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# Inheritance of Characters Involved in Fruit Quality in a Citrus Interspecific Allotetraploid Somatic Hybrid

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The main components of citrus fruit quality (organic acids, sugars, and aromatic compounds) were studied in fruits of a somatic hybrid allotetraploid between Willow leaf mandarin (*Citrus deliciosa* Ten.) + Eureka lemon [*Citrus limon* (L.) Burm.] and the two diploid parents. The somatic hybrid (WLM + EUR) combined both nuclear genomes of the parents, with chloroplasts and mitochondria of mandarin. Variations in sugar and acid content were studied in fruit pulp during the maturing period, and the chemical composition of peel oils was investigated by capillary gas chromatography (GC), GC/mass spectrometry (MS), and $^{13}C$ NMR. The somatic hybrid was close to the lemon parent in the synthesis of organic acids and close to the mandarin parent in fructose content, while sucrose and glucose contents were between the two parents. The aromatic compounds of WLM + EUR were close to mandarin with a non-negligible effect of lemon, which inhibits the methyl N-methylanthranilate, a mandarin-specific compound. Our results lead us to conclude that biosynthesis of compounds involved in citrus fruit quality is not inherited in an additive way in the allotetraploid hybrid. We observed mandarin dominance for fructose and most of the aromatic compounds, lemon dominance for organic acid and methyl N-methylanthranilate, and codominance for sucrose and glucose.

**KEYWORDS:** ***Citrus*** **somatic hybrid; allotetraploid; sugars; organic acids; essential oil; fruit quality**

## INTRODUCTION

Somatic hybridization by protoplast fusion has been used in citrus breeding for the last two decades (*1*). Interspecific and intergeneric hybridizations have produced many allotetraploid hybrids. These tetraploid lines are useful for breeders for the production of seedless varieties, which is a key commercial objective in citrus improvement programs driven by consumer preferences (*2*, *3*). Together with diploid cultivars, they serve as breeding parents for the production of citrus triploid seedless genotypes (*2*, *4*−*6*). Somatic hybridization has resulted in great diversification of the tetraploid gene pool available for sexual hybridization with diploid cultivars, and thousands of triploids have been produced from interploid crosses using somatic hybrid parents (*6*).

Somatic hybridization enables whole nuclear genomes of two genotypes to be added whatever their heterozygosity. It is thus an original tool for analyzing the rules of phenotypic inheritance in citrus. At the international level, evaluations are ongoing for several interspecific and intergeneric hybrids with the aim of understanding the inheritance mechanisms in allopolyploid citrus genomes. Several studies have been made to understand the genetic control of essential oil biosynthesis in the leaf and polyphenols in citrus somatic hybrids (*7*−*10*). The conclusion of these studies was that somatic hybridization did not result in a simple addition of parental traits (*7*, *10*, *11*). For example, Gancel et al. (*10*, *11*) showed that somatic hybrids combining Willow leaf mandarin with sweet orange, grapefruit, and lemon exhibited a leaf aromatic profile close to their mandarin parent. However, an additive behavior was observed in a combination between sweet orange and grapefruit (*12*). Certain authors also mention a general decrease in leaf sesquiterpenoid biosynthesis in somatic hybrids as compared with their parents (*11*). For pulp polyphenols, Tusa et al. (*7*) observed that the metabolic pattern of a somatic hybrid between Valencia sweet orange and Femminello lemon represented an intermediate position between those of the parents.

Sugars, acids, and aromatic compounds are recognized as major components in fruit quality (*13*, *14*). These components vary greatly among the different varieties of citrus. The acidity of

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the citrus fruit results directly from the citric acid cycle. The mitochondria are the site of biosynthesis in the citric acid cycle, but the genes involved are located in the nuclear genome. The biosynthesis of sugars is much more complex because the chloroplast and mitochondria play an important role in photosynthesis and respiration, respectively. We previously studied a cybrid that possessed nuclear and chloroplast genomes from Eureka lemon plus mitochondria from Willow leaf mandarin. These results showed that the main genetic information for biosynthesis of compounds involved in fruit quality is contained in the nucleus (*15*). To evaluate the effects of allotetraploidy on the biosynthesis of fruit quality components, we reported a study on an allotetraploid somatic hybrid (WLM + EUR) between Willow leaf mandarin (*Citrus deliciosa* Ten.) and Eureka lemon [*Citrus limon* (L.) Burm.], produced by symmetric protoplast fusion (*16*). This hybrid is of great interest because it combines mandarin cytoplasm with nuclei from both lemon and mandarin, two genotypes that display very strong differentiation in fruit phenotype (**Figure 1**) and quality. The objective of the present study was to analyze the effects of the coexistence of the two parental nuclear genomes on the biosynthesis of the fruit quality components.

## MATERIALS AND METHODS

**Plant Materials.** The study was conducted on fruits of Willow leaf mandarin SRA 133 (*C. deliciosa* Ten.), Eureka lemon SRA 4 [*C. limon* (L.) Burm.], and their somatic allotetraploid called WLM + EUR (*C. deliciosa* Ten.) + [*C. limon* (L.) Burm.]. WLM + EUR was produced by symmetric protoplast fusion (*16*). WLM + EUR and the two parents were all grafted onto volkameriana rootstock (*Citrus limonia* Osb.) and randomly planted in the same field at the Station de Recherche Agronomique (INRA/CIRAD) in San Giuliano (Corsica, France) in 2002.

**Genetic Characterization.** The ploidy level was determined by flow cytometry (*17*) and by chromosome count. Leaves were harvested from in vitro plants. They were treated in 0.04% hydroxyquinoline for 4 h at room temperature and then for 4 h at 4 °C. They were fixed for 48 h in ethanol: acetic acid (3:1) and stored in 70% ethanol at 4 °C. Chromosome preparation was performed as described by D'Hont et al. (*18*). The chromosomes were counterstained with DAPI (4,6-diamidino-2-phenylindole). Molecular markers were studied by polyacrylamide gel electrophoresis in sequencing gels with silver staining. The nuclear genome was characterized using 26 polymorphic SSR markers (*19*). The chloroplast genome was characterized using four chloroplastic SSR markers (*20*). The origin of the mitochondrial genome was studied using three polymorphic universal polymerase chain reaction primers described by Froelicher et al. (*21*). These new mitochondrial markers displayed indels polymorphism.

**Fruit Sampling for Analysis of Acidity and Sugars.** Fruits were harvested at 15 day intervals from December to February from three trees per variety at stage III of fruit development (*22*). Four fruits were collected from each tree at each sampling. Fruits were peeled, and their pulp was frozen in liquid nitrogen and stored at −140 °C. The frozen fruit pulp was then lyophilized and powdered before analysis.

**Titratable Acidity.** One hundred milligrams of powdered pulp was suspended in 5 mL of bidistilled water. Titratable acidity was determined by titration to pH 8.1 with 0.1 mol $L^{-1}$ NaOH, using an automatic Mettler titrator DL25 (Mettler-Toledo, France). Titratable acidity was expressed as milliequivalents per gram of fresh pulp (mequiv $g^{-1}$ f.p.).

**Analysis of Organic Acids and Sugars.** One hundred milligrams of powdered pulp was dissolved in 5 mL of bidistilled water and centrifuged at 160*g* for 20 min. The supernatant was filtered through 25 mm syringe filters and 0.45 $\mu$m cellulose acetate membranes (VWR). Organic acids and sugars were analyzed with an analytical high-performance liquid chromatography unit (Perkin-Elmer, Series 200, France) as previously described by Albertini et al. (*23*). Organic acid separation was performed using an ion-exclusion column (Spheri-5 RP-18, 220 mm × 4.6 mm, 5 $\mu$m) thermostated at 20 °C. Elution was carried out with a mobile phase made of 25 mM $KH_2PO_4$ solution, adjusted to pH 2.4 with $H_3PO_4$. The flow rate of the mobile phase was 1.0 mL $min^{-1}$. Detection was performed with an UV detector set at 210 nm. Sugar separation was performed with a $NH_2$-bound silica column (Waters, 4.6 mm × 250 mm, 4 $\mu$m) at 35 °C. Elution was carried out isocratically with a mobile phase made of acetonitrile/water (70:30, v/v) at a flow rate of 1.0 mL $min^{-1}$. Detection was performed with a refractometer index detector.

**Figure 1.** Fruits of Willow leaf mandarin (left), allotetraploid hybrid (middle), and Eureka lemon (right).

Data were acquired using TotalChrom software for Windows version 6.2 (Perkin-Elmer Instruments, Shelton, United States). Concentrations of organic acids and sugars were expressed as milligrams per gram of fresh pulp (mg $g^{-1}$ f.p.).

**Sampling of Peel Essential Oils and Analysis of Gas Chromatography (GC), GC/Mass Spectrometry (MS), $^{13}$C NMR.** The peel of at least 30 ripe fresh fruits per genotype, collected at 225 days after anthesis (DAA), was subjected to water distillation using a Clevenger type apparatus for 3.5 h. Yields ranged between 0.20, 0.22, and 0.29% for Willow leaf mandarin, WLM + EUR, and Eureka lemon, respectively.

GC analyses were carried out using a Perkin-Elmer Autosystem apparatus equipped with flame ionization detection and fused-silica capillary columns (50 m × 0.22 mm i.d.; film thickness, 0.25 $\mu$m), BP-1 (polydimethyl siloxane), and BP-20 (poly ethylene glycol). The oven temperature was programmed from 60 to 220 at 2 °C/min and then held isothermally at 220 °C for 20 min; the injector temperature was 250 °C; the detector temperature was 250 °C; the carrier gas was helium (1 mL/min); and a split mode of 1/60 was used. GC/MS analysis was performed on a Perkin-Elmer TurboMass detector, directly coupled to a Perkin-Elmer Autosystem XL equipped with fused-silica capillary columns (60 m × 0.22 mm i.d.; film thickness, 0.25 $\mu$m), Rtx-1 (polydimethylsiloxane). The ion source temperature was 150 °C; the energy ionization was 70 eV; electron ionization mass spectra were acquired oven the mass range 35−350 Da. Other GC conditions were the same as described under GC except the split was 1/80. All NMR spectra were recorded on a Bruker AC 400 Fourier transform spectrometer operating at 100.13 MHz for $^{13}$C NMR, equipped with a 5 mm probe, in deuterated chloroform, with all shifts referred to internal tetramethylsilane. $^{13}$C NMR spectra were recorded with the following parameters: pulse width, 4 $\mu$s (flip angle 45°); acquisition time, 2.7 s for 128 K data table with a spectral width of 25,000 Hz (250 ppm); CPD mode decoupling; and digital resolution, 0.183 Hz/pt. The number of accumulated scans was 5000 for each sample (around 40 mg of the oil in 0.5 mL of $CDCl_3$).

Identification of the components was based on (i) their GC retention indices (RI) on polar and apolar columns determined by comparing the retention times of a series of *n*-alkanes with linear interpolation with those of authentic compounds or literature data (*24*); (ii) computer matching with commercial (*25*−*27*) and laboratory-made mass spectral libraries and by comparing spectra with those of our own library or data in the literature (*24*, *26*, *28*); and (iii) by $^{13}$C NMR spectroscopy, following the method developed and computerized in our laboratories, using homemade software and our spectral data library (*29*).

**Statistical Analysis.** Data were subjected to a one-way analysis of variance (ANOVA) using the GLM procedure of SAS (SAS Institute Inc., 1989) to analyze quantitative differences among the three genotypes.

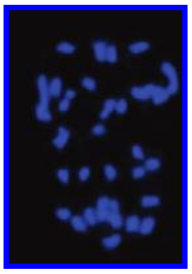

**Figure 2.** Metaphase of the tetraploid somatic hybrid WLM + EUR.

Different amounts of aromatic compounds were used to process cluster analysis with the Ward method on R software (www.R-project.org).

## RESULTS

**Genetic Characterization.** Flow cytometry analysis and chromosome counts showed that WLM + EUR is tetraploid ($2n = 4x = 36$) (**Figure 2**). Molecular markers were chosen to display polymorphism between the parents and enabled us to identify the genomic origin of the somatic hybrid. Analysis of nuclear DNA by 26 SSR markers revealed that WLM + EUR possessed alleles from both Eureka lemon and Willow leaf mandarin (**Figure 3a**). Chloroplast SSR and mitochondrial primers enabled the two parents to be clearly distinguished and revealed that WLM + EUR conserved the chloroplast and mitochondria from Willow leaf mandarin (**Figure 3b**,**c**). Molecular analysis showed that WLM + EUR was allotetraploid with cytoplasm from Willow leaf mandarin.

**Titratable Acidity.** Titratable acidity was analyzed during stage III of fruit maturation from 180 to 260 DAA (**Figure 4**). Eureka lemon and WLM + EUR had the most acidic fruits with an average of 0.80 and 0.64 mequiv $g^{-1}$ f.p., respectively. The allotetraploid was close to Eureka lemon, since Willow leaf mandarin was less acidic with an average of 0.11 mequiv $g^{-1}$f.p.

**Organic Acid Content at the Mature Fruit Stage.** Seven organic acids were detected and identified in pulps of the three genotypes. The dominant acid was citric acid, representing an average of 89.86% of total organic acids for Eureka lemon, 89.34% for WLM + EUR, and 72.27% for Willow leaf mandarin. Malic acid was the second highest with an average of 18% of total organic acids in Willow leaf and 9% in Eureka lemon and the somatic hybrid. Other acids such as oxalic, ascorbic, succinic, tartaric, and quinic acid were present in very low amounts as compared to the two main acids.

The somatic hybrid displayed high amounts of citric acid, 37 mg $g^{-1}$ f.p. on average, still close to its lemon parent, 45 mg $g^{-1}$ f.p. on average (**Figure 5a**). Willow leaf mandarin had very low amounts of citric acid, 7 mg $g^{-1}$ f.p. on average.

The amounts of malic acid decreased gradually from 180 DAA to 260 DAA in Eureka lemon and in the somatic hybrid but remained relatively constant in Willow leaf mandarin (**Figure 5b**). Eureka lemon displayed higher amounts of malic acid (4.63 mg $g^{-1}$ f.p.) than Willow leaf (2.10 mg $g^{-1}$ f.p.). WLM + EUR was closer to Eureka lemon with 3.8 mg $g^{-1}$ f.p. on average. The amounts of ascorbic acid in Willow leaf mandarin decreased gradually from 0.41 mg $g^{-1}$ f.p. and reached 0 mg $g^{-1}$ f.p. at 260 DAA. The somatic hybrid and Eureka lemon displayed the same changing pattern with a constant level of ascorbic acid, 0.44 and 0.40 mg $g^{-1}$ f.p., respectively (**Figure 5c**).

**Sugar Contents at the Mature Fruit Stage.** Three major sugars (sucrose, glucose, and fructose) were identified in pulp of the three genotypes. Willow leaf mandarin fruits had much higher sugar contents than Eureka lemon and WLM + EUR (**Figure 6**). The concentration of sucrose in fruit pulp of Willow leaf mandarin

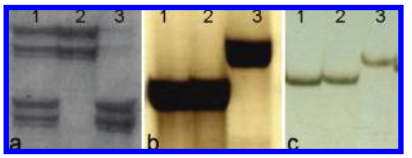


**Figure 3.** Analysis of the genetic structure of WLM + EUR: (**a**) nuclear SSR marker mCrCIR06B07, (**b**) chloroplastic SSR marker ntcp9, and (**c**) mitochondrial primer *rrn*5/*rrn*18-1. Lane 1, WLM + EUR; lane 2, Willow leaf mandarin; and lane 3, Eureka lemon.

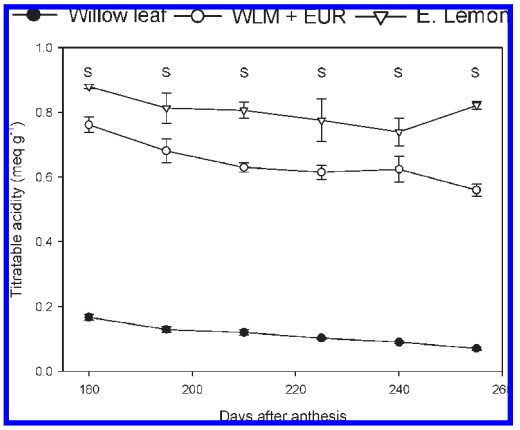


**Figure 4.** Titratable acidity in fruit pulp during the mature stage of Willow leaf mandarin (●), WLM + EUR (○), and Eureka lemon (△). Each point on the graph shows the mean and standard error of three biological replicates. The letter S indicates statistically significant differences between WLM + EUR and his parents ($P < 0.05$).

decreased with maturity from 42.43 to 28.37 mg $g^{-1}$ f.p., whereas in WLM + EUR and Eureka lemon, it remained relatively constant at 12 and 3 mg $g^{-1}$ f.p. (**Figure 6a**). The somatic hybrid showed amounts of sucrose that were intermediate between the two parents. In contrast to sucrose, Willow leaf mandarin showed an increase in glucose from 195 DAA until 260 DAA (**Figure 6b**). The somatic hybrid displayed intermediate values of glucose (7−14 mg $g^{-1}$ f.p.) as compared with those of Willow leaf mandarin (16.78−37.87 mg $g^{-1}$ f.p.) and Eureka lemon (2−4 mg $g^{-1}$ f.p.). The somatic hybrid was closer to Willow leaf mandarin for fructose contents with 9.8 and 9.2 mg $g^{-1}$ f.p., respectively, than to Eureka lemon with 4.5 mg $g^{-1}$ f.p. on average (**Figure 6c**).

**Chemical Composition of Peel Oils.** Thirty-six components were identified in peel essential oils, accounting for 98.8−99.8% of the total amount of oils (**Table 1**). Peel oils consisted almost exclusively of hydrocarbons with limonene as the major component in the three genotypes (65.0% in Willow leaf mandarin, 68.2% in WLM + EUR, and 62.6% in Eureka lemon). The other components were present in lower amounts. γ-Terpinene was present in higher amounts in Willow leaf mandarin (18.7%) and in WLM + EUR (15.2%) than in Eureka lemon (7.7%). However, β-pinene was present in much larger amounts in Eureka lemon peel oil (13.0%) than in peel oils of Willow leaf mandarin (1.7%) and WLM + EUR (4.5%). Methyl N-methylanthranilate represented 3.1% of the total amount of Willow leaf mandarin peel oil but was nearly absent in Eureka lemon (tr) and WLM + EUR (0.2%). Citral (geranial + neral) and α-terpinene were also nearly absent in Willow leaf mandarin and the somatic hybrid peel oils (traces and 0.3%) but represented, respectively, 2.4 and 1.2% of the total amount of Eureka lemon peel oil (**Table 1**). When all aromatic compounds were considered as variables, cluster analysis grouped Willow leaf mandarin and WLM + EUR together separate from Eureka lemon (**Figure 7**).

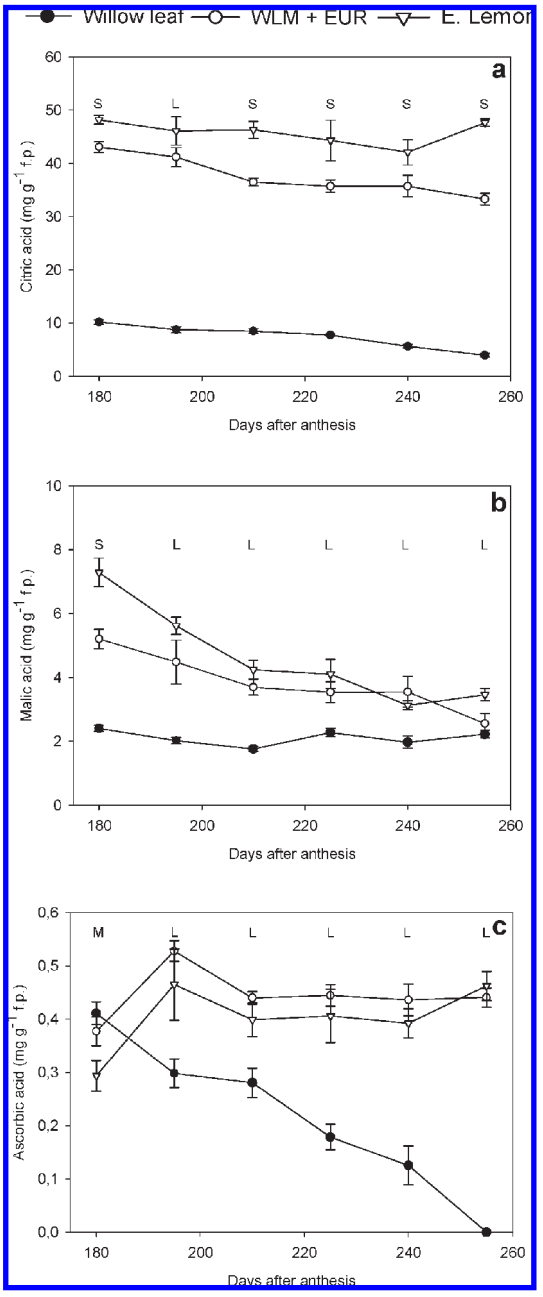


**Figure 5.** Organic acid levels in fruit pulp during the mature stage of Willow leaf mandarin (●), WLM + EUR (○), and Eureka lemon (△). Each point on the graph shows the mean and standard error of three biological replicates. Letters indicate statistical test ($P < 0.05$): S, significant differences between WLM + EUR and his parents; L, no significant difference between WLM + EUR and Eureka lemon; and M, no significant difference between WLM + EUR and Willow leaf mandarin.

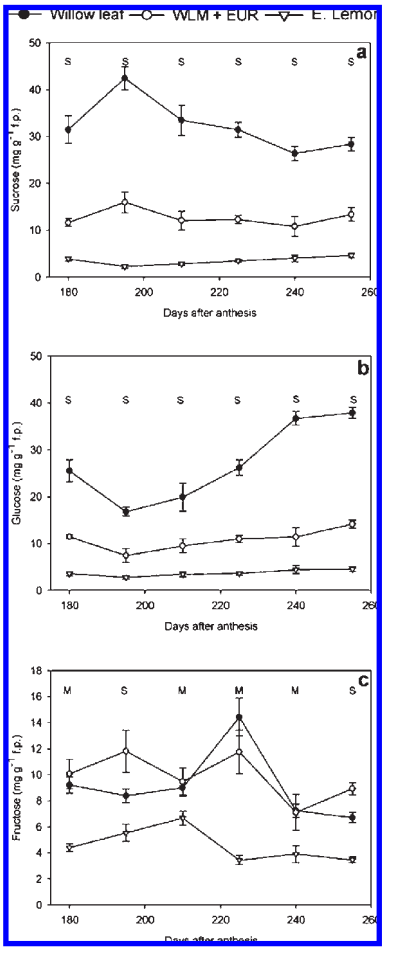


**Figure 6.** Sugar levels in fruit pulp during the mature stage of Willow leaf mandarin (●), WLM + EUR (○), and Eureka lemon (△). Each point on the graph shows the mean and standard error of three biological replicates. Letters indicate statistical test ($P < 0.05$): S, significant differences between WLM + EUR and his parents; and M, no significant difference between WLM + EUR and Willow leaf mandarin.

## DISCUSSION

Genetic analyses revealed that WLM + EUR possessed nuclear genomes from both Willow leaf mandarin and Eureka lemon plus cytoplasm from Willow leaf mandarin. The parents displayed different acid profiles. Eureka lemon had an acidic profile (0.80 mequiv $g^{-1}$ f.p.), while Willow leaf mandarin's profile was less acidic (0.11 mequiv $g^{-1}$ f.p.). In citrus juice, titratable acidity is known to be largely due to citric acid (*23*, *30*, *31*), which is the major organic acid at the mature stage. The somatic hybrid was characterized by an acidic profile (0.64 mequiv $g^{-1}$ f.p.) and also displayed the same high level of citric acid as Eureka lemon (**Figure 5a**). The citric acid cycle takes place in the matrix of the mitochondria of juice cells (*32*). The citrate is then translocated to the vacuole and accumulates to steady-state level in lemon fruit, (or declines in other citrus) and is then released to the cytosol (*31*). The mitochondria of WLM + EUR are from Willow leaf mandarin, which accumulates a lower level of organic acids than Eureka lemon. In a previous work on a lemon cybrid with mandarin mitochondria, we showed that the origin of the mitochondria had only a slight effect on the biosynthesis of organic acids (*15*). Thus, it appears that the level of organic acids in the somatic hybrid fruits is regulated by both lemon and mandarin genomes with a dominance of lemon. By contrast, WLM + EUR showed intermediate amounts of glucose and sucrose as compared to Eureka lemon and Willow leaf mandarin fruits. In fact, lemons and mandarins also displayed significant differences in their characteristic sugar storage patterns. Lemons contained little sugar combined with high amounts of organic acids (*23*), in contrast to mandarins, which contained high amounts of sugar and less organic acids. The amounts of sucrose and glucose in the somatic hybrid fruits may be coregulated by both parent genomes. However, this behavior is not observed for fructose. Indeed, there was no significant difference in the amounts of fructose between Willow leaf mandarin and WLM + EUR. This may be explained by the fact that there is little difference between the amounts of fructose of the two parents as compared to those of glucose and sucrose (**Figure 6**). It should be

**Table 1.** Chemical Composition of Peel Oils[a]

| constituents | apolar | polar | Willow leaf | WLM + EUR | Eureka lemon |
|---|---|---|---|---|---|
| α-thujene | 924 | 1025 | 0.6 | 0.6 | 0.3 |
| α-pinène | 932 | 1025 | 1.6 | 1.6 | 1.6 |
| camphene | 944 | 1066 | | tr | 0.1 |
| sabinene | 966 | 1119 | 0.2 | 0.6 | 1.7 |
| β-pinene | 972 | 1110 | 1.7 | 4.5 | 13.0 |
| octanal* | 980 | 1294 | 0.2 | tr | 0.1 |
| myrcene* | 980 | 1157 | 1.8 | 2.0 | 1.7 |
| α-phellandrene | 997 | 1162 | | tr | |
| α-terpinene | 1010 | 1176 | 0.3 | 0.3 | 1.2 |
| *p*-cymene | 1013 | 1264 | 2.1 | 3.4 | 1.1 |
| limonene* | 1024 | 1200 | 65.0 | 68.2 | 62.6 |
| β-phellandrene* | 1024 | 1209 | 0.3 | 0.3 | 0.4 |
| (E)-β-ocimene | 1038 | 1245 | | tr | 0.1 |
| γ-terpinene | 1052 | 1243 | 18.7 | 15.2 | 7.7 |
| *p*-cymenene | 1074 | 1432 | | 0.1 | |
| terpinolene | 1079 | 1277 | 0.8 | 0.7 | 0.3 |
| nonanal* | 1082 | 1388 | - | tr | 0.2 |
| linalool* | 1082 | 1550 | 0.5 | tr | 0.3 |
| *cis*-limonene-1,2 oxide[#] | 1116 | 1440 | | tr | tr |
| *trans*-limonene-1,2 oxide[#] | 1120 | 1450 | tr | tr | tr |
| terpinen-4-ol | 1161 | 1605 | 0.4 | 0.3 | 0.5 |
| α-terpineol | 1172 | 1701 | 0.4 | 0.2 | 0.5 |
| nerol | 1208 | 1792 | 0.2 | tr | 0.4 |
| neral | 1214 | 1679 | tr | tr | 1.0 |
| geraniol | 1232 | 1837 | | | 0.4 |
| geranial | 1237 | 1731 | tr | | 1.4 |
| thymol | 1266 | 2189 | 0.7 | 0.2 | |
| citronellyl acetate | 1332 | 1654 | | 0.1 | 0.1 |
| neryl acetate | 1343 | 1725 | | | 1.1 |
| geranyl acetate | 1360 | 1748 | | | 0.9 |
| Me N-methylanthranilate | 1378 | 2082 | 3.1 | 0.2 | tr |
| (*E*)-β-caryophyllene | 1424 | 1586 | | 0.1 | 0.1 |
| *trans*-α-bergamotene | 1432 | 1580 | | 0.1 | 0.4 |
| β-bisabolene | 1499 | 1724 | | 0.1 | 0.6 |
| α-sinensal | 1724 | 2323 | 0.2 | 0.2 | |
| nootkatone | 1780 | 2530 | | 0.1 | |
| total | | | 98.8 | 99.1 | 99.8 |

[a] Order of elution and percentages are given on an apolar column (BP-1), except for compounds with an asterisk. *, percentage on BP-20; tr, traces; and [#], methyl vs isopropyl.

kept in mind that WLM + EUR is an allopolyploid in which genes may display dosage dependency and could be expressed additively or nonadditively with possible overdominance, codominance, and repression phenomena (*33*).

The main characteristics of the somatic hybrid are amounts of citric acid similar to those in the lemon parent and amounts of sucrose and glucose that are intermediate between its two parents. We can conclude that the amount of citric acid is obtained directly from sugars, especially glucose, with dominance of the lemon phenotype. We can hypothesize an intermediate influence of the mandarin phenotype on the amount of glucose and sucrose. This hypothesis is supported by previous experiments with a cybrid that possessed nuclear and chloroplast genome from Eureka lemon and showed the same sugar patterns as its lemon parent (*15*). It would be interesting to determine the importance of the glycolytic pathway for the somatic hybrid and its two parents.

The somatic allotetraploid presented similar qualitative chemical composition of peel oil as its two parents. The amounts of the different components in WLM + EUR total peel oil suggest the dominance of mandarin genome, as observed by Gancel et al. (*10*) for leaf aromatic compounds of WLM + EUR. Most of the aromatic compounds in peel oil of the somatic allotetraploid were closer to Willow leaf mandarin than to Eureka lemon. Indeed, amounts of compounds such as α-terpinene, β-pinene, neral, and

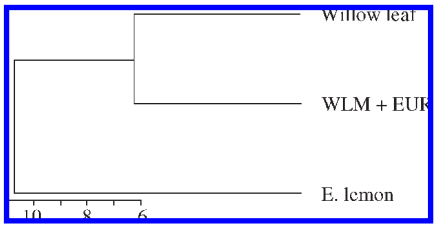


**Figure 7.** Dendrogram of cluster analysis with amounts of aromatic compounds from Willow leaf mandarin, WLM + EUR, and Eureka lemon using the Ward method and Euclidean distance.

geranial in WLM + EUR peel oil were very low and closer to mandarin than to lemon (**Table 1**), although β-pinene, neral, and geranial are characteristic of lemon peel oil (*34*, *35*). However, the synthesis of methyl N-methylanthranilate, a characteristic compound of mandarin (*36*, *37*), was reduced in WLM + EUR peel oil. Global dominance of mandarin for aromatic peel oil content was confirmed by cluster analysis with the Euclidean distance calculated from the amounts of aromatic compounds. WLM + EUR and Willow leaf mandarin were grouped in the same cluster and separate from Eureka lemon (**Figure 7**). Our results are concordant with the results of Gancel et al. (*10*) for the leaf aromatic profile of WLM + EUR. It appears that for these biosynthetic pathways, phenotypic dominance of mandarin is conserved in the different organs of the somatic hybrid.

This study demonstrated that the transmission of characters in the somatic hybrid is complex. Organic acids, sugars, and peel aroma biosynthetic pathways were shown to be coregulated by the genome of the two parents. However, in some cases, there was dominance of one of the parental characteristics, such as lemon for the biosynthesis of organic acids and mandarin for the biosynthesis of aroma. We are currently examining gene expression at fruit maturity in WLM + EUR and its parents with a genome-wide 20K cDNA microarray, developed under the Citrus Functional Genomic Project (CFGP; http://bioinfo.ibmcp.upv.es/genomics/cfgpDB/) (*38*). This will help in the interpretation of the behavior of the somatic hybrid, which is used in the triploid citrus breeding program.

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