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Pomegranate improvement through clonal selection and hybridization in Elche

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Abstract. Spain is one of the most important pomegranate producers and exporters in the Mediterranean Basin. In spite of various pomegranate cultivars grown, sweet and soft seeds 'Mollar de Elche' and 'Valenciana' are the main varieties-population. This paper reports the activities in progress on pomegranate germplasm and breeding in the last ten years at Valencian Agricultural Research Institute (IVIA) in Elche. A collection of 35 accessions collected from different parts of Alicante was established and characterised. The collection has been implemented with new accessions. Hybridization between 'Mollar de Elche' and other foreign varieties was done in order to obtain darker skin and early ripening. Crossbred progenies were selected and they are being examined in an experimental orchard.

Keywords. *Punica granatum* – Germplasm – Accessions – Fruit characteristics.

I – Introduction

Spain is one of the most important pomegranate producers and exporters in the Mediterranean Basin, where the species has a long history. Commercial pomegranate cultivation is concentrated in the South-East, mainly around the South of Alicante province. In this area, pomegranate became a crop in the 19th century. Later, the pomegranate cultivation decayed during the drought period happened at the beginning of 20th century and re-emerged about seventy years ago, when irrigation facilities and water supply were available.

Most of cultivars known today are selections from an unknown origin (Holland, 2009). 'Mollar de Elche' and 'Valenciana` are the most widely spread cultivars in the local area. 'Mollar de Elche' sweet and soft seeded, is the Spanish pomegranate most known in the world. There is variability within this variety-population (Martinez, 2006), therefore it is recommended to select the best clones in order to optimize the potential of the crop in new pomegranates orchards.

Very few varieties have been developed by systematic breeding programmes despite the need of meeting the demands of local and international consumer, processors, growers and exporters. Breeding new varieties are being achieved by conventional approaches through hybridization what generally involves selection of parents, hybridization and selection of superior genotypes. Development of varieties by hybridization and selection has been reported in Israel, India, China, Turkmenistan and Azerbaijan (Bar-Ya'akov, 2009; Jalikop, 2010). The hybrids exhibited distinct morphological features.

Attractive colour is one of the most important sensory characteristics of fruits. For instance, dark colour is a desirable fruit character for fresh consumption and export purpose in Europe. Other desired traits are red peel and early ripening. 'Mollar de Elche' has pink peel and middle ripening time.

The objectives of this study are to find out local pomegranate genotypes, characterize them and to evaluate initial breeding results. In this paper we report the activities in progress carried out on pomegranate germplasm since 2001 at Valencia Agricultural Research Institute (IVIA) in Elche.

II – Materials and methods

Pomegranate accessions were selected from locally grown pomegranate trees found in commercial orchards in 2001. They were raised from cuttings and planted in an experimental orchard in Elche. New accessions have been collected up till now from Valencia, Murcia and Alicante. In addition to the local collection, 11 foreign introduced cultivars were planted. The climate in the South-East region of Spain is semi-arid, dry and hot in summer. Annual precipitation in Elche is less than 280 mm, mainly in October.

Pomological and morphological characteristics for these accessions were recorded. The variables measured were: thorniness, tree vigour, suckering tendency, leaf length, maximum width leaf, fruit weight (g), fruit equatorial diameter (mm), diameter of calyx (mm), total height of fruit without calyx (mm), skin colour, thickness of skin at equatorial area (mm), skin weight plus carpel endocarpic weight (g), seed length (mm), tegmen length (mm), seed width (mm), tegmen width (mm), seed weight (g), tegmen weight (g), seeds firmness (achenes), harvest maturity, eating quality and flavour were investigated. The pH, titrable acidity, and soluble solid content were measured. The pH measurements were performed using a pH-meter (GLP 21; Crison Ltd, Spain). Total titrable acidity (TA) was determined potentiometrically using 0.1 M NaOH to the end point of pH 8.1 and expressed as grams of citric acid per litre. The soluble solid content (SSC) was measured by refractive index as ºBrix with an Atago N1 refractometer (Tokyo, Japan). The ratio, also known as the maturity index, was calculated as the relation between TSS and TA. Peel and juice colour measurements were determined using a Minolta CR-400 tristimulus colour spectrophotometer (Osaka, Japan) according to the CIELAB convention. Juice colour was performed in glass cells of 2 mm path length (CT-A22).

Fertilization is provided through the irrigation system. Trees are grown 4 m apart along the row and 4.5 m between the rows. Four clones per accession are planted.

1. Breeding plot

Following the characterization we selected cultivars for the establishment of a breeding project aimed to improve existing cultivars. Breeding began in 2002 with pollinations between parents with desirable phenotypes. Normal hermaphrodite type flower was effected for crossing purposes after emasculation of flowers shortly before anthesis. Inter-specific hybrids between ‘Mollar’ and ‘Wonderful’ were successfully developed. The seedlings are planted in a distance of 0.5 m from each other. The plot is irrigated from April to October. After examining the tree for at least two successive years, the non promising seedlings are discarded. Once a desirable plant is located it is propagated vegetatively by cuttings.

III – Results and discussion

1. Pomegranate accessions

A collection of 35 accessions collected from Valencia, Murcia and Alicante was established and characterized. These accessions differ from each other by a diverse range of features such as fruit size, peel colour, aril colour, growth habit and yield The characterization resulted in 19 accessions from ‘Mollar de Elche’ , and 6 accessions from ´Valenciana’ . The main traits of the selected types are shown in Table 1. CM49 was the most productive, CM55 has the darker colour arils and CM63 produced the biggest fruit size. Among the Mollar types, the average fruit weight was 330 g, fruit width was 87.25, total soluble solids 16.5%, and acidity 0.21. ‘Valenciana’ clone CV111 has lower weight tegmen/aril ratio.

A molecular genetic identification base on microsatellites markers is being in progress in order to fingerprint all the accessions.

Table 1. Juice yield, total soluble solids (TSS), titrable acidity (TA), index maturity ratio (IM) and pH from selected pomegranate accessions

Accessions	Juice yield (g 100 g^{-1})	TSS (ºBrix, 20ºC)	TA (g citric acid 100 g^{-1})	IM (TSS/TA)	pH	Fruit weight (g)
CM-63	37.37	16.8	0.20	80.89	3.87	369.81
CM-55	37.23	17.53	0.19	89.22	4.22	367.23
CM-49	38.56	17.73	0.21	85.21	3.86	337.21
V-111	42.13	15.43	0.20	74.20	3.94	285.96
V-120	40.37	15.27	0.28	54.62	3.75	280.12
V-116	39.46	15.30	0.21	71.33	3.88	265.69
Wonderful	34.35	16.55	1.41	11.73	3.28	324.86

2. Pomegranate improvement

Breeding began in 2002 through crossing of selected genotypes. Hybridization between 'Mollar de Elche' selected genotypes and other foreign varieties was done in order to obtain sweet fruits, red arils but darker skin and to enlarge the ripening season. Although, sweet fruits with soft seeds, resistance to cracking and sunburn are also requested. Selection of appropriate parents from 'Mollar de Elche' accesions from our collection was done. Inter-specific hybrids between 'Mollar' and 'Wonderful' were successfully developed. The hybrids exhibited variability in colour, pH, maturity index and morphological features (Table 2) . Seedlings were classified as sweet, sweet-sour and sour, juicy and table fruit, soft and hard-seeded. A high degree of variation for fruit characters was observed. Into the progeny, 16% of fruits were sweet, 18% were soft tegmen, 76% were darker peel than Mollar, 78% were more red arils than Mollar and 6% had excellent eating quality. After data, from at least two successive years the non promising seedlings were discarded.

Table 2. Peel colour values (L*,a*,b*,Chroma, Hue angle), pH, total soluble solids (TSS), titrable acidity (TA) and ratio (TSS/TA) from selected pomegranate Mollar x Wonderful hybrids

Accessions	Peel colour					pH	TSS	TA	TSS/TA
	CIE*L**	CIE*a**	CIE*b**	*C**	*h**				
H7/26	40.28	32.41	10.86	34.18	18.53	2.94	14.57	0.39	37.04
NH5/6	48.06	50.19	29.67	58.30	30.59	3.68	14.13	0.40	35.36
NH6/9	58.70	44.27	25.99	51.34	30.42	3.35	14.97	0.53	28.25
H2/19	49.13	49.20	27.93	56.57	29.58	3.29	12.87	0.55	23.57
NH1/9	49.18	48.00	29.33	56.25	31.43	3.38	13.37	0.53	25.23
NH4/7	56.14	41.32	27.95	49.89	34.08	3.55	12.90	0.51	25.12
H10/29	39.77	43.85	20.55	48.43	25.11	2.96	13.07	0.39	33.50
NH1/5	46.54	52.24	27.46	59.02	27.73	3.18	14.90	0.44	34.21
H1/18	43.96	50.46	29.48	58.44	30.29	3.77	12.87	0.28	46.03
H10/18	49.28	52.14	26.02	58.27	26.52	2.91	13.80	0.42	33.17
H10/33	42.74	50.64	27.40	57.58	28.42	3.05	13.10	0.39	33.31
Mollar	64.97	23.96	31.71	39.74	52.93	3.87	16.66	0.21	80.11
Wonderful	54.11	36.23	29.76	46.89	39.40	3.28	16.55	1.41	11.73

The desirable plants are selected and propagated vegetatively by cuttings. The preselected genotypes are under evaluation in multi-location experimental orchards. The seedlings are planted in a distance of 2.5 m from each other. In order to reduce several undesirable traits, backcrosses were carried out. Currently 600 F2 cross pollinated pomegranate seedlings are under evaluation.

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