ANALYSIS AND EXPRESSION OF THE COTTON GENE FOR THE Δ-12 FATTY ACID DESATURASE 2-4 (FAD2-4)

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A genomic clone containing a 16.9-kb segment of cotton DNA was found to encompass a Δ-12 fatty acid desaturases (*FAD2-4*) gene. The *FAD2-4* gene has a single, large intron of 2,780 bp in its 5'-untranslated region, just 12 bp upstream from the ATG initiation codon of the *FAD2-4* opening reading frame. A number of prospective promoter elements, including several light-responsive sequences, occur in the 5'-flanking region. The coding region of the gene is 1155 bp with no introns, and would encode a *FAD2-4* polypeptide of 384 amino acids. The putative protein had four membrane-spanning helices, hallmarks of an integral membrane protein, and would probably be located in the endoplasmic reticulum. The *FAD2-4* gene is indeed a functional gene, since yeast cells transformed with a plasmid containing the coding region of the gene synthesize an appreciable amount of linoleic acid (18:2), not normally made in wild-type yeast cells. The *FAD2-4* gene has many structural similarities to the cotton *FAD2-3* gene that was also analyzed in this laboratory.

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CHAPTER 1

INTRODUCTION

Fatty acids are biomolecules with a hydrophobic, aliphatic tail, which can have from none to five double bonds, and a carboxylic acid head group. Through a series of reactions, the fatty acids are formed from acetyl-CoA for chain elongation on an acyl carrier protein (ACP) (Ohlrogge, 1994). The first double bond in the acyl group is generated while the fatty acid is still attached to the ACP. The activity of a specific thioesterase releases the fatty acyl-ACPs from the ACP, allowing it to cross the plastid envelope membrane through an unknown mechanism and become re-esterified to CoA. There are two pathways of fatty acid biosynthesis in plants (Ohlrogge and Browse, 1995). The prokaryotic pathway is the de novo fatty acid synthesis of 16:0, 18:0, and 18:1 fatty acids, which occurs in chloroplasts. The products of this pathway are exported to the eukaryotic pathway in the form of fatty acyl-CoA esters. The eukaryotic pathway is located in the endoplasmic reticulum in the cytoplasm. The first committed step of the eukaryotic pathway is the hydrolysis of 16:0-ACPs and 18:1-ACPs by acyl-ACP thioesterases to free fatty acids. These free fatty acids are then converted to CoA thioesters by acyl-CoA synthetases to enter the endoplasmic reticulum (Ohlrogge and Browse, 1995). The major difference between the two pathways is that the glycerolipids

obtained from the prokaryotic pathway contain 16:0 fatty acyl chains at position sn-2 while the glycerolipids from the eukaryotic pathway have 18:1 fatty acyl chains at the sn-1 and sn-2 positions.

Desaturation of the fatty acid components of the membrane phospholipids is carried out by membrane-bound desaturases of the chloroplast and endoplasmic reticulum membranes. Seven *Arabidopsis* mutants have been found for the fatty acid desaturase enzymes (designated from FAD2 to FAD8) deficient in a specific fatty acid desaturation step (Topfer and Martini, 1994; Topfer et al., 1995; Ohlrogge and Browse, 1995). The FAD4 to FAD8 enzymes affect chloroplast lipid desaturation, but the FAD2 and FAD3 enzymes primarily desaturate extrachloroplast lipids and occur as integral membrane proteins in the endoplasmic reticulum (Los and Murata, 1998; Shanklin and Cahoon, 1998). The enzymes are phosphatidylcholine desaturases, presumably acting on fatty acids at both the sn-1 and sn-2 positions (Ohlrogge and Browse, 1995; Somerville et al., 2000). From the structure of the *Arabidopsis FAD2* gene (Okuley et al., 1994), the first *FAD2* gene analyzed, the presumptive *Arabidopsis* FAD2 protein was deduced to have two stretches (>45 amino acids each) of hydrophobic amino acids, suggestive of membrane-spanning regions for integral membrane proteins. FAD2 desaturases convert oleate (C18:1) into linoleate (C18:2), and the FAD3 enzymes desaturate linoleate into linolenate (C18:3). Both the FAD2 and FAD3 enzymes have a conserved region at their C-terminal ends, and have three His-containing conserved motifs (Shanklin and Cahoon, 1998).

The *FAD2* gene appears to be especially important in the chilling sensitivity of plants (Miquel and Browse, 1994; Okuley et al., 1994; Ohlrogge and Browse, 1995),

since polyunsaturated membrane phospholipids are essential for maintaining cellular function and plant viability at lowered temperatures (Browse and Xin, 2001). When grown at 22°C, *FAD2* mutant plants are similar in growth and appearance to the wild type. However, at 6°C the leaves of *FAD2* mutant plants gradually deteriorate over several weeks (compared to wild type plants), displaying patches of necrosis and extensive accumulation of anthocyanins, with eventual death of the leaves and whole plants.

Liu et al. (1999) characterized a cotton cDNA clone (designated as *FAD2-1*) encoding a putative FAD2 protein which is apparently expressed only in developing embryos for biosynthesis of storage triacylglycerols in cottonseed. Liu et al. (1999) did not detect *FAD2-1* mRNA transcripts in leaves by Northern blotting. Thus, the cotton *FAD2-1* gene is most likely utilized for seed-specific gene expression and not for constitutive expression in vegetative tissues as leaves. Heppard et al. (1996) analyzed a soybean *FAD2-1* cDNA that is expressed specifically in embryo development for seed-specific expression for the biosynthesis of linoleic acid in soybean oil, similar to the cotton *FAD2-1* cDNA of Liu et al. (1999). A second soybean *FAD2* cDNA (called *FAD2-2)* studied by Heppard et al. (1996) is expressed constitutively throughout the vegetative tissues of the plant. Thus, using soybean as example, the constitutively expressed *FAD2-2* gene should also have an impact on the survivability and viability of cotton at lowered temperatures. Recently, this laboratory reported the structure of the first cotton *FAD2* gene (called the *FAD2-3* gene) (Pirtle et al., 2001), which has a coding region quite distinct from that of the seed-specific *FAD2-1* cDNA clone described by Liu et al. (1999). The *FAD2-3* gene may encode a vegetatively expressed form of the FAD2

protein. The cotton *FAD2-4* gene described in this problems in lieu of thesis is probably a paralog of the *FAD2-3* gene, and likely encodes a vegetatively expressed FAD2 isoform (Pirtle et al., 2002).

The *Arabidopsis FAD2* gene has been over-expressed by two different laboratories in a yeast model system (Covello and Reed, 1996; Kajiwara et al., 1996). Since the *Arabidopsis FAD2* coding region is continuous and has no introns, it was possible for these two groups to generate PCR-derived DNA fragments encoding the *Arabidopsis* FAD2 protein for transformation of yeast strains. Yeast cells are eukaryotic and contain an endoplasmic reticulum, and naturally make oleic acid (18:1), a fatty acid with only one double bond (Stukey et al., 1990). The endoplasmic reticulum is necessary for activity of plant FAD2 enzymes, since they are integral membrane proteins in this cellular organelle (Shanklin et al., 1994; Shanklin and Cahoon, 1998; Los and Murata, 1998). Thus, the expression of the cotton *FAD2* genes in yeast cells should be easily detectable, since the FAD2 proteins would generate linoleic acid (18:2), a fatty acid with two double bonds, as assayed by lipid analysis. Last year, we expressed the cotton *FAD2-3* gene in yeast cells (Pirtle et al., 2001). Since polyunsaturated phospholipids are necessary for maintaining membrane fluidity at lowered temperatures, it may be possible for us to use the expression of the cotton FAD2 polypeptide in yeast as a model system for studying the effects of chilling sensitivity on membrane fluidity at lowered temperatures. In this report, the expression of the cotton *FAD2-4* gene in yeast cells is also confirmed.

Gossypium hirusutum is a cotton cultivar primarily grown for its fiber for use in the textile industry and secondarily grown for its use in the oilseed industry. Thus,

success in designing new cotton oils can provide higher-value products of cottonseed for seed oil production. To better understand the mechanisms that regulate fatty acid desaturation in cotton, a member of the fatty acid desaturase gene family has been isolated and characterized. The regulation of expression of the fatty acid desaturase genes may be a major control point for modification of fatty acid compositions in storage triacylglycerols and membrane phospholipids (Ohlrogge et al., 1994). The expression of the fatty acid desaturase genes could be modified in transgenic cotton plants to improve the nutritional and economic value of cotton oils. The analysis of *cis*- and *trans*-promoter/enhancer regulatory elements for the fatty acid desaturase genes may allow an understanding of the regulation of gene expression for heat tolerance and disease resistance. These regulatory elements may also control the expression of the desaturases during seed development. Thus, in this research for problems in lieu of thesis, a cotton Δ-12 fatty acid desaturase (*FAD2-4*) gene was isolated by screening a cotton genomic DNA library. The *FAD2-4* gene structure was characterized by physical mapping and DNA sequencing, and the expression of the gene in yeast cells was also analyzed (Pirtle et al., 2002). This information will help in the design of experiments to potentially modify *FAD2* gene expression in transgenic cotton plants, so that the modification of cottonseed oils and the modification of the chilling sensitivity of cotton plants (for cold tolerance) by genetic engineering will be possible in the near future.

CHAPTER 2

METHODS AND MATERIALS

I. Isolation of Cotton Genomic Clones Encompassing the *FAD2-4* Gene

A cotton genomic library was screened using a homologous hybridization probe generated by random priming from the 1.2-kb coding region of the *FAD2-3* gene in the clone LCFg24 (Pirtle et al., 2001), using the plaque hybridization procedure of Benton and Davis (1977). Prehybridization of positively-charged nylon membrane filter (Amersham Hybond N^+) replicas was performed at 55°C for 4 hr using a solution consisting of 6xSSC, 0.5% SDS, 5X Denhardt's reagent, and denatured sheared salmon sperm DNA (100 µg/mL). Hybridization was performed overnight using a solution made of 6xSSC, 0.5% SDS, 5X Denhardt's reagent, 20 mM Tris-HCl (pH 8.0), 2 mM Na_2EDTA (pH 7.5), 2.5 mM Na pyrophosphate (pH 8.0), denatured sheared salmon sperm (100 µg/mL) and the ^{32}P-labeled hybridization probe. After hybridization, the nylon membrane replicas were washed at 55°C, once for 2 min in 2XSSC and once for 30 min in 2XSSC, 0.1% SDS and then twice for 30 min in 1X SSC, 1% SDS. The DNA from one genomic clone (called LCFg5b) that intensely hybridized to the *FAD-3* probe was purified by a mini-lysate procedure (Sambrook and Russell, 2001). The LCFg5b DNA was digested with restriction endonucleases and analyzed by agarose gel electrophoresis and alkaline blot hybridization for physical mapping and to select a DNA fragment encompassing the *FAD2-4* gene for sequence analysis. A 8.6-kb *XbaI* fragment of LCFg5b DNA was subcloned into the plasmid pUC19 for fine physical

mapping and for DNA sequence analysis. Drs. Irma and Robert Pirtle of this laboratory did many of the experiments in the library screening and probe preparation.

II. Physical Mapping of the Cotton DNA Segment in the Clone LCFg5b

The physical map of the cotton DNA fragment LCFg5b DNA was generated by analysis of data from single and double restriction endonuclease digestions, agarose gel electrophoresis, and alkaline blot hybridization. In addition, a 8.6-kb *XbaI* fragment of the genomic clone LCFg5b encompassing the hybridizing regions was subcloned into the plasmid vector pUC18 for sequence analysis. The resulting recombinant plasmid (designated pCFg5b) was isolated and purified using the Wizard Plus Minipreps DNA Purification System (Promega)™.

III. DNA Sequence Analysis

For sequencing the 8.6-kb *XbaI* encompassing the putative *FAD2-4* gene in the plasmid pCFg5b, both DNA strands were sequenced using a primer-based approach by semi-automated procedures on an Applied Biosystems Model 377XL DNA Sequencer by Lone Star Labs, Houston, TX. The sequencing reactions were done according to manufacturer protocols by terminator cycle sequencing with BigDye™ using 10% DMSO as denaturant. Analyses and alignments of the DNA and deduced amino acid sequences were done with DNASIS software (Hitachi) by Dr. Irma Pirtle of this laboratory. From the sequence information, the locations of the 5'-and 3'- flanking regions, the *FAD2-4* coding region, prospective promoter elements, and the CAP binding site were tentatively identified by comparison with the structure of the *FAD2-3* gene and cDNA (Pirtle et al., 2001).

IV. Functional Expression of the *FAD2-4* Gene in Yeast Cells

For functional expression of the *FAD2-4* gene in a yeast model expression system, the *FAD2-4* open reading frame was amplified by PCR using Platinum Pfx DNA polymerase (Invitrogen), and the 1.2-kb PCR product was subcloned into the *SacI* and *EcoRI* sites of the yeast-bacterial shuttle vector pYES2 (Invitrogen), which has the yeast *GAL1* promoter for inducible expression of genes in the presence of galactose. A forward amplimer was used to create a *SacI* site adjacent to the *FAD2-4* initiation codon in the open reading frame. A reverse amplimer was designed to provide an *EcoRI* site in the 3'-flanking region of the coding region. Both strands of this construct (called pYES2/FAD2-4) were sequenced to confirm that the coding region was indeed identical to that of the gene and that the construct was in-frame relative to the *GAL1* promoter. *Saccharomyces cerevisiae* strain INVSc1 (Invitrogen) was transformed with both the pYES2/FAD2-4 construct and the pYES2 vector DNA (as control) by electroporation. Drs. Mongkol Nampaisansuk and Robert Pirtle of this laboratory helped in the design and construction of pYES2/FAD2-4.

Both yeast transformants were grown in SC-U (synthetic complete minus uracil) medium at 30°C, washed and suspended in galactose induction medium (SC-U medium containing 2% galactose and 2% raffinose) and grown for three generations (Adams et al., 1998). The cells were pelleted and washed four times with water to remove any media or metabolites that could potentially interfere with the lipid analysis. The fatty acids were extracted and transmethylated with 5% HCl in methanol at 85°C for three hr by Ms. Sylvia Wanjie of the laboratory of Dr. Kent Chapman of this Department. The

fatty acid methyl esters were analyzed by gas chromatography and quantified by flame ionization detection (FID) in comparison to an internal heptadecanoic acid (C17:0) standard.

CHAPTER 3

RESULTS AND DISCUSSION

The genomic clone LCFg5b encompassing a second potential cotton *FAD2* gene, the *FAD2-4* gene, was analyzed by physical mapping, DNA sequencing, and functional expression in a model yeast expression system (Pirtle et al., 2002). A comparison of the architecture and organization of the cotton *FAD2-4* gene and the cotton *FAD2-3* gene (Pirtle et al., 2001) revealed many similarities/ differences in the locations and sizes of the 5'-flanking introns, the promoter/enhancer motifs, and the protein-coding regions of the two genes. The structural analysis may allow insight into how the two *FAD2* genes are related, how they might be regulated for gene expression, and if the 5'-flanking introns have any bearing on transcriptional regulation of the genes.

The cotton genomic insert encompassing the *FAD2-4* gene in the clone LCFg5b was deduced to be 17.9 kb by physical mapping and alkaline blot hybridization. The physical maps of the genomic clones encompassing the *FAD2-3* and *FAD2-4* genes are quite distinct, indicating that the genes are either alleles or paralogs in the allotetraploid cotton genome. Like the coding region of the *FAD2-3* gene (Pirtle et al., 2001), the coding region of the *FAD2-4* gene has 1,155 basepairs, including the termination codon, and is continuous with no introns. The deduced amino acid sequence of the FAD2-4 polypeptide is 384 amino acids, the same as the *FAD2-3* polypeptide. However, the *FAD2-4* gene in clone LCFg5b is distinctly different from the *FAD2-3* gene in clone LCFg24, with minor sequence differences in the coding regions and major differences in the flanking regions. There are 18 nucleotide variations between the DNA sequences of

the protein-coding regions of the *FAD2-3* and *FAD2-4* genes in LCFg5b and LCFg24, respectively, leading to six amino acid differences in the deduced sequences of the two putative FAD2 polypeptides (with an identity of 98%). The other 12 nucleotide differences are simply due to variations in the third codon base due to degeneracy of the genetic code, resulting in no amino acid changes at these positions.

The identities between the deduced amino acid sequence of the FAD2-4 polypeptide and those of the cotton FAD2-3 protein (Pirtle et al., 2001; GenBank AF331163), the cotton FAD2-2 polypeptide (Liu et al., 1997, GenBank Y10112) and the cotton FAD2-1 polypeptide (Liu et al., 1999; GenBank X97016) are 98%, 85% and 74%, respectively. Amino acid identities of 74-78% occur with other FAD2 polypeptides, such as those from soybean, *Arabidopsis*, parsley, *Brassica*, *Borago*, and potato (based on comparisons deduced from the cDNA sequences in the GenBank database). The identities of the DNA sequences of the *FAD2-4* gene with the cotton *FAD2-3* gene (Pirtle et al., 2001), the *FAD2-2* cDNA (Liu et al., 1997), and the *FAD2-1* cDNA (Liu et al., 1999) are 98%, 57%, and 39%, respectively. In addition, the cotton *FAD2-4* gene sequence (Pirtle et al., 2001) showed significant similarities with numerous other plant *FAD2* cDNA sequences, including the *Arabidopsis* FAD2 cDNA (67%) and a *Brassica FAD2* cDNA (63%). There are low sequence similarities between the cotton *FAD2-4* gene and castor bean *FAD2* cDNA (36%) and sunflower *FAD2* cDNA (35%) sequences, with the lowest sequence identity with that of a soybean *FAD2* cDNA sequence (7%).

A hydropathy plot of the cotton FAD2-4 polypeptide, generated by the method of Kyte and Doolittle (1982) in DNASIS software (Hitachi), indicates that there are four tentative membrane-spanning domains that correspond with the predicted membrane-

spanning domains in the desaturase integral membrane protein models of Los and Murata (1998) and Shanklin et al. (1994). Acyl-lipid and acyl-CoA desaturases are hydrophobic proteins that apparently span the membrane four times (Los and Murata, 1998; Shanklin et al., 1994). The enzymes in the endoplasmic reticulum of plant cells accept electrons from an electron transport system composed of cytochrome b_5 and NADH-dependent cytochrome b_5 reductase (reviewed in Los and Murata, 1998; Shanklin and Cahoon, 1998). In addition, all known desaturases are characterized by the presence of three histidine clusters localized at very conserved locations in the protein sequence (Los and Murata, 1998; Shanklin and Cahoon, 1998). These three histidine clusters are thought to comprise the catalytic center of the desaturase enzymes, since they may form ligands to a diiron cluster in the catalytic site (Shanklin and Cahoon, 1998). The amino acid sequence of the putative FAD2-4 polypeptide has the three histidine-rich motifs (Shanklin and Cahoon, 1998) in the conserved locations in the protein structure (Los and Murata, 1998), as does the FAD2-3 protein (Pirtle et al., 2001).

A single, large intron most likely occurs in the 5'-flanking region of the *FAD2-4* gene in LCFg5b, only 12 basepairs upstream from the ATG initiation codon of the protein-coding region. The locations of the tentative cap site for the start site of the *FAD2-4* mRNA product and the tentative 5'- and 3'-intron splice junctions in the 5'-untranslated region (5'-UTR) were deduced from comparison of the *FAD2-4* and *FAD2-3* gene sequences. The 5'-UTR intron of the *FAD2-4* gene is 2,780 basepairs long, which is 187 basepairs shorter than the 2,967-basepair 5'-UTR intron of the *FAD2-3* gene in LCFg24 (Pirtle et al., 2001). The intron has large stretches that are 92-96% identical and of the same length as corresponding regions in the intron of the *FAD2* gene in LCFg24.

However, there is one region (about 360 basepairs from the 3'-end of the intron) which has a gap of 36 basepairs compared to the intron of LCFg24. There are also three other regions in the middle of the intron that have only about 35-59% identity and are shorter than corresponding regions in the intron of LCFg24. Since the intron would be spliced out to generate the mature *FAD2-4* mRNA, the 5'-untranslated region of the mRNA can be estimated to be about 136 nucleotides in length.

The *Arabidopsis FAD2* gene (Okuley et al., 1994) has a large 1,134-basepair intron merely 5 basepairs upstream from its ATG initiation codon. Liu et al. (2001) analyzed the *FAD2*-1 gene 5'-UTR intron structures across 31 species of *Gossypium*, including the *Gossypium hirsutum* A and D genome orthologs. The sizes of the 5'-UTR introns of the *Gossypium FAD2-1* genes are about 1,133 bp, and are located 9 bp upstream from the initiation codon. In contrast, the cotton *FAD2-4* and *FAD2-3* genes have much larger 5'-UTR introns (2,780- and 2,967-basepairs, respectively) in slightly different positions, located 12 bp upstream from the translation start codons. The variations in the relative positions and the substantial differences in the sizes of the 5'-UTR introns are distinguishing structural differences between the *FAD2-4* and *FAD2-3* genes and the *FAD2-1* gene, which could be important in differential expression of the genes. The presence of large introns in the 5'-flanking regions of plant *FAD2* genes could be important in the regulation of their expression. Introns in 5'-UTRs have been reported to have positive effects on the expression of a number of plant genes. For example, Gidekel et al. (1996) found that the gene for *Arabidopsis* elongation factor 1β has a 5'-UTR intron that is required for high levels of expression, and has an enhancer-

like element in this intron. Also, the 5'-UTR intron of a soybean phosphoenolpyruvate carboxylase gene dramatically increases gene expression in plant cells (Kato et al., 1998).

The 5'-flanking region of the *FAD2-4* gene has a number of possible promoter elements that could be positive regulatory elements. A TATA basal promoter element occurs 34 basepairs upstream from the putative cap site. A basic region helix-loop-helix (bHLH) or E box motif (with the consensus sequence CANNTG) occurs 103 basepairs upstream from the potential cap site. The E box motif has been shown to be a seed-specific regulatory element in the French bean β-phaseolin gene (Kawagoe et al., 1994). There are also prospective light-responsive elements in the 5'-flanking region of this gene. For example, there are several consensus GT-1 motifs (GRWAAW) and G-boxes (CACGTG), involved in the light-induced expression of many plant genes (Terzaghi and Cashmore, 1995; Guilfoyle, 1997). A G box occurs 130 basepairs upstream from the tentative cap site. Two consensus GT-1 motifs, general features of light-responsive promoters, occur 82 basepairs and 115 basepairs prior to the supposed cap site. There are two possible *Dof* core recognition sequences at 8 and 57 basepairs upstream from the tentative cap site. *Dof* transcription factors are associated with genes involved in carbon metabolism in maize (Yanagisawa, 2000). Thus, the promoter elements in the 5'-flanking regions of the cotton *FAD2-4* and *FAD2-3* genes are virtually identical in number and in location. The structural comparison of the two cotton *FAD2* genes has revealed great similarities in the 5'-flanking introns, the promoter motifs, and the protein-coding regions of the two genes. This should permit insight into how the two presumptive *FAD2* genes are regulated for gene expression, and if the 5'-flanking introns really have any bearing on transcriptional regulation of the genes.

Yeast cells are eukaryotic and contain an endoplasmic reticulum that is necessary for the activity of plant FAD2 enzymes that are integral membrane proteins in that cellular organelle. Since yeast cells lack a *FAD2*-type gene, they normally do not make linoleic acid (C18:2), a fatty acid with two double bonds. However, yeast cells do convert stearic acid (C18:0), a fatty acid with no double bonds, into oleic acid (C18:1), a fatty acid with one double bond, which accumulates in their membrane phospholipids. Thus, expression of the cotton *FAD2-4* gene in yeast cells would be easily detected, since the FAD2-4 enzyme would convert oleic acid (18:1) into linoleic acid (18:2) when assayed by fatty acid analysis.

Yeast cells were grown in the appropriate media, the fatty acids were extracted from the washed yeast cells, and the fatty acid methyl esters were then analyzed by gas chromatography. The lipid analyses were done in collaboration with Ms. Sylvia Wanjie and Dr. Kent Chapman of this Department. The yeast cells transformed with the plasmid construct pYES2/FAD2-4 were found to have a significant accumulation of linoleic acid (C18:2). The oleic acid (C18:1) peak in the transformed cells was noticeably smaller relative to the corresponding oleic acid peak in the control cells, clearly indicating the conversion of oleate into linoleate in the yeast cells containing the plasmid construct with the *FAD2-4* coding region. No linoleic acid was detected in the control yeast cells transformed with the shuttle vector pYES2 alone. Thus, the *FAD2-4* gene has been functionally identified, since it encodes an enzyme that catalyzes the desaturation of oleate to linoleate. The yeast system was also used to express the cotton *FAD2-3* gene (Pirtle et al., 2001). The expression of the *FAD2-4* and *FAD2-3* genes in the yeast model system will be used for studying the effect of chilling sensitivity on membrane fluidity,

and hence plant viability at lowered temperatures, since polyunsaturated phospholipids are necessary for maintaining membrane fluidity at lowered temperatures (Browse and Xin, 2001).

It is imperative to the long-range understanding of lipid synthesis in cotton to elucidate the mechanisms of regulation of genes for enzymes of fatty acid biosynthesis. This basic information will provide a knowledge base to specifically design the lipid composition of cottonseed oil, but will also enable us to understand the pathways of membrane biosynthesis, important for the improvement of the vigor and vitality of this important crop plant.

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