

S02P16**Mechanism of seedlessness in a new lemon cultivar 'Xiangshui' (*Citrus limon*)**

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Seedlessness is an important economic trait of lemon. Pollen and embryo sac fertility, embryo development, compatibility of self-pollinated 'Xiangshui' lemon and cross-pollinated 'Xiangshui' lemon were studied for investigating mechanism of seedlessness in 'Xiangshui' lemon. The results showed that the fertility of pollen and mature embryo sac was normal. The development of embryo sacs belonged to polygonum type. The mature embryo sac had one egg, two synergids, three antipodal cells and one big central cell containing two polar nuclei. Pollen tube in self- or cross-pollination grew well in stigma. Pollen tube of cross-pollinated 'Xiangshui' lemon could grow normally in style and ovary, and then entered into the embryo sac, double fertilization was accompanied. However, the growth of pollen tube in self-pollination was finally stopped in the bottom of stigma. Embryonic development in cross-pollination was normal. Zygote began to divide 2 weeks after cross-pollination (WACP). Early globular embryos were observed 3 WACP. Globular embryos and heart-shaped embryos were observed 4 WACP. Torpedo-shaped embryos were observed 5 WACP. Cotyledonary embryos were observed 6 WACP and developed into complete seeds. While in self-pollination, ovules began to abort at 2 weeks after self-pollination (WASP), disappeared 5 WASP later and produced seedless fruits. The emasculated flowers without any pollination finally developed into seedless fruits also. Results indicated that parthenocarpy is contributed to seedlessness. However, gametophytic self-incompatibility has a main role in seedlessness of 'Xiangshui' lemon by blocking fertilization in the bottom of stigma.

S02P17**Differences in the genetic structure of citrus triploid hybrids recovered from 2x X 2x and 4x X 2x sexual hybridisations**[Aleza P.](#)¹, Cuenca J.¹, Juárez J.¹, Ollitrault P.², and Navarro L.¹¹Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Spain; and ²Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), BIOS, France. aleza@ivia.es

Citrus triploid hybrids can be recovered by 2x X 2x hybridisations as a consequence of the formation of 2n gametes or by interploidy hybridisation. Most of the 4x parents used in our program are doubled-diploid (DD), also known as autotetraploid. The genetic structure of diploid gametes and particularly the rate of parental heterozygosity restitution (PHR) depend on the meiotic process by which they were originated. Second-division restitution (SDR) is the 2n gamete formation mechanism involved in 'Fortune' and clementines. With SDR, PHR is positively linked with the distance of each locus to the centromere. With DD parents, PHR depends on the rate of preferential pairing and thus the proportion of disomic versus tetrasomic segregations. We have compared the genetic structure of two populations of diploid gametes of clementine (one population of 2n gametes and one of 2x gametes produced by DD) with SNP and SSR markers covering the 9 citrus chromosomes. The DD displays mostly tetrasomic segregation; however three linkage groups present intermediate segregation and one displays a tendency for disomy. The PHR in the 2n gametes is 2/3 of that obtained in 2x gametes produced by DD. The two methods of triploid production appear complementary in terms of genotypic variability. 4x X 2x hybridizations are more efficient than 2x X 2x for developing new cultivars phenotypically closer to the diploid parent of the DD. Conversely, 2x X 2x hybridisations provides a greater opportunity to select innovative products.

S02P18**Efficient haploid production on 'Wilking' mandarin by induced gynogenesis**[Jedidi E.](#)¹, [Kamiri M.](#)², [Pouillet T.](#)³, [Ollitrault P.](#)³, and [Froelicher Y.](#)³¹Tunisian National Agronomic Research Institute (INRAT), Horticultural Laboratory, Tunisia; ²Groupe Kabbage (DAK), Agadir, Morocco; and ³International Center for of Agricultural Research for Development (CIRAD), UMR AGAP, France. jedidi.emy@gmail.com

Seedlessness is a major criterion for the citrus fresh fruit market. Therefore, triploid breeding appears very promising for the selection of new citrus cultivars. CIRAD has developed a method to produce triploid hybrids

by somatic hybridization between diploid and haploid lines, and demonstrates the potential for haploid citrus production by induced gynogenesis in clementine. In this work we analysed the efficiency of induced gynogenesis by irradiated pollen in 'Wilking' mandarin (*Citrus reticulata*). Five doses of gamma irradiation of 'Meyer' lemon pollen were tested (0, 150, 200, 250, 300 Grays). Embryos were extracted from small seeds, obtained in immature fruits, and cultivated *in vitro*. The ploidy level of plantlets was determined by flow cytometry analysis. Irradiated pollen appeared to be efficient for gynogenesis induction in 'Wilking'. A relatively high number of haploids (7 plantlets) were obtained, essentially after hybridisation with 'Meyer' lemon pollen irradiated at 250 Grays. The ploidy level of all haploid plantlets was confirmed by chromosome counts, and molecular markers demonstrated their gynogenetic origin. These haploid lines should be useful for triploid breeding by somatic hybridisation, and also constitute interesting resources for genomic studies.

S02P19

Differences in ploidy levels of interploidal crosses progenies between diploids and tetraploid somatic hybrids in citrus

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One of the main objectives of citrus breeders is to obtain sterile mandarins that produce seedless fruits. Triploids are a response to these criteria. A way for triploid creation is sexual crosses between diploids and tetraploids. However, the scarcity of the natural tetraploid gene pool has been a restriction for using this method. Citrus somatic hybridisation via protoplast fusion allowed the creation of allotetraploid somatic hybrids that can be used as parents to generate triploid cultivars. Several crosses using diploids (female) and allotetraploid somatic hybrids (male) were conducted by CIRAD: a) 'Fortune' mandarin x ('Willow leaf' mandarin 'Star Ruby' grapefruit tetraploid somatic hybrid); b) 'Eureka' lemon x ('Star Ruby' grapefruit 'Corsican' citron tetraploid somatic hybrid); and c) 'Eureka' lemon x ('Mexican lime' 'Shamouti' orange tetraploid somatic hybrid). The majority of progenies were triploid from the 3 crosses (61-76%). However diploid and tetraploid progenies were also obtained in all of them. Molecular analysis of progenies with SSR markers revealed that tetraploids were issued from a diploid male gamete and an unreduced female gamete, while diploids could be issued from a haploid ovule and a haploid male gamete. This study reveals that ploidy variations were owed to meiotic dysfunction of either the tetraploid somatic hybrids or the diploid female parent.

S02P20

Gametic configuration and inheritance of SSR markers in tetraploid interspecific and intergeneric citrus somatic hybrids

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Citrus somatic hybridization has become an integral part of citrus improvement programs worldwide. More than 100 somatic hybrids have been created by CIRAD. Intra and interspecific somatic hybrids will be used mostly for the triploid scion breeding. Intergeneric somatic hybrids can have a direct application as rootstocks or be used as parents for sexual 'tetrazyg' breeding. To optimize the design of such breeding schemes, it is essential to have knowledge of the inheritance mode in such allotetraploid hybrids. The aim of the present study was to investigate the inheritance (disomic, tetrasomic or intermediate) in a citrus interspecific hybrid (*Citrus reticulata* x *Citrus limon*) and an intergeneric somatic hybrid (*C. reticulata* x *Poncirus trifoliata*). Two triploid populations were generated using the somatic hybrids as pollinators in crosses with the diploid 'Chandler' pummelo. The triploid progenies were genotyped with SSRs to infer the allelic constitution of the somatic hybrid gametes. A likelihood-based approach was used to estimate for each locus the proportion of disomic versus tetrasomic segregations. For the two somatic hybrids, intermediate inheritance was observed with a major tendency for tetrasomic segregation in the interspecific hybrid, but a tendency for disomic inheritance in the intergeneric hybrid. The implications of the observed inheritance mode for citrus breeding are discussed with special focus on heterozygosity restitution.