Biotechnological approach for cultivation of *Tanacetum cinerariifolium* (Trevir.) Sch.Bip. (Asteraceae)

Vladimir Metodiev Ilinkin

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Summary

Dalmatian pyrethrum, *Tanacetum cinerariifolium* (Trevir.) Sch. Bip. (Syn. *Pyrethrum cinerariifolium* Trevir., *Chrysanthemum cinerariifolium* (Trevir.) Vis. is a perennial herb of Asteraceae family. It is a Balkan endemic distributed mainly in Croatia, also found in Albania, Bosnia and Herzegovina, and Montenegro. In Croatia, due to increased urbanization, the species is at risk of habitat loss. Phytochemical studies of *T. cinerariifolium* have led to the identification of different types of secondary metabolites. The characteristic bioactive compounds of the species are the pyrethrins, which are associated with the insecticidal action of chrysanthemum and pyrethric acids. The term "pyrethrins" refers to the six insecticidally active ingredients contained in *T. cinerariifolium*: Pyrethrins-I (Pyrethrin-I, Cinerin-I and Jasmolin-I, which are monoterpene esters of chrysanthemum acid, with lethal insecticidal properties), and Pyrethrins-II (Pyrethrin-II, Cinerin-II and Jasmolin-II, monoterpene esters of pyrethric acid, with a knockdown effect on insects). Natural pyrethrins are among the most important insecticides as they possess some of the qualities of an ideal means of controlling harmful insects. They have low toxicity to mammals and other warm-blooded animals because they are easily broken down to inactive forms and are quickly cleared from the body.

The aim of the present dissertation is the experimental determination of suitable conditions for propagation of *Tanacetum cinerariifolium* using methods of plant biotechnology and the selection of promising *in vitro* clones for future testing in agriculture.

T. cinerariifolium seeds were found to be characterized by low viability and low germination, which rapidly decreased with increasing storage period. After 3 years of storage, the seeds completely lost their viability and ability to germinate. Freshly harvested seeds were best suited for initiating *in vitro* cultures. Seed germination was directly related to the physical, physicochemical and chemical characteristics of the soil. The highest percentage of germinated seeds was found in Rendzic Leptosols, followed by Luvic Chernozems and Pellic Vertisols.

The presence of unwanted bacterial microflora, a classic problem in the initiation of *in vitro* cultures and a particularly big problem with *T. cinerariifolium*, was successfully controlled by adding an antibiotic at a concentration of 200 mg/L, which did not adversely affect seed germination. Since its action was bacteriostatic, it had to be added at each subcultivation, but it did not negatively affect morphogenesis. Spontaneous necrosis of *in vitro* rooted and unrooted shoots was successfully minimized by adding Ca to the culture medium at a concentration of 75 ppm, in the form of $CaCO_3$. Temporary Immersion System (TIS) was found to be less effective for *in vitro* propagation of Dalmatian pyrethrum plants compared to agar solidified medium with added antibiotic at optimal concentration. In *in vitro* conditions, the individuals rooted spontaneously without the need for added plant growth regulators, which helped in the subsequent successful *ex vitro* adaptation and acclimatization to the conditions of the *ex situ* collection. A minimal number of dead plants were observed when using a climate chamber with strict control of temperature, lighting and relative air humidity during *ex vitro* adaptation.

Differences were found in the clones regarding the content of some pyrethrins, but also in the ratio of the corresponding esters of chrysanthemum and pyrethric acids. A difference was also found in the clone variation of some of the pyrethrins, which makes it possible to use plant biotechnologies to create agricultural crops with better predictability of yields on the one hand and with a greater possibility of creating commercial products based on the ratio of pyrethrins.