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NOTE

# Novel *FAD3* Mutant Allele Combinations Produce Soybeans Containing 1% Linolenic Acid in the Seed Oil

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**ABSTRACT**

Soybean [*Glycine max* (L.) Merr.] oil typically contains about 7% of linolenic acid, an oxidatively unstable fatty acid that is undesirable for many food applications. For cooking oil, reduction of the linolenic acid content by partial hydrogenation produces *trans* fatty acids, which are now known to increase the risk of coronary heart disease. Genetic reduction of linolenic acid was achieved decades ago, and recently the molecular genetic basis for both 3% and 1% linolenic acid soybean germplasm was revealed to be combinations of mutations in three independent members of the soybean omega-3 fatty acid desaturase (*FAD3*) genes that corresponded to the *fan1*, *fan2*, and *fan3* loci. When the mutant *GmFAD3A* and *GmFAD3C* genes are found in combination in the breeding line CX1512-44, the linolenic acid content is reduced to approximately 3% of the of the seed oil. When the mutant *GmFAD3A* and *GmFAD3B* genes are in combination in the breeding line RG-10, a similar fatty acid profile is produced with approximately 3% linolenic acid. The objective of this work was to determine the ability of different combinations of mutant *FAD3* alleles from CX1512-44 and an RG-10–derived soybean line to produce less than 3% linolenic acid in the seed oil. The results indicated that novel combinations of mutations in the three *FAD3* genes are capable of producing soybean seeds containing only 1% linolenic acid in the seed oil.

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**Abbreviations:** PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

Soybean [*Glycine max* (L.) Merr.] oil is an important vegetable oil used for many foods. The fatty acid profile of soybean oil determines its use in different food and industrial applications. When food labeling requirements changed in 2006 to include the amount of *trans* fats present, the demand for heat and oxidatively stable cooking oils without *trans* fats increased dramatically. While soybean oil was previously partially hydrogenated to reduce the unstable linolenic acid component of the oil, the process also created undesirable *trans* fats. *Trans* fats have been implicated in negative effects on human health, particularly coronary heart disease, and the American Heart Association has recommended limiting *trans* fat intake (Hu et al., 1997; Lichtenstein et al., 2006).

Multiple soybean lines have been identified with reductions in the linolenic acid component of the oil through genetic means, but breeding for the low linolenic acid trait in soybeans has remained complicated. Furthermore, all currently available lines of ultra-low linolenic acid soybeans, containing approximately 1% linolenic acid in the seed oil, trace to a single source (Ross et al., 2000).

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Reduction in linolenic acid content in soybean seed oil to approximately 4% of the fatty acids present in seed oil was first achieved through phenotypic screening of mutagenized lines and germplasm collections (Hammond and Fehr, 1983; Rahman et al., 1996; Rennie et al., 1988; Wilcox and Cavins, 1985). While it was shown that single recessive alleles could reduce linolenic acid content to about the 4% level, additional mutant alleles were necessary to reduce linolenic acid levels below 3% and ultimately to approximately 1% of the seed oil (Fehr et al., 1992; Rahman et al., 1998; Ross et al., 2000; Stojsin et al., 1998; Takagi et al., 1999; Wilcox and Cavins, 1985). Line A29, which contains three recessive alleles at the *fan1*, *fan2*, and *fan3* loci, is the progenitor of the currently available ultra-low (1%) linolenic acid soybean varieties (Fehr, 2007; Fehr and Hammond, 2000; Ross et al., 2000). Other soybean germplasm is available with approximately 3% linolenic acid, and those lines have been shown to contain two genes conditioning the trait (Bilyeu et al., 2005; Fehr et al., 1992; Stojsin et al., 1998; Takagi et al., 1999).

Independent work on the molecular genetic basis for the low linolenic acid trait using different sources of mutant alleles has led to contradictory naming conventions (Anai et al., 2005; Bilyeu et al., 2003). The availability of the soybean genome sequence resulted in an unambiguous, permanent identifier that can be used to link the locus name to the various names representing the genes coding for the soybean FAD3 enzymes, and the annotated soybean genome sequence. *GmFAD3A* corresponds to the *Fan1* locus and is represented as Glyma14 g37350 in the soybean genome sequence; *GmFAD3B* is the *Fan3* locus and is represented as Glyma02 g39230; and *GmFAD3C* corresponds to the *Fan2* locus and is represented as Glyma18 g06950 (Schmutz et al., 2010; W. Fehr, personal communication, 2006). The older linkage group designations would thus be: Glyma14 g37350/*GmFAD3A*/*Fan1* present on B2; Glyma02 g39230/*GmFAD3B*/*Fan3* present on D1b; and Glyma18 g06950/*GmFAD3C*/*Fan2* present on G.

The molecular genetic basis for low linolenic acid soybeans has been demonstrated to be due to mutations in one or more of the three omega-three fatty acid desaturase genes designated *GmFAD3A*, *GmFAD3B*, and *GmFAD3C* (Anai et al., 2005; Bilyeu et al., 2005, 2006, 2003; Chappell and Bilyeu, 2006, 2007; Reinprecht et al., 2009). The FAD3 family of enzymes catalyzes the conversion of linoleic acid precursors into linolenic acid precursors in the endoplasmic reticulum before triacylglycerol synthesis (Yadav et al., 1993). The ultra-low linolenic acid line A29 was shown to possess null alleles of *GmFAD3A* and *GmFAD3B*, in addition to a missense mutation in *GmFAD3C*. In combination, these three mutations were demonstrated to perfectly associate with the 1% linolenic acid phenotype (Bilyeu et al., 2006). Low linolenic acid lines derived from CX1512-44, such as 10-73, contained novel mutant *GmFAD3A* and *GmFAD3C* alleles but wild-type alleles of *GmFAD3B*. As a result, 10-73 contains approximately 3% linolenic acid in the seed oil (Bilyeu et al., 2005). An additional independent low linolenic acid line, designated RG10, also contained less than 3% linolenic acid. RG10 was developed by remutagenesis of the 4% linolenic acid line C1640 (Stojsin et al., 1998; Wilcox and Cavins, 1985). C1640 contained novel null alleles of *GmFAD3A* (Chappell and Bilyeu, 2006). We speculated that line RG-10 and its derivatives contained a second mutant *FAD3* gene, despite the original interpretation that a single locus (*fan-b* alleles at the *Fan* locus) was responsible for the phenotype (Chappell and Bilyeu, 2006; Stojsin et al., 1998). In addition to the *GmFAD3A* mutation from C1640, it was recently demonstrated that line RG-10 contains a novel null allele of *GmFAD3B* (Reinprecht et al., 2009). Other independent 3% linolenic acid soybean lines have also been shown to be due to combinations of mutant alleles of *GmFAD3A* with either *GmFAD3B* or *GmFAD3C* (Anai et al., 2005; Bilyeu et al., 2005).

The objective of the present work was to determine if combining different mutant alleles of the *FAD3* genes present in two 3% linolenic acid lines, 10-73 and line RCAT 0716L, derived from RG10, would create a soybean line containing linolenic acid levels lower than either of the parents. When the project initiated, it was known that both lines contained independent null alleles of the *GmFAD3A* gene coding for the predominant soybean seed omega-3 fatty acid desaturase (Bilyeu et al., 2005; Chappell and Bilyeu, 2006; Stojsin et al., 1998). The *GmFAD3A* allele in the 10-73 line was originally derived from CX1512-44 and is due to a single nucleotide polymorphism (SNP) that disrupts an intron–exon splice site (Bilyeu et al., 2005). The *GmFAD3A* allele in the RCAT 0716L line, derived from C1640, contains an SNP that generates a premature stop codon (Chappell and Bilyeu, 2006). Thus progeny of the 10-73 × RCAT 0716L cross would not segregate for a wild-type version of *GmFAD3A* and were not expected to exceed approximately 4% linolenic acid in the seed oil. Also, at the time this project initiated, the *GmFAD3B* and *GmFAD3C* allele status of RCAT 0716L had not been published. Recently, line RG-10 was characterized molecularly and shown to contain an SNP that resulted in a splice site mutation in *GmFAD3B*, and together with the *GmFAD3A* nonsense allele, combined to reduce the linolenic acid in the seed oil to approximately 3% (Reinprecht et al., 2009). Line 10-73 was known to have a missense mutation in the *GmFAD3C* gene that contributed to further reductions in linolenic acid in the seed oil (Bilyeu et al., 2005). This project describes the effect of segregation of *FAD3* genotypes on fatty acid phenotypes in progeny derived from a cross between two 3% linolenic acid soybean lines, 10-73 and RCAT 0716L.

## MATERIALS AND METHODS

### Parental Selection and Population Development

The population for this study originated from a cross of RCAT 0716L and 10-73 that was made in a greenhouse at Purdue University in

West Lafayette, IN, during the winter of 2007. RCAT 0716L is an experimental line with 3% linolenic acid in the seed oil developed by Dr. Gary Ablett at the University of Guelph. RCAT 0716L was developed from the cross (A2615 × RCAT 9507) × M91-113037. RCAT 9507 is from the cross RG10 × OACShire. RG10, the only source of the low linolenic acid trait in the development of RCAT 0716L, was characterized genetically and more recently at the molecular level (Reinprecht et al., 2009; Stojsin et al., 1998). 10-73 is a selection from 'Williams 82'(Bernard and Cremeens, 1988) × ['Pana' (Nickell et al., 1998) × CX1512-44] containing homozygous mutant alleles of *GmFAD3A* and *GmFAD3C* (Bilyeu et al., 2005). CX1512-44 is a low linolenic acid mutant identified by Dr. Jim Wilcox (retired, USDA-ARS, West Lafayette, IN).

The $F_1$ generation was grown in a greenhouse during the spring of 2008. Approximately 400 $F_2$ seeds were chipped for fatty acid analysis with the remainder of the seed planted and grown in May 2008 in a field nursery at the Purdue University Agriculture Center for Research and Education in West Lafayette, IN. Leaf tissue samples were pressed onto FTA cards (Whatman, Clifton, NJ) for use in molecular marker assays. Five individual $F_2$ plants were identified for each of the four possible homozygous combinations of the mutant alleles for *GmFAD3B* and *GmFAD3C*. The 20 selected $F_2$ plants were single-plant threshed, and a random sample of five $F_3$ seeds from each were analyzed for fatty acid content.

## *GmFAD3A* and *GmFAD3C* Molecular Marker Assays

SimpleProbe assays were developed for *GmFAD3A* and *GmFAD3C* alleles based on the disassociation kinetics of SimpleProbe oligonucleotides (Roche Applied Sciences, Indianapolis, IN). SimpleProbes were designed using the Lightcycler Probe Design Software, version 1 (Roche Applied Sciences), to be exactly complementary to the Williams 82 reference sequence (http://www.phytozome.net/soybean).

The CX1512-44 (10-73) and C1640 (RCAT 0716L) mutations in *GmFAD3A* are separated by only 13 bp, so the SimpleProbe assay is capable of distinguishing among wild-type, CX1512-44, and C1640-derived alleles, as well as all of their heterozygous combinations (Bilyeu et al., 2005; Chappell and Bilyeu, 2006). The *GmFAD3A* probe was: 5′-fluorescein-SPC-GTTA**C**CTTGCCGCGATA<u>C</u>CA-phosphate-3′ blocker, with the position corresponding to the CX1512-44-derived mutation in bold and the C1640-derived mutation underlined. The *GmFAD3C* probe was 5′-fluorescein-SPC-AGGAACCGAC-CAT<u>C</u>CATGGTATGGTACAAGAAT-phosphate-3′, with the location of the CX1512-44 mutation indicated by the underline.

Genotyping reactions used asymmetric polymerase chain reaction (PCR): GMFAD3A, 3AD1:5′-TTGCATCACCATGGTCATCAT-3′ at 0.375 μM final concentration, 3Aix:5′- AGCTATTATCTAGCATTA-ACCTCA-3′ at 0.15 μM final concentration, GmFAD3C, 653Dup:5′-GTCCTTTGTTGAACAGCATT-3′ at 0.375 μM final concentration, and 653T:5′ CTCCTGCAAAAAATC-CATGAGTTGT-3′ at 0.15 μM final concentration.

Reactions were performed in a total volume of 20 μL containing template, primers, 0.2 μM final concentration of SimpleProbe, buffer (40 mM Tricine-KOH [pH 8.0], 16 mM KCl, 3.5 mM $MgCl_2$, 3.75 μg $mL^{-1}$ BSA, 200 μM dNTPs), 5% DMSO, and 0.2× titanium *Taq* polymerase (BD Biosciences, Palo Alto, CA). Genotyping reactions were performed using a Lightcycler 480 II real-time PCR instrument (Roche Applied Sciences), using the following PCR parameters: 95°C for 5 min followed by 40 cycles of 95°C for 20 s, 60°C for 20 s, 72°C for 20 s, and then a melting curve analysis (55 to 75°C for *GmFAD3A*, 60 to 75°C for *GmFAD3C*).

Each genotype produced a characteristic melting profile, as measured by $T_m$ of the negative first derivative of the disappearance of fluorescent signal. *GmFAD3A* yields the following peaks: 64.3°C for wild-type Williams 82 (used as control only), 58.8°C for homozygote C1640-derived lines (RCAT 0716L), and ~54°C for homozygote CX1512-44–derived lines (10-73). Lines containing mutant *GmFAD3A* alleles are represented with lowercase letters (aa). *GmFAD3C* yields the following peaks: 69.3°C for wild-type (represented as CC) and 64.5°C for CX1512-44–derived lines (10-73; represented as cc). Heterozygotes feature two peaks of approximately equal height (represented as Cc).

## *GmFAD3B* Molecular Marker Assay

The *GmFAD3B* molecular marker assay was designed to distinguish RG-10–derived mutant *GmFAD3B* alleles from wild-type *GmFAD3B* alleles (Reinprecht et al., 2009) based on resolution of allele-specific amplification products after the inclusion of GC-rich tails as previously described (Wang et al., 2005). The primers used were FAD4/5(*r*), 5′- CTCATTCATAACTAGTC-CACTTCTAG-3′, FAD4(f), 5′- gcgggcagggcggcCGAAAAAG-TAACAATGTAGGAGTAATTTAGTTAC-3′, and FAD5(f), 5′- gccgcgGAAAAAGTAACAATGTAGGAGTAATTTAGT-TAT-3′. Reactions were performed essentially as described in the preceding section, except the annealing temperature was 65°C and the 15-μL reactions included 0.5 μM final concentration of each primer and 1.25 μM EvaGreen (Biotium, Inc., Hayward, CA). Wild-type *GmFAD3B* alleles (represented as BB) produced a melting curve peak at 77°C, RG-10–derived *GmFAD3B* alleles (represented as bb) produced a peak at 75°C, and heterozygotes (represented as Bb) produced a major peak at 75°C and a shoulder at 77°C. Reactions were performed on a DNA Engine Opticon 2 (MJ Research/Bio-Rad) or Lightcycler 480 II.

Templates for PCR were washed 1.2-mm FTA card presses of $F_2$ leaves prepared according to the manufacturer's directions. The total number of plants containing a particular genotype was distorted by our inability to successfully genotype all of the samples for *FAD3B*. Although fatty acid data were determined for all $F_2$ seed chips, not all samples were completely genotyped due to a lack of germination, poor quality leaf presses, or a focus on homozygous *GmFAD3C* genotypes.

## Fatty Acid Phenotyping

Fatty acid analysis was performed on seed chips from $F_2$ seeds or five individual $F_3$ seeds from five $F_2$ plants per genotype, according to established protocols (Beuselinck et al., 2006; Bilyeu et al., 2005). The results were expressed as relative percentages of each fatty acid component present in the extracted oil fraction.

# RESULTS

To investigate the ability of new combinations of mutant *FAD3* genes to produce low linolenic acid soybeans, we

**Table 1. Fatty acid profiles of triple homozygous mutant *FAD3* genotype lines and the parental lines.**

| Soybean Line | Genotype | Palmitic | Stearic | Fatty Acid Oleic | Linoleic | Linolenic |
|---|---|---|---|---|---|---|
| | | Percent of total oil | | | | |
| $F_2$#15† | aabbcc‡ | 10.7 ± 0.51§ | 5.3 ± 0.4 | 27.4 ± 1.4 | 55.1 ± 1.6 | 1.5 ± 0.4 |
| $F_2$#26 | aabbcc | 12.7 ± 0.41 | 5.1 ± 0.3 | 22.7 ± 1.6 | 58.1 ± 1.8 | 1.4 ± 0.1 |
| $F_2$#51 | aabbcc | 11.6 ± 0.35 | 4.8 ± 0.2 | 25.7 ± 0.72 | 56.6 ± 0.62 | 1.3 ± 0.1 |
| $F_2$#110 | aabbcc | 11.5 ± 0.26 | 4.8 ± 0.3 | 24.1 ± 2.2 | 58.2 ± 2.3 | 1.4 ± 0.1 |
| $F_2$#223 | aabbcc | 11.4 ± 0.42 | 4.9 ± 0.1 | 23.6 ± 1.9 | 58.8 ± 1.8 | 1.3 ± 0.1 |
| All $F_3$ | aabbcc | 11.6 ± 0.75 | 5.0 ± 0.3 | 24.7 ± 2.3 | 57.4 ± 2.1 | 1.4 ± 0.2 |
| 10-73 | aaBBcc | 11.1 ± 0.22 | 3.9 ± 0.1 | 23.2 ± 2.1 | 59.3 ± 2.1 | 2.5 ± 0.2 |
| RCAT 716L | aabbCC | 10.8 ± 0.56 | 4.3 ± 0.3 | 22.6 ± 0.56 | 59.5 ± 0.82 | 2.9 ± 0.1 |

†Five individual $F_3$ seeds from the indicated $F_2$ plants were subjected to fatty acid analysis. All $F_3$ indicates the combination of the data from the individual triple homozygous lines above.

‡The *FAD3* genotype is listed with lowercase letters representing recessive mutant alleles and capital letters indicating functional alleles. Underlined *FAD3a* alleles represent those originally derived from C1640 while non-underlined *FAD3a* alleles represent those originally derived from CX1512-44.

§Mean values plus and minus one standard deviation are indicated.

crossed the parental genotypes 10-73 (*GmFAD3*aaBBcc, Table 1) and RCAT 0716L (*GmFAD3*aabbCC, Table 1). We hypothesized that segregants that inherited all three homozygous mutant alleles (*GmFAD3*aabbcc) would contain the lowest level of linolenic acid in the seed oil. Molecular marker assays were developed or improved from existing assays to distinguish each of the possible mutant *FAD3* alleles from each other, in the case of *GmFAD3A*, or from their wild-type counterparts, as in the case for *GmFAD3B* and *GmFAD3C*. Individual $F_2$ progeny of the 10-73 × RCAT 0716L cross were analyzed for both their fatty acid phenotype and their *GmFAD3A*, *GmFAD3B*, and *GmFAD3C* genotype. The results indicated a strong positive association between the total number of wild-type *FAD3* alleles present and an increase in linolenic acid content (Fig. 1). A step-wise decrease in linolenic acid content was observed as wild-type *FAD3* alleles were replaced by their mutant counterparts. When the triple mutant *FAD3* genotype was recovered (*GmFAD3*aabbcc), the linolenic acid content was the lowest of all of the recovered genotypes, with a mean of 1.4%. The mean of seeds with one wild-type *GmFAD3B* was 2.1% and that of seeds with one *GmFAD3C* allele was 2.2% linolenic acid. As expected, the highest values of linolenic acid were approximately 4.5% of the seed oil instead of the typical approximately 8%, due to the presence of *GmFAD3A* mutant alleles in all of the experimental samples.

The stability of the linolenic acid phenotype was evaluated for the homozygous combinations of *FAD3* alleles. Field-produced $F_3$ seeds from five independent $F_2$ plants for each homozygous genotype along with the parental lines 10-73 and RCAT 0716L were analyzed for their fatty acid phenotype (Fig. 2). Similar to the previous generation, seeds with the triple mutant *FAD3* genotype (*GmFAD3*aabbcc) contained the lowest linolenic acid content at 1.4% of the seed oil. The highest linolenic acid content was for the seeds containing the most wild-type *FAD3* alleles (4.1% for *GmFAD3*aaBBCC). The parental linolenic acid phenotype was recovered for the lines with *FAD3* genotypes identical to the RCAT 0716L line, but lines with *FAD3* genotypes identical to the 10-73 line contained higher average linolenic acid content.

The overall fatty acid profiles were compared for the average of the $F_3$ seeds derived from each of the five triple mutant *FAD3* genotype $F_2$ plants as well as the parental lines (Table 1). Besides the statistically significant lower linolenic acid levels for each of the five *GmFAD3*aabbcc lines compared to the two parental lines, the stearic acid levels were significantly higher for the triple mutant *FAD3* genotype lines compared to the parental lines. Although a concomitant increase in linoleic acid levels would be expected from a decrease in FAD3 activity, the relatively large standard deviations for the linoleic acid data obscured any significant differences among the genotypes for linoleic acid content. Variability of oleic acid levels for the different lines was also noted. There was no significant difference in the linolenic acid content between genotypes that were homozygous for either the 10-73–derived *FAD3A* allele or the RCAT 0716L–derived *FAD3A* allele.

## DISCUSSION

The primary objective of the study was to determine if novel combinations of mutant alleles of soybean *FAD3* genes were capable of producing seeds with approximately 1% linolenic acid levels in the seed oil. Transgressive segregation from a cross of two lines with approximately 3% linolenic acid resulted in some soybean lines that had an average of 1.4% linolenic acid in the seed oil fraction. The lines with the lowest linolenic acid were those that recovered homozygous combinations of mutant alleles of *FAD3A*, *FAD3B*, and *FAD3C*. For the five triple mutant soybean lines evaluated in the $F_3$ generation, two contained homozygous mutant *FAD3A* alleles derived originally from CX1512-44, two contained homozygous mutant *FAD3A* alleles derived originally from C1640, and one line was heterozygous for mutant alleles of *FAD3A*. Although some seeds contained 1.1% linolenic acid, no seeds in the $F_2$ or $F_3$ generation of the experiment contained less than or equal to 1.0% linolenic acid in the

seed oil, a phenotype we have previously observed for some of the *fan1/fan2/fan3* lines such as A29 and IA3017 (data not shown). Additional genetic factors may be responsible for the minor differences in accumulation of linolenic acid between these genotypes. A fourth *FAD3* gene (Glyma11 g27190) exists in the soybean genome as well as multiple plastid-targeted omega-3 fatty acid desaturases that have not yet been fully investigated individually for their roles in linolenic acid accumulation in the seed oil.

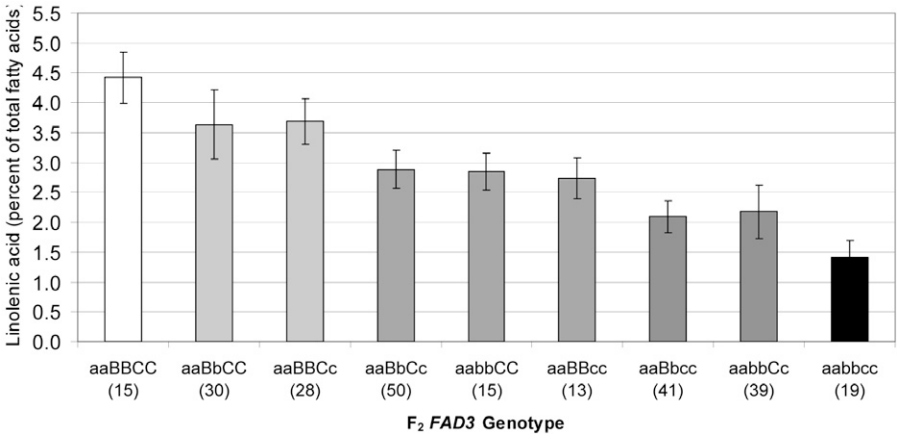


Figure 1. Association between seed oil linolenic acid phenotype and *GmFAD3* genotype in the $F_2$ generation. The columns represent the mean linolenic acid content of the oil from seed chips for each *FAD3* genotype listed with capital letters representing functional alleles and lowercase letters representing mutant alleles. Error bars represent plus and minus one standard deviation from the mean. The numbers in parentheses, below each column, represent the number of seed samples from each genotypic class. Columns are shaded based on their relative number of functional *FAD3* alleles present with darker columns representing samples with fewer functional alleles.

We were able to detect very small differences in linolenic acid content dependent on the number of functional *FAD3B* or *FAD3C* genes present; *FAD3A* was mutant in all cases. For the $F_2$ generation grown in the greenhouse, it did not appear that there were differences in the quantitative reduction in linolenic acid for lines containing the same number of *FAD3B* or *FAD3C* mutant alleles (compare aaBbCC and aaBBCc; aaBbCc, aabbCC, and aaBBcc; and aaBbcc and aabbCc in Fig. 1). In the field-grown $F_3$ generation, there were significant differences detected, with the linolenic content differing between the parental line 10-73 (FAD3aaBBcc), the $F_3$ progeny that recovered the parental genotype (aaBBcc), and the $F_3$ progeny that recovered the alternate parental genotype (aabbCC) (Fig. 1). The relative contribution and magnitude of the genotypic and environmental impacts responsible for the minor differences in linolenic content found in combinations of mutant *FAD3A* and either homozygous mutant *FAD3B* or *FAD3C* alleles will require further study.

We recovered two novel combinations of mutant *FAD3* alleles capable of producing approximately 1% linolenic acid soybeans, one in which the *FAD3A* allele was derived from CX1512-44 and one in which the *FAD3A* allele was derived from C1640. In regard to linolenic acid levels, no statistically significant difference was detected between the effect of the CX1544-12 (10-73)–derived mutation and the C1640 (RG10)-derived mutation, for *GmFAD3A,* in the *GmFAD3*aabbcc progeny. Since both sources of *FAD3A* mutations are null alleles (Bilyeu et al., 2005; Chappell and Bilyeu, 2006; Reinprecht et al., 2009), this result was not surprising. All confirmed sources of *FAD3A* mutations reported to date are null alleles (Anai et al., 2005; Bilyeu et al., 2005, 2003; Byrum et al., 1997; Chappell and Bilyeu, 2006, 2007). The *FAD3B* mutation in this work is due to a mutation that disrupts a splice site, and the *FAD3C* mutation used in this work is due to a missense mutation in a conserved amino acid residue (Bilyeu et al., 2005; Reinprecht et al., 2009). One avenue for future investigation is to identify null mutations in the *FAD3C* gene to

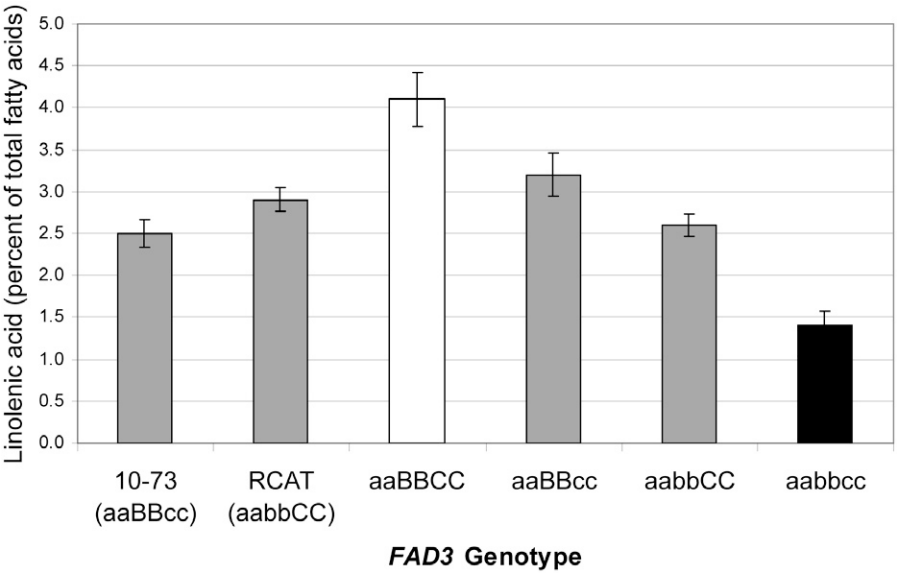


Figure 2. Stability of $F_3$ seed oil linolenic acid phenotype in different *GmFAD3* genotypes. Each column represents mean linolenic acid content, expressed as a percentage of the oil fraction, from whole $F_3$ seeds, with error bars representing plus and minus one standard deviation from the mean. Five independent homozygous $F_2$ plants for each *FAD3* genotype were analyzed, along with the parental lines 10-73 and RCAT 0716L, grown in the same field environment. Capital letters in the genotype represent functional alleles and lowercase letters represent mutant alleles. Columns are shaded based on their relative number of functional *FAD3* alleles present with darker columns representing samples with fewer functional alleles.

incorporate the mutant alleles into a line with null alleles of the *FAD3A* and *FAD3B* genes and evaluate the fatty acid phenotype of the seed oil.

The results of this work demonstrated the ability to produce novel 1% linolenic acid soybean germplasm containing unique combinations of mutant *FAD3A*, *FAD3B*, and *FAD3C* alleles. The 1% linolenic acid phenotype was stably inherited. In addition, the availability of molecular marker assays to select for the desired alleles will accelerate the development of new low linolenic acid soybean cultivars with the novel gene combinations.

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