Scientia Horticulturae xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Pomological diversity of the Italian blood orange germplasm

Marco Caruso[a,*], Filippo Ferlito[a], Concetta Licciardello[a], Maria Allegra[a], Maria Concetta Strano[a], Silvia Di Silvestro[a], Maria Patrizia Russo[a], Donata Pietro Paolo[a], Paola Caruso[a], Giuseppina Las Casas[a,b], Fiorella Stagno[a], Biagio Torrisi[a], Giancarlo Roccuzzo[a], Giuseppe Reforgiato Recupero[a], Giuseppe Russo[a]

[a] *Consiglio per la Ricerca in agricoltura e l'analisi dell'Economia Agraria – centro di ricerca per l'Agrumicoltura e le Colture Mediterranee (CREA-ACM), Corso Savoia 190, 95024 Acireale, Italy*
[b] *Dipartimento D3A, University of Catania, Via Valdisavoia 5, 95123 Catania, Italy*

ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form 27 October 2016
Accepted 28 October 2016
Available online xxx

Keywords:
Anthocyanin
Pigmented oranges
Clonal selection

ABSTRACT

Blood oranges have been cultivated in Italy since the early 19th century. Growers and breeders were able to identify and collect many somatic mutants, which differed in their major pomological traits, such as fruit size and firmness, pulp and peel pigmentation, and ripening period.

Recently, in the framework of a genetic resource conservation program, most of the accessions collected at CREA in recent decades were planted in a single block to evaluate their characteristics. The presence of many selections in the same block, of the same age and grafted on the same rootstock is extremely important for a reliable characterization of the pomological characters, particularly pigmentation, which is strongly influenced by both the environment and the rootstock. The collection consists of 88 genotypes, including old lines and nucellar selections. Some commercial Tarocco, Moro and Sanguinello varieties were also included in the collection to facilitate comparison among clones. Moreover, the pomological features of 20 elite Tarocco clones cultivated nearby in the same experimental station were used as a further reference. The pomological characterization, based on 13 traits and performed in two consecutive years, revealed significant variability. Some genotypes showed strong year-to-year variability, mainly in fruit pigmentation, probably due to different climatic conditions during winter. Similarly, other traits such as fruit size, total soluble solids and acidity also varied among and within the varietal groups. These findings have a potential utility in identifying promising clones, which might fulfil the growing market needs such as higher anthocyanin content and longer shelf life.

1. Introduction

Sweet orange [*Citrus sinensis* (L.) Osbeck] originated from a complex admixture of unknown parents derived from pummelo [*C. grandis* (L.) Osbeck] and mandarin (*C. reticulata* Blanco) hybridizations (Wu et al., 2014; Velasco and Licciardello, 2014). All the sweet orange varieties cultivated worldwide are derived from somatic mutations of a single ancestor, accumulated during centuries in the different growing areas. Sweet oranges are usually categorized into few varietal groups (common, navel, blood), with a diversification in terms of agronomical features within each group.

Blood oranges were typically cultivated in the Mediterranean area, but recently, their cultivation has spread to other citrus growing areas, such as China, Australia and United States. It is very likely that the ancestors of blood oranges originated in southeast Asia and were later introduced into Europe (Chapot, 1963; Hodgson, 1967). The first reference to pigmented orange in European literature is in Ferrari's Hesperides (1646), who described a purple-fleshed fruit ("*Aurantium indicum purpurei coloris medulla*") reported by a Jesuit monk from the Philippines. Moreover, a report by De Loureiro (1790) describes a citrus fruit pigmented both inside and outside ("*intus and foris rubra*"), which was present in *Conchicina* (southern Vietnam). However, the described fruit size was twice that of sweet orange; hence, we could not exclude the possibility that it was a pink-fleshed pummelo.

After their introduction in Europe, blood oranges were included in historical gardens and mainly used as ornamentals. Later, they started to be grown in orchards for fresh fruit consumption. It is likely that many selections have been lost with the changes in destination from ornamental to fresh use. In the descriptions in the

* Corresponding author.
E-mail address: marco.caruso@crea.gov.it (M. Caruso).

http://dx.doi.org/10.1016/j.scienta.2016.10.044
0304-4238/

Mediterranean area from Gallesio (1811) and Risso and Poiteau (1818–1822), at least 7 pigmented genotypes are described. Later, Inzenga (posthumously published by Savastano, 1915) and Casella (1935) made the most exhaustive descriptions of selections grown in Italy. Casella can be considered the first citrologist citing the current pigmented varieties. He distinguished blood oranges into different varieties, the most diffused being Sanguinello, Moro and Tarocco (defined as aristocratic for its excellence).

It is reported that Tarocco arose from a spontaneous bud mutation in a Sanguinello orchard in the early 1900s in the Siracusa province, Sicily (Casella, 1935), while the origin of Moro, which was already cultivated in Sicily in the early 20th century, is unknown. Since then, we assisted to further increase the cultivated selections, derived from spontaneous mutations or nucellar selections. Hence, it is more appropriate to consider Sanguinello, Moro and Tarocco as varietal groups rather than single varieties, with many clonal selections characterized by different agronomical features. The spread of Tarocco compared to Moro and Sanguinello resulted in the isolation of a larger number of vegetative mutations with extremely divergent characteristics.

The molecular basis of the blood orange pigmentation is retrotransposon-mediated transcriptional activation of the Ruby Myb-like transcription factor (Butelli et al., 2012). The study demonstrated that commercial blood orange varieties have a common origin. The sequence upstream of the transcription factor differs exclusively in Moro, which specifically contains only the 3′LTR portion of the TCS1 retrotransposon. Butelli's results were later confirmed by Licciardello (unpublished data), who found no differences in the sequence of Ruby locus in many Tarocco and Sanguinello clones of the CREA germplasm. Despite the common genetic basis, blood orange selections display different degrees of anthocyanin pigmentation in pulp and peel, varying from a few red vesicles to a deep red-purple color (Fig. 1A–E). This indicates that additional and currently unknown molecular mechanisms control the quantity of pigments. Moreover, pigmentation is cold dependent, as was proven by molecular analyses (Butelli et al., 2012). This trait is also influenced by the rootstock and cultural practices, so the level of pigmentation may vary greatly in the same clone cultivated in different environments. Therefore, the exhaustive phenotypic characterization of each clone and the subsequent selection of the best performing clones are hampered by such extreme influences of external factors.

The present study describes the major pomological features of 88 blood orange accessions grafted on same rootstock and planted in the same block. All clones under evaluation are part of the CREA-ACM germplasm that has been isolated by Italian breeders and growers for many decades.

2. Materials and methods

2.1. Origin of plant material

The collection consists of (i) old clones of blood oranges cultivated in the past, which are rarely found and are at the risk of extinction; (ii) clonal selections, discovered in different orchards over decades from CREA-ACM breeders or indicated by growers, having interesting agronomical features, such as deeper pigmentation, fruit firmness, or different degrees of earliness or lateness; (iii) nucellar selections, generated at CREA with the main aim of obtaining virus-free clones showing in some cases distinctive features compared to the mother plant; (iv) mutants of no specific agronomical interest but having some peculiar feature, such as variegation, corrugated peel, lack of acidity, yellowish pulp and peel, or reduction of pulp pigmentation compared to the original variety. The classification of each accession is reported in Supplemental Table 1.

Some commercial varieties, representative of each varietal group and used as a reference were also included in the collection, namely Tarocco '1E' (an early-medium variety), Tarocco 'Meli' (late), Moro '58 8D 1′ and Sanguinello '49 5 5'. As a further reference, a common orange ('Cadenera') and a navel orange ('Washington CRC 3033′) were included in the block.

The criterion used was to exclude from the collection all the elite clones that are widespread in Italian citriculture and already planted in a young block nearby (block 7), as their yield and fruit quality characteristics are well known.

2.2. Establishment of the germplasm block

Eighty-eight clones, replicated twice, were grafted in May 2010 on rootstock seedlings planted in 2006 in block 2b of Palazzelli experimental farm (37° 20′N, 14° 53′E, 48 m a.s.l.), with spacing of 6 × 4 m. Genotypes belong to the Moro (4), Sanguigno and Sanguinello (23) and Tarocco (61) groups. Regarding the rootstock choice, the outbreak of CTV in Sicily (Davino et al., 2003) required the replacement of sour orange with CTV-tolerant rootstocks. Conversely, many clones were probably affected by exocortis, cachexia, or other viruses and viroids. In this context, we decided to graft all clones onto 'Swingle' citrumelo due to its tolerance to viroids and CTV (Wutsher, 1974; Hutchison, 1974). However, this rootstock is sensitive to iron chlorosis in our soil condition, where active lime is above 3–4%. For this reason, the regular application of iron chelates was needed to maintain the plants in a good nutritional state. The soil was a sandy loam (sand 72.0%, clay 15.4%), with pH 8.42 (1:2.5 H_2O), and EC 1.87 dS m^{-1}. The total and active lime contents were 93.30 g kg^{-1} and 31.71 g kg^{-1}, respectively. Mean organic carbon content was 10.42 g kg^{-1}, total Kjeldahl nitrogen (TKN) 1.68 g kg^{-1}, available P 35 mg kg^{-1}, and exchangeable K 325 mg kg^{-1}.

A few clones whose grafting failed in 2010 were re-grafted in spring 2011. Plants were subjected to standard culture techniques, according to best agricultural practices. Most genotypes began to produce in 2013. Since the agronomic performance of blood oranges is influenced by cold during fruit development, air temperatures were recorded by an automated weather station located nearby the collection field.

2.3. Fruit sampling and pomological analysis

Fruits for pomological characterization were sampled during two consecutive seasons (March 2015 and March 2016), except for 7 accessions (Sanguinello 'Moscato Romeo III'; Tarocco 'Fisichella'; Tarocco 'Giardinaro Motta S A'; 'Tarocco M (marze Maugeri ISA)'; Tarocco 'Variegato ornamentale', Tarocco 'Candido Scuderi', Tarocco 'Meli C8150′) for which fruits were missing from both plants or not representative in the first or second year of samplings (i.e., low number of fruits, atypical fruits developed from late blooming). In the same years, the pomological analysis was also performed on 20 commercial varieties, not included in the germplasm block but cultivated in block nearby the same experimental farm (block 7). This second collection was planted in 2003 on 'Carrizo' citrange. The data, being from non-coetaneous plants grafted on a different rootstock, were not used for a direct comparison with the accessions of block 2b but were considered as a reference to evaluate year-to-year stability of the most important traits, such as fruit size, pigmentation, and TSS, and to compare germplasm accessions with valuable cultivars. Based on the maturity indices (°Brix and acidity) recorded during the first season (March 2015), in December 2015 we performed additional samplings on a subset of putative early varieties.

Please cite this article in press as: Caruso, M., et al., Pomological diversity of the Italian blood orange germplasm. Sci. Hortic. (2016), http://dx.doi.org/10.1016/j.scienta.2016.10.044