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Soybean Fatty Acid Desaturation Pathway: Responses to Temperature Changes and Pathogen Infection

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1. Introduction

Soybean [Glycine max (L.) Merr] is the largest oilseed crop produced and consumed worldwide, accounting for 58% of the world oilseed production (SoyStats, 2011), yet the oil produced from most available cultivars is still lacking in several quality characteristics. For example, the oil is too low in oleate and/or too high in linolenate content with resulting negative impacts on oil stability and human nutrition. Three fatty acid metabolism enzymes, the stearoyl-acyl carrier protein-desaturases (encoded by the *GmSACPD* genes), the omega-6 desaturases (*GmFAD2s*), and the omega-3 (*GmFAD3s*) desaturases largely determine the relative degree of unsaturated fatty acids and the content of the C_{18} fatty acids stearate (18:0), oleate (18:1), linoleate (18:2), and linolenate (18:3) in vegetative and seed lipids. In vitro studies have shown that it is possible to redesign soluble fatty acid desaturases from plants for altered fatty acid substrate and double bond position (Cahoon et al., 1997, Whittle et al., 2005) and in that way potentially alter the fatty acid content of plant lipids. Since the fatty acid composition of seed lipid is such an important determinate of oil quality, intensive efforts have also been mounted to select advantageous desaturase alleles (Wilson et al., 2001, Rajcan et al., 2005) and to manipulate molecularly desaturase expression and activity (Buhr et al., 2002), the goal being to produce elite soybean varieties with enhanced oil traits for the needs of industry and for improved human nutrition.

Both field and growth chamber experiments have shown that the fatty acid composition in soybean tissues is responsive to environmental temperature. In field studies, temperatures during the growing season affected seed linolenic content most clearly (Hou et al., 2006). Experiments to model climate change by increasing temperatures and [CO_2] in controlled environment chambers (Thomas et al., 2003) showed that exposure to increasing [CO_2] had no measurable effect, but higher temperatures (greater than 32/22°C day/night) reduced total seed oil concentration while oleate increased and linolenate decreased with increasing temperature. Transcripts of β-glucosidase, a gene expressed during seed development, was detected in seeds grown at 28/18°C but not detected in seeds grown at 40/30°C. This observation suggested that one mechanism by which climate change may affect soybean seed development is through the regulation of gene transcription. The ability to adjust membrane lipid fluidity by changing the levels of unsaturated fatty acids is provided mainly by the regulated activity of fatty acid desaturases (Iba 2002). Through this

mechanism, the modification of membrane fluidity in response to temperature stress results in the maintenance of a membrane environment suitable for the function of critical integral proteins, such as the photosynthetic machinery in chloroplasts (Nishiuchi et al., 1998). The fatty acid composition in soybean tissues is, in addition, responsive to biotic (pathogen) attack (Iba, 2002, Upchurch, 2008) and fatty acids and fatty acid-derived compounds act as signals of plant defense gene expression (Kachroo et al., 2001, Weber, 2002). Evidence suggests that the levels of 18:0 and 18:1 are critical for defense against pathogens in soybean as they have been shown to be in *Arabidopsis thaliana* (Kachroo & Kachroo, 2009). Moreover, the oleate and linoleate content of soybean seeds appears to influence the course of seed colonization by a fungal pathogen (Xue et al., 2008).

Plants often encounter temperatures that are stressing, as well as pathogens and insects in the environment, sometimes simultaneously. Thus, the current worldwide situation of diminishing farm land and the heightened effects of global climate change on the productivity of agriculture (Garrett et al., 2006) increase the need to understand stress responses in crop plants such as soybean. More complete knowledge of fatty acid metabolism and its regulation in this and other important oilseed crops may significantly aid the development of effective strategies for managing abiotic and biotic stresses in the agricultural environment. This chapter focuses on a concise description of three fatty acid desaturase gene families and their contributions to the acclimation of soybean and other plants to high and low temperature and pathogen infection. Investigations of the regulation of desaturase expression and activity by temperature and pathogens are relatively recent in soybean and current results suggest complexity, yet a basic understanding of these phenomena are required if varieties are to be developed that possess stable and durable expression of desirable stress-acclimation traits.

2. Soybean delta-9 stearoyl-acyl carrier protein-desaturases

The Δ-^{9} stearoyl-acyl carrier protein-desaturases are soluble enzymes localized to the stroma fraction of plastids that introduce the first double bond into stearoyl-ACP (18:0-ACP) to produce oleoyl (18:1$^{\Delta 9}$)-ACP. Delta 9-stearoyl-ACP-desaturases thus occupy a key position in C_{18} fatty acid biosynthesis since perturbation of SACPD gene expression and/or enzyme activity may modulate the relative cellular content of both stearate and oleate. Three alleles of *SACPD* have been identified and characterized from soybean (Table 1).

Enzyme function	Gene name	GenBank accession	Chromosome, Linkage Group	Transcript expression	References
Δ^{9}-Stearoyl-ACP-Desaturase	*GmSACPD-A*	AY885234	7, M	Vegetative and seeds	Byfield et al., 2006 Zhang et al., 2008 Ha et al., 2010
	GmSACPD-B	AY885233	2, D1b	Vegetative and seeds	
	GmSACPD-C	EF113911	14, B2	Highly in seeds	

Table 1. Soybean (*Glycine max* L.) Δ^{9}-stearoyl-acyl carrier protein-desaturase genes including putative chromosome and linkage group assignment and tissue transcript expression.

Transcripts of the *GmSACPD-A* and *-B* were detected in developing seeds and other tissues, but differences in transcript abundance between *-A* and *-B* were not dramatic (Byfield et al., 2006). Translation of the 1158-bp transcript of *SACPD-A* or *-B* predicts a protein of 411 amino acids with a molecular mass of 47.2 kDa. The enzyme is a homodimer with each mature subunit containing an independent binuclear iron cluster. Soybean *SACPD*s contain two characteristic histidine box motifs. High transcript levels of a unique third allele, *GmSACPD-C*, is expressed only in developing seeds (Zhang et al., 2008). Structurally, *SACPD-C* is composed of two exons, not three as for *SACPD-A* and *-B*, separated by an 846-bp intron. Thus, *SACPD-C* differs from the *SACPD-A* and *-B* alleles in that it lacks their large intron located immediately after the putative transit peptide-encoding region. Mutations at *SACPD-C* in two soybean germplasm sources, the mutants A6 (30% 18:0) and FAM94-41 (9% 18:0) (Pantalone et al., 2002), have decreased *SACPD-C* expression and elevated seed stearic acid levels. This finding suggests, conversely, that germplasm with high SACPD-C gene expression and/or enzyme activity would produce elevated 18:1 levels. Polymerase chain reaction-based CAPS (Cleaved Amplified Polymorphism) gene probes (Zhang et al., 2008) were developed to screen soybean germplasm for mutations at *SACPD-C*, since varieties with elevated stearate are desirable for certain industrial uses such as food shortening and soap making.

The effect of increasing temperatures (from 22/18^{o}C to 30/26^{o}C) during seed development on 18:0 accumulation and *SACPD-A* and *-B* transcript accumulation has been measured in growth chamber environments (Byfield and Upchurch, 2007A). At the cool temperature, transcript accumulation of both *SACPD-A* and *-B* was significantly elevated and significantly decreased at the warmer temperature. Decreased *SACPD-A* and *-B* transcript accumulation at the warmer temperature was positively associated with a significant increase in the level of seed 18:0, but only in the high stearate mutant A6. It was suggested that temperature modulation of 18:0 content in wild type soybeans may be more complex, potentially involving in addition to the *SACPDs*, plastid thioesterase *FAT* genes, or warm-temperature post-translational modulation of SACPD enzyme activity.

The role of fatty acid desaturation pathways in mediating pathogen defense signaling has been, until recently, examined mainly in *Arabidopsis*. The *SSI2* gene cloned from *Arabidopsis* was shown to encode an (*At*) Δ^9-stearoyl-ACP-desaturase. Plants with the recessive mutation *ssi2* had a 10-fold reduction in SACPD enzyme content resulting in elevated 18:0 and reduced 18:1 content. Reduced SACPD activity in the *ssi2* mutant lead to induction of a salicylic acid-signaled defense response to the oomycete *Peronospora parasitica,* plant dwarfing and spontaneous leaf lesion formation, but also to inhibition of the jasmonic acid-signaled defense response to the fungus *Botrytis cinerea* (Kachroo et al., 2001, Nandi et al., 2003, Kachroo et al., 2005, Kachroo et al., 2007). In a situation similar to that of *Arabidopsis*, suppression of the rice fatty-acid desaturase gene *OsSSI2* (a rice Δ^9-stearoyl-ACP-desaturase) by transposon insertion or RNAi-mediated knockdown increased 18:0 and reduced 18:1 in plants and markedly enhanced resistance to the blast fungus *Magnaporthe grisea* and the leaf blight bacterium *Xanthomonas oryzae* pv. *oryzae* (Jiang et al., 2009). On the other hand, multiple stresses imposed on avocado fruits including inoculation with the fungal pathogen *Colletotrichum gloeosporioides*, exposure to ethylene, $C0_2$, fruit wounding, and low temperature exposure increased transcript abundance of avocado (*Av*) Δ^9-stearoyl-ACP-desaturase. The up-regulation of *AvSACPD* was accompanied by increases in the concentration of 18:2 (presumably from increased 18:1), increase in an antifungal diene volatile and enhanced resistance to fungal infection (Madi et al., 2003). In soybean as in

Arabidopsis, silencing of the *SACPD* genes (-*A*,-*B*, and -*C*) by a *Bean pod mottle virus*-based vector resulted in plants with reduced 18:1, elevated 18:0, the formation of spontaneous lesions, increased salicylic acid accumulation, and constitutively expressed pathogenesis-related genes. These plants also exhibited enhanced resistance to bacterial and oomycete pathogens (Kachroo et al., 2008, Kachroo & Kachroo, 2009).

3. Soybean omega-6 oleate fatty acid desaturases

The soybean ω-6 oleate fatty acid desaturases (FAD2s) are microsomal enzymes that initiate the primary route of polyunsaturated lipid biosynthesis by catalyzing the first extra-plastidal desaturation to convert 18:1 esterified to phosphatidylcholine to α-18:2 (Heppard et al., 1996). Omega-6 desaturase enzymes are typical of other microsomal desaturases in that they contain three histidine box motifs, possess a C-terminal signal for endoplasmic reticulum retention (Li et al. 2007) and have four predicted transmembrane spanning domains (Tang et al., 2005). Four different soybean ω-6 desaturase genes comprise the soybean *FAD2* gene family (Schlueter et. al., 2007) including *GmFAD2-1* and *GmFAD2-2* and their alleles (Heppard et al., 1996, Tang et al., 2005, Bachlava et. al., 2009), *GmFAD2-3* (Li et al. 2007), and *GmFAD6* (Heppard et al., 1996, Bachlava et al., 2009) (Table 2).

Enzyme function	Gene name	GenBank accession	Chromosome, linkage group	Transcript expression	References
Omega (ω)-6 Fatty Acid Desaturase	*GmFAD2-1A*	AB188250	20, I	Highly in seeds	Heppard et al., 1996, Tang et al. 2005, Bachlava et al. 2009, Li et al., 2007, Ha et al., 2010
	GmFAD2-1B	AB188251	10, O	Highly in seeds	
	GmFAD2-2A	AB188252	19, L	Vegetative and seeds	
	GmFAD2-2B	AB188253	19, L	Vegetative and seeds	
	GmFAD2-2C	AC166742.25	15, E	Vegetative and seeds	
	GmFAD2-2D	AC166091.3	3, N	Vegetative and seeds	
	GmFAD2-3	DQ53237	3 ,N	Vegetative and seeds	
	GmFAD6	L29215	2, D1b	chloroplasts	

Table 2. Soybean (*Glycine max* L.) omega-6 fatty acid desaturase genes including putative chromosome assignment and tissue transcript expression.

GmFAD2-1 genes have a short intron immediately after the start ATG which is spliced out and their mature transcripts encode proteins of approximately 387 amino acids (Tang et. al., 2005). *GmFAD2-1s* are highly expressed during lipid synthesis in developing seeds and not in vegetative tissues, while *GmFAD2-2*s are constitutively expressed in both vegetative tissue and developing seeds. Although the FAD2-2s contribute to the production of 18:1 in all tissues, transcript expression analysis suggests that the FAD2-1s play the major role in

the conversion of 18:1 to 18:2 in developing seeds. Two seed specific isoforms of FAD2-1, FAD2-1A and FAD2-1B, have been described that differ in stability at elevated temperature (Tang et al., 2005). Recent soybean genomic analysis has shown that *FAD2-2* exists as four alleles, *GmFAD2-2A, 2-2B*, *2-2C*, and *2-2D* (Schlueter et al., 2007, Bachlava et al., 2009, Ha et al., 2010). The expression level of *GmFAD2*-2C has been shown to increase eightfold in developing pods grown at 18/12°C in comparison to those grown at 32/28°C. The third gene, *GmFAD2-3*, is also constitutively expressed in both vegetative and developing seed tissues but shows no significant changes in transcript abundance in cold stressed leaves (Li et al., 2007). The fourth gene, *GmFAD6,* encodes an omega-6 desaturase that localizes to the plastid membrane. The expression pattern of the FAD6 gene does not suggest changes in transcript abundance in response to different temperatures (Heppard et al., 1996).

Significant efforts have been expended to select soybean varieties that produce higher seed oil 18:1 content, for example, mid-oleic soybean line N98-4445A which produces 50-60% 18:1 as a percent of total seed lipid fatty acids (Burton et al., 2005). Our understanding of the phenomena of elevated seed oleate and efforts to develop soybeans with this phenotype have been facilitated by the isolation and characterization of the X-ray induced mutant M23 and others with similar oleate phenotypes (Takagi, Rahman, 1996, Anai et al. 2008) and the earlier molecular characterizations of *FAD2-1* in high-oleate producing peanut mutants (Martinez-Rivas et al., 2001, Lopez et al., 2002). M23 was found to contain a large genomic lesion that completely deleted *GmFAD2-1A* (Alt et al., 2005, Sandhu et al., 2007) and mutant KK21 has a deletion of 232-bp downstream of the *FAD2-1A* ATG initiation codon (Anai et al., 2008). Both mutants produce 50-60% 18:1 in their seed lipid compared to approximately 20% 18:1 for conventional soybean cultivars. Many of the higher oleate soybean lines under development are progeny of crosses with the M23 mutant. Field trials have uncovered environmental instability in the expression of this trait in the M23-derived lines (Oliva et al., 2006, Scherder et al., 2008), as well as reductions in seed yield, protein, and oil (Scherder & Fehr, 2008). Possibly, the large genomic deletion in M23 (which extends outside of *FAD2-1A*) or additional X-ray induced mutations in M23 may be responsible for some or all of these additional phenotypic alterations. To develop soybean lines with more stable expression of elevated 18:1 without yield penalty, additional approaches involving reverse genetics have been applied. Ribozyme termination cassettes were employed with the aim of producing transgenic soybean with down-regulated GmFAD2-1 gene expression. Soybean transformants were recovered that stably displayed 18:1 levels in seed lipids of over 75% (Buhr et al., 2002). An intron sense suppression construct of *GmFAD2-1A* was employed with the aim of specifically reducing *FAD2-1* transcripts in developing seeds (Mroczka et al., 2010). Single copy transformants were recovered in which both *FAD2-1* alleles were suppressed that produced seeds with 18:1 levels elevated to 65 to 70% and corresponding reduction of 18:2. Targeting Induced Local Lesions In Genomes (TILLING) was employed with the aim of producing mutations in *GmFAD2-1A*. A missense amino acid mutation was recovered that resulted in an increase in seed 18:1 and a decrease in 18:2 compared to the wild type Williams 82 cultivar (Dierking & Bilyeu, 2009). Recently, soybean lines were identified that contain a single missense mutation in *GmFAD2-1A* or in *GmFAD2-1B* as a result of unique single nucleotide polymorphisms (SNPs) that were predicted to alter seed 18:1 content. Crosses were made to combine the two mutant FAD2-1 alleles from these otherwise conventional lines (Pham et al. 2010). Progeny homozygous for both mutant alleles consistently produced 80% seed 18:1 at different geographic locations, two in Missouri in the US and one in Costa Rica.

In both soybean seed and leaf tissues, the levels of 18:2 and 18:3 gradually increase as temperature decreases to 18/12°C, but the levels of *GmFAD2-1*, *GmFAD2-2*, and *GmFAD6* transcripts were found not to increase at low temperature. This suggests that the elevated 18:2 and 18:3 in developing seeds grown at low temperature are not due to enhanced expression (transcriptional control) of these ω-6 genes (Heppard et al., 1996). On the other hand, in developing soybean seed, the levels of 18:2 and 18:3 decreases as temperature increases to 30/26°C and higher, and the levels of *GmFAD2-1A* and *2-1B* transcripts were found to decrease. This suggests transcriptional down-regulation of the *GmFAD2-1* genes does occur as growth temperatures increase (Byfield & Upchurch, 2007A). Substantial evidence suggests that post-translational regulatory mechanisms likely play an important role in modulating FAD2-1 enzyme activities. The FAD2-1A isoform was found to be more unstable than FAD2-1B, especially at elevated growth temperatures. In addition, the FAD2-1s were phosphorylated during seed development. Evidence suggests that phosphorylation may down regulate FAD2-1 enzyme activity. Thus, growth at elevated temperature results in increased 18:1 and decreased 18:2 and 18:3 because the FAD2-1 oleate desaturase enzymes are substantially inactivated (Tang et al. 2005).

Evidence for the participation of microsomal ω-6 fatty acid desaturases in the responses of plants to pathogen infection is not plentiful. Treatment of cultured parsley cells with the Pep25 peptide elicitor derived from the soybean oomycete pathogen *Phytophthora sojae* resulted in a strong local resistance response. Omega-6 fatty acid desaturase transcripts accumulated rapidly and transiently in elicitor-treated cells, protoplasts, and leaves, suggesting that 18:1 desaturation is an early component of the response of parsley to pathogen infection (Kirsch et al. 1997). Growth chamber experiments (Thomas et al., 2003, Xue et al., 2008) have shown that elevated growth temperatures (34/26 versus 22/18°C) during seed development results in higher 18:1 and reduced 18:2 content in seed lipid. Mature soybean seeds with higher ratios of 18:1 to 18:2 that were inoculated with the fungal pathogen *Cercospora kikuchii* were colonized more heavily by the fungus than inoculated seeds with lower 18:1 to 18:2 ratios (Xue et. al., 2008).

4. Soybean omega-3 linoleate fatty acid desaturases

The membrane lipids of higher plants including soybean are characterized by a high proportion of polyunsaturated fatty acids, in particular, fatty acids in the plastidic galactolipids in most plant species are made up of about 70-80% of the trienoic fatty acids, hexadecatrienoic and α-linolenic acids (16:3 and 18:3) (Harwood 1980). In soybean phosphatidylglycerol (PG) is the only lipid synthesized by the prokaryotic type pathway, one of the two glycerolipid synthetic pathways in plants. The other leaf glycerolipids, monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), digalactosyldiacylglycerol (DGDG), and sulphoquinovosyldiacylglycerol (SQDG) are synthesized through the eukaryotic lipid pathway. Soybean lacks hexatrienoic acid (16:3) and contains α-linolenic (18:3) as the only trienoic fatty acid (Browse, Somerville, 1991). Omega-3 fatty acid desaturases are microsomal enzymes that catalyze the insertion of a third double bond into α-linoleic acid ($18:2^{\Delta 9,\ 12}$) to produce α-linolenic acid ($18:3^{\Delta 9,\ 12,\ 15}$). They, like the microsomal ω-6 desaturases, are characterized by the presence of a diiron cofactor that interacts with three conserved histidine motifs (Byfield & Upchurch, 2007B). Three soybean microsomal ω-6 desaturase genes have been isolated: *GmFAD3A*, *GmFAD3B*, and *GmFAD3C* (Bilyeu et al., 2003, Anai et al., 2005). *GmFAD3A* was found to be highly

expressed in seeds and *FAD3B* and *FAD3C* in both vegetative tissues and seeds. *GmFAD3A*, *B*, and *C* encode proteins that lack N-terminal chloroplast signal peptides. Soybean lines have been identified that produce low (2.8% compared to 8% for wild type) levels of 18:3 in their seed lipid. Low 18:3 in soybean seed lipid is a desired trait since 18:3 contributes to oil instability and rancidity. Molecular characterization of the low 18:3 line showed that a missplice mutation was present in *FAD3A* and also a single SNP altering a codon glycine to glutamic acid was present in *FAD3C (*Bilyeu et al., 2005). Molecular identity probes (CAPS markers, SNPs) were developed for all three soybean FAD3 genes and deployment of these probes for screening combinations of *FAD3* mutant alleles have allowed the development of new soybean lines with 1% 18:3 (Bilyeu et al., 2006, Beuselinck et al. 2006). Chloroplast localized soybean ω-6 fatty acid desaturase genes, designated *GmFAD7* and *GmFAD8* (after *Arabidopsis* chloroplast ω-6 desaturase functional nomenclature) have been partially characterized (Collados et al., 2006) and they do possess N-terminal chloroplast signal peptides (Table 3).

Enzyme function	Gene name	GenBank accession	Chromosome, linkage group	Transcript expression	References
Omega (ω)-3 Fatty Acid Desaturase	*GmFAD3A*	AY204710	14, B2	Seeds highly, vegetative	Bilyeu et al., 2003, Collados et al., 2006, Ha et al., 2010, Upchurch & Ramirez, 2011
	GmFAD3B	AY204711	2, D1b	Vegetative and seeds	
	GmFAD3C	AY204712	18, G	Vegetative and seeds	
	GmFAD7	HM769340	18, G and 7, M	chloroplasts	
	GmFAD8	HM769341	3, N and 1, D1a	chloroplasts	

Table 3. Soybean (*Glycine max* L.) omega-3 fatty acid desaturase genes including putative chromosome and linkage group assignment, and tissue transcript expression.

The discussion that follows focuses mainly on regulation of ω-3 FAD activity at the level of transcription control. A recent report has provided compelling evidence for a temperature-sensitive post-translational regulation of FAD3 protein abundance that involves a combination of cis-acting degradation signals and the ubiquitin-protease pathway that modulates FAD3 protein amounts in response to temperature (O'Quin et al., 2010). The half-life of FAD3 protein is greater at cooler temperatures and protein degradation required specific components of the endoplasmic reticulum protease pathway.

Most of our understanding of ω-3 FAD activity and stress acclamation in plants, including temperature change and pathogen infection, comes from research with *Arabidopsis* and other plants. Characterization of *AtFAD7* gene sequence revealed an open reading frame of 1338 bp comprised of 8 exons that encoded a deduced 446 amino acid peptide of 51.1 kDa. Growth temperature had no apparent effect on the steady-state levels of *FAD7* transcripts in wild-type plants (Nishiuchi & Iba, 1998). The *AtFAD8* sequence was found to code for a 435 amino acid peptide of 50.1 kDa that also contained a consensus chloroplast transit peptide. The coding region of *AtFAD8* shared 75% nucleotide identity with *AtFAD7*. Transcript

abundance of *AtFAD8* strongly increases in plants grown at low temperatures suggesting that the role of *FAD8* in *Arabidopsis* is to provide increased chloroplast membrane 18:3+16:3 in plants that are exposed to low growth temperature (Nishiuchi & Iba, 1998). The temperature dependent regulation of *AtFAD8* expression is not due to the *FAD8* 5′ flanking region (promoter and untranslated region), but to the exon/intron structure that is inherent in the *AtFAD8* gene (Iba, 2002). Examination of *GmFAD7* and *GmFAD8* at NCBI GenBank accession numbers HM769340 and HM769341 revealed that both soybean genes have a similar structure containing 8 exons ranging in size from 67 to 521 nucleotides and 7 introns ranging in size from 90 to 393 nucleotides. The soybean *FAD7* and *FAD8* intron/exon structure is similar to *FAD7* and *FAD8* structures of other higher plants except that the rice *OsFAD8* contains 7 exons. For both soybean genes, an exonic sequence of 1362 base pairs encodes a predicted protein of 453 amino acid residues with molecular masses of 51.3 and 51.4 kDa, respectively, for *GmFAD7* and *GmFAD8*. Using *GmFAD7* and *GmFAD8* genomic sequences as queries to interrogate the Williams 82 genome database (Schmutz et al., 2010) revealed that each gene was present in the Williams 82 genome as two complete copies located on different chromosomes, one *GmFAD7* copy located on chromosome 18 and a second on chromosome 7. A recent report has shown that the second *GmFAD7* gene, designated *GmFAD7-2*, and located on chromosome 7 is paralogous to *GmFAD7-1* located on chromosome 18 (Andreu et al., 2010). The paralogous nature of *GmFAD7-1* and *GmFAD7-2* is supported by the finding of specific gene-related FAD7 protein conformations in soybean seeds. The FAD7 protein conformations were differentially affected by *in vitro* changes in redox conditions and iron availability suggesting the existence of tissue-specific post-translational mechanisms that affect the distribution and activity of the FAD7 enzymes. Two complete copies of *GmFAD8* were also present in the Williams 82 genome sequence and they may, as well, be paralogous. One is located on chromosome 3 and the second on chromosome 1. Recent studies have characterized soybean FAD7 chloroplast localization and the transcript expression patterns in response to light of both microsomal and plastidal ω-3 soybean desaturases. In situ analysis using confocal microscopy with FAD7 antibody and chlorophyll auto fluorescence has shown that the soybean FAD7 protein is preferentially localized to the chloroplast thylakoid membranes suggesting that not only the chloroplast envelope, but also the thylakoid membranes could be sites of lipid desaturation in higher plants (Andreu et al., 2007). *GmFAD3, GmFAD7*, and *GmFAD8* transcription and transcript stability have been found to be differentially regulated by light (Collados et al., 2006). In soybean cell suspension, darkness leads to an overall decrease in 18:3 levels and *GmFAD3* and *GmFAD8* transcripts are undetectable, but after reillumination *FAD3* and *FAD8* transcript abundance increased concomitant with an increase in 18:3 accumulation. *GmFAD7* transcript levels were remarkably similar under dark or light conditions and *GmFAD7* mRNA stability dramatically increased in the dark as well. FAD7 protein levels were also very stable in either light or dark conditions, suggesting that an additional post-translational regulatory mechanism may control the activity of FAD7 in response to light.

Numerous studies have shown that temperature regulates the transcript expression of plastid ω-3 and microsomal ω-3 desaturases and leaf trienoic fatty acid levels in plants. In *Brassica napus* leaf 16:3/18:3 levels increase in MGDG during low temperature acclimation (Williams et al. 1996). In birch (*Betula pendula*) seedlings exposed to low temperatures (+ 4 to -24^{o}C) increased 18:3 in the chloroplast membrane lipids (MGDG, DGDG and PG) were found in the leaves at colder temperatures. The higher 18:3 levels were associated with

upregulated expression of the birch ω-3 desaturases, *BpFAD3*, *BpFAD7* and *BpFAD8* (Martz et al. 2006). Transgenic tomato plants in which the microsomal omega-3 desaturases have been silenced have greatly reduced *LeFAD3* transcripts, contain low levels of 18:3, higher levels of 18:2, and exhibit long-term heat tolerance at 36°C (Wang et al. 2010). Heat stressed *LeFAD3*-suppressed plants produced greater fresh weight of aerial plant parts and had a more intact chloroplast membrane structure than did heat stressed wild-type plants. Growth of soybean plants at a cool temperature (22/18°C, D/N) during seed development resulted in elevated seed 18:3 and elevated Gm*FAD3A*, *FAD3B* and *FAD3C* transcript expression, and conversely, decreased 18:3 and transcript expression of these microsomal omega-3 desaturase genes at a warm temperature (30/26°C, D/N) during seed development (Byfield and Upchurch 2007B). A general conclusion to be drawn from experiments with *Arabidopsis* and other plants is that transcript expression of *FAD8* and *FAD3* change in response to changes in ambient temperature, and *FAD8* is cold-inducible whereas expression of *FAD7* is not affected by changes in temperature (McConn et al., 1994, Berberich et al., 1998, Iba, 2002, Upchurch & Byfield, 2007, Nair et al., 2009, Wang et al., 2010). Another conclusion is that the increased 18:3 level in chloroplast membranes due to upregulated *FAD8* expression is associated with low temperature tolerance in *Arabidopsis* and other plants. Presumably, temperature regulation of soybean *GmFAD 7* and *FAD8* follows a similar pattern.

Upregulation of *FAD7* and increased 18:3 levels in chloroplasts have physiological roles in modulating plant defense responses to pathogens in several plant-pathogen systems. For instance, *FAD7* has been shown to be required to provide 18:3 for the synthesis of a long-distance signal (not jasmonic acid) that is required for the induction of systemic acquired resistance (SAR) in *Arabidopsis* and tomato (Chaturvedi et al. 2008). The *A. thaliana FAD7 and FAD8* double mutation prevents the synthesis of trienoic acids in chloroplast lipids, causing a reduction in the production and accumulation of reactive oxygen intermediates in leaves, reduced levels of programmed cell death, and compromised resistance to several avirulent *Pseudomonas syringae* strains (Yaeno et al. 2004). On the other hand and in contrast, disease resistance to compatible and incompatible races of the rice blast fungus *Magnaporthe grisea* is enhanced in 18:2 accumulating and 18:3-deficient transgenic rice (F78Ri) in which *OsFAD7* and *OsFAD8* were suppressed. The 18:3 Jasmonate-mediated wound responses were suppressed, but the expression of jasmonate-responsive PR genes, PBZ1 and PR1b were induced after inoculation. In rice F78Ri mutant plants, the 18:2-derived hydroperoxides and hydroxides (HPODEs and HODEs) increased significantly and these molecules inhibited the growth of *M. grisea* more strongly than their 18:3-derived counterparts (Yara et al., 2007, 2008). In *Arabidopsis*, local mechanical wounding and pathogen attack causes a rapid rise of *AtFAD7* transcripts in the basal rosette leaves and induces *AtFAD7* expression in the roots. Inhibitors of the oxylipin octadecanoid pathway strongly suppress wound activation of the *FAD7* promoter in roots but not in leaves and stems (Nishiuchi et al., 1997). A specific region of the *AtFAD7* promoter is required for wound-activated expression of this gene in leaves and stems, while another region is necessary for wound-activated, jasmonic acid-responsive expression of the gene in roots (Nishiuchi et al., 1999) suggesting that a jasmonate-independent wound signal may induce the activation of the *FAD7* gene in leaves and stems. In tomato *(Lycopersicon esculentum*) containing a mutation in *Spr2* (which encodes the chloroplast ω-3 FAD gene, *LeFAD7*), the 18:3 content of the leaves was less than 10% of wild-type levels. The accumulation of hexadecatrienoic acid was also abolished and both wound-induced jasmonic acid biosynthesis and the production of a long-distance signal for expression of defensive genes were reduced such that *Spr2* plants were compromised in

defense against attack by tobacco hornworm (Li et al. 2003). Recently it was reported that silencing of the three soybean GmFAD3 genes enhanced the accumulation of *Bean Pod mottle virus* (BPMV) in plant tissues and enhanced susceptibility to virulent *Pseudomonas syringae* bacteria (Singh et al. 2011). Silenced plants exhibited increased levels of jasmonic acid and slightly reduced levels of 18:3 indicating that loss of microsomal ω-3 activity enhances jasmonate accumulation and thereby susceptibility to *BPMV* in soybean.

5. Conclusions

Stearoyl-ACP-desaturase, omega-6, and omega-3 desaturases are diiron cofactor, histidine box motif enzymes that introduce, respectively, the first, second or third double bond into the specific C_{18} fatty acid substrate to yield oleate (18:1), linoleate (18:2), or linolenate (18:3). The expression and activity of these enzymes significantly determines the fatty acid composition and overall quality of soybean oil, and also contributes to the physiological adaptation to environmental temperature and the induction of defense responses to pathogens. Investigations of the regulation of desaturase expression and activity by temperature and pathogens in soybean are relatively recent, but initial findings suggest similarities with *Arabidopsis* and other plants. Down regulation of the *SACPD* gene expression results in plants with reduced 18:1, elevated 18:0, the formation of spontaneous lesions, increased salicylic acid accumulation, and constitutively expressed pathogenesis-related genes (Kachroo & Kachroo 2009). These plants exhibit enhanced resistance to bacterial and oomycete pathogens. In both soybean seed and leaf tissues, the levels of 18:2 and 18:3 gradually increase as temperature decreases, but the transcript levels of the omega-6 desaturases do not increase at low temperature, suggesting that post-translational regulatory mechanisms likely play an important role in modulating the omega-6 (FAD2-1) enzyme activities. Transcript expression of the omega-3 desaturases *FAD8* and *FAD3* do change in response to changes in ambient temperature. *FAD8* is cold-inducible and the increased 18:3 level in chloroplast membranes due to upregulated *FAD8* expression is associated with low temperature tolerance. Upregulation of *FAD7* and increased 18:3 levels in chloroplasts modulate plant defense responses to pathogens through increased production of oxylipin antimicrobial and signaling molecules. SACPD, ω-6, and ω-3 fatty acid desaturase genes are present as multiple copies in the soybean genome as expected given the evidence (Schmutz et al. 2010, Ha et al., 2010) from cytogenetics, genetic mapping, and genomic sequencing that soybean is a paleopolyploid species that underwent at least two major genome duplications. The soybean genome possesses tissue-specific alleles for all three of C_{18} desaturase enzymes involved in the biosynthesis of triacylglycerols. The occurrence of seed-specific alleles of these genes provides for the accommodation of the great increase in lipid biosynthesis that occurs as the developing soybean seeds produce storage lipid reserves (Tang et al., 2005). Genomic (Schmutz et al., 2010) and gene expression analysis (Upchurch & Ramirez, 2010) using the Williams 82 soybean genome database is expected to expand knowledge of soybean gene regulatory sequences and their interaction with transcription complexes. Development of soybean SNP markers (Ha et al., 2010), mapping and dissection of Quantitative Trait Loci (Bachlava et al., 2008, Bachlava et al., 2009A, Bachlava et al., 2009B) and gene silencing analyses (Singh et al., 2011) may lead to the discovery of new genes for fatty acid biosynthesis and stress adaptation, and the potential epigenetic interactions between them. Since the capacity to induce host pathogen defenses is associated with specific desaturase-mediated changes in the levels of unsaturated C_{18} fatty

acids in plant lipid, global climate change (Garrett et al. 2006) may potentially negatively impact plant defenses.

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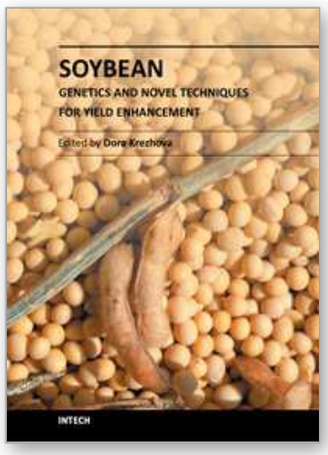

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