S02O01

The triploid mandarin breeding program in Spain

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Production of seedless citrus fruits is required for the fresh market because consumers do not accept seedy fruits. Development of new seedless mandarin cultivars has a high priority for many citrus industries worldwide. The recovery of triploid hybrids is the most promising approach to achieve this goal, since triploids have a very low pollen and ovule fertility and usually are seedless or produce very low number of seeds, and do not induce the formation of seeds in other cultivars by cross pollination. In Spain we are carrying a triploid breeding program since 1996 based on 2x X 2x, 2x X 4x and 4x X 2x pollinations followed by embryo rescue and flow cytometry, and the use of different approaches to produce new tetraploid parents. The objective is to produce new high quality easy peeling and seedless mandarin cultivars. More than thirty spontaneous autotetraploid apomictic genotypes to be used as male parents have been selected from seedbeds by flow cytometry. Also nine autotetraploid non apomictic genotypes mainly to be used as female parents have been obtained by chromosome duplication with treatments of micrografted shoot tips with colchicine. Symmetric protoplast fusion is also being used to produce allotetraploid genotypes. So far, we have obtained more than 5,500 triploid hybrids from 130 parental combinations by 2x X 2x pollinations, more than 4,300 triploid hybrids from 100 parental combinations by 2x X 4x pollinations, and more than 5,600 triploid hybrids from 103 parental combinations by 4x X 2x pollinations. Recently we have released the first cultivars originated in the program, the seedless late maturing mandarins 'Garbí' and 'Safor', obtained from 2x X 2x crosses. More than 400,000 trees of these varieties have been planted by growers during the last three years.

S02O02

Triploid seedless mandarin breeding in France

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Small citrus is an increasing component of the world citrus industry. New high quality, parthenocarpic, sterile mandarin varieties will play a pivotal role for its sustainable development. To produce seedless varieties, the CIRAD breeding program is focused on triploid hybrid selection. The first method to develop triploid progenies exploits 2n gametes that are naturally produced by diploid cultivars to obtain triploids in 2x X 2x crosses. The second method is based on interploid crossings (2x X 4x and 4x X 2x). For the last strategy the parental tetraploid gene pool has been diversified by selecting spontaneous tetraploids in apomictic cultivars, generating doubled diploids by colchicine treatments and production of allotetraploids by somatic hybridization. Several thousand triploid hybrids have been created by CIRAD using these strategies with the support of embryo rescue and ploidy evaluation by flow cytometry. This breeding program is supported by basic and methodological research performed in collaboration with IVIA (Spain), DAK (Morocco) and INRA (France) in the following topics: (1) citrus germplasm management and characterization, (2) studies of polyploidization mechanisms, tetraploid meiosis and its implications on the genetic and phenotypic structure of triploid progenies, and (3) studies of the implication of polyploidy on genomic and phenotypic expression.

S02O03

Mechanism of 2n gametes formation and centromere mapping in citrus

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Citrus triploid hybrids can be recovered by 2x X 2x hybridisations as a consequence of 2n gamete formation. Two main meiotic processes are at the origin of such gametes, the first division restitution (FDR) and the second division restitution (SDR). These two mechanisms lead to very different gamete genetic structures.

Particularly, the opposite feature of parental heterozygosity restitution (PHR) is observed in relation with the distance to the centromere. Without previous knowledge on centromere location, the determination of the restitution mechanism requires an analysis of the frequency of PHR within a large population with numerous codominant markers. With this method we determined that SDR is the mechanism involved in clementines and 'Fortune' mandarin. With SDR, PHR is a direct function of the distance of the considered locus to the centromere. Therefore the analysis of the evolution of PHR, within a linkage group (LG) allows mapping of the centromere position. Centromere has been located in the 9 LGs of the clementine genetic map by genotyping triploid hybrids with SSR and SNP markers. Analysis of the 2n gamete allelic configuration with loci close to the centromere can shed light on the restitution mechanism at the individual level and open the way for simplified study of this mechanism in a large range of genotypes. We are applying this approach to analyse the 2n gamete origin in several genotypes of mandarin and one genotype of sweet orange.

S02O04

Experiences in the development, release and commercialization of new irradiated citrus varieties from the citrus breeding program at the University of California Riverside

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The use of irradiation in the mutation breeding scheme of citrus breeding programs is now quite widespread with most programs in the major citrus producing countries actively pursuing new selections. Irradiation, used principally to reduce the seed content of normally seedy varieties, represents a more rapid method of achieving these goals than does hybridization. Some programs, notably in Israel and California, have a longer history with these techniques, not only in the development of new varieties but especially as relates to the release and commercialization of the newly developed selections. The experiences in the Citrus Breeding Program at the University of California Riverside have included the development, release and commercialization of several mandarin selections including 'Tango', 'DaisySL', 'KinnowLS', and 'FairchildLS' along with others in the 'pipeline'. The presentation will discuss the specific approaches used by the UCR Citrus Breeding Program during the irradiation process, the criteria and standards used in the selection of promising new candidates and the general experiences and problems encountered during the release and commercialization phases.

S02O05

Citrus breeding program in Chile

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Mandarin production and exports from Chile have shown a huge growth in the last ten years. During the 90's the most important cultivar was 'Clemenules' and since 2005 mandarin hybrids are being planted to supply late season markets. This has resulted in the production of fruit with seeds due to cross pollination causing big losses to Chilean producers. In 2007 citrus breeding program was initiated in the Pontificia Universidad Católica de Chile with the support of CIRAD (France) and University of California Riverside. The aim of this program was to obtain seedless mandarin and lemon cultivars. Strategies used are induced mutation by gamma irradiation, *in vitro* triploid rescue obtained from diploid crosses, production of auto-tetraploid plants and the establishment of a protoplast fusion protocol. Irradiation technique has resulted in the establishment of 5700 mandarins and 2500 lemons in a field trial located in Pomaire, Chile (33°39'S, 71°10'W). Field evaluation resulted in the selection of 164 lemon and 14 mandarin seedless selections, 4 lemon thornless selection, and 2 ornamental types. Our methodology allowed an early selection of seedless genotypes and quickly reduced the number of plants under evaluation. 118 triploids were obtained through *in vitro* rescue from diploid mandarins cross pollinations. These genotypes are currently under agricultural practices to induce flowering and are prone to be planted in commercial trials. 9 tetraploid of reduced juvenility were obtained with the use of colchicine in axillary buds. They will be used in cross pollinations in 2012 spring. Finally a protoplast fusion protocol has been developed under our conditions. The results of this program have a high impact for the Chilean citrus industry and the academy, since they have allowed creating varieties adapted to local conditions, a new line of research and training for new professionals.