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Carotenoid compositions are correlated with the genetic diversity organization of *Citrus* genus



Citrus fruits are complex sources of carotenoids with more than 100 kinds of pigments reported in this genus. To understand the origin of the diversity of carotenoid compositions of *Citrus* fruits, 25 genotypes that belong to the 8 cultivated *Citrus* species were analyzed.

Materials and Methods

Juice extracts of mature fruits were analyzed by HPLC: carotenoids were separated along a C₃₀ column; the mobile phases were H₂O as eluent A, methanol as eluent B and MTBE as eluent C; absorbance was followed at 290, 350, 400, 450, and 470 nm using a photodiode array detector; carotenoids were identified using retention times, absorption spectra, and co-injection with authentic standards; quantification of carotenoids was achieved using calibration curves.

For each genotype at least 3 extractions-saponifications-HPLC analyses were performed. The statistical comparison of data was performed by ANOVA using the GLM procedure of SAS (SAS Institute Inc., 1989). @Darwin 4.0 software (CIRAD, Montpellier, France) was used for dissimilarity analysis and tree construction.

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Diversity of carotenoid profiles in pulp of *Citrus* fruits

25 carotenoids were detected and their chromatographic and spectral characteristics were reported in table 1.

Mandarins, oranges and clementine were the richest species in carotenoid contents (total contents ≥ 22.481 mg.L⁻¹. Mandarins and clementine accumulated high amount of β -cryptoxanthin whereas oranges accumulated high quantity of *cis*-violaxanthin the β -cryptoxanthin/*cis*-violaxanthin ratio > 1.5 for mandarin and clementine varieties and < 0.3 for oranges varieties.

The comparison of data by ANOVA showed that the 25 genotypes were significantly different for all carotenoid pigments ($P \leq 0.01$ %).

The main carotenoid in Satsuma mandarin was the β -cryptoxanthin with 17.507 ± 0.897 mg.L⁻¹ (peak 17).

Diamante citron accumulated low amounts of carotenoids (total contents = 0.179 ± 0.026 mg.L⁻¹).

Star Ruby grapefruit was the richest variety in lycopene with 10.072 ± 0.652 mg.L⁻¹ (peak 25) (see figure 1 and 2).

Table 1. Chromatographic and spectral characteristics of 25 carotenoids found in juices

RT n° (min) \pm SD ^a	Tentative identification	Peak I	λ_{max} (nm) (observed)		
			Peak II	Peak III	% III/II
1 15.76 \pm 0.09	<i>cis</i> -apocarotenoid	408	430	458	
2 16.33 \pm 0.04	<i>cis</i> -neoxanthin	416	439	465	76
3 17.35 \pm 0.04	neochrome	399	422	448	75
4 17.96 \pm 0.05		400	422	448	
5 18.71 \pm 0.07	<i>cis</i> -violaxanthin	412	436	464	81
6 19.59 \pm 0.04	luteoxanthin	396	418	443	75
7 19.92 \pm 0.04	mutaxanthin	404	426	448	31
8 20.57 \pm 0.09	Lutein ^b	422	444	472	48
9 20.78 \pm 0.06		407	428	451	
10 21.89 \pm 0.04	zeaxanthin ^b	426	450	476	17
11 22.55 \pm 0.04	<i>cis</i> -antheraxanthin	417	440	468	47
12 24.25 \pm 0.08		422	446	473	44
13 25.40 \pm 0.09	<i>cis</i> - β -cryptoxanthin	413	444	470	
14 25.89		406	428	452	40
15 26.10 \pm 0.04	zeinoxanthin	422	445	473	47
16 26.96 \pm 0.06	phytoene	276	296	298	
17 28.16 \pm 0.08	β -cryptoxanthin ^b	427	450	477	20
18 28.59 \pm 0.03	phytofluene	331	348	368	6.8
19 32.25 \pm 0.09	ζ -carotene	379	400	424	90
20 33.35 \pm 0.09	α -carotene	422	444	470	34
21 35.51 \pm 0.09	β -carotene ^b		452	477	12
22 36.84 \pm 0.09	<i>cis</i> - β -carotene	425	449	475	
23 38.82 \pm 0.04		433	456	488	
24 45.95	<i>cis</i> -lycopene	441	466	490	
25 55.57 \pm 0.06	lycopene ^b	446	472	502	71

ART, retention time. \pm SD, standard deviation. ^bidentified using authentic standards.

Organization of the carotenoid diversity

The genotypes were classified in 3 clusters: the mandarin cluster, the citron cluster and the pummelo cluster on the basis of the presence/absence of each compound (figure 3).

These 3 species are the 3 basic taxa of cultivated *Citrus*. 2 carotenoids strongly determined this classification: the β -cryptoxanthin and the *cis*-violaxanthin. Quantitative data revealed more information at the intraspecific level (figure 4).

For example, in the mandarin group, mandarins and clementine were separated from oranges. Sour oranges, Rangpur lime, Volkamer lemon and Huang pi Chen orange were closer to the citron cluster; these genotypes having low juice contents in carotenoid.

Conclusion

The carotenoid diversity is highly influenced by genetic factors when other sources of variation such as growing conditions, geographical origin, and fruit maturity are minimized.

As for other phenotypical traits, the general evolution of cultivated *Citrus* has been the main factor of the organization of carotenoid diversity.

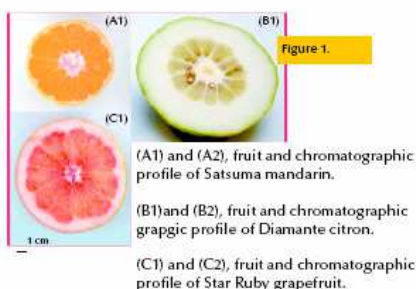


Figure 1.

(A1) and (A2), fruit and chromatographic profile of Satsuma mandarin.

(B1) and (B2), fruit and chromatographic profile of Diamante citron.

(C1) and (C2), fruit and chromatographic profile of Star Ruby grapefruit.

Figure 3. Structuration of the 25 genotypes based on the presence/absence of each carotenoid.

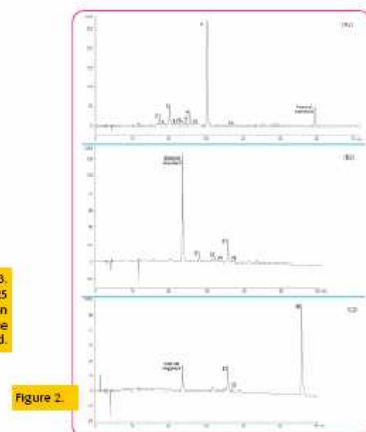
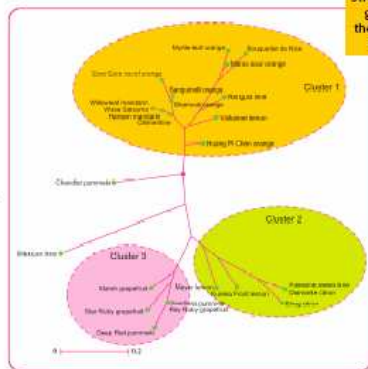


Figure 2.

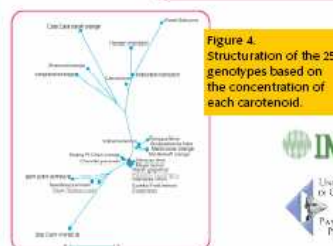


Figure 4. Structuration of the 25 genotypes based on the concentration of each carotenoid.

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