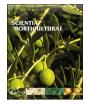
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Characterization of tropical mandarin collection: Implications for breeding related to fruit quality

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ABSTRACT

During the last decade, the worldwide market for fresh citrus fruits, mainly those that are seedless with easypeeling and nice orange-colored skin, greatly increased. The elaboration of organoleptic quality is strongly defined by genetic and environmental components and their interaction. While several mandarin breeding programs have been developed worldwide, very few focus on the issues of tropical areas, and few data are available for citrus germplasm evaluation in a tropical environment. To optimize the mandarin production system, research related to the exploration of existing citrus germplasm, the expansion of the genetic base of orchards and the evaluation of the agronomic potential of new genotypes must be conducted. The objective of this study was to characterize and evaluate 25 mandarin varieties from typical Brazilian tropical citrus germplasm according to genetic, morphological and physicochemical traits over two consecutive years. Simple correlation coefficient analysis showed significant positive and negative correlations among characteristics (e.g., fruit diameter and total soluble solids). Principal component analysis showed that fruit diameter, fruit weight, total soluble solids, total titratable acidity, fruit surface texture and adherence of albedo to pulp were the variables that most contributed to phenotypic variability. Genetic diversity analysis grouped accessions in four main clusters corresponding to traditional edible Chinese mandarins, Mediterranean mandarins, tangelos and tango hybrids and nonedible mandarins. A multiple factorial analysis showed that phenotypic diversity was associated with genetic diversity mainly for taste, shape and color traits. The results of this study will be used for the conservation of the indigenous germplasm, identification of varieties with valuable traits related to fruit quality and subsequent exploitation in citrus breeding programs.

1. Introduction

Worldwide, citrus are among the most consumed fruits, as both fresh (*in natura*) and processed products (e.g., juice) (Fao, 2017). Mandarins constitute an important citrus horticultural group that includes *C. reticulata*, one of the four ancestral taxa with *C. maxima*, *C.*

medica and *C. micrantha* from which all *Citrus* cultivated species were generated by reticulate evolution (Barkley et al., 2006; Nicolosi et al., 2000; Wu et al., 2014). The classification of mandarins is still controversial. Swingle and Reece (1967) categorized all mandarins in three taxa (*C. reticulata, C. tachibana* and *C. indica*), the majority being in *C. reticulata*, while Tanaka (1977) distinguished 36 mandarin species. An

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intermediary classification was proposed by Hodgson (1967), who divided mandarins into the following natural subgroups: common mandarin (*C. reticulata* Blanco), Satsuma (*C. unshiu* Marcovitch), King (*C. nobilis* Loureiro), Mediterranean mandarin (*C. deliciosa* Tenore), small citrus (*C. indica, C. tachibana* and *C. reshni*), clementine (*C. Clementina* Hort. ex. Tan) and hybrids such as tangor (*C. reticulata* x *C. sinensis*) and tangelo (*C. reticulata* x *C. paradisi*). Recent genetic and genomic studies (Curk et al., 2015; Oueslati et al., 2017; Wu et al., 2014) revealed that cultivated mandarins have a complex genome structure with introgression of *C. maxima* genome in a predominant *C. reticulata* genomic background.

Mandarins are mainly consumed as fresh fruits: they are a source of vitamins and fiber and also contain secondary metabolites-including antioxidants like ascorbic acid, phenolic compounds, flavonoids and limonoids-important for human nutrition and health (Jayaprakasha and Patil, 2007). During the last decade, some changes have been observed in the fresh citrus market: consumers and supermarkets prefer easy-to-peel and tasty fruits with nice skin color (e.g., reddish orange) such as mandarins, in contrast to the decreased consumption of other citrus that are difficult to peel or colorless (e.g., yellow) such as oranges, pomelos and grapefruits (Goldenberg et al., 2018). As a result, the global production of mandarins increased 30% during the last 5 years (from 22 million tons in 2010 to 29 million tons in 2015) (Fao, 2017; Goldenberg et al., 2018; Usda, 2017). Actually, the mandarin world market reaches 29 million tons per year: China is the major producer with 20 million tons, followed by the European Union (2.9 million tons), Japan (1.1 million tons), Morocco and Turkey (1 million tons each) and Brazil (938,000 tons). Some countries such as Spain, Italy, Israel, South Africa, the United States (California), Uruguay and Argentina are ranked as leaders in production of fruits with high physicochemical quality attributes such as attractive skin color, seedlessness and balanced sweetness and acidity (Goldenberg et al., 2018; Tietel et al., 2011).

Despite the great diversity of citrus genera, species and varieties, the range of cultivars used in commercial orchards is still restrained. Genetic uniformity makes them more vulnerable to pathogens and insects (Gmitter et al., 2007; Ollitrault et al., 2003). For this reason, a better understanding of the genetic variability distribution as well as the relationship between varieties is necessary to expand genetic diversity (Gmitter et al., 2007). Besides genetic data, phenotypic and agronomic characterization of citrus germplasm may directly result in the selection of new cultivars for commercial production. This information could support citrus breeding programs by identifying desirable traits such as those related to mandarin fruit quality (Coletta Filho et al., 2000; Gmitter et al., 2007; Krueger and Navarro, 2007). These traits depend on the plant genetic background but also on environmental conditions such as orchard localization and culture practices (Dhuique-Mayer et al., 2005; Dhuique-Mayer et al., 2009; Mouly et al., 1999; Zhang et al., 2011). In tropical conditions, the color and acidity of mandarins are less intense (Dhuique-Mayer et al., 2009), which could prejudice their acceptance by consumers. However, few studies and references exist on mandarin germplasm evaluation for fruit quality in tropical conditions.

Brazil has three large citrus germplasms; among them, the germplasm located in the Embrapa Cassava and Tropical Fruit Culture (CNPMF) located in Cruz das Almas (Bahia State) is second in terms of number of citrus accessions (813) and is the only one located in a typical tropical climate (between the equator and the Tropic of Capricorn) (Passos et al., 2007). The diversity present in this germplasm needs to be characterized, evaluated and studied with respect to its applications for fresh fruit production, as well as to select new promising genotypes for inclusion in breeding programs for tropical areas. Such characterization involves a wide range of data including various measured descriptors (Ipgri, 1999; Krueger and Navarro, 2007). The objective of this study was to characterize and evaluate 25 mandarin varieties of the tropical CNPMF germplasm according to genetic, morphological and physicochemical traits. The results of this study will be used for conserving the indigenous germplasm, identifying varieties with valuable traits related to fruit quality and subsequently exploiting in citrus breeding programs.

2. Material and methods

2.1. Plant material

The present study was carried out on 25 mandarin varieties (Table 1) from the citrus germplasm of the CNPMF located in Cruz das Almas, Bahia, Brazil (12°40'39"S latitude, 39°06'23"W longitude and 225 m elevation). Each accession was represented in the citrus germplasm by 2–12 plants (grafted on Rangpur Lime); 2 plants by variety were used for the subsequent analyses. Varieties used as scions as well as rootstock varieties were evaluated (Table 1). Scions are naturally intended for fruit production, and good fruit quality was expected for these varieties. Rootstock varieties generally presented nonedible fruits, but their analysis here allowed for some comparisons between high and low fruit quality. Rootstock varieties also could carry interesting traits useful for breeding programs even if these varieties will not be used for fruit production.

2.2. Phenological stage evaluation

Phenological stages of citrus varieties were monitored from October 2012 to January 2016. Initially, 50 to 100 flowers were labeled at the beginning of the flowering period for each accession, and then field observations were made every 3 weeks. The presence of the following structures was evaluated according to Stoller (2016): flowers (Flo), fruitlet (FLet), immature green fruit (IG), mature green fruit (MG), breaker stage fruit (B), full ripe fruit (Full) or no structure (NS) (Table 2).

2.3. Qualitative and physicochemical analysis of the fruits

The physicochemical analyses of the fruits were conducted in the post-harvesting laboratory of Embrapa CNPMF. Analyses were conducted on 20 full ripe fruits (10 fruits/tree; 2 trees for each variety) harvested from different areas of the treetop, from 2014 to 2015 (two consecutive fructification periods). The qualitative descriptors were fruit shape (FS), shape of fruit base (SFB), shape of fruit apex (SFA), fruit skin color (FSC), fruit surface texture (FST), adherence of albedo to pulp (AAP) and fruit pulp color (FPC), as previously described (Ipgri, 1999). The physical parameters evaluated were (i) fruit weight (FW) measured with an analytical balance (Mettler Toledo); (ii) fruit diameter (FD) measured with a digital pachymeter (Stainlees/Hardened), and (iii) number of seeds (SN). The skin coloration and pulp coloration (SC and PC, respectively) were evaluated using the Minolta CR400 Chroma Meter that recorded the L* (luminosity), a* (green to red) and b* (blue to yellow) values (Mendonça et al., 2003; Minolta Co., 1998); three measures on skin and two on pulp were made. The Citrus Color Index (CCI) was calculated according to the following formula: CCI = 1000. a/L. b (Jimenez Cuesta et al., 1983). The chemical parameters evaluated were (i) total titratable acidity (TTA) of the juice titrated with NaOH 0.1 N and phenolphthalein indicator; (ii) total soluble solids (TSS) measured with a digital refractometer (°Brix) and (iii) the ratio TSS/TTA (Table 2). Statistical analyses were performed using the SAS (Sas, 1988) and Genes (Cruz, 2006) software. The results were submitted to variance analysis and the media were compared by the Scott-Knott ($P \le 0.05$) test. Correlations between the traits were determined using the Pearson correlation coefficient. For the qualitative descriptors the frequency of each category and the entropy (H) level of the traits were calculated using the Renyi entropy (Renyi, 1961):

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