

S02P21**Haploid and polyploid hybrids obtained from cross of diploid citrus**

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Ploidy breeding is an effective and economical method in citrus to create seedless cultivars. Haploid, triploid and tetraploid hybrids were obtained and evaluated in our cross-breeding program. 'Tangor 15-1', a selection of local natural hybrid of *Citrus reticulata* × *Citrus sinensis*, was used as a female parent to cross with 'Red Tangerine' (*C. reticulata*) in 2000. Both parents are diploid. The obtained seeds were sowed in 2001 and seedlings began to flower and fruit successively since 2005. Traits of the seedlings were evaluated. During the evaluation process four seedlings were suspected to be variant for ploidy level. Further identification was performed by chromosome count and flow cytometry. Identification confirmed that one seedling was haploid, which was weak in growth with very small and long-shaped leaf; one was tetraploid, which was strong in growth with thick and dark-green leaf; the other two were triploid, which were strong in growth with seedless fruit. Bud sticks of these plants were taken for propagating conservation in the breeding nursery, and the biological/botanical characteristics of these haploid and polyploid plants were recorded and their fruit quality were analyzed. The tetraploid hybrid has been used as a new male parent in our subsequent breeding program.

S02P22**Chromosome redundancy and phenotypic variation in autotetraploid trifoliolate orange seedlings**

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The study was conducted to evaluate the phenotypic variation in autotetraploid seedlings occurred spontaneously from diploid trifoliolate orange (*Poncirus trifoliata*). Tetraploid nucellar seedlings were selected by flow cytometry and then ploidy level without aneuploid was confirmed by chromosome analysis. These young tetraploid seedlings were transplanted in open field and phenotypic variation such as tree vigor, growth habit, and photosynthesis activity was investigated. Tree vigor such as tree height, trunk diameter, internode length, and thorn length varied at juvenile stage. Specially, distinctively morphological difference was observed in growth habit including branch number and branching angle. Also, there was variation in photosynthesis activity such as leaf size, stomata density, photosynthesis rate, and chlorophyll index (SPAD value). These phenotypic variations had no correlation among phenotypic traits. The effect of chromosome redundancy on phenotypic variation was analyzed by karyotyping chromosomes with chromomycin A3 (CMA) staining and analyzing DNA methylation level. The results indicated that phenotypic variations of autotetraploid seedlings might be related to epigenetic effect.

S02P23**New cybrids resulting from asexual pathway: a promise of the cybridization for creating new rootstocks and varieties**

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Different breeding strategies have been developed by CIRAD to overcome agronomic and economical issues of the citrus industry in intertropical, subtropical and Mediterranean conditions. The present work is focused on the selection of new cybrids through different approaches of protoplast fusion. Two main objectives were targeted: (i) the manipulation of the nucleocytoplasmic male sterility in the framework of our seedless mandarin breeding project and (ii) the development of 'Rangpur' lime and 'Rough' lemon lines tolerant of *Alternaria*. 22 diploid cybrids have been obtained by targeted cybridization or as by-products of symmetric somatic hybridization, from interspecific and intergeneric combinations. For targeted cybridization, 4 genotypes 'Eureka' lemon, 'Volkamer' lemon, 'Rough' lemon and 'Boukhobza' orange, were combined via the cytoplasts method with 'Star

Ruby' grapefruit callus-derived cytoplasts. These combinations regenerated some diploid and tetraploid cybrids. The nuclear origin was confirmed by SSR markers to have arisen from the leaf-derived parent, while universal chloroplastic and InDel PCR markers exhibited respectively random chloroplast segregations but systematic inheritance of grapefruit mitochondria. The analysis of nucleocytoplasmic interaction should open in the near future a new pathway for rootstock improvement and sterility management for new seedless cultivar breeding.

S02P24

Several seedless citrus hybrids selected from tangor x 'Ponkan' cross

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Seedlessness is important for the consumption and marketing of fresh citrus fruit. 'Ponkan' (*Citrus reticulata*) is easy to peel and has excellent inner quality, but many 'Ponkan' cultivars in China are seedy (13~14 seeds per fruit). The goal of this breeding program is to breed new cultivars with the advantages of 'Ponkan' and less seeds. In 2000, 'Ponkan' was used as male parent to cross with 'tangor 15-1', a selection of local natural hybrid (*C. reticulata* × *C. sinensis*). 'Tangor 15-1' produces big fruit without seeds, deep orange-red rind, reddish albedo without cracks, and 'Ponkan' produces fruit with light orange rind, white albedo with cracks. The obtained seeds were sowed in 2001 and seedlings began to flower and fruit successively since 2005. Traits of the seedlings were evaluated. Six seedlings were found to be seedless or low seed. All of these six plants produced fruits of big size, but the color of rind and albedo as well as albedo crack, looked more like 'Ponkan'. The average seed numbers per fruit of six hybrids were 2.45, 2.86, 3.33, 4.33, 1.4 and 3.0. The biological/botanical characteristics of these hybrid plants were recorded and their fruit quality was analyzed for 3 years. Preliminary observations show that some of them are promising for the citrus industry.

S02P25

New citrus hybrids: selection and genetic studies

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The tools of biotechnology have enabled the improvement of citrus with significant reduction in cycle identification and selection of new materials, considering that the genetic studies and breeding in *Citrus* sp. sometimes has been hampered because of characteristics related to the reproductive biology of these species, i.e. high interspecific fertility, apomictic reproduction, polyembryony, a long juvenile phase and others. The Centro de Citricultura Sylvio Moreira-IAC has a breeding program for generating populations of scions and rootstocks hybrids. Phenotypic evaluations and genetic studies are underway to select promising materials, mainly for resistance to major diseases of citrus. The objectives of this study were to identify triploid plants and to construct genetic map with molecular markers using a backcrossing population of TMxLP163 ('Murcott' tangor x 'Pêra sweet' orange), a hybrid pre-selected in the breeding program, crossed with 'Pêra April' orange. Of the approximately 700 plants evaluated, 66 triploid (9.1%) were identified by flow cytometry. We evaluated 239 microsatellite loci, developed from EST library and 34 (14,2%) loci were selected with potential for genetic mapping. With this work, we expect to obtain new combinations resistant to Citrus Variegated Chlorosis (CVC) and with excellent fruit characteristics for the juice industry. Financial support: FAPESP, CNPq, INCT-Citros.

S02P26

Characterization of fruits of hybrids between 'Sunki' mandarin (*Citrus sunki*) and Sour orange (*Citrus aurantium*)

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The Centro de Citricultura Sylvio Moreira, Instituto Agronômico, since 1990, has been developing a breeding program of rootstocks, via hybridization, aiming to increase the number of varieties used in Brazilian citriculture and to improve the genetic variability as a way to overcome biotic and abiotic problems. In this work, we have