ANTHER CULTURE OF TETRAPLOID SOMATIC HYBRIDS AS A CRUCIAL TOOL FOR STARTING INTROGRESSION BREEDING FROM ALLIED SPECIES IN EGGPLANT (S. MELONGENA L.)


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Solanum spp, protoplast fusion, sexual hybridization, Verticillium dahliae, Fusarium oxysporum

The major limits for practical exploitation of the somatic hybrids between eggplant and its wild relatives have been their sterility and tetraploidy which have prevented their incorporation into breeding programs. We successfully employed anther culture to bring back the ploidy level of tetraploid interspecific hybrids between eggplant and the allied species, S. integrifolium (=S. aethiopicum gr aculeatum) and S. aethiopicum gr gilo, to the diploid status. Both the relative species are resistant to Fusarium oxysporum f. sp. melongenae and to some strains of bacterial wilt (Ralstonia solanacearum) which are very destructive diseases of eggplant. Dihaploid androgenetic plants were obtained from the somatic hybrid between eggplant and S. integrifolium and from tetraploid backcrossed plants between the somatic hybrid with S. aethiopicum and eggplant. Phenotypical, molecular, biological and biochemical characterization, and also artificial inoculation with Fusarium oxysporum are consistent with a recombination between the genomes of the species involved in the hybridizations. Dihaploids resistant to Fusarium have been successfully backcrossed with eggplant and incorporated in our breeding program. After 4-6 cycles of backcrosses, lines with improved features of fruits and plant characteristics were obtained. The subsequent 2-4 selfing cycles, allowed to fix superior lines with interesting agronomical traits. The fruit weight increased, reaching that of the recurrent eggplant line employed in the backcrosses. Selected pure lines were completely resistant to Fusarium wilt. In the last years, the best advanced introgressed lines from the two single allied species above mentioned have been inter-crossed each other and with the Verticillium tolerant lines derived from sexual crosses between eggplant and S. sodomaeum (= S. linneanum).

Besides their utility as potential valuable breeding materials, the lines obtained are going to be utilized in genetic and molecular studies about the resistance/tolerance to Fusarium and Verticillium wilts. Biochemical and nutritional studies are also in progress in order to discover other possible useful genes and features introgressed from the allied species into eggplant.
RESULTS OF AN ASPARAGUS BREEDING BASED ON IN VITRO ANther Culture

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Asparagus breeding, in vitro androgenesis, all-male hybrids

The breeding methods applied to asparagus (Asparagus officinalis L.) must take into consideration that it is a dioecious (female and male plants conventionally are X/X and X/Y) and a perennial species, moreover at least five years of field test are necessary to evaluate genotypes. Since male plants are more productive, long-lived and resistant to diseases respect to female plants, all-male hybrids are preferred to dioecious hybrids and high homozygosity of hybrid parents are requested for the greatest heterosis expression.

The in vitro anther culture technique to obtain homogygous female (X/X) and male (Y/Y) clones (DH) and release F1 all-male hybrids has been applied at our research Institute during the last thirty years. As anther donors male plants of Italian local cultivars and genotypes from abroad were utilized. The anther response to in vitro androgenesis ranged from 0.1% to 20% according to anther donor genotypes. From about 300,000 anthers cultured in vitro 4,160 androgenetic embryos were obtained, but 1,050 dead or remained as undifferentiated callus. Out of 3,110 regenerated clones 58%, 25%, 13% and 4% were diploid, tetraploide, triploid and haploid respectively. Cytological observation evidenced that DNA endoreduplication could occur in the microspore at the first mitosis.

Following at least three years of grow in a soil naturally infected with Fusarium spp, seventy female and eighty male DH clones were selected on the basis of plant survival, vigour and fertility. When DH clones were recloned in vitro and replanted in the same soil, they showed the same phenotipic behaviour and percentage of plant dead.

All-male two ways (both DH parents) and three ways hybrid (heterozygous female and DH male parents) were included in multilocation field trials. In Northern Italy out of 400 all-male so far tested nine were selected and four (Eros, Marte, Ercole, Zeno) showed commercial interest. At present Eros and Ercole give near the total green asparagus production in the Po Valley (about 1,000 ha), Marte and Zeno are rapidly expanding in Veneto Region for white spear production; Ercole is appreciated in Southern Spain for spear quality and resistance to rust (Puccinia asparagi). In South Italy about 40 all-male hybrids were tested and one, named “Italo”, appears to be better than the dioecious hybrids UC 157, Grande, Atlas for spear quality and rust resistance. Furthermore new experimental hybrids have been selected for ealiness, spear quality and resistance to abiotic stresses.

Our DH clone collection is recognized to be an excellent germplasm for new F1 all-male hybrids, but the future choice of DH clone combinations will have to be based on DNA marker.
APPLICATION OF IN VITRO ANther CULTure METHODS TO A PEPPER BREEDING PROGRAM FOR DISEASE RESISTANCE

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pepper breeding, anther culture, doubled-haploids, disease resistance

Pepper (Capsicum spp. L.) is one of the most important vegetable crops in the world, used as a comestible condiment, spice and vegetable. In Italy the cultivation has spread mainly in the Southern and Central Regions (Sicilia, Campania and Lazio), even if pest and diseases as Phytophthora capsici, Verticillium dahliae, nematodes and virus (TMV, PVY CMV and TSWV) increasingly damage the crops and are among the main constraints of pepper production.

At our Research Institute a breeding program for disease resistance has been supported by the Italian Agriculture Ministry. The resistant accessions Serrano Criollo de Morelos 334’ (SCM334), C. chinense (PI 152225) and C. frutescens were crossed with some traditional Italian cultivars. The F1 and F2 progenies obtained showed different susceptible/resistant plants ratios, depending from the resistance mechanism and the genetic bases. After several cycles of selfing and inter-cross between partially resistant plants, followed by selection for the resistance, several breeding lines were selected. Some of these lines were very interesting for number, shape and weight of berries, and showed different level of resistance to P. capsici, nematodes and virus. Considering that the main advantage of using haploids in a breeding program is the production of homozygous plants in the shortest possible time, we used the most interesting resistant lines as plant material. Anthers of several partially resistant pepper genotypes and hybrids were cultured following the method of Dumas de Vaulx et al (1981), with some modifications with regard to the composition of the induction media (Nervo et al. 1994). A total of 605 embryos were derived from 11.349 in vitro cultured anthers of 25 genotypes and 256 (5.3%) doubled-haploid (DH) plants were obtained; among the regenerated androgenetic plants about an equal number of haploid and diploid plants were found.

The DH lines were multiplied by selfing and evaluated both in greenhouse for resistance to P. capsici and virus and in open field for the performance under different conditions. Some of the DH lines showed very good agronomic characters and will be used for developing new multiple disease resistant cultivars. It also confirms the possibility of transferring the high level of disease tolerance from resistant DH lines into traditional pepper cultivars.
DI-HAPLOID PLANT PRODUCTION FROM TETRAPLOID ASPARAGUS GENOTYPES

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Asparagus maritimus, Asparagus acutifolius, interspecific hybrids, di-haploid

Two tetraploid (2n=40 chr.) dioecious asparagus species (A. maritimus Miller and A. acutifolius L.), spontaneous in the Mediterranean Basin, are known to be good source of resistance to pathogen fungi (Puccinia asparagi and Stemphylium vesicarium), salt and drought. Such valuable traits could be introgressed into the cultivated species A. officinalis. To this purpose the tetraploid A. officinalis cultivar Violetto d’Albenga was chosen for interspecific hybridizations and the bridge-cross “A. officinalis x A. maritimus” was necessary to overcome the sexual incompatibility between A. officinalis and A. acutifolius. The partially sterile interspecific plant obtained from the combination of the three parental species was crossed to tetraploid A. officinalis; the progeny obtained was fertile, but could not be extensively utilized in the breeding because tetraploid. The cv Violetto d’Albenga was also considered useful to improve spear quality and adaptation to high plant density of normal diploid genotypes, but the F1 plants obtained from the sexual cross were triploid and gave offsprings with unbalanced chromosome number. Therefore, di-haploid plants (n=20 chr.) obtained from in vitro anther culture of tetraploide genotypes were thought to be the best parents in the sexual crosses with normal diploid asparagus genotypes.

In vitro anther culture was performed following the procedure systematically applied at our lab to A. officinalis. One male plant of the following genotypes was utilized as anther donor: 1) A. maritimun; - 2) Violettto d’Albenga; 3) - [(A. officinalis x A. maritimus) x A. acutifolius] x V. Albenga; 4) - 3) x V.Albenga. All genotypes gave positive results. The percentage of anthers yielding at least one androgenetic embryo were 2.2, 1.7, 2.7, 1.6 respectively for genotypes 1), 2), 3) and 4); however during the regeneration phase about 70% of the embryos degenerated and died. The number of clone at present grown in greenhouse conditions were: 13, 7, 14 and 6 respectively for genotypes 1), 2), 3), and 4). The chromosome number computed in root tip cells at meiosis revealed that near 50% of the androgenetic clones were di-haploid and the remaining 50% tetraploid. Since the percentage of di-haploid clones from anther culture was much higher than that of haploid clones from anther culture of diploid asparagus genotypes (50=9 against 4%), it is supposed that in Asparagus gender the di-haploid status is preferred to haploid.

In 2006, the observations revealed strong morphological differencies among di-haploid clones regenerated from the same anther donor, but not all plants flowered. Two vigorous and fertile di-haploid male clones regerated from anther culture of plant 3) were successfully crossed with two
selected doubled haploid; the plants of the four hybrid obtained will be evaluated for resistance to
diseases and those resistant back-crossed to recurrent doubled haploid clones.

Isoenzyme and ISSR analysis allowed to estimate the genetic similarity of *A. officinalis, A. acutifolius, A. amarus*, to verify the interspecific hybrid condition, to evidence both the segregation after a backcross to *A. officinalis* and the androgenetic origin of anther derived plants.
The main goals of research on fruit breeding are: to obtain new varieties with a shorter juvenile non-fruiting period, an increased yield, a longer ripening season, regular bearing, seedlessness and improved external and internal quality of the fruits. Another important aim in fruit tree improvement research is to make available new scions and rootstocks selected for resistance or tolerance to biotic and abiotic stresses.

Fruit species breeding is based on either conventional (hybridization, selection, mutation) or biotechnological methods employing embryo culture, regeneration from protoplasts, somatic hybridization, in vitro mutant selection, genetic transformation and haploid production. Using an integrated approach with both biotechnological tools and conventional ones it is possible to obtain good results in a short time.

The interest of fruit breeders in haploids and doubled haploids (DHs), lies in the possibility of shortening the time needed to produce homozygous lines compared to conventional breeding. Haplo-diploidization through gametic embryogenesis allows single-step development of complete homozygous lines from heterozygous parents. In a conventional breeding programme, a pure line is developed after several generations of selfing.

With fruit crops, characterized by a long reproductive cycle, a high degree of heterozygosity, large size, and, sometimes, self-incompatibility, there is no way to obtain haplodization through conventional methods.

The current status of research on doubled haploid production in the main fruit crops is reviewed.
**ANEMONE CORONARIA ANDROGENETIC PLANTS DERIVED THROUGH ANther CULTURE**

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*Anemone coronaria, haploids, androgenesis, microspore embryogenesis, F1 hybrid breeding*

The genus *Anemone* (*Ranunculaceae*) includes many species cultivated for ornamental purposes. Most cut flower cultivars belong to *A. coronaria* L. and are multiplied by seed and sold for cultivation as 1-year-old tubers. As cultivars represent a population of hybrid individuals derived from crosses between heterozygous parents, the use of a true F1 hybrid would improve the uniformity and quality of the product. As a first step towards the development of pure-breeding lines, anther cultures were established from elite cultivars of *A. coronaria*. Somatic embryos and plantlets were regenerated from all five elite cultivars, and up to 16.9 regenerants per 100 cultured anthers were rescued. Cytological analysis shows that 11 of 19 regenerants had either a 2x = 16 karyotype, or were mixoploids (diploid plus haploid cells). These plants were compatible for breeding diploid F1 hybrids. Triploid and tetraploid cells were also identified. RAPD-based DNA fingerprinting showed that all the regenerants tested differed genetically from their anther donor, confirming their androgenetic origin. In *A. coronaria*, breeding pure line by self pollination is time consuming (at least six years required) and complicated by partial progeny sterility. Shortening to 15 months the time required to produce homozygous lines may convince seed companies to invest in F1 hybrid breeding. A new scheme to breed F1 hybrid crossing androgenetic pure line, maintained *in vitro* and evaluated for their combinatory aptitude, is proposed and compared to the conventional method.