Araştırma Makalesi
(Research Article)

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The Effect of Cross-Pollination on Fruit Set and Quality in 'Robinson' and 'Fremont' Mandarins

'Robinson' ve 'Fremont' mandarin çeşitlerinde yabancı tozlanmanın meyve verim ve kalitesine etkisi

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ABSTRACT

Previous studies on cross-pollination of C*itrus* varieties have shown the existence of a wide variation in fruit set ratio, fruit size, and number of seeds per fruit with different pollinators. In the current study, the effects of four pollinator cultivars ('Rhode Red Valencia', 'Midknight Valencia', and 'Valencia Late' oranges and 'Rio Red' grapefruit) on the fruit set, and some fruit quality characters of 'Robinson' and 'Fremont' mandarin cultivars were determined. The experimental setup was a complete randomized design with three replications of each combination. The effects of cross-pollination on fruit set and pomological characters, such as fruit weight, total soluble solids (TSS), TSS:TA (total acids) ratio, and number of seeds per fruit were statistically significant. At maturity, female parent cultivars' flowers that were cross-pollinated with 'Valencia Late' orange, had the highest fruit set with 23.08% and 21.57%, respectively. The heaviest fruits of 'Robinson' mandarin were obtained by cross-pollination with 'Rhode Red Valencia' orange. Avarege fruit weight from open-pollinated was higher than those from cross-pollination in 'Fremont' mandarin. According to the results, 'Rhode Red Valencia' and 'Valencia Late' oranges for 'Robinson' mandarin and 'Midknight Valencia' orange, and 'Rio Red' grapefruit for Fremont mandarin, were determined as the best crossings.

Anahtar Sözcükler:

Yapay melezleme, C*itrus reticulata*, meyve tutumu, meyve kalitesi

ÖZET

Farklı turunçgil tür ve çeşitlerinde yapılan tozlama çalışmaları, farklı tozlayıcıların meyve tutum oranı, meyve iriliği ve meyve başına tohum sayısı üzerine etkilerinin oldukça geniş bir varyasyon gösterdiğini ortaya koymuştur. Bu çalışmada, 'Robinson' ve 'Fremont' mandarin çeşitlerinin meyve tutumu ve bazı meyve kalite özellikleri üzerine dört farklı tozlayıcı çeşidin ('Rhode Red Valencia', 'Midknight Valencia' ve 'Valencia Late' portakalları ile 'Rio Red' altıntopu) etkileri araştırılmıştır. Denemede her bir çeşit Tesadüf Parselleri Deneme Desenine göre 3 tekerrürlü olarak yer almıştır. Meyve tutumu ile meyve ağırlığı, suda çözünebilir kuru madde (SÇKM) miktarı, SÇKM/Asit oranı, meyve başına tohum miktarı gibi pomolojik özellikler üzerine tozlayıcıların etkisi istatistiksel olarak önemli bulunmuştur. Olgunluk döneminde her iki ana ebevyn çeşidinde de en yüksek meyve tutumu sırasıyla %23.08 ve %21.57 ile 'Valencia Late' portakalı ile melezlendiğinde elde edilmiştir. 'Robinson' mandarininde en ağır meyveler 'Rhode Red Valencia' melezinden elde edilmişken, 'Fremont' mandarininde serbest tozlanma koşularında melezlemeye göre daha büyük meyveler alınmıştır. Elde edilen sonuçlara göre, en iyi tozlayıcılar 'Robinson' mandarini için 'Rhode Red Valencia' ve 'Valencia Late' çeşitleri, Fremont mandarini için ise 'Midknight Valencia' ve 'Rio Red' çeşitleri olmuştur.

INTRODUCTION

Citrus is one of the most important fruit genera in rendering economical benefits in Turkey and around the world. Turkey's citrus production reached 3681158 tons in 2013, with an increase of 36% in the last 10 years. The statistics from 2013 onwards demonstrated that tangerine is the second most voluminous (942226 tons) species of the total citrus production in Turkey (Anonymous, 2013). In the Mediterranean region of Turkey, the most commonly grown tangerine cultivars are 'Owari' and 'Okitsu' Satsumas, 'Clementine', 'Fremont', 'Nova', and 'Robinson' mandarins (Demirkeser et al., 2009).

'Robinson' mandarin is derived from crossing between 'Clementine' mandarin and 'Orlando' tangelo. The quality of fruit is excellent (Saunt, 1990), and it possesses the typical mandarin characteristic of an easily separable peel (Davies and Albrigo, 1994). It requires cross-pollination with compatible cultivars, but fruits are also parthenocarpic (Tuzcu, 1990). 'Fremont' mandarin is a hybrid between the 'Clementine' and 'Ponkan' mandarins. The fruit size is small and the peel color is deep orange. The fruit has high juice content and seed number. This cultivar has a high yield per tree and is self-compatible (Saunt, 1990; Tuzcu, 1990; Kaygisiz and Aybak, 2005).

Self-incompatibility is a problem with several of the citrus hybrid varieties. The problem is due to slow pollen tube growth and/or resultant inadequate cross-pollination. Fruit set is often quite low. One means of overcoming self-incompatibility is cross-pollination with a compatible pollen; however, this results in seedy fruit. A good pollenizer should produce large amounts of pollen, produce flowers every year, and produce commercially marketable fruit (Futch and Jackson, 2003).

In *Citrus* species and varieties, many studies have been carried out in order to determine good pollenizer. It was reported that 'Bhadri Lemon' proved to be a good pollinizer for higher yield of quality 'Kagzi Kalan' lemon fruits (Thomas et al., 2000). The fruit sets from selfing of 'Fina', 'Marisol', 'Fina Sodea', 'Nules' Clementines and 'Afourer' mandarin were very low or near 0. Fruit set was highest (20% to 40%) in cross-pollination between two Clementines, 'Nules' and 'Fina Sodea', and 'Afourer' mandarin (Cho, 2005). Demir et al. (2015) found the effects of self-pollination, naturel open pollination and reciprocal pollination on fruit set of limon varieties ('BATEM Pınarı', 'BATEM Sarısı', 'Interdonato', 'Kütdiken', 'Italyan Memeli', 'Meyer', 'Lamas'). According to results, 'Italyan Memeli' lemon with 35.17% was determined the highest rate on account of percentage of fruits at harvest time. Similar studies were also obtained in the other *Citrus* cultivars by Eken (2006), Waqar et al. (2007), Papadakis et al. (2009), Stephen and Larry (2009) and Seday (2010).

Many researchers (Futch and Jackson, 2003; Chao, 2005) reported that using appropriate pollenizers is one of the most efficient and environmental-friendly agricultural practice to improve yield and fruit quality of self-incompatible citrus varieties. The aim of this study was to investigate the yield and fruit quality of 'Robinson' and 'Fremont' mandarin as well as of 'Valencia' orange varieties and 'Rio Red' grapefruit when they were self-pollinated in solid plantings or cross-pollinated in mixed variety plantings.

MATERIAL and METHODS

Plant material

This research was conducted on 'Robinson' and 'Fremont' mandarins in the 2010 and 2011 growing seasons in the regions of Dörtyol (Hatay), Turkey (36°09′E, 36°51′N; 9 m above the sea level). The climate, according to Köppen climate classification, is of the Csa type (subtropical with moderate and rainy winters, hot and dry summers), with 28.5°C maximum temperature, 11.8°C minimum temperature, 20.1°C average temperature, and 729.6 mm rainfall. Plant materials were planted at a spacing of 7 m × 7 m, and grafted on sour orange (*Citrus aurantium*) rootstock in November 1998.

Pollination

'Rhode Red Valencia', 'Midknight Valencia', and 'Valencia Late' orange cultivars and 'Rio Red' grapefruit cultivar were used as pollinators for 'Robinson' and 'Fremont' mandarins. All previously opened flowers and small, immature buds in 'Robinson' and 'Fremont' mandarins used as female parent were removed. The remaining buds were mechanically opened and the stamens removed. Pollen were applied to the pistil of each emasculated flower, and the branch was labeled. Each pollination treatment was applied to flowers on three trees (about 100 flowers per treatment). For open pollination, opened flowers tagged and recorded, as for small, immature buds removed.

Fruit set and quality

Fruit set per tree was separately calculated by the relation: the number of fruits/the number of blossoms hand-pollinated at 60 days after pollination and at maturity. Fruits were harvested in early December of each year. In two of the experimental years, 20 fruits per cross-combination were collected to evaluate fruit

quality. Fruits were weighed, and fruit juice was extracted with an electrical squeezer and number of seed per fruit was counted. The juice content (percentage) was calculated by the relation juice weight:fruit weight. TSS content was determined by direct reading in a hand refractometer (Atago ATC-1E model). Ratio was calculated by the relation TSS:TA concentration. Fruit rind color was determined with a Minolta Chroma Meter CR-300 (Osaka, Japan). Color measurements were recorded using the CIE L*a*b* color space. Fruit skin color was expressed as lightness (L) and hue angle ($h°$). From these values, hue angle was calculated as $h° = \tan^{-1}$ (b*/a*). The hue angle is expressed in degrees and is a measure of color that, for example, from 0 to 90°, spans from red to orange to yellow. Lightness separates color into bright and dark.

Statistical analysis

The experimental setup was a complete randomized design with three replications of each combination. Fruit set and fruit quality characters were evaluated by variance analysis using GLM procedure of SAS software (SAS Institute Inc., North Carolina, USA), and means were separated by Tukey test at 5% significance.

RESULTS and DISCUSSION

The fruit set was affected by different pollinators for both cultivars (Table 1). At the 60th day after pollination, the highest percentage of fruit set for both 'Robinson' and 'Fremont' mandarins was obtained by cross-pollination with 'Rhode Red Valencia' and 'Valencia Late' orange cultivars. 'Robinson' and 'Fremont' mandarin flowers cross-pollinated with 'Valencia Late' cultivar had the highest fruit set at maturity. When both 'Robinson' and 'Fremont' mandarin flowers were pollinated by 'Midknight Valencia' orange, fruit set at maturity had the lowest compared with other pollen sources. Open pollination had lower fruit set than cross-pollination. According to the average of cross-pollination, fruit sets at maturity of 'Robinson' and 'Fremont' mandarins were about 5.1 and 2.5 times higher than those on open pollination, respectively.

Table 1. Variation in fruit set among cross-pollinators in 'Robinson' and 'Fremont' mandarins (%)

Pollen variety	Robinson		Fremont	
	After 60 days	At maturity	After 60 days	At maturity
Rhode Red Valencia	29.76 a(1)	21.54 ab	31.25 a	17.61 ab
Midknight Valencia	11.21 c	8.95 c	21.00 b	13.50 bc
Valencia Late	24.77 a	23.08 a	30.80 a	21.57 a
Rio Red	18.82 b	18.82 b	24.75 b	17.58 ab
Open-pollination	6.16 c	3.50 d	8.26 c	7.06 c
HSD	5.33	4.10	4.75	7.49

(1): Means with a different letter in each column are significantly different at p≤0.05 (Tukey test).

Citrus trees produce an abundance of flowers, but all flowers not set fruit. A good crop may be borne if only 3–7% of the flowers that are set yield mature fruit (Keogh et al., 2010). In 'Robinson' mandarin, Eti et al. (1989) observed 3–4% fruit set under open pollination which is in line with the present results. This cultivars' requirement of cross-pollination was shown by previously reported experiments (Reece and Register, 1961). Further, Futch and Jackson (2003) reported that cross-pollination in respect of self-incompatible types increased fruit set. Similar results were obtained for 'Ellandale' tangor (Vithanage, 1991), 'Minneola tangelo' (Ozkan and Eti, 1992), 'Nova' tangelo (Demirkeser et al., 2001; Ferraro et al., 2006; Papadakis et al., 2009), 'Oroval' Clementine (Wallace, 2004), 'Fina', 'Marisol', 'Fina Sodea', 'Nules' and 'Afourer' (Chao, 2005), 'Nules', 'Fina Sodea', and 'Murcott' (Fang et al., 2008). 'Fremont' mandarin, which is used as pollinator for 'Clementine' and its hybrids in Turkey, has self-compatibility. This experiment showed that the fruit set increased by cross-pollination compared with the open pollination. Hossain and Rabbani (2011) similarly observed that in the case of cross-pollination, the tree had higher fruit set percentages than open pollination in the other citrus genus. Nevertheless, the low fruit setting seemed to be due to cross incompatibility among the parents. It might be due to genetical factor. Domingues and Tulmann (1999) observed that 68% varieties set fruit under open pollination, 15% under self-pollination and 35% and %15 by cross-pollination with 'Minneola' and 'Troyer' pollinators, respectively, in 34 representative varieties of sweet orange.

The effects of pollinators on fruit quality characteristics of 'Robinson' and 'Fremont' mandarins are shown in Tables 2 and 3. The heaviest fruits of

'Robinson' mandarin were obtained through cross-pollination by 'Rhode Red Valencia' orange. The fruit weight from open pollination in 'Fremont' mandarin was higher than those from cross-pollination combinations. Small fruit size is particularly common in mandarins. Fruit size is a function of cell division and cell enlargement processes. Competition affects final fruit size in citrus. There is an inverse relationship between fruit size and flower number and fruit number per tree. Consequently, flower and fruit thinning, both manually and chemically, have been used to improve fruit size. Fruit size is mainly determined by the genetic make-up of the cultivar, but can be affected by cultural practices, factors related to the tree condition, and climatic factors (El-Otmani et al., 2000).

Table 2. Effect of different pollinators on some fruit quality characters of 'Robinson' mandarin

Pollen variety	Fruit weight (g)	Number of seeds per fruit	Juice content (%)	TSS (%)	TSS:TA ratio	Fruit rind color	
						L*	hue
Rhode Red Valencia	137.49 a[(1)]	15.80 b	49.62	11.32 b	8.72 c	65.42	69.24
Midknight Valencia	116.49 cd	8.18 c	51.60	11.37 b	9.27 bc	67.62	71.83
Valencia Late	129.76 ab	27.81 a	51.66	11.79 b	9.58 b	65.79	69.57
Rio Red	124.77 bc	15.58 b	50.43	11.40 b	9.28 bc	66.69	70.80
Open-pollination	115.49 d	8.33 c	51.57	12.65 a	10.65 a	66.65	70.61
HSD	9.05	2.31	NS[(2)]	0.56	0.65	NS	NS

(1): Means with a different letter in each column are significantly different at p≤0.05 (Tukey test).
(2): NS: Non-significant.

For 'Robinson' mandarin, the crosses with 'Valencia Late' orange had an average of 27.81 seeds per fruit. The 'Robinson' mandarin × 'Midknight Valencia' orange combination had similar number of seeds per fruit with open pollination. In 'Fremont' mandarin, although the highest number of seeds per fruit was observed from 'Rhode Red Valencia' orange used as pollinator, the number of seeds obtained from 'Midknight Valencia' orange and 'Rio Red' grapefruit pollens decreased. This result is important in reducing the number of seeds per fruit of 'Fremont' mandarin because this cultivar has a high number of seeds under open pollination (Akgul and Tuzcu, 1993). In the hand cross-pollination study, a much larger quantum of pollen was applied to the stigma than would occur naturally. However, Chao (2005) reported that the maximum seed number per fruit from field sampling is similar to that of hand cross-pollination.

Table 3. Effect of different pollinators on some fruit quality characters of 'Fremont' mandarin

Pollen variety	Fruit weight (g)	Number of seeds per fruit	Juice content (%)	TSS (%)	TSS:TA ratio	Fruit rind colour	
						L*	hue
Rhode Red Valencia	64.29 bc[(1)]	12.89 a	51.60 ab	9.42 c	10.36 b	65.09	66.44 b
Midknight Valencia	65.27 ab	7.10 c	48.01 c	10.03 ab	9.94 b	65.40	68.22 ab
Valencia Late	57.64 c	11.35 ab	49.89 bc	9.44 c	8.18 d	66.59	71.66 a
Rio Red	65.12 a-c	8.08 c	48.94 c	9.75 bc	9.08 c	66.50	68.07 ab
Open-pollination	72.03 a	9.57 bc	52.34 a	10.45 a	11.74 a	65.60	69.57 ab
HSD	7.55	3.15	2.03	0.54	0.66	NS[(2)]	4.00

(1): Means with a different letter in each column are significantly different at p≤0.05 (Tukey test).
(2): NS: Non-significant.

Cross-pollination can significantly increase the fruit set and number of seeds per fruit in many citrus varieties, such as 'Robinson', 'Nova', 'Lee', and 'Page' (Hearn et al. 1968); 'Ellendale' tangor (Vithanage, 1991); 'Robinson' (Eti et al., 1989); 'Murcott', 'Imperial' and 'Ellenor' (Wallace and Lee, 1999); 'Nova' (Papadakis et al., 2009; Demirkeser et al., 2001) and 'Oroval' Clementine (Wallace, 2004) mandarins. These studies showed that there are different levels of compatibility among different citrus types, and some combinations can give very high seed numbers. Although we found a positive relationship between numbers of seeds per fruit versus fruit weight for 'Robinson' mandarin, this status did not appear in 'Fremont' mandarin (data not shown). Seedless fruits are often more sought-after, demanding higher retail prices in comparison with seeded varieties (Keogh et al., 2010). However, seedless fruits, which are in

demand by the market, are usually smaller in size and weight. Thus, in many mandarin-producing countries, plant growth regulators are used to enhance fruit size of seedless mandarins and mandarin hybrids (El-Otmani et al., 2000).

The effect of cross-pollination on juice content was found to be statistically significant for 'Fremont' mandarin, but not 'Robinson' mandarin. In 'Fremont' mandarin, the juice content decreased by cross-pollination compared with the open pollination. Eken (2006) on 'Robinson' mandarin and Seday (2010) on 'Clementine' types found that the effect of the cross-pollination on juice content were significant. Our results disagreed with the findings of Thomas et al. (2000), who reported that juice content was significantly reduced upon cross-pollination. On the other hand, Papadakis et al. (2009) resulted that juice content of mandarin fruits was not affected by the cross-pollination.

TSS content and TSS:TA ratio of cross-pollination for both 'Robinson' and 'Fremont' mandarin fruits were lower than those of open pollinated fruits. Although the lowest TSS value was obtained for 'Rhode Red Valencia' and 'Valencia Late' oranges used as pollinators in 'Fremont' mandarin, it ranged from 11.32% to 11.79% in 'Robinson' mandarin. Among different pollinators, the highest TSS/TA ratio was obtained from cross-pollination by 'Valencia Late' orange in 'Robinson' mandarin, and by 'Rhode Red Valencia' in 'Fremont' mandarin. These soluble solids are, on average, made up of 70% sugars. Citric acid and minerals in the juice also contribute to the soluble solids (Siddiqui, 2015). Total acidity has an extremely wide range between different species, but also rootstock, growing area, climate, fruit load, tree age, position of fruit on tree, irrigation, and nutrition affect acidity (Zekri et al., 2009). Also, fruit juice acidity (percentage of citric acid), and juice volume are considerably affected by fruit size. Small fruits have high acid and low TSS contents compared with larger fruits (El-Otmani et al., 2000).

The effects of pollen source on rind color L* and hue values of 'Robinson' mandarin fruit, were not found to be statistically significant (Table 2). In 'Fremont' mandarin, although the best colored fruits were harvested from crossings with 'Rhode Red Valencia' orange, the poorest colored ones were observed in 'Valencia Late' orange used as pollinator. The rind color L* value in 'Fremont' mandarin fruit was not significantly different among cross-pollinated fruits (Table 3). Similar results regarding the fruit color not affected by pollinator were registered in 'W. Murcott Afourer' mandarin (Wright, 2007). In citrus fruit, rind color is important for its aesthetic value because consumers of fresh fruit prefer brightly colored fruit and are ready to pay a high price for them. Color development results from changes in carotenoid and chlorophyll levels with concomitant changes in plastids (El-Otmani et al., 2000). Rind color is an important cosmetic preference of consumers when purchasing citrus fruit. Citrus rind color is primarily a genetic trait and is secondarily affected by climatic and other growing conditions. The major factors affecting rind color are temperature, light, nutrition, plant water relations, rootstock, and phytohormones. Other important factors include tree age, soil conditions, and crop load. Besides the direct effects of some of these factors on rind color, various indirect effects may also be important to development of rind color (Barry and Roux, 2010).

CONCLUSIONS

The results of this study demonstrate that cross-pollination increased the fruit set compared with open pollination for both mandarin cultivars. In addition, the number of seeds per fruit of 'Fremont' mandarin decreased by cross-pollination between 'Midknight Valencia' orange and 'Rio Red' grapefruit. 'Rhode Red Valencia' and 'Valencia Late' oranges could be used as suitable pollenizers for 'Robinson' because of increasing fruit set and fruit weight. However, these cultivars resulted in a high number of seeds per fruit, so fruit quality was affected negatively.

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