Workshop on Plant Genome Dynamics and Evolution

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A phylogenomic study based on Genotyping By Sequencing unravels the interspecific mosaic structures of the cultivated Citrus genomes.

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Reticulate evolution, including hybrid speciation, introgression and lateral gene transfer is frequent in plant species. When coupled with vegetative propagation, it results in mosaic genomes of large genomic fragments, from different species or sub species. These complex inter(sub)-specific genomic structures can support a major part of the phenotypic diversity organization. Cultivated Citrus are a good example of such genepool issued from reticulate evolution with limited number of further interspecific recombination. It is generally agreed that four ancestral taxa (Citrus maxima: pummelos, Citrus reticulata: mandarins, Citrus medica: citrons and Citrus micrantha: papedas) are the ancestors of all the cultivated citrus species. These four species, have undergone allopatric evolution. This has led to a strong genetic differentiation which is also found for many phenotypic characters. The so-called secondary species (sweet and sour oranges, grapefruits, lemons and limes) are the result of a reticulate evolution between these four ancestral taxa. Facultative apomixis (nucellar polyembrionny) then limited the number of interspecific meiosis cycle.

The application of NGS on reduced genome representation with methods such as GBS (Genotyping By Sequencing) coupled with the availability of a clementine reference sequence open the way for pangenomic studies of large populations. The objective of this work was to validate a GBS approach on citrus in order to identify a pangenomic panel of diagnostic markers (DSNPs) of each ancestral taxa and to decipher the phylogenomic structures of 56 citrus varieties representative of the ancestral taxa and secondary species. The DSNP panel was also used to analyse the phylogenomic structures of diploid and triploid recombining populations of the Cirad-Inra breeding programs.

GBS library were prepared with Apeki and a selective PCR to improve the depth of the analysis. 56 accessions were pooled and sequenced in one line of Illumina HISEQ-2000 (single reads). Diversity structure analysis showed that the varieties are distributed among the four ancestral taxa in perfect consistency with the previous studies carried out with SSRs, Indels and SNPs markers. The GBS approach is thus validated. 14926 DSNPs were identified. These diagnostic markers allowed to infer efficiently the majority of the phylogenomic karyotypes of the 56 Citrus accessions and revealed the interspecific recombination point in the diploid hybrids of breeding populations. For polyploid germplasm and hybrids, needing the evaluation of allelic doses, we were not able to infer these doses from the relative reads number at individual locus level. The potential of analysis at genome fragment level, covering numerous DSNPs, is under study.

This work demonstrate the potential of GBS for deciphering the phylogenomic structure of the modern citrus varieties and recent hybrids of breeding populations. It bring new insights on the origins of citrus fruit and open the way for genetic associations studies and QTLs analysis based on phylogenomics and further to genomic selection.

**Key words:** Citrus, evolution, interspecific structure, GBS, SNPs, phylogenomic inference