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# Breaking Seed Dormancy of Strawberry Tree (*Arbutus unedo*)

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## ABSTRACT

This study was conducted to break dormancy and enhancing germination of *Arbutus unedo* seeds. With this aim, seeds prior to sowings were treated as follows: stratification of the seeds at 4°C for 15, 30, 60 and 90 days, soaking in 50 and 100 mg per 100 mL Polystimulin (PS) or indole butyric acid (IBA) for 48 h. Results indicated that the highest germination rate, which was 92%, was obtained by stratification at 4°C for 60 days treatment and soaking the seeds in 50 mg per 100 mL PS for 48 h treatment. The stratification temperature of 9ºC was more effective than 4ºC. Breaking dormancy for *A. unedo* seeds should be used to stratification for 60 days and application to PS hormone at lower doses. © 2010 Friends Science Publishers

**Key Words:** *Arbutus unedo*; Germination; Hormone treatment; Ornamental plant

## INTRODUCTION

The Strawberry tree (*Arbutus unedo* L.) is one of 12 species, which belongs to the genus *Arbutus* of Ericaceae family. In generally, it is native to Lebanon, Greece, Southern Europe and Anatolia (Anşin & Özkan, 1993; Karadeniz & Şişman, 2003). It is used in several domains; the strawberry tree produces the forage of good energizing value, leaves and fruits can be used in the pharmaceutical industry (Hammami *et al.,* 2005). Also *A. unedo* is an ornamental bush; its beauty resides in the mixture of its foliage green obstinate brightness with its white flowers and its red fruits decorating the bush all along of year. *A. unedo* distribution ranges from an altitude of 700 to 1000 m. The tree can grow on alkaline and relatively acidic soils (pH 5–7.2) (Çelikel *et al*., 2008).

Propagation of the strawberry tree is difficult by seed. Seed included in their fruit have a very low rate of germination not passing 4.2%, caused by the presence of certain inhibitory substances diffused by the fruit (Hammami *et al*., 2005). The seeds require pretreatment to overcome dormancy. Dormant seeds can be stimulated to germinate using treatments that emulate natural conditions or satisfy certain physiological requirements. Stratification leaching, scarification, light and, plant growth regulators [especially gibberellic acid ($GA_3$) & cytokinin] are effective dormancy releasing treatments (Bradbeer, 1988; Bonner *et al.,* 1994; Nowag, 1998; Rahman *et al*., 2006). Several germination stimulators have been used to improve the seed germination, e.g., $GA_3$ (Dhankhar *et al.,* 1996; Vijaya *et al.,* 1996; Rahman *et al*., 2006; Soyler & Khawar, 2007), benzyladenin (Shafi *et al.,* 1991), polystimulins (Kırdar & Ertekin, 2001). The role of $GA_3$ in promoting seed germination has been described by several authors (e.g., Lewak, 1985; Karssen, 1995). The promoting effect of $GA_3$ treatment is often attributed to the mobilization of stored reserves (Bewley & Black, 1994; Soyler & Khawar, 2007) and acceleration of the disappearance of abscisic acid (ABA)-regulated polypeptides, which are abundant in dormant seeds (Nicolas *et al.,* 1997).

In developed countries, the synthesis of new phytoactive compounds that control or regulate plant growth or protect plants against environmental stress is highly advanced. Analogues to auxin or cytokinin that have high biological activity have been synthesized. The synthetic high-molecular weight plant growth regulators polystimulin-A6 (PS-A6), which is similar to auxin and polystimulin-K (PS-K), which is similar to cytokinin, have various effects on plant growth and development. PS-A6 and PS-K have high biological activity, similar to 2, 4-dichlorphenoxyacetic acid and 6-benzylaminopurine, respectively and were defined as plant growth regulators by Tsatsakis *et al.* (1993). Polystimulins resemble compounds found in plants and promote growth, photosynthesis and enzyme activity (Allahverdiev, 1988; Tsatsakis *et al.,* 1993). Kırdar and Ertekin (2001) state that, when using together, PS-K and PS-A6 hormones have a greater effect than that one on germinating and PSA6+ PS-K with 100 ppm concentration gave the best result on seed germination in *Magnolia grandiflora.* Stratification of *Arbutus unedo* seeds at 4ºC for 9 or 12 weeks or treatment of seeds with $GA_3$ successfully overcomes dormancy (Tilki, 2004). Thus, our objectives were to investigate: (1) the effects of cold-moist stratification duration and temperatures on germination and (2) the effects of plant growth regulators on breaking seed dormancy.

## MATERIALS AND METHODS

**Seed sources:** *Arbutus unedo* seeds were collected from Bartın, Turkey in the fall of 2008. Fruits were soaked in water before seeds were extracted by hand. Seeds were stored at approximately 4-6ºC in a cool-room prior to treatment in February 2009. Seed fill was determined by floatation-filled seeds sunk, while empty seed floated. The number of filled seeds was counted after floating test was performed.

**Hormone treatment:** The polystimulin (PS) and indole butyric acid (IBA) were used to break dormancy and improve seed germination. PS-A6 and PS-K solutions (using 25 & 50 mg) were prepared separately and dissolved in 5-6 drops of alcohol, and 100 mL of distilled water was added. IBA was used to 50 or 100 mg. Filled seeds were immersed in the solution (50 or 100 mg 100 $mL^{-1}$) for 48 h at room temperature (approx. 20ºC) before cold-moist stratification.

**Cold-moist stratification:** After hormonal treatment for 48 h, seeds were stratified in moistened sand in a cold room or warm room at 4, 9 or 20±0.5ºC for 15, 30, 60, 90 days in February 2009. A total of 300 seeds were not stratified, but soaked in water for 48 h as a control. No controls with any stratification conducted, because of seed dormancy. Following were the stratification treatments:

a. Stratification at 4ºC
   Stratification for 15 days
   Stratification for 30 days
   Stratification for 60 days
   PS (50 mg 100 $mL^{-1}$)+Stratification for 60 days
   PS (100 mg 100 $mL^{-1}$)+Stratification for 60 days
   IBA (50 mg 100 $mL^{-1}$)+Stratification for 60 days
   IBA (100 mg 100 $mL^{-1}$)+Stratification for 60 days
   Stratification for 90 days
b. Stratification at 9ºC (stratification for 60 days)
c. Different Stratifications Temperatures (stratification at 4ºC for 30 days later stratification at 20ºC for 30 days)
d. Nonstratified (Soaked in water for 48 h)

**Germination test:** After stratification, seeds were separated from the moistened sand, watered and exposed to a gentle air stream at room temperature (approx. 20ºC). Seeds were germinated in 12 cm Petri dishes with two layers of filter paper moistened with distilled water. Petri dishes were placed in a plant growth chamber (MMM Clima Cell) at 18-24°C variable temperature, under 16 h photoperiod. Every 1-2 days, germination was checked and water was added as needed during the period of germination test.

**Statistical analyses:** All experiments were based on a completely randomized design with three replicates and 100 seeds per replicate. Seeds showing radicle emergence were recorded as germinated and removed from Petri dishes. Percentage data was arcsine transformed before analysis (Zar, 1996). To determine the significant differences in the treatments, transformed means were subjected to multifactor analysis of variance using the software package SPSS and the least significant differences for all pairs were compared at $P<0.01$ using Duncan's multiple range test.

## RESULTS

ANOVA results showed that the germination percentage (GP) was significantly affected by cold moist stratification temperature, duration and hormone application ($P<0.001$). There were significant differences among stratification date in GP. Only 5% of non-treated seeds germinated and stratification of 15 days significantly increased germination. Stratification for 90 days (pretreatment 8) was more effective than others (Table I). Also the GP was affected by stratification temperatures (Table II).

The stratification temperature of 9ºC was more effective than 4ºC or 4 to 20ºC. An increase in stratification temperature from 4 to 9ºC increased the GP from 72 to 84%. Germination response of seeds with hormone application kept under stratification for different durations and stratification temperatures (Table III).

All hormone treatments improved to germination. Stratification for 60 days with 50 mg per 100 mL PS application for 48 h (pretreatment 4) was successful in breaking dormancy resulting in 92% germination. In additional, 50 mg per 100 mL IBA application effected dormancy and obtained high GP (86%). However concentration of hormone effected to GP negatively. High PS and IBA doses reduced GP (73% & 75%, respectively). This shows that PS application increased the germination from 72% to 92% (Table III) and which lover doses had a large effect on enhancing *A. unedo* seed germination. When combined with stratification, PS application at 50 mg per 100 mL was successful in breaking dormancy and enhancing germination values resulting in 92% germination.

## DISCUSSION

Ceccherini *et al*. (1998) noted that it is common nursery practice to chill imbibed seeds of a wide range of species at 1-10ºC to initiate the release from dormancy. Huxley *et al.* (1992) informed that *Arbutus* seeds require four to six weeks of stratifications, while Tilki (2004) reported that *A. unedo* seeds required 9 weeks. In present study, cold stratification for 60 days was sufficient but stratification temperature must be 9ºC. Our results for *A. unedo* seed germination could be successfully incorporated into commercial nursery operations if the management methods currently used for this species are changed.

PS is biologically active compound and meets the water and food needs of the seeds at normal level by activating the metabolism of *A. unedo* seeds. It also was the most effective in *Magnolia grandiflora* and *Abies nordmanniana* (Kırdar & Ertekin, 2001, 2008).

**Table I: Effect of stratification (at 4 ± 0.5ºC) time on seed germination**

| Stratification days | GP% |
|---|---|
| 0 | 5 e[1] |
| 15 | 30 d |
| 30 | 53 c |
| 60 | 72 b |
| 90 | 85 a |

[1] Numbers not followed same letters significantly different at 1% level. Duncan criterion

**Table II: Influence of stratification temperatures on seed germination for 60 days**

| Stratification temperature | GP% |
|---|---|
| 4 ± 0.5ºC | 72 b[1] |
| 9 ± 0.5ºC | 84 a |
| 4 ± 0.5ºC + 20 ± 0.5ºC | 55 c |

[1] Numbers not followed same letters significantly different at 1% level. Duncan criterion

**Table III: Influence of hormone treatment on seed germination for 60 days**

| Hormone treatment | GP % |
|---|---|
| Control | 72 c[1] |
| PS (50 mg 100 $mL^{-1}$) | 92 a |
| PS (100 mg 100 $mL^{-1}$) | 73 c |
| IBA (50 mg 100 $mL^{-1}$) | 86 b |
| IBA (100 mg 100 $mL^{-1}$) | 75 c |

[1] Numbers not followed same letters significantly different at 1% level. Duncan criterion

Concentration of hormone application had negative effective on GP in the present study. This reason is accordance with results of *A. andrachne* (Karam *et al*., 2001). Hormones not only are use for getting deficiency of hormones in the seeds but breaking dormancy, accelerating the disappearance of abscisic acid (ABA)-regulated polypeptides also, which are abundant in dormant seeds (Nicolas *et al.,* 1997). PS enhanced the germination of *A. unedo* seeds, as reported previously for same and other species with $GA_3$ treatment (Lewak, 1985; Bewley & Black, 1994; Karssen, 1995; Dhankhar *et al.,* 1996; Vijaya *et al.,* 1996; Kırdar, 2002). According to Tilki (2004), the best germination percentage for *A. unedo* is 84% at 300 mg $L^{-1}$ $GA_3$, whereas in the present study the highest germination was 92% at 50 mg per 100 mL PS hormone.

The dormancy breaking treatment is especially important in early spring and longer pretreatment, especially at lower temperatures, results in higher germination rates (Kolotelo, 1998). However if the pretreatment time is prolonged and sowing is delayed, the seeds are more likely to suffer from fungal attack (Edwards, 1982; Leadem, 1986; Tanaka & Edwards, 1986). In present study, it's determined that PS treatment reduced stratification time. In conclusion, stratification for 60 days combined with 50 mg per 100 mL PS hormone can successfully breaking dormancy in *A. unedo*.

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