

PERSPECTIVES OF PRODUCING BIOPESTICIDES THROUGH OVER-EXPRESSION OF A TRITERPENE CYCLASE GENE

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Environmental contamination caused by synthetic pesticides is a well known problem in agriculture. An alternative pest control can be managed by replacing synthetic pesticides with biopesticides, compounds of natural origin, many of which produced by plants against pests and diseases. In *Asteraceae*, a rich chemical diversity exists to such an extent that the evolutionary success of the family was suggested to be due to the highly diversified chemical defence system. In particular, triterpenoid saponins, abundant in this family, have an important role for their activity against fungi, bacteria, insects and nematodes.

A phytochemical analysis of leaves from *Aster sedifolius* led to the isolation of three novel triterpenoid saponins which showed inhibitory activity against different pathogenic fungi, *Rhizoctonia solani*, *Fusarium solani*, *Sclerotium rolfsii* and *Sclerotinia spp.*, as well as against a bacterium, *Xanthomonas campestris*. In this context, our research group isolated in *A. sedifolius* *OXA1* gene encoding a key enzyme of the triterpene pathway performing cyclization of 2,3 oxidosqualene into pentacyclic carbon skeleton. To increase the production of triterpenoid saponins, a metabolic engineering strategy based on the over-expression of *OXA1* was designed. An expression vector useful for transformation by *Agrobacterium* was constructed with *OXA1* under the control of the constitutive promoter CaMV35S. Strains of *A. tumefaciens* (LBA4404 and EHA105) with CaMV35S::*OXA1* have been utilized to transform different species: *Aster sedifolius* and *A. caucasicus*, as well as *Arabidopsis thaliana* and *Nicotiana tabacum* as model plants. Moreover, *A. rhizogenes* was used for *A. sedifolius* transformation. It is expected that *OXA1* could increase the production of bioactive compounds in *Aster*, and particularly in *A. caucasicus* (species close to *A. sedifolius*) that spontaneously produces saponins inhibiting the growth of pathogenic fungus *Botrytis cinerea*. In *A. thaliana* saponins are absent, but it is known that a multifunctional lupeol synthase gene is expressed and lupeol as well as small amounts of β -amyrin are produced. For this reason, a modification in terpene pathway products could be expected following *OXA1* expression. The results obtained from transformation experiments will be discussed.