

## Effects of pollen source on fruit set and pollen tube growth of sweet cherry (*Prunus avium* L.) CV. 'Tak Daneh Mashhad'

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### ABSTRACT

In this experiment, effects of pollen source of 'Siah Mashhad', 'Sorati', 'Zodras' and 'Stella' cultivars on fruit set and pollen tube growth of sweet cherry (*Prunus avium* L.) CV. 'Tak Daneh Mashhad' was studied as randomized complete block design (RCBD) in triplicate statistically. The results showed that 'Siah Mashhad', 'Sorati', 'Pishras' and 'Stella' cultivars are the suitable pollinizers for 'Tak Daneh Mashhad' cultivar, and also 'Tak Daneh Mashhad' cultivar is a self-incompatible cultivar, which produced the most fruit in cross-pollination, it should be used as mixture for orchard establishment. Results of this research showed that fruit set in pollination combinations was different. So that the highest fruit set was in 'Tak Daneh Mashhad' (38%) using 'Pishras' pollen grains, while the lowest was fruit set (22%) in this cultivar by 'Sky' pollen grains. Also, this investigation showed that the pollen tube growth in style with different treatments was different. But, only compatible pollen tube reached to base of style and the time required 120 hours after pollination was determined. These microscopic examinations and observations confirmed the obtained results from controlled hybridization in garden.

**KEYWORDS:** cherry, self incompatibility, fruit set, pollen tube growth.

### 1. INTRODUCTION

Cherry (*Prunus avium* L.), belonging to rosaceae and sub-family of prunoideae, is always in the focus of attention as one of the most popular fruits of temperate zone fruits, but most of cherry cultivars have always had difficulties of fertilization and fruit set because of self-incompatibility, and since a kind of cross-compatibility is also seen among different cultivars, sometimes inter cultivating two different cultivars will not solve the problem of fertilization and fruit set. Perhaps a lot of orchards have been established due to unawareness of orchard owners from this issue that encounter the problem of fruit set after a few years and they need improvement using pollinizer cultivars [4, 5, 10, 11, 14, 20, 21].

In the world, the failure of fertilization and fruit set has been known since old times and has been showed that all the cherry cultivars are self-incompatible [9]. Furthermore, it has been reported that most of the important cultivars like Bing, 'Sorati' and 'Lambert' are cross incompatible and are in the same group of incompatibility. It was founded that six S-alleles are responsible for incompatibility of cherry cultivars [9, 24]. So that Tehrani and Lay (1988) [22] could suggest 14 groups of incompatibility for cherry cultivars. The recent researches have shown that each group produces a certain S-RNase. These S-RNases have been known for studying of S-alleles variety in *Prunus* genus (Sonneveled *et al.*, 2001) [18] and sweet cherry [7, 26]. According to fruit set reports in the results of Tosun and Koyuncu's (2007) [23] show that when Bekazyo, Bing, Nobel, Starscold, Stella and Vesta cultivars are used as pollinizers for female Ziavat 0900, a significant difference of fruit set is observed among pollinators and the best combination fruit set has been acquired in the combination of Starcold and Zivarat. On the other hand, investigation of the germination percent of the above mentioned cultivars is statistically different so that the highest germination percent was 51.50 in Picario cultivar and the least germination percent was 36.25 in Ziavat cultivar.

In Iran, the pollination compatibility of cherry cultivars like Shabestar, Sorati Lavasan, 'Sorati' and Cilege Blamarka with Siah Mashhad cultivar has been investigated (Arzani, 1998) [3]. Mahmoodi, *et al* (2006) [16] investigated the compatibility or incompatibility of 'Zarde Daneshkadeh', 'Protiva', 'Sorati Lavasan', 'Haj-Yosef' and 'Meshkin Shahr' cultivars with 'Red Rezaeieh' as mother parent and their results showed the mean of fruit set in 'Red Rezaeieh' with use of pollen grain of 'Zarde Daneshkadeh', 'Protiva', 'Sorati Lavasan', 'Haj-Yosefy' and 'Meshkin Shahr' were 0.54, 40.49, 0.24, 0.80 and 61.36%, respectively. Therefore 'Protiva' and 'Meshkin Shahr' cultivars were selected as the best pollinizer for 'Red Rezaeieh'. Recent studies of Tosun and Koyuncu (2007) [23], Ipek *et al* (2011) [13] and Sutyemez (2011) [20] in relation to determining the cherry genotypes and cultivars self-incompatible groups existing in Turkey showed that not only most of cherry cultivars existing in Turkey are self-incompatible, but also some cultivars are cross-incompatible with each other. In this regard, the present study has been done in order to effects of pollen source on fruit set and pollen tube growth of sweet cherry (*Prunus avium* L.) CV. Tak Daneh Mashhad.

### 2. MATERIALS AND METHODS

In order to effects of pollen source of 'Siah Mashhad', 'Sorati', 'Zodras' and 'Stella' cultivars on fruit set and pollen tube growth of sweet cherry (*Prunus avium* L.) CV. 'Tak Daneh Mashhad', an investigation was performed in Kraj orchard complex dependent to Agricultural Jihad Ministry and it is located in Kraj, Alborz Province. This complex is

located in 25 kilometers from Karaj. For selecting branches and isolating flowers, after frequent visits from orchard a few days before anthesis of selected cultivars for hybridizing and determining their compatibility status, branches which had enough flower buds were selected in four different parts of parent trees and were specified with labels. In order to prevent from open pollination, before anthesis the selected branches were covered with jaconet cloth bags of 70 centimeters long and 30 centimeters wide which had been designed and prepared beforehand. Meanwhile, in order to prevent from direct contact of cloth bag with flowers, the branches were put in wire shields. This experiment was carried on the basis of randomized complete block design (RCBD) in 10 replicate statistically. Before pollination, to make sure of germination power of the collected pollens and showing their viability, solution containing 15 percent sucrose and 20 milligram per liter of boric acid was provided and became jelly by adding 1 percent agar, was cultured. The practice of pollination was done by using pollens which had been collected before two days after flowers flourishing. The pollens were put on the stigma by a small brush. An especial brush was used for pollination of each cultivar. Hands and devices were infected by ethyl alcohol during all the processes so that the pollens of different cultivars wouldn't be mixed with each other. Pollination was done again by opened flowers of paternal parent which had been covered by jaconet cloth bags before so as to be sure of pollination. For studying the pollen tube growth, at least 5 flowers of each branch were selected in different hours after pollination and were put in FAA fixation (%5 Aldehyde, %5 Glacial acetic acid, %24 water of distilled twice, % 65 alcohols). Then samples were brought out of FAA fixation and washed several times and were put in the glass vials containing 15 milliliter of alcohol %65 at the temperature of 4 °C until the time of observation were kept. To prepare the samples for microscopic observation (samples coloring), we used %1 aniline blue and %1 potassium phosphate using Cerovic and Ruzic method (1992). The average germination of pollen in stigma and pollen tube in higher and middle part of style were recorded for each pistil. The pistil percent with pollen tube obtained of pollination at the end of style and the average of the number of pollen tube obtained of pollination at the end of style were recorded with fluorescence microscope (Leitz & Wetzler).

In orchard, treated branches, all the flowers were counted before and after pollination. Falling of flowers and fruit and the number of acquired fruit and fruit set percent were determined. Finally the compatibility results of the studied cultivars were distinguished. The statistical analysis was performed using Microsoft Excel (2007) and SAS software [SAS Institute Inc., 1990] and means were compared using Duncan's Multiple Range Test (DMRT).

### 3. RESULTS AND DISCUSSION

#### 3.1. Fruit set

In order to determine the fruit set percent and also pollinated flowers fall, the obtained results were recorded three times after pollination of the cultivar of 'Tak Daneh Mashhad', in an order that bags were taken out, the pollinated flowers were counted again and the rate of flower fall was specified. According to the number of pollinated flowers on each branch, the formed fruit percent was the base of statistical analysis. Fruit counting was carried out 3 times after pollination. The first counting was done in the time of pollination (2012/4/23), the second counting was carried out 15 days after the first counting (2012.5.8), the third counting was performed at the time of harvest (2012. 7.8). The number, the percent of each trial unit fruit and each treatment were investigated in the mentioned dates. Also, in order to be sure of the tested pollens living, the pollens of every four cultivars were cultured before pollination. The obtained results showed that the germination power of the above mentioned cultivars pollens were over %70. This test specified that the pollens were strong and healthy and has viability and the next steps in this investigation were carried out with more certainty.

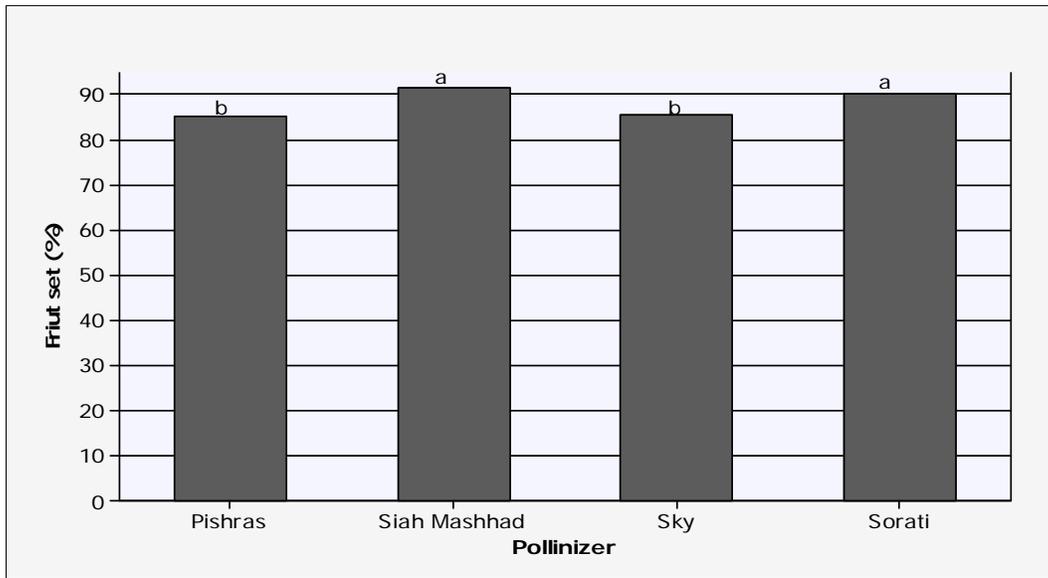
The results of the variation analysis of the data related to the fruit set percentage in different countings (1 to 3) in Table 1 show that there is no significant difference of fruit set percent among the studied cultivars in the first counting and it is also obvious that there is significant difference among treatments in the difference countings.

**Table1.** Variation analysis of the data pertaining to the fruit set in different countings

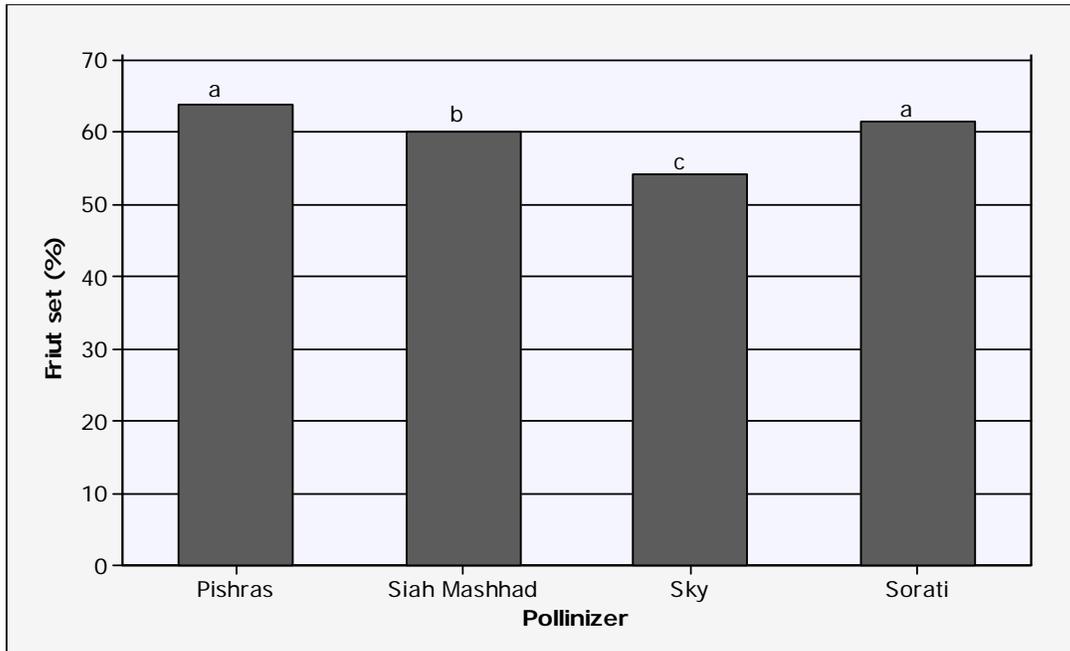
S.V	df	MS		
		1 <sup>rd</sup> counting	2 <sup>nd</sup> counting	3 <sup>st</sup> counting
<b>Pollinizer</b>	3	102.09**	170.433**	639.53**
<b>Replication</b>	9	21.67	14.722	4.10
<b>Error</b>	27	14.94	5.544	2.48
<b>Cv%</b>	39	4.39	3.94	5.21

\*: Significant at the possibility level of %5 \*\*: significant at the possibility level of %1 ns: not significant

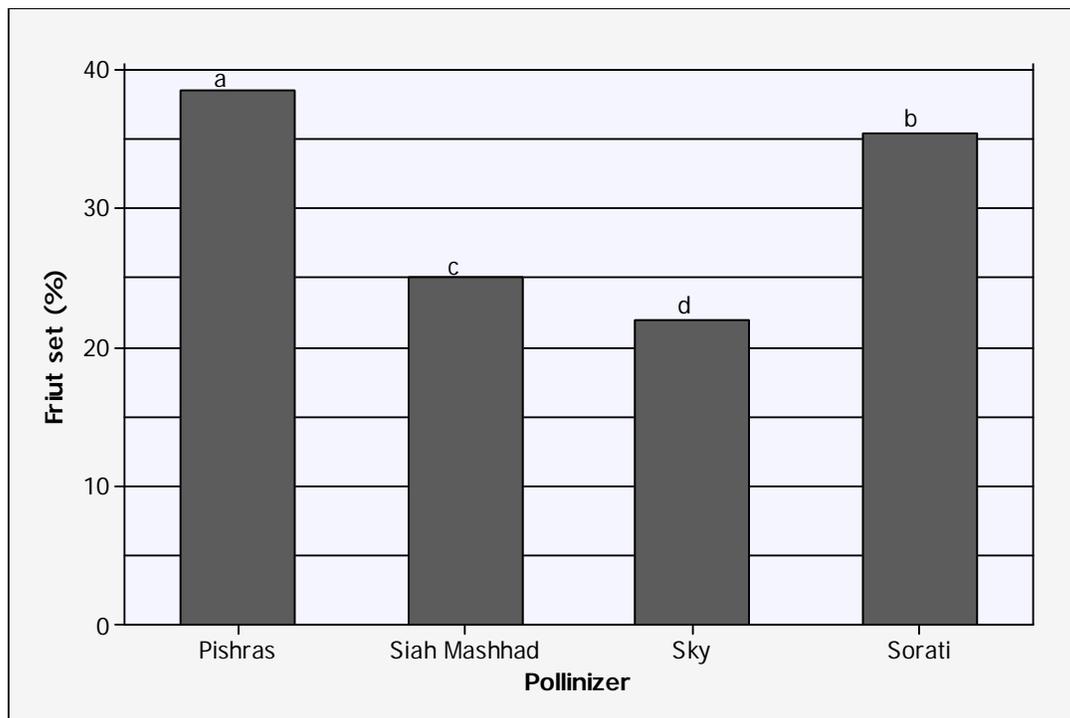
The comparison of the average effects of pollinizers on the number of fruit set in different countings is shown in Figure 1 to 3.



**Fig. 1.** Effects of male cultivars on the percent of fruit set in 'Tak Daneh Mashhad' in first counting  
\*Mean values marked with the same letter do not differ significantly at  $p = 0.05$  according to Duncan's multiple range tests.



**Fig. 2.** Effects of male cultivars on the percent of fruit set in 'Tak Daneh Mashhad' in second counting  
\*Mean values marked with the same letter do not differ significantly at  $p = 0.01$  according to Duncan's multiple range tests



**Fig. 2.** Effects of male cultivars on the percent of fruit set in 'Tak Daneh Mashhad' in final counting

\*Mean values marked with the same letter do not differ significantly at  $p = 0.01$  according to Duncan's multiple range tests.

The averages comparison results of different pollinizers pollen on the fruit set of 'Tak Daneh Mashhad' in Figure 1 show that 'Siah Mashhad' and 'Sorati' pollinizers had the most effect on the fruit set while 'Sky' and 'Pishras' pollinizers had the least effect on the fruit set in 1<sup>st</sup> counting and this shows that 'Siah Mashhad' and 'Sorati' cultivars may be the best pollinizers for this cultivar.

Also, the results of the averages comparison in Figure 2 show that 'Pishras' and 'Sorati' pollinizers had the most effects on the 'Tak Daneh Mashhad' cultivar for fruit set in the 2<sup>nd</sup> countings whereas 'Siah Mashhad' and 'Sky' pollinizers had the least effect for fruit set respectively.

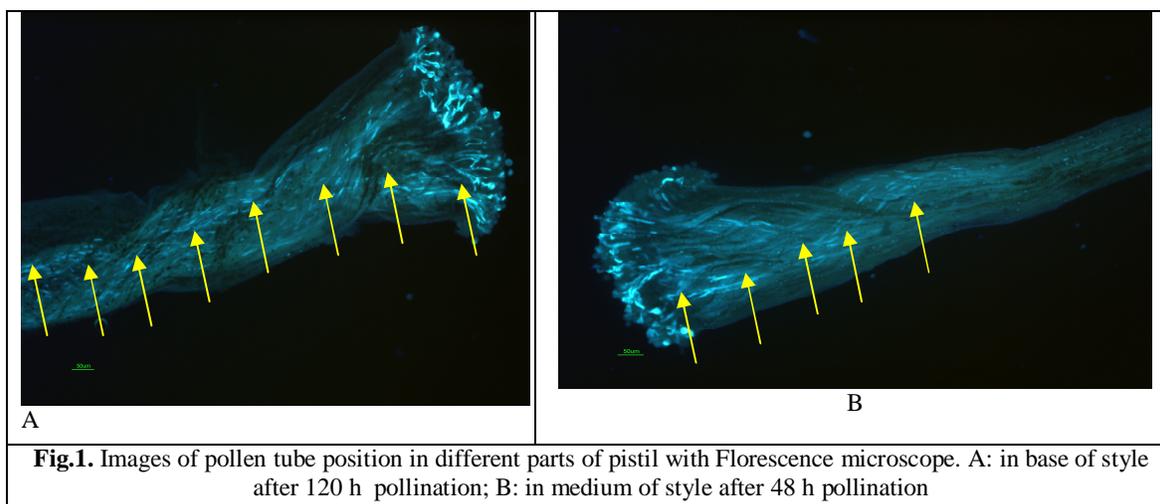
Finally, The effect of the 'Pishras' pollinizer on the 'Tak Daneh Mashhad' cultivar was the most for fruit set in the 3<sup>rd</sup> countings and this shows the good compatibility of these two cultivars and the effect of the 'Sky' pollinizer on the 'Tak Daneh Mashhad' cultivar was the least for fruit set in the 3<sup>rd</sup> 'Tak Daneh Mashhad' and this shows the less incompatibility of this cultivar with the 'Tak Daneh Mashhad' cultivar. So that the highest fruit set was in 'Tak Daneh Mashhad' (38%) using 'Pishras' pollen grains. while the lowest was fruit set (22%) in this cultivar by 'Sky' pollen grains. Similar results were reported by other researchers [2,5,7,13,16]. In their results, ratios of fruit set were different depending on various factors especially the pollinizer [20].

### 3.2. The study of pollination by using florescence microscope

For studying of pollination with florescence microscope (UV), the pollen tube growth in different parts of the style after 36, 48, 56, 72 and 120 hour's pollination according to method of Alonso and Socias I company (2005) were investigated. A sample of microscopic images has been shown in figure 1. This investigation showed that the pollen tube growth on the stigma and on style with different treatments was different (Table 5.). But, only compatible pollen tube reached to base of style. In 'Pishras' pollen treatment on the 'Tak Daneh Mashhad' 120 hours after pollination, the average of the number of germinated pollens on the stigma was approximately 90 percentage and the 80 percentage pollen tube growth has reached base of style and this is in case that after 48 hours the pollen tube growth percolated up to one third of upper of style that shows the pollen growth speed in different parts of the style are influenced by time and the type of pollen (compatible and incompatible) and environmental conditions. Compatible pollen tubes reached the ovary of self-incompatible 'Tak Daneh Mashhad' in 120 h when it was pollinated with 'Pishras' cultivar. Similar results in cherries (Crane and Brown, 1937) [9], almond (Grigs and Iwakari, 1975) [12], apricot (Mahanoglu *et al.*, 1995) [17] and sweet cherry (Sutyemez, 2011) [20] have reported.

**Table5.** Pollen tube growth in different parts of the style after 36, 48, 56, 72 and 120 hour's pollination of 'Tak Daneh Mashhad'

pollinizer	After pollination (hours)	Less than 25% pollen tube in base of Style(self incompatible)	25% to 50% pollen tube in base of Style(doubly self compatible)	50% to 75% pollen tube in base of Style(self compatible)	more than 75% pollen tube in base of Style(complete self compatible)
'Siah Mashhad'	36				
'Sorati'	36				
'Pishras'	36				
'Sky'	36				
'Siah Mashhad'	48				
'Sorati'	48				
'Pishras'	48				
'Sky'	48				
'Siah Mashhad'	56				
'Sorati'	56				
'Pishras'	56				
'Sky'	56				
'Siah Mashhad'	72				
'Sorati'	72				
'Pishras'	72				
'Sky'	72				
'Siah Mashhad'	120			*	
'Sorati'	120				*
'Pishras'	120				*
'Sky'	120		*		



**Fig.1.** Images of pollen tube position in different parts of pistil with Florescence microscope. A: in base of style after 120 h pollination; B: in medium of style after 48 h pollination

**4. Conclusion**

Results of this research showed that fruit set in pollination combinations was different. So that the highest fruit set was in 'Tak Daneh Mashhad' (38%) using 'Pishras' pollen grains, while the lowest was fruit set (22%) in this cultivar by 'Sky' pollen grains. Also, this investigation showed that the pollen tube growth in style with different treatments was different. But, only compatible pollen tube reached to base of style and the time required 120 hours after pollination was determined. These microscopic examinations and observations confirmed the obtained results from controlled hybridization in garden. 'Tak Daneh Mashhad' cultivar was founded self- incompatible and it is better to inter-cultivate with suitable pollinizer cultivars for orchard establishment.

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