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Contribution to a better knowledge of the quality of pomegranate pollen (*Punica granatum* L.)

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SUMMARY – The aim of the present work has been to determine reasons for low fertilisation in some pomegranate cultivars. The studied cultivars were ME5, ME16 and ME17 cultivated in homogeneous conditions in the collection grown at Escuela Politécnica Superior of Orihuela (EPSO). The investigation was carried out during 1996 and 1997. Pollen viability has been studied with acetic carmine dye weekly throughout flowering. This parameter was also studied in pollen that has been conserved at 5°C and 28°C. Germination ability was researched with pollen of male and hermaphrodite flowers grown in a culture medium at 15°C and 28°C which were observed 24 and 48 hours after sowing. Different pollination types were studied (natural, anemophilous, entomological and self-pollination). The stigma receptivity period, ranging from two days before anthesis to four days after it, was also determined. To summarise it could be deduced that there are different flowering abilities and effective pollination types. It was also confirmed that lower temperature conservation allowed pollen to be conserved for longer, and the effective pollination period varied from anthesis up to three days later.

Key words: Pomegranate tree, floral biology, pollen, pollination.

RESUME – *"Contribution à une meilleure connaissance de la qualité du pollen de grenadier (*Punica granatum *L.)". Le but de la présente étude a été de déterminer les raisons de la faible fertilisation chez certains cultivars de grenadier. Les cultivars étudiés étaient ME5, ME16 et ME17 cultivés en conditions homogènes dans la collection plantée à la Escuela Politécnica Superior de Orihuela (EPSO). La recherche a été menée pendant 1996 et 1997. La viabilité du pollen a été étudiée avec une teinture au carmin acétique hebdomadairement tout au long de la floraison. Ce paramètre a également été étudié chez le pollen qui avait été conservé à 5°C et 28°C. La capacité de germination a été étudiée avec du pollen de fleurs mâles et hermaphrodites cultivées dans un milieu de culture à 15°C et 28°C qui ont été observées à 24 et 48 heures après semis. Différents types de pollinisation ont été étudiés (naturelle, anémophile, entomologique et autopollinisation). La période de réceptivité du stigmate, qui va de deux jours avant l'anthèse à quatre jours après, a également été déterminée. En résumé, on peut en déduire qu'il y a différentes capacités de floraison et types de pollinisation effective. Il a également été confirmé qu'une conservation à plus basse température permettait de conserver le pollen pendant plus longtemps, et que la période effective de pollinisation variait de l'anthèse jusqu'à trois jours plus tard.*

Mots-clés : *Grenadier, biologie florale, pollen, pollinisation.*

Introduction

Floral biology studies, which have been intensified in recent years, have often shown the close relationship with fructification problems.

The quantity and quality of available pollen, receptivity of the stigma, pollen-pistil compatibility and relationship with environmental factors, etc., are now being studied in three cultivars, ME5, ME16 and ME17, in order to find out to what extent lack of fructification is related to one or several of these phenomena.

We believe it necessary to increase studies on floral biology of the pomegranate (*Punica granatum* L.), as up to now all the existing bibliography consists of a few articles which do not study the subject in depth, and mainly deal with varieties grown outside Spain.

During the prospecting for and pre-selection of pomegranate vegetable matter, we were able to appreciate the great variety which exists in our plantations, resulting from a rapid growth in cultivation without sufficient knowledge about this, or about the chosen matter.

This varietal diversity has led to various general problems in plantations, such as lack of productivity, inadequate ripening time, deficient fruit colour, etc. There is now a need to carry out a pomological characterisation of the species matter, and then, within this, a floral biology study characterisation.

National bibliography on this subject is non-existent except for what has been written during the last four years at Escuela Politécnica Superior de Orihuela (EPSO).

International bibliography is also scarce, and refers to very specific aspects of the problem. In any case, the studies have been carried out on different varieties from our own. We can cite studies carried out by Nalawadi *et al.* (1973), Josan *et al*. (1979), Singh *et al.* (1981), Game (1987), Purohit (1987), El-Sese (1988) and Keskar *et al*. (1993).

Materials and methods

The vegetable matter used was extracted from the experimental plot of the EPSO. The varieties studied were three sweet pomegranate cultivars: ME5, ME16 and ME17.

Pollen extraction

Pollen was extracted from flowers at phenological stage F (Melgarejo *et al*., 1997), when the stamen, and therefore also the pollen, had reached their full morphological and physiological development. Pollen was extracted with a different No. 1 brush for each variety and each sex, in order to avoid mixing the different types of pollen and causing errors in the results. It was then placed in sterilised Petri dishes and labelled. A sample of each cultivar was placed in a refrigerator at 5°C, and another in a cabinet at 28°C, to study pollen conservation. Samples were taken this way weekly to carry out viability and germination potential tests.

Viability analysis

The dye used to study the pollen grain viability was acetic carmine, because, being a protoplasmic dye, it is easily identifiable and does not colour abnormal grains, whereas those which are normal appear red. An Olympus BH2 microscope with a magnifying power of 10, and a binocular microscope, also with a magnifying power of 10, were used in the test.

Once the pollen was extracted as indicated above, the brush was used to sprinkle it onto a slide, and a drop of acetic carmine was added. A slide cover was placed on top, avoiding bubble formation, and the grains were counted immediately.

To obtain the corresponding viability percentage for each variety, an average of 1000 pollen grains per slide were counted in each case. The viability tests were carried out periodically every seven days.

Pollen conservation

To measure the pollen conservation time, a generous pollen sample was taken at the start of flowering; part of the sample was kept in cold storage at 5°C, and the rest was kept in a cabinet at 28°C. Throughout the period, weekly samples were taken to measure the viability.

Study of germination potential

The culture medium used consists mainly of a saccharose solution, agar and micronutrients in distilled water, sterilised in an autoclave (at 120°C for 15 minutes), and deposited in a fine film on a sterile Petri dish. Once cooled down and solidified, the pollen is sown here. This process is carried out in a laminar flow chamber, and with an alcohol burner, to avoid possible contamination.

The culture medium composition for a solution of 100 g is 81.5 g of distilled water, 17.5 g of saccharose, 1 g of agar, 22 mg of dihidrated calcium chloride and 2 mg of boric acid.

This test was carried out on pollen from staminate or "male" flowers, and hermaphrodite flowers, at two different temperatures, 15°C and 28°C, and with light. The dishes were stored at these temperatures and were observed 24 and 48 hours after sowing. The number of pollen grains counted on each dish was 1000.

Types of pollination

To measure the percentage of different types of pollination, the following criteria were met:

(i) In order to study the self-pollination percentage, a large number of flowers at phenological stage E2 were chosen, i.e. before the flower opened, as described by Melgarejo *et al*. (1997). The flowers were then put in bags, and those that had set were counted.

(ii) The anemophilous pollination percentage was obtained by putting flowers at stage E2 in bags with anti-mosquito nets, and after two months the number of set flowers was counted.

(iii) The natural pollination percentage was obtained by marking the control flowers, without obstructing pollination at all.

(iv) The entomological pollination percentage was obtained by subtracting from natural pollination the results of self-pollination and anemophilous pollination.

Stigma receptivity

The stigma receptivity period, and therefore also the pollination period, were measured by selecting a series of hermaphrodite flowers at stage E2 and castrating them. This consists in cutting the upper part of the sepals with a scalpel to remove the anthers before they dehisce; vaseline is then applied to the cut to reduce flower dehydration as much as possible. After this, the flower was pollinated by touching the stigma lightly with a brush impregnated with pollen which stayed on the stigma. The flower was immediately protected by a paper bag, to prevent different pollen reaching its stigma, insect attacks, etc. The bag was labelled for identification, and to study its setting.

Results and discussion

Pollen viability

Viability evolution throughout flowering is shown in Fig. 1.

As can be seen, all the cultivars maintain a high viability percentage during the months of June and July, coinciding with the maximum flowering period, with the exception of cultivar ME5 during the 1996 season, which showed brusque changes.

The average viability of the pollen grains throughout the weeks of the test is shown in Table 1.

According to Josan *et al*. (1979), pollen viability varied from 64.44% for the Afghanistan seedling cultivar, to 98.99% for the Bedana cultivar. The results obtained in our case are not comparable, because there have been changes in the culture medium, incubation time, temperatures and, most importantly, different varieties. However, the results are very close to those mentioned, varying between 60.66% for the ME5 cultivar and 77.05% for ME16.

Pollen conservation

Pollen conservation was studied by analysing the viability weekly, and the results were as

expected, as it progressively lost its capacity to be viable in both of the studied cabinets over the 70 days of the test.

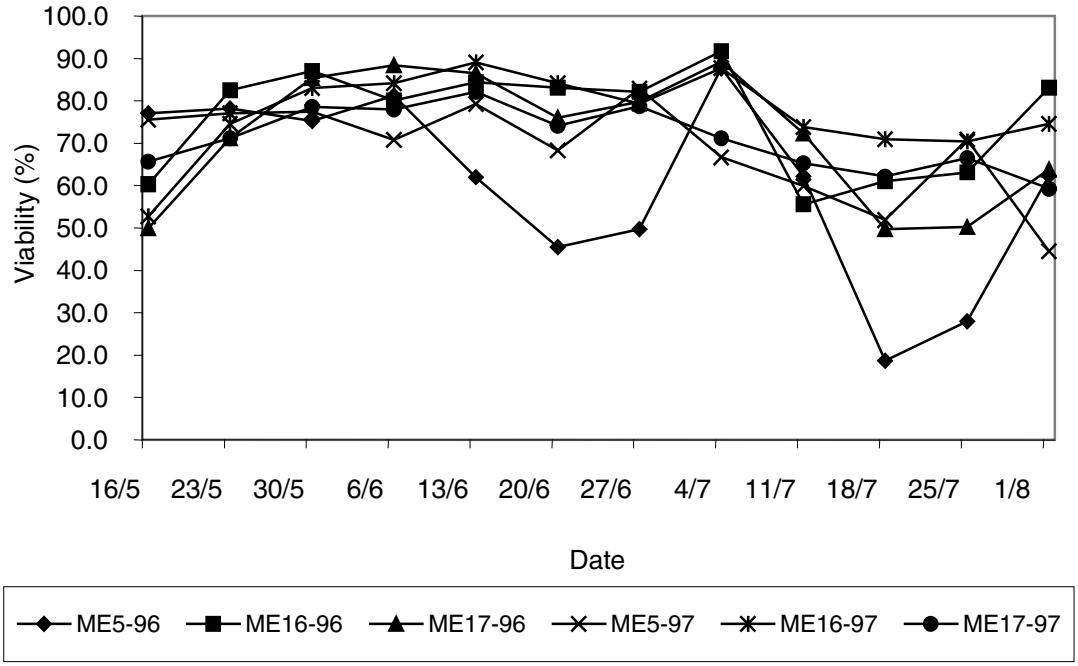

Fig. 1. Pollen viability evolution in time.

Table 1. Average viability percentages of the pollen

	ME5 (%)	ME16 (%)	ME17 (%)
1996	60.66	76.18	71.87
1997	68.76	77.05	71.08
Average	64.71 ± 5.72	76.61 ± 0.62	71.47 ± 0.56

Fernández (1995) observed a rapid loss in pollen viability with cultivars PTO2 and PTO7 stored at 5°C and 28°C, which lost their viability after 40 and 30 days respectively, which contrasts with our results as shown below.

Figure 2 shows the viability evolution of the stored pollen in time, and at the two tested temperatures.

Pollen germination potential

The germination potential of the pollen grains is calculated for the viable grains, considering these to be 100% of the pollen. The pollen grains are incubated for 48 h at 28°C and at 15°C. The results are shown in Table 2.

According to Josan *et al.* (1979), the highest pollen germination was obtained from the Kazkai cultivar (96.55%) and the lowest from Bashi Kalinski cv. (65.05%). The germination ability of these pollen grains was studied in a 20% saccharose solution after 24 hours.

The tests on cultivars ME5, ME16 and ME17 gave notably lower germination percentages, which

could be due to the different physiological and climatic conditions, or to the different vegetable matter used.

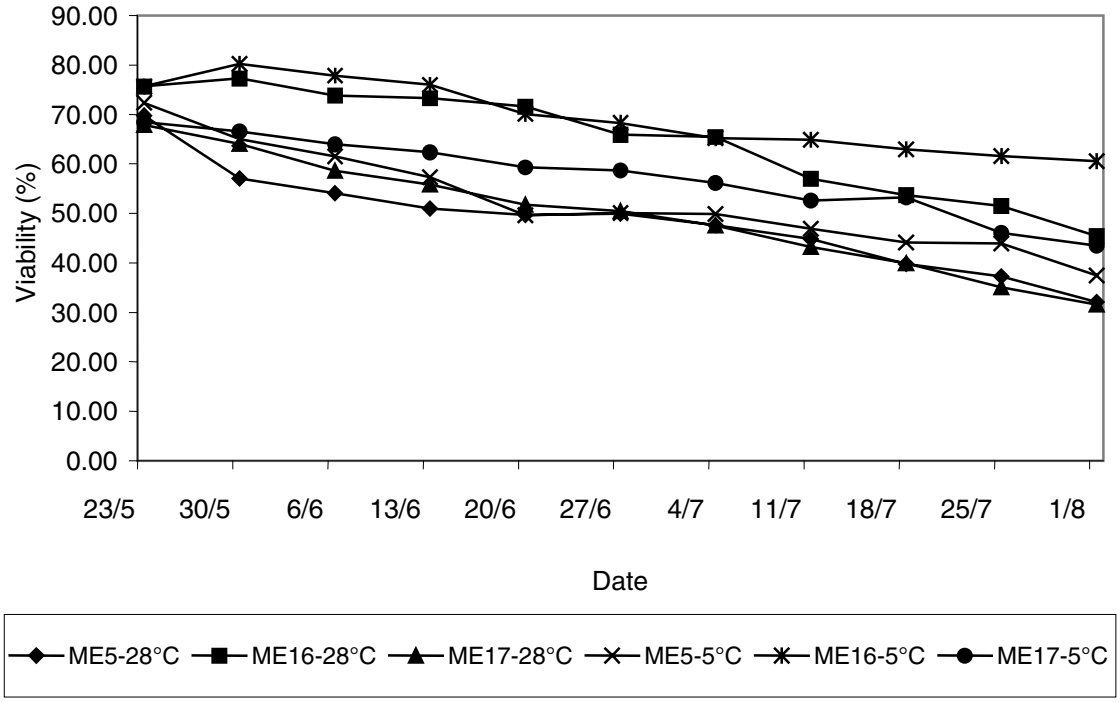

Fig. 2. Viability evolution of pollen stored at 5°C and 28°C.

Table 2. Pollen germination percentage at 15°C and 28°C

		ME5 (%)	ME16 (%)	ME17 (%)
1996	15°C	10.59	24.49	17.45
	28°C	25.24	35.35	30.75
1997	15°C	13.42	20.19	15.7
	28°C	16.49	22.52	17.79

Types of pollination

The results obtained with natural pollination (control), controlled natural self-pollination (flowers in paper bags) and controlled pollination (flowers in net bags) are shown in Table 3.

Figure 3 shows the average values reached by the different types of pollination for the three cultivars tested, in years 1996 and 1997.

As is shown, natural pollination has the highest values in the three cultivars which were studied.

According to Fernández (1995), the percentage of natural setting in the three cultivars studied (PTO1, PTO2 and PTO7) was approximately 50%.

For our cultivars ME5 and ME17 the results were similar (50% and 54.71% respectively) whereas ME16 shows a high percentage of natural setting (68.96%).

For clones ME5 and ME17, the values of entomological pollination are very considerable and

anemophilous pollination is low. The ME5 cultivar's incompatibility with its own pollen should be noted.

Table 3. Percentages of the different types of pollination

	1996			1997		
	ME5 (%)	ME16 (%)	ME17 (%)	ME5 (%)	ME16 (%)	ME17 (%)
Natural P.	50.00	70.00	56.66	50.00	67.92	52.77
Anemophilous P.	18.42	2.12	21.44	12.37	14.63	16.72
Entomological P.	31.58	24.55	27.21	30.96	14.27	29.25
Self-pollination	0.00	43.33	8.01	6.67	39.02	6.80

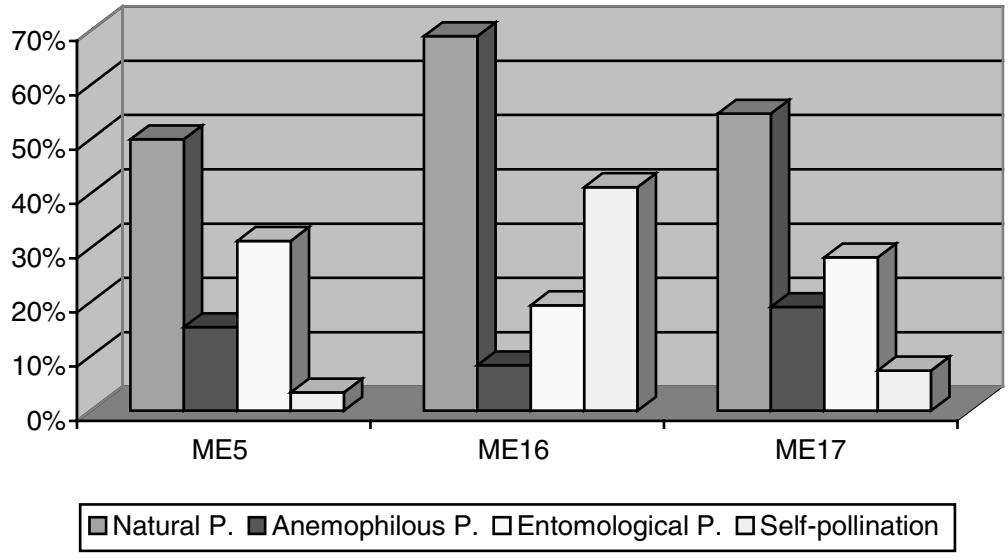

Fig. 3. Average values reached for each pollination type.

Stigma receptivity

In Table 4, the average percentage of stigma receptivity is shown from two days before to four days after anthesis. The results show that the time when the stigma is receptive varies from two days before to three days after anthesis, with highest receptivity being on the day of anthesis and the following day.

Table 4. Stigma receptivity percentages

	Days in relation to anthesis†						
	-2	-1	0	1	2	3	4
ME5	21.5	34	48.5	55	42.5	17.5	0
ME16	21.5	42.5	56.5	44	31.5	36	0
ME17	26.5	32	54.5	62	38.5	34	0

†Negative numbers indicate days before anthesis and positive numbers indicate days after;
zero shows the day when the flower opens.

According to Nalawadi *et al.* (1973), stigma receptivity is from one day before to three days after anthesis, which coincides with our results, although we observed that it is also receptive two days before.

Conclusions

(i) All the varieties, except ME5, show maximum pollen viability coinciding with the moment of maximum flowering.

(ii) Pollen conservation is much greater at 5°C than at 28°C.

(iii) Germination ability is greater with incubation at 28°C than at 15°C.

(iv) Self-pollination is very considerable in variety ME16, whereas ME5 is practically incompatible with its own pollen.

(v) The period during which the stigma is receptive goes from two days before to three days after anthesis.

(vi) The effective pollination period is from the day of anthesis to three days after this.

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