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Study of the floral morphology of the pomegranate clones: PTO8, CRO1 and ME14

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SUMMARY – We study in depth some aspects of the floral morphology of the pomegranate clones, PTO8, CRO1 and ME14, the first two from the province of Murcia and the third from the province of Alicante (both in SE Spain). The study was carried out in 1996 and 1997. Twenty-five flowers were taken from each clone and analysed in the second week of May in both years. We study the types of flower in pomegranate (hermaphrodite or male) and their percentage in each clone studied. The importance of this aspect is underlined by the fact that only hermaphrodite flowers produce fruit. The number of petals and sepals is also of importance in understanding the pomegranate flower, the number of each usually coincide between 5 and 9, with 6 or 7 being the most usual. The height and width of the petals can also differ between clones. Finally, we consider the number of stamens, the height of the pistil and the relation between the level of the anthers and stigma of the flower, and observe that heterostyly is a common occurrence in the clones studied.

Key words: Floral morphology, flowers, petals, sepals, stamens, pistil.

RESUME – *"Etude de la morphologie florale des clones de grenadier PTO8, CRO1 et ME14". Nous avons étudié en profondeur certains aspects de la morphologie florale des clones de grenadier, PTO8, CRO1 et ME14, les deux premiers de la province de Murcie et le troisième de la province d'Alicante (tous deux dans le SE de l'Espagne). L'étude a été menée en 1996 et 1997. Vingt-cinq fleurs ont été prélevées sur chaque clone et analysées lors de la deuxième semaine de mai pour les deux années. Nous avons étudié les types de fleurs du grenadier (hermaphrodites ou mâles) et leur pourcentage dans chaque clone étudié. L'importance de cet aspect est souligné par le fait que seules les fleurs hermaphrodites ont produit des fruits. Le nombre de pétales et de sépales est également important pour comprendre la fleur de grenadier, dont le nombre coïncide habituellement entre 5 et 9, le plus courant étant 6 ou 7. La hauteur et la largeur des pétales peuvent également différer entre clones. Finalement, nous considérons le nombre d'étamines, la hauteur du pistil et la relation entre le niveau des anthères et le stigmate de la fleur, et nous observons que l'hétérostylie est un évènement commun chez les clones étudiés.*

Mots-clés : *Morphologie florale, fleurs, pétales, sépales, étamines, pistil.*

Introduction

The bibliography on flowering periods, flowering density, percentage of male and hermaphrodite flowers, floral morphology, in short, the topics of pomegranate floral biology, is rather sparse. In the revision that we have made, we have not found a single study dealing specifically with pomegranate floral morphology. At most there are brief sections within other wider-scope articles. Besides, these studies are carried out on very different varieties from those we grow in south-east Spain and in different agro-climatic conditions.

Therefore, we consider it necessary to carry out a more intensive study of the floral morphology of the pomegranate that can provide knowledge on these aspects of varieties grown in Levant, Spain.

El-Sese (1988), in Egypt, carried out trials to study the flowering periods, distribution of whole and partial flowers during the flowering season, initial fruit set and final fruit retention. The cultivars studied are Manfalouty, Nab El-Gamal and Arabi. Among other results, this author has observed that the percentage of perfect flowers in Manfalouty and Nab El-Gamal is higher than those in the Arabi cultivar. This data shows a positive correlation between the percentage of perfect flowers and the production capacity of the tree. There was no alternation in production in the Manfalouty cultivar, a slight alternation in Nab El-Gamal and a greater alternation in the Arabi cultivar.

Nalawadi *et al*. (1973), in a study on the floral biology of the pomegranate carried out in the state of Mysore, presents a table reflecting the percentages of male, intermediate and hermaphrodite flowers of the Dholka, G.B-1 and Local varieties. The number of floral buds observed was 55, 50 and 40 respectively. The maximum number of male flowers was observed in Dholka (47.27%). The maximum number of intermediate flowers was observed in G.B-1 (20%). The greatest number of hermaphrodite flowers was observed in the Local variety (60%), being 56 and 38.18% in the G.B-1 and Dholka varieties respectively.

Josan *et al*. (1979), in a study carried out in India, devotes the last section to some aspects of floral morphology of 21 varieties of pomegranate. The parameters counted or measured when appropriate were: flower size, sepal, petal and stamen numbers. They also observe that in the pomegranate cultivars studied, heterostyly is quite common. In 13 cultivars the style length was shorter than that of the stamens, in 1 case the style length was greater than that of the stamens and in 7 cultivars both lengths were equal.

The main objective of this study is to contribute to improving the different aspects of floral morphology of the pomegranate. In order to do so we have chosen 3 clones of autochthonous Spanish pomegranates (Murcia and Alicante). These clones are: PTO8, CRO1 and ME14.

Material and methods

The plant material used in this study is made up of pomegranate trees of the clones PTO8, CRO1 and ME14, described by Melgarejo (1993). These were grown on the estate of the Higher Polytechnical School of Orihuela (University Miguel Hernández), obtained by vegetative propagation, planted in a 4×3 m layout. The estate is situated in the catchment area of Orihuela (Alicante), and drip-irrigated. The trees are goblet-trained.

The soil is loam-clay, rainfall is scarce, with an annual precipitation of approximately 300 mm, falling in spring and autumn. The average annual temperature is 19°C, with mild winters and hot summers, classified as subtropical in the Papadakis climate index (MAPA, 1986). The agro-climatic area satisfies the crop requirements.

The clones were 4 years old at the beginning of the trial and therefore the plantation was still young. The study was conducted over the period 1996-1997.

Sex ratio

In order to determine the proportion of male and hermaphrodite flowers, the southern half of one tree of each clone was monitored. The tree chosen was in a good state of health and was representative of the clone in question. A count was made of the flowers in E3 advanced stage or F initial stage (Melgarejo *et al*., 1996) every 3 days, taking note of which were male and which were hermaphrodite. This was done for the first year from 3 June to 4 August and the second year this was done on the PTO8 and ME14 clones from 14 April to 12 August. Clone CRO1 was likewise monitored from 30 April to 8 August. Thus all the flowers reaching phenological stage F were studied. At this stage it is very easy to distinguish the sex of the flower. From this information we can determine, not only the percentage of male and hermaphrodite flowers but we can also represent the evolution of the flowering period of both sexes.

Petals, sepals, stamens and the style size / stamen length ratio

In order to study these aspects, samples were taken from each clone of at least 25 flowers. These flowers were taken to the laboratory where the number of petals and sepals were counted. The length and diameter of the flower were measured, as well as the width and height of each petal of each flower, taking note of the stamen/pistil height ratios. Finally the stamens of each flower were counted.

Statistical analysis

The results obtained were submitted to the following statistical tests:

(i) Comparison of length and diameter of the flowers in different clones using an analysis of variance, with a later comparison by multiple rank test.

(ii) Comparison of length and width of petals in different clones, using an analysis of variance, with a later comparison by a multiple rank test.

(iii) Comparison of the number of stamens of the flowers in different clones, using an analysis of variance, with a later comparison by a multiple rank test.

Results and discussion

The results obtained are expressed in Tables 1 and 2, and separated into years to make the presentation clearer.

Table 1. Types of flower: male (M) and hermaphrodite (H), year 1996

Clones	M	H	Total	% of M	% of H
ME14	168	154	322	52.2	47.80
PTO8	624	62	686	91	9.00
CRO1	602	96	698	86.2	13.80

Table 2. Types of flower: male (M) and hermaphrodite (H), year 1997

Clones	M	H	Total	% of M	% of H
ME14	321	483	804	39.9	60.10
PTO8	378	312	690	54.8	45.20
CRO1	333	87	420	79.3	20.70

In Tables 1 and 2 there are notable differences from one year to the next in the total number of flowers produced by each clone. This can be due to various causes but moreover it is because the study is conducted on young trees in the development phase. We should not forget that at the beginning of the trials the trees were only 4 years old.

In the second year of the experiment, there was an increase in the percentage of hermaphrodite flowers. This increase was slight in the ME14 and CRO1 clones, but much bigger in the PTO8 clone. Even though this is what really happened, we do not have enough experience with this clone in order to be able to affirm that this variability is normal or just a chance occurrence, even though in both years, the production was much lower than that of the other two clones studied. The PTO8 clone to date has shown to be more vigorous, more erect and thornier than the CRO1 clone, which in turn is more vigorous, erect and thornier than the ME14.

Nalawadi *et al*. (1973), when studying the Dholka, G.B-1 and Local varieties distinguishes between male, intermediate and hermaphrodite flowers. The local variety produced most hermaphrodites with 60%, and the Dholka produced the least, with 38.18%.

El-Sese (1988) also studies the ratio between flowers of different sexes, however this author calls them perfect flowers, hermaphrodites with reproductive capacity and imperfect flowers – those that cannot reproduce – which would correspond to the male and intermediate flowers described by

Nalawadi *et al*. (1973), or the "male" flowers we have described in our study. El-Sese conducted this type of study during 1985, 1986 and 1987, with the Manfalouty, Nab El-Gamal and Arabi varieties. The highest percentage of perfect flowers was obtained in the Nab El-Gamal variety with 35.4% during the second year of the experiment whereas the lowest percentage was obtained in the same variety in the third year of the experiment with a value of 19.5%. This author also studies the correlation between the percentage of perfect flowers and production. The result is that the correlation is very high in the Manfalouty (r = 0.914) and Nab El-Gamal varieties (r = 0.899), whereas it is quite low in the Arabi variety (r = 0.232).

In our study, the greatest percentage of hermaphrodite flowers was obtained in the ME14 clone with 60.10%, and the lowest value (not counting the PTO8 which, as we have mentioned, was highly variable) in clone CRO1 with 13.80%, which is why our results are in line with those obtained by El-Sese (1988) and Nalawadi *et al.* (1973).

The number of sepals is, in the majority of cases, the same as the number of petals. The mean values obtained for both coincide, as can be observed in Tables 3 and 4. In the three clones studied, the number of sepals and petals is 6 in the first year. In the second year it remains stable for clones ME14 and CRO1, however the PTO8, as before, shows a higher variability giving an average value of 7.64, which differs significantly from the other two clones and from itself in comparison with the previous year.

Table 3. Floral morphology. Mean values, year 1996

	Clone ME14	Clone PTO8	Clone CRO1
No. sepals	6.20	6.20	6.10
No. petals	6.20	6.20	6.10
Length (L)† male flower	2.20	2.00	2.00
Length (L)† hermaph. flower	2.50	2.20	2.20
Diameter (D)† male flower	1.80	1.60	1.60
Diameter (D)† hermaph. flower	2.10	1.70	1.70
(L/D)×100, male flower	122.22	129.40	129.40
(L/D)×100, hermaph. flower	119.04	125	125
No. male stamens	252	254	239
No. hermaph. stamens	230	242	232
No. stamens (medium)	241	248	235
No. stamens (maximum)	299	314	293
No. stamens (minimum)	184	145	166
Length† "male pistil"	0.90	0.80	0.70
Length† hermaph. pistil	1.80	1.80	1.90

†Lengths and diameters in cm.

Regarding the flower length and number of stamens, the PTO8 clone once again shows differences in the second year in comparison with the first year and with the other two clones, as can be observed in Tables 3 and 4.

The denomination used in this study of "male and hermaphrodite flowers", means that those called hermaphrodites are hermaphrodites to all effects whereas those called male are hermaphrodite flowers with a somewhat atrophied pistil, clearly visible but unviable. Therefore it is logical that there is an important difference in the pistil length of the hermaphrodite flowers and those which we have incorrectly called "male".

The lowest number of stamens occurred with clone ME14 (108), whereas the highest number was obtained with PTO8 (416). The average number of stamens for the two years was 235 for clone ME14, 274 for clone PTO8 and 230 for clone CRO1.

The results that we have obtained for length and width of the flower, as well as the number of stamens, are along the same lines as those published by Josan *et al*. (1979).

Table 4. Floral morphology. Mean values, year 1997

	Clone ME14	Clone PTO8	Clone CRO1
No. sepals	6.03	7.64	6.05
No. petals	6.03	7.64	6.05
Length (L)† male flower	2.02	3.17	1.77
Length (L)† hermaph. flower	2.48	3.59	2.01
Diameter (D)† male flower	1.71	1.07	1.29
Diameter (D)† hermaph. flower	2.09	1.36	1.61
(L/D)×100, male flower	118.71	296.26	137.31
(L/D)×100, hermaph. flower	118.24	263.97	125.20
No. male stamens	219.32	313.40	228.70
No. hermaph. stamens	241.33	287.47	219.90
No. stamens (medium)	230.32	300.43	224.30
No. stamens (maximum)	283	416	331
No. stamens (minimum)	108	214	175
Length† "male pistil"	1.1	0.86	1.00
Length† hermaph. pistil	1.89	2.30	2.30

†Lengths and diameters in cm.

We have also made observations of the size of the style in relation to the length of the stamens. Heterostyly is quite common in these pomegranate clones, occurring in the same clone flowers in which the style is shorter than the stamens, longer and even the same length.

With all data we have carried out a statistical analysis, whose results are represented in Figs 1-7. In the first place, we indicate the results referring to length, width and length/width of the flowers of the three clones with the joint data of the two years.

Figure 1 shows the result of comparing flower width at ovary height. There are significant differences at 95% confidence between the three clones, the ME14 being outstanding with the greatest ovary diameter. This result was expected to a certain extent since it was also the clone with the highest percentage of hermaphrodite flowers.

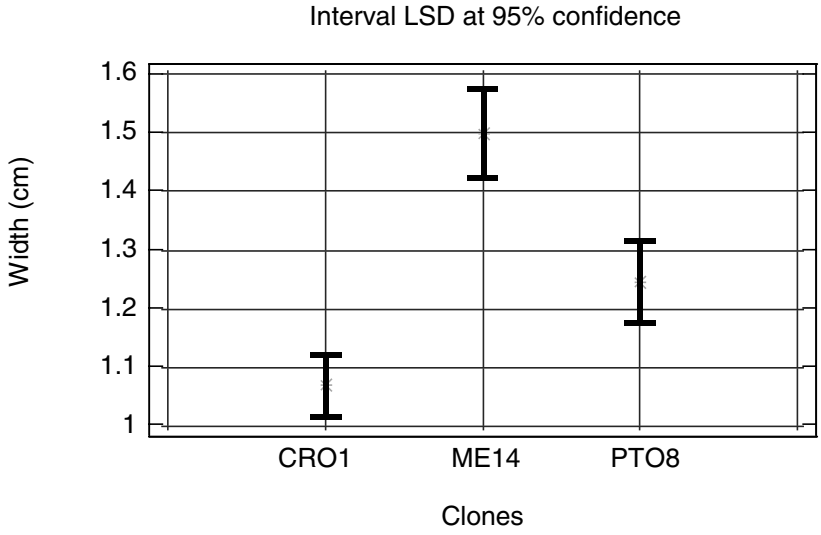

Fig. 1. Flower width.

The longest flowers belonged to the ME14 and PTO8 clones, that show a clear difference in this sense to the CRO1 clone. Based on the results shown in Figs 1 and 2, CRO1 has flowers with a smaller ovary diameter and are not so high, which in fact supposes that the flowers are smaller than those of the other two clones.

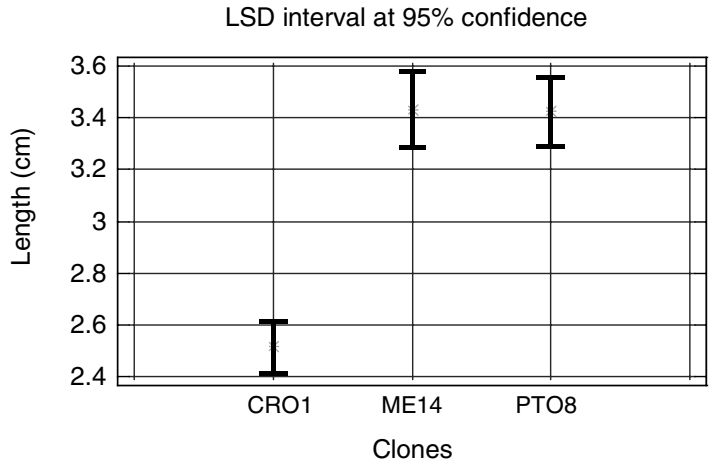

Fig. 2. Flower length.

Regarding the length/width ratio of the flowers (Fig. 3), this is significantly greater in the PTO8 clone than in the other two clones. The clone with the smallest L/W ratio is ME14, due mostly to the fact that this clone has a greater proportion of hermaphrodite flowers, which present a much more developed ovary region than the male flowers.

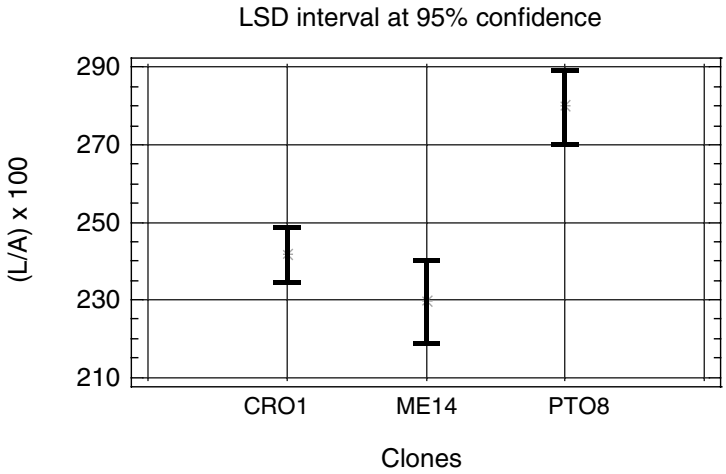

Fig. 3. Flower length/width ratio.

In second place we compared the petals of clones ME14, PTO8 and CRO1 for the parameters width, length and length/width ratio.

Figure 4 shows significant statistical differences between the three clones. From the biggest to the smallest width of petals, the order of the clones is: ME14 > PTO8 > CRO1. Even though they are two independent measurements, the width of the flowers follows the same order.

Regarding petal length (Fig. 5), the clone with the longest petals is ME14, followed by CRO1 and

PTO8. For the latter two clones, the statistically significant differences have a level of significance of 10%.

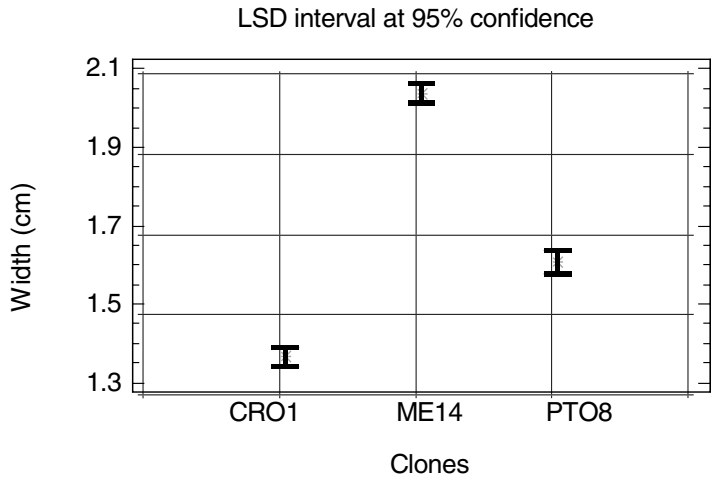

Fig. 4. Petal width.

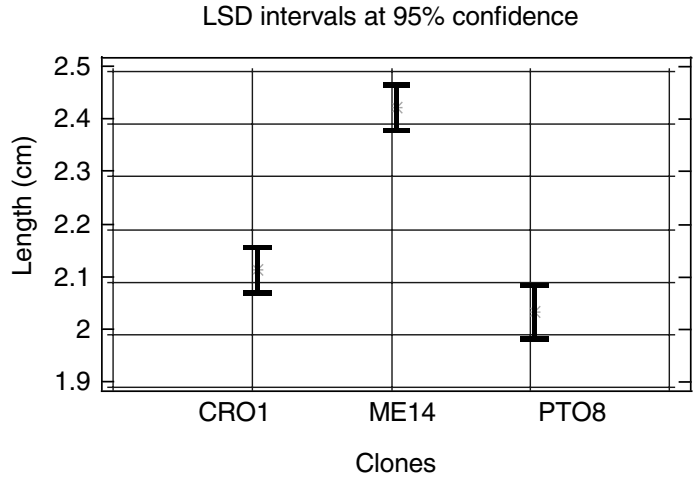

Fig. 5. Petal length.

Figure 6 shows statistically significant differences between the three clones studied, at a confidence level of 95%. We can also appreciate in this graph that the clone ME14 has rounder petals than the PTO8 clone, and CRO1 has the longest petals.

In third place we compared the number of stamens of the clones ME14, PTO8 and CRO1 reaching the results shown in Fig. 7.

With a level of significance of 5%, the PTO8 clone has more stamens than the other two clones, which did not present significant differences at this level of confidence. When we calculate for a confidence level of 90%, the differences between all clones are significant. In a decreasing order, the number of stamens is: PTO8 > ME14 > CRO1.

Conclusions

In view of the results, the most important conclusions drawn are the following:

(i) The ME14 clone produces the greatest percentage of hermaphrodite flowers and CRO1 produces the lowest number. The PTO8 clone shows a great variability between the two years of study and therefore we do not have sufficient criteria to draw conclusions about it.

(ii) The size of the flowers of clones CRO1, ME14 and PTO8 is not the same in all three cases. We have:

- In decreasing order, the width (or ovary diameter) is: ME14, PTO8 and CRO1.
- The order of the clones for flower length is: ME14 ≅ PTO8 > CRO1.
- The longest flowers belong to clone PTO8 and the shortest to clone ME14. This is logical as the greatest percentage of hermaphrodite flowers is produced by clone ME14.

(iii) There are statistically significant differences in the size and form of the petals of the flowers of the clones studied.

- In decreasing order of petal width the clones are the following: ME14-PTO8-CRO1.
- In decreasing order for petal length, the order of clones is: ME14-CRO1-PTO8.
- The longest petals are those of clone CRO1 and the roundest petals are produced by clone ME14.

(iv) The PTO8 clone produces the highest number of stamens and the CRO1 produces the lowest number.

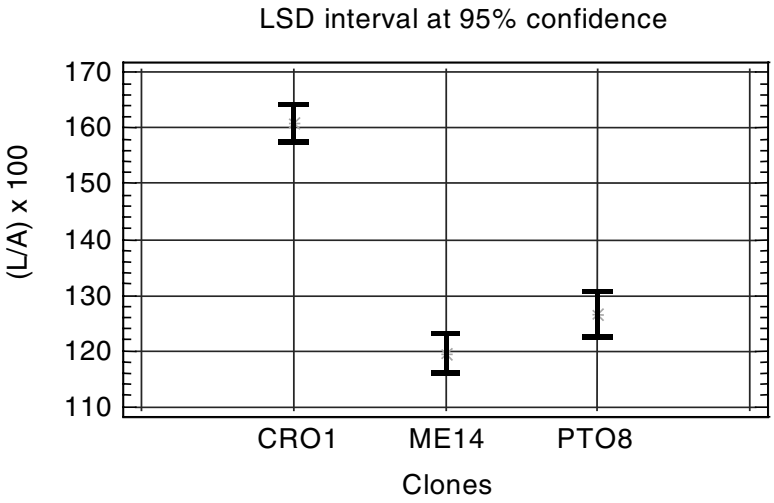

Fig. 6. Height/width ratio of petals.

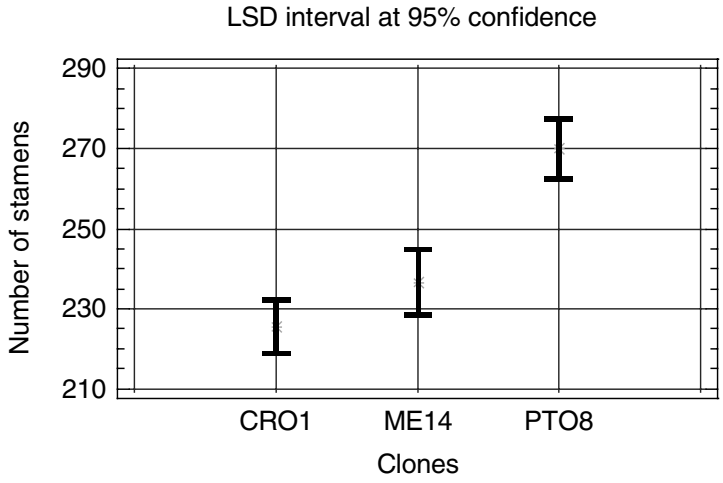

Fig. 7. Number of stamens.

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