

DISCOVERY OF NUCLEAR MALE-STERILITY IN RED CHICORY: GENETIC ANALYSIS AND METHODS FOR THE MARKER-ASSISTED BREEDING OF F1 HYBRID VARIETIES

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The present research deals with the discovery and genetic analysis of male sterile mutants of red chicory (*Cichorium intybus*, $2n=2x=18$). Four distinct mutants¹, which to the best of our knowledge are the first spontaneous male sterile mutants ever discovered and described in the genus *Cichorium*, were characterized in great details for the developmental pathway of microsporogenesis and gametogenesis, and the inheritance pattern of the gene, here named *Cims-1*, underlying the male-sterility trait. A quick molecular diagnostic assay was also developed for the early marker-assisted selection of the genotype associated to male sterile plants. Overall data clearly support a nuclear origin and a monogenic control of recessive type for the male-sterility trait in each of the red chicory mutants. Male gametogenesis was documented to arrest at the stage of uninucleate microspores. In particular, cytological observations revealed that microspores degenerate before their release from the tetrads, later showing a collapse of the exine. In the mutants, the totality of microspores proved to be shrunken and much smaller than wild-type ones. In fact pollen grains were never detected in mature anthers, demonstrating a full expressivity of the trait with mutants being 100% male sterile. Moreover, the fine mapping of the mutant locus was attempted by molecular markers using F₂ and BC₁ populations segregating for male-sterility. The gene responsible for male-sterility was found tightly linked to a microsatellite of the TC/GA type whose full sequence was recently deposited in the NCBI databases under the accession no. JF748831. A molecular diagnostic assay was then developed to be profitably adopted as a tool of marker-assisted breeding and exploited for an early screening of male-sterile plants within segregating progenies stemmed from back-crosses, with a genotyping error lower than 3%. Four new hybrid varieties of radicchio *Rosso di Chioggia* with different earliness, spanning from 80 to 110 days, were bred during 2011 by crossing male sterile partially inbred clones, used as seed parents, with wild type highly inbred lines, used as pollen donors. The parental lines to be used in large-scale pair-wise crosses were selected on the basis of their specific combining ability (SCA) assessed by means of molecular marker analysis (*i.e.* genetic distances) and agronomic progeny tests (*i.e.* field performances). Hybridization between parental genotypes so chosen produced vigorous and uniform F₁ hybrids, presenting detectable effects of heterosis. On the whole, the constitution of F₁ hybrids seems profitable in a practical breeding scheme and it is also feasible on a large commercial scale by the selection of self-compatible genotypes, for the production of inbred lines, and the identification of male sterile genotypes, to be used as seed parents for the hybridization with unrelated pollen donors. The discovery of non-engineered male-sterility in red

chicory will open new frontiers for maximizing crop productivity in this important cultivated vegetable species through the breeding of heterotic F₁ hybrid varieties.

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