

# Determination of Pollen Quality, Quantity and Effect of Cross Pollination on the Fruit Set and Quality in the Pomegranate

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**Abstract:** This research was carried out to determine the pollen quality (pollen viability and germination), quantity (pollen production) and the role of cross pollination on the fruit set in Hicaz and 33 N 26 pomegranate cultivars under Adana ecological conditions in 1997. Pollen viability levels with TTC and FDA, pollen germination level with agar in the petri method, and pollen production capacity with the hemacytometric method of these cultivars were determined in both male (A type-unfertile) and bisexual (B type-fertile) flowers. In addition to these experiments, the changes in the fruit set and fruit quality in open-, self- and cross-pollination (with pollen taken from both bisexual and male flowers) were examined. These studies showed that the highest pollen viability and germination rate were obtained from male flowers of Hicaz (75.24% viability in TTC, 82.45% viability in FDA, 61.50% germination in 1% agar + 10% sucrose medium in agar plate method). Regarding pollen production capability per anther, the best results were obtained from male flowers (3055 pollen/anther in Hicaz, 2701 pollen/anther in 33 N 26). The rates of fruit set in self- and open pollination were found to be lower than those of cross-pollination.

**Key Words:** pomegranate, self- and cross-pollination, fruit set, fruit quality

## Narda Çiçek Tozu Kalitesi ve Üretim Miktarının Belirlenmesi ile Meyve Tutumu ve Kalitesi Üzerine Yabancı Tozlanmanın Etkisi

**Özet:** Bu çalışma, 1997 yılında Adana koşullarında Hicaz ve 33 N 26 nar çeşitlerinin çiçek tozu kalitesi (canlılık ve çimlenme) ve üretim miktarları ile meyve tutumu üzerine yabancı tozlanmanın etkisini araştırmak amacıyla yapılmıştır. Erkek ve hermafrodit çiçeklerde Hemasitometrik metot ile çiçek tozu üretim miktarları, TTC ve FDA testleri ile çiçek tozu canlılık düzeyleri ve Petride Agar yöntemi kullanılarak da çiçek tozu çimlenme kapasiteleri belirlenmiştir. Buna ek olarak serbest tozlanma, kendileme, erkek ve hermafrodit çiçeklerden alınan çiçek tozları ile yabancı tozlanmanın meyve tutumu ve kalitesi üzerindeki etkileri incelenmiştir. Denemeden elde edilen sonuçlara göre en yüksek çiçek tozu canlılık ve çimlenme oranı Hicaz çeşidinin erkek çiçeklerinden (TTC: % 75.24, FDA: % 82.45 ve % 1 agar + % 10 sakkaroz: % 61.50) elde edilmiştir. Çiçek tozu üretim miktarı bakımından ise en olumlu sonuçlar erkek çiçeklerde (Hicaz: 3055 adet / anter ve 33 N 26: 2701 adet / anter) belirlenmiştir. Kendileme ve serbest tozlanma uygulamaları sonucunda yabancı tozlanma uygulamalarına göre daha düşük meyve tutma değerleri elde edilmiştir.

**Anahtar Sözcükler:** nar, kendileme ve yabancı tozlanma, meyve tutumu, meyve kalitesi

## Introduction

The pomegranate (*Punica granatum* L.) is known as an exotic fruit in European countries, and as "paradise fruit" in Arabic countries. Turkey has different pomegranate types and forms because it is one of the origins of the pomegranate. Therefore, various selection breeding studies have been carried out and a quite number of pomegranate types have been determined (1).

Pomegranates are mainly grown in the Aegean, Mediterranean and Southeastern Anatolian regions of Turkey. Nowadays, pomegranate growing is increasing, especially in Southeastern Anatolia because of its suitable ecological conditions. Pomegranate production in Turkey

is about 56,000 tons and the main production area is İçel with 8,160 tons (2). Hicaznar (07 N 08), Çekirdeksiz-VI (33 N 26), Silifke Aşısı (33 N 16), Katırbaşı (31 N 07), Mayhoş-IV (07 N 14), Lefan (31 N 06) and Ekşi Göknar (33 N 12) are the main varieties grown in Mediterranean region of Turkey (3).

The pomegranate is andromonoecious, in other words, male (A type - unfertile) and bisexual flowers (B type - fertile) develop on the same plant. Flowers have an attractive red calyx and corolla. One flower has about 200-350 anthers. Bisexual flowers contain 400-1000 ovules. Pollen transfer is accomplished by insects. The principal pollinator is the honeybee. To meet export market standards, the fruit has to be of a certain size;

this is related to pollination success and consequently to seed number. To ensure a reasonable level of pollination, it is usual to place beehives in orchards during flowering (4-7).

There are only a few experimental studies on the reproduction biology of pomegranate; hence, the viability, germination and production of pollen and the fruit set with other cultivars are not known exactly. The objective of this study is to investigate the viability, germination and pollen production level of the pomegranate and to determine the effect of cross-pollination on the fruit set and quality.

### Materials and Methods

This study was conducted on two important pomegranate cultivars, namely, "Hicaz" and "33 N 26", at Çukurova University's experimental farm in 1997. Some fruit characteristics of these cultivars are given below:

**Hicaz:** The origin of this variety is Alanya (Antalya). It has red-yellow rind and dark red arils with sweet-sour taste. The seed hardness is intermediate.

**33 N 26:** The origin of this variety is Anamur (İçel). The fruit rind is red-yellow and the arils are pink or red. It has a sweet taste and soft seeds.

In this research, the viability, germination and production level of pollen, the fruit set and some pomological characteristics of two pomegranate cultivars were studied. The pollen viability level was determined with TTC (8) and FDA tests (9) and the pollen germination capability by the Agar Plate method (10) in 1% agar + 0, 5, 10, 15, 20 and 25% sucrose media for both bisexual and male flowers. The amount of pollen production per anther and per flower was determined using the Hemacytometric method. The morphological homogeneity level of pollen was also investigated with the same method (11). Flower samples for pollen viability, germination and production tests were taken at the middle of the flowering period. In addition, the changes in the fruit set and fruit quality in open-, self- and cross-pollination were investigated. Bisexual flowers were pollinated with pollen taken from both bisexual and male flowers. All fruits obtained were analyzed pomologically. In these fruits, average fruit weight, 100 aril weight, juice percentage, TSS and acidity were measured.

Six trees were used in each combination and each of these was taken as a replicate. In each replication, 50

flowers were used. The experiment was established according to the randomized design with six replications, and the values were evaluated by the Tukey test.

### Results and Discussion

The results of TTC and FDA tests for pollen viability are shown in the Figure. The rates of viable pollen in TTC and FDA were higher in Hicaz compared with in 33 N 26 cv. In addition, male flowers of both cultivars produced higher viable pollen rates than bisexual flowers. In the TTC test, the highest percentage of viable pollen grains (stained dark red) was found to be 75.24% in male flowers of Hicaz. This ratio was 72.36% in male flowers of 33 N 26 and 70.31 % in bisexual flowers of Hicaz. Bisexual flowers of 33 N 26 had the lowest pollen viability level (68.61%).

The rates of viable pollen grains in the FDA test appeared to be higher than in the TTC test. The pollen viability level varied from 70.11% (bisexual flowers of 33 N 26) to 82.45% (male flowers of Hicaz) in the FDA test. Bisexual flowers of Hicaz and male flowers of 33 N 26 produced 78.05% and 76.75% pollen viability rates, respectively.

The germination percentages of pollen grains of the two pomegranate cultivars are given in Table 1. The highest percentages were observed from male flowers of Hicaz in all tested media. The cultivars used in the experiments produced high pollen germination rates parallel to the results of TTC and FDA tests. For the germination tests, 10% or 15% sucrose concentration + 1% agar were found to be suitable combinations. The best germination percentage was found to be 68.50% in Hicaz male flowers (1% agar + 10% sucrose) and 63.20% in Hicaz bisexual flowers (1% agar + 15% sucrose). In the case of 33 N 26 cultivar, the best germination medium was found to be 1% agar + 15% sucrose for male flowers (61.50%) and 1% agar + 10% sucrose for bisexual flowers (56.95%).

From the results given above, it is clear that the results from the germination and viability tests are in good agreement with each other. The flowering, pollination, fertilization, embryo development, fruit development, fruit set and growing characteristics of the Hicaz cultivar were studied by Gözlekçi (7). Pollen germination studies in this research were performed in a medium consisting of 1% agar + 10% sucrose using agar plate method. According to Gözlekçi (7), the

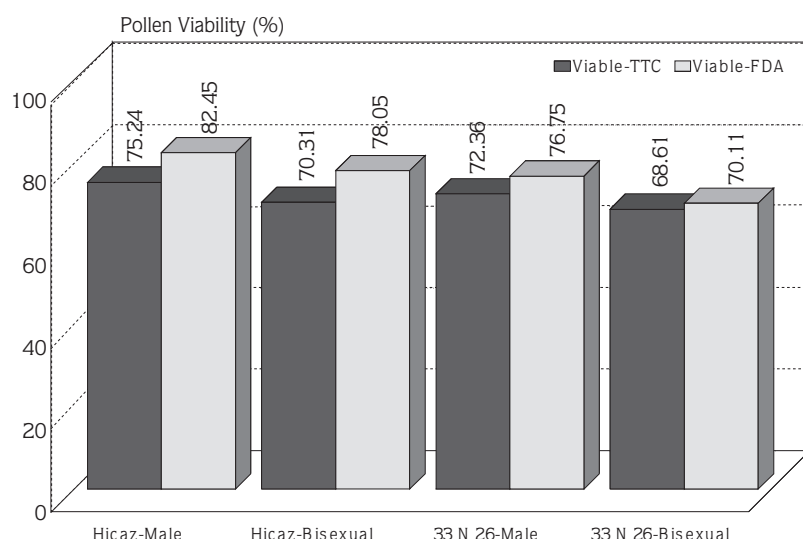


Figure Pollen viability level of the tested pomegranate cultivars.

Pollen germination and production capacity	HICAZ		33 N 26		D*5%
	Male	Bisexual	Male	Bisexual	
1% agar + 0% Sucrose	2.36	2.05	2.10	2.50	n.s.**
1% agar + 5% Sucrose	3.25	3.20	3.06	3.00	n.s.**
Agar 1% agar +10% Sucrose	68.50 a	62.15 b	60.48 b	56.95 c	3.04
Plate 1% agar + 15% Sucrose	65.29 a	63.20 ab	61.50 b	56.00 c	3.58
1% agar + 20% Sucrose	55.75 a	54.78 a	55.10 a	50.05 b	3.50
%1 agar + 25% Sucrose	52.16 a	48.40 ab	50.52 a	45.25 b	3.91

Table 1. Pollen germination rates (%) in tested pomegranate cultivars.

\*: mean significant differences

\*\*.: n.s.: non-significant

highest pollen germination rates were 69.5% in bisexual flowers and 68.1% in male flowers. The pollen germination capability was found to be above 90% in another experiment (12). Nalavadi et al. (13) found that the most suitable medium for pollen germination under *in vitro* conditions was 10% sucrose concentration; however, it was found to be 20% by Chitale and Deshpande (14). Similar results were obtained by Nath and Randhava (5). According to those researchers, the pollen viability level was not lower than 82% in all of the cultivars tested. 12.5% sucrose + 0.5% agar medium was found to be suitable for pomegranate pollen germination.

The number of anthers per flower varied from 213 (33 N 26 bisexual flowers) to 325 (male flowers of Hicaz). Generally, male flowers in the two cultivars gave higher anther numbers (325 in Hicaz and 287 in 33 N 26) than bisexual flowers did (218 in Hicaz and 213 in 33 N 26) (Table 2).

The pollen production tests showed that male flowers in both cultivars provided the best results (Table 2). The highest amount of pollen per anther was found in Hicaz male flowers (3055). Male flowers of 33 N 26 and bisexual flowers of Hicaz followed Hicaz male flowers with 2701 and 2166 pollen grains per anther respectively. Bisexual flowers of 33 N 26 gave the lowest pollen production with 2096 grains per anther. Similar results were obtained concerning pollen production per flower. The best pollen production capacity was found in Hicaz male flowers with 992,875 grains per flower. Bisexual flowers of 33 N 26 with 446,448 grains per flower had the lowest pollen production.

Besides the amount of pollen production in the flowers of a variety, the rate of morphologically normal pollen grains is also important. The data in Table 2 reveal that the morphological homogeneity in male and bisexual flowers of examined cultivars is quite satisfactory,

Pollen production and Morphological Homogeneity	HICAZ		33 N 26		D*5%
	Male	Bisexual	Male	Bisexual	
Anther number per flower	325 a	218 c	287 b	213 c	18.34
Pollen production per anther	3055 a	2166 c	2701 b	2096 c	182.11
Pollen production per flower	992875 a	472188 c	775187 b	446448 c	99302
Morphological homogeneity (%)	90.35 a	71.10 c	81.50 b	68.45 d	2.24

Table 2. Pollen production and morphological homogeneity (%) capacity of pollens in tested cultivars.

\*: mean significant differences

although higher values were obtained in male flowers (90.35% in Hicaz and 81.50% in 33 N 26).

The results of the fruit set after self-, cross- (with pollen taken from male and bisexual flowers) and open pollination are given in Table 3. The rates of fruit set in self- and open pollination were lower than in cross-pollination. The lowest fruit set was obtained from open pollination in 33 N 26 with 46.02% and the highest rate (78.92%) was observed in Hicaz pollinated with pollen taken from 33 N 26 male flowers. The fruit set level was found to be 70.75% in Hicaz x 33 N 26 bisexual flower combinations, 68.50% in 33 N 26 x Hicaz male flower combinations and 61.75% in 33 N 26 x Hicaz bisexual flower combinations. In both Hicaz and 33 N 26 cultivars, the fruit set was increased considerably by pollination with pollen taken from male flowers. Cross-pollination with other cultivars increases the fruit set level in pomegranates, and thus, in order to establish a new

orchard, at least two different pomegranate cultivars should be used.

In the Hicaz cultivar, 77.68% - 86.42% of the total flowers on a single tree were A type (male) flowers, and 13.80%-22.32% were B type (bisexual) flowers. The fruit set percentage as a whole ranged between 7.59% to 16.07%, but it was between 44.0% and 71.99% in B type flowers (7). Similar results were obtained by Onur (15) and by Tibet and Baktır (16).

The fruit quality characteristics of the cultivars tested are shown in Table 3. Different sources of pollen did not play an important role in the quality parameters, namely, juice percentage, total soluble solids (TSS) and titrable acidity contents. On the other hand, fruit weight and 100 aril weight were increased considerably by cross-pollination with pollen taken from other cultivars' male flowers. The best results in terms of fruit weight and 100 aril weight were obtained in 33 N 26 x Hicaz male flower

Combination	Fruit set (%)	Fruit weight (g)	100 Aril weight (g)	Juice Percentage	TSS (%)	Acidity (%)
Hicaz O.P.*	56.25 d	280.1 d	19.6 c	31.9 b	14.2	2.5
Hicaz x Hicaz M. **	61.14 c	298.5 c	21.7 b	32.5 b	14.2	2.4
Hicaz x Hicaz B.* **	57.55 d	285.8 d	19.5 c	32.3 b	14.0	2.5
Hicaz x 33 N 26 M.	78.92 a	325.3 a	24.3 a	34.2 a	14.0	2.5
Hicaz x 33 N 26 B.	70.75 b	302.9 b	23.2 a	34.3 a	14.0	2.3
D 5%	3.26	7.71	1.36	1.55	n.s.	n.s.
33 N 26 O.P.	46.02 d	401.9 d	26.0 b	38.6	16.0	0.5
33 N 26 x 33 N 26 M.	50.33 c	420.2 c	26.6 b	38.4	16.2	0.5
33 N 26 x 33 N 26 B.	46.40 d	406.8 d	25.8 b	38.4	16.2	0.5
33 N 26 x Hicaz M.	68.50 a	446.7 a	28.9 a	39.2	16.0	0.4
33 N 26 x Hicaz B.	61.75 b	435.5 b	28.4 a	39.1	16.2	0.4
D 5%	3.48	8.51	1.41	n.s.	n.s.	n.s.

Table 3. Rates of fruit set and some pomological characteristics after open- self- and cross-pollination in tested pomegranate cultivars.

O.P.: Open Pollination  
M. : Male  
B.: Bisexual  
n.s.: Non-significant  
D% 5: mean significant differences  
TSS: Total soluble solid

combinations (fruit weight: 446.7 g, 100 aril weight: 28.9 g). The effects of self-, cross- and open pollination on juice percentage, TSS and acidity were not found to be statistically significant except for juice percentage in the Hicaz cv. Juice percentage, TSS and acidity varied from 31.9% (Hicaz open pollination) to 39.1% (33 N 26 x Hicaz bisexual flowers), 14.0% (Hicaz x Hicaz bisexual flowers, Hicaz x 33 N 26 male flowers and Hicaz x 33 N 26 bisexual flowers) to 16.2% (33 N 26 x 33 N 26 male flowers, 33 N 26 x 33 N 26 bisexual flowers and 33 N 26 x Hicaz bisexual flowers), and 0.4% (33 N 26 x Hicaz male flowers and 33 N 26 x Hicaz bisexual flowers) to 2.3% (Hicaz x 33 N 26 bisexual flowers). Gözlekçi (7) found that there was a positive correlation between diameter, weight and volume of the fruit, especially between fruit volume and fruit weight in the Hicaz cultivar. In that experiment, the average fruit weight, 100 aril weight, TSS and acidity were 329.87 g, 29.89 g, 15.88% and 1.91% respectively. In different adaptation

studies of the pomegranate, similar results related to fruit quality have been obtained (3,7,15,17-19).

## Conclusion

The pomegranate carries its male and bisexual flowers separately on the same tree. Both male and bisexual flowers produced sufficient high quality pollen. Although bisexual flowers have viable and germinable pollen, self- and cross-pollination with male flowers' pollen results in a significant increase in the fruit set as found in this research. Moreover, cross pollination increased fruit quality, besides expanding fruit set level. In conclusion, it is recommended that at least two different cultivars should be planted in an orchard.

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