

TRANSIENT EXPRESSION OF A GRAPEVINE STILBENE SYNTHASE GENE IN TOBACCO PROTOPLASTS

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Resveratrol (trans-3,5,4'-trihydroxy-stilbene) is a naturally occurring phytoalexin and antioxidant produced by a restricted number of plants such as grapes, peanuts and mulberry.

Stilbenes are phenylpropanoid derivatives and their biosynthesis is induced by fungal infection and abiotic stress such as wounding and UV irradiation. The physiological functions of most stilbenes, especially resveratrol, are well recognized. The health-related properties of resveratrol were indeed investigated intensively over the past two decades and numerous biological activities have been attributed to this molecule rendering very interesting its production by metabolic engineering. The last step of resveratrol biosynthesis is catalyzed by the action of stilbene synthase multigenic family (STS). Likewise chalcone synthase (CHS), the key enzyme in flavonoid biosynthesis in plants, stilbene synthase uses the same substrates but produces different products. Therefore, a single STS gene is sufficient to synthesize resveratrol in heterologous plant species.

Tobacco has a long history as a successful crop system for molecular farming and is therefore one of the strongest candidates for the large-scale production of pharmaceutical macromolecules.

The major advantages of tobacco include the well-established technology for gene transfer and expression, high biomass yield, selfing reproduction and existence of a large-scale processing infrastructure.

The aim of our research is the production of stable transgenic tobacco plants to be used as bioreactors for the molecular farming of resveratrol.

To this target an accurate study of stilbene synthase multigenic family was performed. We used transient expression to verify the expression construct and for localization analysis of STS in tobacco leaves before proceeding to produce transgenic plants. For this purpose a GFP-construct with a stilbene synthase gene isolated from a grapevine cultivar showing high resveratrol production was generated by Gateway[®] technology. The construct was utilized to transform *Nicotiana tabacum* protoplasts where it was expressed at the cytosolic level.

Future goal will be the agroinfiltration of tobacco leaves and the regeneration of transformed plants. The expression and activity of the stilbene synthase gene will be analyzed by determining mRNA accumulation and the detection of resveratrol and resveratrol derivatives in the transgenic lines will be carried out by HPLC analysis.