World mandarin production rose from 18.3 million tons to 27.9 million tons between the years 2000 and 2007 (FAO, Food and Agriculture Organization, 2009). In Spain, 87% of mandarin production in the last 5 years has been destined to the fresh fruit market (Intercitrus, Interprofessional Citricola Española, 2008), which demands high-quality, seedless fruits throughout the marketing season. Therefore, the production of seedless varieties is very important.

Triploid plants are generally considered an evolutionary dead end, because they generally give rise to aneuploid gametes with very low fertility (Otto and Whitten, 2000). Predominantly trivalent and high numbers of bivalent and univalent associations are formed during meiosis of citrus triploid hybrids (Frost and Soost, 1968). Moreover, abortion of the megasporeogenesis in the period between the embryo-sac first divisions and the fecundated egg cell is common (Fatta Del Bosco et al., 1992). For this reason, citrus triploid hybrids are generally sterile, although they can occasionally produce fruits with very few seeds and induce seed formation in fruits of other cultivars.

Citrus triploid hybrids can be obtained by means of $2 \times 2x$ hybridizations through the production of unreduced gametes by the female parent (Esen and Soost, 1971). The frequency of unreduced gamete formation depends on the genotype (Esen and Soost, 1971; Luro et al., 2004). Triploid embryos are preferentially found in seeds between one-third and one-sixth smaller than normal seeds and these small seeds generally do not germinate under conventional greenhouse conditions. Embryo rescue from these small seeds is required to reach high germination rates (Navarro et al., 2002). Luro et al. (2004) proposed that Second Division Restitution is the mechanism controlling unreduced gamete formation in clementines, whereas in sweet orange, Chen et al. (2008) proposed First Division Restitution to be the mechanism involved. In these two genetic mechanisms, only one part of the maternal heterozygosity is transmitted to the triploid hybrid and the rate of maternal heterozygosity varies among the loci in relation with the rate of single crossing over between the centromere and a given locus (Ollitrault et al., 2008).

Ploidy level determination by histological methods is too laborious for large-scale analyses. However, ploidy level can be accurately determined relatively rapidly in large populations by flow cytometry (Ollitrault and Michaux-Ferrière, 1992). embryo rescue and flow cytometry are two indispensable techniques for extensive triploid citrus breeding programs. These techniques allow the efficient recovery of plants from embryos contained in small seeds and enable the ploidy level of regenerated plantlets to be determined quickly and easily with just a small piece of leaf while the plants are still in the test tube (Navarro et al., 2002).

In Spain, there are numerous problems associated with the production season of mandarin cultivars, which include satsumas [Citrus unshiu (Mak.) Marc.], clementines (C. Clementina Hort. ex Tan.), and mandarin hybrids. Satsumas and clementines are traditionally harvested from the beginning of September until mid-February. Satsumas produce seedless fruits because they have sterile pollen and ovules. Clementines, the most widely growing mandarins in Spain, are self-incompatible and also produce seedless fruits if grown in isolation. However, their pollen and ovules are viable, and consequently they are able to pollinate and be pollinated by other sexually compatible cultivars. As a result of the demand for late-season mandarins by international markets, several mid- and late-maturing mandarin hybrids were introduced in Spanish citrusiculture. These hybrids are self-incompatible, but their pollen and ovules are viable and cross-pollinate with clementines, producing fruits with seeds in both groups of cultivars, which causes substantial economic losses.

With a view to solving this problematic situation, a triploid breeding program was started in Spain in 1996. The main objective was to produce new mid- and late-maturing triploid cultivars through sexual hybridization, embryo rescue, and ploidy analysis by flow cytometry (Navarro et al., 2002). Recently we released the late-maturing triploid variety Garbi [C. Clementina × C. tangerina] × (C. reticulata × C. sinensis) (Aleza et al., 2010), which reaches optimum maturation during the second half of March. This variety of mandarin is replacing ‘Fortune’ mandarin (C. Clementina × C. tangerina), which reached a peak production of 300,000 tons but is currently being replaced rapidly as a result of its high susceptibility to Alternaria alternata. Another problem is the low fruit quality of ‘Hernandina’ clementine, our latest maturing clementine, when grafted on ‘Carrizo’ citrange (Citrus sinensis × Poncirus trifoliata), which is by far the most predominant rootstock. Fruit peel deteriorates quickly after mid-January, and in practice, no clementine fruits are available by February.

In this article, we describe a new triploid hybrid named ‘Safor’ mandarin [(C. Clementina × C. tangerina) × (C. unshiu × C. nobilis)] characterized by its high quality, seedless fruits, and mid-late ripening season.

**Origin**

‘Safor’ is a new triploid hybrid obtained from a cross between diploid ‘Fortune’ mandarin and diploid ‘Kara’ mandarin (C. unshiu × C. nobilis). Anthers of ‘Kara’ mandarin 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 until needed for pollination, usually less than 1 month. Controlled cross-pollination was done by applying one anther from the paternal parent to receptive stigma of ‘Fortune’ mandarin flowers in Spring 1996. Approximately 100 flowers of ‘Fortune’ mandarin

Table 1. Summary of fruit quality characteristics of ‘Safor’ mandarin.

| Year | 2007 | 2008 | 2009 | 2010 | Mean |
|------|------|------|------|------|------|
| Diameter (mm) | 54 ± 2 | 60 ± 2 | 50 ± 2 | 62 ± 4 | 56 ± 5 |
| Height (mm) | 50 ± 3 | 54 ± 1 | 45 ± 2 | 57 ± 4 | 52 ± 5 |
| Weight (g) | 86 ± 9 | 114 ± 6 | 65 ± 8 | 128 ± 25 | 98 ± 28 |
| CTT | 16 ± 2 | 18 ± 1 | 15 ± 1 | 21 ± 3 | 17 ± 2 |
| Rind thickness (mm) | 2.2 ± 0.3 | 2.6 ± 0.4 | 2.5 ± 0.3 | 2.6 ± 0.2 | 2.5 ± 0.2 |
| Segments per fruit | 9.4 ± 0.3 | 10.1 ± 0.7 | 10.7 ± 0.4 | 10.3 ± 0.4 | 10.1 ± 0.6 |
| Soluble solids (%) | 15.9 ± 1.1 | 13.8 ± 1.1 | 16.8 ± 0.2 | 13.7 ± 0.9 | 15.1 ± 1.5 |
|酸 (%) | 1.9 ± 0.2 | 1.6 ± 0.3 | 2.6 ± 0.2 | 1.6 ± 0.2 | 1.9 ± 0.5 |
| Juice content (%) | 43 ± 2 | 42 ± 4 | 39 ± 2 | 43 ± 3 | 42 ± 2 |

*Table 1. Summary of fruit quality characteristics of ‘Safor’ mandarin.*
were pollinated and 50 fruits were collected, which contained 81 small seeds produced by
2x × 2x crosses. Eighty-one embryos were isolated from these seeds and cultured in vitro
in the culture medium described by Murashige and Skoog (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 pyridox-
ine hydrochloride, 1 mg L⁻¹ nicotinic acid, 0.2 mg L⁻¹ thiamine hydrochloride, 4 mg L⁻¹ gly-
cine), and 8 g L⁻¹ Bacto agar [Murashige and Skoog (MS) culture media]. After germina-
tion, 81 plantlets were subcultured in an elongation medium, which consisted of the
MS culture media without malt extract. Cultures were maintained at 24 ± 1 °C, 60%
humidity, and 16-h daily exposure to 10 μE
m⁻²·s⁻¹ illumination. Ploidy level of all plants
was analyzed by flow cytometry in the flow
cytometer Ploidy Analyzer (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 nuclei extraction buffer, stained with
DAPI, and analyzed in the cytometer. Seventy-
eight plantlets were triploid, were transplanted
to the greenhouse, and in 1998, 1 year later,
were grafted onto ‘Carrizo’ citrange rootstock
for field evaluation at IVIA plots (Instituto
Valenciano de Investigaciones Agrarias, lo-
cated in Moncada, Valencia, Spain). ‘Safor’
mandarin flowered for the first time in Spring
2001. After 3 years of production, this variety
was selected because of its high fruit quality
and propagated in two additional evaluation
plots to confirm its uniformity and stability.
Ploidy level of ‘Safor’ mandarin was con-
firmed by cytology using the hematoxylin
staining technique of Sass (1958) and modified
by Tusa et al. (1990).

Description

Descriptions are based on data taken from
five trees: the original hybrid tree, grafted on
‘Carrizo’ citrange growing at an IVIA plot,
was used for the first screening of triploid
progenies. Two trees, top-worked on 3-year-
old ‘Carrizo’ citrange rootstock growing at
a different IVIA plot, were used for second-
ary evaluation of the selected hybrids, and
two trees top-worked on 20-year-old ‘Valen-
cia Late’ sweet orange [C. sinensis L. (Osb.)]
grafted on ‘Troyer’ citrange (C. sinensis ×
P. trifoliata) growing at a plot located in
Museros, Valencia, belonging to the citrus
cooperative ANECOOP. Data were collected
essentially following the “Guidelines for the
Conduct of Tests for Distinctness, Unifor-
mity and Stability,” Citrus L. Group 1
Mandarins, from the International Union for
the Protection of new Varieties of Plants
(UPOV, International Union for the Protec-
tion of New Varieties of Plants, 2009). No
significant differences were found in any
parameter among trees growing in the differ-
ent plots. Furthermore, the description was
confirmed by comparison with first-year
fruits produced by three other trees of ‘Safor’

![Fig. 1. (A) Fruits of ‘Safor’ mandarin and its parents. (B) Fruits of triploid ‘Safor’ mandarin.](image1)

![Fig. 2. Comparison of the ratio solids/acids evolution between ‘Safor’ mandarin, its parents, and ‘Garbı’
mandarin. Data are the average of three seasons and the bars represent the sd.](image2)
mandarin top-worked on 26-year-old ‘Newhall’ sweet orange grafted on ‘Carrizo’ citrange growing in an experimental plot in Villarreal, Castellón. Data of ‘Safor’ mandarin was compared with ‘Garbi’, ‘Fortune’, and ‘Kara’ mandarins.

The ‘Safor’ mandarin tree is vigorous, oboid in shape, and exhibits drooping growth. Main branches have thorns of ≈16 mm in length, although new branches do not have thorns.

‘Safor’ mandarin fruits are seedless in an open-pollinated environment. They reach optimum maturity in the second half of February, when the ratio of solids/acid of the fruits can reach eight, although they can be harvested from mid-February until the end of March. Fruit characteristics are described in Table 1. Fruits are easy to peel like ‘Kara’ mandarin, oboid in shape with the proximal part slightly rounded with a diameter between 51 and 61 mm, height between 47 and 57 mm, weight between 70 and 126 g, and the broadest part is toward the distal end, circular shape in the transverse section with an absent neck (Fig. 1A–B). Fruits do not have areola or persistent style nor do they have radial grooves at the distal end. Fruit rind is orange–red in color (Citrus Color Index = 17) (Jiménez-Cuesta et al., 1981), similar to ‘Nova’ mandarin with reduced thickness (average 2.5 cm). The albedo is white and the flesh is orange in color. Fruits lack a navel and have absent or weak rudimentary segments and an intermediate number of well-developed segments (nine to 11 segments per fruit). At maturity, the fruits have high total soluble sugars and acidity content with 15.1 °Brix and ≈1.9% of acid concentration. The solids/acid ratio increases through the season (Fig. 2), primarily because of the increase in solids and maintenance of acids. If we compare the ratio of ‘Safor’ mandarin with its parents and ‘Garbi’ mandarin at the optimum ripening stage (second half of February), the ratio of ‘Safor’ mandarin (8.7) is higher than ‘Garbi’ (7.8), ‘Fortune’ (8.3), and ‘Kara’ mandarins (7.7) mainly as a result of the lower quantity of acids in ‘Safor’ mandarin. The flavor is slightly acidic like ‘Fortune’ mandarin with medium strength of fiber, easy-to-eat texture of the segments, and a pleasant aroma, resembling ‘Fortune’ mandarin.

The leaves of ‘Safor’ mandarin are evergreen and simple with a long leaf blade (average length 13.2 cm) and broad width of the leaf blade (average 4.7 cm). The margin of the leaves have crenate–fascinate incisions and acute-shaped apex. The petiole is medium in length (average 14.7 mm) with very small wings. Leaves are characteristic and very similar to ‘Kara’ mandarin.

‘Safor’ mandarin flowers are hermaphrodite and white. Flowering occurs in April in Moncada (Valencia, Spain) and many borne flowers are produced like ‘Garbi’ mandarin. ‘Safor’ mandarin pollen fertility is very low with only 0.2% of pollen grains germinating in vitro (lower fertility than ‘Garbi’ mandarin) as compared with ‘Fortune’ mandarin pollen grains, of which over 82% germinated.

Controlled cross-pollinations have been carried out among ‘Safor’ mandarin, ‘Loretetina’ clementine, and ‘Fortune’ mandarin. Fifty flowers of ‘Loretetina’ clementine with receptive stigma were pollinated by applying one anther from ‘Safor’ mandarin and only seedless fruits were obtained. Also, 50 flowers of ‘Safor’ mandarin were pollinated with pollen from ‘Fortune’ mandarin and no seeds were obtained. In open pollination, seeds were very rare in fruits of ‘Safor’ mandarin. These results confirm that this new triploid cultivar is seedless and that seed formation in clementines is not induced by cross-pollination.

Trees of ‘Safor’ mandarin and several mandarin varieties are planted in experimental plots at IVIA that have a high level of A. 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 in a 6-year observation period, no symptoms have ever been observed in the leaves or fruits of ‘Safor’ mandarin.

Table 2. Genetic analysis with simple sequence repeat markers of ‘Safor’ mandarin and different cultivars of mandarin.

| Genotypes | Fortune | Murcott | Ellendale | Kara | Willow leaf | Clementine | Garbi | Safor |
|-----------|---------|---------|-----------|------|-------------|------------|-------|-------|
| Locus     |         |         |           |      |             |            |       |       |
| Ci06A12   | 96      | 96      | 96        | 96   | 96          | 100        |       |       |
| C108C05   | 157     | 157     | 151       | 165  | 173         | 157        |       |       |
| Ci02D09   | 248     | 248     | 248       | 248  | 248         | 248        |       |       |
| Ci05A04   | 264     | 264     | 264       | 264  | 264         | 264        |       |       |
| Ci05A05   | 144     | 152     | 152       | 152  | 144         | 144        | 152   | 152   |
| Ci06B07   | 105     | 105     | 105       | 105  | 105         | 105        | 107   | 107   |
| TAA 41    | 138     | 138     | 138       | 138  | 138         | 138        |       |       |
| TAA 15    | 188     | 188     | 186       | 188  | 188         | 186        |       |       |
| TAA 19    | 192     | 192     | 192       | 192  | 192         | 192        |       |       |
| Mest 458  | 215     | 215     | 215       | 215  | 215         | 215        |       |       |
| Ci07D06   | 166     | 152     | 152       | 227  | 227         | 227        |       |       |
| Ci03G05   | 199     | 196     | 196       | 196  | 188         | 196        |       |       |
| Mest 123  | 254     | 254     | 254       | 254  | 254         | 254        |       |       |
| Mest 15   | 260     | 260     | 260       | 260  | 260         | 260        |       |       |
| Ci07F11   | 152     | 152     | 152       | 152  | 152         | 152        |       |       |

*Numbers indicate the size, in nucleotides (nt), of the alleles for each simple sequence repeat marker.
‘Safor’ mandarin was analyzed with 14 simple sequence repeat (SSR) markers by capillary electrophoresis. Table 2 shows the size of alleles in nucleotides for each SSR marker (Froelicher et al., 2008; Kijas et al., 1997; Luro et al., 2008) and these results clearly distinguish ‘Safor’ mandarin from other cultivated commercial cultivars and from triploid ‘Garbi’ mandarin. Genetic analyses with the microsatellite loci Ci05A05, TAA 15, Mest 123, Ci03G05, and Ci07F11 indicated that the origin of ‘Safor’ mandarin was the unreduced gamete of ‘Fortune’ mandarin. This was indicated by the presence of two alleles of this cultivar and one allele of ‘Kara’ mandarin. Also, molecular markers help protect breeders’ rights and control traceability of nursery-propagated plants.

The overall characteristics of ‘Safor’ mandarin would indicate that its fruits could be collected during February and even up until the first week of March. This will fill an important gap in the production calendar of seedless mandarins for the Spanish citrus industry.

**Availability**

Protection of Plant Breeders’ Rights for this cultivar have 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 of 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 ‘Safor’ mandarin have been obtained by shoot-tip grafting in vitro according to the methodology described by Navarro et al. (1975) and healthy budwood was released in Summer 2008 to 22 Spanish nurseries that signed propagation agreements to produce certified plants. Approximately 50,000 certified plants of ‘Safor’ mandarin will be planted by growers in 2010, which is the first year it has been available at nurseries.

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