

S01P06**Diversity analysis of citrus fruit pulp acidity and sweetness: toward to understand the genetic control of the fruit quality parameters**Luro F., Gatto J., [Costantino G.](#), and Pailly O.Unité de Recherche 03 Génétique et Ecophysiologie de la Qualité des Agrumes (GEQA), Station de Recherche INRA, France. costantino@corse.inra.fr

Acidity level is the main criteria of quality and physiological maturity evaluation for citrus fruit, especially for mandarins and oranges. The acidity level varies during fruit development but also among species and varieties, and also depends upon rootstock and environmental factors such as temperature and rainfall. Acid metabolism is linked to sugar metabolism. To better control fruit maturity and harvest time, it is necessary to understand the genetic control and the parameters influencing the variation of acidity and sweetness. *Citrus* diversity was investigated by selecting 87 varieties belonging to the 8 major *Citrus* species grown under the same environmental and cultivation conditions. The juice acidity and sweetness were analysed by assessing pH Na-neutralization and by refraction index, respectively, and their components by HPLC. The sequence polymorphisms of 9 candidate genes encoding for key enzymes of sugars and organic acids metabolic pathways were investigated by SSCP (Single Strand Conformation Polymorphism). Whatever the biochemical or molecular analysis, the observed structure of the *Citrus* diversity was organized around the ancestral species (mandarin, pummelo and citron). As expected, the secondary species were closely related to their putative species progenitors. The biochemical diversity was strongly correlated to molecular SSCP diversity without having any certainty on the cause and effect relationship.

S01P07**Screening a core collection of citrus genetic resources for resistance to *Fusarium solani***[Krueger R.R.](#)¹, and Bender G.S.²¹USDA-ARS National Clonal Germplasm Repository for Citrus & Dates (USDA-ARS-NCGRCD), USA; and ²University of California Cooperative Extension, San Diego County (UCCE-SD), USA. robert.krueger@ars.usda.gov

A causal agent for Dry Root Rot (DRR) of citrus has not been definitively identified, but the organism most consistently associated with DRR is *Fusarium solani*. To efficiently screen a citrus germplasm collection for resistance to *F. solani*, a core subset of the collection was evaluated. Seedlings of forty five accessions were wounded by girdling. Treatment consisted of inoculation with *F. solani*, with uninoculated controls. Three parameters were evaluated: recovery from girdling (0 - 3); amount of DRR (0 - 4); and percentage of healthy feeder roots. Thirty three accessions showed no significant difference in recovery from girdling between inoculated and uninoculated seedlings and 11 of these accessions showed good recovery from girdling (> 2.7). Twenty four accessions showed no significant difference in DRR between inoculated and uninoculated seedlings, and 12 of these accessions showed low development of DRR (< 1). Twenty four accessions showed no significant difference in % healthy feeder roots between inoculated and uninoculated seedlings and 8 of these showed a high percentage (> 80%) of healthy feeder roots. Five accessions were superior in all three parameters: 'Fremont' mandarin (PI 539507), 'Lamas' lemon (PI 539226), 'Mato Buntan' pummelo (PI 529398), 'Olivelands' sour orange (PI 539164), and 'South Coast Field Station' citron (PI 539435). These accessions would probably represent the best candidates for further study of *F. solani* resistance.

S01P08**Nuclear phylogeny of *Citrus* and four related genera**[Garcia-Lor A.](#)¹, [Curk F.](#)², [Snoussi H.](#)³, [Morillon R.](#)⁴, [Ancillo G.](#)¹, [Luro F.](#)², [Navarro L.](#)¹, and [Ollitrault P.](#)⁴¹Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal Y Biotecnología, Spain; ²INRA, UR1103 Génétique et Ecophysiologie de la Qualité des Agrumes (INRA UR GEQA), DGAP, France; ³Institut National de la Recherche Agronomique de Tunisie (INRAT), Laboratoire d'Horticulture, Tunisie; and ⁴Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. angarcia@ivia.es

Despite considerable differences in morphology, the genera representing "true citrus fruit trees" are sexually compatible, but their phylogenetic relationships remain unclear. Most of the important commercial species

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 papada (*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.