Investigations of suitable pollinator for 0900 Ziraat sweet cherry cv.: pollen performance tests, germination tests, germination procedures, *in vitro* and *in vivo* pollinations

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ABSTRACT: The objective of this study was to determine suitable cultivars to be used as pollinators for 0900 Ziraat. 0900 Ziraat was used as a female cultivar; Bigarreu Gaucher, Bing, Noble, Starks Gold, Stella, Van, and Vista were used for pollination in the experiments. Starks Gold had the highest values in terms of anther number, average number of pollens per anther, number of pollen per flower and the morphological homogeneity. The pollen viability rates showed significant differences according to stain tests. *In vitro* pollen germination in 0.5% agar + 15% sucrose + 5 ppm boric acid medium increased with increasing incubation period, and the highest germination was obtained after 48 hours for all cultivars. In orchard trials parallel to pollen tube growth experiments in the laboratory, 0900 Ziraat × Starks Gold combination gave the best fruit set results.

Keywords: sweet cherry; *Prunus avium* L.; pollen; fertilization biology

The first condition of formation of seed and fruit is developing healthy male and female organs of the flower and cells, except for an apparent partenocarpy of some cultivars. Pollen performance includes pollen produced in a flower, pollen morphological homogeneity, pollen germination, pollen tube growth and pollen competition; it is an important component of fertilization success in fruit trees (Thompson 2004; Janick, Moore 1996; Hedly et al. 2004). Although environmental factors and genetic characteristics are effective in the case of cultivation of fruit trees, an abundant crop of cherries is dependent upon a successful completion of a sequence of reproductive events. Failure or deficiency of any aspect of this process can result in a crop reduction or total loss. The first requirement is the availability of an adequate source of viable and compatible pollen. Secondly, there must be an effective transfer of pollen when stigmas are receptive. Thirdly, pollen tubes must grow down the style and enter the ovule when embryo sacks have matured. And finally, double fertilization and subsequent growth of embryo and endosperm must occur to provide the necessary stimulus for fruit development (Thompson 2004). The phenomenon of incompatibility prevents inbreeding and promotes crossing of natural populations and cultivars of cherries. Pollen grains are simple structured plant cells. Pollen tube formation is a good, simple model of growth and development (Taylor, Hepler 1997). Thus, pollen germination and growth of pollen tubes are important research materials for morphological, physiological, biotechnological, ecological, evolutional, biochemical and molecular biological studies (Ottavio 1992; Dane et al. 2004). The viability, morphological homogeneity and tube growth of pollen are the most important properties in cherry tree fertilization. However, an easy method for determining pollen viability is required to increase the efficiency of the breeding program and selection of suitable pollinizer while the orchard is being established (Tehrani, Lay 1991; Brown et al. 1996). Successful fertilization of sweet cherries depends on the transfer of compatible pollen by honeybees, as most commercial cultivars are self-

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incompatible (Janick, Moore 1996; Thompson 2004). Pollen viability could be assessed by different methods like staining with nuclear or non-vital dyes or *in vitro* germination tests (Parfitt, Ganeshan 1989; Heslop-Harrison et al. 1984; Koyuncu et al. 2000; Voyiatsiz, Paraskevopoulou-Paroussi 2002). The aim of the staining techniques is to determine the pollen enzymatic activity, membrane integrity and stainability of the nucleus (Norton 1966; Vizintin, Bohonec 2004). Stain tests are faster and easier than pollen germination tests but, in some cases, different results may be obtained with them in many fruit species or cultivars. Therefore, to determine the actual amount of viable pollen, germination tests are necessary. The 'hanging drop' and 'agar in plate' tests are generally used to determine the *in vitro* pollen germination rate (Koyuncu et al. 2000; Vizintin, Bohonec 2004).

The objectives of this study were to investigate the pollen performance of some sweet cherry cultivars and to determine a suitable pollinator for 0900 Ziraat, which is the major cultivar produced in Turkey (also for export). For this purpose, pollen viability, pollen production, pollen germination rate, and pollen tube lengths were assessed under *in vitro* conditions; hand pollination was also done *in vivo*. The experiments were conducted twice over a two-year period with consistent results and the data presented here were obtained in the second experimental year.

MATERIALS AND METHODS

This work was carried out on a sweet cherry collection maintained at the Uluborlu Municipality Orchards located in the northwest of Isparta, Turkey. The latitude is 38°10´, longitude is 30°37´, and altitude is 1,050 m. Bigarreu Gaucher, Bing, Noble, Starks Gold, Stella, Van, and Vista cultivars were used as pollinators and 0900 Ziraat was used as a female cultivar.

Phenological observation

The phenological stages were determined by a direct observation in the orchard.

Pollen performance

Pollens were obtained from flowers of the above-mentioned cultivars at balloon stage. The flowers were transferred to the laboratory immediately. Anthers were removed and placed into the dark-colored bottle to promote dehiscence at room temperature. The pollen production amount and morphological homogeneity percentages of sweet cherry cultivars were assessed with the haemocytometer (Marienfeld, Germany) slide (Eti 1990). Imperfectly shaped pollen grains were considered as aborted pollen. The final percentage of morphological homogeneity was defined as:

$$MH = \frac{\text{(number of normal shaped pollen) – (number of aborded pollen) per field}}{\text{total number of pollen field}} \times 100$$

For the *in vitro* test, pollen grains were sowed in the medium containing 0.5 agar + 15% sucrose + 5 ppm H_3BO_3 (boric acid) and incubated at the constant temperature of 25°C. Pollen tube long at least as its diameter was considered to be 'germinated'. The percentage of pollen germination was determined after 4 h, 12 h, 24 h and 48 h incubation period.

In the stain tests, pollen viability was estimated by using TTC (2, 3, 5-triphenyl tetrazolium chloride) and FDA (fluorescein di acetat) stains. Pollens were scattered onto TTC and FDA solutions, and stained pollens were counted after 2 hours and 15 minutes, respectively. To determine the pollen viability, pollens of each cultivar (of four different areas) were observed onto two slides under a light microscope (×100 magnification). The stained pollen was considered as viable in these tests.

Pollen tube growth investigations in the laboratory

The pollen tube growth was evaluated in the laboratory, while the parallel hand pollination was also done in the orchard. Emasculated female organs were put on a medium consisting 0.5% agar-agar + 15% sucrose + 5 ppm boric acid. They were pollinated by hand and placed in an incubator with 25°C. Preparations of pollen tube were made according to Kho and Baer (1971), 48, 96, 144 and 192 hours after the hand pollination. Preparates stained with aniline blue were examined and a photo of them was taken with Nicon Eclipse E600 microscopy using the filter NCB 11 (UV-2A).

Orchard trials

Emasculation was done in the orchard at the beginning of the flowering stage. 400 buds in total were emasculated on four different branches. 0900 Ziraat was chosen as a female cultivar and to determine a suitable pollinator it was pollinated by hand with

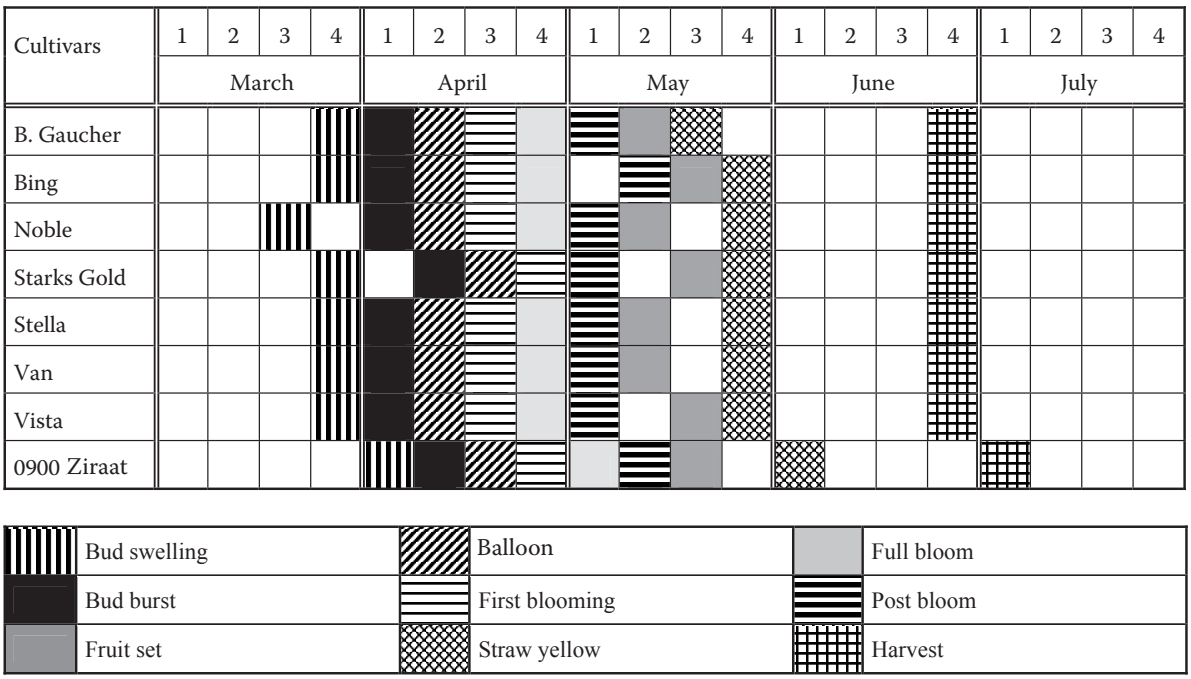

Fig. 1. Phenological stages of cherry trees

pollens of Bigarreu Gaucher, Bing, Noble, Stella, Starks Gold, Van and Vista cultivars. To determine fruit sets with free pollination, 100 flowers were counted and released. To prevent a probability mistake, hand pollination was replicated in two days. The fruit set with free pollination was evaluated as counting 100 flowers on each branch and the results were evaluated according to Öz (1977).

Less than 2% fruit set; incompatibility.
2–4% fruit set; high probability of incompatibility.
4–6% fruit set; high probability of compatibility.
Higher than 6% fruit set; compatibility.

Statistical analyses

Statistical analyses were performed with the General Linear Model using SPSS (V.10; Statistical software, SPSS. Inc., USA). The percentage data were first transformed to arcsine square root transformation, and an analysis of variance was performed. The differences among means were analyzed using the Duncan's multiple range test at $P < 0.05$ significance.

RESULTS AND DISCUSSION

Phenological stages

Phenological phases of cherry trees are shown in Fig. 1. The beginning of bud swelling of cultivars occurred in the fourth week of March. The bud burst appeared during the first and the second week of April. The balloon stage started in the second half of April. The flowering period took approximately

Table 1. Some quantitative characteristics of pollen of sweet cherry cultivars (average values of 2 experimental years)

Cultivars	Number of anthers in a flower	Mean pollen number in an anther	Pollen number in a flower	Morphological homogeneity
B. Gaucher	39.0	233.40	9,106.5	93.10
Bing	37.0	164.55	6,150	89.15
Noble	38.5	120.60	6,415	96.25
S. Gold	41.0	378.40	15,495	97.25
Stella	38.0	164.70	6,272.5	93.50
Van	38.5	178.05	6,860	91.15
Vista	37.5	140.35	5,274	88.70
0900 Ziraat	37.0	131.70	4,875	82.10
Max	41.0	378.40	15,495	97.25
Min	37.0	120.60	4,875	82.10

Table 2. Pollen viability ratios of sweet cherry cultivars with TTC and FDA

Stains	B. Gaucher	Bing	Noble	S. Gold	Stella	Van	Vista	0900 Ziraat
FDA	87.00	78.00	86.50	72.50	80.00	77.00	64.50	79.00
TTC	82.00	89.50	89.25	91.00	83.00	93.00	79.00	80.50
Mean	84.50 ab	83.75 ab	87.87 a	81.75 b	81.50 b	85.00 ab	71.75 c	79.79 b

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

11–17 days. Starks Gold and Vista cultivar's blooming time was similar to 0900 Ziraat. Small fruits were seen in the second week of May. The fruits turned straw yellow in the third and the fourth week of May. Harvest time was at the end of June except for 0900 Ziraat. Noble was the earliest cultivar while 0900 Ziraat was the latest. The phenological dates can change with temperature. Our results were similar to those of Ülger (1988). In his study the cultivar's flowering time ranged from 17 (Salihli) to 23 (Hedelfinger) days. With respect to weather conditions, the flowering time can show alteration. An earlier cultivar cannot be recommended as a pollinator for the latest cultivar (Kiriş 1992).

Pollen production

Starks Gold ranked first as to the number of anthers, pollen production capacity and morphological homogeneity (Table 1). Morphological homogeneity was generally high for all cultivars. High pollen viability and germinating as well as pollen production capacity are important to decide on a suitable pollinator, since not all pollen grains germinated on stigma can reach ovule. Gerçekçioğlu et al. (1999) reported that high pollen production, morphological homogeneity and pollen viability are important for fertilization.

Staining tests

FDA and TTC staining tests were used for pollen viability. The pollen viability values of sweet cherry cultivars are given in Table 2. In the FDA test, Pollens of Stella showed the highest (80%) value of viability, whereas the lowest values were obtained from Vista (65.5); in the TTC test, pollen viability values were between 79% (Vista) and 91% (Starks Gold). The minimum pollen viability was determined for Vista cultivar in both staining test. Koyuncu (2006) studied strawberry pollens using TTC and reported that pollen viability ratios reached 82% (Allstar and Elvira) and 86.5% (Chandler). Quarta et al. (1985) observed that the pollen viability of apricot was very high (95%) for all selections, but the germinability was never higher than 10%. Koyuncu and Tosun (2005) used TTC, FDA and IKI stain tests for the same sweet cherry cultivars. They reported that the pollen viability differed according to stain and cultivars.

Pollen germination tests

In vitro pollen germination increased with increasing incubation period. Germination began after 4 h and a noticeable increase was observed after 12 h. All cultivars increased as much as 2-fold between 4 h and 12 h. Despite being non-significant an increase was observed between 24 and 48 h (Fig. 2).

The highest germination was obtained from Starks Gold (67.62) while the lowest was from Bigarreu Gaucher (57.00%) at 48 h of incubation (Table 3). Results of incubation duration experiments were similar to the findings of Yildiz and Yilmaz (2002), who reported that the germination pollen of strawberry

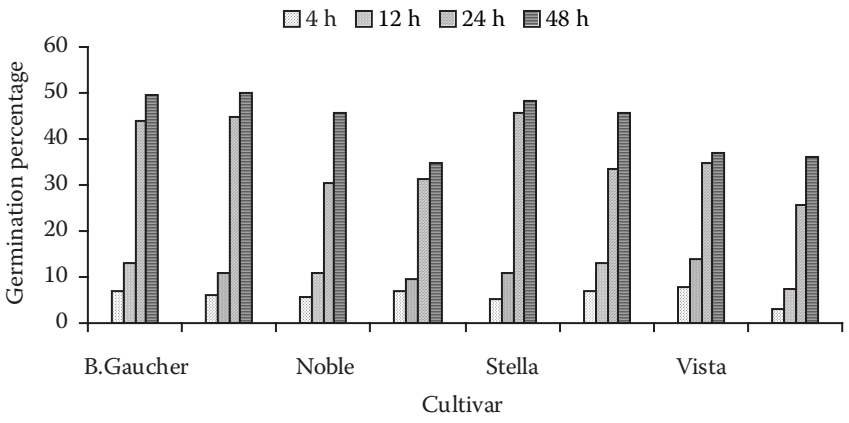

Fig. 2. *In vitro* pollen germination percentages of eight sweet cherry cultivars at 4, 12, 24 and 48-hour incubation periods; $P < 0.05$

Table 3. Pollen germination rates at 48-hour incubation period (%)

Cultivar	Germination (%)
B. Gaucher	51.50 ab
Bing	59.25 a
Noble	40.00 cd
Starks Gold	34.75 d
Stella	48.25 bc
Van	53.25 ab
Vista	36.75 d
0900 Ziraat	36.25 d

*Values within a column followed by different letters are significantly different ($P < 0.05$)

Table 4. Fruit set ratio obtained from hand pollination in the orchard (%)

	B. Gaucher	Bing	Noble	S. Gold	Stella	Van	Vista	0900 Ziraat	Free pollination
0900 Ziraat ×	12.5	12.5	14	26.5	10	9.5	19.6	4.0	58.5

cultivar Tufts began within 1 h at 24°C. Similarly, the pollen of Tsakoniki pear started to germinate after 1 h incubation at 10°C (Vasilakakis, Porlingis 1985). In this study, it was found that an increase in incubation period resulted in an increase on rate *in vitro* pollen germination rate of all cultivars. Koyuncu and Tosun (2005) also reported that the germination rates increased with incubation period.

Pollinations in orchard

The fruit set results of hand pollinations are summarized in Table 4. The highest fruit set was obtained from free pollination with 58.5%; after that 0900 Ziraat × Starks Gold combination gave 26.5% fruit set. Fruit sets exceeding 6% can be considered as compatible according to Öz (1977) scale. No incompatible combination was found, except for 0900 Ziraat × 0900 Ziraat (4%).

Pollen tube growth in laboratory

0900 Ziraat was pollinated with pollens of other cultivars and with its own pollen. The pollen tube was observed after 48, 96, 144 and 192 hours. In 48 h observation, the pollens of all cultivars were germinated on the stigma, and a few short pollen tubes occurred. After that some of the pollens showed an advance in style. After 144 hours, tube elongation was seen in all the combinations (Fig. 3a). Pollen tubes reached ovary in the combination of 0900 Ziraat × Starks Gold after 192 hours (Fig. 3b). Koyuncu (1992) carried out studies with some pear cultivars and reported that 48 hours after hand pollination there were some germinated pollens on the

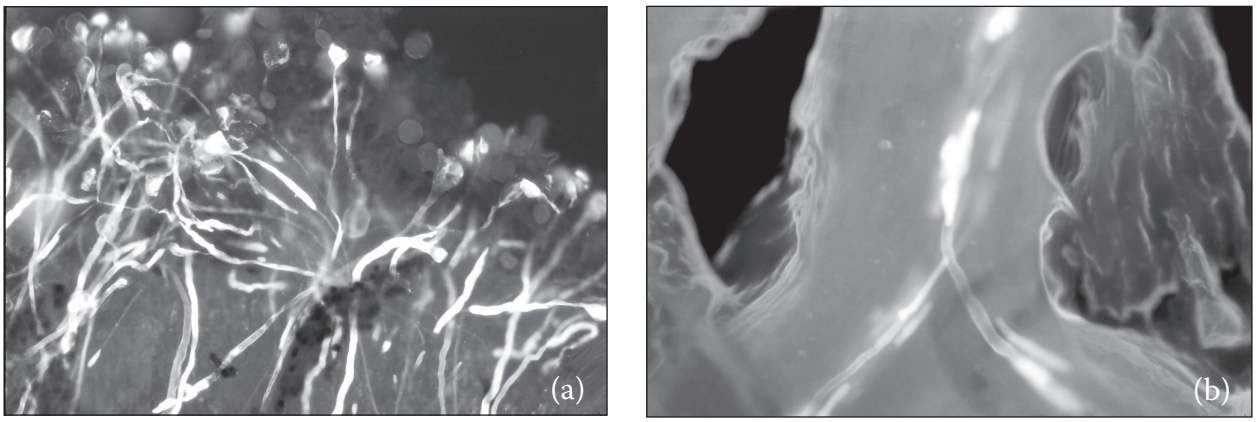

Fig. 3. (a) Pollen tube growth in 0900 Ziraat × Noble combination 144 hours after hand pollination; (b) pollen tubes that could reach to ovary 192 hours after hand pollination in 0900 Ziraat × Starks Gold combination

stigma; 96 hours later some of pollen tubes reached a half of style.

CONCLUSIONS

The viability, morphological homogeneity and tube growth of pollen are the most important properties in cherry tree fertilization. Breeding experiments should be conducted to get quality fruit. Testing pollen performance could be helpful for a fruitful cultivation of genetic progeny for breeding purpose, and especially for selecting which cultivars should be used by growers. In this context, this study may provide useful information for facilitating the evaluation of sweet cherry cultivars based on their pollen performance.

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Hledání vhodného opylovače pro odrůdu třešně 0900 Ziraat: testy kvality pylu, testy klíčivosti, metody hodnocení klíčivosti, opylování v podmínkách *in vitro* a *in vivo*

ABSTRAKT: Cílem studia bylo určení vhodné odrůdy, která by mohla být použita jako opylovač kultivaru 0900 Ziraat. V založených pokusech byl kultivar 0900 Ziraat použit jako mateřská odrůda, zatímco jako její opylovače byly použity odrůdy Bigarreu Gaucher, Bing, Noble, Starks Gold, Stella, Van a Vista. Odrůda Starks Gold dosáhla nejvyšších hodnot, pokud jde o počet prašníků, průměrný počet pylových zrn připadajících na jeden prašník, počet pylových zrn připadajících na jeden květ a morfologickou homogenitu pylu. Použité testy barvení životaschopného pylu se vzájemně průkazně odlišovaly ve výsledných hodnotách. Klíčení pylu v podmínkách *in vitro* na médiu, které obsahovalo 0,5 % agaru + 15 % sacharózy + 5 ppm kyseliny borité, prodlužovalo inkubační období, protože v této variantě byla nejvyšší klíčivost u všech odrůd zaznamenána až po 48 hodinách. Jak v pokusech založených v sadu, tak i v paralelních pokusech se sledováním růstu pylových láček v laboratoři kombinace odrůd 0900 Ziraat × Starks Gold vedla k nejvyšší násadě plodů.

Klíčová slova: třešeň; *Prunus avium* L.; pyl; biologie oplodňování

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