Carotenoid composition are correlated with the genetic diversity organization of Citrus genus

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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.

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Materials and Methods

Juice extracts of mature fruits were analyzed by **HPLC** : carotenoids were separated along a C_{30} column; the mobile phases were H_2O 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 Institue Inc., 1989). ©Darwin 4.0 software (CIRAD, Montpellier, France) was used for dissimilarity analysis and tree construction.

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

n°	RT (min)±SD[a]	Tentative identification	λ_{max}(nm) observed				
				Peak I	Peak II	Peak III	% III/II
1	15.76±0.09	*cis*-apocarotenoid	cis328	408	430	458	
2	16.33±0.04	*cis*-neoxanthin	cis328	416	439	468	76
3	17.35±0.04	neochrome		399	422	448	75
4	17.96±0.05			400	422	448	
5	18.71±0.07	*cis*-violaxanthin	cis328	412	436	464	81
6	19.59±0.04	luteoxanthin		396	418	443	75
7	19.92±0.04	mutatoxanthin		404	426	448	31
8	20.57±0.09	Lutein[b]		422	444	472	48
9	20.78±0.06			407	428	451	
10	21.89±0.04	zeaxanthin[b]		426	450	476	17
11	22.55±0.04	*cis*-antheraxanthin	cis330	417	440	468	47
12	24.25±0.08			422	446	473	44
13	25.40±0.09	*cis*-β-cryptoxanthin	cis338	420	444	470	
14	25.89		406	428	452	40	
15	26.10±0.04	zeinoxanthin		422	445	473	47
16	26.96±0.06	phytoene		276	286	298	
17	28.16±0.08	β-cryptoxanthin[b]		427	450	477	20
18	28.59±0.03	phytofluene		331	348	368	68
19	32.25±0.09	ζ-carotene		379	400	424	90
20	33.35±0.09	α-carotene		422	444	470	34
21	35.51±0.09	β-carotene[b]			452	477	12
22	36.84±0.09	*cis* -β-carotene	cis342	425	449	475	
23	38.82±0.04			433	456	488	
24	45.95	*cis* -lycopene	cis355	441	466	490	
25	55.57±0.06	lycopene[b]		446	472	502	71

aRT, retention time. ±SD, standard deviation. bidentified using authentic standards.

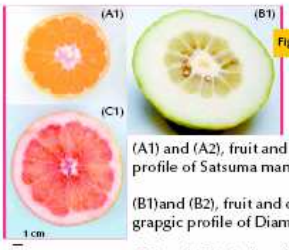

Figure 1.

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

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

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

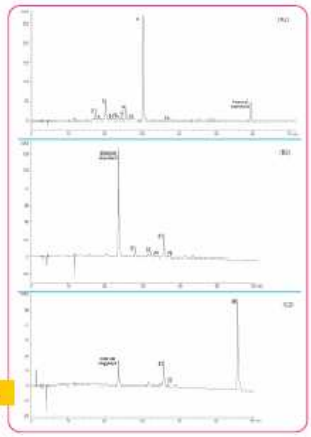

Figure 2.

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 exemple, 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.

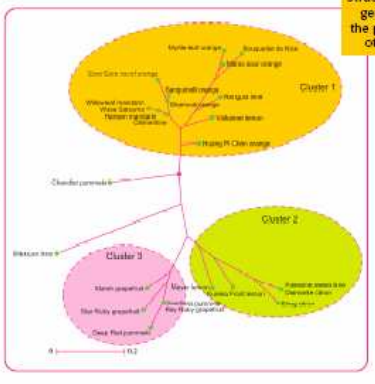

Figure 3. Structuration of the 25 genotypes based on the presence/absence of each carotenoid.

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

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