variation in germination ability across provenances. The Adana provenance required only 8-week stratification to maximize germination percentage whereas the Izmir and Artvin provenances required more than 8 weeks.

Stratification requirements and seed germination can vary greatly among seed lots (Edwards and El-Kassaby 1996, Jull and Blazich 2000). Seeds generally have an inherent high genetic variability, which results in great heterogeneity in their behavior and, in particular, in their germinability following stratification procedures. Kose (1998) found that 2 months of stratification resulted in 100% germination but additional stratification (75 days) decreased germination percentages significantly (66%) in a provenance of *A. andrachne*. Karam and Al-Salem (2001) stated that 12 weeks were needed to break dormancy and to maximize germination percentage in *A. andrachne* seeds. In the present study, it was found that 8 or 10 weeks of stratification was needed to maximize germination percentage depending on the provenances.

For untreated seeds (control) mean germination time (MGT) was the highest (29 days), and a significant reduction in mean germination time (increase in germination rate) was achieved under GA_3 application (200-1000 mg l^{-1}) and stratification treatments (4-12 weeks). Mean germination time, combined over three provenances, was the lowest after 10 or 12 weeks of stratification and GA_3 applications (400 or 600 mg l^{-1}) (MGT~15 days).

The effects of light and temperature on GP% and MGT were evaluated and no significant effects of light and temperature were observed on GP% of the seeds treated with 400 mg l^{-1} GA_3, averaged over three provenances (Table 4). Generally, temperature and light requirements for seed germination vary among and within species (Phartyal et al. 2003, Escudero et al. 2002, Cicek and Tilki 2007). In the present study germination was faster under light condition at 20 or 24°C although temperature didn't significantly affect MGT. There was a remarkable direct effect of population on the germination percentage and germination rate, but population effects on GP% and MGT were not significant in interaction with light and with temperature. Karam and Al-Salem (2001) stated that *A. andrachne* seeds do not require light since KNO_3 and thiourea was not effective in enhancing germination. In the present study, however, the highest germination rate was seen under light.

The study reveals that *A. andrachne* seeds have a physiological dormancy, which may be due to insufficient development of the embryo, presence of chemical inhibition, or failure of chemical reactions as stated by Karam and Al-Salem (2001). Since chemical scarification with sulfuric acid or hot water scarification does not overcome the seed dormancy in the present study, we

Table 4. Effects of temperature and photoperiod on seed germination (%) and mean germination time (days) of *Arbutus andrachne* seed treated with 400 mg l^{-1} GA_3 combined over three provenances (MGT in parenthesis).

Temperature (°C)	Photoperiod (h)	
	0	8
20	86 (15.7 b)	90 (13.1 a)
24	87 (14.8 b)	89 (12.4 a)

Values in the same row followed by the same letter are not significantly different at $p \leq 0.05$.

can state that the seed is not hard coated. Physiological dormancy in the seeds has been broken with equal success by stratification (8-10 weeks) or GA_3 application (200-800 mg l^{-1}). But the duration of stratification requirement necessary to maximize seed germination varies according to the provenances. For these three sources of *A. andrachne* seeds treated with 400 mg l^{-1} GA_3, light impacted significantly the germination rate, rather than germination percentage although temperature (20 or 24°C) did not affect germination rate and germination percentage.

REFERENCES

Bewley J. D., Black M. (1994). Seeds: Physiology of development and germination. Plenum Press, New York, 445 pp.

Bonner F. T., Vozzo J. A., Elam W. W., Land S. B. (1994). Tree seed technology training course. USDA Forest Service GTR-SO-106. New Orleans, LA, 160 pp.

Bradbeer J. W. (1988). Seed dormancy and germination. Blackie and Son Ltd. London, 146 pp.

Cicek E., Tilki F. (2007). Seed germination of three *Ulmus* species from Turkey as influenced by temperature and light. Journal of Environmental Biology, 28: 423-425.

Edwards D. G. W. (1980). Maturity and quality of tree seeds – a state-of-the-art review. Seed Science and Technology, 8: 625-657.

Edwards D. G. W., El-Kassaby Y. A. (1996). The effect of stratification and artificial light on the germination of mountain hemlock seeds. Seed Science and Technology, 24: 225-235.

Escudero A., Perez-Garcia F., Luzuriaga A. L. (2002). Effects of light, temperature and population variability on the germination of seven Spanish pines. Seed Science Research, 12: 261-271.

Gosling P. G., Samuel Y. K., Jones S. K. (1995). A systematic examination of germination temperature, chipping and water temperature/soak duration treatments on the seeds of *Leucaena leucocephala*. Seed Science and Technology, 23: 521-532.

Groot S. P. C., Karssen C. M. (1987). Gibberellins regulate seed germination in tomato by endosperm weakening: A study with gibberellin-deficient mutant. Planta, 171: 525-531.

Hartmann H. T., Kester D. E., Davies F. T. (1990). Plant propagation: Principles and practices. Fifth edition, Prentice Hall, USA, 647 pp.

Jull L. G., Blazich F. A. (2000). Seed germination of selected provenances of Atlantic white-cedar as influenced by stratification, temperature, and light. HortScience, 35: 132-135.

Karam N. S., Al-Salem M. M. (2001). Breaking dormancy in *Arbutus andrachne* L. seeds by stratification and gibberellic acid. Seed Science and Technology, 29: 51-56.

Kayacik H. (1982). The systematic of trees. I.U. Yayin No: 3013. Istanbul, 333 pp. (In Turkish).

Kose H. (1998). Studies on the germination of some woody ornamental plants existing in Turkish flora. Anadolu Journal of AARI, 8: 55-65. (In Turkish).

Leadem C. (1996). A guide to the biology and use of forest tree seeds. Land Management Handbook 30. Province of British Columbia. Victoria, BC, Canada, 21 pp.

Msanga H. P., Maghembe J. A. (1986). Effect of hot water and chemical treatments on the germination of *A. schimperiana* seeds. Forest Ecology and Management, 17: 137-146.

Phartyal S. S., Thapliyal R. C., Nayal J. S., Rawat M. M. S., Joshi G. (2003). The influences of temperatures on seed germination rate in Himalayan elm (*Ulmus wallichiana*). Seed Science and Technology, 31: 83-93.

Roy D. F. (1974). *Arbutus menziesii* Pursh. *In*: Schopmeyer C. S. (Eds.). Seeds of Woody Plants in the United States. Agricultural Handbook 450. USDA Forest Service, Washington, DC: 226-227.

Soyler D., Khawar K. M. (2006). Effects of prechilling, scarification, incubation temperature, photoperiod, KNO_3 and GA_3 treatments on germination of Caper (*Capparis ovata* Desf. var. *palaestina* Zoh.) seeds. Propagation of Ornamental Plants, 6: 159-164.

Tigabu M., Oden P. C. (2001). Effect of scarification, gibberellic acid and temperature on seed germination of two multipurpose *Albizzia* species from Ethiopia. Seed Science and Technology, 29: 11-20.

Tilki F. (2004). Improvement in seed germination of *Arbutus unedo* L. Pakistan Journal of Biological Sciences, 7: 1640-1642.

Tilki F., Dirik H. (2007). Seed germination of three provenances of *Pinus brutia* (Ten.) as influenced by stratification, temperature and water stress. Journal of Environmental Biology, 28: 133-136.

Yousheng C., Sziklai O. (1985). Preliminary study on the germination of *Toona sinensis* (A. Juss.) Roem. seed from eleven Chinese provenances. Forest Ecology and Management, 10: 269-281.

Zar J. H. (1984). Biostatistical analysis. Prentice Hall, Englewood Cliffs. NJ, 718 pp.