Multilocus SNPs analysis allows phylogenetic assignation of DNA fragments to decipher the interspecific mosaic genome structure of cultivated citrus
Franck Curk, Gema Ancillo, Andres Garcia-Lor, François Luro, Luis Navarro, Patrick Ollitrault

To cite this version:
Franck Curk, Gema Ancillo, Andres Garcia-Lor, François Luro, Luis Navarro, et al.. Multilocus SNPs analysis allows phylogenetic assignation of DNA fragments to decipher the interspecific mosaic genome structure of cultivated citrus. Plante genome evolution, Sep 2011, Amsterdam, Netherlands. 2011. hal-02746454
Multilocus SNPs Analysis Allows Phylogenetic Assignation of DNA Fragments to Decipher the Interspecific Mosaic Genome Structure of Cultivated Citrus

Introduction

All current studies seem to support the theory that four basic taxa (C. medica, C. maxima, C. reticulata and C. microcarpa) have generated all cultivated Citrus species (Figure 1). It is supposed that the genomes of most of the modern Citrus cultivars, vegetatively propagated, are interspecific mosaic of large DNA fragments issued from a limited number of inter-specific meioses. In the present work we analyzed how multilocus study of closely linked SNPs allows a phylogenetic assignation of DNA fragments of the main cultivated species.

Genotype Assignation (Sanger)

The average SNP rate was about 20 SNPs/kb. Citrus reticulata was the most polymorph basic taxa with an average of 4.2 SNPs/kb.

For each amplified fragment, the polymorphism was enough for multilocus differentiation of the basic species and assignation of a phylogenetic origin for the secondary species. A preliminary reconstitution of phylogenetic structure of the chromosome 3 is proposed for clementine, sweet orange, sour-orange, grapefruit, lime and lemon (Figure 4). However only haplotype analysis would allow definitive assignation. A Parallel pyrosequencing (454 Roche) for multilocus haplotyping of heterozygous genotypes has been done.

Consensus haplotype sequences were successfully obtained from 454 sequencing with genotype sequence in total agreement with Sanger control.

Each haplotype sequence of Clementine was univocally assigned to one of the haplotype clusters of the basic taxa. As an example, we can see with the Figure 5 (NJ tree of the locus 5.5) that both haplotypes belong to the Mandarin group.

Material and Methods

Genomic fragments of 25 genes dispersed in the different chromosomes covering more than 12,5 Kb were amplified by PCR and sequenced (Sanger) for 24 accessions representative of 10 species of Citrus.

Moreover we checked the potential of parallel pyrosequencing (454 Roche) for direct multilocus haplotyping of heterozygous genotypes. Amplified fragments from 7 genes in 8 genotypes of Citrus were obtained by using an original new method based on universal primers (Figure 2).

C. clementina (clementine) was used as model for secondary species.

Haplotype Assignation (454)

The Figure 6 (NJ tree of the locus 9.2) that one haplotype belongs to the Mandarin group and the other one to the Pumelo group.

Conclusion

Phylogenetic origin of specific DNA fragments can be assigned from multilocus analysis of closely linked SNPs. Multilocus haplotyping by parallel sequencing of individual DNA molecule will be a very powerful tool to decipher the interspecific mosaic genome structure of cultivated Citrus.