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Effect of Bottom Heating, Germination Medium and Gibberellic Acid Treatments on Germination of Isabella (*Vitis labrusca* L.) Grape Seeds

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Abstract: Seeds collected from naturally grown Isabella grape cultivar (*Vitis labrusca* L.) were treated with 0, 500, 1000 and 1500 ppm GA_3, sown into perlite + silt (2:1), peat moss and soil mixture (1:1:1–sand : manure : soil) in the beds maintained at 25°C, 30°C and 35°C bottom heating with control (no bottom heating). The first germination occurred on 17^{th} day after sowing of the seeds treated with 0 and 500 ppm GA_3 and sown into the peat moss having a bottom heating temperature of 35°C. Germination ended within 35 days. Bottom heating caused to germinate the Isabella grape seeds earlier than the control application but did not affect the final germination rate. Finally, bottom heating had no positive effect on seed germination while perlite + silt (53.21%) and 1500 ppm GA_3 treatments (51.72%) promoted the germination of Isabella grape seeds.

Key words: Grape, seed, heating, gibberellic acid, medium, germination

Introduction

Because of the great variation of grape seedlings, seed can not be relied upon to produce a vineyard of the desired variety. Commercial varieties of grapes are propagated by cuttings and grafting and sometimes by budding and layering. Seedlings derived from the same vine strongly differ from each other due to the heterozygotous genetic structure originated from foreign fertilization of grapes (Celik, 1998). Besides this, F1 population gained from cross pollination had less values for vigour, growing, fruitfulness, fruit and wine quality than parents. Therefore, sexual propagation of grapes has no practical value in modern viticulture (Celik *et al.*,1998). However seeds obtained from combination breeding programme are too important propagation materials to produce new grape varieties for special purposes (Winkler *et al.*, 1974; Altintoprak and Agaoglu, 1999). For genetic resources conservation, it is necessary to be able to remove dormancy to ensure that the genetic heterogeneity of an accession is maintained for use by breeders and to avoid confounding dormancy with non-viability in germination tests designed to monitor the viability of accessions in long-term storage (Ellis *et al.*, 1983). Pandey and Singh (1988) stated that germination of grape seeds also differ to berry maturation and temperature. Maeda *et al.* (1986) concluded that grape seeds intended for purposes of selection, if collected from ripe berries, may be stored for a year without serious deterioration. According to Celik *et al.* (1998), grape seeds easily germinate when stratified under low temperatures (±5°C) and dumpy conditions for several months in a proper medium. A favourable temperature is another important requirement for grape seed germination. It not only affects the germination percentage but also the rate of germination (Manivel and Weaver, 1974; Hartmann *et al.*, 1990). Ellis *et al.* (1983) showed that a temperature of 25°C is the best constant temperature for rapid and full germination. The importance of maintaining proper medium temperature to achieve maximum germination percentages cannot be over-emphasized. Exposure of grape seeds to low temperature for long periods is another successful method in breaking dormancy (Celik *et al.*, 1998). Various temperatures and treatment periods have been used by many workers previously. One of those reported as successful is 8 weeks at 5°C (Chohan and Dhillon, 1976). Satisfactorily germination of grape seeds usually occurs only after a period of ripening at about 18°C followed by a cold stratification treatment at about 5°C (Considine, 1983). Various plant growth regulators and chemicals such as gibberellic acid, thiourea, indoleacetic acid, kinetin, ethephon and cyanamid were found to be effective for substituting completely or partially the conditions needed for improving seed germination (Pal *et al.*, 1976; Forlani and Coppola, 1978; Ellis *et al.*, 1983; Spiegel-Roy *et al.*, 1988; Pandey and Singh, 1988; Yeh *et al.*, 1990; Toledo-Gaytan *et al.*, 1992; Bordelon and Moore, 1994; Wilde and Darne, 1996; Ergenoglu *et al.*, 1997; Herrera *et al.*, 1998; Chuanli and Jing, 1999). Selim *et al.* (1981) stated that soaking the seeds in IAA, IBA or Ether at different concentrations before sowing failed completely to produce normal seedling and the best results with respect to germination and normal seedlings were obtained with 5000 ppm GA_3 application. Moreover there was little evidence to suggest that the combination of treatment in GA_3 and a short prechill promotes the grape seed germination sufficiently. The best combined treatment of Kachru *et al.* (1972) was the treatment of 2000 ppm GA_3 for 48 h followed by prechill at 5°C for 30d. An artificial, soilless mix also provides the desired qualities of a good germination medium. The basic ingredients of such a mix are sphagnum peat moss and vermiculite, both of which are generally free of diseases, weed seeds, and insects (Celik *et al.*, 1998; Ming *et al.*, 1998).

The objective of this study was to evaluate the effects of bottom heating, medium and GA_3 application on the germination of recently-harvested Isabella (*Vitis labrusca* L.) grape seeds.

Materials and Methods

The required seeds of Isabella grape (*Vitis labrusca* L.) were obtained from vines grown naturally at the Black Sea Coastal Region around the Samsun Province in 1999. The seeds were extracted by hand, cleaned and immersed in 2% solution of sodium hypochlorite for 5 min to avoid micro organism infection then washed with sterile distilled water for several times. The seeds were air-dried until they reached a level of 15% humidity dry base and were stored in 0.17 mm-thick polyethylene bags.

In the experiment, seeds were kept in cold conditions (5±1°C) for 8 weeks as indicated by Chohan and Dhillon (1976) then immersed in 500, 1000 and 1500 ppm GA_3 solutions for 24 h. The seeds for control application (0 ppm) were immersed into distilled water for the same duration. For each GA_3 treatment, 1800 seeds were soaked in twice their volume of solution. After GA_3 application, seeds were washed with sterile distilled water. They were sown in peat moss, perlite+silt (2:1) and soil mixture (sand : manure : soil, 1:1:1) in 10 m long and 1 m wide trays maintained at 25°C, 30°C and 35°C constant bottom heating temperature. The trays were placed on an iron-frame, 1 m above the ground under greenhouse conditions. The Holland Box with no bottom heating and settled on iron frame at one meter height from ground under greenhouse were used as control. The constant temperature was tested as bottom heating in the medium controlled by thermostatically active base units during day and night. The Previcur 607 SL having 722 g pure Promacarb-HCl per litre was used as fungicide. It was applied to the medium every day by adding to irrigation water until the end of experiment. The inside temperatures of the greenhouse were also measured after seed sowing. Seedling emergence percent was recorded at a day intervals when the first germination took place.

The Randomized Complete Block Design for the factors was used as experimental model with three replicates. Each replicate had 50 seeds. Totally 7200 seeds were sown. Data collected from the application as percentage were transformed using the arcsin$\sqrt{x}$ transformation and statistical analyses were applied over these transformed data using MSTAT-C pocket programme (Russell D. Freed, Crop and Soil Sciences Department, Michigan State University). The Duncan's Multiple Range test was used to indicate the differences among average data.

Results

The data represent the effect of bottom heating, germination medium and GA_3 applications on the germination rate of Isabella (*Vitis labrusca* L.) seeds are given in Table 1. It can be seen that the earliest germination appeared from the seeds sown in peat moss, with 500 ppm GA_3 application and control at 35°C bottom heating. This was followed by the seeds sown in perlite+silt medium having 25°C and 35°C bottom heating with 1000 ppm GA_3 application. While peat moss hastened germination, soil and perlite+silt mixture delayed it. There were 17 days between seed sowing and first germination in the combination of peat moss and 0, 500 ppm GA_3 at 35°C. Whereas this duration took 29 days to the seeds sown in soil mixture with the application of 1000 ppm and 1500 ppm GA_3 (control and 25°C bottom heating) and perlite+silt mixture with all GA_3 applications at 30°C bottom heating. In all combinations, the germination rate showed a stable increase until 5th May and then it showed a sharp increase. This stable period took 39 days after sowing. Concerning the effect of combined treatments, seeds treated with 500 ppm GA_3, sown into perlite+silt without bottom heating gave the highest germination rate (88.33%) (Table 1).

The germination rate obtained from average data for bottom heating, medium and GA_3 application are given in Table 2. Germination rate of Isabella grape seeds did not require a bottom heating. Because the control application gave the highest germination rate (59.33%) and there were significant differences between control and bottom heating temperatures. However, within the temperatures, bottom heating application at 35°C followed the control with a germination rate of 49.50% . On the other hand, gibberellic acid, especially for 1500 ppm, gave the best results with respect to germination percentage (51.72%) although all the concentrations of GA_3 showed to be in the same group statistically and did not appear to be more significant than others. There was a considerable variation in the medium. As shown in Table 2, perlite+silt mixture seed bed gave the highest germination rate (53.21%) and was followed by peat moss (48.75%). The germination first began in the seeds sown in the trays having a bottom heating temperature of 35°C (Fig. 1). This was followed by 25°C and control applications. There was a sharp increase in germination rate in all trays on 25.04.2000 that took 13 days after first germination appeared. This increment was also shown on the date of 05.05.2000 that took 25 days after first germination. According to the medium, the first germination appeared in the peat moss and was followed by perlite+silt and soil mixture. Seeds sown in peat moss began to germinate 17 days after sowing although perlite+silt and soil mixture delayed the germination that appeared 18 and 21 days after seed sowing, respectively. In all medium, the germination rate showed a rapid increase at 13 and 25 days after the first germination appeared (Fig.2). The effect of GA_3 doses on the earliness of germination of Isabella seeds can not be out looked since the first germination began at the control and 500 ppm GA_3 application. These were followed by 1000 and 1500 ppm GA_3 applications. The germination showed to

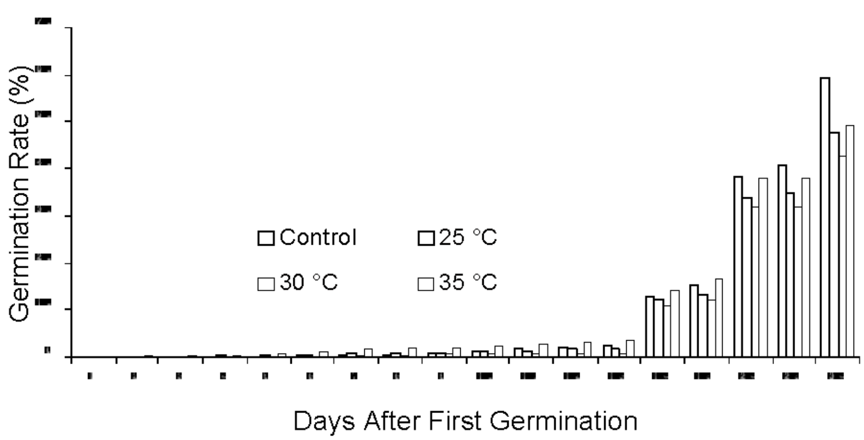

Fig. 1: The variation of the germination rate of isabella grape seed according to the bottom heating temperature

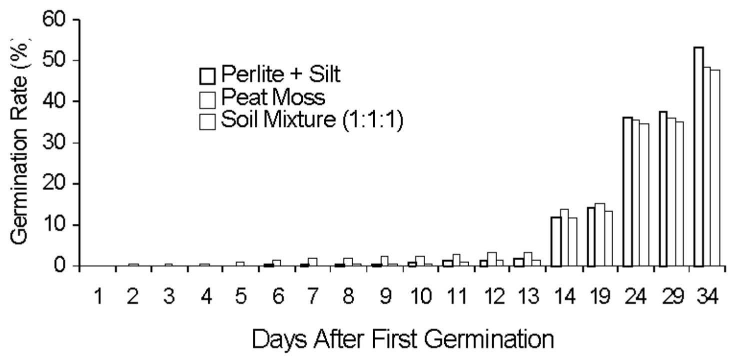

Fig. 2: The variation of the germination rate of Isabella grape seed according to the medium

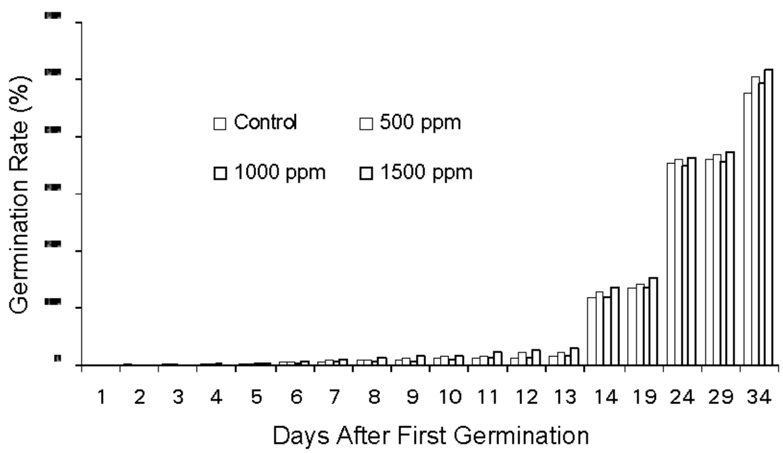

Fig. 3: The variation of the germination rate of Isabella grape seed according to the gibberellic acid treatments

be affected by the same progress as in bottom heating and medium application (Fig. 3).

Discussion

Dormancy breaking applications are too important to overcome the deep dormancy of grape seeds thus breeding programs could be ended more effectively and earlier. There are several reports in literature which demonstrate that the use of prechill under cool conditions, various chemicals and diurnal or constant temperatures for breaking the grape seed dormancy (Pal *et al.*, 1976; Forlani and Coppola, 1978; Ellis *et al.*, 1983; Spiegel-Roy *et al.*, 1988; Pandey and Singh, 1988; Yeh *et al.*, 1990; Toledo-Gaytan *et al.*, 1992; Bordelon and Moore, 1994; Wilde and Darne, 1996; Ergenoglu *et al.*, 1997; Herrera *et al.*, 1998; Chuanli and Jing, 1999). But none of them represents the interactive effect of temperature-medium and GA_3 applications. This experiment identified that all applications stimulating different levels of Isabella (*Vitis labrusca* L.) grape seeds germination. Here are the best results obtained from combination treatments of control (no bottom heating), perlite+silt and 500 ppm GA_3 (Table 1). Although Cupidi and Barba (1991) reported the maximum germination of Isabella grape seed under *In vitro* conditions (64%), the three-factor treatment as mentioned above gave 83.33% germination rate. There are no cumulative results in literature by the application of GA_3 for overcoming the seed dormancy. However, gibberellins are indispensable for germination and dormancy release treatments can induce either their synthesis or a change in their compartmentation or a change in sensitivity of the tissue. According to Yeh *et al.* (1990) treatments of Golden Muscat seeds with 250, 500 or 1000 ppm GA_3 had no effect on seed germination. Conversely Selim *et al.* (1981) found that the high concentration of GA_3 (e.g. 5000 ppm) promoted the grape seed germination. Toledo-Gaytan *et al.* (1992) stated that the most viable method for propagation of wild grape is sexual propagation and the germination rate is just 54% and gibberellic acid treatment did not increase this rate. Bordelon and Moore (1994) affirmed these findings by stating that plant growth regulators had little effect on seed germination. However our findings showed that the 1500 ppm GA_3 application promoted the Isabella seed germination up to 51.72% although GA_3 doses were classified into the same group, statistically. Ergenoglu *et al.* (1997) stated that 1000 or 2000 ppm GA_3 application increased germination percentage and shortened the germination period as compared to control. This may be

Table 1: The effect of bottom heating, medium and gibberellic acid treatments on Isabella grape seed germination rate (five day intervals)

Bottom Heating (°C)	Medium	GA_3 (ppm)	Germination Rate (%)						
			12.04.2000	20.04.2000	25.04.2000	30.04.2000	5.5.2000	10.5.2000	15.5.2000
Control	Perlite +	0	-	-	10.67	14.00	38.00	44.00	58.00 c*
	Silt	500	-	-	12.00	17.33	44.00	48.00	83.33 a
		1000	-	-	12.67	16.00	43.33	47.33	77.33 ab
		1500	-	1.33	15.33	17.33	41.33	45.33	72.67 b
	Peat moss	0	-	2.00	12.67	14.67	37.33	38.67	53.33 cde
		500	-	2.00	15.33	17.33	38.00	42.00	54.00 cde
		1000	-	2.00	16.67	18.67	38.67	38.67	53.33 cde
		1500	-	2.00	16.00	18.00	40.00	41.33	58.00 c
	Soil	0	-	1.33	12.00	14.00	38.67	39.33	54.67 cd
	mixture	500	-	-	13.33	14.00	36.00	37.33	51.33 cdefgh
		1000	-	-	10.00	11.33	35.33	35.33	52.00 cdefg
		1500	-	-	10.00	10.67	31.33	31.33	44.00 fghijk
25	Perlite	0	-	-	12.00	13.33	35.33	35.33	46.67 defghijk
		500	-	0.67	14.67	17.33	37.33	39.33	52.67 cdef
		1000	-	0.67	10.67	12.67	33.33	34.67	47.33 defghijk
		1500	-	0.67	10.00	11.33	32.00	33.33	48.67 defghijk
	Peat	0	-	2.00	12.67	12.67	32.67	32.67	42.67 hijk
		500	-	1.33	12.67	13.33	33.33	33.33	43.33 ghijk
		1000	-	1.33	11.33	11.33	31.33	32.67	44.67 efghijk
		1500	-	3.33	15.33	17.33	37.33	40.67	52.00 cdefg
	Soil	0	-	0.67	11.33	14.00	34.67	36.00	50.67 cdefghi
	mixture	500	-	-	12.00	12.00	34.00	34.00	50.67 cdefghi
		1000	-	-	10.00	10.00	30.00	30.00	44.00 fghijk
		1500	-	0.67	11.33	13.33	36.00	36.00	52.00 cdefg
30	Perlite	0	-	-	10.00	10.00	30.00	30.00	40.67 k
		500	-	-	10.00	10.00	30.67	30.67	40.67 k
		1000	-	-	10.00	10.67	30.67	30.67	40.67 k
		1500	-	-	10.00	10.67	30.67	30.67	43.33 ghijk
	Peat	0	-	1.33	12.00	12.00	30.67	30.67	40.67 k
		500	-	1.33	11.33	11.33	31.33	31.33	42.00 ijk
		1000	-	-	10.67	11.33	31.33	31.33	42.67 hijk
		1500	-	1.33	12.00	12.00	32.00	32.67	44.67 efghijk
	Soil	0	-	0.67	10.67	11.33	31.33	31.33	41.33 jk
	mixture	500	-	0.67	10.67	12.00	32.00	32.00	42.00 ijk
		1000	-	-	11.33	13.33	34.67	34.67	44.67 efghijk
		1500	-	2.67	14.67	18.67	38.67	38.67	50.67 cdefghi
35	Perlite	0	-	0.67	12.00	16.00	38.67	38.67	48.67 defghijk
		500	-	2.67	15.33	17.33	37.33	37.33	47.33 defghijk
		1000	-	2.00	14.00	16.67	37.33	37.33	50.00 cdefghij
		1500	-	2.67	14.67	20.00	40.67	40.67	53.33 cde
	Peat	0	0.67	4.00	14.67	18.00	38.67	38.67	50.00 cdefghij
		500	0.67	4.67	14.67	16.67	40.67	40.67	54.00 cd
		1000	-	4.00	16.67	19.33	40.00	40.00	52.00 cdefg
		1500	-	2.67	16.67	17.33	37.33	37.33	52.67 cdef
	Soil	0	-	0.67	12.67	14.00	36.00	36.00	46.00 defghijk
	mixture	500	-	0.67	12.67	14.67	37.33	37.33	47.33 defghijk
		1000	-	-	10.67	11.33	33.33	34.00	44.00 fghijk
		1500	-	2.00	16.67	18.00	38.00	38.00	48.67 defghijk

* There are no significant differences (LSD %1 = 4,307) between the data having the same letter given into the last column.

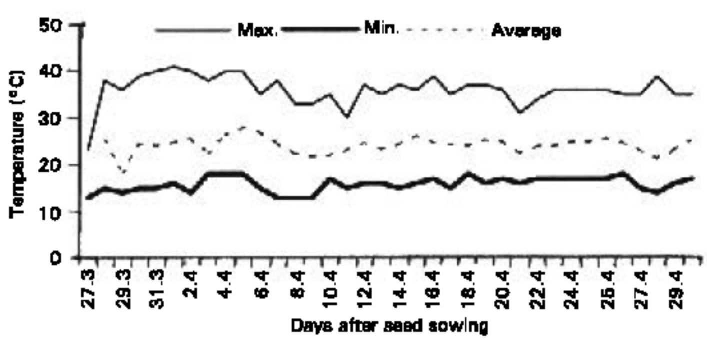

Fig. 4: The greenhouse inner temperature (max. min. and average) changing after seed sowing

Table 2: The average germination rate of Isabella grape seeds according to the treatment

Treatments		Germination rate (%)
Bottom heating (°C)		
	Control	59.33 a*
	25	47.94 b
	30	42.83 c
	35	49.50 b
	$LSD_{1\%}$ =	1.243
Medium		
	Perlite + Silt	3.21 a*
	Peat Moss	8.75 b
	Soil mixture	47.75 b
	$LSD_{1\%}$ =	1.243
GA_3(ppm)		
	0	47.78 b*
	500	50.72 a
	1000	49.39 ab
	1500	51.72 a
	$LSD_{1\%}$ =	1.243

*There are no significant differences between the data having the same letter given into the column, separately.

the cultivar effect. The experimental findings from the present study showed that all GA_3 applications had the same effect on Isabella seed germination as statistically but 1500 ppm GA_3 gave the highest germination rate (51.72%) (Table 2). The constant temperatures as bottom heating started the germination more earlier but they did not effect the rate of germination. The control application (no bottom heating) gave the highest germination rate (59.33%). This may be the result of higher inner temperatures of the greenhouse during Isabella grape seed germination tests (Fig. 4). Much of the work on overcoming seed dormancy in *Vitis* spp. apparently used uncontrolled environments for the subsequent germination tests. But the temperature of the germination regime influences the expression of seed dormancy quite considerably in a wide range of species (Ellis *et al.*, 1983). We found that the best temperature for Isabella grape seed germination is 35°C followed by unheated trays. Elis *et al.* (1983) reported that 25°C constant temperature is the best for rapid germination. But 25°C and 35°C bottom heating treatments had no statistical difference as shown in Table 2. We also found that raising the bottom heating temperature with the use of light weight and pore materials like peat moss affected Isabella grape seed germination earlier than soil including medium. There are significant differences between the medium. While the first germination appeared in the peat moss, perlite+silt gave the highest germination rate (53.21%). These results affirm the findings of Celik *et al.* (1998) and Ming *et al.* (1998). They emphasized that the grape seed germination could vary according to medium. Germination rates showed a sharp increase within 13 and 25 days after seed sowing in all factors used to promote the Isabella grape seed germination (Fig. 1, 2 and 3).

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