

## DIFFERENT APPROACHES TO DEVELOP NOVEL MOLECULAR MARKERS FOR SEX DETERMINATION IN *ASPARAGUS OFFICINALIS*

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Cultivated asparagus (*Asparagus officinalis* L.) is a dioecious species ( $2n=2x=20$ ) with a haploid genome size of 1,323 Mb. With respect to sex expression female, male, supermales, andromonoecious and hermaphrodite plants are known. As male plant is agronomically superior respect to female one, a breeding goal is all-male hybrids constitution. In the present study, novel molecular markers have been developed with different approaches, in order to isolate candidate genes associated with sex expression.

In asparagus up to date genetic maps were originally based on isozymes, RFLP, AFLP and RAPD. Using next Generation Sequencing technologies as GSFLX 454 pyrosequencing, thousands of codominant markers (SNPs and SSRs) have been isolated from male and female cDNA libraries. Thus, codominant molecular markers are used to map a large BC<sub>1</sub> population for sex traits. Linkage analysis using MAPMAKER with a LOD score of 3.0 and max distance of 5.0cM provided a map with coverage for 19 linkage groups consisting of 73 markers, starting from a first panel of 144 SNPs. Sex-linked SNPs were identified in linkage group 12 (LG-12). Further experiments are underway to place additional EST-SNP and EST-SSR markers isolated from a BAC library.

The large number of independent shifts between dioecy and hermaphroditism at different times since the origin of flowering plants, makes angiosperms the group of plant useful for testing a general model explaining the evolution of dioecy. In this frame, asparagus is considered a model for studying the evolution of sex chromosomes. Starting from flanking region sequences of the M-locus (the region controlling sexual dimorphism) obtained from three BAC clones (2 from males and 1 from female), three different regions around this site have been identified: one recombinant region between male and female, one non recombinant region and then a male specific region. These sequences were BLASTed against a large asparagus database and novel sex-specific markers (from a region linked 0.25cM to M-locus) have been developed to investigate genes and regulatory networks involved in the origin and early diversification of flowering plants.