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DETERMINATION OF THE DEGREE OF HYBRIDIZATION IN EXPERIMENTAL PROGENIES OF LETTUCE (*LACTUCA SATIVA* L.) USING MOLECULAR MARKERS

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Lettuce (*Lactuca sativa* L., 2*n*=2*x*=18), one of the major crops of the group of leafy vegetables, reproduces mainly by selfing whereas out-crossing, naturally ranging from 0.1% up to 6.0%, is strongly limited by cleistogamy. In this species, basic populations genetically improved for a number of traits, including F2 and BC1 progenies, are usually developed by selfing and backcrossing experimental F1 hybrid plants. Hence hybridization between different pure lines is a process frequently adopted in lettuce breeding programs and it can be achieved using hand-made emasculation and pollination protocols. Unfortunately, this breeding approach is time-consuming and technically demanding, and requires genetic markers for screening progeny plants and assessing their hybridity.

The most recent breeding programs for lettuce improvement integrate genetic data deriving from the evaluation of morphological traits and investigation of molecular markers. In particular, marker-assisted selection is becoming a routine component in lettuce breeding programs to rapidly assess selfing and crossing rates in experimental F1 progenies.

The aim of the present study was to assess selfing and crossing rates in 102 experimental populations, stemmed from a total of 71 parental lines, by using a panel of microsatellite (SSR) markers. To this end, 14 highly informative microsatellite loci uniformly distributed throughout the genome were selected on the basis of their estimated polymorphism information content and observed marker allele composition in the parental accessions and individual progenies. Each of the pairwise combinations analyzed in this study was represented by 8-12 progeny plants. Overall molecular data were used to evaluate and compare the performance of two different emasculation and hybridization methods. The traditional emasculation technique consists of washing anthers with water in the early hours of the morning, before pollen grains can naturally settle on the stigmatic outermost surface of pistils. Then manual pollination is performed by rubbing the anthers of the pollen parent on the stigma of the seed parent. The alternative technique includes an incision of the calyx and corolla of flowers, so that it is possible for breeders to perform a better wash of the anthers for removal of pollen grains. Flowers are artificially dried and then used for manual pollination, which is simplified by the absence of a part of sepals and petals. Overall, the mean hybridization rate of the two crossing methods was estimated as 38.6 ± 29.1% and 76.5±29.5%, respectively. Only hybrid plants were maintained and used for selfing or backcrossing strategies in order to develop segregating F2 and BC1 populations, respectively.

In conclusion, we demonstrated that our simple SSR-based genotyping method could be employed to increase the efficiency of breeding programs in lettuce by providing a reliable and

reproducible information, not always retrievable by morphological descriptors, crucial for assessing selfing and crossing rates in experimental progenies.

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