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Characterization of the fruit of five pomegranate (*Punica granatum* L.) clones cultivated in homogeneous soils

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SUMMARY – We have made a morphological study of the fruit and seeds of five pomegranate clones, PTO9, ADO4, ME15, MO2 and BA1, and analysed the chemical characteristics of the juice. Mean fruit weight was greatest in ADO4, followed by MO2 and PTO9, while the fruits of ME15 were the smallest. There was a clear correlation between the overall production of these trees and mean fruit size and weight, with ME15 being the most productive (46.7 kg/tree) and ADO4 the least (12 kg/tree). The greatest seed production (70.69%) was obtained with ADO4, followed by the 65.1% of ME15. The lowest values were those of BA1 (48.52%). The largest seeds were those of ADO4 (0.53 g) and the smallest those of ME15 (0.31 g). Maximum values for juice were obtained with BA1 and PTO9 (50 and 55 ml/100 g of seeds). Clones ME15 and ADO4 showed intermediate values (44 and 47 ml/100 g of seeds respectively), while MO2 only produced 35 ml of juice/100 g of seeds. The clones BA1 and ADO4 showed the most acidic juice, the maturity index (MI) values being 7.70 and 16.27, respectively. The higher MI values 77.23, 56.93 and 53.73 were obtained for MO2, ME15 and PTO9, respectively.

Key words: Morphological characterization, fruits, seeds, pomegranate tree.

RESUME – *"Caractérisation du fruit de cinq clones de grenadier (*Punica granatum *L.) cultivés dans des sols homogènes". Nous avons fait une étude morphologique du fruit et des graines de cinq clones de grenadier, PTO9, ADO4, ME15, MO2 et BA1, et analysé les caractéristiques chimiques du jus. Le poids moyen du fruit était plus élevé chez ADO4, suivi par MO2 et PTO9, tandis que les fruits de ME15 étaient les plus petits. Il y avait une corrélation claire entre la production globale de ces arbres et la taille et le poids moyens des fruits, ME15 étant le plus productif (46,7 kg/arbre) et ADO4 étant le moins productif (12 kg/arbre). La plus grande production de graines (70,69%) a été obtenue avec ADO4, suivie par 65,1% pour ME15. Les plus faibles valeurs ont été celles de BA1 (48,52%). Les graines les plus grandes ont été celles de ADO4 (0,53 g) et les plus petites celles de ME15 (0,31 g). Les valeurs maximales pour le jus ont été obtenues avec BA1 et PTO9 (50 et 55 ml/100 g de graines). Les clones ME15 et ADO4 ont montré des valeurs intermédiaires (44 et 47 ml/100 g de graines respectivement), tandis que MO2 n'a produit que 35 ml de jus/100 g de graines. Les clones BA1 et ADO4 ont montré le jus le plus acide, les valeurs de MI étant de 7,70 et 16,27 respectivement. Les valeurs de MI les plus élevées 77,23, 56,93 et 53,73 ont été obtenues pour MO2, ME15 et PTO9, respectivement.*

Mots-clés : *Caractérisation morphologique, fruits, graines, grenadier.*

Introduction

The pomegranate is originally from Vavilov's Centre of Origin IV, the Near East (Sánchez-Monge, 1974). It is a warm-climate species, needing high summer temperatures to mature well. For this reason the south-east of Spain is a very appropriate climate to grow and produce high quality fruit. The Mediterranean Basin is one of the areas best adapted to pomegranate cultivation. The great adaptation of this species to the Mediterranean climate has enhanced its dispersion, which has, over time, led to a large number of new individuals that are sometimes grouped together under the same name, as is the case of the Mollar de Elche or Piñón Tierno de Ojós varieties, among others, that have common characteristics (Melgarejo and Martínez, 1992).

This wide adaptation of the pomegranate to the area of Alicante and Murcia, in particular, has given rise to a great variability of this species that on many occasions affects not only the quality but also the productivity of commercial plantations. For this reason, in 1986, individual trees and plots from the provinces of Alicante and Murcia were pre-selected in order to carry out a complete classification of the pomegranates. This work concluded in 1992, which is when the classification under homogeneous conditions began. For this purpose, selected clones were vegetatively

propagated on the estate of the Higher Polytechnical School of Orihuela where their study continues (Melgarejo, 1993). The climate of the selection area is subtropical Mediterranean, according to the Papadakis classification (MAPA, 1986), satisfying the agroclimatic requirements of the crop.

This work studies some morphological and chemical characteristics of fruits from clones grown under homogeneous conditions.

Material and methods

The plant material studied is made up of pomegranate trees of the following clones: PTO9 (Piñón Tierno de Ojós 9), ADO4 (Agridulce de Ojós 4), ME15 (Mollar de Elche 15), MO2 (Mollar de Orihuela 2) and BA1 (Borde de Albatera 1). Ojós is a town in the province of Murcia, whilst Elche, Orihuela and Albatera are towns in Alicante. These clones were described by Melgarejo (1993), who studied them *in situ* then planted them out after vegetative propagation, in homogeneous conditions on the estate of the Higher Polytechnical School of Orihuela (EPSO - University Miguel Hernández). The pomegranates used for this study were harvested on the EPSO estate, where the plantation layout was 4x3 m. The estate is situated in the catchment area of Orihuela (Alicante), and the trees are drip-irrigated. The trees are goblet-trained and are six years old. The fruit was harvested in 1997.

The soil is loam-clay, typical of this region. Rain is scarce, falling in spring and autumn with an annual rainfall of 300 mm. The mean annual temperature is 19°C, being 11°C in January and 28°C in August, with mild winters and hot summers.

In order to harvest the samples, 10 mature fruits were taken at random from each clone for morphological analysis. Some internal and external characteristics of the fruit were studied, such as the seeds. The results were studied statistically.

Morphological characterization of fruit and seeds

In order to make a morphological characterization of the fruit and seeds, the following parameters were measured:

(i) In fruit: fruit weight (Fw), equatorial diameter (D1), calyx diameter (D2), length of fruit without calyx (L1), length of fruit with calyx (L2), length of calyx (L3), number of carpels measured in the equatorial section (NC), weight of rind plus weight of carpellary membranes (Pr + Cm), rind thickness (Tr) and seed yield (SY). The fruits were peeled by hand.

(ii) In whole seeds: the seeds from the 10 fruits of each clone were mixed in a bowl, then 25 were taken as a sample for study.

- The following parameters were studied in the whole seeds: weight (Ws), length (L) and maximum width (W).
- After the measurement of the previous parameters, the pulp was removed by hand from each of the 25 seeds and the following characteristics were determined of the woody part (tegmen): weight of the woody part (Wwp), length (l) and maximum width (w).
- Knowing the weight of the whole seeds (Ws) and of the woody part (Wwp) the index of the woody part was determined (Melgarejo, 1993), defined as $Wpi = (Ws/Wwp) \times 100$.

(iii) 100 g of seeds was collected from each clone and expressed. The following data was obtained from the juice: volume of total juice from the 100 g of seeds, pH of juice, total soluble solids (TSS) obtained with a refractometer and expressed as °Brix, acidity percentage expressed as citric acid (valuated with sodium hydroxide 0.1 N and brought to pH 8.1), and finally the maturity index (MI) was obtained by dividing the total soluble solids by total acidity.

(iv) Finally, a tasting panel of ten assessed the following parameters: seed hardness (from 1, softest to 10, the hardest), seed colour, gustative and taste quality (classified as sweet, bitter and bitter-sweet).

Statistical analysis

The data of each parameter of Tables 1 and 2, between the different clones, was subjected to a multiple comparison with an analysis of variance, followed by a multiple rank test LSD at 95% confidence.

Results

Fruit morphology

Table 1 shows the arithmetic mean of each clone for the parameters considered in order to study the dimensions and other characteristics of the pomegranates. The means correspond to 10 fruits per clone. Thus a vision may be provided of the mean morphological characteristics of the fruits and seeds of each clone. An analysis of variance was then made, followed by a multiple rank test between all the clones studied and for each parameter. For this purpose, various homogeneous groups of clones have been formed for each parameter, which are shown in the table with small letters after each arithmetical mean. Within each column, different letters indicate statistically significant differences at 95% confidence, whereas the clones with the same letter belong to the same homogeneous group and do not present statistically significant differences at 95% confidence.

Table 1. Fruit morphology[†]

Clone	Fw (g)	D1 (mm)	D2 (mm)	L1 (mm)	L2 (mm)	L3 (mm)	NC	Rw+Cm (g)	Tr (mm)	SY (%)
PTO9	404.76b	91.2b	23.13c	79.54bc	99.80cd	20.26c	6.6a	163.88bc	1.70a	40.41c
ADO4	524.02c	102.14c	18.31a	84.72c	104.23d	19.51bc	6.7a	157.63bc	1.98a	29.30a
ME15	271.68a	80.75a	20.48abc	72.16a	86.63a	15.64a	6.6a	93.61a	3.77b	34.89b
MO2	414.32b	92.92b	19.97ab	78.71bc	95.34bc	16.63ab	7.3b	147.12b	3.06b	35.43bc
BA1	369.51b	89.07b	21.54bc	77.61ab	93.15b	15.35a	6.3a	189.14c	3.74b	51.84d

[†]Fw: fruit weight; D1: equatorial diameter; D2: calyx diameter; L1: length of fruit without calyx; L2: length of fruit with calyx; L3: calyx length; NC: number of carpels; Rw+Cm: rind weight plus carpellary membrane weight; Tr: thickness of rind; SY: seed yield.
[a,b,c,d]Different letters indicate statistically significant differences at 95% confidence.

From this data it can be observed that the ADO4 clone has the largest mean weight, showing significant differences from other clones at 95% confidence; clone ME15 also presented significant differences from the other clones, with the smallest mean weight of all 5 clones. The other 3 did not present significant differences between each other at 95% confidence, but were different from the other two previously specified clones. Therefore, 3 homogeneous groups were established according to the multiple rank test at 95% confidence for the fruit weight among the 5 clones studied. However, it is important to consider that the total mean productions per tree vary widely. Thus, ME15, which had the smallest fruits, had the greatest production, 46.7 kg/tree, whilst clone ADO4, with the biggest fruit weight, presents the lowest production with only 12 kg/tree. The other 3 clones, without significant differences between each other had the following productions: PTO9 13 kg/tree, MO2 37.9 kg/tree and BA1 38 kg/tree. Clone ME15 presents a maximum production, which may be the reason why its fruit are lighter and clone ADO4 has a minimum production which may explain why the fruits are bigger. The 3 intermediate clones lie between these two.

The next parameter studied is the maximum equatorial diameter (D1), whose results are totally parallel with the weight, showing 3 homogeneous groups according to the multiple rank test at 95% confidence and which correspond with the same clones and in the same positions (maximum, intermediate and minimum) as in the previous parameter. These results are to be expected, as, together with L1, they intervene in the volume and therefore the weight of the fruit. These statistical results are repeated for parameter L1 – the length of the fruit without the calyx.

As for the parameter D2 – the diameter of the calyx – the statistical results do vary, even if only

slightly. In this case, there are 3 homogeneous groups according to the multiple rank test, with the following order: ADO4 < MO2 < ME15 < BA1 < PTO9.

Regarding calyx length (L3), there are also 3 homogeneous groups, where clones BA1 and E15 present the minimum L3 and MO2 and ADO4 the intermediate length. Finally PTO9 presents the maximum L3.

Finally parameter L2 is directly related with L1 and L3, since it studies the total maximum length of the fruits, including the calyx. It can be observed that for this parameter there are 4 homogeneous groups according to the multiple rank test, with the following order: BA1 < MO2 < PTO9 < ADO4.

Of all these results, it can be concluded that clone ME15 presents the smallest fruits, which therefore weigh less, with a minimum equatorial diameter and a minimum total length, its calyx having a small length with an intermediate calyx diameter.

Clones BA1, PTO9 and MO2 form a homogeneous group, intermediate between ME15 and ADO4, without statistically significant differences between them at 95% confidence for weight, equatorial diameter and length with and without calyx. However, there are statistically significant differences for the calyx shape. The order for diameter, in decreasing order is: PTO9, BA1 and MO2, and for calyx length: PTO9, MO2 and BA1.

Clone ADO4 has the biggest fruits, which explains why they are the heaviest, with the largest equatorial diameter, the longest, with and without calyx, but they have the smallest calyx diameter and a large calyx length but not the maximum.

As for the parameter No. of carpels (NC), the differences between clones are very slight, ranging between 6 and 8 carpels. According to the multiple rank test there are two homogeneous groups, clone MO2 presents a mean number of carpels of 7.3 and which clearly shows significant differences at 95% confidence with the rest of the clones studied. There is a second group that includes the other clones, among which there are no statistically significant differences and which presents a mean of 6.6 carpels per fruit.

The parameter rind weight plus carpellary membrane weight (Rw + Cm) was later studied, to be used, together with thickness of rind (Tr), to calculate seed yield (SY). This data is very useful to determine the quality of the pomegranates as there are very large fruits but the edible part may be very small or viceversa. In this sense it is noteworthy that for Tr there are two statistically homogeneous groups: that of PTO9 and ADO4 with thin rinds and the other group formed by MO2, BA1 and ME15 with very thick rinds. As for Rw + Cm however, there are 3 homogeneous groups according to the multiple rank test. On one hand the ME15 clone has the smallest mean, followed by MO2, ADO4 and PTO9. Finally, BA1 forms another group statistically different from the rest with a maximum Rw + Cm.

On calculating the seed yield (SY), there are 3 groups that are homogeneous with each other, where the increasing order of the clones is the following: ADO4 < ME15 < MO2 < PTO9 < BA1.

In conclusion regarding the morphology of the fruits, it can be observed that clone ADO4 presents the biggest fruit but which has the lowest seed yield. Clone ME15 is the smallest and lightest fruit with an intermediate seed yield. BA1, MO2 and PTO9 are of intermediate sizes, however the BA1 has the maximum SY, which is intermediate in the other two clones.

Seed morphology

Table 2 shows the arithmetic means and the letters indicating the homogeneous groups at 95% confidence for the parameters established for studying the morphology of seeds. It is known that the pomegranate seeds are made up of an edible sac that contains a woody seed that is difficult to chew (Melgarejo, 1993). This is why the juice sac is the most interesting part of the pomegranate to study in order to determine the quality of the fruit.

Table 2. Mean dimensions of seeds and woody parts†

Clone	Sw (g)	L (mm)	W (mm)	Wwp (g)	l (mm)	w (mm)	Iwp (%)
PTO9	0.48 b	9.97 ab	4.83 a	0.05 c	5.97 b	1.01 a	11.29 b
ADO4	0.53 c	12.17 c	7.31 d	0.04 b	7.12 c	2.21 c	8.13 a
ME15	0.31 a	10.28 ab	5.67 bc	0.029 a	6.41 b	2.04 c	9.89 ab
MO2	0.43 b	9.91 a	5.19 ab	0.03 b	5.18 a	1.38 b	8.68 a
BA1	0.46 b	10.78 b	6.21 c	0.06 d	7.64 d	2.70 d	14.38 c

†Sw: seed weight; L: seed length; W: maximum seed width; Wwp: weight of woody part; l: length of woody part; w: maximum width of woody part; Iwp (%): index of woody part.
[a,b,c,d]Different letters indicate statistically significant differences at 95% confidence.

As for the total weight of the seed (Ws), there are 3 statistically homogeneous groups, according to the multiple rank test at 95%; the ME15 clone presents the minimum value and ADO4 the maximum value, whereas the other clones studied from a homogeneous group with statistically significant differences from other groups, formed by MO2, BA1 and PTO9.

As for the seed length, there are three statistically homogeneous groups MO2, PTO9 and ME15 < BA1 < ADO4.

Finally, the seed width parameter has 4 homogeneous groups. The minimum value corresponds to the group formed by PTO9 and MO2; the second group, of intermediate value is formed by ME15; the third group by BA1; and finally ADO4 that presents the maximum value for seed width.

After conducting this study, the tegmen has been measured, in weight, length and width, and in all three cases, there were 4 statistically homogeneous groups. In all cases, clone BA1 presents the maximum values, as its differences from the values of other clones are statistically significant, whereas the minimum and intermediate values correspond to different clones for each parameter.

The index of the woody part is the parameter that summarizes all those related with seed morphology, and that we consider to be the most important, as it indicates the yield in woody proportion and therefore, without considering the hardness, measures the non-gustative palatability. There are 3 homogeneous groups for this parameter: ADO4, MO2, ME15 < PTO9 < BA1 (without significant differences between the three).

Chemical analysis of seed juice

The results presented in Table 3 were obtained from a sample taken after homogenizing the seeds of 10 fruits taken at random. We can observe that the clone with the greatest volume of juice per 100 g seeds is PTO9, followed by BA1, ADO4, ME15 and finally MO2. The highest pH value of juice corresponds to clone PTO9, pH 4.97, which presents a clear difference in pH from the MO2 clone, with a value of 4.00. However, the latter clone does not present statistically significant differences from the ME15 clone, whose pH is 3.97. The pH values were later found of the clones ADO4 (3.29) and BA1 (2.89). Logically this data corresponds with that obtained from the % of acidity, the increasing order being the following: MO2 < PTO9 < ME15 < ADO4 < BA1. The study of the total soluble solids (TSS) shows that the maximum value corresponds to clone MO2, followed by ME15, ADO4 and finally BA1 and PTO9. The ratio °Brix/% acidity is a good exponent of the state of maturity of the pomegranates (Artés, 1992), and is expressed by the parameter MI, where great differences are observed between the different clones, the maximum value being for clone MO2 with 77.23, followed by ME15 with 56.93 and PTO9 with 53.73. The MI values are much lower for the other two clones, ADO4 has a MI of 16.27, and finally BA1 with 7.70.

This data indicates that the only edible clones are MO2, ME15 and PTO9 as they are sweet, whereas the ADO4 and BA1 clones are bitter and therefore not edible.

Table 3. Chemical analysis of the juice from the pomegranate seeds†

Clone	Volume/100g	pH juice	TSS	% Acidity	MI
PTO9	55	4.97	13.97	0.26	53.73
ADO4	47	3.29	14.97	0.92	16.27
ME15	44	3.97	15.37	0.27	56.93
MO2	35	4.00	16.22	0.21	77.23
BA1	50	2.89	14.09	1.83	7.70

†Volume/100g: volume juice in 100 g of pomegranate seeds;
TSS: total soluble solids; MI: maturity index.

Tasting panel

Finally, Table 4 shows the results of a tasting panel. With the seed hardness parameter, the purpose was to find the degree of edibility of the seed, a parameter related with lwp (%). The data is related for BA1 and TO9 that are very hard and have a maximum lwp (Table 2), according to the tasting panel, ADO4 is also hard, although its lwp is slightly lower and so this data is not concordant.

Table 4. Results of pomegranate tasting panel

Clone	Seed hardness	Seed colour	Gustative quality	Taste
PTO9	3.5 (soft kernel)	Reddy-pink	Good	Sweet
ADO4	8 (hard kernel)	Red	Poor	Bitter-sweet
ME15	3 (soft kernel)	Intense red	Good	Sweet
MO2	3 (soft kernel)	Reddy-pink	Good	Sweet
BA1	8 (hard kernel)	Intense red	Poor	Bitter

The seed colour was also studied as this is an important parameter for the consumer. The clone that presents the most intense red colour is ME15 followed by MO2 and ADO4. Finally PTO9 presents a reddy-pink and white colour for BA1, which would not be so attractive to consumers. All this means that the gustative quality of the clones for the tasters was only found for MO2, ME15 and PTO9 and poor for the rest, which also coincides with the taste of the pomegranate as the only three clones that are sweet, are those mentioned previously that have a maximum maturity index as shown in Table 3.

Conclusions

Of all the data put forward, we can conclude that although the biggest, heaviest and those with most seed yield are ADO4 and PTO9, they have the smallest yield per tree. The clone ADO4, together with BA1 have the bitterest seeds and the lowest maturity index. However, MO2 presents a good fruit size but has a small production per tree. Nevertheless, it has the highest MI, followed by ME15 and PTO9. ME15 has the smallest-size fruit but a maximum yield per tree, which means that the latter three clones are best for fresh consumption.

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