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Rootstock-modulated yield performance, fruit maturation and phytochemical quality of ‘Lane Late’ and ‘Delta’ sweet orange

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ABSTRACT

Graft combinations of sweet orange cultivars Delta and Lane Late on six rootstocks were established on calcareous clayey soil and evaluated for compatibility, yield, and fruit physicochemical quality attributes at three stages of harvest maturity. Citrumelo proved a deleterious rootstock to both scions, while GouTou was deleterious to Delta and had low compatibility with Lane Late. Most compatible rootstock for Delta was Sour Orange, whereas all rootstocks except GouTou were equally compatible with Lane Late. Yield and fruit weight were highest on Volkameriana. Titratable acidity was highest on Sour Orange and Carrizo and lowest on Volkameriana. Dominant acids were citric (8.5–11.3 g/L), malic (1.8–1.9 g/L) and succinic (1.3–1.4 g/L). Citrate/malate ratio was lower in Lane Late and declined further with maturation. Acids were highest on Sour Orange and Carrizo. Increased levels of malate (2.07–2.21 g/L) elicited by Volkameriana may constitute an index of inferior sensory quality. Soluble solids were highest on Sour Orange, Carrizo and Cleopatra (9.6–10.6%) and lowest on Volkameriana (9.3–9.7%). Sucrose was the most abundant soluble carbohydrate (33.9–39.9 g/L) and sugar ratios were stable across rootstocks, however sucrose concentration in Delta increased with maturity at the expense of glucose and fructose. Increase in chroma paralleled increase in soluble solids during maturation of Delta but not Lane Late. Fruit phenolics were highest with the least compatible GouTou and lowest with the highly productive Volkameriana. Maximal ascorbate levels were attained at mid-maturity (380.4–450.2 mg/L) and were lowest on Volkameriana (323.4–398.8 mg/L). Considering its overall performance and resistance to Citrus Tristeza Virus, Carrizo appears as a promising rootstock alternative to Sour Orange particularly on calcareous soils.

1. Introduction

Several known and less familiar species of the genus *Citrus* have been cultivated worldwide since antiquity, concentrated mainly in niches of tropical, subtropical or Mediterranean climate. Citrus fruits draw their popularity not only from their attractive appearance and palatability but also from their acclaimed bioactive value (Liu et al., 2015). They serve versatile uses as table fruit, fresh or concentrated juice, components of non-alcoholic and alcoholic beverages, and even as flavor enhancers and food additives. Promoting value-adding uses for citrus as a commodity of prime nutritional, industrial and pharmaceutical importance is a major objective for the citrus industry which holds an important position in the agroindustry spectrum.

Citrus cultivars selected in pursuit of the above uses are asexually propagated through grafting onto rootstocks appraised for superior performance under specific edaphoclimatic conditions or for disease tolerance (Forner-Girner et al., 2014). Numerous rootstocks are currently available, yet few have found extensive use. One of the most popular worldwide is the Sour Orange (*Citrus aurantium* L.), owing to its adaptability to wide-ranging soil conditions and the excellent fruit quality it imparts (Louzada et al., 2008). It remains top-ranked across Mediterranean citrus industries, including Cyprus (Georgiou, 2009), Egypt (Bassal, 2009), Greece (Elena et al., 2006), Israel (Benjamin et al., 2013) and Italy (Castle, 2010). An excellent rootstock for fresh fruit production in areas free of Citrus Tristeza Virus (CTV) (Castle, 1987), Sour Orange is progressively replaced by other rootstocks precisely because of its susceptibility to CTV. Among its outstanding features is tolerance to highly alkaline and saline soil conditions (Wutscher, 1979). Sour Orange tends to raise the soluble solids content (SSC) but also the titratable acidity (TA) of the scions’ fruit juice, which consequently delays attainment of commercial maturity compared to other rootstocks (Davies and Albrigo, 1994). Carrizo Citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliate* (L.) Raf.] is a CTV-tolerant rootstock that augments productivity and fruit quality but is susceptible to salinity and

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lime-induced chlorosis (Legua et al., 2011). It is currently the most extensively used rootstock in Spain (Cardeñosa et al., 2015; Forner-Giner et al., 2003; Legua et al., 2011). Cleopatra Mandarin (*Citrus reshni* Hort. *ex* Tan.) is a rootstock tolerant to CTV, Citrus exocortis viroid (CEVd), Hop stunt viroid (HSVd) known as Xyloporosis or Cachexia, salinity, cold and calcareous soils, however it retards initial scion growth and precocity and results in scion fruit of reduced size but excellent sensory quality (Castle, 1987). Citrus scions grafted onto Volkameriana (*Citrus volkameriana* Ten. and Pasq.) produce large, vigorous trees, yielding large quantities of moderate to poor quality fruit, they are not susceptible to CTV and xyloporosis and are tolerant to malesco and phytophthora under wide-ranging conditions, but susceptible to blight and nematodes (Castle, 1987). GouTou Chen is probably a sour orange (*Citrus aurantium* L.) hybrid, used as a citrus rootstock in China and reported in Florida trials as liable to low productivity and inferior fruit quality (Wutscher and Bowman, 1999). Swingle Citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] is tolerant to CTV and phytophthora, has low sensitivity to exocortis and xyloporosis but low tolerance to iron chlorosis (Hutchison, 1974). It is evident from the above that citrus yield performance, growth habit, precocity and fruit quality are substantially rootstock-modulated and dependent on the dynamics of rootstock-scion interactions (Cardeñosa et al., 2015; Forner-Girner et al., 2003; Dubey and Sharma, 2016; Dubey and Sharma, 2016; Legua et al., 2013; , 2014).

Studies dealing with citrus fruit ripening behavior are scarce (Lin et al., 2015; Obenland et al., 2009), moreover the potential effect of rootstocks thereon remains largely unexplored (Cardeñosa et al., 2015; Bermejo and Cano, 2012). Introducing novel rootstocks is a pressing need posed by the risks for severe pest and disease outbreaks against the currently narrow genetic pool of established rootstocks. Additional pressure on the citrus industry stems from growing demand for land for alternative crops and not least by urban development, especially on coastal areas which are the most suitable for early crop production. These terms corroborate the growing need for extending citriculture to more marginal lands characterized by extremes in alkalinity, salinity and soil structure all of which can potentially be ameliorated through proper rootstock selection (Castle, 2010).

Accordingly, the objective of the current study was to examine the response of two orange scion cultivars grafted onto six prospective rootstocks and grown under soil conditions of high alkalinity and high clay content. Evaluations were performed for four productive years and comprised yield performance characteristics, plant and fruit morphometric characteristics, and fruit physicochemical composition with respect to fruit harvest maturity.

2. Materials and methods

2.1. Plant material and experimental conditions

Scions of Sweet Orange (*Citrus sinensis* L.) juice type cv. Delta and navel type cv. Lane Late were grafted on Sour Orange (*Citrus aurantium* L.), Carrizo Citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliate* (L.) Raf.], Cleopatra Mandarin (C. *reshni* Hort. *ex* Tan.), *Citrus volkameriana* Ten. and Pasq. (*Citrus limonia* Osb.), GouTou Chen (*Citrus aurantium* L.) and Swingle Citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] and planted in the field in 2004. Ten replicate trees per graft combination were established in a fully randomized design. Trees were spaced 4.20 m apart within rows and 6.50 m apart between rows amounting to a plant density of 366 plants ha^{-1}. The study was conducted at the Zygi Experimental Station (34° 44′ 00′′ N; 33° 20′ 15′′ E) of the Agricultural Research Institute, Cyprus. The prevailing climate is typical Mediterranean, with most precipitation (c. 280 mm) received between November and March. The soil had uniform 40% clay content throughout its profile, approximately 50% calcium carbonate content, and pH 8.0–8.3. Trees were drip irrigated on a frequency cycle of 3–15 days depending on season and on estimates of irrigation requirements based on Class A pan evaporation (Papazafiriou, 1999). The N, P and K applications were based on the guidelines of Agustí (2003), corrected on the basis of annual soil and tissue analyses and not exceeding 30-8-15 kg ha^{-1}. Combined top-dressing fertilization and fertigation were applied for efficient nutrient distribution. Iron chelate (Fe-EDTA) was applied by fertigation, while other micronutrient requirements were addressed by foliar applications. Standard pest and weed control practices were applied.

2.2. Trunk index, yield and fruit sampling

Estimation of Trunk Index was based on the ratio of scion to rootstock circumference at 10 cm above and below the graft union, respectively. Cumulative yield per tree was measured for the cultivation periods 2012–2013, 2013–2014, 2014–2015, 2015–2016. Three sequential harvests (S1, S2, S3) were performed on November 24, December 15, and January 19 for Lane Late Orange, and on January 4, February 1 and February 28 for Delta Orange. Each replicate sample for fruit quality assessment consisted of ten representative healthy fruits, free of visible defects, collected from all external sides of the canopy at the harvester's height. Fruit morphometric measurements and juice sample preparation were conducted within 24 h from harvest.

2.3. Fruit morphometric characteristics and juice content

Unit fruit weight was determined to ± 0.01 g on a Precisa XT 4200C electronic balance (Precisa Gravimetrics, Dietikon, Switzerland). Shape Index was calculated as the ratio of meridian to equatorial diameter measured using an electronic caliper, used also for measuring rind thickness at two representative points of cross-sectioned fruit. Juice was extracted using an electric Classic Mod. 11 citrus juicer (Santos, Lyon, France) and juice content calculated as the percentage ratio of juice volume (ml) to fruit weight (g). Juice samples for phytochemical analyses were transferred to 50 ml Falcon tubes and maintained at −80 °C.

2.4. Titratable acidity, soluble solids and optical assessment

The titratable acidity (TA), soluble solids content (SSC) and color of the juice were determined as previously described (Kyriacou et al., 2016), using respectively a Mettler Toledo T-50 automatic titrator (Mettler-Toledo International Inc.), an Index TCR 15–30 digital refractometer (Index Instruments, Ramsey, England) and a Minolta CR-410 Chroma Meter (Minolta, Osaka, Japan).

2.5. Total phenols, ascorbic acid, organic acids and carbohydrate content

The total phenols content of the juice was determined according to the method of Singleton et al. (1999), slightly modified as previously described (Kyriacou et al., 2016). Quantification was performed on a Jasco V-550 UV–vis spectrophotometer (Jasco Corp., Tokyo, Japan) equipped with Spectra Manager ™ II Software against linear calibration with external gallic acid standards over the range of 50–500 mg l^{-1}, yielding a regression coefficient (R^2) greater than 0.99. The total phenols content of the juice was expressed in gallic acid equivalents μg ml^{-1}.

The total ascorbic acid of the pulp was determined according to the method of Klein and Perry (1992), based on the reduction of 2,6-dichloro-indolphenol by pulp samples diluted by 1:10 (w/v) in 1% metaphosphoric acid. Quantification was performed at 515 nm against linear calibration with external L-ascorbate standards over the range of 10–100 mg l^{-1}, yielding regression coefficients (R^2) greater than 0.99. Ascorbic acid content of the pulp was expressed in μg g^{-1} f.w.

Analysis of non-structural carbohydrates (glucose, fructose, and sucrose) in the juice was performed by liquid chromatography on an Agilent HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a 1200 Series quaternary pump and a 1260 Series