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BIOTECHNOLOGICAL APPROACHES TO THE GENETIC IMPROVEMENT OF *CHRYSANTHEMUM CINERARIAEFOLIUM* L.

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The interest in sustainable agriculture has increased the demand of plant-derived compounds which can be less toxic both to mammals and to the environment than the synthetic agrochemicals.

Chrysanthemum cinerariaefolium L. (Asteraceae), commonly termed pyrethrum, is an economically important crop from highlands of tropical and subtropical regions of the world. It is grown for the extraction of pyrethrins, natural insect repellents of plant origin. Pyrethrins are a mixture of six compounds produced by esterification of two acids (chrysanthemic and pyrethric acid) with three mono-terpene-alcohols (pyrethrolone-5, jasmolone-3 and cinerolone-4). The principale source of pyrethrins are the dried flowers of *Chrysanthemum cinerariaefolium*. Thanks to the low toxicity to mammals and other warm blooded animals, pyrethrum is the only plant species whose metabolites are currently commercially exploited in insecticides, and its worldwide demand exceeds the supply.

Asteraceae species are considered as recalcitrant to successful growth in *in vitro* condition. Recalcitrance in root or shoot formation or in regeneration are associated to endogenous bacterial contamination, hyperhydricity, and tissue browning, hence studies on *in vitro* culture of *Chrysanthemum cinerariaefolium* have been rather limited. The aim of this study was to establish highly reproducible *in vitro* regeneration systems for an efficient multiplication of *Chrysanthemum cinerariaefolium,* since the pointing out of an efficient regeneration system could play an important role for the industrial exploitation of this plant.

Petiole explants and leaf segments were used for micropropagation, callus induction and protoplast isolation. Sterile seeds were used as plant material source. The ability of petiole cuttings to produce direct shoot buds varied depending upon the different media composition tested for the experiments. Growth rate of shoots as well as root induction from shoots have been periodically analyzed. The *in vitro* raised plantlets were acclimatized and transferred to greenhouse with 60% success.

For protoplast isolation and culture, young leaves of *in vitro* grown plants were used as initial plant material. Different enzymatic combinations were tested to achieve the highest protoplast release. We were able to recover a reasonable number of viable protoplast for further manipulation at the ploidy level with the aim to enhance the biological activity of *Chrysanthemum cinerariaefolium* and for the regeneration of novel insecticidal plant germplasm.