Europ.J.Hort.Sci., **71** (3). S. 125–128, 2006, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Response of *In Vitro* Pollen Germination and Pollen Tube Growth of Strawberry Cultivars to Temperature

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Summary

Effects of different temperatures (7, 15, 25 and 30 °C) on *in vitro* pollen germination and pollen tube growth were evaluated in six strawberry cultivars, including 'Allstar', 'Cavendish', 'Chandler', 'Elsanta', 'Elvira' and 'Selva'. Except for 'Elsanta', the viabilities of pollen from all cultivars were found considerably high in 2, 3, 5-triphenly tetrazolium chloride (TTC) stain test. Temperature has a significant effect on pollen germination and pollen tube growth. The highest pollen germination occurred at 30 °C for 'Chandler' and 'Selva' but at 25 °C for 'Elsanta', 'Cavendish', 'Allstar' and 'Elvira'. A longer incubation period increased the rate of *in vitro* pollen germination and all cultivars showed the highest germination percentage after 24 hours of incubation. *In vitro* elongation of pollen tubes was clearly affected by incubation temperature, and cultivars reached their maximum pollen tube growth at different temperature.

Key words. Pollen viability – pollen germination – pollen tube growth – strawberry – temperature

Introduction

Fruit production of tree plants is highly affected by environmental conditions. Effects of temperature on pollen germination have been studied extensively in many fruit species. Poor pollen quality and germination capacity may significantly reduce the yield in strawberry. Although vegetative and reproductive development of strawberry (*Fragaria* x *ananassa* Duch.) are controlled by genetic factors, they are also influenced by environmental conditions (Braun and Kender 1985; Paroussi et al. 2002). Photoperiod and temperature are two of the major environmental factors influencing strawberry flowering, and they interact in the regulation of different stages of the process (Taylor 2002). Long-day conditions may improve pollen quality to some extent and increase productivity (Lieten 1994). On the other hand, temperature controls flowering (Guttridge 1985). Studies on pollen germination showed that pollen performance of different strawberry cultivars can vary considerably depending on the temperature. (Hortynski and Zebrowska 1991; Risser 1997; Leech et al. 2002). Risser (1997) observed that low temperature (below 16 °C) negatively affected pollen germination of 'Chandler'. Poor pollen performance and stamen sterility may significantly decrease the fruit setting and cause unfavourable fruit formation in strawberries (Voyiatzis and Paraskevopoulou-Paroussi 2002a). Despite a large number of studies conducted on growth, flowering physiology and yield of cultivated strawberry (Paroussi et al. 2002; Taylor 2002), there is only scarce and minor information about *in vitro* pollen germination and tube growth at different temperatures (Leech et al. 2002; Voyiatzis and Paraskevopoulou-Paroussi 2002b).

The objective of this study was in this respect to gain deeper insight.

Materials and Methods

Different strawberry cultivars (*Fragaria* x *ananassa* Duch.) including 'Allstar', 'Cavendish', 'Chandler', 'Elsanta', 'Elvira' and 'Selva' grown under natural outdoor conditions in Isparta (West Mediterranean, Turkey) were used. Flowers at the balloon stage were collected from plants in early morning on May 6, 2003, and quickly transferred to the laboratory. Anthers were removed and placed into dark coloured bottles to promote dehiscence at room temperature. Pollen viability was determined by 2, 3, 5-triphenly tetrazolium chloride (TTC) stain test (Norton 1966). Pollen grains stained red were considered as viable in this test. Morphological homogeneity (MH) of pollen grains was determined by a haemocytometer (Marienfeld, Germany) slide (Eti 1990). Pollen grains with shrunken and shapely defective appearance were considered aborted. The final percentage of morphological homogeneity was calculated as

$$MH = \frac{(\text{number of normal shaped pollen}) - (\text{number of aborted pollen}) \text{per field}}{\text{Total number of pollen per field}} \times 100$$

The pollen germination and pollen tube growth rates were assessed using the 'agar in plate' method. Pollen scattered on the medium consisting of 15 % sucrose and 1 % agar (Koyuncu et al. 2000) were incubated at 7, 15, 25 and 30 °C in the dark. Pollen was considered as 'germinated' when the length of pollen tube was at least as

Table 1. Pollen viability and morphological homogeneity of six strawberry cultivars.

Parameter	'Allstar'	'Chandler'	'Cavendish'	'Elsanta'	'Elvira'	'Selva'
Pollen viability (%)	82.0 a	86.5 a	82.5 a	71.0 b	82.0 a	82.5 a
Morphological Homogeneity (%)	91.5 b	91.4 b	94.6 a	89.2 b	91.6 b	91.5 b

Values within a row followed by different letters are significantly different (P<0.05).

long as the diameter of the pollen grain. For each temperature treatment, the percentage of germinated pollen was calculated after 1, 3, 6 and 24 h of incubation. *In vitro* elongation of pollen tubes was measured on germinated grains within 24 h of incubation. Pollen tube length was measured by an ocular micrometer under a light microscope at a 40-times magnification.

For each cultivar, three Petri dishes were used for germination and pollen tube growth experiments. Pollen grains were counted from 4 different microscope fields (100–150 pollen grains per field) in each Petri dish. For all experiments, treatments were arranged according to a randomised design. Statistical analyses were performed with General Linear Model using SPSS (V.10; Statistical software, SPSS. Inc., USA). The percentage data were first transformed to arcsine root square transformation, and analysis of variance was performed. The differences among means were analysed by Duncan's multiple range test at P<0.05 level of significance.

Results

Pollen viability

The lowest pollen viability was observed in 'Elsanta' (71 %) which was significantly different from the remaining cultivars. The other tested cultivars did not show significant difference in pollen viability and their viability ranged from 82 to 86.5 % (Table 1). There is a variation among cultivars regarding the percentage of morphological homogeneity of pollen grains. Homogeneity among strawberry cultivars ranged from 89.2 to 94.6 %. The pollen homogeneity did not vary significantly among cultivars except for 'Cavendish' (Table 1).

Pollen germination

Observed values for pollen germination of six strawberry cultivars in response to different temperature treatments are shown in Fig. 1. Pollen began to germinate *in vitro* after 1 h of incubation, and, at all temperatures, the rate of pollen germination increased as the incubation period extended. Pollen germination rates of 'Chandler', 'Elvira' and 'Selva' showed as much as two fold increase between 6 h and 24 h of incubation at all temperatures, but 'Elsanta', 'Allstar' and 'Cavendish' did not show any differences at 25 °C and 30 °C, respectively (Fig. 1).

In vitro pollen germination rates showed statistically significant changes depending on the incubation temperatures at P<0.05. The optimum temperature for *in vitro* germination of 'Cavendish', 'Allstar', 'Elsanta' and 'Elvira' was close to 25 °C while that of 'Selva' and 'Chandler' was 30 °C at the end of incubation. Increasing the temperature from 7 to 30 °C resulted in up to 139 % increase in germination for 'Selva' and 57 % increase for 'Chandler'. The lowest germination rate was obtained for 'Elvira', 'Selva' and 'Chandler' at 7 °C and for 'Cavendish', 'Allstar' and 'Elsanta' at 15 °C (Fig. 1).

In vitro germination performances of strawberry pollen incubated at different temperatures for 24 h are presented in Table 2. There were significant differences among the *in vitro* pollen germination of all strawberry cultivars for each incubation temperature at P<0.05. The germination percentage of 'Chandler' in all incubation temperatures was statistically higher than the other cultivars. The mean germination percentage of 'Chandler' and 'Selva' was 64.2 % and 50.3 %, respectively. The other cultivars showed the mean germination percentage between 25.3 % and 27.9 %. In general, overall germination rate of 'Allstar' was lower than the others.

Pollen tube growth

In vitro elongation of pollen tubes was affected by incubation temperatures (Table 3). The response of tested

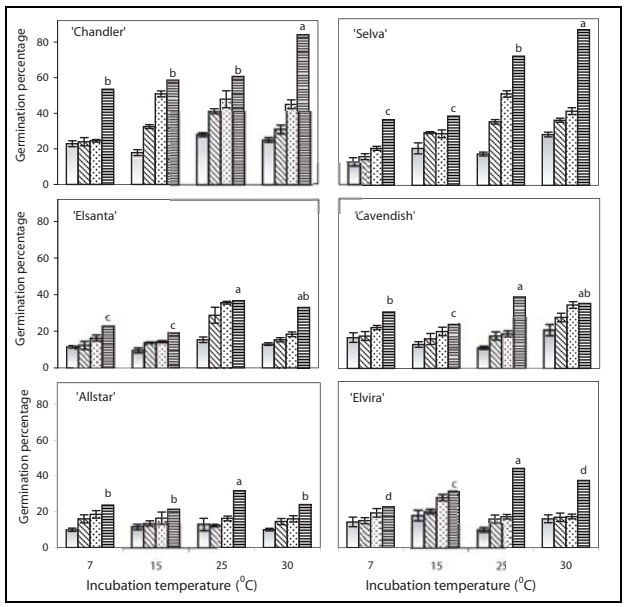


Fig. 1. *In vitro* pollen germination percentages at different temperature during 1, 3, 6 and 24 hours incubation of six strawberry cultivars. 24 hours bars of each cultivar having different letters indicate significant differences according to Duncan's multiple range test (p<0.05). 1, 3 and 6 hours bars represent with mean ± SD from 4 replicates.

Table 2. *In vitro* germination (%) of pollen of six strawberry cultivars at different temperatures after 24 hours incubation, in a medium containing 15 % sucrose + 1 % agar.

Cultivar	Incubation temperature				
	7 °C	15 °C	25 °C	30 °C	mean
'Allstar'	23.8 c	21.4 d	31.8 b	24.0 d	25.3
'Chandler'	53.5 a	58.6 a	60.6 a	84.1 a	64.2
'Cavendish'	26.6 c	20.8 d	33.6 b	30.6 c	27.9
'Elsanta'	22.8 c	19.1 d	36.6 b	33.0 c	27.9
'Elvira'	19.6 c	26.9 c	38.1 b	32.9 c	27.6
'Selva'	31.4 b	33.3 b	62.0 a	74.6 b	50.3

Different letters in the same column indicate significant differences, according to Duncan's multiple range test (P<0.05).

Table 3. The effect of incubation temperature on the pollen tube growth (μm) of six strawberry cultivars *in vitro* after 24 hours.

Temperature	'Allstar'	'Chandler'	'Cavendish'	'Elsanta'	'Elvira'	'Selva'
7 °C	93.5 b	147.8 b	121.3 a	179.3 b	162.5	203.5
15 °C	115.1 a	153.5 b	100.3 b	183.5 b	182.3	202.8
25 °C	93.8 b	204.3 a	113.5 ab	169.0 b	155.8	204.0
30 °C	93.5 b	153.1 b	99.8 b	219.3 a	167.3	203.5
Mean	98.9	164.7	108.7	187.8	166.9	203.4

Different letters in the same column indicate significant differences, according to Duncan's multiple range test (P<0.05).

cultivars to different temperatures was statistically significant at P<0.05. The effect of incubation temperatures at 7, 15, 25 and 30 °C on *in vitro* pollen tube growth of 'Elvira' and 'Selva' was not significant. Moreover, 'Selva' showed almost equal pollen tube elongation at all temperatures. Pollen tube growth of 'Elsanta' increased progressively from 7 to 30 °C (179.3 and 219.3 μm, respectively). In contrast, pollen tube growth of 'Cavendish' was inhibited by increased incubation temperature. The maximum pollen tube elongation was obtained at 15 °C for 'Allstar' (115.1 μm) and 25 °C for 'Chandler' (204.3 μm).

Discussion

The pollen performance of six strawberry cultivars was evaluated in this study. The percentage of pollen viability, morphological homogeneity, pollen germination and the pollen tube growth were examined *in vitro*. In general, high viability of pollen indicates that pollen is capable of germination under favourable conditions. In this study, the pollen viability of tested cultivars was higher than the pollen germination rate. Eti and Stösser (1988) reported that there is no direct and consistent relationship between these two parameters. Pollen deformation is a cause of inadequate pollination and fertilization leading to poor fruit setting. Morphological homogeneity of pollen may constitute a good characteristic for a progeny (Voyiatzis and Paraskevopoulou-Paroussi 2002b).

Pollen germination of tested strawberry cultivars was affected by incubation duration in a similar manner. Germination of pollen started in the first hour of incubation period, and reached maximum percentage at 24 h. Results of incubation duration experiments were similar to findings of Yildiz and Yilmaz (2002), who reported that germination of 'Tufts' pollen began within 1 h at 24 °C. Similarly, the pollen of 'Tsakoniki' pear started to germinate after 1 h incubation at 10 °C (Vasilakakis and Porlingis 1985). In this study, it was found that increase in the incubation period resulted in an increase on pollen germination rate *in vitro* for all cultivars at all temperatures. Consequently, incubation duration should be considered as an important factor affecting *in vitro* pollen germination which is a reliable method for assessing pollen performance of strawberry.

Results clearly showed that incubation temperatures had a significant effect (P<0.05) on pollen germination capacity and pollen tube growth, but the responses of six cultivars were not uniform. Temperature requirement of *in vitro* pollen tube growth was equal to or lower than that of the pollen germination in all cultivars except for 'Cavendish'. Incubation temperatures between 25 and 30 °C seem to be appropriate for the *in vitro* pollen germination of all strawberry cultivars tested. Findings of this study are comparable to germination temperatures reported for other strawberry cultivars. Hortynski and Zebrowska (1991) reported that optimum temperature range of 'Dukat', 'Red Gauntlet' and 'Senga Sengana' strawberry cultivars was 15 to 25 °C. Leech et al. (2002) found that 10 °C for 'Florence', and 20 °C for 'Pegasus', 'Tamella' and 'Marmolada' strawberries were optimum temperatures for pollen germination. Low temperature

had an adverse effect on pollen germination of 'Chandler' (RISSER 1997). On the other hand, VOYIATZIS and PARASKEVOPOULOU-PAROUSSI (2002b) found no significant difference, on average, between 20 and 25 °C for pollen germination rate of seven strawberry cultivars. All these results may contribute to a better understanding of the phenomenon of temperature which is an important factor in strawberry pollen germination *in vitro.* In this study, 'Chandler' gave the highest germination percentage at all incubation temperatures. Based on our results and the others, it may be concluded that pollen germination rate is also cultivar dependent.

Strawberry may be cultivated in controlled environments or under temperate climates out of the season. In these cases, a better performance may be provided by specific treatments depending on cultivars and environmental conditions (LIETEN 1991). On the other hand, deformed pollen production was observed under early spring conditions, this pollen deficiency probably induced misshapen fruit, in early production in Southern Europe (RISSER 1997). The establishment of a reliable method for testing pollen performance could also be helpful in breeding and selecting of suitable cultivars. In this context, this study may provide useful information for facilitating the evaluation of strawberry cultivars by their pollen performance.

Acknowledgements

I would like to thank Dr. Kahraman Kepenek (Horticulture Department) for providing the plant materials used in this study.

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Received June 9, 2004 / Accepted September 15, 2005

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