Acta Biochimica Polonica

Vol. 49 No. 1/2002

29–42

QUARTERLY

# Yellow lupine gene encoding stearoyl-ACP desaturase — organization, expression and potential application✪

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Received: 19 September, 2001; revised: 3 December, 2001; accepted: 4 February, 2002

**Key words***:* stearoyl-ACP desaturase, fatty acids, yellow lupine, nitrogen fixation, root nodules

**A gene for the $\Delta^9$ desaturase specific to stearoyl-ACP (acyl carrier protein) was identified from yellow lupine (*Lupinus luteus*) cDNA and genomic libraries through the differential display method. The desaturase transcript appears in plants infected with *Bradyrhizobium* sp. *(Lupinus)* as revealed by Northern hybridization, RT-PCR and expression of $\beta$-glucuronidase under the desaturase promoter. A small amount of desaturase transcript was also detected in uninfected plants, which suggests that the gene does not belong to the strict nodule-specific sequences. The desaturase provides unsaturated fatty acids for additional cell membrane synthesis. During nodule and symbiosome development a peribacteroid membrane is formed and the requirement for membrane surface increases, thus the level of desaturase expression is also higher. Transgenic plants of *Nicotiana tabacum* with overexpression of the full-length lupine stearoyl-ACP desaturase sequence were obtained. They revealed higher content of unsaturated fatty acids (especially oleic acid) in comparison with control plants.**

Desaturases catalyze the conversion of saturated fatty acids to unsaturated acids introducing the first double bond into saturated fatty acids (Harwood, 1980; Stumpf, 1980). This group of enzymes has been identified in all eukaryotes, cyanobacteria and in some *Bacillus* bacteria (Bloomfield & Bloch, 1960; Fulco, 1974). Three types of desaturases are distinguishable depending on the kind of compounds esterified to fatty acid. The fatty acid can be attached to acyl carrier protein (ACP), to coenzyme A (CoA) or to lipid molecules. The only known soluble desaturase is the plant stearoyl-ACP desaturase specific to

✪This research was supported by the Polish-French Plant Biotechnology Center and by the UNESCO-PAS Network for Molecular and Cellular Biology.

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**Abbreviations:** ACP, acyl carrier protein; GUS, $\beta$-glucuronidase.

stearic acid, localized in plastids. Desaturases identified in plants, animals and yeast are membrane-bound proteins with acyl chain attached to CoA or lipids.

Desaturation proceeds under oxidizing conditions (Bloomfield & Bloch, 1960; Mudd & Stumpf, 1961) and requires a short electron transport chain. Two electrons are necessary for the formation of one double bond. There are two systems of electron transport. One is present in plastids, while the other is localized in the endoplasmic reticulum. In plants, the role of an electron donor is played by NADPH, while ferredoxin-$NADP^+$ oxidoreductase functions as a flavoprotein and ferredoxin with its iron-sulfur center is the electron carrier (Nagai & Bloch, 1966; Schmidt & Heinz, 1990; Wada *et al.,* 1993). The source of electrons in photosynthetic tissues under light condition is photosystem I (Jacobson *et al.,* 1974). In the endoplasmic reticulum, the electron donor is NADH and cytochrome $b_5$ reductase plays the role of flavoprotein, while cytochrome $b_5$ (a heme protein) functions as an electron carrier (Dailey & Strittmatter, 1979; Hackett & Strittmatter, 1984; Spatz & Strittmatter, 1971).

The chemical bond between carbon and hydrogen in a fatty acid chain is one of the most stable bonds. A metal cofactor is essential for the breakage and further modification of this bond. Crystallization of plastid $\Delta^9$-18:0-ACP desaturase from *Ricinus communis* has revealed that this enzyme requires two iron atoms for its catalytic activity (Shanklin & Sommerville, 1991; Thompson *et al.*, 1991; Fox *et al.*, 1993). The conservative region $(D/EX_2H)_2$ was identified in soluble plant desaturases and other di-iron proteins, such as methane monooxygenase and ribonucleotide reductase (Fox *et al.,* 1993). The stearoyl-ACP desaturase from *Ricinus communis* is a homodimer consisting of two 41.6 kDa units. The secondary structure of the desaturase consists of 11 $\alpha$ helices and two $\beta$ strands. The active site is localized within a tetrahelix core bundle. A hydrophobic channel is necessary for substrate orientation in the vicinity of di-iron center. Glutamic acid and histidine residues coordinate two iron atoms essential for catalytic activity.

Plant desaturases have gained a wide interest due to their potential biotechnological significance. These enzymes expressed in transgenic crops can modify fatty acid spectrum, which might be useful for the production of healthy edible oils with an increased level of unsaturated fatty acids.

## MATERIALS AND METHODS

### Identification of stearoyl-ACP desaturase cDNA, screening of a genomic library and Southern hybridization

Identification of stearoyl-ACP desaturase cDNA clone (Gen Bank acc. no. AF 139377) and sequence alignment with other plant desaturases were published earlier (Swiderski *et al.,* 2000). RNA differential display reactions were carried out according to (Liang *et al.,* 1993; Liang & Pardee, 1992) as described elsewhere (Swiderski *et al.,* 2000). A genomic library of yellow lupine DNA constructed in $\lambda$ EMBL-3 (Stratagene) was screened using an [$\alpha$-$^{32}$P]dATP labeled desaturase cDNA probe. Nitrocellulose membranes were hybridized at 60°C for 16–20 h and washed two times in 4 × SSC/0.1% SDS at 60°C, two times in 2 × SSC/0.1% SDS at 60°C and two times in 1 × SSC/0.1% SDS at room temperature.

### Northern hybridization

Total RNA isolated from lupine tissues was separated on 1.2% agarose gel with 2% formaldehyde. After nucleic acid transfer to a nylon membrane it was hybridized with an [$\alpha$-$^{32}$P] dATP labeled desaturase cDNA at 42°C for 48 h. The membrane was washed two times in 2 × SSC/0.1% SDS at room temperature, once in 1 × SSC/0.1% SDS at 68°C and once in 0.1 × SSC/0.1% SDS at 68°C.

### RT-PCR reaction

Two micrograms of total RNA isolated from yellow lupine tissues was mixed with reverse transcriptase buffer and 50 pmol of oligonucleotide primer specific to the 3′ end of desaturase cDNA. The mixture was incubated at 65°C for 10 min and finally at 42°C for 1 h. Reverse transcription reaction was started with the addition of 20 units of MMLV transcriptase and 1 mM of each dNTP. The reaction was performed at 37°C for 1 h. The enzyme was inactivated at 65°C for 10 min.

### Plant transformation

***Alfalfa transformation.*** Alfalfa transformation was obtained by wound inoculation. Leaf explants of alfalfa were cocultivated with a bacterial suspension of *Agrobacterium tumefaciens* EHA105 strain grown overnight (YEB medium, kanamycin and rifampicin resistance). Leaf fragments were incubated for 72 h in the dark on a nonselective medium and for 4–5 weeks in the dark on the selective medium SHMab with kanamycin and carbenicylin. Then the growth conditions were changed to light/dark (16 h/8 h) and after 3 weeks an embriogenic callus was obtained on SHM2 medium with kanamycin and carbenicylin. Growing plants were transferred to soil. After 3 weeks the plants were inoculated with *Bradyrhizobium meliloti* bacteria to induce symbiotic root nodule formation. Alfalfa roots, stems, leaves, flowers, seeds and nodules between 3–4 weeks after rhizobial inoculation were used for further analyses.

***Tobacco transformation.*** Transformation of tobacco was performed by cocultivation of leaf explants with a bacterial suspension of *A. tumefaciens* EHA105 and LBA4404 strain grown overnight (YEB medium, BASTA herbicide and rifampicin resistance). Leaf fragments of tobacco were grown on MS medium for two days. Then the explants were transferred onto T1 medium with BASTA herbicide and rifampicin for transgenic plant selection. Leaves were used for lipid content analysis using gas chromatography.

## RESULTS

### cDNA clone encoding stearoyl-ACP desaturase from yellow lupine

The yellow lupine desaturase cDNA was identified by the differential display as a sequence expressed in root nodules after inoculation with symbiotic bacteria (Swiderski *et al.,* 2000). Alignment, based on putative amino-acid sequence allowed us to classify the lupine enzyme as a desaturase specific to stearic acid attached to acyl carrier protein. Two di-iron motifs separated by 100 amino acids were found within the active center of the enzyme. These motifs are essential for catalytic activity and their amino-acid sequence is characteristic of a soluble group of acyl-ACP desaturases (Fox *et al.,* 1993).

### Screening of a genomic library

The yellow lupine desaturase cDNA clone was labeled with [$\alpha$-$^{32}$P]dATP and used as a probe to screen genomic library constructed in $\lambda$ EMBL-3 phage. Strong single hybridization signals were used as a material to phage DNA isolation and restriction analysis (Fig. 1A). Southern hybridization with labeled desaturase cDNA revealed several hybridization signals providing additional indication that the analyzed sequence encodes the desaturase (Fig. 1B).

### Organization and nucleotide sequence of the genomic clone encoding stearoyl-ACP desaturase

To establish the orientation of the genomic clone, PCR (Expand Long Template PCR System) was performed using phage template and two oligonucleotide primers specific to the right and left arms of $\lambda$ EMBL-3 phage and a

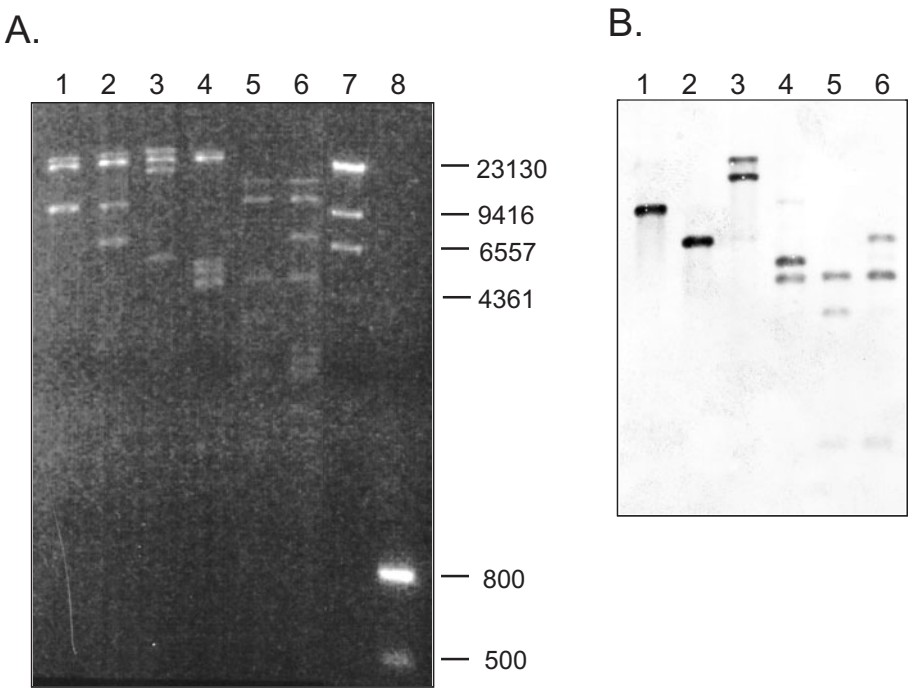


**Figure 1. Southern hybridization of a yellow lupine desaturase genomic clone in phage vector with digoxygenin labeled desaturase cDNA.**

A. Agarose gel electrophoresis with separated restriction fragments of a desaturase genomic clone digested with the following enzymes: Lane 1, *Sal*I; Lane 2, *Sal*I/*Eco*RI; Lane 3, *Eco*RI; Lane 4, *Sal*I/*Hin*dIII; Lane 5, *Sal*I/*Pst*I; Lane 6, *Pst*I; Lane 7, $\lambda$ *Hin*dIII DNA marker; Lane 8, DNA marker consisting of two bands: 800 and 500 nucleotides. B. Nylon membrane with digested DNA fragments of phage genomic clone isolated from $\lambda$ EMBL 3 library. Hybridization signals appeared after incubation with a digoxygenin labeled desaturase cDNA clone.

primer specific to the 5′ end of desaturase cDNA clone. The reaction revealed that the 5′ end of the genomic clone of desaturase is localized close to the left arm of the phage. Sequencing results indicated that the clone did not contain the complete sequence of the stearoyl-ACP desaturase gene and included only the coding region with promoter. To obtain the complete genomic sequence, PCR was performed using yellow lupine genomic DNA template (Fig. 2). Figure 3 presents the organization of the cDNA and the genomic clone of the stearoyl-ACP desaturase gene from lupine.

### Northern blot and RT-PCR analyses of lupine desaturase gene expression

Total RNA preparations isolated from different organs of yellow lupine (stem, petiole, leaf, flower, pod, green seeds, root, and nodule-like structures or nodules 4, 6, 9, 12, 19, 28, 36, 45 days after inoculation with *Bradyrhizobium* sp. (*Lupinus*) were transferred on a nylon membrane and hybridized with radioactive desaturase probe (Fig. 4A). Control hybridization was performed with radioactive labeled leghemoglobin probe that served as a marker of effective symbiosis (Fig. 4B). Northern hybridization confirmed nodule specificity of the desaturase. Its transcript appears on day 12 after symbiotic bacteria inoculation and the highest expression is observed 19 days after the infection. The expression pattern of the desaturase gene as revealed by Northern blot hybridization is similar to that of leghemoglobin gene. RT-PCR analysis revealed that desaturase mRNA appears 9 days after inoculation. Moreover, from this analysis it follows that this enzyme cannot be qualified as a strict nodulin since a low amount of the transcript was detected in stem, pod, leaf as well as in uninfected root (Fig. 4C).

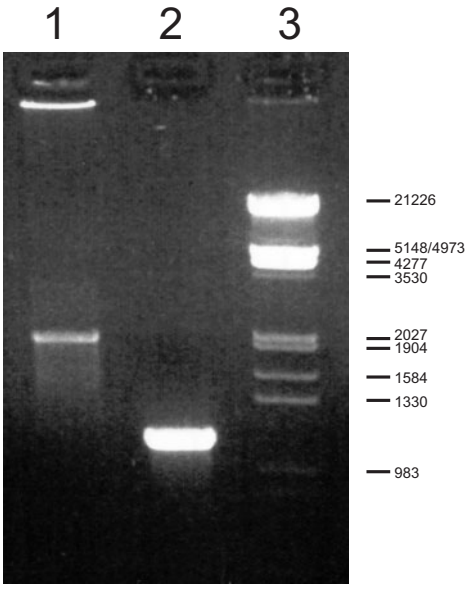


**Figure 2. Agarose gel electrophoresis of PCR amplification product of the desaturase gene with primers specific to the 5′ and 3′ ends of desaturase coding sequence.**

Lane 1. PCR product including a full genomic clone encoding desaturase. Genomic DNA isolated from yellow lupine plants used as a template. Lane 2. PCR control reaction with desaturase cDNA clone in pBluescript vector as a template. Lane 3. λ *Eco*RI/*Hin*dIII DNA marker.

## Functional analysis of lupine desaturase promoter in transgenic *Medicago truncatula* plants

The lupine desaturase gene promoter region was introduced into the transformation vector pPR97 and the construction was used to transform *Medicago truncatula* plants *via A. tumefaciens* strain EHA 105. Transgenic plants were analyzed by PCR with oligonucleotide primers specific to the desaturase promoter. Fragments of transgenic alfalfa plants were then tested for the β-glucuronidase (GUS) reporter gene activity. Blue color that confirmed GUS expression was observed in the nodules, root meristems, seeds and pollen grains (Fig. 5).

## The lupine stearoyl-ACP desaturase encoding sequence was used to modify fatty acids content in transgenic tobacco plants

To investigate whether over expression of the lupine desaturase encoding sequence affects the ratio of saturated and unsaturated

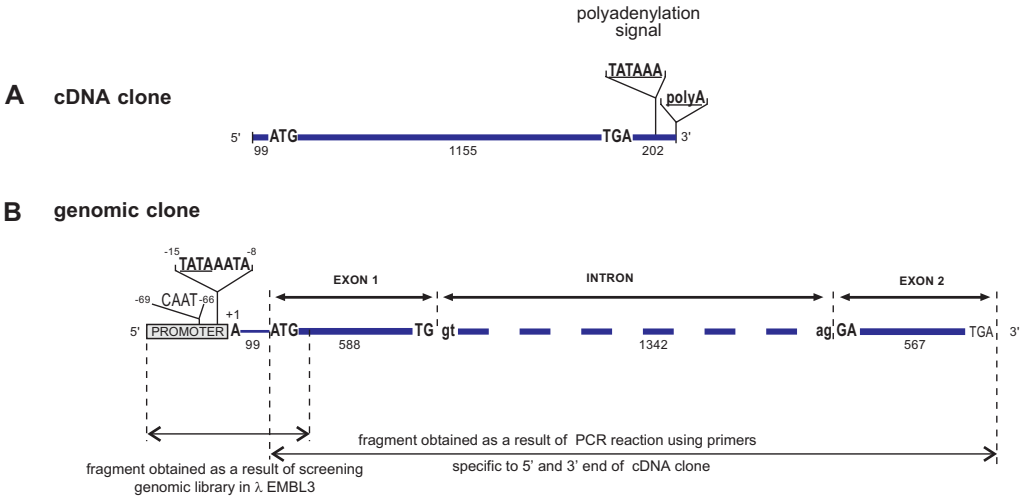


**Figure 3. Yellow lupine stearoyl-ACP desaturase gene organization.**

A. cDNA clone coding for desaturase with start (ATG) and stop (TGA) codon, and polyadenylation signal (TATAAA). Numbers below the scheme indicate the lengths in nucleotide pairs. B. Genomic clone of desaturase consists of two exons and a centrally located single intron. Desaturase promoter sequence includes elements typical of eukaryotic promoters: CAAT and TATA boxes.

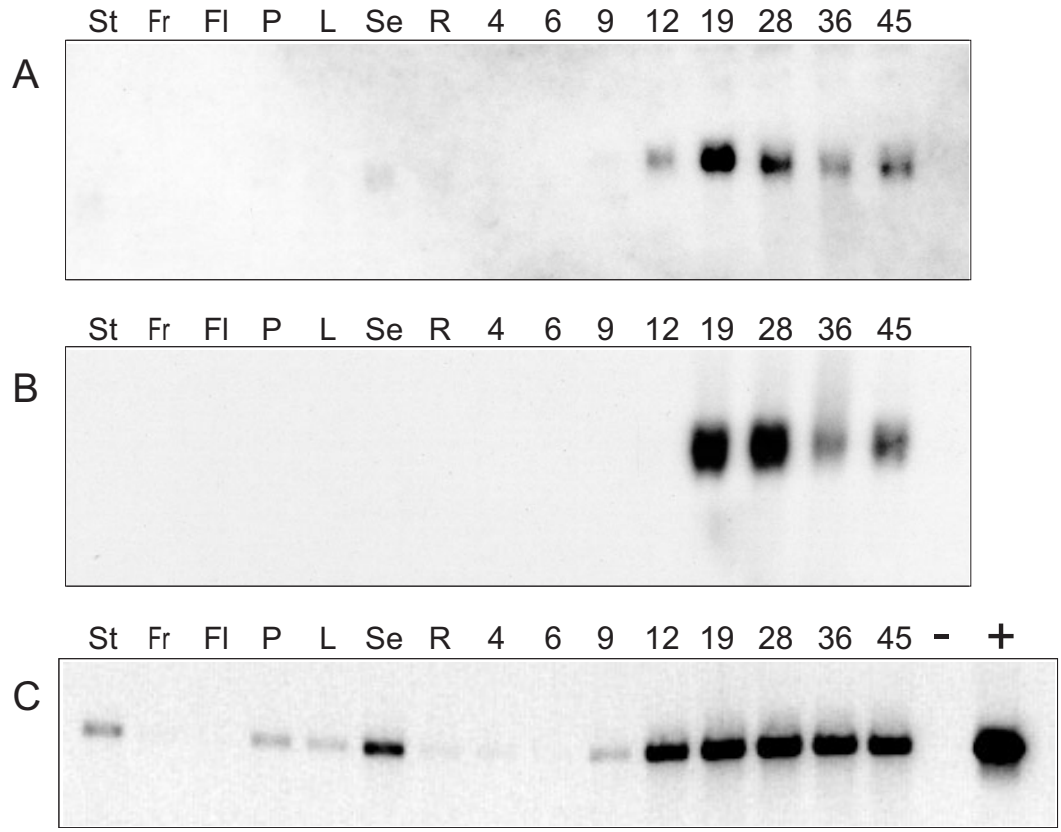


**Figure 4. Northern hybridization and RT-PCR analysis of yellow lupine desaturase gene expression.**

A. Nylon membrane showing hybridization signals detected after incubation with radioactive labeled desaturase clone as a probe. B. Control Northern hybridization with radioactive labeled leghemoglobin probe as a marker of effective symbiosis. C. Agarose gel showing mRNA bands appearing after RT-PCR analysis in different yellow lupine organs. St, stem; Fr, fruit; Fl, flower; P, pod; L, leaf; Se, seed; R, root; nodules 4, 6, 9, 12, 19, 28, 36, 45 days after *Bradyrhizobium* infection; (–), control PCR without any template (negative control); (+), control PCR with desaturase cDNA as a template (positive control).

fatty acids, it was placed into the pGPTV-BAR vector with the *bar* gene conferring BASTA herbicide resistance under the constitutive 35S promoter and introduced into tobacco plants by *Agrobacterium tumefaciens*-mediated transformation. Transgenic plants were analyzed by PCR with oligonucleotide primers specific to 5′ and 3′ regions of the coding sequence. Fatty acid fractions were extracted from leaf samples of transgenic tobacco for esterification. Methyl esters of fatty acids from twenty transgenic and four control tobacco plants were analyzed by gas chromatography. An increased amount of oleic acid and a decreased amount of stearic and linolenic acid in the transgenic plants were observed. Three groups of transgenic plants were distinguished depending on the content of oleic acid (Fig. 6). The first group with more than 60% of oleic acid content was characterized by a decreased content of saturated fatty acids (16 : 0, 18 : 0) and linolenic acid (18 : 3) and by an increased level of linoleic acid (18 : 2). A similar tendency was observed in the second group with a 40–60% oleic acid content although the amount of not only linolenic, but also linoleic acids decreased. A decreased amount of palmitic, linoleic and linolenic acid and an increased level of oleic acid were characteristic for the third group of tobacco plants with the amount of oleic acid below 40%. A comparison of the average content of fatty acids in transgenic tobacco plants indicates a decreased level of saturated fatty acids, polyunsaturated fatty acids and an increased level of the monounsaturated oleic acid. Yellow lupine

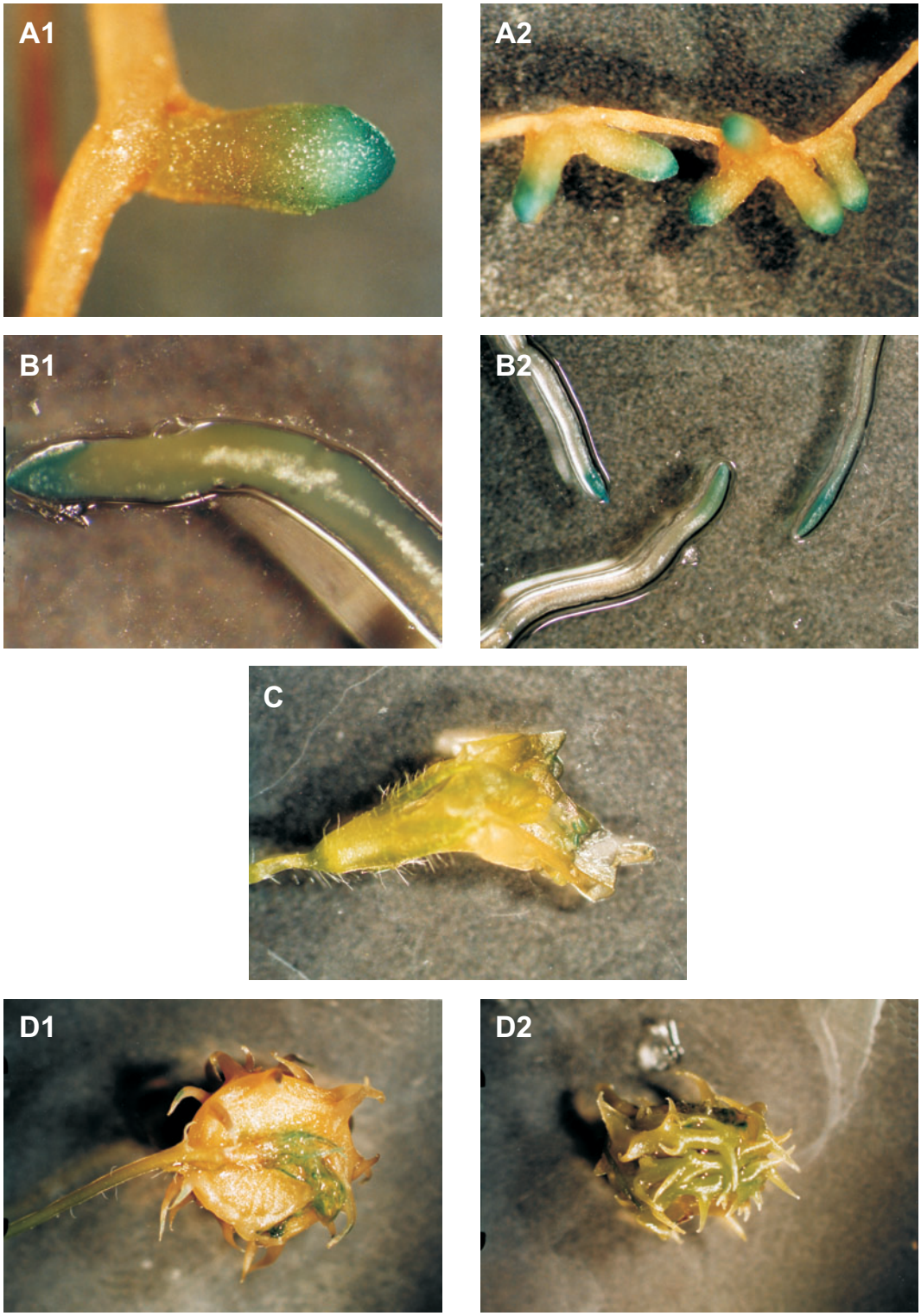


**Figure 5. Organ-specific expression of the $\beta$-glucuronidase gene under desaturase promoter in fragments of transgenic alfalfa plants.**

Blue color is present in nodule structure (A1, A2), meristematic tissue of roots (B1, B2), pollen grains (C) and seeds (D1, D2).

stearoyl-ACP desaturase catalyses the conversion of saturated stearic acid to unsaturated oleic acid, thus the increased amount of oleic acid and the decreased amount of stearic acid

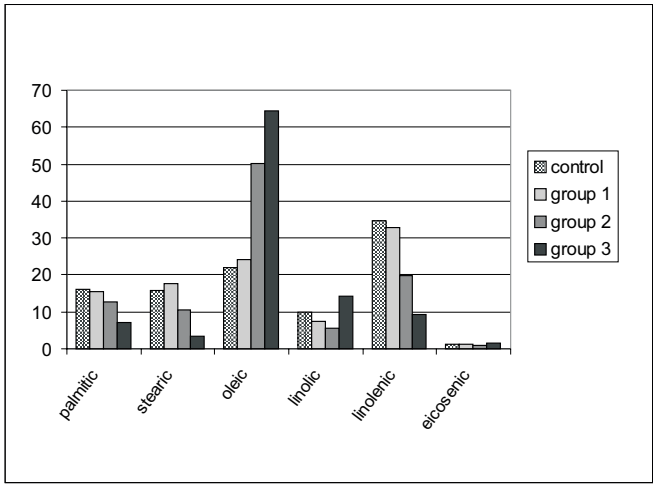


**Figure 6. Correlation between the level of oleic acid and the content of other fatty acids analyzed in control and transgenic tobacco plants.**

The percentage content of oleic acid in transgenic plants showed significant differences, thus three groups of transgenic plants were distinguished. Group 3 was characterized by the highest expression of the introduced stearoyl-ACP desaturase gene and the plants showed the lowest level of saturated fatty acids and the highest content of the monounsaturated oleic acid. Group 1 includes transgenic plants with the average content of oleic acid < 40%. Group 2 includes transgenic plants with the average content of oleic acid = 40–60%. Group 3 includes transgenic plants with the average content of oleic acid > 60%.

**Table 1. Percentage content of fatty acids in transgenic *Nicotiana tabacum* plants**

| Plant No. | Palmitic acid 16:0 | Stearic acid 18:0 | Oleic acid 18:1 | Linolic acid 18:2 | Linolenic acid 18:3 | Eicosenic acid 20:1 |
|---|---|---|---|---|---|---|
| 1 | 17.1 | 21.3 | 24.5 | 3.4 | 32.1 | 1.5 |
| 2 | 13.2 | 10.4 | 49.2 | 6.5 | 19.1 | 1.7 |
| 3 | 13.1 | 9.9 | 59.9 | 2.4 | 13.4 | 1.1 |
| 4 | 15.6 | 15.7 | 32.6 | 6.9 | 27.1 | 1.6 |
| 5 | 16.7 | 17.4 | 7.7 | 9.8 | 48.4 | 0 |
| 8 | 16.3 | 19.6 | 25.8 | 5.1 | 30.3 | 2.8 |
| 9 | 12.7 | 13.0 | 30.3 | 9.8 | 31.3 | 2.9 |
| 10 | 7.6 | 3.8 | 62.7 | 14.3 | 10.1 | 1.4 |
| 11 | 13.2 | 11.9 | 32.5 | 8.7 | 33.1 | 0.3 |
| 12 | 14.2 | 14.4 | 39.7 | 6.9 | 23.8 | 0.9 |
| 13 | 16.4 | 18.2 | 19.2 | 5.8 | 36.0 | 4.2 |
| 14 | 15.3 | 14.4 | 19.8 | 9.9 | 40.6 | 0 |
| 15 | 16.7 | 20.4 | 18.8 | 11.8 | 32.2 | 0 |
| 16 | 18.9 | 25.1 | 13.7 | 9.1 | 32.5 | 0 |
| 17 | 16.7 | 17.2 | 21.7 | 5.2 | 37.9 | 1.1 |
| 18 | 6.4 | 3.0 | 66.3 | 13.9 | 8.6 | 1.6 |
| 20 | 13.3 | 11.8 | 40.7 | 6.0 | 27.6 | 0.4 |
| 21 | 16.6 | 19.3 | 26.9 | 6.9 | 28.8 | 1.4 |
| 22 | 11.7 | 9.9 | 51.3 | 7.0 | 19.0 | 0.9 |
| 27 | 18.6 | 21.2 | 25.5 | 7.5 | 27.2 | 0 |
| Average | 14.515 | 14.895 | 33.44 | 7.845 | 27.955 | 1.19 |

in transgenic tobacco plants are consistent with expectations. The decreased level of polyunsaturated fatty acids and the increased amount of monounsaturated fatty acids were

**Table 2. Percentage content of fatty acids in control *Nicotiana tabacum* plants**

| Plant No. | Palmitic acid 16:0 | Stearic acid 18:0 | Oleic acid 18:1 | Linolic acid 18:2 | Linolenic acid 18:3 | Eicosenic acid 20:1 |
|---|---|---|---|---|---|---|
| K1 | 16.9 | 15.4 | 21.6 | 7.1 | 36.6 | 2.3 |
| K3 | 14.8 | 14.7 | 29.2 | 12.4 | 27.5 | 1.4 |
| K7 | 16.2 | 16.0 | 16.1 | 12.3 | 38.6 | 0.7 |
| K10 | 17.0 | 17.5 | 21.3 | 8.0 | 36.0 | 0 |
| Average | 16.225 | 15.9 | 22.05 | 9.95 | 34.675 | 1.1 |

**Table 3. Average levels of fatty acids in the three groups of transgenic plants differing by oleic acid content**

| Average content of fatty acids | Palmitic acid 16:0 | Stearic acid 18:0 | Oleic acid 18:1 | Linolic acid 18:2 | Linolenic acid 18:3 | Eicosenic acid 20:1 |
|---|---|---|---|---|---|---|
| Group 1 (oleic acid content < 40%) | 15.54 | 17.8 | 24.2 | 7.4 | 32.9 | 1.2 |
| Group 2 (oleic acid content 40–60%) | 12.82 | 10.5 | 50.3 | 5.5 | 19.7 | 1.02 |
| Group 3 (oleic acid content > 60%) | 7 | 3.4 | 64.5 | 14.1 | 9.35 | 1.5 |
| Control plants | 16.225 | 15.9 | 22.05 | 9.95 | 34.675 | 1.1 |

probably connected with the required balance between the contents of monounsaturated and polyunsaturated fatty acids.

## DISCUSSION

### Differential display

Differential display is a very useful method for identification and isolation of single genes characteristic of some physiological states or developing phases (Liang & Pardee, 1992). These genes are differentially expressed in two or more relevant cell types (Liang *et al.,* 1993). Isolated RNAs were compared to each other after gel electrophoresis, and specific RNA products appeared only in mRNA preparations isolated from different cells or under altered conditions. In this way many genes have been identified, for example nodulins, with their specific expression during symbiosis between soil bacteria and legume plants (Cohn *et al.,* 1998; Swiderski *et al.,* 2000). Nodulins represent a distinct group of proteins encoded by plant genes and appear exclusively during nodule formation (Nap & Bisseling, 1990).

### Organ specificity of the yellow lupine stearoyl-ACP desaturase

#### Northern hybridization and RT-PCR analysis

Northern hybridization and RT-PCR analyses demonstrated a high level of desaturase gene expression in nodules and a low amount of transcripts in stem, pod, leaf and uninfected root. This may be an indication that desaturase functions normally in other yellow lupine organs, not only in nodules, and during symbiosis an increased desaturase expression is observed. Desaturase transcripts appear 9 days after infection and are probably involved in the symbiosom peribacteroid membrane formation.


**Figure 7. Nucleotide sequence of the yellow lupin stearoyl-ACP desaturase gene promoter.**

Blue indicates regions specific to promoters of nodulin genes. Pink indicates sequence specific to organ-specific expression. Green indicates TATA and CAAT motifs typical of eukaryotic promoters.

The symbiosom consists of the bacteroid cell and the surrounding peribacteroid membrane (Roth *et al.,* 1988). The bacteroid membrane is formed from plasmalemma during endocytosis of symbiotic bacteria from the infectious strand inside the plant cell. The surface of the peribacteroid membrane increases 20–40 times during bacteroids cell division and becomes larger by including, for example, Golgi structure. Stearoyl-ACP desaturase introduces a double bond into stearic acid and provides an unsaturated fatty acid that may be essential for the membrane structure, fluency and functions.

In our earlier studies on characterization of the lupine plant-*Bradyrhizobium* sp. *(Lupinus)* symbiotic system we have detected and described plant genes that were induced during endosymbiotic interaction (Strozycki *et al.,* 2000; Strozycki & Legocki, 1995) and those

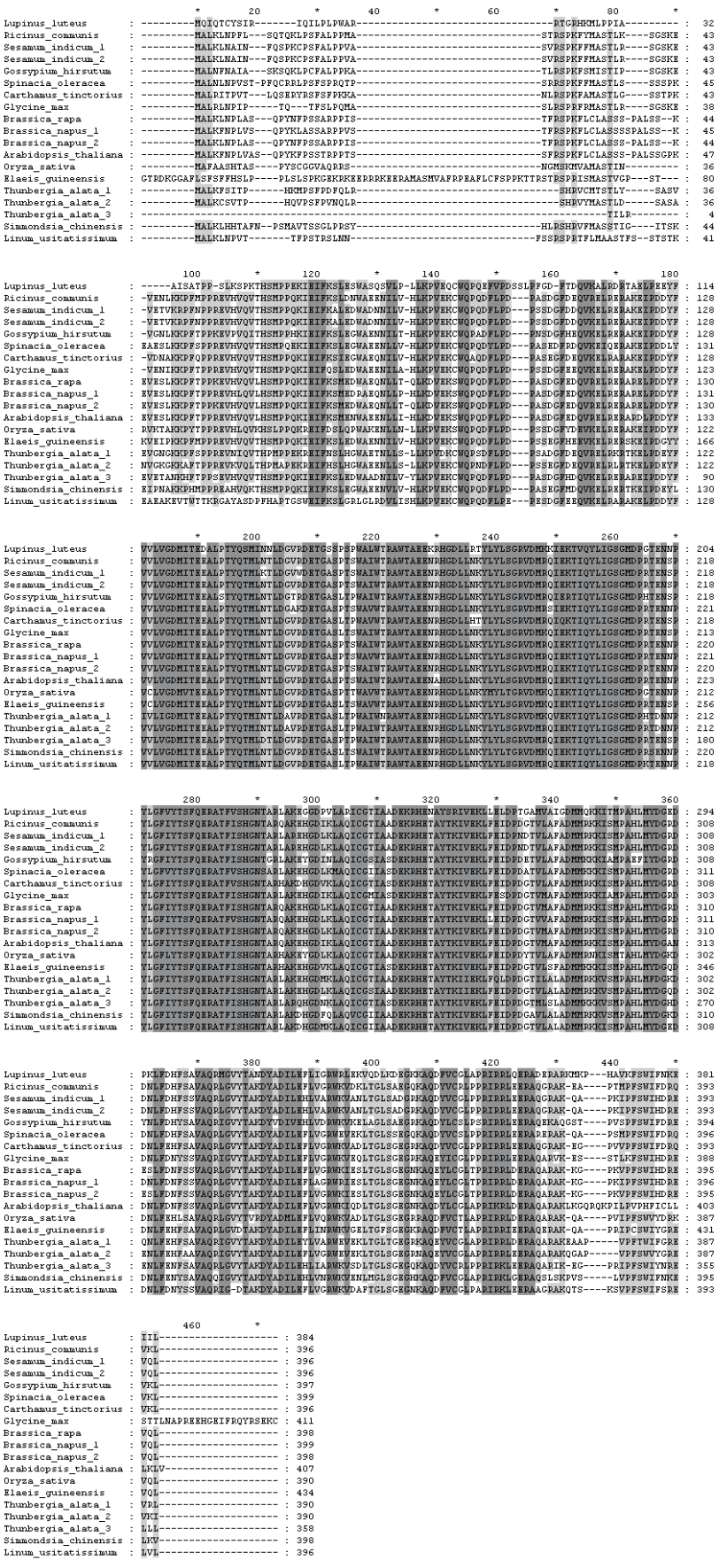


**Figure 8. Alignment of stearoyl-ACP desaturases amino-acid sequences.**

which were down regulated (Biesiadka *et al.*, 1999; Sikorski *et al.*, 1999). Although symbiotic regulation of plant genes seems to be highly specific, some of nodulin genes are expressed at a low level in other organs than the root nodule.

Stearoyl-ACP desaturase promoter sequence

Stearoyl-ACP desaturase promoters are the most active in developing tissues (Slocombe *et al.,* 1994). Expression of desaturase genes is temporally regulated and organospecific. An analysis of canola stearoyl-ACP desaturase promoter in transgenic tobacco plants has revealed high expression of $\beta$-glucuronidase reporter gene in developing seeds, flowers and pollen grains (Slocombe *et al.*, 1994). Expression of the $\beta$-glucuronidase gene under yellow lupine stearoyl-ACP desaturase promoter was observed in nodules, root meristems, seeds and pollen grains. This suggests an important role of desaturase in division processes requiring unsaturated fatty acids essential for the construction of the cell membrane. The promoter sequence of yellow lupine stearoyl-ACP desaturase contains regions specific to other nodule gene promoters (Fig. 7).

**Alignment of yellow lupine stearoyl-ACP desaturase with other known plant desaturases**

Stearoyl-ACP desaturase in stroma plastids converts stearic acid connected with acyl carrier protein to oleic acid (Slocombe *et al.,* 1994; Stumpf, 1980). Oleic acid is transported to tylakoid membranes or to the cytoplasm and then is attached to lipids and desaturated (Roughan, 1987; Roughan & Slack, 1982). The first double bond can be generated at the $\Delta$4, $\Delta$6 and $\Delta$9 position. Each stearoyl-ACP desaturase requires two iron atoms essential for reactive complex formation with oxygen (Fe–O–Fe) necessary for the catalytic activity (Fox *et al.*, 1994; Shanklin *et al.*, 1994). A crystallographic analysis of stearoyl-ACP desaturase from *Ricinus communis* has revealed that desaturase forms an active di-iron cluster (Lindqvist *et al.,* 1996). One of these iron atoms interacts with side chains of E196 and H232 residues, whereas the other with side chains of E105 and H146 (Lindqvist *et al.,* 1996). A di-iron cluster was identified also within the active center of yellow lupine stearoyl-ACP desaturase (Swiderski *et al.,* 2000). This motif characteristic of acyl-ACP desaturases contains two EXXH sequences separated by about 100 amino acids. In yellow lupine stearoyl-ACP desaturase the di-iron cluster includes two EKRH motifs separated by 82 amino-acid residues.

Plant stearoyl-ACP desaturases are the only known soluble desaturases. These enzymes are transported to the stroma plastid by the presence of a signal peptide localized at the N-end of the protein, consisting of about 30 amino acids – most of them hydrophobic or positively charged. After a hydropathy analysis, the signal peptide was also identified at the amino end of yellow lupine stearoyl-ACP desaturase. An alignment of stearoyl-ACP desaturase amino-acid sequences indicates a high percentage of identity (Fig. 8).

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