cDNA CLONING AND EXPRESSION OF CAROTENOID BIOSYNTHETIC GENES IN SUNFLOWER


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The biochemistry of carotenoid biosynthesis has been well established. The fundamental steps of carotenoid biosynthesis are the assembly of C40 backbone, the desaturation and cyclization and finally, the xanthophyll formation. The first committed step of carotenoid synthesis, the head-to-head condensation of geranylgeranyl diphosphate molecules to produce phytoene (colourless), is mediated by the enzyme phytoene synthase. Membrane-localized enzymes carry out subsequent steps of the pathway leading to the coloured carotenoids (Sandmann, 2002, Physiol. Plant., 116: 431-440). Genes encoding some of the carotenogenic enzymes have been isolated in bacteria, algae, fungi and higher plants (Sandmann, 2002, Physiol. Plant., 116: 431-440; Giuliano et al. 2002, Trends Plant. Sci., 7: 427-429). However, the regulatory mechanisms that control carotenoid biosynthesis are poorly understood. In this study, we report the isolation of cDNAs corresponding to phytoene synthase (HaPsy) and z-carotene desaturase (HaZds) genes of sunflower and the analysis of their expression in relation to different organs and light-dependent pigment accumulation in chloroplasts.

The reconstructed full-length sequence (1916 bp) of the HaZds cDNA contains a 1761 bp CDS, 62-nucleotides of 5'-untranslated region (UTR), and 77-nucleotides of 3'-UTR (Conti et al., 2004, Plant & Cell Physiol. 45: 445-455). The predicted protein (64.9 kDa) consists of 587 amino acid residues with a putative transit sequence for plastid targeting in the N-terminal region and a typical amino oxidase domain that includes the flavin adenosine dinucleotide (FAD) binding motif. The sunflower Zds gene comprises 14 exons and 13 introns scattered in a ca. 5.0 kb region. Also, sunflower Zds showed a high conservation of the distribution and size of the exons with rice Zds gene (Conti et al., 2004). The full-length sequence (1598 bp) of the HaPsy cDNA contains a 1242 bp CDS, 172-nucleotides of 5'-untranslated region (UTR), and 170-nucleotides of 3'-UTR (Salvini et al., 2004, J. Plant Physiol., in press). The predicted protein (46.8 kDa) displayed a sequence of 414 amino acid residues with a putative transit sequence for plastid targeting in the N-terminal region. The phylogenetic analysis demonstrated that both HaPsy and HaZds were clustered to marigold (Tagetes erecta) Psy and Zds genes, for which showed an overall amino acidic identity of 93.7 % and 96.6%, respectively.

Both HaZds and HaPsy were highly expressed in cotyledons and leaves; by contrast, their transcript levels were comparatively lower in both stems and roots. In addition, HaZds and HaPsy transcript levels were influenced by leaf expansion, which suggested that their expression are regulated during the process of leaf development. The light-dependent enhanced carotenoid production in sunflower...
chloroplasts is concurrent with an increase of both *HaZds* and *HaPsy* transcript accumulation. These results stand in contrast to those obtained in pepper (Simkin et al. 2000, J. Agric. Food Chem., 48: 4676-4680) and in tomato (Simkin et al. 2003, Z. Naturforsch., 58c: 371-380). A possible explanation for these species to species differences may result from the very dissimilar experimental conditions used in these studies. Moreover, although the control of gene expression at the transcriptional level is a key regulatory mechanism, one or more post-transcriptionally control points must be decisive in the regulation of carotenogenesis; therefore one can suggest that some of these mechanisms are prevalent over others in some species.