‘Garbí’ Mandarin: A New Late-maturing Triploid Hybrid

Pablo Aleza, José Cuenca, José Juárez, José A. Pina, and Luis Navarro1

Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias. Ctra. Moncada a Náquera Km. 4.5, Apartado Oficial 46113, Moncada, Valencia, Spain

Received for publication 24 Aug. 2009. Accepted for publication 2 Oct. 2009.

This work is jointly financed by the AGL2008-00596-MCI and Prometeo 2008/121 Generalitat Valenciana projects.

1To whom reprint requests should be requested; e-mail lnavarro@ivia.es.

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 than half 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 of 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 polyplodyization 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 ‘Ortanque’ tangor (n Nuclear hybrid between mandarin and C. sinensis), were introduced in the Spanish citrus trade 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 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 cm², 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 nucleus 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/acid 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) (Jimenez-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
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\(^\circ\)Brix. Solids/acids ratio increased through the season (Fig. 2), primarily because of the increase in solids. If we compare the ratio of ‘Garbı´mandarin with its parents at the optimum ripening stage (second half of March), the ratio of ‘Garbı´ 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 ‘Garbı´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 ‘Garbı´ 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. ‘Garbı´mandarin flowers are hermaphro-dite 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 ‘Garbı´ 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 ‘Garbı´mandarin, ‘Loretina’ clementine, and ‘Fortune’ mandarin. Fifty flowers of ‘Loretina’ clementine with receptive stigma were pollinated by applying one anther from ‘Garbı´ mandarin and seedless fruits were obtained. Also, 50 flowers of ‘Garbı´ 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 ‘Garbı´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 ‘Garbı´ 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 ‘Garbı´ mandarin.

‘Garbı´ 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 ‘Garbı´mandarin from other cultivated commercial cultivars on an international level. Genetic analysis with the microsatellite loci C05A05, C06B07, TAA 41, and Mest 15 indicated that origin of ‘Garbı´mandarin was 2n

Table 1. Summary of fruit quality characteristics of ‘Garbı´mandarin on ‘Carrizo’ citrange rootstock.

<table>
<thead>
<tr>
<th></th>
<th>2007(^z)</th>
<th>2008</th>
<th>2009</th>
<th>Mean(^y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (mm)</td>
<td>64 ± 3</td>
<td>66 ± 2</td>
<td>59 ± 4</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>Rind thickness (mm)</td>
<td>2.4 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>2.6 ± 0.3</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Segments per fruit</td>
<td>10.3 ± 0.2</td>
<td>10.5 ± 0.5</td>
<td>10.6 ± 0.5</td>
<td>10.5 ± 0.2</td>
</tr>
<tr>
<td>Soluble solids (%)</td>
<td>16.2 ± 0.8</td>
<td>16.9 ± 0.8</td>
<td>17.8 ± 0.6</td>
<td>17.0 ± 0.1</td>
</tr>
<tr>
<td>Acids (%)</td>
<td>1.8 ± 0.1</td>
<td>1.9 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Juice content (%)</td>
<td>46 ± 3</td>
<td>45 ± 2</td>
<td>43 ± 5</td>
<td>45 ± 3</td>
</tr>
</tbody>
</table>

\(^z\)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.

\(^8\)Citrus Color Index according methodology described by Jiménez-Cuesta et al. (1981).

Fig. 1. Fruit of triploid ‘Garbı´’ mandarin.

Fig. 2. Comparison of the ratio solids/acids evolution between ‘Garbı´’ mandarin and its parents. Data are the average of three seasons and the bars represent the sd.