GENETIC TRANSFORMATION OF TOBACCO AND GRAPEVINE FOR EXPRESSION OF 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE 1 (dxs-1) GENE


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Muscat flavor in grapevine is well-known to be determined by the accumulation of monoterpenic compounds in the ripening berry. In addition, several studies have shown that 1-deoxy-D-xylulose-5-phosphate synthase 1 (DXS-1) is an important regulatory enzyme of the mevalonate-independent pathway involved in the biosynthesis of carotenoids, chlorophyll phytol and terpenoids in bacteria and plants. As a result of a structured association study, we identified a heterozygous non-synonymous mutation of dxs-1 which is strongly associated with Muscat genotypes. In order to validate the role of the gene and to evaluate the possible effects of this mutation, we cloned and sequenced two full-ORF cDNA alleles of the dxs-1 gene from Muscat Blanc berries. Each allele was placed under the strong constitutive 35S promoter of the Cauliflower mosaic virus and inserted in the plasmid pK7WG2 together with the neomycin phosphotransferase gene (nptII) as a selection marker. Embryogenic calli of three cultivars of Vitis vinifera (‘Chardonnay’, ‘Müller Thurgau’ and ‘Brachetto g.l.’) and leaf discs of Nicotiana tabacum were subjected to genetic transformation. Plant materials were co-cultivated with Agrobacterium tumefaciens EHA105 harboring the described plasmids. After two months of culture 8 T₀ plants of tobacco were regenerated from leaf segments and analysed by PCR and Southern blot to confirm the transformation. After a six months culture, embryogenic calli of grapevine are maintained under kanamycin selection onto an embryo differentiation medium to stimulate the regeneration of transgenic plants. Chlorophyll and monoterpenoid contents are currently being quantified in transgenic T₁ plants of tobacco obtained from controlled self-pollination of T₀ lines and will be later determined in transgenic regenerated grapevine plants.