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Structure and Expression of a Perilla (*Perilla frutescens* Britt) Gene, *PfFAD3*, Encoding the Microsomal ω-3 Fatty Acid Desaturase

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ABSTRACT : Microsomal ω-3 fatty acid desaturase (FAD3) is an essential enzyme in the production of the n-3 polyunsaturated fatty acid α-linolenic acid during the seed developing stage. To understand the regulatory mechanism of the gene encoding the ω-3 fatty acid desaturase, a genomic fragment corresponding to the previously isolated perilla seed *PfFAD3* cDNA was amplified from perilla (*Perilla frutescens* Britt) by GenomeWalker PCR. Sequence analysis of the fragment provided with identification of a 1485-bp 5'-upstream region and a 241-bp intron in the open reading frame. To determine the tissue-specificity of the *PfFAD3* gene expression, the 5'-upstream region was fused to the β-glucuronidase (GUS) gene and incorporated into *Arabidopsis thaliana*. Histochemical assay of the transgenic plants showed that GUS expression was restricted to seed and pollen, showing that *PfFAD3* gene was exclusively expressed in those tissues.

Key words : ω-3 fatty acid desaturase gene, promotor, seed, pollen

INTRODUCTION

Polyunsaturated fatty acids are not synthesized de novo in vertebrates, since they lack the fatty acid desaturase enzymes required for the production of linoleic acid and linolenic acid from oleic acid (Wallis *et al.*, 2002). Hence, polyunsaturated fatty acids are essential nutrients for vertebrates and vegetable oil is one of the most important dietary source of the n-3 highly unsaturated fatty acids (Simapoulos, 1991). Recent studies have shown that an adequate dietary intake of α-linolenic acid has many clinical benefits for our health and considerable information on ω-3 fatty acid desaturase (FAD) involved in the production of α-linolenic acid has accumulated (Crawford *et al.*, 2000). The cDNAs encoding ω-3 fatty acid desaturase have been cloned from various plants including Ababidopsis (Arondel *et al.*, 1992), soybean (Yadav *et al.*, 1993), tobacco (Hamada *et al.*, 1994), mung bean (Yamamoto, 1994), rice (Kodama *et al.*, 1997), and perilla (Chung *et al.*, 1999) and their expression was extensively studied. Especially, the identification of the *PfFAD3* cDNA clone from perilla generated great interest, since perilla seeds contain α-linolenic acid at levels of 40~50% of the total fatty acid content (Lee *et al.*, 1998). Therefore, the objective of this study was to isolate the corresponding genomic clone and assess the tissue-specificity of gene expression.

We isolated a genomic fragment from perilla by GenomeWalker PCR and determined the presence of the fragment of a 1485-bp 5'-upstream region containing putative *cis*-elements, and a 241-bp intron. In order to test the tissue-specificity of gene expression, the 5'-upstream region was fused to the β-glucuronidase (GUS) reporter gene. The fusion gene construct was incorporated into Arabidopsis and the transgenic plants were subjected to histochemical assay. We report here the determination of the tissue-

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specific expression profile of the perilla *PfFAD3* gene.

MATERIALS AND METHODS

DNA analysis : Genomic DNA was extracted from perilla (*Perilla frutescens* Britt) leaves as described (Doyle & Doyle, 1990). Genomic walking was performed on *P. frutescens* DNA to isolate *PfFAD3* 5' upstream fragment according to the GenomeWalker kits (Clontech, CA) using 5'-GCGGCGACGT CCCAAACGAC GTAGCTCAA-3' and 5'-AACCTCTCCA TCAGCGCCAC TCTTCGAGA-3' for gene-specific primers GSP1 and GSP2, respectively. PCR product was extracted from a gel using a gel extraction kit (Q-Biogene, Korea) and cloned into pCR2.1kb-TOPO vector (Invitrogen). Nucleotide sequence was carried out by the dideoxy chain-termination method (Sanger *et al.*, 1977). Analysis of the nucleotide sequence was performed at the NCBI using the BLAST network service. To obtain the 5'-terminal sequence of perilla *PfFAD3* transcript, 5'-RACE was performed using the SMART RACE cDNA amplification kit (Clontech). p*PfFAD3*, a 1485-bp fragment of *PfFAD3* promoter, was obtained from the genomic fragment by PCR and inserted into the *Hind*III-*Xba*I sites of pBI101, forming p*PfFAD3*::GUS.

***Agrobacterium*-mediated transformation of *Arabidopsis* and GUS assay** : The p*PfFAD3*::GUS construct was introduced into *Agrobacterium tumefaciens* strain GV3101 and subsequently transferred into *Arabidopsis* using a floral dip method (Clough & Bent, 1998). Transgenic plants were selected on media containing 30 ㎍/㎖ of kanamycin. Various tissues of transgenic *Arabidopsis* plants were subjected to histochemical GUS assay as described (Jefferson *et al.*, 1987). Samples were stored in 70% ethanol before microscopic observation.

RESULTS AND DISCUSSION

Structure of the *PfFAD3* gene : Four different Genome Walker libraries were constructed using genomic DNA of which each was digested with four different restriction enzymes, *Dra*I, *Eco*RV, *Pvu*II or *Stu*I (Fig. 1). Gene-specific primers GSP1 and GSP2 for genome walking were prepared from the sequence information for the *PfFAD3* cDNA that was published previously (Chung *et al.*, 1999). A 1598-bp genomic fragment that was amplified from a *Pvu*II library was eluted from a gel and subcloned into pCR2.1kb-TOPO vector. The insert was subjected to DNA sequencing and the sequence was compared with that of the *PfFAD3* cDNA. The isolated genomic fragment was found to contain a 1485-bp 5'-upstream region and also an intron of 241 bp in the open reading frame. In the 5'-upstream region, there were apparently few short sequences homologous to plant *cis*-elements. Among them, endosperm-specific elements such as GCN4 motif, RY repeat motif and skn-1 motif, and ABA-inducible elements such as ABRE motif and CE-1 box were identified as putative elements that might act as regulatory elements directing definite and specific spatial expression patterns of the perilla *PfFAD3* gene (Table 1). The endosperm-specific *cis*-elements have been well recognized in other seed-specific genes (Chandrasekharan *et al.*, 2003; Opsahl-Sorteberg *et al.*, 2004). For example, the RY elements were found to mediate high levels of expression in embryos (Chandrasekharan *et al.*, 2003). The

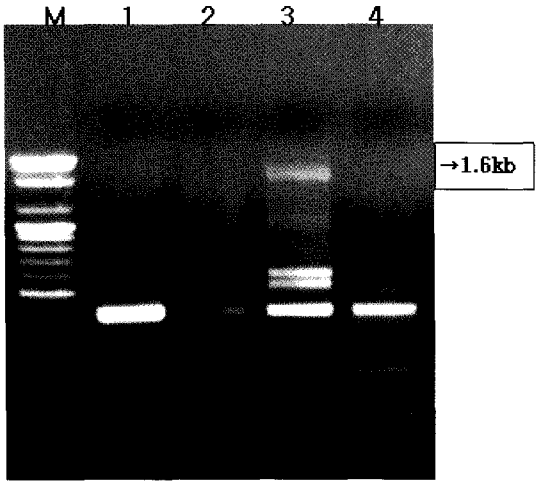

Fig. 1. PCR products generated by genome walking with *P. frutescens* GenomeWalker libraries. Lane M, DNA size marker; lanes 1 to 4, Genome Walker libraries constructed from genomic DNA of which each was digested with *Dra*I, *Eco*RV, *Pvu*II or *Stu*I, respectively. The arrow indicates the PCR product amplified from the *Pvu*II library.

Table 1. Putative *cis*-elements of the 5'-upstream region in *PfFAD3* gene.

Category	Motif	Consensus sequence	Description
Endosperm specific elements and ABA-inducible elements	ABRE motif	ACGTG	ABA responsive element
	CE-1	TGCCACCGC	ABRE associated element, involved in ABA responsiveness
	GCN4_motif	TTAGTCA	Endosperm specific expression
	RY repeat motif	CATGCATT	seed-specific response element
	Skn-1_motif	GTCAT	Required for Endosperm expression
gibberellin-inducible elements	TATC-box	TATCNCA	Gibberellin-responsiveness
	P-box	CCTTTTN	Gibberellin-responsive element

ABA-inducible elements were also found to be very common in other seed-specific genes and might be involved in the developmental regulation of *PfFAD3* expression in seeds (Chung *et al.*, 2004). It was not investigated whether the 241-bp intron was involved in the regulation of the *PfFAD3* gene expression. There was no well-defined regulatory elements found in the intron.

Expression of the p*PfFAD3*::GUS in transgenic *Arabidopsis* plants : Previous work has shown *PfFAD3* expression to be seed-specific (Chung *et al.*, 1999). To further localize gene expression for *PfFAD3*, we used the 5'-upstream region for this gene to construct a reporter gene fusion (p*PfFAD3*::GUS) for stable transformation of Arabidopsis (Fig. 2). At the whole seedling level, GUS activity, detected by histochemical staining, was not visible in any organs or tissues from transgenic Arabidopsis seedlings harboring the p*PfFAD3*::GUS construct (data not shown). However, the *PfFAD3* promoter directed GUS expression exclusively in seed and pollen from a 4-week-old plant while p*CaMV35S*::GUS fusion

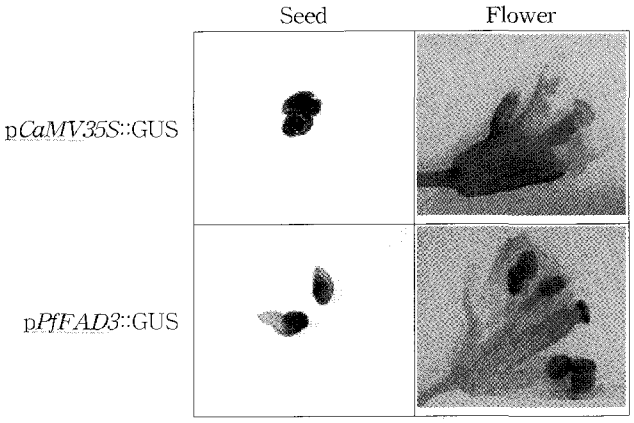

Fig. 3. Histochemical assay of the GUS activity in *Arabidopsis* seedlings transformed with the p*PfFAD3*::GUS fusion construct. Seeds and flowers of transgenic Arabidopsis plants harboring p*CaMV35S*::GUS or p*PfFAD3*::GUS as a transgene were subjected to histochemical GUS assay.

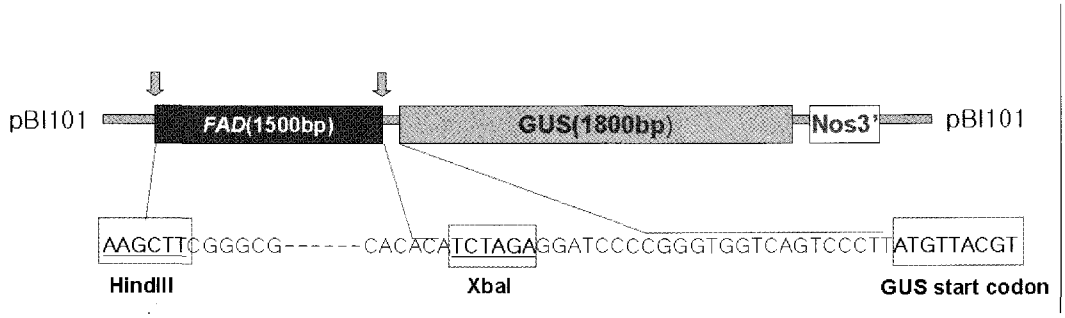

Fig. 2. The physical map of the *PfFAD3* promoter::GUS fusion construct. p*PfFAD3*, 1485-bp 5'-upstream region in the *PfFAD3* gene was amplified by PCR and inserted into *Hind*III-*Xba*I sites of pBI101, forming p*PfFAD3*::GUS.

gene directed GUS expression in all the tissues including seed and pollen from a transgenic plant (Fig. 3).

In conclusion, p*PfFAD3*, the promoter of the *PfFAD3* gene displays stringent spatial regulation, being very highly expressed in seed and pollen and completely silent in other vegetative tissues. Further functional analysis is required to identify a role that the *PfFAD3* gene encoding ω-3 fatty acid desaturase may play in pollen.

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