Isolation and Characterisation of Fatty Acid Desaturase Genes of Cotton (*Gossypium hirsutum* L.)

Qing Liu[1,2], Surinder Singh[1], Peter Sharp[2], Allan Green[1] and D.R. Marshall[2]

[1]Commonwealth Scientific and Industrial Research Organisation, Division of Plant Industry, GPO Box 1600, ACT 2601, Australia

[2]University of Sydney, Plant Breeding Institute, Cobbitty, PMB11, Camden, NSW 2570, Australia

Introduction

Australian production of all oilseeds is approximately 1 million tonnes per annum, about half of which is cottonseed (Burden, 1995). While importing soybean, sunflower, olive, palm and coconut oil, Australia is a surplus producer of cottonseed. Oil content of cottonseed varies between 17 to 26%, with palmitic acid (24%), oleic acid (18%), linoleic acid (54%) being the principal fatty acids. The relative levels of oleic acid and linoleic acid are controlled by the activities of the desaturase enzymes that sequentially insert double bonds into the C18 chain. Δ9 stearoyl-ACP desaturase converts stearate to oleate by inserting a double bond at the Δ9 position, and Δ12 (or ω-6) desaturase introduces the second double bond into oleic acid to form linoleic acid. Δ9 and ω-6 fatty acid desaturases are the two key enzymes controlling the unsaturation and hence the quality and value of cottonseed oil.

Oils having very high levels of oleic acid have superior stability and are valuable for frying and other high temperature applications. Oils with equal proportion of palmitic acid, oleic acid, stearic acid, namely, the POS type oil may be suitable starting material for the development of cocoa butter substitute. Our project aims to produce cottonseed oil with elevated levels of stearic and/or oleic acid by means of antisense inhibition of both the Δ9 stearoyl-ACP and microsomal ω-6 desaturase genes.

Results and Discussion

To isolate the cDNAs for fatty acid desaturases, a λ ZAPII cDNA library was constructed by using developing embryos of cotton (*Gossypium hirsutum* L.).

One full length cDNA clone, designated as pgh9-1, was obtained by screening the cDNA library with a PCR fragment corresponding to the entire coding region of the cotton Δ9 stearoyl-ACP desaturase cDNA (Liu et al., 1996a). This 1557bp long cDNA clone encodes an almost 1.2 kb open reading frame corresponding to a polypeptide of 398 amino acid residues, similar to other stearoyl-ACP desaturases (396-399 amino acids). As is the case with other Δ9 stearoyl-ACP desaturase genes in plants, this clone contains a pair of several conserved amino acids (D/E-E-X-R-H) separated by about 100 amino acids within the open reading frame (Fig.1a). These domains have been found in diiron-oxo proteins and may be essential for the function of stearoyl-ACP desaturase which requires molecular oxygen and reduced ferredoxin as an electron donor.

We have also isolated two different genes encoding the microsomal ω-6 desaturase, designated as pghD12-1 and pghD12-2 respectively (Liu et al., 1996b), by screening a cottonseed cDNA library using a heterologous cDNA probe from *B. juncea* (Singh et al., 1995). The coding sequences of both pghD12-1 and pghD12-2 were conserved, but the 3' nontranslated regions of the two sequences were unique, suggesting that they are two distinct members of the cotton microsomal ω-6 desaturase gene family. Within the open reading frame, there are three histidine-rich domains that are conserved in all known ω-6 and other membrane bound desaturases in plants (Fig.1b). Although the function of these domains is not clear, the first histidine-rich domain (at amino acid 105-110), HECGH, has similarity to zinc-binding domains of a class of metalloproteins.

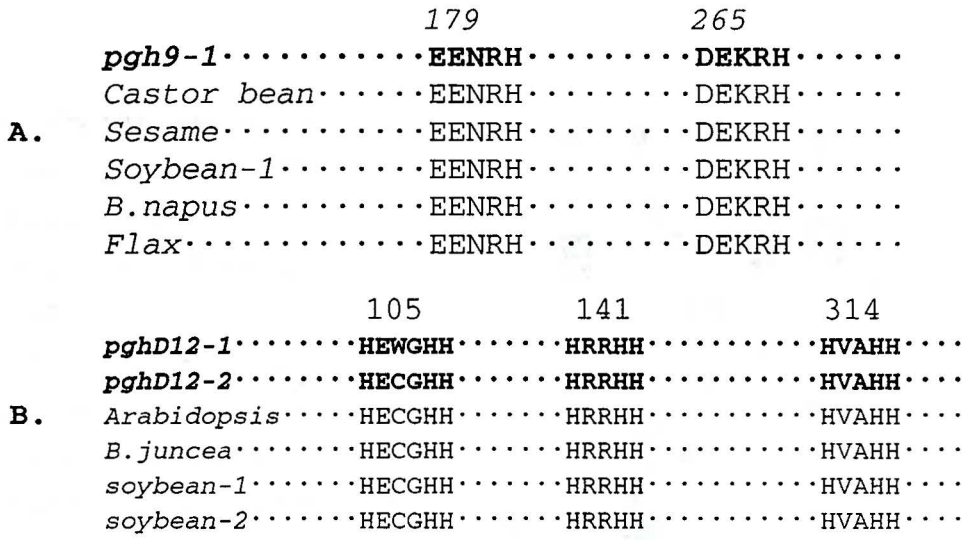

Fig.1 Alignment of the derived amino acid sequences of plant fatty acid desaturases (a, Δ9 stearoyl-ACP desaturase; b, microsomal ω-6 desaturases) showing the characteristic conserved domains. The numbers on the top indicate the their positions within the open reading frames.

To assess the copy number of the Δ9 stearoyl-ACP desaturase gene in cotton, genomic Southern blot analysis was carried out by using pgh9-1 as a probe. As shown in Fig.2 (lane1-2), three bands were revealed and this indicates that there are three copies of this gene in cotton (*G. hirsutum*).

Genomic Southern blot analyses using pghD12-1- and pghD12-2-specific probes confirmed that these genes were nonallelic. Both EcoRI and HindIII digested genomic DNA from *G. hirsutum* revealed two bands when probed with pghD12-1- (Fig.2, lane 3-4) while three (EcoRI, Fig.2, lane 5) or two (HindIII, Fig.2, lane 6) bands when probed with pghD12-2- suggesting the presence of at least two copies each of these genes in *G. hirsutum* genome. This is consistent with the tetraploid nature of *G. hirsutum* genome.

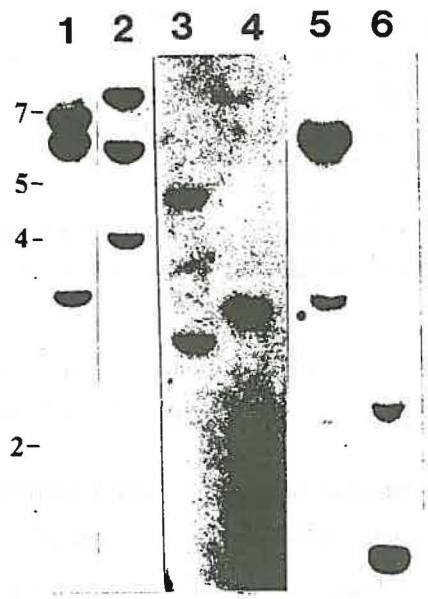

Fig.2 Southern blots of genomic DNA probed with pgh9 (lane 1-2), pghD12-1- (lane 3-4) and pghD12-2-specific (lane 5-6) sequence fragments respectively. The enzymes used to cut the DNA are: lane 1, BglII; lane 2, speI; lane 3&5, EcoRI; lane 4&6, HindIII. Molecular size (kb) of DNA ladder is indicated on the left.

Northern data with developing embryos of 25, 30, 36, 45 days after fertilisation as well as young leaves (Fig.3a), indicated that the Δ9 stearoyl-ACP desaturase gene is expressed throughout embryo development with the highest expression being at 30 days after fertilisation. No transcripts were detected in leaf tissues. By comparison with previously published data (Galau et al., 1983) stearoyl-ACP desaturase appears to be expressed before storage protein synthesis in cottonseed. This result is in consistent with reports that in *Brassica napus*, Δ9 stearoyl-ACP desaturase gene is expressed earlier than that of seed storage proteins, e.g. cruciferin and napin (Slocombe et al., 1992).

As illustrated in Fig.3b, pghD12-1 is specifically induced during seed development and as such is likely to play a major role in controlling conversion of oleic to linoleic acid during seed development. In contrast, pghD12-2 was constitutively expressed in both vegetative tissues and throughout the seed development at very low levels (Fig.3c). This is similar to the situation reported for soybean (Heppard et al., 1995).

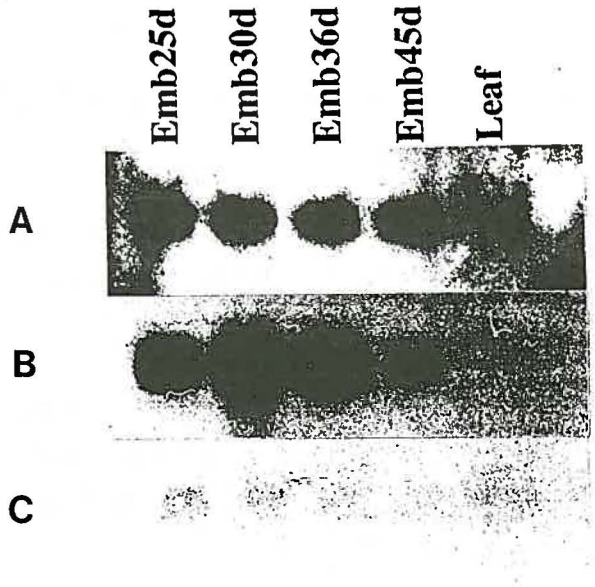

Fig.3 RNA gel blot analyses of the developmental and tissue-specific expression of cotton Δ9 stearoyl-ACP (a) and microsomal ω-6 desaturase genes pghD12-1 (b), pghD12-2 (c). Emb25d, Emb30d, Emb36d, Emb45d indicate RNA isolated from developing embryos of 25, 30, 36, and 45 DAF respectively.

To find a suitable promoter for the antisense expression of fatty acid desaturase genes in cottonseed, a number of seed specific promoters, including napin, vicillin, and lectin were examined. Constructs involving the various promoters driving the GUS gene were introduced into developing cotton embryos using particle bombardment techniques and expression of the GUS gene was monitored by histochemical staining. Lectin promoter (Cho et al., 1995) was selected and antisense constructs with Δ9 stearoyl-ACP desaturase (Fig.4a) and ω-6 microsomal desaturase (Fig.4b) have been made and will be transformed into elite Australian cotton cultivars.

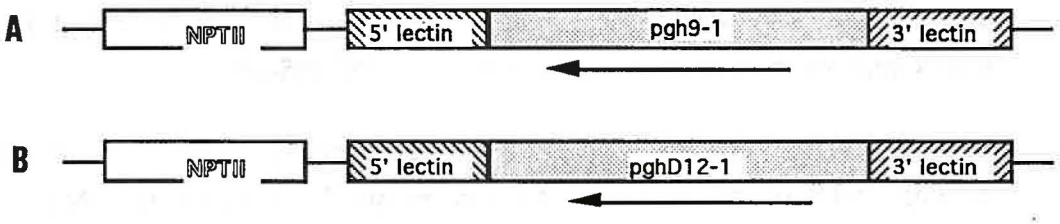

Fig.4 Diagrams showing the Lectin-desaturase antisense constructs. (a) Δ9 stearoyl-ACP desaturase (pgh9-1); (b) ω-6 microsomal desaturase (pghD12-1). Arrow shows the direction of normal transcription of the fatty acid desaturase genes, pgh9-1 and pghD12-1.

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