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Temperature Responses of Pollen Germination in Walnut (*Juglans regia* L.)

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ABSTRACT

This study was carried out in order to determine the effects of different temperatures (16±1 °C, 19±1 °C and 27±1 °C) with various sucrose concentrations (10, 15 or 20% sucrose) on the pollen germination of 'Şebin', 'Kaplan 86', 'Yalova 3', 'Pedro', 'Hartley' and 'Franquette' walnut cultivars in 2007 and 2008. Pollens were also subjected to tetrazolium (TTC) test to determine their viability level. Temperature had significant effect on the germination percentage. Pollen germination rates increased significantly with increasing temperatures in walnut cultivars studied. The highest germination rates were recorded at 27 ±1 ˚C temperature in both years (26.94-73.98%; 22.78-70.86%), respectively. The rate of pollen viability of all the cultivars tested was high (> 75%). The highest pollen germination percentage was obtained from 15% and 20% sucrose concentrations in both years.

Key Words: ***Juglans regia*, pollen viability, pollen germination, temperature.**

INTRODUCTION

Juglans regia L. (Persian walnut) is a monoecious, wind-pollinated and, apparently, self-compatible species. Despite of its self-compatible nature, breeding and research programs encounter difficulties having sufficient quantities of desired pollen at the time pistillate flowers receptivity because of the dichogamous nature of the species which has protandrous or protogynous mating types (Luza and Polito 1985). Because of the life span of walnut pollen appears to be short under natural conditions its handling requires care with temperature, relative humidity and maturity, as each of these factors can affect viability (Luza and Polito 1985). Presumably, the effects noted involved factors associated with rates of pollen rehydration. Fanner and Barnett (1974) found that variation in germinability was broad for both fresh and stored pollen of black walnut (*Juglans nigra*). Todd and Brethcrick (1942) reported that water content of 3.91% for pollen of this species; this was the lowest value of 36 collections of pollen from different species that they examined. For this reason walnut pollens lost their viability soon after leaving their teka tissues. Also, pollen of walnut (*Juglans* spp.) has been considered difficult to germinate *in vitro* (Griggs et al. 1971).

Usually, pollen germination is negatively affected by high or low ambient temperatures (Westwood 1978). The effects of temperature on pollen germination and pollen tube growth have been reported by several workers in various fruit species (Cerovic and Ruzic 1992; Egea et al. 1992; Garcia and Egea 1979; Godini et al. 1987; Luza et al. 1987; Mellenthin et al. 1972). Pırlak (2002) investigated different temperatures (5,10,15 and 20 °C) on pollen germination and pollen tube growth rate of different apricot and sweet cherry cultivars and observed that pollen germination was low at 5 °C and optimum germination at 15 and 20 °C. Luza et al. (1987) observed that no germination of pollen from any of the *J. regia* or *J. nigra* cultivars occurred below 14 °C. At 14 to 15 °C only pollen from early blooming varieties (Serr, Manregian, and Early Ehrhardt) germinated with 16.4%, 2.2% and 2.1%, respectively. In addition, maximum germination obtained at 28 °C in *J. regia* and 32 °C in *J. nigra.* In almond (*Prunus dulcis*) Griggs and Iwakiri (1975) observed pollen germination at temperatures between 4.4 and 10 °C. Vachun (1981) reported that optimum temperature required for pollen germination in apricot was about 15 °C, and that temperatures above 25 °C caused a decrease in pollen germination rates. Egea et al. (1992) observed that pollen germination and tube growth speed was low at 5 °C for apricot varieties and the germination rate increased with increasing temperature. In cherimoya, Rosell et al. (1999) found 20-25 °C to be optimum for pollen germination, while germination decreased at 30 and 35 °C; and at 10 °C, only 1.8% of the pollen grains germinated. In mango, optimum temperature was found to be 15-25 °C, while at 10 and 30 °C pollen germination was reduced and that 25 °C to be optimum for *in vivo* incubation (Sukhvibul et al. 2000).

Temperature requirement for the pollen germination and pollen tube growth in fruit species and cultivars may differ in early or late flowering period. For example, the desired temperature for optimum pollen germination of walnut was lower in early flowering cultivars than late flowering walnut counterparts (Luza et al. 1987). A somewhat similar pattern of temperature responses by pollen was noted by Weinbaum et al. (1984) in a comparison of almond (*Prunus dulcis*) and peach (*P. persica*), two closely related species that

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differ in bloom time. Pollens from the earlier blooming almond had higher germination percentages at low temperatures and a lower temperature for maximum germination was needed.

The objective of this study was to determine the effects of different temperatures on pollen germination rate of different walnut cultivars. Effects of various sucrose concentrations on the pollen germination and pollen viability rates with Tetrazolium (TTC) test were also determined.

MATERIALS AND METHODS

Studies were carried out on pollen grains of six cultivars of walnut 'Şebin', 'Kaplan 86', 'Yalova 3', 'Pedro', 'Hartley' and 'Franquette'. Pollens collected during the spring of 2007 and 2008 were used in these experiments.

Pollen viability and germination tests in vitro

Samples of pollens were collected in the morning between 8:00 and 9:00 a.m. Pollens were collected at the time of the first staminate flowers of the catkins had begun to shed their pollens. Catkins were brought into the laboratory and laid on clean black paper. Staminate catkins were kept under the laboratory conditions to shed their pollens for 1-2 hours and then the experiment was soon established before the pollens lost their viability.

The germination tests were conducted by hanging drop method with 10, 15 or 20% sucrose. The concentrations of sucrose were determined by a previous research (Sütyemez 2007). To determine the effect of temperature on germination, all of the treatments (10, 15 or 20% sucrose) were kept 16 ±1 °C 19 ±1 °C and 27±1 °C for 24 h under dark conditions. Data were obtained from three slides with three fields each. The percentage of pollen germination was determined under a light microscope. A pollen grain was considered to be germinated when the length of pollen tube was equal to or exceeded its diameter.

The viability of the pollen was determined on 1% 2,3,5-triphenyl tetrazolium chloride (TTC). One or two drops of TTC solution was put on a clean microslide and pollen grains were sprinkled on these drops with a brush. Then, the drop was carefully covered by a cover glass without trapping air and kept for 4 h at ambient conditions. For this assay, two lamella for each cultivars and three regions of each lamella were investigated; viable, semi-viable and dead pollen numbers and their percentages were determined. Viable pollen was dyed in red, semi-viable pollen dyed in light red-pink and dead pollen not dyed at all. Examples of viable, semi-viable and dead pollens are presented in Figure 1.

Statistical analysis

The data were analysed using MSTAT-C statistical software, and means were compared using Duncan's Multiple Range Test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Pollen viability

Pollen viability rates of the cultivars tested with TTC is presented in the Table 1. Within each year, no significant differences were found among the pollen viability rates of the walnut cultivars except viable pollen rate in 2007 year. Viable, semi-viable and dead pollen as shown in Figure 1. If we evaluate the viable and semi viable pollens together with the viability range varied between 75.67-87.33% in 2007 and 77.99-89.36% in 2008. Therefore, regarding pollen viability, all the walnut cultivars can be considered as good pollinizers. Sütyemez (2007) reported that the pollen viability rates of 32 different walnut cultivars varied between 81% and 94. In another study pollen viability ratio of selected 19 walnut cultivars varied between 77-92% (Sütyemez and Eti, 2006). In general, in some previous studies, similar results for pollen viability were reported in hazelnut (49-97%) by Beyhan and Odabas (1995), in chestnut (8.8-35.8%) by Beyhan and Serdar (2009), in apricot (76-86%), sweet cherry (67-81%), and sour cherry (71%) by Bolat and Pırlak (1999). Our results are in agreement with the previous studies in various species.

Pollen germination

Effects of different temperatures on pollen germination at various sucrose concentrations are presented in the Table 2.

Effects of sucrose concentrations on the pollen germination percentages was different but the differences were non-significant among the cultivars (Table 2, and Figs. 2,3,4,5,6 and 7). Generally, the best pollen germination rates were obtained from 15% and %20 sucrose concentrations (Table 2). However, in both 2007 and 2008, 'Pedro' with 10% sucrose concentration had the best germination rates (Table 2 and Figure 6). Sütyemez (2007) found that a 15% sucrose concentration gave the highest germination rates for walnut cultivars. Other researchers also noticed that, 15% and 20% sucrose concentration gave the highest germination rates in various fruit species (Eti 1991; Pırlak and Güleryüz 1997; Sütyemez and Eti 2006; Mert and Soylu 2007; Asma 2008).

Table 1. Percentage of pollen viability of walnut cultivars in 2007 and 2008 (%).

Cultivars	2007			2008		
	Viable	Semi-viable	Dead	Viable	Semi-viable	Dead
Şebin	38.49 c[z]	41.36[y]	20.14[y]	45.46[y]	43.90[y]	10.63[y]
Yalova 3	45.00 abc	36.37	18.61	28.06	49.93	21.99
Kaplan	40.18 bc	35.49	24.32	35.67	47.22	17.10
Hartley	56.94 a	26.42	16.62	48.018	31.48	20.49
Pedro	53.71 a	27.55	18.72	43.62	35.25	21.12
Frenquette	51.77 ab	35.56	12.66	50.02	33.10	16.87

[z] Mean separation in columns by Duncan's multiple range test at $P \leq 0.05$ lowercase letters.

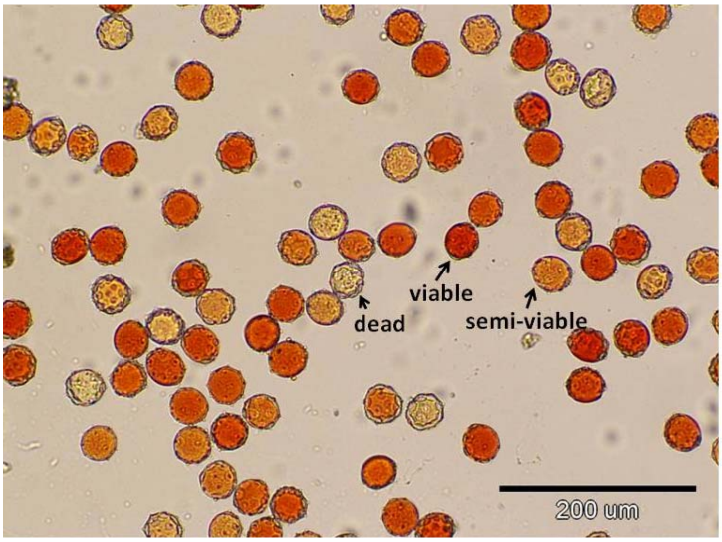

Figure 1. Views of viable, semi-viable and dead pollen grains in 'Şebin' walnut cultivars. Pollens stained with TTC and photographed using light microscopy.

Temperature significantly affected *in vitro* pollen germination of the walnut cultivars studied in both years. Pollen germination rates increased significantly with increasing temperatures in all cultivars (Table 2, Figures 2,3,4,5,6,7). The pollen germination occurred at 16±1 °C but was lower. The pollen germination rates at this temperature were 1.97-8.29% in 2007 and 2.71-8.92% in 2008 (Table 2). The pollen germination rates increased with temperatures up to 19±1°C. The pollen germination rates at this temperature were 11.73-46.26% in 2007 and 10.20-39.25% in 2008 (Table 2). Maximum germination rates obtained from 27±1 °C in all cultivars studied in both years (26.94-73.98%; 22.78-70.86%) (Table 2, Figures 2,3,4,5,6,7). ***These results*** indicate that the optimum temperature for pollen grain germination appeared to be 27±1 °C. Luza et al. (1987) indicated that no germination of pollen from any of the *J. regia* or *J. nigra* cultivars occurred below 14 °C. At 14 to 15 °C only pollen from early blooming varieties ('Serr', 'Manregian', and 'Early Ehrhardt') germinated. Pollens of the later-blooming *J. regia* cultivars, 'Idaho', 'Chico', 'Sharkey', 'Amigo', 'S. Franquetta', and 'Meylan' did not germinate at temperatures below 16 to 18 °C and germination percentages ranging from 1.4 to 6.2% in that temperature range. They found that maximum germination occurred at 28 °C in *J. regia* and 32 °C in *J. nigra.* Similarly, in our study, we obtained maximum germination at 27±1 °C in both years.

Table 2. Effects of different temperatures and sucrose concentrations on the rate of pollen germination (%) of walnut cultivars in 2007 and 2008 year.

Cultivars	Sucrose concentrations	Pollen germination rates (%)					
		2007			2008		
		Temperatures °C			Temperatures °C		
		16±1	19±1	27±1	16±1	19±1	27±1
Şebin	10%	2.58 b	23.89 abc	40.07 cd	3.19 a	24.30 ab	40.95 ab
	15%	3.13 ab	25.01 abc	39.97 cd	6.05 a	26.04 ab	44.65 ab
	20%	2.23 b	24.09 abc	51.37 abcd	6.36 a	25.95 ab	41.84 ab
Yalova 3	10%	5.08 b	16.91 bc	26.94 d	5.34 a	15.03 ab	22.78 b
	15%	3.4 ab	20.08 abc	55.39 abcd	5.02 a	17.97 ab	63.55 ab
	20%	5.53 ab	24.13 abc	61.85 abc	7.89 a	18.41 ab	64.39 ab
Kaplan 86	10%	1.97 b	14.02 bc	41.96 bcd	3.34 a	22.07 ab	35.76 ab
	15%	2.85 b	46.26 a	40.41 bcd	8.92 a	31.08 ab	40.71 ab
	20%	3.00 ab	21.05 abc	63.87 abc	6.44 a	26.69 ab	39.81 ab
Hartley	10%	2.85 b	11.73 c	64.27 abc	5.54 a	10.20 b	57.74 ab
	15%	6.79 ab	25.70 abc	71.76 a	6.23 a	25.70 ab	70.86 a
	20%	5.43 ab	32.01 abc	70.56 ab	4.67 a	20.04 ab	65.36 ab
Pedro	10%	8.29 a	39.24 ab	71.71 a	5.60 a	39.25 a	64.75 ab
	15%	4.46 ab	22.09 abc	56.11 abcd	3.27 a	24.60 ab	50.84 ab
	20%	3.07 ab	23.63 abc	68.68 abc	4.11 a	19.47 ab	62.97 ab
Franquette	10%	4.79 ab	22.86 abc	54.93 abcd	2.71 a	20.32 ab	47.82 ab
	15%	2.38 b	21.00 abc	73.98 a	3.10 a	22.28 ab	63.56 ab
	20%	3.59 ab	25.57 abc	64.27 a	3.92 a	17.58 ab	63.17 ab

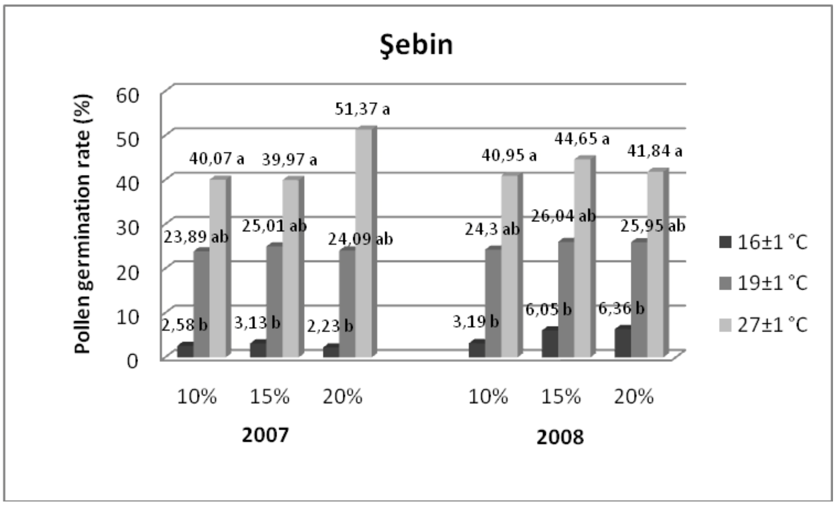

Figure 2. Effects of different temperatures and sucrose concentrations on the rate of pollen germination of 'Şebin' walnut cultivar in 2007 and 2008.

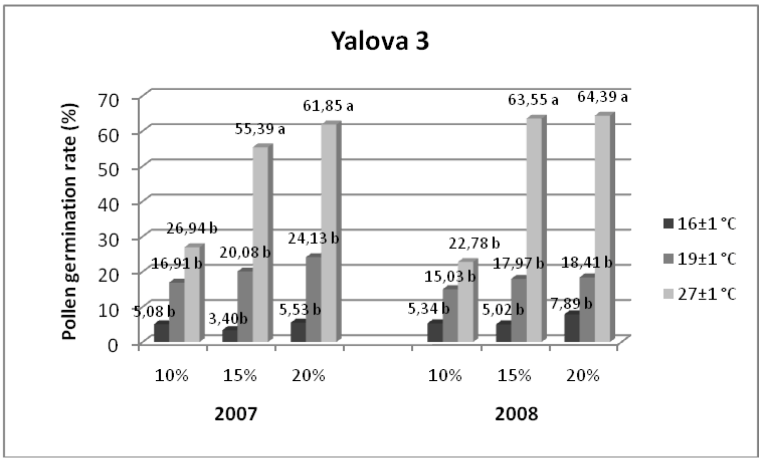

Figure 3. Effects of different temperatures and sucrose concentrations on the rate of pollen germination of 'Yalova-3' walnut cultivar in 2007 and 2008.

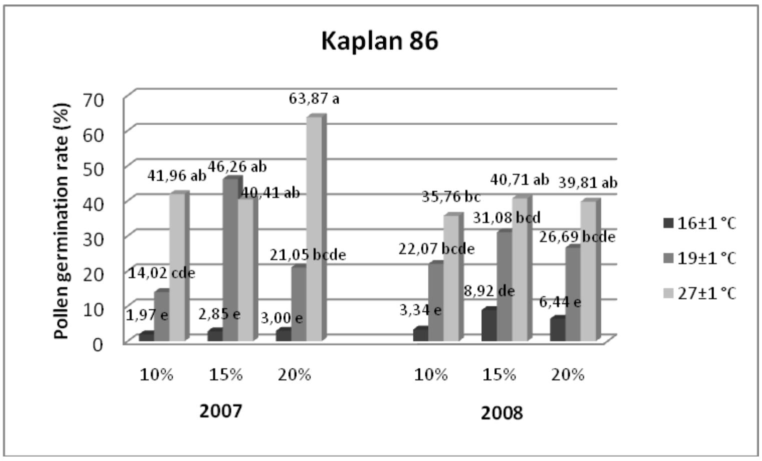

Figure 4. Effects of different temperatures and sucrose concentrations on the rate of pollen germination of 'Kaplan 86' walnut cultivar in 2007 and 2008.

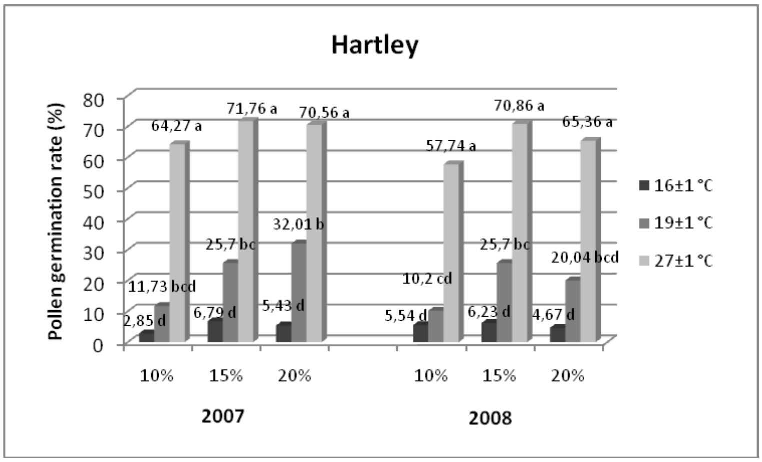

Figure 5. Effects of different temperatures and sucrose concentrations on the rate of pollen germination of 'Hartley' walnut cultivar in 2007 and 2008.

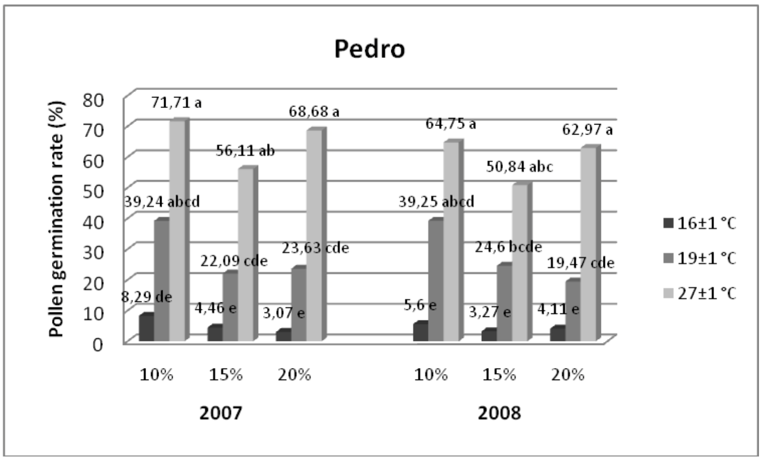

Figure 6. Effects of different temperatures and sucrose concentrations on the rate of pollen germination of 'Pedro' walnut cultivar in 2007 and 2008.

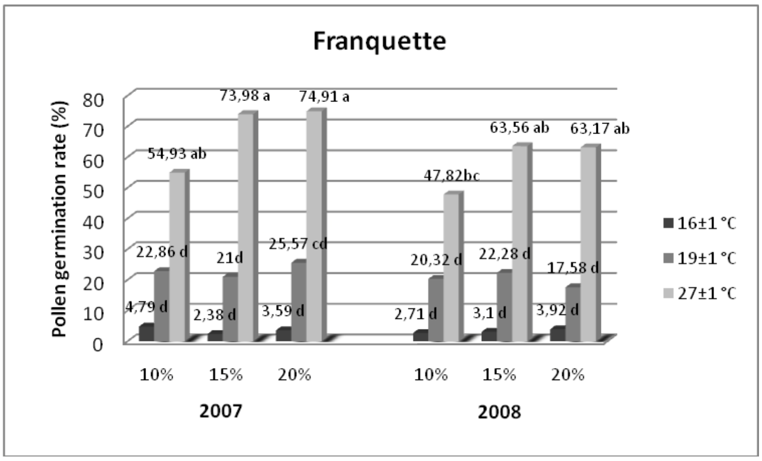

Figure 7. Effects of different temperatures and sucrose concentrations on the rate of pollen germination of 'Franquette' walnut cultivar in 2007 and 2008.

In another fruit species optimum pollen germination temperatures were found to be different. Namely some of the species required comparatively lower temperatures. For example apricot (15 °C) (Vachun 1981), apricot and sweet cherry (15 and 20 °C) (Pırlak 2002), mango (15-25 °C) (Sukhvibul et al. 2000), cherimoya (20-25 °C) (Rosell et al. 1999), papaya (*Carica papaya* L.) (Cohen et al. 1989). This study indicate that temperature effects on pollen grain germination are seem to be cultivar or species dependent.

The following pollen germination rates were observed among walnut cultivars; 'Şebin' cultivar as 2.23-51.37% in 2007 and 3.19-44.65% in 2008, 'Yalova-3' cultivar as 3.4-61.85% in 2007 and 5.02-64.39% in 2008, 'Kaplan 86' cultivar as 1.97-63.87% in 2007 and 3.34-40.71% in 2008, 'Hartley' cultivar as 2.85-71.76% in 2007 and 4.67-70.86% in 2008; 'Pedro' cultivar as 3.07-71.71% in 2007 and 3.27-64.75% in 2008, and 'Franquette' cultivar as 2.38-73.98% in 2007 and 2.71-63.56% in 2008. Although germination rates shown close rates in all the cultivars in both years, germination rates were a bit lower in 2008 except 'Yalova-3' cultivar (Table 2). Sütyemez (2007) reported that the highest pollen germination percentages were found as 42.09% for 'Şebin', as 38.61% for 'Yalova 3', as 45.27% for 'Kaplan 86', as 44.46% for 'Hartley', as 44.63% for 'Pedro' and as 34.71% for 'Franquette'. These rates were lower than our results which obtained from 27±1 °C. These results show us the importance of temperature effect on walnut pollen germination. Luza et al. (1987), reported that the pollen germination of *J regia* was 100% at 25-29 °C.

In conclusion, different temperature treatments were effective on pollen germination and pollen germination rates. Germination rates increased significantly with increasing temperatures in walnut cultivars studied. The highest germination rates were recorded at 27 ±1 ˚C temperature in both years. The rate of pollen viability of all the investigated cultivars was high (> 75%). The highest pollen germination percentage was obtained from 15% and 20% sucrose concentrations in both years.

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