Do chloroplastic PCR markers fit with Aurantioideae evolution ?

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Genetic information on plant chloroplastic DNA presents a great interest because their uniparental origin and theoretical low evolution rate make it particularly adapted for phylogenic studies at interspecific and intergeneric levels. Moreover, in citrus the making of numerous somatic hybrids required tools to characterize their cytoplasmic genome, and the development of new PCR markers appeared very suitable. The application of Cleaved Amplified Polymorphic Sequences (CAPS) method with universal primers has been recently demonstrated to be efficient at the interspecific level but it displays weak diversity at the infraspecific one (Lotfy et *al.*, 2003a). Genetic markers based upon simple sequence repeats (SSR) in chloroplastic genomes (CpSSR) have been shown to be useful markers in several plant species such as rice (Ishii and Couch, 2000) and Solanaceous (Bryan *et al.*, 1999; Weising and Gardner, 1999). These CpSSR are characterized by mononucleotide repeats. The transportability to citrus of primers defined from rice and tobacco has been recently proven (Lotfy *et al.*, 2003b). In the present work, we compare the traditional botanical classifications of Aurantioideae subfamily (Figure 1) with the ones obtained with these two kinds of chloroplastic markers PCR.

Organization of chloroplastic CAPS diversity among Aurantioideae subfamily

Four couples of chloroplastic universal primers (Demesure *et al.*, 1995) revised for citrus by Lotfy et al. (2003a) have been combined with two to four restriction enzymes [psaA/trnS3 (HindIII, EcoRI, Hin6I), trnT3/trnD2 (DraI, Bsp143I), trnC2/trnD1 (HaeIII, EcoRI), trnM/rbcL (MvaI, Eco130I), trnH/trnK3 (Mva1, AvaII, HaeIII, DraI)] and analyzed in agarose gels (Figure 2). NJ tree was established from Sokal and Michener's distances based on the profiles observed for theses 13 primers/enzymes combinations (Figure 3).

Organization of CpSSR diversity among Aurantioideae subfamily

Eight couples of primers from tobacco [ccmp1, ccmp2, ccmp4, ccmp5, ccmp6, NTCP7, NTCP9, NTCP28 (Bryan *et al.*, 1999; Weising and Gardner, 1999) have been used for a diversity analysis among 50 species of Aurantioideae sub-family (germplasm from SRA and IVIA collections). 5'-end γP^{33}]-radiolabelled primers have been used for PCR, and migrations were done in sequencing gels (Figure 4). We observed that NTCP7 and ccmp2 primers amplify a same cpSSR locus, so NJ tree was established from Sokal and Michener's distances based only on seven locus (Figure 5).

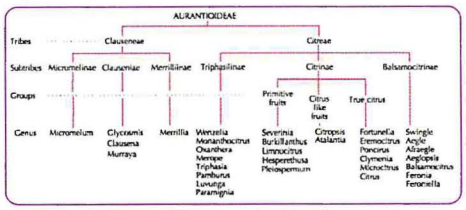


Figure 1. Botanical classification of Aurantioideae subfamily.

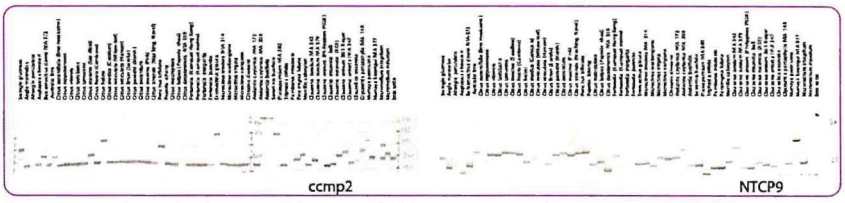


Figure 4. Autoradiography of two CpSSR locus amplified from 50 Aurantioideae species.

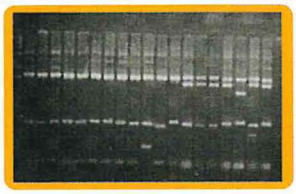
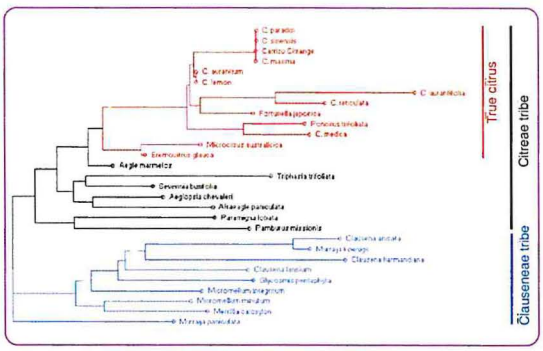


Figure 2. Agarose electrophoresis of citrus DNA amplified with trnH/trnK3 primers and restricted with Mva1 enzyme.

Figure 3. NJ tree analysis established from thirteen chloroplastic CAPS profiles.



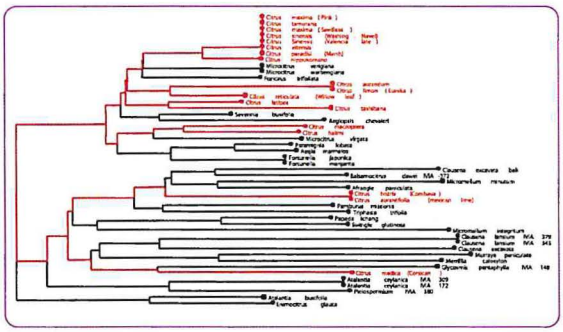


Figure 5. NJ tree analysis established from seven CpSSR locus.

Discussion

The two kinds of markers display similar genetic organizations for the cultivated species of the *Citrus* genus. No differentiation was possible between *C. limon* and *C. aurantium* and no more between *C. maxima*, *C. paradisi* and *C. sinensis*. The differentiation between *C. medica*, *C. reticulata* and *C. maxima* is in agreement with the one observed for the nuclear genome. With respect of the generally admitted status of these species as ancestors of the cultivated forms, it appears that *C. maxima* has been implied as the female parent in the genesis of *C. sinensis* and *C. paradisi*. The high differentiation of *C. aurantifolia* with all other cultivated *Citrus* demonstrated that an additional species has been implied in the lime evolution. *C. hystrix* displays the same profile than *C. aurantifolia* for CpSSR markers as in the Nicolosi *et al.* (2000) chloroplastic CAPS analysis. These authors suggested that a third species with the same CAPS chloroplastic profiles, *C. micrantha*, was a progenitor of limes. At the intergeneric level, the structuration of CAPS diversity is very coherent with the botanical classification with a cluster grouping the true citrus genus and some clear differentiation between the Citreae tribe and Clauseneae tribe. At the opposite, CpSSR clustering is not in agreement with traditional taxonomy. *Citrus* species appear dispersed in the different clusters of the NJ tree. The evolution mode of this kind of markers associates microsatellite evolution but also insertion or deletion. The first one having a much higher evolution rate than the other ones, it is not suitable to infer genetic distances directly from fragment size variations. CpSSR fragments should be sequenced to allow a better phylogenetic interpretation. It is also possible that CpSSR evolution is too rapid to use to this kind of markers for broad intergeneric studies. CAPS analysis should be preferred for such applications. CpSSR should be recommended to differentiate chloroplastic genomes of related species and as routine tool for the chloroplastic characterization of somatic hybrids.

References

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