Encoding of a Cytochrome P450-Dependent Lauric Acid Monooxygenase by CYP703A1 Specifically Expressed in the Floral Buds of Petunia hybrida

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The cDNA clone of novel cytochrome P450 CYP703A1 from petunia floral buds was isolated by RT-PCR. The nucleotide sequences of this cDNA clone contained the open reading frame that has been predicted to encode polypeptides consisting of 539 amino acid residues. A significant high level of the transcript of the cyp703A1 gene was found in the early stage of petunia flower buds, but not in the leaves, stems and roots. The 3′41bp 5′-flanking sequences of the cyp703A1 gene contained the conserved motifs of ATHB-1, AGAMOUS, MYB.Ph3, P and SBF-1 binding boxes. CYP703A1 cDNA was expressed in yeast Saccharomyces cerevisiae A22 cells under the control of an alcohol dehydrogenase 1 promoter and terminator. The recombinant yeast microsomes containing the CYP703A1 hemoprotein were found to metabolize lauric acid. Based on these results, CYP703A1 was specifically expressed in the early stage of flower development and appeared to participate in the monooxygenation of fatty acids.

Key words: cytochrome P450; cDNA cloning; floral bud; Saccharomyces cerevisiae; Petunia hybrida

Cytochrome P450 monooxygenases consisting of a number of cytochrome P450 (P450 or CYP) species and a generic NADPH-cytochrome P450 oxidoreductase (P450 reductase) play an important role in the biosynthesis of a variety of secondary metabolites, as well as in the metabolism of xenobiotics in higher plants. Estimates from current genome projects imply that the number of P450 genes exceeds 400 in Arabidopsis thaliana.2 Over 360 P450 cDNA sequences from higher plants are currently known, but the functions of very few have been identified. Important P450 enzymes whose functions remain elusive or poorly understood are related to the biosynthesis of sterols,3 glucosinolates,4 phenylpropanoids,5 salicylic acid,6 jasmonic acid,7,8 gibberellins,9 abscisic acid,10 brassinosteroids11 and alkaloids,11 and to the metabolism of herbicides including chlorotoluron, bentazone and dioclolfop-methyl.11

It has been reported that flavonoid 3′,5′-hydroxylase (CYP75A3) involved in the biosynthesis of flower pigments12 was expressed in flowers. Nadeau et al. have reported that CYP78A2 was specifically expressed in the pollen tubes of Phalaenopsis.13 These reports have suggested that some P450 species may play important roles in the flowering or flower development process. On the other hand, it has been reported that the presence of a large amount of the co-hydroxy derivative of the C18 fatty acid family exist in plants.13 Furthermore, it has also reported that lipids were the essential factor needed for pollen tubes to penetrate the stigma.14 These reports have suggested that P450 species may play important roles in the recognition of stigma by pollen or in the initial stage of pollen-tube growth during flower development. Although cDNA cloning of the plant P450 species which catalyze the metabolism of fatty acids has been reported,15,16 there are no reports about P450 species specifically expressed in the early stage of flower development which catalyze the monooxidation of fatty acids.

In this study, we attempted to isolate and characterize P450 cDNA encoding the fatty acid monooxygenase which is specifically expressed in the early stage of floral buds in Petunia hybrida. Expression of the novel P450 cDNA clone in Saccharomyces cerevisiae cells and the metabolism of endogenous substrates with microsomal fractions from recombinant yeast cells were examined.

Materials and Methods

Plant materials. Seeds of Petunia hybrida (cv Blue Star) were obtained from Sakata Seeds Co. Japan and grown for nine weeks with a 16-h light and 8-h dark cycle at 25°C in a controlled chamber.

RT-PCR cDNA cloning. PCR primers were designed according to the sequences of five plant P450 species reported as shown in Fig. 1. Poly(A)+ RNA was isolated from a mixture of Petunia flower buds from stages 1 to stages 5 by using a Quickprep Micro mRNA purification kit (Pharmacia Biotech, U.S.A.) (see Fig. 2A). First-strand cDNA was synthesized from 100 ng of poly-(A)+ RNA by using a First-Strand cDNA synthesis kit (Pharmacia Biotech, U.S.A.) according to the protocol of the manufacture. From this cDNA, 10% was used for PCR reactions (10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.5 mM MgCl2, 0.25 mM dNTP each, 2.5 pmol of primers, 2.5 units Taq-polymerase (Parkin Elmer,..
U.S.A.). The temperature program used was 30 sec at 94°C, 2 min at 40°C, 3 min at 72°C and 30 cycles. For the first PCR, the primers used were (primer A) 5'-ATGAAATTCAAGAAAGCTTTGGAAACGCCACCC-3' and (primer D) 5'-ATAAGCTTAAATTTCTTCGTC- CAGCACCCCAATGG-3'. After the first PCR reaction, the second nested PCR was carried out on an aliquot of the first PCR reaction mixture as template DNAs. The PCR primers used for the second PCR were (primer B) 5' - AACTCGAGGTATTCTATATACCCAAAAAC- AC-3' and primer D. The second PCR reaction mixture was digested with restriction enzymes EcoRI and XhoI and then run on 2.0% agarose gel. Around 190-bp bands were extracted by using GeneElute (SUPELCO, U.S.A.). The cDNA fragments purified from the agarose gel were cloned into the pBluescript II SK (+) vector (Stratagene, U.S.A.) and transformed into Escherichia coli strain JM109.

Northern blot analysis. A Poly(A)+ RNA fraction was isolated from petunia flower buds from stage 1 to stage 5 (see Fig 2A). The prepared Poly(A)+RNA fraction was separated on 1.2% (w/v) agarose gel under denaturing conditions (formaldehyde) and then transferred to a Hybond-N+ nylon membrane (Amersham, U.S.A.) with 20 x SSC as a transfer buffer according to the standard procedure. The probes were labeled with 32P-labeled dCTP by the Klenow reaction for hybridization which was carried out at 65°C with 6 x SSC, 7.5 x Denhardt's reagent, 200 µg/ml of salmon sperm DNA and the labeled probes for 18 h. After hybridization, the filters were washed for 30 min in 300 ml of a solution of 1 x SSC and 0.1% SDS at room temperature, and then again for 30 min in a solution of 0.1 x SSC and 0.1% SDS at 60°C. The radioactivity on the filter was measured with a BAS2000 image analyzer (Fuji Film Co., Tokyo, Japan).

Screening of the cDNA library of petunia floral buds. The cDNA library was constructed from the poly(A)+ RNA fractions prepared from Petunia flower buds at stage 2 (see Fig 2A) by using Uni-ZAPII as a library vector. Screening of the library was carried out according to the general method described in the manufacturer's manual. cDNA clone CLONE-2 prepared by RT-PCR was used as a probe for screening the cDNA library. For the first screening, hybridization was performed with cDNA probes of CLONE-2 labeled with a random primer oligo DNA labeling kit (Takara CO., Japan), using 32P-labeled dCTP, at 65°C for 16 h in a hybridization buffer (6 x SSC, 5 x Denhardt’s solution, 0.1% SDS, and 100 µg/ml of salmon sperm DNA). After hybridization, the filters were washed for 30 min in 500 ml of a solution of 1 x SSC and 0.1% SDS at room temperature, and then again for 30 min in a solution of 0.1 x SSC and 0.1% SDS at 60°C. Secondary screening was carried out under the same hybridization and washing conditions as those used for the first screening. After the secondary screening, the positive clones were subcloned into the pBluescript II SK (+) plasmid.

Cloning of the 5’ flanking region of the cyp703A1 gene with IPCR. To clone of the 5’ flanking region of the cyp703A1 gene with IPCR, restriction digestion was carried out by using 1 µg of petunia genome DNA treated with 10 units of XbaI. For circularization, 0.5 µg of an appropriate restriction fragment was diluted to a concentration of 1 ng/ml in a ligation buffer (50 mM Tris-HCl at pH 7.4, 10 mM MgCl2, 10 mM dithiothreitol and 1 mM adenosine triphosphate). The ligation reaction was initiated by adding T4 DNA ligase to a concentration of 20 units/ml, and the reaction was allowed to proceed for 12 h at 15°C. The PCR reaction was performed with a sample containing 0.5 ng of circularized DNA obtained as just described in the presence of 50 pmol of each primer and 500 mM dNTPs. Two PCR reactions were carried out with PCR primers corresponding to the N-terminal region of cloned CYP703A1 cDNA. At first, primer 2-1 and primer 2-2 were used for PCR reactions, and then a second nested PCR reaction was performed with primer 2-3 and primer 2-4, and 5 µl of the sample from the first PCR. The same conditions for the PCR reactions were used, the positions of the PCR primers being shown in Fig. 3. The nucleotide sequences of the primers used were as follows: primer 2-1, 5’-AAACAAGATTGGGACATATC- GACC-3’; primer 2-2, 5’-TAGTTCAATTTTTCTCCC- ATCGAGAGG-3’; primer 2-3, 5’-TATACGCAGAATAGGACCTAGAATAATGG-3’; and primer 2-4, 5’-AACAGAAAATCATCGACTCCCTCC- TG-3’. The temperature program involved 1 min at 94°C, 2 min at 60°C, 3 min at 72°C, and 30 cycles. The PCR products from the second PCR reaction were separated on 0.7% agarose gel, and the DNA bands were purified and ligated to the T-vector (Novagen, U.S.A.).

Southern blot analysis. Genomic DNA was isolated from 5 g of the leaves of Petunia hybrida by using ISOPLANT from Wako Chemical Co., (Osaka, Japan). For the Southern blot analysis, 20 µg of the DNA fraction was digested with the restriction enzymes indicated in the Results section, separated on 1.2% (w/v) agarose gel and then transferred to a Hybond-N+ nylon membrane (Amersham, Japan) with 10 x SSC. For hybridization, the probes were labeled with 32P-labeled dCTP by the Klenow reaction. Hybridizations were conducted with at 65°C 6 x SSC, 7.5 x Denhardt’s reagent, 200 µg/ml of salmon sperm DNA and the 32P-labeled probes for 18 h. After hybridization, the filters were washed for 30 min in 300 ml of a solution of 1 x SSC and 0.1% SDS at room temperature, and then again for 30 min in a solution of 0.1 x SSC and 0.1% SDS at 60°C. The radioactivity on the filter was measured by a BAS 2000 image analyzer.

Construction of expression plasmid pAHPF20 for CYP703A1 cDNA in the Saccharomyces cerevisiae yeast. Expression vector pAAH5 was used for expressing CYP703A1 cDNA in S. cerevisiae. The PCR methods was used to add the HindIII sites to the DNA sequences corresponding to both the N- and C-terminal regions of IMT-2 cDNA. Expression plasmid pAHPF20
was constructed by ligating the digested modified PCR products with HindIII and digesting pAAH5 with HindIII. Transformation of S. cerevisiae AH22 cells with expression plasmid pAHPF20 was carried out by the lithium chloride method as described previously. Reduced CO-difference spectra of microsomal fractions prepared from the transformed yeast cells were measured according to the method of Oeda et al. The P450 hemoprotein contents in the microsomal fractions were determined from the spectra by using an extinction coefficient of 91 mM−1 cm−1.

**Assay for monoxygenase activity.** The metabolism of lauric acid was assayed for microsomal fractions prepared from the recombinant yeast strains of both AH22/pAAH5 and AH22/pAHPF20 cells. The reaction mixture in 200 µl of a 100 mM potassium phosphate buffer (pH 7.4) contained 0.5 mM NADPH, 60 pM [1-14C]lauric acid (50 µCi/ml) and 50 pM P450 protein. The reaction was carried out at 30°C for 1 h and then stopped by adding 1 ml of ethyl acetate. The ethyl acetate layers were collected and dried. The resulting residues were dissolved in 10 µl of methanol and then spotted on to a thin-layer silica gel plate (Kieselgel 60F254, Merck, Darmstadt, Germany). The thin-layer plate was developed by a solvent system of diethyl ether/petroleum ether/formic acid (70:30:1), and the radioactivity on the TLC plate was measured with a BAS 2000 image analyzer.

**DNA sequence and computer analysis.** DNA sequences were analyzed with ABI 310 (PE Applied Biosystems, Foster, CA, U.S.A.) and 5500L (Hitachi, Tokyo, Japan) instruments. The sequence data were analysed by GENETYX-MAC, version 7.3 (Software Development Co., Tokyo, Japan), and the resulting DNA sequences were searched for in the DDBJ, EMBL and GenBank Nucleotide Sequences databases.

**Results**

cDNA cloning of the partial sequence of novel P450 with RT-PCR

We isolated the cDNA clone of novel P450 expressed in the flower buds of petunia by RT-PCR. The PCR primers used were designed according to the sequences of five reported P450 plant species. Figure 1 shows the region between the 360th and 450th amino acid residues of the five P450 species that were aligned. The region was divided into the four domains of A, B, C and D based on their sequence similarity. The PCR primers were synthesized according to the amino acid sequences of domains A, B and D. PCR amplification was carried out with these primers and with template first-strand cDNA that had been synthesized from a mixture of the poly(A)+RNA fractions prepared from petunia flower buds at five different stages as shown in Fig. 2A. The amplified DNA fragments migrated on 2.0% agarose gel as diffuse bands ranging between 250bp and 270bp. When we used primer B and primer D for nested PCR, DNA bands ranging between 180bp and 210bp were obtained. The PCR products of 190bp obtained from nested PCR were cloned into pBluscript II SK(+) . Two hundred randomly selected clones were each sequenced, and the sequences were compared with those of the reported P450 plant species reported by using the BLAST search program. CLONE-2 from nested PCR was found to encode a novel P450-like sequence. Five CLONE-2 clones were obtained from a sequence analysis of randomly selected clones.

**Northern blot analysis**

The Northern blot analysis was carried out by using CLONE-2 as a DNA probe for stages 1 to 5 of flower development and for the roots, stems and leaves of petunias (Fig. 2A). As shown in Fig. 2B, the level of transcripts hybridized with CLONE-2 was the highest at stage 2. On the other hand, no transcripts of CLONE-2 were detected in the leaves, stems and roots (Fig. 2B). Thus, the genes corresponding to CLONE-2 were found to be specifically expressed in the early floral bud stage of petunias.

cDNA cloning

The CLONE-2 clone was used as a probe for screening the cDNA libraries prepared from the poly(A)+RNA fractions from stage 2 of the petunia flower. After the secondary screening, cDNA clones designated as IMT-2 were obtained by using CLONE-2 as a DNA probe. Based on the DNA sequence, IMT-2 cDNA was found to contain 1,617bp inserts with open reading frames encoding polypeptides consisting of 536 amino acid residues. The cDNA sequence of IMT-2 is shown in Fig. 3. A computer search of the SWISS PROT database revealed that the deduced amino acid sequences encoded on IMT-2 contained domains A, B, C and D which are highly conserved among the P450 gene families. D.R. Nelson and the Committee for Standardized Cytochrome P450 Nomenclature have officially named IMT-2 as CYP703A1.

Cloning of the 5’ flanking region of the cyp703A1 gene

Use of the sequence information on the N-terminal region of CYP703A1 for designing the IPCR primers enable us to clone the 1,041bp 5’-flanking region of the cyp703A1 gene from petunia genome DNA. As shown in Fig. 4, a putative TATA box was found at -45bp in the 5’-flanking region of the cyp703A1 gene. The putative binding motifs and binding positions of ATHB-1, AGAMOUS, MYB, P3' and SBF-1 were also found in the 5’-flanking region of the cyp703A1 gene (Fig. 4).

Southern blot hybridization

Southern blot hybridization was also carried out to examine the number of cyp703A1 genes in the genome of the petunia. One band, three bands and two bands were observed after restriction digestion of petunia genomic DNA with restriction enzymes EcoRI, HindIII and Dral, respectively. These results indicate that the petunia genome contains only a few of these genes, although a high degree of sequence similarity was found in the cyp703A1 gene (Fig. 5).
### Table: P450 species and Domain

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Fig. 1. Multiple Alignment of the Amino Acid Sequences of CYP71A1, CYP71A2, CYP73A2, CYP75A3 and CYP80 for Designing the PCR Primers.

Four domains A, B, C and D located between the 340th and 450th amino acid residues were separated from each other by three short segments with limited sequence similarity as shown in a line. An asterisk shows the position of cysteine conserved in the heme-binding region of the P450 species. PCR primers for RT-PCR are shown with arrows, and amino acids are designated by a single-letter code. The accession numbers of the P450 genes in GeneBank are CYP71A1, M32885; CYP71A2, X71654; CYP73A2, L07634; CYP75A3, Z22544; CYP80, U09610.

![Stage 1-5](image)

Stage 1 2 3 4 5

![Stage 1-8](image)

1 2 3 4 5 6 7 8

Fig. 2. Northern Blot Analysis of the Poly (A)+ RNA Fractions Prepared from Different Stages of Petunia Floral Buds.

A. Floral development was divided into 5 stages: stage 1, floral buds with a length of 1-2 mm.; stage 2, floral buds with a length of 2-4 mm.; stage 3, floral buds with a length of 5-12 mm.; stage 4, floral buds with a length of 12-30 mm.; stage 5, mature flower. B. Northern blot analysis. Poly(A)+ RNA fractions from stages 1-5 and from the leaf, stem and root were electrophoresed and transferred to a Hybond-N membrane. Two micro gram of the poly(A)+ RNA fraction was applied to each lane. Lane 1, mRNA in stage 1; lane 2, mRNA in stage 2; lane 3, mRNA in stage 3; lane 4, mRNA in stage 4; lane 5, mRNA in stage 5; lane 6, mRNA in leaf; lane 7, mRNA in stem; lane 8, mRNA in root. The cDNA clone of CLONE-2 was used as a DNA probe.

**Sequence analysis**

Multiple alignment of the amino acid sequences of CYP703A1 with those of the reported P450 plant species was carried out. The similarity scores for the amino acid sequences show that the highest sequence similarity of CYP703A1 was 39% with CYP71A1 (related to the ripening process of avocado fruit),[20] and the similarity with CYP75A3[11] and CYP92A2 (preferentially expressed in tobacco during the hypersensitive reaction)[32] was 33% and 37%, respectively. The N-terminal region of CYP703A1 contained a hydrophobic domain corresponding to the membrane anchor sequences of the microsomal P450 species. A dendrogram showed that CYP703A1 belongs to the same cluster of P450 species CYP84A1 (ferulate-5-hydroxylase),[33] CYP73A1 (cinnamate 4-hydroxylase),[34] CYP80A1 (berbamanine synthase),[23] CYP75A3, CYP75A1 (flavanoid 3', 5' hydroxylase),[11] and CYP75A2 (flavanoid-3', 5'-hydroxylase),[35] which are related to the phenylpropanoid biosynthesis pathway (Fig. 6).

**Expression of CYP703A1 cDNA in yeast**

We attempted to express the cDNA clones of CYP703A1 in yeast. pAHPF20, a multicopy plasmid, carried the coding regions of CYP703A1 corresponding to the first methionine at the C-terminal between the alcohol dehydrogenase (ADH) I promoter and terminator of expression vector pAAH5. Figure 7A shows the reduced CO-difference spectra of a microsomal fraction prepared from transformed yeast AH22/pAHPF20 cells with a peak at 447 nm, the content of a p450 molecule being estimated to be 47 pmol P450 equivalent/mg of protein in the microsomal fraction. As shown in Fig. 7B, lactic acid being used as a substrate, metabolites-1, metabolites-2 and metabolites-3 were found. Thus, CYP703A1 was found to metabolize lactic acid.

**Discussion**

We isolated the cDNA clones of novel P450 CYP703A1 from petunia flower buds by RT-PCR, using three primers specific to the domains conserved among the P450 plant species that have been reported. The cyp703A1 gene was found to be expressed specifical-
ly at the early stage of flower development of the petunia, but not in its leaves, stems or roots. The level of transcripts hybridized with CYP703A1 was the highest at stage 2 (Fig. 2B). The 5’-flanking region of the cyp703A1 gene contained the conserved DNA-binding motifs of plant genes expressed in the flower (Fig. 4). ATHB-1 is a homeodomain-binding protein in Arabidopsis thaliana, and may be involved in the differentiation and/or development of higher plants.

AGAMOUS is a floral homeotic gene required for normal flower development in Arabidopsis thaliana. It is known that ATHB-1 and AGAMOUS may play important roles during flower development. On the other hand, MYB-Ph3 is involved in regulating the flavonoid biosynthetic genes in Petunia hybrida. The Zea mays P gene has been identified to regulate the biosynthetic pathway of a flavonoid-derived pigment in floral tissues. SBF-1 binding sites have previously been identified in
the promoter region of the chalcone synthase gene in beans. These results indicate that MYB.Ph3, P and SBF-1 can control the expression of genes encoding the intermediate of plant pigments in flowers. Therefore, under the control of these DNA-binding proteins, the cyp703A1 gene may be specifically expressed in the early stage of the petunia flower. Many of the P450 plant genes involved in normal flower morphogenesis, and in the biosynthesis of flower pigments and plant hormones, are expressed at the early or middle stage of flower development. These results imply that the function of the CYP703A1 enzyme may be involved in the process at the early stage of flowering or flower development in the petunia.

CYP703A1 shared the highest similarity of 39% in amino acid sequence with that of CYP71A1 from avocado fruit. CYP71A1 may be related to the ripening process of avocado fruit. The branching patterns of the dendrogram in Fig. 6 reveal that CYP703A1 belongs to a different branch of CYP51 (obtusifoliol 14 alpha-demethylase), CYP90 (teasterone-23-hydroxylase), CYP88A1 (13-hydroxylase in the gibberellin biosynthesis pathway) and CYP74A1 (allene oxide synthase). Therefore, CYP703A1 is likely to have evolved with the P450 family that is involved in the biosynthesis and metabolism of secondary metabolites, including phenylpropanoids rather than steroids and terpenoids. In the phenylpropanoid biosynthesis pathway, cell wall constituents (lignin), pigments (flavonoids), ultraviolet protectants (coumarins and flavonoids), and plant defense compounds (phytoalexins) are included. Since the hypersensitive response in plants is associated with the activation of a wide range of defense-related genes in the biosynthesis process of plant secondary metabolites, CYP703A1 may also play a role in the biosynthesis of plant defense compounds.

In order to analyze the enzymatic function of CYP703A1, we used a yeast expression system and subsequent enzyme assay. In general, in order to improve the expression levels of P450 proteins in yeast cells or E. coli, modification of the N-terminal sequences of cDNA has been used. Although modifications of the N-terminal sequences of cDNA is effective to improve the expression levels of P450 proteins, the substrate specificity of P450 proteins may be changed by a modification of the N-terminal amino acid sequence. To prevent such a problem, CYP703A1 was expressed in the yeast microsomal fraction without any modification of the N-terminal sequence of CYP703A1.

It was found that CYP703A1 in yeast microsomes metabolized lauric acid. The biological roles and substrate specificity of P450 plant cytochrome species involved in fatty acids are poorly understood. The presence of a large amount of the α-hydroxy derivative of the C18 fatty acid family in plant stigmas suggests...
Fig. 7. Reduced CO-differential Spectrum of the Recombinant Yeast Microsomes and TLC Analysis of the Lauric Acid Metabolites.

(a) Reduced CO-differential spectrum of the microsomal fraction from recombinant yeast strain AH22/pAHPF20. The microsomal fraction (10 mg of protein) was reduced by adding sodium dithionite. After bubbling the reduced microsomal fraction with CO for 1 min, the spectrum was measured. (b) TLC analysis of [1-14C]laurie acid and its metabolites. The metabolism of laurie acid was assayed in microsomal fractions prepared from the recombinant yeast strains of both AH22/pAAHS and AH22/pAHPF20. A reaction mixture in 200 μl of a 100 mM potassium phosphate buffer (pH 7.4) contained 0.5 mM NADPH, 60 mM [1-14C]laurie acid (50 μCi/ml) and 50 mM P450 protein. After extracting with ethyl acetate, each sample was spotted on a thin-layer silica gel plate and then developed by a solvent system of diethyl ether/petroleum ether/formic acid (70:30:1). The radioactivity on the TLC plate was measured by a BAS 2000 image analyzer. Solid arrows show [1-14C]lauric acid, metabolite-1, metabolite-2, metabolite-3, and the starting point for the TLC analysis. Each lane shows samples as follow: lane 1, without microsomal protein; lane 2, microsomal proteins (100 μg) from recombinant yeast strains of AH22/pAAHS; lane 3, microsomal proteins (100 μg) from recombinant yeast strains of AH22/pAHPF20.

that they may play a role in recognition of the stigma by pollen. It has recently been reported that lipids are the essential factor needed for pollen tubes to penetrate the stigma. Thus, CYP703A may play a role in stigma recognition or in the initial stage of pollen-tube growth during flower development. Moreover, cutins and suberins, which prevent water loss and chemical penetration and protect plants from microbial attack, are mainly constituted of hydroxylated fatty acids. These reports suggest that CYP703 proteins may also participate in the biosynthesis pathways of plant defence compounds.

Further studies on the petunia cyp703A gene should lead to a better understanding of its role in the process of floral bud development.

Acknowledgments

We thank Dr. David R. Nelson of the Cytochrome P450 Nomenclature Committee for assistance in assigning the CYP703A1 sequence to the cytochrome P450 gene family.

References


