

Determination of Blooming, Pollen and Fruit Set Characteristics in *Punica granatum*

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Abstract

The objective of this study was to investigate flowering, pollen, and fruit set characteristics of locally registered pomegranate cultivars between two consecutive years. 'Dr. Ercan 35', 'Efenar 35', 'Kamilbey 35', and 'Tezeren 35' cultivars have been developed by cross-breeding programs. Open-, cross-, and self-pollination studies were performed with these cultivars using three trees in each replicate in the field. Number of anthers per flower, number of pollen grains per anther, viability, and germination tests were conducted in the laboratory. Fruit set ratio of self-pollinated combinations were lower than open-pollination. The fruit set ratio from self-pollination was obtained from 'Dr. Ercan' (60.3%), 'Efenar 35' (62.4%), 'Kamilbey 35' (50.5%), and 'Tezeren 35' (25.2%). The highest fruit set ratio (49.7%) was obtained from 'Kamilbey 35' × 'Dr. Ercan 35' combination. The highest number of characteristics was as follows: 'Tezeren 35' (402 anthers/hermaphrodite flowers), 'Kamilbey 35' (8550 pollen/anther, male flowers in the beginning of flowering), 'Efenar 35' (84.7% pollen viability, hermaphrodite flowers), 'Kamilbey 35' (71.2% pollen germination, hermaphrodite flowers). In conclusion, at least one, two or more, pollinator cultivars are necessary for commercial plantations using with these recently released pomegranate cultivars.

Keywords: breeding; pollination; pollen number; pomegranate; viability and germination ratio

Introduction

Pomegranate (Lythraceae, syn. Punicaceae, $2n=2x=16$), is one of the vitamin, nutrition, and antioxidant sources in human health (Mars, 1998; Jalikop, 2010; Gözlekçi *et al.*, 2011; Melgarejo *et al.*, 2012). Pomegranate has been known as one of the first cultivated plants around Fertile Crescent dated back to 11 million years ago in fossil records (Gözlekçi, 2014). As a temperate-zone fruit crop, it was believed that pomegranate was domesticated around 3,000-4,000 B.C. in north Iran, southeast Turkey, Caucasus, and India, among figs, dates, olives, and grapes (Onur, 1988; Stover and Mercure, 2007). Turkey was in the fourth place with 445,750 t in pomegranate production while China (1,200,000 t), Iran (1,009,890 t), and India (743,000 t) were other leading countries (Anonymous, 2017). Turkey exported 137,000 t pomegranate fruit in 2013. Besides fresh consumption, pomegranate fruit can be used for juice, patisserie, spice, pharmaceutical, staining, oil, animal feed, tannin, pectin, and vinegar industry (Onur, 1988; Yılmaz, 2007; Yazıcı and Şahin, 2016).

Pomegranate has andromonoecious type flowers, which means both hermaphrodite and functionally male flowers

occur on the same tree (Holland *et al.*, 2009; Wetzstein *et al.* 2011). While hermaphrodite flowers set fruit, male flowers serves as pollen source for adequate pollination.

The number of male flowers is 1.5-4.5 times more than hermaphrodite flowers (El-Kassas *et al.*, 1998; Engin and Hepaksoy, 2003). In contrast, the pollen viability of hermaphrodite flowers is lower than that of male flowers (Gözlekçi and Kaynak, 2000). For these reasons, the quantity and quality of male flowers are important for sufficient pollination and fruit set in modern pomegranate orchards. During a relatively long flowering season, from April to July, there are more hermaphrodite flowers than male flowers in the beginning of the flowering (Onur, 1988; Tibet and Baktır, 1991). In general, the fruit set was found 3-9% (Onur, 1988). The fruit set was observed as 5.9-10.6% in 'Canernar' and 5.7-12.1% in 'Hicrannar' (Dalka, 2010). Insects, especially honeybees, play an important role in pollination (Derin and Eti, 2001).

The objective of this study was to investigate flowering, pollen number, viability and germination, and fruit set characteristics of some registered 'Dr. Ercan 35', 'Efenar 35', 'Kamilbey 35', and 'Tezeren 35' pomegranate cultivars. The pollination biology of these pomegranate cultivars was determined at the first time with this research.

Materials and Methods

Biological material

'Dr. Ercan 35' (İzmir 1513' × İzmir 1445'), 'Efenar 35' (İzmir 1445' × İzmir 23'), 'Kamilbey 35' (İzmir 23' × İzmir 1445') and 'Tezeren 35' (İzmir 1513' × İzmir 23') pomegranate (*Punica granatum* L.) cultivars (registered in 2015) located at Aegean Agricultural Research Institute (ETAE), Menemen, İzmir, Turkey were used as plant material in the experiments conducted in 2016. 'Efenar 35' and 'Kamilbey 35' cultivars were previously obtained from the reciprocal cross of the same parents. The experimental plants were 8 years old and planted 4.0 × 2.5 m distance between and within rows. In each treatment, there were three replicate trees were used.

Phenological observations

The beginning of flowering, full flowering and end of flowering dates was recorded in both hermaphrodite and male flowers.

Self-pollination, cross- and open-pollination

The self-pollination experiments were conducted under 3.2 × 3.0 × 3.3 m dimension cabinets made by white nylon mesh to prevent insect entry from outside (Küçük, 2003). In each cabin, one bumblebee (*Bombus fervidus*) beehive was replaced. For the controlled cross-pollination (hybridization) experiments, the flowers were emasculated by hand, removing sepals, petal and stamen with sharp razor blade without disturbing the pistil at balloon stage. Three branches approximately 1.5-2.0 m above the ground were chosen from four directions of the plants. The flowers from each cultivar were collected at balloon stage and brought to the laboratory. The anther and pollen preparation was performed according to Engin and Hepaksoy (2003) and Üstüntaş *et al.* (2019). Pollen of each cultivar was used as a male parent for other three cultivars (full-sib design). Open-pollination experiments were conducted by choosing and labeling three branches in each tree. The fruit set of hermaphrodite type of flowers were counted at harvest.

Anther numbers, pollen number, viability, and germination rate of pomegranate cultivars

Both hermaphrodite and male flowers were collected at the balloon stage at three different times namely, the beginning, full, and end of flowering. Anthers were counted as three replicates from ten flowers. The anthers were burst overnight spreading onto a white paper under a lamp at 25 °C in the laboratory. Pollen grains were stored in a small glass vials in the refrigerator until they are used for pollination. The pollen number, viability and germination tests were performed according to Eti (1991).

The number of pollen grains was calculated using hemocytometer modified from Eti (1990). The viability test of pollen grains was performed by TTC (2, 3, 5 triphenyl tetrazolium chloride) according to Eti (1991). The red-colored pollen grains were recorded as viable after 2 h incubation observed at 10 × 10 magnification.

Pollen grain germination test was performed with agar + sucrose method using 15% sucrose, 25 ppm boric acid with 1% agar according to Engin and Hepaksoy (2003). The germination was recorded 24 h after pollen plating. The

germination ratios were determined by dividing the number of pollens germinated by the total number pollens in the field of light microscope view according to Günver-Dalkılıç and Dayı-Doğru (2003). Data were analysed as completely randomized design with three replicates. Differences between means were determined by LSD test at $p < 0.05$ level.

Results and Discussion

Phenological observations

Although bud swelling times were very close in all cultivars, bud burst times were different in the field (Table 1). The bud burst in 'Dr. Ercan 35' and 'Efenar 35' were observed one week before 'Kamilbey 35' and 'Tezeren 35' cultivars. While hermaphrodite flowers in 'Efenar 35' and 'Kamilbey 35' opened earlier than male flowers, that of in 'Dr. Ercan 35' and 'Tezeren 35' opened later than male flowers. Corresponding flowering period for cultivars lasted approximately one month allowing for cross-pollination to each other.

Self-, cross- and open-pollination

The fruit set ratios from different pollination experiments were changed between 9% and 85% (Table 2). The highest fruit set was obtained from open pollination from 60.1% in 'Tezeren 35' to 85.0% in 'Dr. Ercan 35'. In self-pollination, the fruit set ratios were changed from 25.2% in 'Tezeren 35' and 62.4% in 'Efenar 35'. While the highest fruit set ratio was obtained as 49.7% in 'Kamilbey 35' × 'Dr. Ercan 35' combination, the lowest of that was obtained as 9% in 'Efenar 35' × 'Dr. Ercan 35' combination (Table 2). This means that more than one cultivar existence in the pomegranate orchard should be preferred (Derin and Eti, 2001).

In open pollination, the fruit set ratio was between 4.1% in 'İzmir 1445' and 94.2% in 'İzmir 1513' cultivars (Küçük, 2003). The fruit set ratios were obtained as 56.3% in 'Hicaz' open pollination, 61.1% in 'Hicaz' × 'Hicaz' (male flower) selfing, 57.6% in 'Hicaz' × 'Hicaz' (hermaphrodite flower) selfing, 78.9% in 'Hicaz' × 33 N 26 (male flower), 70.8% in 'Hicaz' × 33 N 26 (hermaphrodite flower), 46.0% in 33 N 26 open pollination, 50.3% in 33 N 26 × 33 N 26 (male flower), 46.4% in 33 N 26 × 33 N 26 (hermaphrodite flower), 68.5% in 33 N 26 × 'Hicaz' (male flower), 61.8% in 33 N 26 × 'Hicaz' (hermaphrodite flower) (Derin and Eti, 2001). In another study with seven pomegranate cultivars at ETAE, the fruit set ratios were obtained as 82.8% in selfing and 94.2% in open pollination (Küçük 2003). In this current study, the fruit set ratios of the progeny were between those of their parents. In most cases, self-pollination gives lower fruit set ratio than open pollination. The reason for that could be self-, cross-, or group-incompatibility among cultivars.

Anther numbers

The number of anthers was the same (337) in both hermaphrodite and male pomegranate flowers (Table 3). The number of anthers per hermaphrodite and male flowers was changed between 284 in male flowers of 'Efenar 35' and 402 in hermaphrodite flowers in 'Tezeren 35' cultivars, respectively.

Table 1. Phenological stages of hermaphrodite and male flowers in pomegranate cultivars in 2015

Cultivar	Beginning of flowering		Full flowering		End of flowering	
	Hermaphrodite	Male	Hermaphrodite	Male	Hermaphrodite	Male
'Dr. Ercan 35'	11 May	05 May	30 May	25 May	23 June	15 May
'Efenar 35'	08 May	27 May	25 May	30 May	07 June	15 June
'Kamilbey 35'	05 May	12 May	17 May	25 May	09 June	16 June
'Tezeren 35'	14 May	14 May	02 June	19 May	23 June	15 June

Table 2. Fruit set in different cross experiments in pomegranate cultivars (%)

Cultivar	Pollinator	Open*	Dr. Ercan 35	Efenar 35	Kamilbey 35	Tezeren 35	Average
	'Dr. Ercan 35'		85.0 a	60.3 a-d	13.9 fg	20.4 fg	38.3 c-f
'Efenar 35'		82.6 a	9.0 g	62.4 a-c	13.7 fg	13.9 fg	36.3
'Kamilbey 35'		75.0 ab	49.7 b-e	32.8 e-g	50.0 b-e	16.0 fg	44.7
'Tezeren 35'		60.1 a-d	38.6 c-f	35.1 d-g	29.8 e-g	25.2 e-g	37.8
Average		75.7 a	39.4 b	36.1 bc	28.5 bc	23.3 c	
LSD _(0.05) (Cultivar)		ns					
LSD _(0.05) (Pollinator)		13.8					

*: Different letters within the same column indicate significant differences (LSD test, $p < 0.05$)

Table 3. Number of anthers per flower in pomegranate cultivars

Cultivar	Number of anthers / flower ^{ns}	
	Hermaphrodite	Male
'Dr. Ercan 35'	290	341
'Efenar 35'	309	284
'Kamilbey 35'	347	368
'Tezeren 35'	402	353
Average	337	337

^{ns}: Non significant (LSD test, $p < 0.05$)

Pollen numbers

The number of pollens per anther and per flower was changed between 1,459 and 583,333 in hermaphrodite flowers in 'Tezeren 35', and 8,550 and 3,083,333 in male flowers in 'Kamilbey 35' cultivars in the beginning of flowering, respectively (Table 4). The highest number of pollen grains was obtained in full flowering. In general, male flowers (4,841) had more pollen than hermaphrodite flowers (3,511).

The number of pollen grains per anther and flower was 1,061 (hermaphrodite in 'Eksilik' cultivar) and 1,008 (male in 'Aşınar' cultivar) and 215,625 (hermaphrodite) and 198,750 (male), respectively, (Gözlekçi and Kaynak, 2000). The highest pollen grain per anther in male flowers was 3,055 and 2,701 in 'Hicaz' and 33 N 36 pomegranate cultivars, respectively (Derin and Eti, 2001). The number of pollen grains was 472,188 per hermaphrodite and 992,875 per male flowers (Küçük, 2003). Şahin (2004) determined the number of pollen grains per anther as 485 in hermaphrodite and 1,353 in male flowers in 'Hicaz' pomegranate cultivar. The highest number of pollen grains per anther and flower as 300 and 330, and 1,036,380 and 2,143,700, in hermaphrodite and male flowers, respectively (Üstüntaş *et al.*, 2019).

Pollen viability

The ratio of pollen viability was changed from 39.4% in hermaphrodite flowers at the end of flowering in 'Tezeren 35' to 84.7% in hermaphrodite flowers at the beginning of

flowering in 'Efenar 35' (Table 5). The differences were significant in cultivar \times time \times type, cultivar \times time, time \times type, cultivar, and time at $p < 0.05$. According to the cultivars, the highest pollen viability was recorded in 'Kamilbey 35' (67.2%) and 'Efenar 35' (65.5%). According to the flowering time, the highest of that was observed in the beginning of flowering (74.5%) followed by full flowering (63.2%) and end of flowering (51.4%). According to cultivar \times time interaction, the highest pollen viability was observed in 'Dr. Ercan 35' in the beginning of flowering (81.7%). According to the type \times time interaction, the highest pollen viability was observed in the hermaphrodite flowers in the beginning of flowering (79.0%). According to cultivar \times time \times type interaction, the highest pollen viability was observed in 'Efenar 35' \times beginning of flowering \times hermaphrodite flower combination (84.7%).

The pollen viability ratios in TTC were found as 70.3% and 68.6% in hermaphrodite flowers, and 75.2% and 72.4% in male flowers in 'Hicaz' and 33 N 26 cultivars, respectively, (Derin and Eti, 2001). In India, the highest pollen viability was observed as 65.2-62.2% in 1% TTC test (Prakash *et al.*, 2010). The highest number of pollen viability and germination was 75.2% (TTC) and 61.5% (1% agar+10% sucrose), respectively, in male flowers in 'Hicaz' which is a dominated pomegranate cultivar in Turkey (Küçük, 2003). The highest pollen viability in TTC test was recorded in 95.0% in hermaphrodite flowers in the end of flowering and 92.4% in male flowers in the beginning of flowering (Üstüntaş *et al.*, 2019).

Pollen germination

The ratio of pollen germination was changed from 14.9% in the hermaphrodite flowers at the end of flowering in 'Kamilbey 35' to 71.2% in the hermaphrodite flowers at the beginning of flowering in 'Kamilbey 35' (Table 6). The differences were significant in cultivar × time × type, cultivar × time, cultivar × type, time × type, cultivar, time, and type at $p < 0.05$. According to the cultivars, the highest pollen germination was recorded in 'Dr. Ercan 35' (44.9%) and 'Efenar 35' (42.9%). According to the flowering times, the highest of that was observed in the beginning of flowering (53.2%) followed by full flowering (40.9%) and end of flowering (28.6%). According to the flower types, the pollen germination ratios were observed in hermaphrodite flowers (42.7%) followed by male flowers (39.1%). According to cultivar × time interaction, the highest pollen germination was observed in 'Efenar 35' in the beginning of flowering (59.9%) and 'Dr. Ercan 35' in the beginning of

flowering (59.8%). According to cultivar × type interaction, the highest pollen germination was observed in both types of flowers in 'Dr. Ercan 35', 'Efenar 35', and hermaphrodite flowers in 'Tezeren 35'. According to the time × type interaction, the highest pollen germination was observed in the hermaphrodite flowers in the beginning of flowering (65.4%). According to cultivar × time × type interaction, the highest pollen germination was observed in 'Kamilbey 35' × beginning of flowering × hermaphrodite flower combination (71.2%). The best pollen germination ratio (22.8%) in male flowers of pomegranate clone ME15, a Mollar type, was obtained from 1.5% agar + 10% sucrose (Melgarejo *et al.*, 2000). Derin and Eti (2001) recommended 12.5% sucrose + 0.5% agar for the best pollen germination medium. Engin and Hepaksoy (2007) found that 15% and 20% sucrose gave better pollen germination ratios than 10% sucrose.

Table 4. Pollen numbers per anther and per flower in pomegranate cultivars

Cultivar	Flowering Time	Pollen / Anther		Pollen / Flower	
		Flower type*		Flower type	
		Hermaphrodite	Male	Hermaphrodite	Male
'Dr. Ercan 35'	Beginning	4,369 c-f	3,306 e-k	1,250,000	1,083,333
	Full	5,849 bc	7,112 ab	1,666,667	2,416,667
	End	4,050 c-i	2,773 f-k	1,166,667	916,667
'Efenar 35'	Beginning	2,160 i-k	5,450 b-d	666,667	1,416,667
	Full	4,388 c-f	5,689 bc	1,333,333	1,583,333
	End	4,647 c-f	5,136 b-e	1,416,667	1,416,667
'Kamilbey 35'	Beginning	1,911 jk	8,550 a	666,667	3,083,333
	Full	4,608 c-f	4,343 c-f	1,583,333	1,583,333
	End	4,193 e-h	3,401 e-k	1,416,667	1,250,000
'Tezeren 35'	Beginning	1,459 k	3,565 d-j	583,333	1,250,000
	Full	2,193 h-k	4,512 c-f	833,333	1,583,333
	End	2,301 g-k	4,252 c-g	916,667	1,500,000
Average		3,511	4,841	1,125,000	1,590,278

*: Significant (LSD test, $p < 0.05$)

Table 5. Pollen viability (%) in pomegranate cultivars

Cultivar	Av. (C)*	Flowering Time	Flower Type		Av. (C×Time)*	Av. (Time)*	Time
			Hermaphrodite	Male			
			C×Time×Type*				
'Dr. Ercan 35'	59.75 b	Beginning	83.1 ab	80.2 a-c	81.7 a	74.5 a	Beginning
		Full	40.5 j	57.7 g-i			
		End	48.2 ij	48.8 ij			
		Av.(C×Type) ^{ns}	57.3	62.2			
'Efenar 35'	65.50 a	Beginning	84.7 a	62.1 e-h	73.4 b	63.2 b	Full
		Full	59.0 f-i	77.7 a-d			
		End	57.4 g-i	52.1 hi			
		Av.(C×Type) ^{ns}	67.0	64.0			
'Kamilbey 35'	67.17 a	Beginning	78.8 a-c	64.2 e-g	71.5 bc	51.4 c	End
		Full	71.8 b-c	70.3 c-f			
		End	55.5 g-i	62.4 e-h			
		Av.(C×Type) ^{ns}	68.7	65.6			
'Tezeren 35'	59.75 b	Beginning	69.4 c-f	73.2 a-e	71.3 bc	63.0	Av.
		Full	62.6 e-h	66.3 d-g			
		End	39.4 j	47.6 ij			
		Av.(C×Type) ^{ns}	57.1	62.4			
Av.(Type) ^{ns}			62.5	63.6		63.0	Av.
Time×Type*	H	M					
Beginning	79.0 a	69.9 b					
Full	58.5 c	68.0 b					
End	50.1 d	52.7 cd					

^{ns}: Non significant for type, cultivar × type; *: Significant (LSD test, $p < 0.05$) for cultivar, time, cultivar × time, time × type, cultivar × time × type
Av: Average, C: Cultivar, H: hermaphrodite, M: male

Table 6. Pollen germination (%) in pomegranate cultivars

Cultivar	Av. (C)*	Flowering Time	Flower Type		Av. (C×Time)*	Av. (Time)*	Time
			Hermaphrodite	Male			
			C×Time×Type*				
'Dr. Ercan 35'	44.9 a	Beginning	62.7 ab	56.9 bc	59.8 a	53.2 a	Beginning
		Full	29.4 gh	51.6 cd	40.5 bc		
		End	35.8 e-g	33.2 fg	34.5 cd		
		Av.(C×Type)*	42.6 AB	47.2 A			
'Efenar 35'	42.9 ab	Beginning	63.4 ab	56.4 bc	59.9 a	40.9 b	Full
		Full	29.0 gh	44.7 de	36.9 cd		
		End	36.7 e-g	27.0 gh	31.9 d		
		Av.(C×Type)*	43.0 AB	42.7 AB			
'Kamilbey 35'	36.4 c	Beginning	71.2 a	21.9 hi	46.6 b	28.6 c	End
		Full	34.2 fg	43.0 d-f	38.6 cd		
		End	14.9 i	33.2 fg	24.1 e		
		Av.(C×Type)*	40.1 B	32.7 C			
'Tezeren 35'	39.4 bc	Beginning	64.4 ab	28.9 gh	46.7 b	40.9	Av.
		Full	43.4 d-f	52.3 cd	47.9 b		
		End	27.3 gh	20.3 hi	23.8 e		
		Av.(C×Type)*	45.0 AB	33.8 C			
Av.(Type)*			42.7 a	39.1 b			
Time×Type*	H	M					
Beginning	65.4 a	41.0 c					
Full	34.0 d	47.9 b					
End	28.7 c	28.4 e					

*: Significant (LSD test, $p < 0.05$) for cultivar, time, type, cultivar × time, time × type, cultivar × time × type (indicated by small letters), cultivar × type (indicated by capital letters)

Av: Average, C: Cultivar, H: hermaphrodite, M: male

In India, the highest pollen germination ratio in the beginning and full flowering periods were 45.8% and 42.3%, respectively, in 15% sucrose (Prakash *et al.*, 2010).

The highest percentage of germination (70%) was obtained for 'Alktorshabrizi' cultivar in 1.5% agar + 10% sucrose + 50 mg L⁻¹ NAA and IBA + 100 mg L⁻¹ boric acid (Imani and Nazarian, 2013). The highest ratio of pollen germination was observed as 29.1% and 19.5% in hermaphrodite and male flowers, respectively, in the beginning of flowering in 1% agar + 20% sucrose + 25 ppm boric acid (Üstüntaş *et al.*, 2019).

Conclusions

The number of anthers per flower was not different among cultivars and flower types. The highest number of pollen grains per flower was in male flowers in the beginning of flowering in 'Kamilbey 35'. The highest pollen viability was in hermaphrodite flowers at the beginning of flowering in 'Efenar 35'. The highest pollen germination in hermaphrodite flowers at the beginning of flowering in 'Kamilbey 35'. The highest fruit set in self-pollination was in 'Efenar 35' and 'Dr. Ercan'. The highest fruit set was in 'Kamilbey 35' × 'Dr. Ercan 35' cross combination. These new pomegranate cultivars, 'Dr. Ercan 35', 'Efenar 35', 'Kamilbey 35', and 'Tezeren 35', are recommended in the Mediterranean climate to be used both fruit production and as pollinators to the international and national standard pomegranate cultivars.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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