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Investigations on pollen production and quality in some standard pomegranate (*Punica granatum* L.) cultivars

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SUMMARY – In this study, the capacity of pollen germination and viability of Fellahyemez, Eksilik, Ernar, Hicaznar, Katirbasi and Asinar cultivars, which were determined after the selection studies conducted on Mediterranean pomegranates, and the amount of pollen production per flower and anther were examined. Unfertile (type A) and fertile (type B) flowers belonging to each cultivar were examined individually. While the pollen viability was determined in 2,3,5 Triphenyl Tetrazolium Chlorid (TTC), Fluorescein Diacetat (FDA) and Trypan Blue (TB) solutions by staining, the pollen germination experiments in *in vitro* conditions were made by using agar method in a petri dish. The pollen production capacities were determined by hemacytometric method. Results indicated that there were some differences among cultivars and between fertile and unfertile flowers of each individual cultivar in terms of pollen production, pollen germination and viability.

Key words: Pomegranate, *Punica granatum* L., pollen viability, pollen germination, pollen production.

RESUME – *"Recherches sur la production et la qualité du pollen chez quelques cultivars standards de grenade (*Punica granatum *L.)". Dans cette étude, on a examiné la capacité de germination et la viabilité du pollen des cultivars Fellahyemez, Eksilik, Ernar, Hicaznar, Katirbasi et Asinar, qui ont été déterminées après des études de sélection menées sur des grenadiers méditerranéens, ainsi que la quantité de pollen produit par fleur et anthère. Les fleurs non fertiles (type A) et fertiles (type B) appartenant à chaque cultivar ont été examinées individuellement. Tandis que la viabilité du pollen a été déterminée dans des solutions de 2,3,5 Chlorure de Triphényl Tétrazolium, Diacétate de Fluorescéine et Bleu de Trypane par teinture, les expériences de germination de pollen dans des conditions* in vitro *ont été faites en utilisant la méthode agar en boîtes de Pétri. Les capacités de production de pollen ont été déterminées par la méthode hémacytométrique. Les résultats ont indiqué qu'il y avait quelques différences parmi les cultivars et entre les fleurs fertiles et non fertiles de chaque cultivar individuel en termes de production de pollen, de germination et de viabilité du pollen.*

Mots-clés : *Grenadier,* Punica granatum *L., viabilité du pollen, germination du pollen, production de pollen.*

Introduction

The number of pomegranate orchards has been increasing rapidly recently, because of gaining high profit in Turkey. In recent years, the total pomegranate production of Turkey has reached 56,000 tons/year. The Mediterranean region produces about 35% of total production (Anonymous, 1996). Antalya in the Mediterranean region plays an important role in pomegranate production in Turkey. Since there are several pomegranate types and cultivars in Turkey, Turkey is considered as one of the centres of origin. There is an increasing pomegranate demand, especially in outside markets. However, desired pomegranate characteristics such as: (i) high fruit setting; (ii) high and regular yield; (iii) large, red and thin skinned fruits; (iv) large, red, juicy, aromatic, sweet and sweet-sour, soft seeded arils; and (v) resistant to cracking and storage, may change according to both domestic markets and outside markets.

Therefore, breeding experiments should be conducted to get quality fruit. For this purpose crossing studies are conducted by using selected standard pomegranate cultivars. However, there are some problems faced in breeding programmes. Previous studies conducted on six different cultivars showed that there were some problems with fertilisation and fruit setting (C. Onur, pers. comm.). Fertilisation and fruit setting problems may occur between different flower types of a cultivar or between flower types of different cultivars.

This present study was conducted in order to shed more light on problems concerning fruit setting on pomegranate.

Materials and methods

Experiments were carried out in Antalya Citrus and Greenhouse Crop Research Institute, Serik-Kayaburnu Station, and Physiology Laboratory of Department of Horticulture, Faculty of Agriculture, University of Akdeniz, in 1998.

The research was conducted on Asinar, Eksilik, Ernar, Fellahyemez, Hicaznar and Katirbasi cultivars which are selected from the Mediterranean region pomegranates (Onur, 1982).

These cultivars have the following important properties. Katirbasi (516.88 g) and Asinar (505.01 g) cultivars have big fruits. Cultivars with dark red skin colour are Hicaznar and Ernar cultivars. Fellahyemez (59.78 g) and Katirbasi (47.74 g) cultivars have the largest and heaviest arils. The sourest cultivar is Eksilik (4.98%) according to titratable acid. The sour-sweet cultivars are Hicaznar (1.90%), Katirbasi (1.37%) and Asinar (1.24%) cultivars in terms of titratable acidity. The sweetest cultivars, which have also the lowest acidity, are Ernar (0.33%) and Fellahyemez (0.22%) cultivars. Fellahyemez cultivar has faint pink coloured arils and other cultivars have red and dark red coloured arils. Fellahyemez and Asinar cultivars have soft seeded aril.

In this experiment, flower samples were taken from six trees of each individual cultivar during flowering period and were divided into two groups. In the first group, flower samples were A type (unfertile flowers), and the other group of flower samples were B type (fertile flowers). In this study, the pollen viability, germination and production capacity of A and B type flowers in the above stated cultivars were determined.

In order to test pollen viability, 2,3,5 Triphenyl Tetrazolium Chloride (TTC), Fluorescein Diacetat (FDA) and Trypan Blue (TB) were used (Norton, 1966; Heslop-Harrison and Heslop-Harrison, 1970; Kruse and Patterson, 1973).

The pollen germination rates were determined by using agar in petri method in *in vitro* conditions with 10% sucrose + 0.2% agar + 5 ppm boric acid (H_3BO_3) (Charles and Harris, 1972).

For determining the amount of pollen produced by the flowers of the experimental cultivars, hemacytometric method was used (Eti, 1990).

The results of the experiments were analysed in a completely randomised design with 3 replications. LSD multiple range test was applied for means separation at 5% level. In statistical analyses percentages of values were used by angular transformation. The means used in the tables were both the original and arc sin values.

Results and discussion

The pollen viability values of pomegranate cultivars used in this experiment, according to flower types and pollen viability tests are given in Table 1. Total amount of A type viable pollen in TTC test varied between 30% and 65%. The highest pollen viability was found in Katirbasi cultivar (56.50%) for A type flowers. The amount of pollen viability on Ernar (40.89%) and on Hicaznar (40.45%) cultivars were found in the same group statistically. The lowest pollen viability was seen in Fellahyemez cultivar (28.14%). In TB test for A type flowers, the highest pollen viability value was found on Katirbasi (95.49%) and Asinar (95.19%) cultivars and the lowest one was on Eksilik cultivar (81.65%). The viability levels of other cultivars were around 85-90%. In FDA test, the highest pollen viability value was determined on Hicaznar cultivar (83.15%), and the lowest one was on Eksilik cultivar (16.85%) for A type flowers. The viability levels of other cultivars were found to be around 50-80%. Pollen germination test for A type flowers in sucrose gave the highest pollen viability on Hicaznar cultivar (68.25%) and the lowest one was on Eksilik cultivar (27.43%) as it was observed in FDA test. While pollen viability values for A type flowers of all cultivars in other tests varied between 16-27%, none of the cultivars for A type flowers in TB test gave pollen viability values below 82%.

Total amount of B type viable pollen in TTC test dispersed between 22% and 61%. The highest pollen viability was found on Eksilik cultivar (61.10%) and the lowest ones were on Fellahyemez (22.89%) and on Hicaznar (24.47%) cultivars were found in the same group statistically. In TB test

according to B type, the highest pollen viability was obtained from the Asinar (91.07%) and Katirbasi (90.75%) cultivars and the lowest one was on Fellahyemez (33.55%) cultivar. Pollen viability levels of the other cultivars were around 50-65%. In FDA test for B type flowers, the highest pollen viability was found on Fellahyemez cultivar (60.90%) and the lowest one was on Ernar cultivar (40.25%). Pollen germination test for B type flowers in sucrose and the highest viability value was obtained on Hicaznar cultivar (58.88%), the lowest one was on Eksilik cultivar (38.70%). Similarities and dissimilarities in terms of flower types and viability tests were obtained between different cultivars. The pollen viability of flowers on each single cultivar varied according to flower type and viability test. Similar results were reported for other crops by other researchers (Seilheimer and Stosser, 1982; Mahanoglu *et al.*, 1993). Pollen viabilities of unfertile flowers were higher than the fertile flowers of each cultivar.

Table 1. Pollen viability levels of cultivars according to flower types and viability tests (%)†

Flower type	Tests	Cultivars						LSD (5%)
		Asinar	Eksilik	Ernar	Fellahyemez	Hicaznar	Katirbasi	
A	TTC	31.09 (18.114)^{a}	42.95 (25.434)^{b}	40.89 (24.136)^{c}	28.14 (16.345)^{e}	40.45 (23.859)^{c}	56.50 (34.406)^{a}	0.85
	TB	95.19 (72.191)^{a}	81.65 (54.755)^{d}	88.21 (61.902)^{b}	89.22 (63.159)^{e}	84.97 (58.179)^{c}	95.49 (72.729)^{a}	1.48
	FDA	50.67 (30.443)^{e}	16.85 (9.701)^{f}	78.79 (51.996)^{b}	53.76 (32.520)^{d}	83.15 (56.258)^{a}	74.19 (47.896)^{c}	0.85
	Sucrose	48.74 (29.173)^{d}	27.43 (15.923)^{e}	56.54 (34.433)^{c}	48.60 (29.080)^{d}	68.25 (43.045)^{a}	58.73 (35.974)^{b}	1.67
B	TTC	46.95 (28.005)^{c}	61.10 (37.667)^{a}	52.54 (31.699)^{b}	22.89 (13.235)^{e}	24.47 (14.157)^{e}	39.42 (23.220)^{d}	1.22
	TB	91.07 (65.609)^{a}	60.44 (37.185)^{c}	53.52 (32.364)^{d}	33.55 (19.605)^{e}	64.05 (39.837)^{b}	90.75 (65.164)^{a}	1.12
	FDA	48.95 (29.311)^{b}	41.79 (24.701)^{d}	40.25 (32.364)^{e}	60.90 (37.521)^{a}	41.48 (24.510)^{d}	46.28 (27.569)^{c}	0.62
	Sucrose	48.23 (28.769)^{b}	38.70 (22.769)^{e}	45.73 (27.216)^{c}	43.37 (29.080)^{d}	59.88 (36.794)	49.8 (29.398)^{b}	1.15

†Numbers in brackets are angular transformation values.
^{a,b,c,d,e,f}Values with same letter do not differ significantly.

Pollen viability levels of cultivars and A and B flower types according to viability and germination tests are given in Table 2. In unfertile flowers of Asinar cultivar, the highest pollen viability was found by TB test (95.19%) and the lowest one was by TTC test (31.09%). In fertile flowers belonging to the same cultivar, the highest pollen viability was found by TB test (91.07%) and the lowest pollen viability was determined by TTC test (46.95%) as it was observed in unfertile flowers of Asinar cultivar. The highest pollen viability according to A flower type on Eksilik cultivar was obtained by TB test (81.65%) and the lowest pollen viability value was found by FDA test (16.85%). The highest pollen viability for B type flowers on Eksilik cultivar was found by TTC test (61.10%) and by TB test (60.44%) and the lowest one was in sucrose germination test (38.70%). The highest pollen viability for A type flowers of Ernar cultivar was obtained by TB test (88.21%) and the lowest one was by TTC test (40.89%). In B type flowers of Ernar cultivar the highest pollen viability was found by TB test (53.52%) and by TTC (52.54%), and the lowest one was by FDA (40.25%). In A type flowers of Fellahyemez cultivar the highest pollen viability value was determined by TB test (89.22%) and the lowest one was by TTC (28.14%). In B type flowers belonging to the same cultivar the highest pollen viability was found by FDA test (60.90%) and the lowest pollen viability value was obtained by TTC test (22.89%). In unfertile flowers on Hicaznar cultivar the highest pollen viability was obtained by TB (84.96%), and the lowest one was by TTC test (40.45%). In fertile flowers belonging to same cultivar the highest pollen viability was determined by TB test (64.05%) and the lowest one was by TTC test (24.47%). The highest pollen viability for A type flowers on Katirbasi cultivar was obtained by TB (95.49%) and the lowest one was by TTC test (56.50%). In B type flowers on the same cultivar the highest pollen viability was

determined by TB test (95.49%) and the lowest pollen viability value was found by TTC test (39.42%) as it was observed in A type flowers.

Table 2. Pollen viability levels of cultivars and flower types according to viability and germination tests (%)[†]

Cultivars	Flower type	Flower tests				LSD (5%)
		TTC	TB	FDA	Sucrose	
Asinar	A	31.09 (18.114)^{d}	95.19 (72.191)^{a}	50.67 (30.443)^{b}	48.74 (29.173)^{c}	1.19
	B	46.95 (28.005)^{c}	91.07 (65.609)^{a}	48.95 (29.311)^{b}	48.23 (28.839)^{bc}	0.59
Eksilik	A	42.95 (25.434)^{b}	81.65 (54.755)^{a}	16.85 (9.701)^{d}	27.43 (15.923)^{c}	1.97
	B	61.10 (37.667)^{a}	60.44 (37.185)^{a}	41.79 (24.701)^{b}	38.70 (22.769)^{c}	0.75
Ernar	A	40.89 (24.136)^{d}	88.21 (61.902)^{a}	78.79 (51.996)^{b}	56.54 (34.433)^{c}	0.88
	B	52.54 (31.699)^{a}	53.52 (32.364)^{a}	40.25 (23.733)^{c}	45.73 (27.216)^{b}	1.02
Fellahyemez	A	28.14 (16.345)^{d}	89.22 (63.159)^{a}	53.76 (32.520)^{b}	48.60 (29.080)^{c}	1.03
	B	22.89 (13.235)^{d}	33.55 (19.605)^{a}	60.90 (37.521)^{a}	43.37 (25.706)^{b}	1.10
Hicaznar	A	40.45 (23.859)^{d}	84.96 (58.179)^{a}	83.15 (56.258)^{b}	68.25 (43.045)^{c}	1.19
	B	24.47 (14.157)^{d}	64.05 (39.837)^{a}	41.48 (24.510)^{c}	59.88 (36.794)^{b}	1.57
Katirbasi	A	56.50 (34.406)^{d}	95.49 (72.729)^{a}	74.19 (47.896)^{c}	58.73 (35.974)^{c}	1.41
	B	39.42 (23.220)^{d}	90.75 (65.164)^{a}	46.28 (27.569)^{c}	49.8 (29.398)^{b}	1.33

[†]Numbers in brackets are angular transformation values.
^{a,b,c,d}Values with same letter do not differ significantly.

As can be seen from Table 2, the pollen viability levels of cultivars and flower types changed according to viability and sucrose germination tests. In general, the highest pollen viability for all cultivars and their flower types was found by TB test and the lowest one was by TTC test. In each cultivar results obtained from FDA test showed similarities with sucrose germination test. Some pollens of Eksilik, Ernar, Fellahyemez and Katirbasi cultivars did not germinate, although they were found to be viable. It is assumed that variable outer factors such as humidity, temperature and ingredients of the substrate used for germination may have an effect on pollen germination. So, it is possible to say pollen viability tests give more reliable results than germination tests, as was stated by Stanley and Linskens (1985). On the other hand, some researchers stated that pollen viability and germination tests should be conducted together in order to determine the pollen quality (Parfitt and Ganeshan, 1989).

The pollen viability levels in cultivars according to flower types, germination and viability tests are given in Table 3 according to flower types and cultivars. There were statistically significant differences between A and B type flowers for each cultivar in every pollen viability and pollen germination tests except for Asinar cultivar which did not give any differences in germination test with sucrose.

Table 3. Pollen viability levels of cultivars according to flower types, germination and viability tests (%)†

Tests	Flower type	Cultivars					
		Asinar	Eksilik	Ernar	Fellahyemez	Hicaznar	Katirbasi
TTC	A	31.09 (18.114)[a]	42.95 (25.434)[a]	40.89 (24.136)[a]	28.14 (16.345)[a]	40.45 (23.859)[a]	56.50 (34.406)[a]
	B	46.95 (28.005)[b]	61.10 (37.667)[b]	52.54 (31.699)[b]	22.89 (13.235)[b]	24.47 (14.157)[b]	39.42 (23.220)[b]
LSD (5%)		0.75	1.01	0.81	1.41	0.96	2.39
TB	A	95.19 (72.191)[a]	81.65 (54.755)[a]	88.21 (61.902)[a]	89.22 (63.159)[a]	84.97 (58.179)[a]	95.49 (72.729)[a]
	B	91.07 (65.609)[b]	60.44 (37.185)[b]	53.52 (32.364)[b]	33.55 (19.605)[b]	64.5 (39.837)[b]	90.75 (65.164)[b]
LSD (5%)		1.99	2.17	1.43	1.75	1.81	0.34
FDA	A	50.67 (30.443)[a]	16.85 (9.701)[a]	78.79 (51.996)[a]	53.76 (32.520)[a]	83.15 (56.258)[a]	74.19 (47.896)[a]
	B	48.95 (29.311)[b]	41.79 (24.701)[b]	40.25 (23.733)[b]	60.90 (37.521)[b]	41.84 (24.510)[b]	46.28 (27.569)[b]
LSD (5%)		0.61	0.68	1.01	0.79	1.24	0.93
Sucrose	A	48.74 (29.173)[a]	27.43 (15.923)[a]	56.54 (34.433)[a]	48.60 (29.080)[a]	68.25 (43.045)[a]	58.74 (35.974)[a]
	B	48.23 (28.839)[a]	38.70 (22.769)[a]	45.73 (27.216)[b]	43.37 (25.706)[b]	59.88 (36.794)[b]	49.8 (29.398)[b]
LSD (5%)		0.53	2.49	1.20	1.05	2.53	2.12

†Numbers in brackets are angular transformation values.
[a,b]Values with same letter do not differ significantly.

The number of anthers and pollen production capacity according to flower types for experimental pomegranate cultivars are given in Table 4. Amount of pollen and number of anthers per flower showed variation between different cultivars and between A and B type flowers of each cultivar. In addition, according to A type flowers of all cultivars, the maximum average number of anthers was found on Katirbasi cultivar (331.78) and the lowest one was on Asinar cultivar (197.17).

The number of anthers for other cultivars took place in between above stated values (Table 4). B type flowers of all cultivars, the highest average number of anthers was determined on Katirbasi cultivar (320.17) and the lowest one was on Eksilik cultivar (203.17). The number of anthers for other cultivars varied between 320.17 and 203.17. No literature has been found related to this subject in pomegranates. However, similar results were reported for other crops by other researchers. For example, it was reported that in strawberries the amount of pollen per flower varied between 80,847 and 162,927 (Eti *et al*.,1995). Similar results were also stated for apple, peach, plum and grape (Oberle and Goertzen, 1952).

While Asinar cultivar gave the highest average (1008.00) amount of pollen per anther for A type flowers, Katirbasi cultivar gave the lowest one (484.81). For the amount of pollen per anther, the highest average value was found on Eksilik cultivar (1061.30) and the lowest one was on Katirbasi cultivar (529.56) for B type flowers. Especially, amount of pollen per anther on Eksilik and Asinar were found to be higher than the other cultivars. A good pollinator cultivar should have high pollen viability and germination as well as producing more number of pollen per anther. So, these two above stated cultivars can be considered as important pollinators.

Conclusions

Pollen viability, pollen germination and pollen production capacity of different type of flowers on

pomegranate cultivars were determined. Results obtained in this present study may guide the future studies and can be useful for plant breeders.

Table 4. Pollen production capacity and number of anthers in different flower types and cultivars

Cultivars	Flower type	Number of anthers	Amount of pollen per flower	Amount of pollen per anther
Asinar	A	197.17[a]	198,750[a]	1008.00[a]
	B	207.03[b]	138,600[b]	669.5[b]
LSD (5%)		2.27	3,555.92	3.93
Eksilik	A	223.00[a]	193,400[a]	867.3[a]
	B	203.17[b]	215,625[b]	1061.3[b]
LSD (5%)		2.74	2,043.91	5.07
Ernar	A	321.67[a]	159,420[a]	495.60[a]
	B	290.67[b]	179,460[b]	617.40[b]
LSD (5%)		5.07	2,648.69	3.20
Fellahyemez	A	331.00[a]	184,575[a]	557.63[a]
	B	256.00[b]	166,440[b]	650.16[b]
LSD (5%)		3.93	1,787.12	3.20
Hicaznar	A	257.65[a]	159,300[a]	618.23[a]
	B	238.67[b]	129,450[b]	542.38[b]
LSD (5%)		3.51	3,569.77	5.31
Katirbasi	A	331.78[a]	160,850[a]	484.81[a]
	B	320.17[b]	169,550[b]	529.56[b]
LSD (5%)		3.31	3,192.12	3.58

[a,b]Values with same letter do not differ significantly.

Pollen viability of B type flowers of every cultivar, except Eksilik cultivar, was found to be lower than A type flowers. A and B type flowers of Fellahyemez cultivar gave the lowest level of pollen viability in a comparison to other cultivars. The highest pollen viability for both type of flowers and for all cultivars, except B type flowers of Fellahyemez, was obtained by using TB viability test. FDA viability and sucrose germination tests gave approximately the same level of pollen viability for all cultivars and flower types. In TTC viability test, pollen viability level was low for all cultivars except Eksilik and Ernar cultivars. There were also statistically significant differences between A and B type flowers of all cultivars.

Pollen production capacity varied according to cultivar and flower type. The highest number of pollen production per anther was found in Eksilik and Asinar cultivars. This result means that Eksilik and Asinar can be used as pollinator.

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