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Isolation and characterization of the bHLH transcription factor and putative casein kinase interacted with *FAD2* promoter in sesame (*Sesamum indicum*) developing seeds

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Objectives

A study for global regulation factors involved in seed-specific gene expression of the *SeFAD2* gene

Materials and Methods

1. Material

: Plant - sesame (*Sesamum indicum*)

2. Methods:

: Yeast one-hybrid screening, Northern analysis, Cellular localization, EMSA, and Yeast two-hybrid.

Results and Discussion

Microsomal oleic acid desaturase (*FAD2*) catalyzes the first extra-plastidial desaturation in plants, converting oleic acid to linoleic acid. The regulatory mechanism controlling sesame (*Sesamum indicum*) seed-specific *FAD2* expression has been investigated because the content of polyunsaturated fatty acids plays a crucial role in the quality of vegetable oils. It was previously reported that -179 to -53 regions of the *SeFAD2* promoter functions as a positively regulatory element, which contains potential endosperm-specific or ABA related *cis*-elements. Two components showing good-matched sequence homology with the basic region/helix-loop-helix proteins and putative casein kinase II were isolated by yeast one-hybrid screening with the -179 to -53 regions, and named as *SebHLH* and *SePCK*, respectively. The *SebHLH* and *SePCK* transcripts were most abundantly expressed in sesame developing seeds. The *SebHLH::GFP* fusion protein was localized in the nucleus and the *SePCK::GFP* fusion protein was shown in the intracellular space including nucleus. EMSA showed that the recombinant *SebHLH* protein binds E-box (CANNTG) and G-box (CACGTG) elements in -179 to -53 region of the *SeFAD2* promoter. The interaction between the *SebHLH* and *SePCK* proteins was demonstrated by yeast two-hybrid system, suggesting that these two components are probably involved in the regulation of the seed-specific *SeFAD2* gene expression.

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