



Genetic relationships between some Tunisian *Citrus* species based on their leaf volatile oil constituents



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ABSTRACT

Volatile oil constituents of Tunisian sweet orange (*Citrus sinensis* Osbeck) cv. Meski (MES), Valencia Late (VAL), Thomson Navel (THN) and Maltese Blanc (MAB); mandarin (*Citrus reticulata* Blanco); sour orange (*Citrus aurantium* L. cv. Amara (AM)) and pummelo (*Citrus grandis* Osbeck) were obtained by hydrodistillation and analysed by gas chromatography (GC) combined with a flame ionisation detector (FID) and mass spectrometry (MS). A total of 41 components accounting for more than 95% of the total essential oils were identified, and oxygenated monoterpenes (69.5–99.9%) were found as the most prominent fraction in all oil samples. The main constituents were linalool (3.1–73%), isoborneol (0–55.8%) and tepinen-4-ol (1.1–19.2%). A high degree of inter- and intraspecific chemical variability between species and cultivars was found to be genetically determined, and a set of distinctive traits (chemical markers) in the essential oils profile was established. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) of all identified components grouped the oils into two main chemotypes (linalool/isoborneol and isoborneol/linalool).

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1. Introduction

Citrus plants are the most important fruit tree crop in the world, with an annual production of approximately 1.02 hundred million tons (Hwang et al., 2012). Citrus fruits are the most popular for consumers throughout the world due to their pleasant flavours and nutritional value (Qiao et al., 2008). Fruits are mainly used for dessert, juice, jam production and provide a variety of functional components including vitamin C, folic acid, flavonoids, dietary fibres and essential oils, among others (Senevirathne et al., 2009). Unequivocally, essential oils are the most important citrus by-products usually obtained from the peels. They are widely used in a variety of industrial fields, ranging from food and beverages, to cosmetics, from pharmaceuticals, onto their extensive employment in fragrances (Tranchida et al., 2012).

The chemical composition of citrus essential oils has been extensively studied and different compositional patterns owing to the species/cultivars, origin, climate, season, ripening stage, extraction and analytical methods have been published (Minh Tu et al., 2002; Buettner et al., 2003; Fanciullino et al., 2005; De Pasquale et al., 2006; Chutia et al., 2009; Hosni et al., 2010).

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These studies have reported that the basic components of citrus essential oil were monoterpene (limonene), sesquiterpene hydrocarbons and their oxygenated derivatives including aldehydes (citral), ketones, acids, alcohols (linalool) and esters (Fisher and Phillips, 2008). Most of these studies were focused on peel essential oil, while the volatile oil constituents of leaves have not received much attention. The few published data on leaf volatile oils revealed the presence of sabinene, linalool, (*E*)- β -ocimene and terpinen-4-ol as the major component of *Citrus reticulata* (Lota et al., 2000). Two years later, the same authors analysed the leaf essential oils of *Citrus limon* and *Citrus aurantifolia* and found that limonene, β -pinene, neral and geranial were the most prominent compounds in *C. limon*, while limonene, neral and geranial were the main components in the leaf essential oils of *C. aurantifolia* (Lota et al., 2002). Linalool and linalyl acetate were reported as the main volatile compounds of leaf essential oils of *Citrus aurantium* (De Pasquale et al., 2006). The aforementioned studies also revealed remarkable chemical polymorphism in volatile oil constituents in different cultivars from various locations. Therefore, comprehensive evaluation of poorly characterized cultivars and/or species is an important task for the selection and including some species/cultivars in breeding programs directed at improvement of essential oil composition.

In Tunisia, citriculture has existed traditionally and the citrus varieties have been naturally selected. The area under citrus cultivation was estimated to be about 19,250 ha with a yearly production of over 230,000 tons. Such high production inevitably satisfies the fresh fruit market, the agro-food industry and the exportation demands. Among the different species, orange and mandarin are the most popular fruits in Tunisia. In Tunisian cuisine, the fresh exocarp of *Citrus sinensis* and the leaf hydrolat of numerous citrus species are widely used for flavouring tea and the preparation of some traditional dishes. Despite the socio-economic relevance of citrus and their by-products, there is dearth of information about the leaf essential oil composition.

With regard to this topic, the present contribution aimed at identifying the chemical composition of the essential oil of the leaves of four selected citrus species and to assess the genetic differences between them. Such information would be fundamental to promote the citrus processing industry in Tunisia via the selection of the most valuable source (in term of quality and quantity) of fragrant compounds. On the other hand, it enabled us to enrich the flavour-bank and to extend our knowledge on volatile constituents of the national citrus germplasm. Moreover, they could provide a basis for including the studied species and cultivars in breeding programs aimed at optimizing the production of high added value products.

2. Materials and methods

2.1. Plant materials

Leaves were collected from healthy trees (10 trees for each species and cultivars), cultivated under the same pedo-climatic and cultural conditions in the experimental station of the "École Supérieure d'Agriculture de Mograne, Zaghouan, Tunisia" (latitude 36°25'38"N; longitude 10°05'41"E; altitude 149 m). Selected species and cultivars were: sweet orange (*C. sinensis* Osbeck) cultivars Meski (MES), Valencia Late (VAL), Thomson Navel (THN) and Maltaise Blanc (MAB); mandarin (*C. reticulata* Blanco); sour orange (*C. aurantium* L. cv. Amara (AM)) and pummelo (*Citrus grandis* Osbeck). Plant materials were botanically identified by Prof. Nadia Ben Brahim (Department of botany, National Institut of Agronomic Research, Tunis, Tunisia) where voucher specimens were deposited. Leaves were air dried (Yadav et al., 2004; Rehman, 2006) at room temperature (20 ± 2 °C) for one week, and subsequently assayed for their essential oil composition.

2.2. Essential oil isolation

The air dried materials (400 g) were ground using a Retsch blender mill (Normandie-Labo, Normandy, France), sifted through 0.5 mm mesh screen to obtain a uniform particle size and submitted to hydrodistillation for 3 h using a Clevenger type apparatus. The oils obtained were recovered, weighed, dried over anhydrous sodium sulphate and stored in amber and air-tight sealed vials at 0 °C until required.

2.3. GC and GC-MS analyses

Gas chromatography analyses were carried out on a Shimadzu HRGC-2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with flame ionisation detector (FID), Auto-injector AOC-20i and auto-sampler AOC-20s. An apolar column Rtx-1 (30 m \times 0.25 mm, 0.32 μ m film thickness; Restek Corporation, Bellefonte, PA, USA) was used. The oven temperature was held at 50 °C for 10 min then programmed at 2 °C/min to 190 °C. The injector and detector temperature were programmed at 230 °C. The flow of the carrier gas (N_2) was 1.2 mL/min and the split ration was 1:60. Injection volume for all samples was 0.5 μ L of diluted oils in *n*-pentane (LabScan Dublin, Ireland).

The GC-MS analyses were performed on a gas chromatograph HP 6890 (II) interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, Ca, USA) with electron impact ionization (70eV). A HP-5MS capillary column (60 m \times 0.25 mm, 0.25 μ m film thickness; Supelco, Bellefonte, PA, USA) was used. The column temperature was programmed to rise from 40 to 280 °C at a rate of 5 °C/min. The source temperature was 270 °C and the carrier gas was helium with a flow rate of 1.2 mL/min. Scan time and mass range were 1 s and 50–550 *m/z*, respectively. The injection volume and the split ratio were 1 μ L and 1:20, respectively.

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