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Pollen quality, quantity and fruit set of some self-compatible and self-incompatible cherry cultivars with artificial pollination

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The fertilization biology of eight sweet cherry cultivars (0900 Ziraat, Lambert, Sunburst, Lapins, Starks Gold, Bing, Chelan and Summit) was studied. Pollen viability was determined with triphenyl tetrazolium chloride (TTC) and fluorescein diacetate (FDA) tests. Pollen germination tests were carried out by hanging drop method on 0, 5, 10, 15 and 20% sucrose solutions. Pollen quantity and pollen morphological homogeneity were determined by hemocytometry method. Pollen tube growth and fruit set ratios of open-pollinated and self-pollinated flowers were also explored. Using TTC and FDA tests, the highest pollen viability rate was obtained with cultivar Sunburst; however, higher pollen germination ratios were obtained with cultivar Lapins and Bing. Starks Gold and Summit cultivars produced more amount of pollen than the other cultivars. A high pollen morphological homogeneity was found for all the cultivars. In self, cross and open pollinations, pollen tubes reached the ovules in 2 to 5 days. The pollen tubes of the self pollinated self-fertile cultivars reached the ovule in a less time. Fruit set ratios of the cross-pollinated combinations varied between 0 and 40%. In comparison with self-incompatible cultivars, fruit set ratios of self-fertile cultivars were higher.

Key words: Sweet cherry, pollination, pollen tube growth, fruit set.

INTRODUCTION

In many fruit species, especially in cherries, sufficient pollination and fertilization are important factors that affect the rate of fruit set and fruit quality. The stigma is the first female structure on which the pollen grains have to germinate and pollen tubes have to face on their way to the female gametophyte to achieve double fertilization. The morphology and structure of stigma have been studied in detail in a variety of species. It provides an adequate environment for pollen grain germination (Heslop-Harrison and Shivanna, 1977; Stösser and Anvari, 1982). One of the most important features of stigmas is stigmatic receptivity defined as the ability of the stigma to support pollen germination. The receptivity of stigma is a decisive stage in fertilization success and has a large variability among plant species (Eti et al., 1995; Heslop-Harrison, 2000).

As it is the case in many deciduous fruit species, sufficient pollination and successful fertilization are essential for fruit set and high yield in sweet cherry growing. The factors that affect fertilization and yield of the sweet

cherry are temperature, rainfall during blooming period, self and cross compatibility status of cultivars and the period of overlapping blooming of the main and pollinizer cultivars. Other environmental factors such as bees are also required as pollen vectors (Eti et al. 1996; Sutyemez and Eti, 1999; Choi and Andersen, 2001; Pirlak, 2002; Bekefi, 2004; Tosun and Koyuncu, 2007). Most of the sweet cherry cultivars except a few self fertile cultivars are self incompatible and some cultivars are cross incompatible (Stancevic, 1971; Heslop-Harrison, 1975; Öz, 1985; Stösser and Anvari, 1981; Choi et al., 2002; Sonneveld et al., 2003; Wünsch and Hormaza, 2004). For this reason, cultivars with overlapping blooming periods and that are compatible with each other should be employed in commercial sweet cherry orchards.

Fertilization success is highly dependent on male-female interaction. The pollen genotype may have a significant effect on pollination and pollen performance. Also, the female genotype may modulate the landing of pollen grain and final result of its performance. The

coexistence of these two forces might make it difficult to obtain clear-cut results. Although, the effects of male and female genotypes are not simply additive, the interaction between pollen and pistil may significantly affect pollen behavior *in vivo* (Hormaza and Herrero, 1999).

The incompatibility occurs when S allele, in addition to pollen grain, is present in the pistil as well. It can be gametophytic which is manifested in the style during the growth of pollen tubes or it can be sporophytic, where the germination of pollen grain does not occur on the surface of the stigma (Way, 1968; Öz, 1985; Shivanna, 2003).

In cherries, fruit set depends on the genotype, whether the cultivars are self cross compatible or incompatible, stigmatic receptivity; the presence of pollen vectors such as bees and favorable weather conditions and temperature during blooming period (Roversi et al., 1984; Eti, 1991; Choi and Andersen, 2001). There are differences among cultivars in the level of tolerance to low temperatures during the blooming period (Eti, 1987; Choi and Andersen, 2001). Thus, choosing the right cultivars when establishing the orchard is very important. Main and pollinizer cultivars should be cross compatible, have an overlapping blooming period and produce high amount of viable pollen.

The aim of this study was to investigate the pollen quantity and quality, pollen viability, pollen germination ability and pollen tube growth of some self fertile and self incompatible sweet cherry cultivars to find out the best cultivar combinations for hybridization and high fruit set.

MATERIALS AND METHODS

The experiment was conducted in 2008. Nine years old cherry (*Prunus avium* L.) trees growing in Kahramanmaraş Sutcu Imam University (KSÜ) and Prof. Dr. Nurettin Kaska Nuts Research Center in Kahramanmaraş, Turkey, were used as the material. Self fertile 'Sunburst, Lapins' and self incompatible '0900 Ziraat and Lambert' were used as the main cultivars. Starks Gold, Bing, Chelan and Summit were used as the pollinizer cultivars. Starks Gold is the standard pollinizer for '0900 Ziraat' and Bing is the standard pollinizer for 'Lambert'.

Self-cross and open pollination experiments

Pollination experiments were performed according to the methods described by Arzani and Khalighi (1998), Sutyemez and Eti (1999) and Choi and Andersen (2001). Hand cross pollinations were carried out in order to determine the ratios of fruit set. '0900 Ziraat, Lambert, Sunburst and Lapins' cultivars were pollinated with '0900 Ziraat, Lambert, Sunburst, Lapins Starks Gold, Bing, Summit and Chelan' cultivars.

For each combination of cultivars, four trees belonged to the main cultivars and from each tree, 5 branches with similar size were selected. At least 300 flowers from each tree were hand pollinated. Female flowers were emasculated at the white balloon stage (Eti et al., 1996). The remaining non-emasculated small flowers and previously opened flowers were removed from the selected branches. Thus, only mature buds of the same phenological stage (at the balloon stage just before the petals expanded) were used on the selected branches. After emasculations, selected branches were

isolated with cotton tissue bags to prevent possible bee visitation and entry of unwanted pollen. The pollens were collected from the picked flowers at the white balloon stage of the pollinizer cultivar and were preserved at refrigerated temperature ($2\pm 4^{\circ}\text{C}$) in small glass. Emasculated flowers were pollinated when the stigma became receptive.

Pollination was carried out by using either a small bristle brush or with bare fingers. Hand pollinations were repeated daily when possible in order to uphold the effective pollination period of the single flower. Also, self pollination and open pollination experiments were performed. Pollinated flowers were counted and isolated again. Isolation bags were removed after petal fall.

Pollen tube growth and fruit set

24 h after pollination, pollen germination ratios on stigma and pollen tube growth were analyzed every six hour intervals and for six days. For each combination of cultivars, seven flowers were used. Sample flowers were placed in small glass vials containing 5 ml of FAA (fixing solution consisting of 1:1:18 ratio of 40% (v/v) formaldehyde, 90% (v/v) glacial acetic acid and 70% (v/v) ethanol). The pistils were washed and autoclaved for 30 min at a pressure of 1 kg/cm^2 in a solution of 5% sodium sulphite to soften the tissue and enhance staining with 0.1% aniline blue in 0.1 N potassium phosphate. Pistils carefully stripped of their pubescence and then, they were placed on slides, crushed and examined by fluorescence microscopy (Anavri and Stösser, 1978; Stösser et al., 1985). After 24 h, pollen germination was observed using a light microscope at 40 magnifications. Pollen germination was determined when the length of a pollen tube exceeded pollen diameter. Ten fields per genotype were observed (five fields; two dishes) and both germinated and ungerminated pollen grains were counted.

The basal ends of the branches taken from trees used in pollination experiments were stored in 5% sucrose solution at 25°C . Each variety was self-pollinated or cross-pollinated one day after emasculating. The pistils were put into a fixer (FAA) 24 to 102 h after pollination and were prepared for observation with fluorescence microscopy (Martin, 1959; Kho and Baer, 1970).

The number of pollen grains on the stigma and the number of tubes in the first, second and third part of the style and in the ovary were counted. A total of 800 pistils were analyzed. Fruit set ratios were determined 45 days after pollination in the field.

Pollen viability and germination tests, pollen production

Pollen viability and germination tests were performed according to Eti (1990). Triphenyl tetrazolium chloride (TTC) and fluorescein diacetate (FDA) tests were used to determine the pollen viability ratios. TTC and FDA solutions were prepared according to Norton (1966) and Heslop-Harrison and Heslop-Harrison (1970), respectively. For pollen germination, hanging drop method (Stanley and Linskens, 1985) was conducted in 0, 5, 10, 15 and 20% sucrose solution.

For each cultivar, 40 flowers were randomly sampled and they were used to determine the amount of pollen produced. Number of pollen grains per flower (PF) was determined with hemacytometric method (Eti, 1990).

The amount of pollen per anther was determined using the following formula;

$$\text{PA} = \text{PF}/\text{AF}$$

Where, PA is the number of pollen grain per anther, PF is the number of pollen grains per flower, AF is number of anthers per flower and DP is percentage of well-developed pollen.

Experimental data were analyzed using analysis of variance and the means were statistically grouped by Tukey's (HSD) test. Data containing percentage values were angle transformed before the statistical analysis.

RESULTS AND DISCUSSION

Pollen viability

The results of TTC and FDA tests for the averages are summarized in Table 1. In TTC tests, 'Sunburst' had the highest (85.75%) pollen viability followed by 'Starks Gold' (84.51%), 'Bing' (84.47%) and 'Summit' (84.28%) respectively. Pollen viability determined by FDA test was 89.26% for 'Sunburst' and 89.18% for 'Chelan'. The pollen viability ratios of the other cultivars were over 75% in both tests. Therefore, regarding pollen viability, all the sweet cherry cultivars can be considered good pollinators. TTC and FDA tests gave similar results. These findings were in accordance with previous studies (Seilheimer ve Stösser, 1982; Eti, 1991; Mahanoğlu et al., 1995; Sütyemez ve Eti, 1996).

Pollen germination

The germination percentages of the pollen grains of the eight sweet cherry cultivars used in this study are presented in Table 2. In the hanging drop test, sucrose concentration affected the pollen germination. The higher sucrose concentration (10 and 15%), in general, improved the germination rates of the sweet cherry pollens (Table 3). The highest pollen germination rate was for 'Lambert' (58.26%), followed by 'Sunburst' (58.17%) in 15% sucrose concentration. The lowest (18.21%) value was obtained for 'Lapins' in 0% sucrose concentration (Table 3). Özçağiran (1996) found that, 15% sucrose concentration gave the highest germination rates for sweet cherry cultivars. Other researchers also noticed that, 15 and 20% sucrose concentration gave the highest germination rates in the various fruit species (Eti, 1991; Sütyemez and Eti, 2006; Mert and Soyulu, 2007; Asma, 2008).

The results of germination and viability tests clearly indicated that, most sweet cherry cultivars have a high viable pollen ratios and pollen germination capacities.

Pollen production

For a cultivar to be used as a pollinizer, in addition to high pollen viability and pollen germination rates, it is important that its anthers produce high amounts of pollens. Because not all pollens germinated on stigma reach the carpels, thus, for successful fertilization, pollinizer cultivars producing high amount of pollens are desired (Stösser and Anvari, 1981; Stösser, 1984). The pollen production tests showed that the male flowers of the eight

cultivars produced sufficient amount of pollen (Table 3).

In general, the results obtained from the pollen production study have potentially practical uses in fertilization biology. 'Starks Gold' had the highest AF (38.60) and PF (110.692), 'Bing' had the highest PA (3.244) and 'Chelan' and 'Starks Gold' had the highest DP (91.86 and 90.13%).

It can be seen from the amount of the observed pollen production that, most of the tested sweet cherry cultivars were above the satisfactory level for sweet cherry. Besides, the amount of pollen production in the flowers of a cultivar and the rate of production of morphologically normal pollen grains is also important (Derin and Eti, 2001). Sütyemez ve Eti (1996) reported that, the values of pollen productions were between 71.000 and 201.000 in different fruit species and cultivars. Eti (1991) reported that, the values of morphological homogeneity were between 52 and 100% in different fruit species and cultivars. The highest values of morphological homogeneity can be evaluated as a good property in terms of fertilization biology. Ülkümen (1973) and Dokuzoguz (1964) reported that, there is a relationship between the ratio of pollen germination and morphological structure of pollen grains and the pollen germination rate is low in the pollen grain not having morphological homogeneity. Gerçekcioğlu et al. (1999) reported that, high pollen production, morphological homogeneity and pollen viability are important for fertilization.

Pollen tube growth

Pollen tube growth was observed 24 to 102 h after pollination. Following pollination, pollen germination on stigma was observed for all the cultivars and some germination developed short pollen tubes within 42 h. The pollen tubes continued growing in the style (Figure 1).

The pollen tubes reached the ovary of '0900 Ziraat' in 54, 90 and 102 h, when the male parents were 'Starks Gold, Lambert and Sunburst', respectively (Figure 1). Pollen tube reached the ovary of self compatible 'Lambert' in 66 h, when it was self-pollinated or pollinated with cultivar 'Bing' (Figure 1). However, pollinating Lambert with other cultivars resulted in unsuccessful pollen tube growth.

Pollen tube reached the ovary of self-compatible 'Sunburst' in 42 h when it was self-pollinated, in 84 h when it was pollinated with 'Chelan', in 96 h when it was pollinated with 'Starks Gold and 'Summit' and in 102 h when it was pollinated with '0900 Ziraat'. Although, pollen tube grew and passed the style, they did not reach the ovary when 'Sunburst' was pollinated with the other cultivars (Figure 1).

Pollen tube reached the ovary of self-compatible 'Lapins' in 54 h when it was self-pollinated, and in 102 h when it was pollinated with 'Bing, Chelan or Summit' cultivars. Pollen tube grew and passed the style but bud did not reach the ovary when 'Lapins' was pollinated with

Table 1. Pollen viability (%) of sweet cherry cultivars determined by TTC and FDA tests (%).

Cultivar	TTC Test		FDA Test	
	Viable	Dead	Viable ^{ns}	Dead ^{ns}
0900 Ziraat	81.63 ^{bc}	10.28 ^d	88.70	11.30
Lambert	78.41 ^c	21.59 ^a	85.82	14.18
Sunburst	85.75 ^a	14.25 ^c	89.26	10.74
Lapins	82.47 ^{ab}	17.53 ^b	87.59	12.41
Starks Gold	84.51 ^{ab}	15.49 ^{bc}	86.43	13.57
Bing	84.47 ^{ab}	15.53 ^{bc}	88.15	11.85
Chelan	82.93 ^{ab}	17.07 ^b	89.18	10.82
Summit	84.28 ^{ab}	15.72 ^{bc}	88.42	11.58

Data followed by the same letters are not significantly different (1%); ns, non-significant.

Table 2. Pollen germination (%) of sweet cherry cultivars determined by hanging drop method (%).

Cultivar	0%	5%	10% ^{ns}	15%	20%
0900 Ziraat	23.21 ^a	31.40 ^a	45.57	52.03 ^{bcd}	41.13 ^b
Lambert	19.73 ^b	29.17 ^{cd}	49.93	58.26 ^a	44.92 ^b
Sunburst	19.64 ^b	28.74 ^d	48.29	58.17 ^a	45.27 ^b
Lapins	18.21 ^b	26.81 ^f	52.06	55.84 ^{ab}	53.86 ^a
Starks gold	19.05 ^b	27.38 ^e	48.84	55.14 ^{abc}	42.68 ^b
Bing	25.42 ^a	29.62 ^c	51.33	55.29 ^{abc}	53.63 ^a
Chelan	23.10 ^a	30.48 ^b	50.27	49.10 ^d	47.14 ^{ab}
Summit	24.86 ^a	25.19 ^g	44.68	51.78 ^{cd}	42.07 ^b

Data followed by the same letters are not significantly different (1%).

Table 3. Pollen production parameters and pollen homogeneity.

Cultivars	AF*	PF*	PA*	DP (%)*
0900 Ziraat	35.40 ^{ab}	84.163 ^e	2.377 ^b	75.48 ^c
Lambert	34.70 ^{ab}	98.710 ^d	2.844 ^a	87.68 ^a
Sunburst	35.20 ^{ab}	104.018 ^b	2.955 ^a	85.06 ^{ab}
Lapins	36.40 ^{ab}	103.814 ^b	2.852 ^a	88.72 ^a
Starks gold	38.60 ^a	110.692 ^a	2.867 ^a	90.13 ^a
Bing	30.90 ^b	102.557 ^{bc}	3.244 ^a	88.53 ^a
Chelan	34.10 ^{ab}	101.019 ^{cd}	2.962 ^a	91.86 ^a
Summit	34.70 ^{ab}	104.248 ^b	3.004 ^a	79.17 ^{bc}

Data followed by the same letters are not significantly different (1%). AF*, number of anthers per flower; PF*, number of pollen grains per flower; PA*, number of pollen grains per anther = PF/AF; DP*, percentage of well-developed pollen. Means represented with the same letter in a column are not significantly different.

the other cultivars.

It has been reported that, in almond (Pimienta and Polito, 1983) and in peach (Arbeloa and Herrero, 1987) the pollen tubes grow in a discontinuous fashion. The pollen tubes grew in the base of the style within 4 day after pollination in almond and 7 days in peach. Then, the pollen tubes growth stop for 3 days for almond, for 5 days in peach until the embryo sac developed further or until

suitable conditions occurred for fertilization.

Following pollination, the time required for fertilization was reported to be 2 to 4 days for cherries (Crane and Brown, 1937), 4 to 5 days for almond (Grigs ve Iwakari, 1975) and 4 to 8 days for apricot (Mahanoglu et al., 1995).

Oukabli et al. (2000) reported that, pollen tubes grew at similar speed in the pistil of 'Tuono', after self- and crosspollination, although, they observed a delay in the

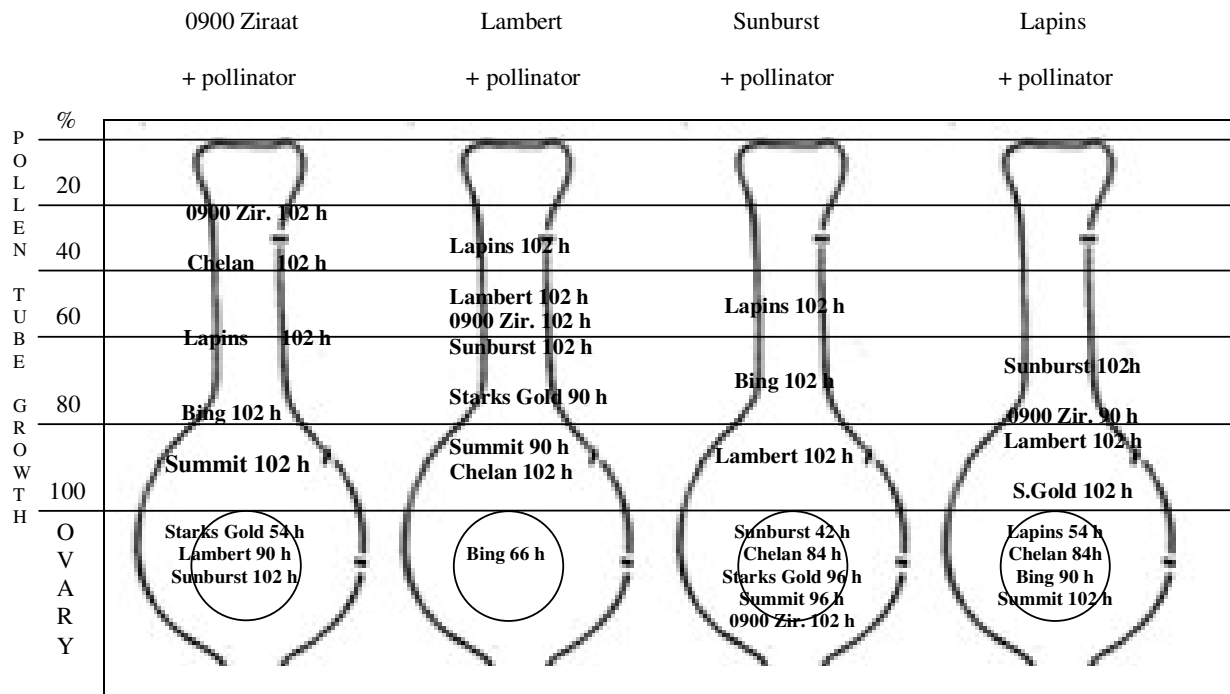


Figure 1. Pollen tube growth in the pistil 24 to 102 h after self and cross pollination of the sweet cherry cultivars (0900 Ziraat, Lambert, Sunburst and Lapins) pollinated with various pollinizers. The pause of pollen tube growth of unsuccessful pollinizers is indicated above dashed line.

Table 4. Means, pollen tube (%) that reached the ovary and fruit set (%) of sweet cherry cultivars pollinated with various pollinizers.

Pollinator cultivar	Main cultivar							
	0900 Ziraat		Lambert		Sunburst		Lapins	
	A (%)	B (%)	A (%)	B (%)	A (%)	B (%)	A (%)	B (%)
0900 Ziraat	0.00 ^c	0.00 ^c	0.80 ^b	1.60 ^b	6.25 ^c	4.15 ^{cd}	5.00 ^e	2.45 ^b
Lambert	21.25 ^b	10.80 ^b	0.80 ^b	1.13 ^b	3.75 ^c	2.14 ^{de}	3.75 ^e	3.40 ^b
Sunburst*	17.50 ^b	10.14 ^b	1.25 ^b	1.88 ^b	62.25 ^a	39.42 ^a	3.75 ^e	2.28 ^b
Lapins*	0.00 ^c	1.30 ^b ^c	0.00 ^b	0.92 ^b	5.00 ^c	3.79 ^{cd}	56.25 ^a	36.97 ^a
S.Gold**	47.50 ^a	36.79 ^a	6.25 ^b	2.74 ^b	23.75 ^b	10.36 ^b	6.25 ^d ^e	3.64 ^b
Bing***	0.00 ^c	0.80 ^b ^c	45.00 ^a	33.57 ^a	1.25 ^c	1.10 ^e	25.00 ^b	12.19 ^b
Chelan	0.00 ^c	0.00 ^c	3.75 ^b	1.09 ^b	7.50 ^c	5.30 ^c	12.50 ^{cd}	8.17 ^b
Summit	2.50 ^c	3.09 ^{bc}	7.50 ^b	2.23 ^b	18.75 ^b	5.68 ^c	13.75 ^c	3.38 ^b
Free pollination	-	34.26 ^a	-	33.78 ^a	-	36.80 ^a	-	33.56 ^a
Mean	11.09	10.79	8.16	8.72	16.06	12.08	15.78	11.78

Data followed by the same letters are not significantly different (1%). *Self- fertile cultivars; **, control for 0900 ziraat; ***, control for Lambert. A, Pollen tube (%) that reached the ovary; B, fruit set (%).

elongation of the embryo sac in the case of the selfed ovule. Pollen tube growth was expressed as the percentage of the total style length traversed by the longest pollen tube (Lewis, 1942).

In compatible combinations, pollen tubes usually reached ovaries within 2 to 3 days after pollination. In self-compatible cultivars, pollen tubes reached the ovaries in a less time when self-compatible cultivars were selfed

pollinated.

Fruit set

The results of pollen tube growth and fruit set of self, cross and free pollinated flowers are given in Table 4. The highest fruit set of 36.79% (after 45 day pollination)

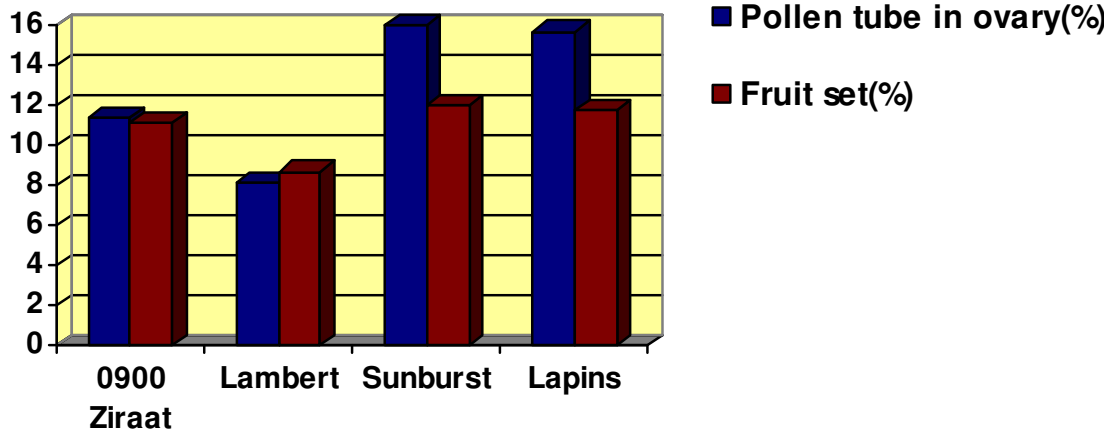


Figure 2. Comparison of means for fruit set and pollen tubes number in ovary percentage of crosses.

was obtained with 0900 Ziraat x Starks Gold combination followed by free pollinated Sunburst (36.80%), free pollinated Lambert and 'Lambert x Bing' combination which gave final fruit set of 33.78 and 33.57%, respectively. Self pollinated, 'Sunburst' and Lapins', control trees had the highest fruit set with 39.42 and 36.97%, while free pollination resulted in 36.80 and 33.56% fruit set, respectively. The means of results for pollen tube growth and fruit set were given in figure 2.

Bekefi (2004) reported that in sweet cherries, the fruit set was above 30%, between 20 and 30%, between 10 or 20% and below 10%, depending on the cultivars. These ratios of fruit set were described as extremely high, high, medium and low, respectively. Other studies reported that, fruit set ratios differed between 0 and 70% (Öz, 1985; Ülger and Özçağiran 1989; Eti et al., 1996; Paydaş et al., 1998; Arzani and Khalighi, 1998; Sütyemez and Eti, 1999; Pirlak, 2001; Tosun and Koyuncu, 2007, Beyhan ve Karakaş, 2009). Fruit set ratios of 15.5, 19.4 and 26.5%, were found for the combination of '0900 Ziraat x Starks Gold' by Öz (1985), Sütyemez and Eti (1999) and Tosun and Koyuncu (2007), respectively. Oukabli et al. (2000) observed some negative effects of self-fertilization on the ovule viability, although considering the low number of samples examined; the differences with cross-fertilization were insignificant.

Conclusion

The results were obtained from self, cross and free pollination of eight cherry varieties at KSU Prof. Dr. Nurettin Kaska Nuts Research and Application Center in year 2008. TTC and FDA pollen viability tests indicated that, 'Sunburst' had the highest values, while pollen germination test using hanging drop method (0, 5, 10 and 15% sucrose) indicated that, 'Lapins' and 'Bing' showed the highest numbers. 'Starks Gold' showed the highest pollen production while pollen homogeneity was found high in all the varieties.

In some pollination applications, pollen tube reached the ovary but could not fertilize them. Pollen of self-fertile varieties reached the ovary in less time (42 h) than those of the self-incompatible varieties. Pollen tube of well-adjusted combinations required more time (2 to 3 days) to reach the ovary. This study confirms that the fruit set in cherries could be satisfactory when the right combination of the main and pollinizer cultivars are chosen.

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