P 66 Analysis of Endosperm-specific Δ^4-palmitoyl-ACP Desaturase and Acyl Carrier Protein Promoters from *Coriandrum sativum*

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Objectives

Petroselinic acid is an unusual monounsaturated fatty acid which constitutes 85% of total fatty acid composition of coriander (*Coriandrum sativum*) seeds. Endosperm-specific Δ^4-palmitoyl-ACP desaturase and acyl carrier protein were reported to be involved in the biosynthesis of petroselinic acid. To improve our understanding of the overall regulation of petroselinic acid synthesis, we isolated Δ^4-palmitoyl-ACP desaturase and acyl carrier protein genes from *Coriandrum sativum* and their promoters were analyzed in coriander and *Arabidopsis thaliana*

Materials and Methods

1. Materials: Coriander (*Coriander sativum*), *Arabidopsis thaliana*.
2. Methods: Southern and northern blot analyses, isolation of Δ^4-palmitoyl-ACP desaturase and acyl carrier protein genes and their promoters by using PCR and IPCR. Identification of transcriptional initiation sites of two genes by 5′-cRACE. Transient expression in developing coriander seeds by particle bombardments. *Arabidopsis* transformation, Histochemical and fluorometric analyses of β-glucuronidase (GUS) activity, Exogenous application of ABA, Isolation of nuclear proteins and electrophoretic mobility shift assay (EMSA).

Results and Discussion

In previous reports, two Δ^4-palmitoyl-ACP desaturase (4DE) and acyl carrier protein (ACP) were more abundantly expressed in endosperm than leaf tissues of coriander. Southern blot analyses showed that both endosperm-specific ACP and 4DE genes are present as a single copy gene. ACP and 4DE genes including their 5′ flanking region were isolated from coriander by inverse PCR and their transcriptional initiation sites were identified by 5′-cRACE. The isolated 5′ flanking regions of ACP and 4DE were fused to GUS reporter gene, and then transiently expressed in developing coriander endosperm tissues by particle bombardment. To identify cis-elements involved in endosperm-specific expression of coriander ACP and 4DE genes, 5′-flanking regions and their deletion constructs were fused to GUS reporter gene and introduced into *Arabidopsis thaliana* plants by floral dipping method. Histochemical and quantitative analyses of GUS activity in *Arabidopsis* transgenic plants demonstrated that coriander 4DE and ACP genes were highly active in 13 - 17 days after flowering, which is coincident with the period of storage oil accumulation during seed development. ACP promoter was also active in flower tissues except petals, whereas 4DE promoter was seed-specific. *In vivo* activity of coriander ACP promoter was approximately 5-fold higher than that of CaMV35S promoter in developing seeds. Deletion construct analyses indicated that -117/+74 region of ACP and -334/-188 region of 4DE were sufficient to seed-specific expression. By an application of exogenous 50 μM ABA, *in vivo* activity of ACP promoter was induced by approximately 3-fold, whereas *in vivo* activity of 4DE was not significantly induced in developing seeds. EMSA revealed that the -312/-161 region of 4DE is involved in DNA-protein interaction. Further analysis about the effect of ABA on the expression of ACP and 4DE is being carried out and cis-elements involved in ABA signalling will be presented.

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