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AN EFFICIENT AND AFFORDABLE PTOTOCOL TO PRODUCE DOUBLED HAPLOIDS IN CAULIFLOWER (BRASSICA OLERACEA VAR. BOTRYTIS L.) BY ISOLATED MICROSPORE CULTURE

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Doubled Haploid (DH) is an efficient and rapid technique for developing genetically stable and pure homozygous inbred lines suitable for F1 hybrid variety production and population genetics studies. The inbred lines are developed by the production of haploid plants through various methods followed by chromosomal doubling aimed at obtaining homozygous lines in a single generation.

In the present work we focuses on the production of double haploids in cauliflower (*Brassica oleracea* var. *botrytis* L.), an economically and nutritionally important horticultural crop. Breeding to obtain inbred lines is difficult in this species due to its long-life cycle and the occurrence of self-incompatibility and male sterility. Traditionally, to obtain homozygous inbred lines several generations of self-pollination are required, which is expensive and time consuming.

In particular, we developed a simplified and affordable protocol to obtain DH plants through isolated microspore culture. Buds containing microspores at late uninucleate/early binucleate stage were sterilized and then used for microspore isolation. Isolated microspores were cultivated in vitro in a liquid medium and microspore derived embryos were obtained. These embryos were used to regenerate shoots that were rooted to form plantlets. The ploidy levels of these plantlets were evaluated by chromosome counting. Using a set of molecular markers we discriminated between plantlets regenerated from diploid cells of the mother plants and DH plantlets regenerated from microspores that had spontaneously doubled their genome.

DH plantlets were acclimatized to in vivo conditions and used in breeding programs while the obtained haploid plants were treated with colchicine to induce chromosome doubling to obtain other/more DH lines.