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PRODUCTION AND QUALITY OF POLLEN IN TERMS OF FRUIT SET ON SOME SELF-POLLINATED POMEGRANATE CULTIVARS

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Abstract

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Pomegranate is well-adapted fruit species to arid and semi-arid conditions. This species have male (A type - unfertile) and hermaphrodite flowers (B type - fertile) on the same plant. Because of several problems, appear for fertilization and fruit set, researches on pollen quality and fruit set may be essential. In the present study pollen viability, germination, pollen quantity of A and B type flowers of seven pomegaranate cultivars were investigated. Pollen viability assessment was carried out using 1% 2, 3, 5 triphenyl tetrazolium chloride (TTC) test. Pollen germination capability was investigated agar in petri method by using four different media. Pollen production per anther and per flower was assayed by Hemacytometric method. In addition, fruit set of these cultivars under self-pollination condition was determined. The highest pollen viability level obtained from 'Mayhos VIII' and 'Hicaz' cultivars. Pollen germination was assayed using 1% agar and different sucrose concentrations (5, 10, 15 and 20%). In general, 15% and 20% sucrose concentrations determined as the best germination medium. Anther number per flower ranged between 275 and 410 according to the cultivars. 'Katirbasi' had the highest pollen number per flower whereas 'Cevlik' had the lowest. Self-pollination of "Hicaz" with B type flowers showed the best fruit set. Besides, "Eksi Goknar" did not provide any fruit on self-pollination treatments. For that reason, this cultivar can determine as self-incompatible. This study showed that, some cultivars need pollinators to set fruit and this should be considered for new plantations.

Key words: pollen viability, pollen germination, self-compatible, *Punica granatum*

Introduction

Punica granatum L. commonly known as "Pomegranate", 'Anar' (in India) and 'Nar' (in Anatolia) belongs to family Punicaceae. The genus includes two species *Punica granatum* L. and *Punica protopunica* Balf. f.. *Punica protopunica* is endemic to Socotra Islands (Yemen) and is the only one relative of the cultivated pomegaranate (Yilmaz, 2007; Verma et al., 2010). Pomegranate is considered as an excellent fruit tree for growing in arid and semi-arid areas and especialy outside of suitable areas and saline waters without special conditions (Mars and Marrakchi, 1999). This fruit is an ancient, mystical, unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, in Southeast Asia, and in California and Arizona in the United States. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of aliments. The Mediterranean Basin is one of the areas best adapted to pomegranate cultivation. The great adaptation ability of this species to the Mediterranean climate has enhanced its dispersion in large areas (Amoros et al., 2000; Jurenka, 2008). People preference pomegranate fruits for its attractive and refreshing characteristics. Growing of this species is incrasing for quality of fruits with fresh use and such processed materials like juice, syrup, squash and wine (Jalikop, 2010).

In Turkey, pomegranate has been produced in the Mediterranean, Aegean, Southeastern Anatolian and Bilecik-Eskisehir regions. Pomegranate growing is increasing in all regions and the highest increasing expected to be happen in Mediter-

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ranean region (Yilmaz, 2007). Total pomegranate production of Turkey was nearly 170 thousand tons (TUIK, 2009).

Because of presence of andromonoecious form in pomegranate flowers, male (A type - unfertile) and hermaphrodite flowers (B type - fertile) develope on the same plant. Flowers have an imposing red calyx and corolla. Approximately, 200-350 anthers exist in a flower and hermaphrodite flowers contain 400-1000 ovules. The insects realize pollination of pomegranate and major pollinator is the honeybee. To meet correspond export market standards, the fruit has to be of a certain size; this is related to pollination success and consequently to seed number (Derin and Eti, 2001). Sometimes several problems appear in fertilization and fruit set between different flower types of a cultivar or between flower types of different cultivars (Gozlekci and Kaynak, 1998).

Several studies have carried out pollen viability and germination of pomegranate cultivars because of its importance in fertilization. Engin and Hepaksoy (2003) investigated pollen germination of type A flowers of 11 İzmir series pomegranate varieties and according to results, media containing 15 and 20% sucrose offered higher polen germination ratio. Derin and Eti (2001) determined the pollen quality (pollen viability and germination), quantity (pollen production) of 'Hicaz' and '33 N 26' pomegranate cultivars. The highest pollen viability, germination rate and production capability were observed in 'Hicaz'. Besides, Gozlekci and Kaynak (1998) found pollen viability of six pomegranate cultivars between 22% and 61% according to triphenyl tetrazolium chlorid (TTC) test.

The aim of this study is determining the pollen production and quality of seven important Turkish pomegranate cultivars. Furthermore, to find the existing fruit set problems, self-pollination treatments were investigated on B type flowers.

Materials and Methods

Seven pomegranate cultivars selected from Mediterranean region of Turkey were used for this study (Onur and Kaska, 1985). Among them, 'Hicaz', 'Mayhos I', 'Katırbasi', and 'Silifke Asisi' have sourish, 'Cevlik' have, 'Eksi Goknar' and 'Mayhos VIII' have sour taste (Onur and Kaska, 1985; Yilmaz, 2007). All trees were seventeen years old and planted in field of Alata Horticultural Research Institute, Erdemli, Mersin, Turkey. 'Hicaz' is the most produced cultivar in Turkey and 'Katirbasi' and 'Silifke Asisi' were produced mostly in Hatay and Mersin province respectively.

Flower samples of seven cultivars were collected from three plants of each cultivar just before anthesis. For each assay, male (A type - unfertile) and hermaphrodite (B type - fertile) flowers were used separately. Pollen viability assessment was carried out using 1% 2, 3, 5 triphenyl tetrazolium chloride (TTC) test (Norton, 1966). Pollen germination capability was investigated agar in petri method (Stanley and Liskens, 1985) by using four different media. All media included 1% agar and each of them included 5%, 10%, 15% and 20% of sucrose. Pollen grains were distributed uniformly with a brush on the media poured petri. Pollen production per anther and per flower was assayed by Hemacytometric method (Eti, 1990). For pollen viability of cultivars three lamella and five regions of each lamella were assayed. Numbers of viable (stained dark red), semi-viable (stained red-pink) and non-viable (not stained) pollens were investigated. At the same way, for pollen germination capability, three petri and five regions of each petri were assayed. Besides, fruit setting of each cultivar by self-pollination was determined. For this, approximately 100 hermaphrodite (B type) flowers of each cultivars cultivar were self pollinated with brush and were covered with air permeable fabric. Fruit numbers were recorded at harvest time in order to investigate fruit set ratios of each cultivar by self-pollination.

The study was carried out according to randomized design. Data were analyzed using a SAS based programme and means were grouped by using Tukey's test ($P < 0.05$).

Results and Discussion

Pollen viability showed significant differences for cultivars and flower types (A, B) (Table 1). Rate of viable pollen of A type flowers varied between 64.35% ('Mayhos VIII') and 29.44% ('Cevlik'). Gozlekci and Kaynak (1998) observed similar findings for pollen viability of A type flowers of some pomegranate cultivars (28-56%). In another two studies, 'Hicaz' had the highest ratio of viable pollen as 77.52% (Gozlekci and Onursal, 2005), and 75.24% (Derin and Eti, 2001). In this study, "Mayhos VIII" and Hicaz showed the highest polen viability respectively. On the other hand, for B type hermaphrodite flowers of studied cultivars, while the highest pollen viability ratio was found in 'Mayhos VIII' (54.93%), the lowest rate determined in 'Silifke Asisi' (27.14%). Semi-viable pollen ratio showed lower variation than viable ones for A type flower. The highest percentage of semi-viable pollen of A type flower was observed in 'Cevlik' (38.75%) whereas the lowest value obtained from 'Mayhos I' (17.87%). Semi-viable pollen for hermaphrodite B type flowers ranged between 51.30% ('Silifke Asisi') and 23.19% ('Katirbasi'). In terms of non-viable pollens, "Mayhos I" had the highest (35.39 %) while "Mayhos VIII" the lowest (12.39 %) ratio for A type flowers. On the other hand, non-viable pollen rates of B type flowers varied between 36.64% ('Katirbasi') and 9.10% ('Mayhos VIII') (Table 1).

In all cultivars except 'Cevlik' A type (male) flowers had higher viable pollen than B type flowers. Similar results also found in previous studies (Derin and Eti, 2001; Gozlekci and Onursal, 2005). In addition, it was reported that number of A type flowers on a pomegranate plant are 1.5–4.5-fold higher than B type flowers (Engin and Hepaksoy, 2003). For these reasons, A type flowers may play more important role in fruit setting.

Pollen germination tests were carried out by using 1% agar + and different sucrose concentrations (5, 10, 15 and 20%). Although there were no statistically differences for the pollen germination among sucrose concentrations, the germination rates of cultivars were varied to sucrose concentrations (Table 2). Sucrose level of 5% showed slightly lower germination rates than the other concentrations but not significantly so. In general, the highest germination ratio was obtained from 15% and 20% sucrose concentrations for 2 flower types of all cultivars. Pollen germination percentages of some pomegranate cultivars in 10, 15 and 20% saccharose concentrations found as 13.76%, 25.16% and 23.15% respectively in previous study (Engin and Hepaksoy, 2003). Their results were consistent with our present study. On the other hand, Derin and Eti (2001) reported that the most suitable sucrose concentrations were 10% and 15% for pollen germination of pomegranate cultivars. Melgarejo et al. (2000) notified that 10% saccharose concentration provided higher percentage of pollen germination for 'ME15' pomegranate. As summarized above, there were little differences between sucrose concentarions in terms of germinated pollen ratio. It was assumed, variable outer factors such as humidity, temperature and ingredients of the substrate used for germination may have an effect on pollen germination (Gozlekci and Kaynak, 1998). At the same time, different ecological conditions and cultivars may affect the pollen germination tests.

Table 1
Pollen viability levels of A and B type flowers of some pomegranate cultivars, %

Cultivar	Viable		Non-viable		Semi-viable	
	A	B	A	B	A	B
Mayhos VIII	64.35 a	54.93 a	12.39 b	9.10 d	23.27 bc	35.98 ab
Katirbasi	49.08 ab	40.18 ab	27.99 ab	36.64 a	22.93 bc	23.19 b
Mayhos I	46.75 ab	47.46 ab	35.39 a	15.56 cd	17.87 c	36.98 ab
Eksi Goknar	46.15 ab	45.73 ab	33.77 a	26.50 ab	20.08 bc	27.77 ab
Cevlik	29.44 b	32.55 b	31.81 a	27.27 ab	38.75 a	40.18 ab
Hicaz	61.02 a	41.19 ab	17.39 ab	20.13 bc	21.59 bc	38.67 ab
Silifke Asisi	47.29 ab	27.14 b	23.88 ab	21.56 bc	28.83 ab	51.30 a
D 5%	21.65	21.52	18.57	10.28	10.85	28.02

Mean separation within columns by Tukey's multiple range test. $P < 0.05$.

Table 2
Pollen germination levels under 1% agar and different sucrose concentrations for A and B type flowers of some pomegranate cultivars, %

Cultivar	Sucrose concentrations							
	5%		10%		15%		20%	
	A	B	A	B	A	B	A	B
Mayhos VIII	23.35 b	22.35 b	35.70 bc	30.20 c	25.70 bc	44.90 a	32.25 b	38.60 c
Katirbasi	38.40 a	41.05 a	54.05 ab	50.80 a	50.10 a	50.20 a	54.40 a	53.85 ab
Mayhos I	45.50 a	40.20 a	48.15 abc	40.05 b	51.65 a	41.15 a	54.75 a	58.80 a
Eksi Goknar	19.85 bc	27.75 ab	34.15 c	31.60 c	50.15 a	33.90 a	33.45 b	42.95 bc
Cevlik	8.75 c	3.90 c	14.20 d	2.70 d	15.15 c	2.45 b	11.10 c	4.50 e
Hicaz	42.20 a	23.00 b	56.70 a	27.60 c	48.00 a	35.50 a	61.90 a	22.75 d
Silifke Asisi	38.35 a	28.70 ab	34.35 c	46.15 ab	38.55 ab	48.15 a	33.50 b	57.35 a
Mean	30.91^{ns}	26.71^{ns}	39.61^{ns}	32.73^{ns}	39.90^{ns}	36.61^{ns}	40.19^{ns}	39.83^{ns}
D 5%	12.07	14.41	18.58	7.78	15.91	17.18	11.44	13.86

Mean separation within columns by Tukey's multiple range test. $P < 0.05$, ns: insignificant.

On the other hand, significantly differences among cultivars for pollen germination also found (Table 2). For 1% agar + 5% sucrose concentration of A type flower the highest pollen germination was obtained from 'Mayhos I' (45.50%) whereas 'Cevlik' had the lowest ratio (8.75%). In B type flowers for 1% agar + 5% sucrose concentration, germination ratios ranged between 41.05% ('Katirbasi') and 3.90% ('Cevlik). Among A type flowers for 1% agar + 10% sucrose concentration 'Hicaz' showed the highest ratio of polen germination (56.70%) and 'Cevlik' provided the lowest ratio (14.20%). In B type flowers of same media, pollen germination ratio varied between 50.80% ('Katirbasi') and 2.70% (Cevlik). For 1% agar + 15% sucrose concentration of A type flowers the best pollen germination was observed in 'Mayhos I' (51.65%) whereas the lowest ratio was found in 'Cevlik' (15.15%). Among all germination medias 1% agar + 15% sucrose concentration was the most suitable for pollen germination of A type flowers of 'Eksi Goknar' with ratio of 50.15%. Pollen germination ratio of B type flowers studied in 1% agar + 15% sucrose concentration ranged between 50.20% ('Katirbasi') and 2.45% ('Cevlik). Most of cultivars showed higher pollen germination in 20% sucrose. For A type flowers, the highest value obtained from 'Hicaz' (61.90%) and the lowest from 'Cevlik' (11.10%). Besides, germination percentages ranged between 58.80% ('Mayhos I') and 4.50% ('Cevlik').

Pomegranate pollen germination rate of A type flower found higher than B type flower in previous researches (Derin and Eti, 2001; Gozlekci and Onursal, 2005). In this study, A type flowers of 'Hicaz' and 'Cevlik' showed higher percentage of germination than B type flowers for all sucrose concentrations. It was a remarkable finding that B type flowers germination percentage of 'Hicaz' clearly lower (about 50%) than A type flowers. In contrast, B type of 'Silifke Asisi' had higher level of pollen germination than A type flowers except 5% of sucrose. On the other hand, A type flowers of 'Mayhos I' showed higher germination percentages than B type flowers except 20% sucrose concentration. There was no correlation between germination of A or B type flowers and sucrose concentartions for 'Katirbasi', 'Mayhos VIII' and 'Eksi Goknar'. Consistently with pollen viability tests, 'Cevlik' had the lowest germination rate, which clearly separated from other cultivars for all sucrose concentrations. Furthermore, pollen germination rates can vary by different ecological and growing conditions. For example, in a study made in west Mediterranean region of Turkey, A and B type flowers of 'Mayhos VIII' and 'Hicaz' were investigated higher pollen germination rates than the existing results (Gozlekci and Onursal, 2005).

There were significant differences among cultivars in terms of anther number and pollen production (Table 3). Whereas anther numbers of A type flowers varied between 410 (Mayhos I) and 275 (Mayhos VIII), for B type flowers it ranged between 352 (Cevlik) and 276 (Mayhos VIII). This results were consistent with previous study made in Adana conditions (213-325 anthers/flower) (Derin and Eti, 2001). The highest number of pollen per anther obtained from 'Katırbasi' and 'Hicaz' cultivars for A type flowers. 'Hicaz' also had the highest pollen number per anther for B type flowers. On the other hand 'Mayhos I' showed the lowest pollen number in both of A and B type flowers. Pollen number per anther of all cultivars except 'Katirbasi' was higher in B type flowers than A type. In contrast, pollen viability and germination level of most cultivars were higher in A type flowers than B type. Oppositely with our results, higher level of pollen production was observed in A type flowers compared to B type for some pomegranate cultivars (Derin and Eti, 2001; Gozlekci and Onursal, 2005).

Pollen number per flower ranged between 889 760 (A type of 'Katirbasi') and 503 800 (A type of 'Mayhos VIII'). As pollen number per anther, B type flowers also had higher pollen number per flower for all cultivars except 'Katirbasi'.

Table 3
Number of anther and pollen production levels in different flower types and cultivars

Cultivar	Anther number		Number of pollen per flower		Number of pollen per anther	
	A	B	A	B	A	B
Mayhos VIII	275.00 c	276.60 b	503.800 c	553.200 b	1.832 bc	2.000 cd
Katirbasi	332.00 b	307.00 ab	889.760 a	704.872 a	2.680 a	2.296 abc
Mayhos I	410.20 a	310.20 ab	607.096 bc	528.580 b	1.480 c	1.704 d
Eksi Goknar	310.00 bc	291.80 ab	523.280 c	747.008 a	1.688 c	2.560 ab
Cevlik	284.00 c	352.00 a	433.952 c	765.952 a	1.528 c	2.176 bcd
Hicaz	282.60 c	286.00 b	743.803 ab	787.072 a	2.632 a	2.752 a
Silifke Asisi	332.20 b	347.60 a	773.361 ab	820.336 a	2.328 ab	2.360 abc
D 5%	47.30	60.62	178.469	148.869	574.79	508.42

Mean separation within columns by Tukey's multiple range test. $P < 0.05$.

In addition, the fruit set ratios of cultivars under the self-pollination conditions were observed. For this trait, there were significant differences among cultivars (Table 4). The highest fruit set was found in 'Hicaz' (61.20%), whereas no fruit set observed in 'Eksi Goknar'. Consistent with their high level of pollen viability and germinaton ability, 'Hicaz' had the highest fruit set in self-pollination. In previous study, B type flowers of 'Hicaz' were self-pollinated and 57.55% fruit set was investigated (Derin and Eti, 2001). From these results, it can be concluded that 'Hicaz' is a self-compatible cultivar. On the other hand, although 'Eksi Goknar' had moderate pollen number, viability and germination level, there was no fruit set in self-pollination. For that reason, 'Eksi Goknar' can be accepting as a self-incompatible cultivar. For new plantations, there must place suitable pollinator cultivars in same orchard to provide higher fruit set ratios. Concordant with low level of pollen germination of 'Cevlik', there was a very low fruit set (3.40%) in self-pollination of this cultivar. In addition, 'Mayhos VIII' had low level of fruit set ratio (8.70%) to assess as self compatible. In this case, using pollinator cultivars may increase fruit set of both cultivars? These reports are important to know whether pollinator cultivars are necessary or not for new orchards.

Conclusion

Knowledge of pollen production level, viability and germination ratio of pollen and fruit set by self-pollination in pomegranate may help to decide cultivars for growing and obtain higher yield. Especially it's important to understand the self-(in) compatiblity feature of cultivars for establishing new plantations. We believe this study may offer valuable information ragarding to these subjects.

Table 4
Fruit set ratios in self-pollination of some pomegranate cultivars, %

Cultivar	Fruit set	
Mayhos VIII	8.70	d
Katirbasi	36.90	b
Mayhos I	31.10	bc
Eksi Goknar	0.00	e
Cevlik	3.40	de
Hicaz	61.20	a
Silifke Asisi	26.40	c
D 5%	8.22	

Mean separation within columns by Tukey's multiple range test. $P < 0.05$.

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