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The Effect of Some Treatments on Breaking Seed Dormancy and Seed Germination in *Vaccinium arctostaphylos* L. (*Caucasian whortleberry*)

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Abstract

Caucasian whortleberry (*Vaccinium arctostaphylos* L.) is a useful medicinal plant with many seeded berries; the limits of germination rate and long dormancy of the seeds are as major barriers to the optimal use of this plant. To evaluation of some treatments on breaking seed dormancy and increasing germination rate, the experiment carried out using factorial design with three replications in research lab in Faculty of Agriculture in 2013. Treatments were including gibberellic acid, Nitrate potassium, heating, wet and dry chilling each with two levels and transferred to germinator 15 °C and 25 °C (day/night) and 12 hours light. Seeds were counted every two days within one month. The results revealed the highest germination rate were belong to gibberellic acid (500 ppm), potassium nitrate 1% (8 hr) and wet chilling (15 days) while heating had no significant effect on germination. The seeds have physiological dormancy in which could be removed by some treatments.

1. Introduction

Caucasian whortleberry (*Vaccinium arctostaphylos* L.) is a shrub, belonging to Eriaceae family, which grows at maximum to up the height of 2.5 m. The fruit is rich source of anthocyanins and is useful for short-term treatment of cholesterol, fat and blood sugar (Kianbakht *et al*., 2013), and has properties such as anti-diarrheal characteristics, stabilizing the free radicals, protection of cells against chemical damages, including environmental pesticides and protecting the liver against a variety of stresses (Milburi *et al*., 2007; Mirheidar., 1996). The limits of germination rate and long dormancy of the seeds are major barriers to the optimal use of these plants outside their natural habitats (Bradbeer., 1988). Therefore, the researchers are trying to find appropriate ways to break the dormancy and increase the percentage and germination rate of the seeds (Hadi *et al*., 2012, Matus-Cadiz & Hucl., 2005). Sometimes, chilling treatment alone or in combination with other treatments, such as gibberellin is used to break dormancy or increase the seed germination (Nadiafi *et al*., 2006). Cooling (4 °C) leads to increased expression of GA3OXL gene (producing enzyme of the active form of gibberellin) in the radicle and aleurone layer (Yamauchi *et al*., 2004, Chiovecha *et al*., 2005). Thomas and Sambrooks (1985) showed that the gibberellins in celery seeds change the levels of other hormones as well as some of the flow of some ions such as k+ and Ca2+ through the membranes, and such changes lead to specific signals transduction and stimulation of synthesis or metabolites activity and seed germination stimulating enzymes. Kay *et al*., (1988) and Emery (1988) stated that in the *Salvia dorrii*, the dormancy or incubation period disappears with primary drying after ripening and cooling or placing the seed for one hour in the gibberellin at a concentration of 100-500 ppm. Review of sources indicates that chilling leads to reduced amounts of abscisic acid and increased gibberellin quantities of the seeds and stimulates germination in many seeds needing chilling such as hazelnut and Sycamore maple (Bryant., 1996). Ghasemi Pirbaloti *et al.* (2008) in studies on dormancy breaking of pharmaceutical species of *Thymus daenensis* and *Pimpinella anisum* concluded that treatment with potassium nitrate 0.2% and 500 ppm gibberellic acid have significant positive effects on breaking the dormancy of these plants seeds. Matos and Hucl (2005) reported the usefulness of seeds treatment of *Phalaris canariensis* 73 through heating. The natural habitats climate of Caucasian whortleberry provides its cold need, and prompts its fruiting in normal conditions. Testing the germination conditions showed that the

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Caucasian whortleberry seeds after undergoing the cold periods would be capable of germination only in the optical alternation conditions, and the seeds cannot germinate in absolute darkness. Thus, the seed has positive photoblastic reaction (Sedaghathoor *et al*., 2006). Unfortunately, in recent years, the use of improper exploitation methods by local and native people and overgrazing before the seed germination of the plant has led to destruction of its natural areas. Obviously, preservation and protection of natural resources requires that these endangering areas will be renewed. Restoration of natural areas of the plant requires adequate knowledge about the physiology of seed germination and breaking the seeds dormancy. Given the importance of Caucasian whortleberry due to its use as a medicinal plant, this experiment was performed to domesticate this plant.

2. Material and Methods

The whortleberry fruit was collected from one of its natural habitats (Talesh highlands, Shagerdkoh, Asalem, located at an altitude of 1600 meters above sea level and at 37 ° 35 ´ N latitude and 48 ° 42´ longitude. Based on the recommendations by Griffin and Blazich (2002), to extract the seeds, the fruits were first placed in a container with water, and then were completely soaked with being pressed for a short time. After this stage, more water was added so that the pomace is floated in the water, and healthy seeds are deposited. This process may be repeated several times to properly separate the pomace from the seeds. The pomace residue was separated from water and the precipitated seeds. Then, using a suitable method (e.g., the use of a sieve with small holes or using filter paper), the seeds were separated from water and some fine debris; then, they were surface sterilized with 1% sodium hypochlorite and washed several times with distilled water (Sauer and Burroughs., 1986; Griffin & Blazich, 2002). For breaking the dormancy of seeds, different physical, chemical and pre-chilling methods were used:

2.1. Chilling Treatments

2.1.1. Wet chilling

In this experiment, seeds were randomly selected and placed in six sterile petri dishes containing Whatman filter paper and moistened with distilled water (Auld *et al*., 1988). Three petri dishes containing seeds and the other three petri dishes were placed in the refrigerator at temperature of 4 °C for 15 days and 30 days, respectively. During this period, the seeds were monitored, and distilled water was added again to them in case of reduced water content. At the end of treatments, the petri dishes were placed in the germinator with a temperature of 25/15 °C day/night and at dark / light 12/12 h.

2.1.2. Dry Chilling

In this experiment, 150 seeds were randomly selected and separately transferred into 6 sterile petri dishes containing filter paper as 25 seeds per dish. Three petri dishes containing seeds for 15 days and 3 other petri dishes for 30 days were placed in the refrigerator at 4 °C. At the end of treatments, the petri dishes were transferred to a germinator.

2.2. Gibberellic Acid Treatment

The seeds were immersed for 4 hours in gibberellin 1000 and 500 ppm, and then were transported into the germinator.

2.3. Potassium Nitrate Treatment

The seeds were immersed in potassium nitrate 1% and 4%, each for 4 and 8 h and then were transferred to the germinator.

2.4. Heating Treatment

The seeds were placed in the oven at 70 ° C for 12 and 24 hours, and then were transferred into the germinator.

Table 1 ANOVA of treatments on *Vaccinium arctostaphylos* seed germination

Sources of Variation	Df	Mean Square					
		Gibberellic Acid			Heat		
		Max seed germ.	Evenness seed germ.	Seed germ. rate	Max seed germ.	Evenness seed germ.	Seed germ. rate
Treatment	2	220.11**	309.13**	0.029 ^{ns}	4.11^{ns}	151.25	0.03^{ns}
Error	6	3	10.92	0.006	1.77	32.11	0.006
CV (%)	-	15.13	20.14	55.7	42.85	50.72	57.6
Sources of Variation	-	Potassium Nitrate			Chilling		
Treatment	4	88.76**	173.98^{ns}	0.017^{ns}	19.73 *	124.59**	0.021**
Error	10	3.06	67.06	0.012	4.46	9.33	0.003

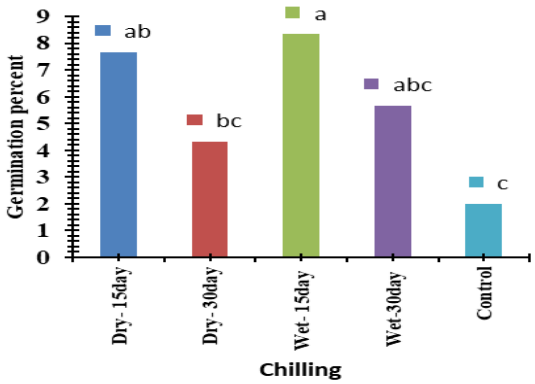

Fig. 1 The effect of chilling on seed germination percentage of *V. arctostaphylos*

2.5. Control Treatment

The examined seeds were placed in a sterile petri dish containing filter paper without any special treatment and were transferred to the germinator.

3. Results

The effects of different treatments of dormancy breaking on germination percentage and rate of cranberry seeds are shown in Table 1. Since the treatments used in this experiment are varied, to avoid the complexity of the issue, their impacts on dormancy breaking and germination of Caucasian whortleberry seed are individually examined and discussed. As mentioned in the introduction, this plant is a useful medicinal plant that its growth is limited to some specific natural habitats and is not cultivated; unfortunately, little research has been done on its dormancy; thus, we compared the results of this research with other studies performed on seeds of some other herbs.

3.1. Chilling

The seeds chilling in two dry and wet levels for 15 and 30 days at 4 °C increased the seeds germination respectively as (53% - 73%) and (64% - 76%) that the increase was significant compared with the controls (Fig. 1). In applying chilling factor, the effect of moist chilling for 15 days had a greater impact on germination percentage. This treatment showed no significant differences between dry chilling for 15 days and moist chilling for 30 days. Chilling had no impact on germination rate, but the most uniformity of germination in chilling factor was related to the dry chilling for 15 days, which had no significant difference with dried chilling treatment for 30 days.

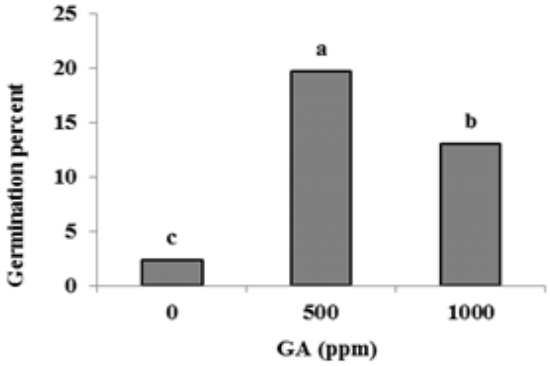

Fig. 2 The effect of Gibberellic acid concentration on *V. arctostaphylos* seed germination Percentage

3.2. Gibberellic Acid

The seeds germination rate under the influence of applying 500 and 1000 ppm gibberellic acid was as 88% and 82%, respectively, which had a significant increase compared with the controls (Fig. 2). Also, among all the treatments used, the highest percentage and uniformity of germination was observed in 500 ppm gibberellic acid treatment, while this acid had no effect on seed germination rate. As can be seen, with increasing concentrations of gibberellic acid from 500 to 1000 ppm, the germination percentage has decreased.

4.3. Potassium Nitrate

The analysis of variance results on potassium nitrate treatment showed that the highest germination percentage occurs at 0.5 grams of potassium nitrate treatment for 8 hours, and then 0.5 mg for 4 hours. However, this factor had no impact on the rate and uniformity of germination of the plant seeds, and increased amount of potassium nitrate (2 g) had no significant effect on the percentage, rate and uniformity of the seeds (Fig. 3).

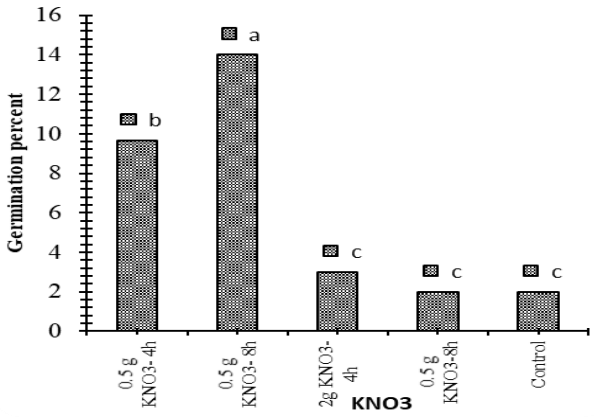

Fig. 3 The effect of Potassium Nitrate on *V. arctostaphylos* seed germination percentage

3.4. Heating Treatment

Heating did not affect the speed and uniformity of germination. As a result, this factor cannot serve as a key factor for breaking the dormancy of this plant. Lack of effect of heat treatment indicates that the dormancy of these seeds cannot be as a result of hardness and impenetrability of the seeds shell.

4. Discussion

4.1. Chilling

Powell (1987) reported that the chilling processes increase the production of some growth stimulating substances (such as gibberellin). Amoaghayee (2007) in a similar experiment on Ferula assa-foetida seed stated that chilling was effective on seeds dormancy breaking, and on average, increased the germination percentage from 4.25% in not- chilled seeds to 42.75% in seeds chilled for 11 weeks. Baskin *et al*. (1995) and Waleck *et al*. (2002) mentioned in their reports that the Erythronium and Osmorhiza species from Apiaceae family have some degree of physiological dormancy, which can be broken by applying the appropriate chilling treatment periods. They believe that such a need to be chilled is related to the seed dispersal ecology. Given that cranberry seeds experience relatively pretty cool winters, it can be assumed that the physiological dormancy of cranberry seeds broken with chilling treatment have been formed as an ecological adaptation in seeds of this plant. Increased seed germination induced by cold pretreatment is caused by seed sheath due to the cold, which reduces the mechanical strength of seed shell on the embryo. Also, it is likely that in addition to endogenous gibberellic acid synthesis, the cold factor activates other stimuli leading to increased rate and percentage of seeds germination. It seems that cold treatment reduces the levels of inhibiting hormones and increases the stimulating hormones levels, and thus, can increase the potential for seed germination. These events occur simultaneously, and germination in seeds is the result of a balance between the hormones (Tipirdamaz and Gomorgan, 2000).

4.2. Gibberellic Acid

One reason for the positive effect of chemical stimuli such as gibberellic acid on germination may 166 be related to hormonal equilibrium ratio in seeds and reduced growth-inhibiting substances such as abscisic acid (Yamauchi *et al*. 2004). In other words, gibberellic acid as a chemical stimulus can break the seed physiological dormancy. Gibberellic acid, through inducing alpha-amylase enzyme synthesis triggers the germination, and thus the seed dormancy breakage. Nabaei *et al*. (2012) in an experiment on seed dormancy breaking of Rhubarb (*Rheum ribes* L.) observed that the highest germination percentage (65%) occurs at a concentration of 500 ppm gibberellic acid that with increasing concentration of hormone up to 1000 ppm, the germination percentage decreased (49%). Also, increasing concentrations of gibberellic acid from 400 to 800 ppm reduced the seed germination percentage of *Xanthium strumarium* L. (Majni *et al*., 2011). Research has shown that the seeds of many plants require light to break the seed dormancy, and the ability to react to the light is associated with absorption of water by the seeds (Villiers, 1978). The seeds are divided into three categories regarding the germination response to the light: Positive photoblastic (requiring light), negative photoblastic (no need to light) and non-photoblastic (indifferent to light) (Copland and McDonald, 1995). According Sedaghathoor *et al*. (2008) studies, the cranberry seeds are among the seeds with positive photoblastic reaction. GA can replace the light requirements of many photoblastic seeds (such as lettuce and tobacco) (Kochaki and Sarmadnia, 2001) that studies conducted on the effects of gibberellic acid leading to broken dormancy confirms such a conclusion.

4.3. Potassium Nitrate

Chemical conditions in the environment of the seed are a crucial factor to prevent or stimulate the germination. The nitrate ion is the most important soil chemical that increase the activity of seed germination in many plant species (Mousavi and Ahmadi, 2009). One reason for the positive effect of chemical stimuli such as potassium nitrate on seed germination of plant species may be related to hormonal equilibrium ratio in seed and reduced growth inhibitors such as abscisic acid. These chemical stimuli will help break the seed physiological dormancy (Farhadi *et al*., 2007). Mousavi (2009) stated that the Physalis seed germination was significantly affected by potassium nitrate treatment. According to Jain and Singh study (1989), the Scoparia dulcis seed germination increased due to potassium nitrate treatment. It was also observed that potassium nitrate caused the breaking of Setaria parviflora seeds dormancy (Federico and Mulard, 2009). Mahmoud Zadeh *et al*. (2003) suggested that potassium nitrate treatment has not been effective on dormancy breakage of yellow alfalfa (*Melilotus officinalis*) seeds. In general, response to potassium nitrate has been attributed to the sensitivity of plants' seeds (Dreaks and Carson, 1993). The positive effects of potassium nitrate on seed germination in plants, including Achillea (Sharifi *et al*., 2003), Amaranthus (Mahmoodzadeh *et al*., 2003),

Medicago scutellata (Shabani *et al*., 2003) and Anison (Ghasemi Pirbalotti *et al*., 2008) have been proved.

5. Conclusion

In conclusion, this study indicated the highest germination rate were belong to gibberellic acid (500 ppm), potassium nitrate 1% (8 hr) and wet chilling (15 days), while heating had no significant effect on germination. Some treatments can remove physiological dormancy in seeds.

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