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ORIGINAL PAPER

Effect of a novel mutation in a Δ9-stearoyl-ACP-desaturase on soybean seed oil composition

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Abstract Soybean [*Glycine max* (L.) Merr.] oil typically contains 2–4 % stearic acid. Seed oil with 20 % stearic acid would be useful for solid fat applications, both for its cooking properties and health benefits. Breeding lines with high stearic acid have been developed, but many suffer from agronomic problems. This study identifies a new source of high stearic acid, determines its relationship with another high stearic locus and presents molecular markers for it is use in breeding. TCJWB03-806-7-19, a 'Holladay' mutant with high stearic acid, was crossed to two FAM94-41-derived lines that contained a point mutation in a seed-specific isoform of a Δ9-stearoyl-acyl carrier protein-desaturase (*SACPD-C*). Fatty acid analysis was performed over two growing seasons with F_2-derived lines and transgressive segregation for stearic acid content was observed. Sequencing of *SACPD* isoforms in TCJWB03-806-7-19 revealed the deletion of an 'A' nucleotide in exon 3 of *SACPD-B,* which results in a protein whose final 28 amino acids are predicted to differ from Williams 82 SACPD-B. Sorting intolerant from tolerant (SIFT) analysis revealed that this frameshift mutation may affect SACPD-B protein function. Allele-specific genotyping for the *SACPD-C* point mutation and *SACPD-B* nucleotide deletion was performed in both populations. Additive effects and R^2 for stearic acid were +3.3 and 0.55 for *SACPD-C* and +1.9 and 0.19 for *SACPD-B*. Average stearic acid in lines homozygous for both mutations was 14.6 %. This *SACPD-B* mutation represents a novel high stearic allele.

Abbreviations

18:1-ACP TE	18:1-Acyl carrier protein thioesterase
KASII	3-Ketoacyl-acyl carrier protein synthase II
KASPar	KBiosciences competitive allele-specific PCR
NEB	New England biolabs
SACPD	Δ9-Stearoyl-acyl carrier protein-desaturase
SIFT	Sorting intolerant from tolerant
TSAP	Thermosensitive alkaline phosphatase

Introduction

Soybean [*Glycine max* (L.) Merr.] is the most widely produced oil seed crop in the world, accounting for over half of worldwide oil crop production (USDA-Economic Research Service 2011). An oxidatively stable oil with a relatively high melting temperature is necessary for solid fat applications (Clemente and Cahoon 2009). Soybean seed oil naturally high in saturates would be suitable for this end use (List and Pelloso 2007; Clemente and Cahoon 2009). Palmitic (16:0) and stearic acid (18:0) are the two saturated fatty acids present in soybean oil. Typical palmitic and stearic acid contents of soybean oil are 11 and 4 %, respectively (Hildebrand et al. 2008).

Short chain saturated fatty acids, such as palmitic acid, are undesirable because their consumption results in an unfavorable lipoprotein profile in blood serum (Mensink and Katan 1990). In contrast, stearic acid appears to have no cholesterolemic effects in humans (Kris-Etherton and

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Yu 1997) and exhibits similar thrombogenic effects as oleic and linoleic acids (Thijssen et al. 2005).

Stearic acid content in soybean typically represents only 2–5 % of total fatty acids (ILSI 2010; USDA-ARS 2012); however, several germplasm lines have been developed with increased stearic acid. All high stearic soybean germplasm lines have been developed using mutagenesis, with the exception of FAM94-41 (Pantalone et al. 2002). FAM94-41 (9 % stearic acid) has a spontaneously occurring mutation in the *SACPD-C* gene, a seed-specific isoform of a Δ9-stearoyl-acyl carrier protein-desaturase (SACPD), which gives rise to the elevated stearic phenotype (Zhang et al. 2008). SACPD is responsible for the desaturation of stearic acid to oleic acid (Ohlrogge and Browse 1995). The stearic acid QTL on linkage group B2 identified by Spencer et al. (2003) is likely due to the *SACPD-C* mutation in FAM94-41, as FAM94-41 was a parent in the mapping population used and the most closely linked marker identified in the study, Satt070, is 0.2 cM (centimorgans) from *SACPD-C*. The elevated stearic acid phenotype in A6 (28 % stearic acid; Hammond and Fehr 1983) is due to the deletion of *SACPD-C* (Zhang et al. 2008) and is allelic with the mutations in A81-606085 (19 % stearic acid) and FA41545 (16 % stearic acid; Graef et al. 1985). RG-7 (12 % stearic acid) and RG-8 (10.6 % stearic acid) both have a point mutation in *SACPD-C* and are presumably allelic with the A6 and FAM94-41 mutations (Boersma et al. 2012). ST1 (29 % stearic acid), ST3 (23 % stearic acid), and ST4 (23 % stearic acid) also have high stearic mutations allelic to the SACPD-C deletion in A6; however, the mutation in ST2 (28 % stearic acid) has been hypothesized to be at a different locus (Bubeck et al. 1989). Other elevated stearic germplasm lines have also been developed with unknown causative loci (Rahman et al. 1995; Hudson 2012).

Two other isoforms of SACPD have been identified in soybean, *SACPD-A* and *SACPD-B* (Byfield et al. 2006), but to date neither has been associated with an elevated stearic acid phenotype. *SACPD-A* and *SACPD-B* transcripts have been detected in developing seed, root, leaf, and flower tissues while *SACPD-C* transcript has been primarily detected in developing seed (Byfield et al. 2006; Zhang et al. 2008; Kachroo et al. 2008).

3-Ketoacyl-acyl carrier protein synthase II (KASII) and 18:1-acyl carrier protein thioesterase (18:1-ACP TE) are other genes in which a mutation or differential expression could lead to elevated stearic acid content (Pantalone et al. 2002). KASII is responsible for the elongation of palmitic acid to stearic acid (Ohlrogge and Browse 1995). A mutation in KASII has been found to increase palmitic acid in soybean (Aghoram et al. 2006). Theoretically, overexpression or a mutation of KASII could lead to elevated stearic acid. 18:1-ACP TE catalyzes the hydrolyzation of 18:1-ACP and 18:0-ACP to release fatty acid in order to facilitate their transport to the cytoplasm (Jones et al. 1995). Increased 18:1-ACP TE activity has been shown to increase stearic acid content in sunflower (Cantisán et al. 2000).

Stearic acid QTL not associated with any known enzyme in the fatty acid pathway have also been reported. In addition to the QTL associated with *SACPD-C*, 12 additional stearic acid QTL have been reported, on 8 different linkage groups (Diers and Shoemaker 1992; Hyten et al. 2004; Panthee et al. 2006; Reinprecht et al. 2006; Li et al. 2011).

Agronomic problems such as lower seed yield, poor germination, and reduced seedling growth rate have been associated with elevated levels of stearic acid (Lundeen et al. 1987; Rahman et al. 1997; Wang et al. 2001a). The effect varies depending on the causative allele, and not every allele results in a yield decrease (Lundeen et al. 1987). The identification of new high stearic QTL and/or novel mutations in known isoforms of SACPD, KASII, or 18:1-ACP TE may provide alternatives to overcome these constraints, and also may lead to a greater ability to fine-tune fatty acid composition (Cardinal 2008). The objectives of this research were to genetically characterize a new source of elevated stearic acid identified in a population developed by mutagenesis, to determine it is relationship to other known high stearic loci, and to develop molecular markers for use in breeding for improved fatty acid composition.

Materials and methods

Plant materials

Two populations were developed by bi-parental crosses performed in 2009 in Clayton, NC. FA-G is a population of 209 F_2-derived lines from the cross LLL-05-1 × TCJWB03-806-7-19. FA-H is a population of 117 F_2-derived lines from the cross LLL-05-14 × TCJWB03-806-7-19. LLL-05-1 (12.0 % stearic acid) and LLL-05-14 (11.7 % stearic acid) are maturity group V F_5-derived selections from the cross FAM94-41-3 × N98-4445A. LLL-05-14 matures 3 days later than LLL-05-1 and both lines differ in pubescence color. FAM94-41-3 (8.8 % stearic acid; Pantalone et al. 2002) is a selection from an elevated stearic germplasm line with a natural mutation in *SACPD-C* (Zhang et al. 2008). N98-4445A (Burton et al. 2006) is a mid-oleic germplasm line. TCJWB03-806-7-19 is a maturity group V elevated stearic selection from a mutagenized population of the cultivar 'Holladay' (Burton et al. 1996) that was developed by exposing seed to 250 grays (Gy) of gamma radiation from a Gammacell 220

(MDS Nordion Inc., Ottawa, Ontario, Canada). TCJWB03-806-7-19 has 7 % stearic acid compared to the 4 % stearic acid of 'Holladay' (unpublished data).

Field evaluation

In 2010, F_2 plants were grown in Clayton, NC and harvested individually. F_2-derived lines were grown in 3.7-m-long one-row plots in 2011. Soils at Clayton, NC were Norfolk Loamy Sands for both years. In 2011, flower color, pubescence color, and maturity date at the R8 reproductive stage (Fehr and Caviness 1977) were recorded.

Seed oil analysis

Fatty acid methyl ester analysis was performed on a 20-seed sample from each F_2 plant in 2010 and from each $F_{2:3}$ line in 2011. Seed samples were crushed and approximately 1 g was extracted for about 24 h in 3 mL of solvent (chloroform:hexane:methanol, 8:5:2 v/v/v) in stoppered glass test tubes. Fatty acid methyl esters of the lipid extracts were prepared and analyzed as outlined by Burkey et al. (2007).

Oil content was measured from a 10-g seed sample from each F_2 plant in 2010 and each $F_{2:3}$ line in 2011. Oil content was determined by pulsed proton nuclear magnetic resonance (NMR) using a Maran pulsed NMR (Resonance Instruments, Witney, Oxfordshire, UK) by the field induction decay-spin echo procedure (Rubel 1994).

SACPD gene isoforms sequencing

Sequencing was performed for the three known *SACPD* gene isoforms (A, B and C) in the three parental lines and Holladay. Primers used for amplification and sequencing are listed in Tables 1 and 2.

Amplification reactions were carried out in 1× New England Biolabs (NEB, Ipswich, Massachusetts, USA) standard reaction buffer with $MgCl_2$ (final $MgCl_2$ 1.5 mM), 1.4 Units NEB Taq polymerase, 208 μM dNTPs, 6 pmol forward primer, 6 pmol reverse primer, and 3 μL of 10 ng/μL DNA for a reaction volume of 15 μL. A touch-down PCR reaction was performed with the following parameters—94 °C for 2 min—13 cycles of: 94 °C for 32 s, 63 °C for 30 s,−1 °C/cycle, 72 °C for 2 min—22 cycles of: 94 °C for 32 s, 50 °C for 30 s, and 72 °C for 2 min—72 °C for 7 min. PCR products were cleaned with a thermosensitive alkaline phosphatase (TSAP)/Exol enzyme mix prepared as follows: 10.4 μL TSAP, 10.4 μL Exol, 187.2 μL H_2O. 2 μL of the TSAP/Exol mix was used per 8 μL of PCR product and incubated at 37 °C for 45 min followed by inactivation at 80 °C for 15 min. Samples were submitted to GeneWiz (Research Triangle Park, NC) with 5 μM of the sequencing primer.

Molecular marker analysis

A single leaf was collected at random from 20 plants from each F_2-derived line in 2011. A single punch was taken from each leaf using a cork borer and the punches for each F_2-derived line were bulked for DNA extraction. DNA was extracted using a CTAB method (Allen et al. 2006). Primers were designed based on the *SACPD-B*-1114 SNP and *SACPD-C*-229 SNP (Zhang et al. 2008) for use in the KBiosciences competitive allele-specific PCR (KASPar) SNP genotyping system (KBiociences, Herts, UK) (Table 3).

The DNA samples were diluted to 2 ng/μL and 3 μL from each sample were transferred to a 384-well plate and dried down at 55 °C for 30 min in an incubator. Total volume per reaction was 4 μL, which consisted of 2 μL 2× KASP reaction mix, 0.11 μL 0.5× assay mix, 0.072 μL 50 mM $MgCl_2$, and 1.818 μL H_2O. The 0.5× assay mix was prepared to a total volume of 20 μl, which consisted of 1.2 μL 100 μM of each allele-specific primer, 3 μl 100 μM common reverse primer, and 14.6 μL 10 mM Tris pH 8.3.

Table 1 Amplification primers for *SACPD* isoforms in *Glycine max*

Primer	Direction	Sequence (5′→3′)
SACPD-A exon 1	Forward	ACACTTGCTCCTCCTCTCTC
SACPD-A exon 1	Reverse	CACACACAAATGAAAGC
SACPD-A exons 2 and 3	Forward	CTATGATGCCATGCAAACTT
SACPD-A exons 2 and 3	Reverse	CTTCCACAGACATCACTACC
SACPD-B exon 1	Forward	CCTTCTTCTTCTGCCTTACATC
SACPD-B exon 1	Reverse	TTAGACACTCAACCACAC
SACPD-B exons 2 and 3	Forward	CTTCTACCAATGATGCCT
SACPD-B exons 2 and 3	Reverse	CTCACATGTTAACGTAGCG
SACPD-C exons 1 and 2	Forward	CCAACCTCTCCACAGTTCCAC
SACPD-C exons 1 and 2	Reverse	GCTAAGCTAATATGCAAGCC

Table 2 Sequencing primers for *SACPD* isoforms in *Glycine max*

Exon sequenced	Direction	Sequence (5′→3′)
SACPD-A exon 1	Forward	CTCCTCCTCTCTCCTTCTAC
SACPD-A exon 2	Forward	CTGTGTTCATGTAATGGTG
SACPD-A exon 3	Forward	CATTCATATTCACACGAGTG
SACPD-B exon 1	Forward	GCCTTACATCACATACTTC
SACPD-B exon 2	Reverse	CATAAATGGGTAGCAAGAG
SACPD-B exon 3	Forward	GAATACGATGACACTTTCC
SACPD-C exon 1	Forward	CTCTCCACAGTTCCACTC
SACPD-C exon 2	Forward	GGGCAGACTTAAATTGAAC

Table 3 KASPar genotyping primers for *SACPD-B* snp 1114 and *SACPD-C* snp 229

Primer	Fluor	Direction	Sequence (5′→3′)
SACPD-B snp 1114 wild type	FAM	Forward	GAAGGTGACCAAGTTCATGCTAAGTGTTGACGACTCCTTGACTCTT
SACPD-B snp 1114 mutant	VIC	Forward	GAAGGTCGGAGTCAACGGATTGTGTTGACGACTCCTTGACTCTG
SACPD-B snp 1114 common	None	Reverse	AGAAGGTTGGAGGAGAGAGCTCAA
SACPD-C snp 229 wild type	FAM	Forward	GAAGGTGACCAAGTTCATGCTGGGAGATATGGTCACCGAGG
SACPD-C snp 229 mutant	VIC	Forward	GAAGGTCGGAGTCAACGGATTGTGGGAGATATGGTCACCGAGA
SACPD-C snp 229 common	None	Reverse	GTTGATCATGGTCTGGTAAGTGGGAA

Thermocycling parameters were—94 °C incubation for 15 min—10 cycles of: 94 °C for 20 s, 65 °C for 60 s, −0.8 °C/cycle—30 cycles of: 94 °C for 20 s and 57 °C for 60 s. Endpoint fluorescence reading was performed using a Roche LightCycler® 480 (Penzberg, Germany). Allele calling was performed using Version 1.5 of the Roche LightCycler® 480 software.

Statistical analysis

Chi-square tests were conducted to determine if the *SACPD-C* and *SACPD-B* loci were segregating according to the expected ratios in each population (Snedecor and Cochran 1956). Analysis of variance (ANOVA) for all fatty acids and total oil was conducted using SAS PROC GLM (SAS Institute, Cary, NC, 2009). Means were calculated for each F_2-derived line, using the years as the replicates, and linear regression analysis of maturity versus fatty acid concentration was conducted using PROC REG to determine whether maturity had an effect on fatty acid concentration.

For each population, the F_2-derived line means were used in a PROC GLM ANOVA for the *SACPD-B* and *SACPD-C* loci and least squares means were calculated for each genotypic class. Since both populations share the same male parent and the female parents are sister lines, a combined ANOVA for both populations was also conducted. In this combined ANOVA, population and genotypic class by population were also considered as factors. Dunnett pairwise comparison was conducted on the genotypic class least square means to determine if the mutant alleles resulted in a different phenotype than the wild type. Contrast statements were used to estimate the additive and dominant effects of each locus and the additive by additive, additive by dominant, dominant by additive and dominant by dominant epistatic interactions between the *SACPD-B* and *SACPD-C* loci (Holland 2001). Additive and additive by additive effects were estimated using the genotypic class least squares means from both years of data (F_2 and $F_{2:3}$ lines) while dominant, additive by dominant, dominant by additive, and dominant by dominant effects were estimated using only the data from F_2 plants.

Results

Sequence analysis of *SACPD* isoforms

The sequencing results were compared to the Williams 82 reference sequence for each isoform (Schmutz et al. 2010). The *SACPD-A* (glyma07g32850) reference sequence was found to be identical to the Williams 82 reference sequence in all three parents and Holladay.

A silent mutation was identified at nucleotide 930 in *SACPD-C* (glyma14g27990) in LLL-05-01, LLL-05-14, TCJWB03-806-7-19, and Holladay. In addition, the SNP previously identified in this locus at nucleotide 229 in FAM94-41 (Zhang et al. 2008) was also found in LLL-05-01 and LLL-05-14.

The *SACPD-B* (glyma02g15600) coding sequence had a silent mutation at nucleotide 76 in TCJWB03-806-7-19 and

Holladay. TCJWB03-806-7-19 had a deletion of the A at nucleotide 1,114 (Fig. 1a) that is predicted to alter the location of the stop codon and result in a longer protein whose final 28 amino acids are predicted to differ from those of the SACPD-B protein in Williams 82 (Fig. 1b) and the SACPDs examined by Byfield et al. (2006). The TCJWB03-806-7-19 *SACPD-B* sequence is available as GenBank no. JQ993842 and an allele designation has been requested to the soybean genetics committee.

To further examine the conservation of this position, the sorting intolerant from tolerant (SIFT) algorithm as described by Ng and Henikoff (2001) was used. The Williams 82 *SACPD-B* reference coding sequence was used as the query sequence and the TCJWB03-806-7-19 amino acid substitutions caused by the nucleotide deletion were the substitutions of interest. The "UniProt-TrEMBL 2009 Mar" protein database was used, with median conservation of sequences specified as 3.00 and sequences >90% identical to the query sequence were removed. 135 related sequences were retrieved by the algorithm and 12 of the 20 amino acid carboxyl-terminus substitutions due to the nucleotide deletion were predicted to affect protein function.

Genetic analysis of the *SACPD-C* and *SACPD-B* mutations

No segregation distortion was detected for the SACPD-C locus or SACPD-B locus in either population (Table 4). There was also no segregation distortion for flower or pubescence color (data not shown). Since the results for both populations were very similar, only the results from the combined analysis are presented. Maturity differed by 16 days among F_2-derived lines and was not a significant factor in the regression analysis; therefore, it was not included as a covariate in the statistical analysis. No significant additive by dominant, dominant by additive, or dominant by dominant epistatic effects were detected. The mutant forms of both genes were associated with an increase in stearic acid and a decrease in oleic acid content in all the analyses (Table 5). The *SACPD-C* nucleotide 229 mutation had a larger positive additive effect (+3.3) on stearic acid levels than the *SACPD-B* nucleotide 1,114 deletion (+1.9), while both mutations had comparable negative additive effects on oleic acid (Table 6). Significant epistatic additive by additive gene action of effect +3.3 on stearic acid was also detected. The *SACPD-C* mutation exhibited a dominance genetic effect (−0.8) on stearic acid levels while the *SACPD-B* mutation did not (Table 7). Both mutations were associated with a small decrease in palmitic acid and a small decrease in total oil (Table 6). Interestingly, both mutations were associated with opposite significant effects on linolenic acid concentration, a decrease of −0.2 with the mutant form of *SACPD-C* and an increase of 0.4 with the mutant form of *SACPD-B*. R^2 values for the *SACPD-C* and *SACPD-B* loci are indicated in Table 6.

The double-mutant genotype resulted in average stearic acid of 14.6 % versus the wild-type average of 4.3 %. This occurred largely at the expense of oleic acid where lines with both mutations had 14.8 % oleic acid versus 25.4 % oleic acid for the wild type. The double-mutant genotype also resulted in a small, but significant decrease in total oil, 19.6 % compared to 20.8 % for the wild type (Table 8).

Discussion

The effect of the *SACPD-C* nucleotide 229 mutation on fatty acid composition was confirmed in FAM94-41-derived lines and a new functional mutation in *SACPD-B* was identified. The effect of this *SACPD-C* mutation on stearic acid in these populations is in agreement with the mapping study performed by Spencer et al. (2003); however, this study provides stronger evidence of a dominance genetic effect, likely because of a larger population size

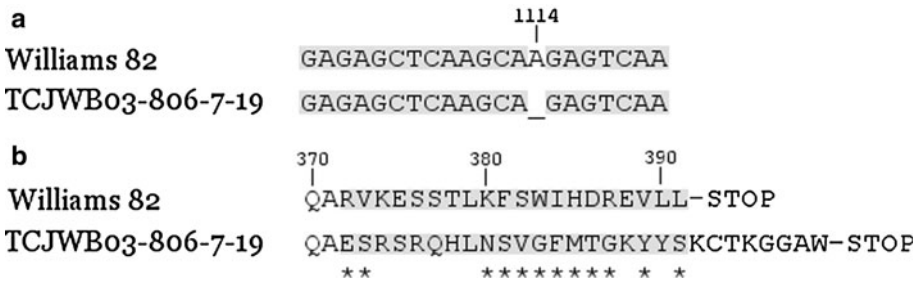

Fig. 1 Frameshift mutation in the *SACPD-B* gene of TCJWB03-806-7-19. **a** Nucleotide sequence comparison of the *SACPD-B* genes from 'Williams 82' (Glyma02g15600) and TCJWB03-806-7-19 (GenBank no. JQ993842) in the region surrounding the 'A' deletion at position 1114 (relative to the start codon). **b** Amino acid comparison showing the frameshift mutation, starting at position 372 (relative to the start amino acid) and predicted altered location of the stop codon in the SACPD-B enzyme from TCJWB03-806-7-19. Amino acids with an *asterisk* are substitutions predicted to affect enzymatic function by sorting intolerant from tolerant (SIFT) analysis

Table 4 Segregation ratios for *SACPD-C* and *SACPD-B* among F_2-derived lines in the LLL-05-01 × TCJWB03-806-7-19 (FA-G) and LLL-05-14 × TCJWB03-806-7-19 (FA-H) populations

Population	Gene	Mutant	Heterozygous	Wild type	Total	χ^2 (1:2:1)	*P* value
FA-G	*SACPD-C*	44	111	54	209	1.77	0.41
	SACPD-B	40	108	61	209	4.45	0.11
FA-H	*SACPD-C*	27	53	37	117	2.74	0.25
	SACPD-B	21	62	34	117	3.31	0.19

Table 5 Fatty acid and total oil least squares means (%) for each genotypic class in the 2-year combined analysis of the F_2-derived populations LLL-05-01 (SACPD-C mutant) × TCJWB03-806-7-19 (SACPD-B mutant) and LLL-05-14 (SACPD-C mutant) × TCJWB03-806-7-19, segregating for *SACPD-C* snp 229 and *SACPD-B* snp 1114

Genotypic class	Palmitic mean	Stearic mean	Oleic mean	Linoleic mean	Linolenic mean	Total oil mean
SACPD-C homozygous mutant	11.3***	11.9***	18.1***	52.0	6.8*	20.2***
SACPD-C heterozygous	11.6***	7.9***	20.7***	52.7	7.1	20.5
SACPD-C homozygous wild type	12.1	5.3	22.8	52.5	7.2	20.5
SACPD-B homozygous mutant	11.5**	10.4***	17.8***	52.8	7.5***	19.9***
SACPD-B heterozygous	11.7	8.2***	20.9***	52.3	6.9	20.5***
SACPD-B homozygous wild type	11.8	6.5	22.8	52.1	6.7	20.8

Mean different from homozygous wild type for a Dunnett pairwise comparison, * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$

Table 6 Additive effect, additive by additive effect estimates, and R^2 associated with *SACPD-C* snp 229 and *SACPD-B* snp 1114 in the 2-year combined analysis of the F_2-derived populations, LLL-05-01 (SACPD-C mutant) × TCJWB03-806-7-19 (SACPD-B mutant) and LLL-05-14 (SACPD-C mutant) × TCJWB03-806-7-19

Gene and parameter estimated	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Total oil
SACPD-C additive effect	−0.4***	3.3***	−2.4***	NS	−0.2**	−0.2***
SACPD-B additive effect	−0.2**	1.9***	−2.5***	NS	0.4***	−0.4***
SACPD-C additive × *SACPD-B* additive	NS	3.3***	NS	−2.8**	NS	NS
SACPD-C R^2	0.12	0.55	0.20	<0.01	<0.01	0.04
SACPD-B R^2	0.01	0.19	0.22	<0.01	0.03	0.18

Estimate significance, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 7 Dominance effect estimates of *SACPD-C* snp 229 and *SACPD-B* snp 1114 in one year analysis of F_2 populations, LLL-05-01 (SACPD-C mutant) × TCJWB03-806-7-19 (SACPD-B mutant) and LLL-05-14 (SACPD-C mutant) × TCJWB03-806-7-19

Gene and parameter estimated	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Total oil
SACPD-C dominance effect	−0.2*	−0.8***	NS	NS	NS	NS
SACPD-B dominance effect	NS	NS	NS	NS	NS	NS

Estimate significance, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

and the use of perfect markers. Boersma et al. (2012) recently reported a line with a SACPD-C mutation whose resulting protein product is predicted to be only 63 amino acids long and a separate line with a point mutation resulting in a predicted proline → leucine substitution at amino acid 286. Interestingly, those two lines, FAM-94-41 and the FAM-94-41-derived lines in this study all exhibit comparable levels of stearic acid. This suggests that all characterized elevated stearic mutations involving *SACPD-C* are due to a complete, rather than partial, loss of function and that one or more additional loci are probably involved in the 28 % stearic acid phenotype observed in A6 (Hammond and Fehr 1983; Zhang et al. 2008). The nucleotide 1,114 deletion in *SACPD-B* is the first functional mutation in this isoform reported in soybean. This deletion causes a frameshift and is predicted to result in a longer protein whose 28 final amino acids are predicted to differ from Williams 82 SACPD-B (glyma02g15600). The SIFT analysis provides support to the notion that this frameshift results in a functional mutation and that the altered fatty acid composition is due to this mutation and not due to a mutation at a linked locus; however, it is

Table 8 Fatty acid and total oil least squares means (%) of two F_2-derived populations, LLL-05-01 (SACPD-C mutant) × TCJWB03-806-7-19 (SACPD-B mutant) and LLL-05-14 (SACPD-C mutant) × TCJWB03-806-7-19 at *SACPD-C* snp 229 and *SACPD-B* snp 1114

SACPD-C	*SACPD-B*	Palmitic mean	Stearic mean	Oleic mean	Linoleic mean	Linolenic mean	Total oil mean
Homozygous mutant	Homozygous mutant	11.1***	14.6***	14.8***	52.1	7.3	19.6***
	Heterozygous	11.4***	11.9***	19.0***	51.3	6.6	20.3**
	Homozygous wild type	11.6**	9.1***	20.4***	52.5	6.4	20.7
Heterozygous	Homozygous mutant	11.4***	9.9***	18.6***	52.7	7.4	20.0***
	Heterozygous	11.7**	7.7***	20.8 ***	52.8*	7.0	20.6
	Homozygous wild type	11.7*	6.1***	22.7***	52.6	6.8	21.0
Homozygous wild type	Homozygous mutant	12.1	6.6***	20.0***	53.6**	7.7*	20.1**
	Heterozygous	12.2	5.0*	23.0**	52.7	7.1	20.7
	Homozygous wild type	12.1	4.3	25.4	51.2	6.9	20.8

Mean different from homozygous wild type/homozygous wild type for a Dunnett pairwise comparison, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

unknown whether the *SACPD-B* nucleotide 1,114 deletion results in a complete or partial loss of enzymatic function. This mutation has a similar effect on phenotype to that observed with the *SACPD-C* nucleotide 229 mutation, which is associated with increased stearic acid, primarily at the expense of oleic acid, and small decreases in palmitic acid and total oil. Additionally, the *SACPD-B* nucleotide 1,114 deletion is associated with a small increase in linolenic acid while the SACPD-C mutation is associated with a small decrease in linolenic acid in these populations. This difference could be due to a low linolenic acid QTL inherited from N98-4445A (Bachlava et al. 2009) being linked to the SACPD-C mutation in the LLL-05-1 and LLL-05-14 parental lines. Although *SACPD-B* expression is not seed specific (Byfield et al. 2006) and *SACPD-B* is not as strongly expressed in seed as *SACPD-C* (Kachroo et al. 2008), this research demonstrates that mutations in this gene do affect seed fatty acid composition and total oil accumulation. There is also significant evidence for an additive by additive epistatic interaction between the *SACPD-C* and *SACPD-B* loci. Biologically, this could be due to the wild-type enzyme of one isoform compensating for the mutant enzyme of another isoform, therefore, the observed additive effect of *SACPD-C* is larger when *SACPD-B* is mutant than when this locus is wild type.

These mutations bring us closer to the 20 % stearic acid necessary for high stearic oil for use in solid fat applications, but another elevated stearic locus will be needed. Combining these two mutations with a *SACPD-A* mutation may reduce 18:0 to 18:1 desaturation activity sufficiently to achieve this goal. Alternatively, these mutant genes could be combined with other mutant genes in the fatty acid metabolic pathway that could result in elevated stearic acid concentration, such as KASII *or 18:1-ACP TE. Also, if the *SACPD-B* nucleotide 1114 deletion only results in a partial loss of function, a mutation with a complete loss of function could also be a candidate for use in developing a high stearic acid soybean oil.

High stearic acid oil, also high in oleic acid, is another oil type desired by industry (Clemente and Cahoon 2009) for its baking qualities and health properties. These data suggest that obtaining both high stearic acid and high oleic acid from a single cultivar may prove difficult due to the increase in stearic acid being obtained at the expense of desaturation to oleic acid. Developing high stearic acid oil and blending it with high oleic acid oil may be a more realistic goal.

Since *SACPD-B* is expressed in a variety of plant tissues, a mutation in it may have agronomic implications not realized in mutations observed in seed-specific desaturases. Silencing all three known isoforms of *SACPD* in soybean has been shown to result in adverse morphological differences (Kachroo et al. 2008). Soybean seed with elevated stearic acid has been shown to have increased triacylglycerol and phospholipid melting temperatures (Wang et al. 2001b). Changes in membrane fluidity are known to affect plant metabolic processes (Quinn and Williams 1978; Yamamato et al. 1981). Decreased membrane fluidity could be responsible for the reduction in total seed oil accumulation associated with these *SACPD-C* and *SACPD-B* mutations. Because the non-seed-specific *SACPD-B* had a larger effect on total oil accumulation, the authors propose that metabolic processes occurring in vegetative tissues, rather than seed, are driving this reduction in total seed oil.

It would be of great value to evaluate this new *SACPD-B* mutation for agronomic effects, such as seed yield, emergence, and cold tolerance to determine if it would be a viable candidate for use in breeding a high-yielding, elevated stearic soybean. The KASPar markers developed in this study would be useful for marker-assisted backcrossing in crosses involving these *SACPD-B* and

SACPD-C mutations to develop a cultivar with elevated stearic acid content.

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