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'Garbí' Mandarin: A New Late-maturing Triploid Hybrid

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Citrus is the most extensively produced fruit tree crop in the world (FAO, Food and Agriculture Organization, 2009). There are two clearly differentiated markets: fresh fruit and processed juice. In 2007, the main citrus fruit-producing countries were China (17.9%), Brazil (17.8%), the Mediterranean countries (17.1%), and the United States (8.5%) (FAO, Food and Agriculture Organization, 2009). These areas account for more of two-thirds of the total production of citrus fruits. In the Mediterranean area, citrus fruits are primarily produced for the fresh fruit market. Spain is the principal producer in the area with a total planted area of 330,000 ha and a production ≈6.3 million tons.

Seedlessness is one of the most important characteristics for mandarin on the fresh fruit market, because consumers do not accept seeded fruits. Parthenocarpy is an essential trait for seedless fruit production, and this characteristic is present in citrus germplasm. Triploidy gives rise to seedless commercial cultivars. However, triploid plants have very low fertility, are generally sterile, and do not induce seeds in other cultivars by cross-pollination (Frost and Soost, 1968). Several methods have been developed to obtain triploid citrus (Navarro et al., 2002; Ollitrault et al., 2008). One exploits natural events of polyploidization such as 2n gametes using embryo rescue and flow cytometry to select triploids in 2x × 2x crosses. Second meiotic division restitution has been proposed for diploid megagametophyte development in clementine (Luro et al., 2004), whereas Chen et al. (2008) proposed first meiotic division restitution in sweet oranges.

In Spain, the structure of mandarin cultivars poses several problems. Mandarins include satsumas, clementines, and mandarin hybrids. Satsumas are harvested from the beginning of September until the beginning of November. They are cultivars with a high degree of parthenocarpy and have sterile pollen and ovules. Clementines are the most representative cultivars of mandarin in Spain. They are picked from mid-September until mid-February. They are self-incompatible, but pollen and ovules are viable, being able to pollinate and to be pollinated by other cultivars. Mandarin hybrids, like 'Nova' [*C. clementina* × (*C. paradisi* × *C. tangerina*)], 'Fortune' (*C. clementina* × *C. tangerina*), and 'Ortanique' tangor (natural hybrid between mandarin and *C. sinensis*), were introduced in the Spanish citriculture to cover the demand of late-season mandarins by international markets. They enable the harvesting period to be extended until May. These hybrids are also self-incompatible, but pollen and ovules are viable and cross-pollinate with clementines, producing fruits with seeds in both groups of cultivars.

A triploid breeding program has been carried out in Spain since 1996 based on sexual hybridization, embryo rescue, and ploidy analysis by flow cytometry (Navarro et al., 2002). The objective is to produce new high–quality, late-season and seedless triploid mandarin hybrids. In this article, we describe 'Garbí' mandarin, a new triploid hybrid developed within this program characterized by its high-quality, late-season ripening and seedless fruits.

Origin

'Garbí' is a new triploid hybrid obtained from a cross between diploid 'Fortune' mandarin and diploid tangor 'Murcott' (*C. reticulata* × *C. sinensis*). Anthers of 'Murcott' tangor were removed from flowers collected in preanthesis and dried in petri dishes over silica gel in a desiccator. Dried dehisced anthers were stored in small petri dishes at –20 °C. Controlled cross-pollination was conducted by applying one anther from the paternal parent to receptive stigma of 'Fortune' mandarin flowers in Spring 1996. Approximately 100 flowers of 'Fortune' mandarin were pollinated and 45 fruits were collected, which contained 129 small seeds produced by 2x × 2x crosses. One hundred twenty-two embryos were isolated from these seeds and cultured in vitro in the culture medium described by Murashige and Skoog (MS) (1962) with 50 g·L^{-1} sucrose, 500 mg·L^{-1} malt extract supplemented with vitamins (100 mg·L^{-1} i-inositol, 1 mg·L^{-1} pyridoxine hydrochloride, 1 mg·L^{-1} nicotinic acid, 0.2 mg·L^{-1} thiamine hydrochloride, 4 mg·L^{-1} glycine) and 8 g·L^{-1} Bacto agar (MS culture media). After germination, 116 plantlets were subcultured in an elongation medium, which consisted of the MS culture media without vitamins. Cultures were maintained at 24 ± 1 °C, 60% humidity, and 16-h daily exposure to 40 μE·m^{-2}·s^{-1} illumination. Ploidy level of all plants was analyzed by flow cytometry in the flow cytometer Ploidy Analyzer (Partec®; Partec GmbH, Münster, Germany). Small pieces of leaves, measuring ≈0.5 cm^2, were taken from the in vitro-growing plants and chopped together with a piece of leaf from a control diploid plant, placed in a Partec® CyStain ultraviolet Precise P nuclei extraction buffer, stained with DAPI, and analyzed in the cytometer. All the plantlets were triploid and were grafted onto 'Carrizo' citrange rootstock (*C. sinensis* × *P. trifoliata*) for field evaluation at IVIA (Instituto Valenciano de Investigaciones Agrarias, located in Moncada, Valencia, Spain) in 1998. 'Garbí' mandarin flowered for the first time in Spring 2002 and in 2004 it was selected because of its high fruit quality and propagated in a second evaluation plot to confirm its uniformity and stability. Ploidy level of 'Garbí' mandarin was confirmed by cytology using the hematoxylin staining technique of Sass (1958) and modified by Tusa et al. (1990).

Description

Description has been done with data taken from five trees growing at IVIA plots essentially following the Guidelines for the Conduct of tests for distinctness, uniformity, and stability for Citrus L. Group 1 mandarins from the International Union for the Protection of New Varieties of Plants (UPOV, International Union for the Protection of New Varieties of Plants, 2009).

The 'Garbí' mandarin tree is vigorous, obloid in shape, and exhibits drooping growth. Main branches have thorns of ≈30 mm in length, although on new branches ≈80% of the nodes have thorns measuring an average of 7.2 mm long.

'Garbí' mandarin fruits are seedless in an open-pollinated environment. They reach the optimum maturity at the end of March when the ratio solids/acids of the fruits is close to nine, although they can be harvested from mid-February to the end of April. Fruit characteristics are described in Table 1. Fruits are easy to peel like 'Nova' mandarin, obloid in shape, with a diameter slightly over 60 mm; the broadest part is central, circular shape in transverse section, absent neck; and the general shape of the proximal part is slightly rounded and very homogeneous (Fig. 1). Fruits do not have areola or persistent style nor do they have radial grooves at the distal end. Fruit rind is medium orange in color (Citrus Color Index = 13) (Jiménez-Cuesta et al., 1981) similar to 'Clemenules' clementine (*C. clementina* Hort. Ex Tan.) with reduced thickness (average 2.5 cm). The albedo is white and the flesh is orange in color. Fruits have absent or weak rudimentary

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segments and a medium number of well-developed segments (10 to 11 segments per fruit) and lack a navel. At maturity, the fruits have high total soluble sugars and acidity content, ≈2% of acid concentration, and 17 °Brix. Solids/acids ratio increased through the season (Fig. 2), primarily because of the increase in solids. If we compare the ratio of 'Garbí' mandarin with its parents at the optimum ripening stage (second half of March), the ratio of 'Garbí' mandarin (8.9) is lower than 'Fortune' mandarin (9.5) and 'Murcott' tangor (10.3) mainly as a result of a major quantity in acids of 'Garbí' mandarin. The flavor is slightly acidic like 'Fortune' mandarin with strong strength of fiber, easy-to-eat texture of the segments, and pleasant aroma, resembling 'Murcott' tangor.

The leaves of 'Garbí' mandarin are evergreen, simple with a long leaf blade (average length 11.5 cm) and medium width of the leaf blade (average 3.8 cm). The margin of the leaves has crenate incisions and an acute shape of the apex. The petiole is medium in length (average 13.6 mm) with small wings. Leaves are very characteristic and different from other mandarin cultivars.

'Garbí' mandarin flowers are hermaphrodite and white. Flowering occurs in April and many borne flowers are produced. Pollen fertility is very low with only 0.4% of pollen grains of 'Garbí' mandarin germinating in vitro culture as compared with 'Fortune' mandarin pollen grains of which over 82% germinated.

Controlled cross-pollinations have been carried out among 'Garbí' mandarin, 'Loretina' clementine, and 'Fortune' mandarin. Fifty flowers of 'Loretina' clementine with receptive stigma were pollinated by applying one anther from 'Garbí' mandarin and seedless fruits were obtained. Also, 50 flowers of 'Garbí' mandarin were pollinated with pollen from 'Fortune' mandarin and an average of 0.3 seeds were obtained per fruit. In open pollination, seeded fruits of 'Garbí' mandarin were very rare. These results confirm that this new triploid cultivar is essentially seedless and that seed formation in clementines is not induced by cross-pollinization.

Trees of 'Garbí' mandarin and several mandarin varieties are planted in experimental plots at IVIA that have a high level of *Alternaria alternata* inoculum. 'Fortune' mandarin and 'Minneola' tangelo (*C. paradisi* × *C. tangerina)* trees display severe *A. alternata* symptoms in leaves and fruits, and 'Nova' mandarin trees display mild to medium symptoms, whereas no symptoms have ever been observed in leaves or fruits of 'Garbí' mandarin.

'Garbí' mandarin was analyzed with 14 simple sequence repeat (SSR) markers by capillary electrophoresis. The size of alleles in nucleotides for each SSR marker is described in Table 2 (Froelicher et al., 2008; Kijas et al., 1997; Luro et al., 2008) and these results clearly distinguish 'Garbí' mandarin from other cultivated commercial cultivars on an international level. Genetic analysis with the microsatellite loci Ci05A05, Ci06B07, TAA 41, and Mest 15 indicated that origin of 'Garbí' mandarin was 2n

Table 1. Summary of fruit quality characteristics of 'Garbí'mandarin on 'Carrizo' citrange rootstock.

	2007[z]	2008	2009	Mean[y]
Diameter (mm)	64 ± 3	66 ± 2	59 ± 4	63 ± 2
CCI[x]	12 ± 1	15 ± 2	11 ± 1	13 ± 1
Rind thickness (mm)	2.4 ± 0.2	2.4 ± 0.1	2.6 ± 0.3	2.5 ± 0.1
Segments per fruit	10.3 ± 0.2	10.5 ± 0.5	10.6 ± 0.5	10.5 ± 0.2
Soluble solids (%)	16.2 ± 0.8	16.9 ± 0.8	17.8 ± 0.6	17.0 ± 0.1
Acids (%)	1.8 ± 0.1	1.9 ± 0.2	2.4 ± 0.2	2.0 ± 0.2
Juice content (%)	46 ± 3	45 ± 2	43 ± 5	45 ± 3

[z]Averaged over five sample dates per year between February to April. Each sample comprised 10 fruits and was collected every 15 d.
[y]Average of 3 years.
[x]Citrus Color Index according methodology described by Jiménez-Cuesta et al. (1981).

Fig. 1. Fruit of triploid 'Garbí' mandarin.

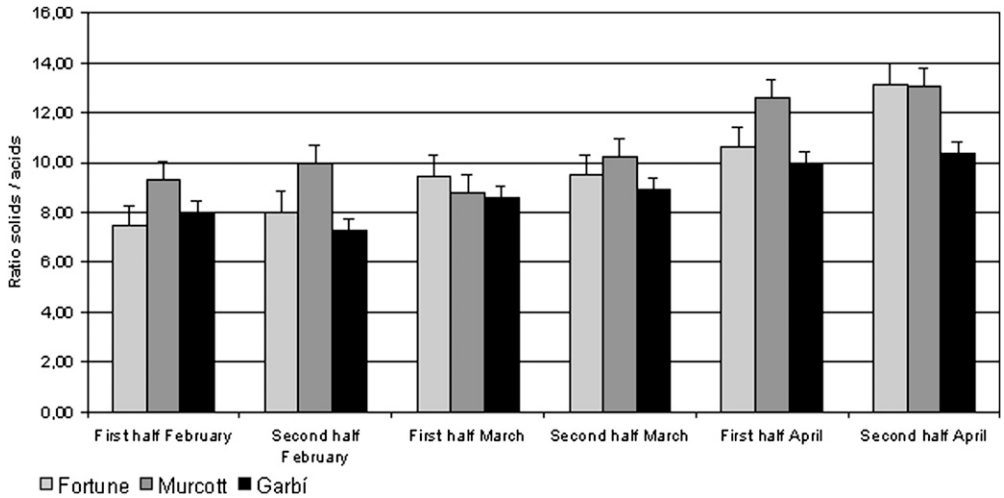

Fig. 2. Comparison of the ratio solids/acids evolution between 'Garbí' mandarin and its parents. Data are the average of three seasons and the bars represent the SD.

Table 2. Genetic analysis with simple sequence repeat (SSR) markers of 'Garbí' mandarin and different cultivars of mandarin.[z]

	Genotypes						
Locus	Fortune	Murcott	Ellendale	Kara	Willow leaf	Clementine	Garbí
Ci06A12	96	96	96	96	96	96	96
					100		
	102		102			102	102
Ci08C05				151			
	157	157					157
					165		
						173	
	175				175		175
			179	179			
Ci02D09	248	248	248	248		248	248
				254	254		
	256				256	256	256
Ci05A04		264		264	264	264	264
	268		268		268	268	268
Ci05A05	144				144	144	144
		152	152	152		152	152
	162		162		162		162
Ci06B07					102		
		105	105	105		105	105
	107			107	107	107	107
	109						109
TAA 41	138		138				138
	148			148	148	148	148
			152	152			
		154				154	154
TAA 15			164				
				186			
		188		188		188	
	192				192	192	192
	204	204	204		204		204
Mest 458					209		
	215	215	215		215	215	215
				218			
		227		227			227
Ci07D06	166					166	166
					178		
	188		188	188	188	188	
		196		196			196
Mest 123	254	254			254	254	254
		260		260			
				270			
	280		280		280	280	280
Ci03G05	199						199
				224			
		227	227	227	227	227	227
	229	229	229		229	229	229
Mest 15		173	173	173			173
	182					182	182
	185				185	185	185
		188	188	188			
					191		
Ci07F11				146			
	152				152	152	152
		160	160	160		160	
			162				
	164	164			164		164

[z]Numbers indicate the size, in nucleotides (nt), of the alleles for each SSR marker.

gamete of 'Fortune' mandarin as a result of the presence of two alleles of this cultivar and one allele of 'Murcott' tangor. Also, molecular markers will help protect breeders' rights and to control traceability of nursery-propagated plants.

Availability

Protection of Plant Breeders' Rights of the cultivar has been requested in the European Union, Morocco, Egypt, South Africa, and Turkey and also a U.S. Plant Patent has been requested. IVIA holds the rights to the cultivar and the Fundación de la Comunidad Valenciana para la Investigación Agroalimentaria (AGROALIMED) is handling these rights for commercial propagation under royalty agreements with licensed nurseries. Pathogen-free plants of 'Garbí' mandarin have been obtained by shoot-tip grafting in vitro according to the methodology described by Navarro et al. (1975) and healthy budwood has been released to 22 Spanish nurseries that have already signed propagation agreements to produce certified plants.

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