

## TOWARD THE METABOLIC ENGINEERING OF BETA-AMYRIN PATHWAY IN ASTER

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A phytochemical analysis of *Aster sedifolius* has led to the isolation from aerial part of three novel triterpenoid saponins, named astersedifolioside A, B and C. These compounds exhibit mycelium growth inhibitory activity against some phytopathogenic fungi such as *Fusarium solani*, *Rhizoctonia solani*, *Sclerotinia spp.* and *Sclerotium rolfsii*. Moreover, *in vitro* tests show that astersedifoliosides B and C inhibit tumoral cell proliferation.

The purpose of the present study is the assessment of a metabolic engineering strategy for genetic manipulation of triterpenoid saponin production in *Aster sedifolius*.

For molecular cloning of 2,3-oxidosqualene cyclase gene, encoding the key enzyme of triterpenoid saponin pathway, a homology-based PCR method was applied by designing two sets of degenerate oligonucleotide primers at the regions which are highly conserved among known oxidosqualene cyclase genes. Nested PCRs were carried out to amplify the core fragment of the putative gene for beta-amyrin cyclase in *A. sedifolius* and PCR-RACE was applied for amplification of 3' and 5'-ends. Sequence comparison of the ORF from aster gene showed a high level of similarity with beta-amyrin synthase of *Panax ginseng*, *Betula platyphylla* and *Lotus japonicus* as well as with cycloartenol and lupeol synthase indicating a close evolutionary relationship between sterol and triterpene biosynthesis. The gene of beta-amyrin synthase isolated in *A. sedifolius*, *OXA1*, will be engineered. In this context, a method of genetic transformation was set up in *A. sedifolius* by *A. rhizogenes* wild type containing agropine type plasmid.