

of *Citrus* are believed to be of interspecific origin. By studying SNP and InDel polymorphisms of 27 nuclear genes on 45 genotypes of *Citrus* and related taxa, the average molecular differentiation between species was estimated, and the phylogenetic relationship between “true citrus fruit trees” was clarified. A total of 16238 bp of DNA was sequenced for each genotype, and 1097 SNPs and 50 InDels were identified. Nuclear phylogenetic analysis revealed that *Citrus reticulata* and *Fortunella* form a clade clearly differentiated from the other two basic taxa of cultivated citrus (*Citrus maxima*, *Citrus medica*). A few genes displayed positive selection patterns within or between species, but most of them displayed neutral patterns. The phylogenetic inheritance patterns of the analysed genes were inferred for commercial *Citrus* species. The SNPs and InDels identified are potentially very useful for the analysis of interspecific genetic structures. The nuclear phylogeny of *Citrus* and its sexually compatible relatives was consistent with their geographic origin. The positive selection observed for a few genes will orient further work to analyze the molecular basis of the variability of the associated traits. This study presents new insights into the origin of *Citrus sinensis*.

## S01P09

### New insights on limes and lemons origin from targeted nuclear gene sequencing and cytoplasmic markers genotyping

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It is believed that *Citrus medica*, *Citrus maxima*, *Citrus reticulata* and *Citrus micrantha* have generated all cultivated *Citrus* species. Depending on the classification, lemons and limes are classified either into two species, *Citrus limon* and *Citrus aurantifolia* (Swingle and Reece) or into more than 30 (Tanaka). In order to study the molecular phylogeny of this *Citrus* group, we analyzed 20 targeted sequenced nuclear genes and used 3 mitochondrial and 3 chloroplastic markers for 21 lemons and limes compared with representatives of the 4 basic taxa. We observed 3 main groups, each one derived from direct interspecific hybridizations: (1) the Mexican lime group (*C. aurantifolia*), including *Citrus macrophylla*, arising from hybridization between papaya (*C. micrantha*) and citron (*C. medica*); (2) the yellow lemon group (*C. limon*) that are hybrids between sour orange (*Citrus aurantium*, which is believed to be a hybrid between *C. maxima* and *C. reticulata*) and citron; and (3) a rootstock lemon/lime group (Rough lemon and Rangpur lime) that are hybrids between the acid small mandarin group and citron. We also identified different probable backcrosses and genotypes with more complex origins. None of the analyzed limes and lemons shared the *C. medica* cytoplasm, while this taxon is the common nuclear contributor of all limes and lemons. Limes and lemons appear to be a very complex citrus varietal group with the contribution of the 4 basic taxa. Neither the Swingle and Reece nor the Tanaka classifications fit with the genetic evidence.

## S01P10

### Diversity of citron (*Citrus medica*) and phylogenetic analysis of related citron hybrids using molecular markers and essential oil compositions

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It is commonly accepted that citron (*Citrus medica*) was the first citrus fruit to reach the Mediterranean area about 3 centuries B.C. The fragrance due to the essential oils is probably the main characteristic that has contributed to the reputation of this fruit in all Mediterranean countries for multiple uses including cosmetics, food, medicinal, ornamental and also as religious symbol. The Citron group is supposed genetically low diversified compared to mandarins and pummelos. To assess the diversity of the citron group we analyzed the molecular polymorphism of nuclear and cytoplasmic genetic markers and the variation in composition of essential oils from leaves and peels of 24 varieties of citron or apparent hybrids of citron.

Our results demonstrate that the citron group has a relatively high allelic diversity, probably due to multiple introductions of several varieties. Self-fertilization was the mode of reproduction, which most frequently led to the development of commercial varieties with specific aromas and flavors such as the cultivar ‘Corsican’, a putative ‘Common Poncire’ self-crossed hybrid. However, some varieties appeared to be interspecific hybrids related to citron but only by male parent. This varietal diversity was probably favored by spreading seeds to extend the cultivation of citron in Mediterranean countries. The chemical diversity does not always correspond to genetic diversity but nevertheless reveals specific profiles for some genotypes.

## S01P11

### Analysis of genetic diversity in Tunisian citrus rootstocks

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Breeding and selection of new citrus rootstocks are nowadays of the utmost importance in the Mediterranean Basin because the citrus industry faces increasing biotic and abiotic constraints. In Tunisia, citrus contributes significantly to the national economy, and its extension is favored by natural conditions and economic considerations. Sour orange, the most widespread traditional rootstock of the Mediterranean area, is also the main one in Tunisia. In addition to sour orange, other citrus rootstocks well adapted to local environmental conditions are traditionally used and should be important genetic resources for breeding. Prior to initiation of any breeding program, the exploration of Tunisian citrus rootstock diversity was a priority. Two hundred and one local accessions belonging to four facultative apomictic species (*Citrus aurantium*, sour orange; *Citrus sinensis*, sweet orange; *Citrus limon*, lemon; and *Citrus aurantifolia*, lime) were collected and genotyped using 20 nuclear SSR markers and four InDel mitochondrial markers. Sixteen distinct Multi-locus genotypes (MLGs) were identified and compared to references from French and Spanish collections. The differentiation of the four varietal groups was well-marked. Each group displayed a relatively high allelic diversity, primarily due to very high heterozygosity. The Tunisian citrus rootstock genetic diversity is predominantly due to high heterozygosity and differentiation between the four varietal groups. The phenotypic diversity within the varietal groups has resulted from multiple introductions, somatic mutations and rare sexual recombination events. This diversity study enabled the identification of a core sample of accessions for further physiological and agronomic evaluations. These core accessions will be integrated into citrus rootstock breeding programs for the Mediterranean Basin.

## S01P12

### Characteristics of ‘Pompia’ a natural citrus hybrid cultivated in Sardinia

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The ‘Pompia’ is a probable citrus natural hybrid sporadically growing in the “Baronia” area (East Sardinia, Italy). The origin and taxonomic classification of this *Citrus* taxon is unknown. The fruit shows a yellow-amber flavedo at maturity and large size (weight from 200 to 700 g), irregular shape, light-yellow pulp, and low juice content. The traditional use as food is limited to the thick albedo after removal of the flavedo tissue and of the acid, non-edible pulp, and candying. This research had as its objective a morphological study of nine accessions selected from four orchards of ‘Pompia’ in order to sample the observed variability of trees, shoots, leaves, and fruits. The results of morphological determinations showed a high similarity of ‘Pompia’ samples to lemon and citron species for thorns, fruit size and colour. The presence of small petiole winglets was observed only on one accession. AFLP, RAPD, and SCAR analysis allowed the comparison of ‘Pompia’ with seven *Citrus* species: citron, lemon, pummelo, grapefruit, bitter orange, and mandarin. The analysis with molecular markers demonstrated a close genetic relationship among ‘Pompia’, lemon, and citron. Moreover, the elaboration of the phenotypic and molecular analysis allowed evidencing a high level of similarity of the nine ‘Pompia’ accessions. Seven of them probably genetically correspond to the same clone.