sour orange seedlings. The embryogenic callus and leaf midveins of 3 month-old regenerated plants were cultured in SP4 and LD8 media, which were used for PCR assays. Results of sanitary tests showed a 30% infection rate in the callus while no infection was detected in the regenerated plants.

S05P27

The study on callus induction of citrus anther culture *in vitro*

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Anther culture has special significance for breeding of citrus. It has been proved as one effective way of obtaining haploid and heteroploid materials. Citrus anthers mostly at middle and late uninucleate stage were cultured to study the influence of genotype, hormone composition of the culture medium, cold pretreatment, culture temperature and light conditions on callus formation. Results showed that the sequence of increasing difficulty on callus induction started with 'Meyer' lemon (*Citrus limon*), followed by '439' tangor (*C. reticulata* × *C. sinensis),* calamondin (*C. madurensis*), 'Changshou' kumquat (*Fortunella obovata*) and 'Zaoju' (*Citrus compressa*), regarding genotype. MT 6-BA 0.5mg/L, TDZ 0.2 mg/L, 2,4-D 0.2 mg/L, sugar 30-40g/L was determined to be a suitable culture medium for anther callus induction of citrus types. Cold pretreatment at 6±2°C for 5-10 d, and then cultivation at 23-25°C were beneficial for callus induction of citrus anthers *in vitro*. Cultivation in darkness was also helpful to mitigate the maturation and browning of anthers, promoting their survival and then callus induction. More than 3000 citrus calli were obtained during 2 years of anther culture, more than 2900 pieces from 'Meyer' lemon, and 35, 39, 2 and 2 callus pieces from '439' tangor, calamondin, 'Changshou' kumquat and 'Zaoju', respectively. 'Meyer' lemon calli is being subcultivated to study ploidy, embryoid induction and plant regeneration.

S05P28

Induction of tangerine embryogenic calli from unfertilized or immature ovules

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Triploid hybrids may be obtained by interploid crosses between diploid and tetraploid individuals, but it is difficult to obtain tetraploid lineages because of the high frequency of chimerical polyploids. It is possible to induce polyploidy in embryogenic calli or cell suspensions by using antimitotics. Embryogenic calli may be obtained from abortive, unfertilized or fertilized ovules, or from nucellus. Aiming to establish suspension cell cultures for further treatment with antimitotics, ovules of 'Ponkan', 'Montenegrina', 'Mexerica-do-Rio' and 'Cravo' tangerines were used. After asepsis in sodium hypochlorite (3:1) solution, the ovules were inoculated on Petri dishes (100 x 15 mm) (20 ovules per plate) containing 20 mL MT basal medium, 500 $mg \cdot L^{-1}$ malt extract, 50 $g \cdot L^{-1}$ saccharose and 5,0 $mg \cdot L^{-1}$ kinetin and maintained in the dark at 25°C ± 2°C, being subcultivated every four weeks. The experimental design was completely randomized with four repetitions. After two months, the percentage of embryogenic calli and somatic embryos was recorded. The data were submitted to variance analysis and mean deviation was calculated. Calli and embryo induction occurred at the 8th week. 'Mexerica-do-Rio', 'Cravo', 'Ponkan' and 'Montenegrina' presented 25%, 35%, 15% and 9% calli, respectively. Somatic embryos developed rapidly. 'Ponkan' and 'Montenegrina' presented the highest percentage of somatic embryos, 45% and 30%, respectively. The different cultivars presented different responses to calli and somatic embryos induction.

S05P29

Chromosome instability in 'Carrizo' citrange × *Citrus macrophylla* somatic hybrids

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Somatic hybridization by protoplast fusion is a very useful technique for citrus rootstock breeding. Indeed, it theoretically allows the accumulation of all genes of the parents, irrespective of their heterozygosity level and

therefore the addition of complementary dominant traits. In this work, protoplasts were isolated from callus of *Citrus macrophylla* and from leaves of 'Carrizo' citrange (*C. sinensis* × *P. trifoliata*). Chemical, electro-chemical and electric fusions were performed and the ploidy of regenerated plants was evaluated by flow-cytometry. Five tetraploid plants, one pentaploid plant, one mixoploid (3x-6x) plant and one heptaploid plant were recovered. All these plants were analyzed with SSR and SNP markers, distributed in the nine chromosomes of citrus. Cytoplasmic genomes were characterized with chloroplastic and mitochondrial markers. Mitochondrial genome was inherited from *C. macrophylla* for all plants while segregation was observed for the chloroplastic genome. Nuclear genome analysis revealed the loss of parental alleles in most of the regenerated plants. In tetraploids, it affected mainly *C. macrophylla* alleles while *P. trifoliata* alleles were mostly lost in 5x and 7x plants. The results indicate chromosome instability in this complex intergeneric combination with apparent non random loss of some chromosome fragments. Two allotetraploid somatic hybrids have been selected for evaluation as potential rootstocks.

S05P30

Preliminary results on biotization of encapsulated *in vitro*-derived propagules of 'Carrizo' citrange (*Citrus sinensis* × *Poncirus trifoliata*)

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The encapsulation technology represents a new tool to integrate micropropagation into the nursery activity. It allows combine the advantages of zygotic or gamic seeds with those of micropropagation. The synthetic seed or artificial seed, described as "artificially encapsulated somatic embryos, shoots or other tissues which can be used for sowing under *in vitro* or *ex vitro* conditions", will be a powerful propagation tool in the nurseryman hands, if the levels of the synthetic seeds conversion will be increased also in the nurseries, without the asepsis of *in vitro* laboratories and with the presence of many parasitic microorganisms, like bacteria and fungi, responsible for contamination and/or for trophic competition. This research has been carried out in order to introduce the biotization to the synthetic seed technology of 'Carrizo' citrange (*C. sinensis* × *P. trifoliata*), one of the most widespread citrus rootstocks, because of its resistance to the *Citrus tristeza virus* (CTV). With this goal, preliminary experiments to set up protocols for biotization, through the introduction of Plant Growth Promoting Bacteria (PGPB) into calcium alginate capsules of 'Carrizo' citrange *in vitro*-derived encapsulated microcuttings, have been carried out, in order to protect the plantlets from abiotic and biotic factors and to promote their growth during the first stages of development. Specifically, the *Sinorhizobium meliloti* wild type strain 1021 and its derivative RD64, that synthesizes 39-fold more IAA as compared to the wild type strain, have been used to evaluate their performances in inducing rooting of synthetic seeds.

S05P31

Biodegradable films made from PLA-limonene blends for food active packaging applications

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Active packaging films are increasingly being investigated for their application in food packaging industry. Among these, an increasing proactive attitude towards a reduction on the environmental impact produced by food packaging materials has focused research on bio-based polymers. In this sense, polylactic acid (PLA) films have increasingly received attention due to its biodegradation, biocompatibility, overall good mechanical property, superior transparency, being obtained from renewable resources, and labeled as Generally Recognized as Safe material by the FDA. Packaging has a prominent role in packaged food products, being a key component in the food preservation. Since active agents could have an important effect on shelf-life extension of foods, a way to develop actives packaging is by adding antioxidants components into the packaging system. Essential oil rich in monoterpens contain natural antioxidants and are recognized as food preservatives. Additionally, natural antioxidants are of great interest as stabilizers for polymers and the fact that natural antioxidants are