Research Article

Soybean Oil-Quality Variants Identified by Large-Scale Mutagenesis

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To identify genetic variation for fatty acid composition in mature soybean seeds, 4566 M₃ generation seed samples from a chemically mutagenized population were subjected to fatty acid profiling by gas chromatography. In the population, a wide range of variation in the content for each of the five major fatty acids was observed. Seventy-nine lines were identified which contained significantly high or low levels of one of the five major soybean fatty acids. These lines were advanced to the subsequent generation. Of the 79 lines showing a variant fatty acid profile in the M₃, 52 showed clear heritability for the oil composition in the seeds of the subsequent generation. These lines are likely to represent 52 distinct genetic mutations. These mutants may represent new loci involved in the determination of soybean seed oil content or could be new isolates or alleles of previously identified genetic variants for soybean oil composition.

1. Introduction

Soybean oil is composed of five major fatty acids, which are synthesized in the seed during development [1]. In general, wild-type soybeans contain 10–12% palmitic acid (16:0), 3–4% stearic acid (18:0), 20–25% oleic acid (18:1), 50–55% linoleic acid (18:2), and 8–10% linolenic acid (18:3). Soybean oil has industrial uses and is also a source of vegetable oil for human consumption. For these divergent applications, a modified fatty acid composition profile is sometimes desirable. For example, oil low in the saturated fatty acid palmitate has health benefits in food oils, and reduction of linolenic acid results in increased oxidative stability without the need for hydrogenation [2]. However, for some industrial applications, increased levels of saturated fatty acids (stearate and palmitate) could be desirable [3]. Additional alleles providing new ways to incorporate new and existing oil composition traits could be of use to breeders for many reasons.

Genetic variation is an inexpensive and nontransgenic way to achieve alterations in oil content, which does not require postharvest processing. Soybean mutagenesis has previously been used for the successful improvement of seed oil composition [4–6]. In addition, novel alleles have been

identified in germplasm collections of natural accessions, and the combination of these two sources of genetic variation has resulted in the identification of a number of soybean genotypes with modified oil content [2, 3]. In some cases genetic lesions underlying these traits have been identified at the molecular level. In all published instances for soybean, the mutations are found in genes encoding enzymes that function within the soybean oil biosynthetic pathway. A number of mutations in one of the major enzymes required for fatty acid biosynthesis in the developing soybean seed have been identified in current composition variants. Mutation in the gene encoding the beta-keto-acyl-ACP synthase (*KASIIa*) results in seeds containing high levels of palmitic acid [7]. The low linolenic acid-containing mutants of soybean carry mutations in the *fatty acid desaturase3* (*FAD3*) genes [8–10]. Lines carrying mutations in the omega-6 fatty acid desaturase (*FAD2-1a*) gene results in increased levels of oleic acid [11]. The A6 and FAM94-41 lines carry mutations in the gene encoding the delta-9-stearoyl-ACP-desaturase (*SACPD-C*), which results in high levels of stearic acid [12].

While many variants with significantly improved oil quality for various applications have been identified and used in breeding programs, previous efforts have focused on screening a population for a specific, favorable altered composition

trait. In this project we characterize and describe a large number of mutants with composition distinct from that of the parental genotype. In some cases variants with an unfavorable oil quality profile (one that is unsuitable for any current downstream applications) may add to the present body of knowledge about other genes required for normal oil deposition and soybean seed development and offer new avenues for the genetic improvement of oil content. At the time of this writing, all of the known mutants in oil composition traits have implicated biosynthetic enzymes, and it is likely that other genes encoding proteins that affect the activity or localization of these enzymes could impact oil quality. While such mutations may affect quality to a lesser degree, they may offer scope for more dramatic improvements using biotechnological approaches or in combination with other alleles. Here we describe the screening of a large mutant population for oil composition and the identification of a number of heritable mutations that affect the determination of soybean oil quality.

2. Methods

2.1. Plant Materials and Growth Conditions. The mutant population consists of N-nitroso-N-methylurea (NMU) mutagenized *Glycine max* cv. Williams-82 (W82) and was described previously [13, 14]. To screen for new, and as-yet unidentified, mutations that contribute to the fatty acid composition of mature soybean seeds, a forward genetic screen was conducted for seed oil composition. A total of 4566 mutagenized lines were subjected to fatty acid profiling by gas chromatography (GC). The fatty acid composition was initially screened by profiling samples of M_3 seeds (each sample consisting of three seeds from an individual M_2 plant) produced in the field at West Lafayette, IN, USA during the 2005 growing season and comparing these to the W82 wild type grown in parallel. The heritability of the identified variants was subsequently confirmed in the next generation (5 M_4 seeds from 3 to 25 individual M_3 plants) produced in the field in West Lafayette, IN during the 2008, 2009, or 2010 growing seasons. To estimate the level of environmentally induced variation and to calculate statistical significance for plant-to-plant differences in oil content, ten W82 plants were harvested individually from the field (2009 growing season) and their seed was analyzed for oil content. Level of significance was calculated by Welch's *t*-test comparing values from M_4 samples to these ten wild-type seed samples.

2.2. Fatty Acid Composition Profiling. Fatty acid profiling was performed at the National Center for Agricultural Utilization Research (Peoria, IL) using the following protocol: fatty acids were extracted into CHCl_3 :hexane:MeOH (8:5:2) for 4 hours at room temperature from three beans cracked with a small hammer. A volume of 0.1 mL 0.35 M methoxide was added to the samples. Samples were analyzed on an Agilent 6890 GC with an Agilent J&W GC column (DB225, 30 m $\times$ 0.25 mm id $\times$ 0.25 μm film thickness). Data was normalized such that the palmitic, stearic, oleic, linoleic, and linolenic fractions total is 100, and content is expressed as a percentage. Data was analyzed and plots were generated in Microsoft

Excel and R (<http://cran.r-project.org/>). Product names are necessary to report factually on available data. However, the USDA neither guarantees nor warrants the standard of the product and the use of the names implies no approval of the product to the exclusion of others that may also be suitable.

3. Results

Screens for seed fatty acid content were conducted over four years. For each fatty acid, a normal distribution was observed among the 4566 lines profiled. Each distribution showed a number of outliers that potentially represent mutants with extreme levels of one of the major fatty acids (Figure 1). In the population as a whole, a broad range of composition of the five major fatty acids was observed. Specifically, palmitate levels ranged from 5 to 16%, stearate levels ranged from 2% to 15.3%, oleate levels ranged from 14.6 to 46.1%, linoleate levels ranged from 34.6% to 62.6%, and linolenate levels ranged from 4% to 12.9% (Figure 1). For each fatty acid, upper and lower cut-offs were chosen based on the distribution: nine lines with seed palmitate levels above 14% or below 7% were selected for further characterization. Eight lines with stearate content less than 3.5% or greater than 8% were selected, 39 lines with oleate content less than 19% or greater than 30% were selected, 5 lines with less than 40% or more than 60% linoleate were selected, and 18 lines with less than 5% or more than 10% linolenate were selected for further characterization. Mutant M_3 seeds of these 79 selected lines were planted in the field during the 2008–2010 growing seasons, and M_4 seed samples derived from self-pollinated individual plants from these lines were profiled for oil composition. Data from the M_4 seed samples allows determination if the phenotype was transmitted to the following generation, and if the M_3 lines were heterozygous or homozygous. Figure 2 shows the reproducibility of fatty acid levels in the M_3 and corresponding M_4 progeny seed samples for 52 lines that were selected for propagation and crosses on the basis of reproducibility of the phenotype in the M_4 samples, taking into consideration that the mutation may not have been homozygous in the M_3 generation. In particular, in three lines (lines 8, 34, and 36) evidence of both aberrant and wild-type fatty acid levels in the M_4 progeny is apparent, which may indicate that the original M_3 isolate was heterozygous. While a range of variation in the levels of fatty acids are observed in the M_4 progeny of several of the mutant lines, in contrast in the W82 control plants, variation between individual plants for the level of each fatty acid was less than 0.5%, and minimal year to year variation was observed (Figure 2). For the majority of selected lines, fatty acid values from mutant M_4 differed significantly from the wild type ($P < 0.001$).

Complete fatty acid profiles for the M_3 lines that were selected for further study are listed in Table 1, along with statistical significance data for oil content differences in the M_4 seed samples (see Section 2). A total of three (lines 1–3) low-palmitic acid-containing mutants were identified, two of these contained normal levels of oleic acid. Six (lines 4–9) high-palmitic acid-containing mutants were identified (see Section 4). Four low-stearic acid-containing mutants

TABLE 1: Complete fatty acid profiles for selected M₃ mutants.

Key ID	16:0%	18:0%	18:1%	18:2%	18:3%	Significance	
W82	10.6	4.0	22.9	54.8	7.1	NA	Wild type
1	5.9	4.4	31.2	53.2	5.4	***	Low 16:0
2	5.1	3.5	24.5	59.8	7.2	***	
3	6.6	4.6	26.8	55.6	6.4	***	
4	15.1	4.0	26.0	48.7	6.3	***	High 16:0
5	15.0	5.8	20.5	50.8	7.9	***	
6	12.4	5.0	23.3	50.8	8.5	***	
7	15.1	5.7	20.7	49.8	8.6	***	
8	14.9	4.8	22.5	50.8	7.1	NS	
9	16.4	3.2	19.3	52.9	8.3	***	Low 18:0
10	9.6	3.1	21.1	58.2	8.0	***	
11	10.3	3.2	21.0	58.8	6.7	***	
12	9.7	3.2	24.7	55.0	7.5	***	
13	8.1	2.9	23.7	56.0	9.4	***	High 18:0
14	10.9	10.1	19.2	51.7	8.1	*	
15	10.1	11.5	31.8	41.6	5.0	**	
16	8.6	15.3	17.2	50.9	8.0	***	
17	10.2	7.1	25.0	51.0	6.7	***	Low 18:1
18	9.5	3.9	17.4	58.5	10.7	***	
19	10.6	3.6	17.0	59.0	9.8	***	
20	10.1	3.7	16.5	62.6	7.1	***	
21	8.1	4.2	17.3	61.1	9.3	***	
22	7.7	3.6	17.7	60.6	10.3	***	
23	8.6	4.1	17.6	59.0	10.8	***	High 18:1
24	8.4	4.3	46.1	34.0	7.2	***	
25	10.7	5.3	38.0	39.6	6.4	***	
26	10.1	5.0	42.4	37.2	5.5	*	
27	9.7	4.4	34.3	46.0	5.7	***	
28	10.8	4.0	33.4	45.7	6.2	***	
29	8.4	4.8	41.5	39.6	5.7	**	
30	9.2	4.8	36.3	43.7	6.0	***	
31	9.8	4.7	36.4	41.5	7.7	***	
32	8.9	4.5	43.6	35.8	7.1	***	
33	8.9	4.4	35.7	45.4	5.7	**	
34	9.3	5.0	44.5	36.7	4.5	NS	
35	12.6	4.7	31.5	45.1	6.2	***	
36	9.1	4.7	33.2	47.8	5.4	NS	
37	9.2	4.8	38.5	42.0	5.5	***	
38	9.3	5.8	33.4	44.7	6.8	***	
39	9.9	4.3	33.7	44.5	7.7	***	High 18:2
40	6.3	3.5	19.9	60.9	9.4	***	
41	7.6	3.7	20.3	60.1	8.2	**	
42	9.6	3.5	19.9	61.0	6.0	***	Low 18:3
43	10.3	3.7	26.1	55.9	4.1	***	
44	9.0	4.8	37.8	44.4	4.0	***	High 18:3
45	6.9	6.6	21.9	53.5	11.1	**	
46	6.8	4.6	20.6	55.1	12.9	***	
47	10.1	4.4	24.0	50.0	11.5	*	
48	9.3	4.8	20.3	54.9	10.7	***	
49	10.7	4.4	19.2	55.0	10.8	***	
50	8.6	3.1	20.9	56.9	10.5	***	
51	9.4	5.2	16.8	56.1	12.6	***	
52	9.2	5.8	19.6	54.8	10.5	***	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant, NA: not applicable for wild type samples.

FIGURE 1: Distribution of fatty acid levels in the mutant population. Percentage of each fatty acid as measured over 4566 M_3 seed samples from mutant M_2 individuals by gas chromatography for five major fatty acids of soybean seeds. Black diamond on x-axis represents mean fatty acid level for Williams-82 wild type, and error bars represent two standard deviations from the mean. (a) 16:0 Palmitic acid, (b) 18:0 stearic acid, (c) 18:1 oleic acid, (d) 18:2 linoleic acid, and (e) 18:3 linolenic acid.

(lines 10–13) and four mutants with high stearate levels (lines 14–17) were identified. Six low-oleate mutants (lines 18–23) had increased levels of linoleate, and five of these six had elevated levels of linolenate (in all cases these mutants would also have been identified as outliers in the population on the basis of linoleate or linolenate content). The high linoleate mutants (lines 40–42) had marginally reduced levels of oleate. All 16 high-oleate mutants (lines 24–39) had lower than average levels of linoleate. A decrease in linolenate occurred along with an increase in linoleate or oleate in two low-linolenate mutants (lines 43 and 44). All of the mutants with high levels of linolenate (lines 45–52) had reduced levels of oleate.

4. Discussion

Here we have described 52 newly isolated soybean seed composition mutants. These mutants may represent new alleles of previously identified loci that are known to be involved in the determination of seed composition, which will be determined by the sequencing of candidate genes encoding biosynthetic enzymes. Based on the mutagen used to generate these lines, the lesions in these mutants are predicted to be single-base changes. Alternatively, some of these mutants

may carry lesions in genes not previously implicated in the determination of soybean seed oil content.

In this study, three mutants (lines 1–3 in Table 1) containing 5–6% palmitate were identified. Other mutants with low levels of palmitic acid (ranging from 5% to 8%) have been identified in previous studies, and at least three non-allelic loci are thought to be involved: *fap**, *fap1* (C1726), and *fap3* (A22) [15–19]. The allele *fap_{nc}*, which is allelic to *fap3*, has been associated with mutation of the *FAT1B* gene [19, 20]. Lines 4–9 contain high levels of palmitate, ranging from 12.4% to 16.4%. Mutants containing similarly elevated levels of palmitic acid have been described previously. The allele *fap2* has been associated with mutation of the *KASIIa* gene [7]. Other loci exist which confer a high palmitic acid phenotype which include *fap4*, *fap5*, *fap6*, and *fap7*. Therefore, there are likely to be other genes present that when mutated influence palmitic acid levels in the seed [16, 21–23].

Four mutants containing low levels of stearate (lines 10–13) were isolated in this study, however no mutants with this phenotype are characterized at the molecular level. Four mutants (lines 14–17) with reproducibly high levels of stearate ranging from 7 to 15% were identified in this study. Mutations in the *SACPD-C* gene are known to result in

FIGURE 2: Reproducibility for fatty acid levels in selected mutant lines. For each M_3 - M_4 pair the open circle on the left represents the fatty acid level observed in the M_3 seeds, and the black diamonds represent the levels observed in the M_4 seeds descended from M_3 individuals. Key for the labeling of each line is found in Table 1. The dotted horizontal bars on each plot represent the mean level of the fatty acid in wild-type Williams-82 (W82) seed samples. Actual values for a representative W82 sample (as well as values for single plant progeny from this W82 plant) is plotted on the far right of each plot for reference. Level of significance was calculated by Welch's t -test comparing values from M_4 samples to these ten wild type seed samples. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant. (a) Palmitic acid mutants (mean palmitic acid for W82 = 10.6%), (b) stearic acid mutants (mean stearic acid for W82 = 3.9%), (c) oleic acid mutants (mean oleic acid for W82 = 23.2%), (d) linoleic acid mutants (mean linoleic acid for W82 = 54.8%), and (e) linolenic acid mutants (mean linolenic acid for W82 = 7.7%).

increased levels of stearic acid ranging from 9% to 30% [5, 12, 24]. There are thought to be at least two genetic loci that contribute to the high stearate phenotype [25].

Sixteen mutants (lines 24–39) were identified in this study that contain high levels of oleic acid. Plants carrying deletions in the *FAD2-1a* gene contain high levels (up to 50%) of oleic acid in the seeds [11]. A number of other genes affecting oleate levels are known, but have not been characterized at the molecular level at the time of writing. One possible reason for this is that the dependence of oleic acid levels in soybean seeds upon environmental conditions complicates the molecular mapping of these genes [26–28].

RNA interference of *FAD2-1* (which probably silences multiple *FAD* transcripts in the seed) results in even higher oleate levels in the range of 70–80%, therefore it is thought that other *FAD* genes may contribute to the conversion of 18:1 to 18:2 [29, 30]. Six mutants containing low levels of oleic acid (lines 18–23) were also identified in this study.

Two mutants containing low levels of linolenate (line 43 and 44) were identified in this screen. Mutants with low linolenate (designated *fan*) have been identified previously, and two independent low linolenic mutants have been shown to contain lesions in the *FAD3A* gene [5, 10, 31, 32]. When all three *FAD3* homologs are downregulated with RNAi in seeds,

linolenic acid levels can be further reduced, therefore, it is possible that mutation in *FAD3B* or *FAD3C* may also result in low levels of linolenic acid [33]. Eight lines containing 10.5–12.9% linolenic acid were isolated in this study (lines 45–52). One mutant with comparable levels of linolenic acid (12.6%) is known, but the nature of the molecular lesion that causes this phenotype has not been determined [34].

5. Conclusions

Candidate gene sequencing and complementation tests or genetic mapping will be necessary to determine if the lines identified in this study represent new alleles of the known enzymes that control seed oil composition or represent mutations in other genes not yet implicated in the determination of soybean oil quality. These mutations have been isolated from a heavily mutagenized population created for the reverse genetics TILLING approach and carry a number of base changes at additional sites [13]. As with all variants created using a mutation breeding strategy, it will therefore be necessary to introgress these loci into elite cultivars for several generations to eliminate potentially deleterious mutations at other loci. Lines developed in this way will likely be of substantial utility for further development of new soybean varieties with both improved oil content and competitive agronomic characteristics.

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References

- [1] J. Ohlrogge and J. Browse, "Lipid biosynthesis," *Plant Cell*, vol. 7, no. 7, pp. 957–970, 1995.
- [2] T. E. Clemente and E. B. Cahoon, "Soybean oil: genetic approaches for modification of functionality and total content," *Plant Physiology*, vol. 151, no. 3, pp. 1030–1040, 2009.
- [3] W. R. Fehr, "Breeding for modified fatty acid composition in soybean," *Crop Science*, vol. 47, pp. S72–S87, 2007.
- [4] S. M. Rahman, Y. Takagi, K. Kubota, K. Miyamoto, and T. Kawakita, "High oleic acid mutant in soybean induced by X-ray irradiation," *Bioscience, Biotechnology and Biochemistry*, vol. 58, no. 6, pp. 1070–1072, 1994.
- [5] E. G. Hammond and W. R. Fehr, "Registration of A5 germplasm line of soybean," *Crop Science*, vol. 23, article 192, 1983.
- [6] J. R. Wilcox, J. F. Cavins, and N. C. Nielsen, "Genetic alteration of soybean oil composition by a chemical mutagen," *Journal of the American Oil Chemists' Society*, vol. 61, no. 1, pp. 97–100, 1984.
- [7] K. Aghoram, R. F. Wilson, J. W. Burton, and R. E. Dewey, "A mutation in a 3-keto-acyl-ACP synthase II gene is associated with elevated palmitic acid levels in soybean seeds," *Crop Science*, vol. 46, no. 6, pp. 2453–2459, 2006.
- [8] A. S. Chappell and K. D. Bilyeu, "The low linolenic acid soybean line PI 361088B contains a novel GmFAD3A mutation," *Crop Science*, vol. 47, no. 4, pp. 1705–1710, 2007.
- [9] J. R. Byrum, A. J. Kinney, K. L. Stecca, D. J. Grace, and B. W. Diers, "Alteration of the omega-3 fatty acid desaturase gene is associated with reduced linolenic acid in the A5 soybean genotype," *Theoretical and Applied Genetics*, vol. 94, no. 3–4, pp. 356–359, 1997.
- [10] A. S. Chappell and K. D. Bilyeu, "A GmFAD3A mutation in the low linolenic acid soybean mutant C1640," *Plant Breeding*, vol. 125, no. 5, pp. 535–536, 2006.
- [11] T. Anai, T. Yamada, R. Hideshima, T. Kinoshita, S. M. Rahman, and Y. Takagi, "Two high-oleic-acid soybean mutants, M23 and KK21, have disrupted microsomal omega-6 fatty acid desaturase, encoded by GmFAD2-1a," *Breeding Science*, vol. 58, no. 4, pp. 447–452, 2008.
- [12] P. Zhang, J. W. Burton, R. G. Upchurch, E. Whittle, J. Shanklin, and R. E. Dewey, "Mutations in a $\Delta 9$ -stearoyl-ACP-desaturase gene are associated with enhanced stearic acid levels in soybean seeds," *Crop Science*, vol. 48, no. 6, pp. 2305–2313, 2008.
- [13] J. L. Cooper, B. J. Till, R. G. Laport et al., "TILLING to detect induced mutations in soybean," *BMC Plant Biology*, vol. 8, article 9, 2008.
- [14] R. Ritchie, H. Wiley, D. Szymanski, and N. Nielsen, "Targeting induced local lesions in genomes," in *Legume Crop Genomics*, R. F. Wilson, H. T. Stalker, and E. C. Brummer, Eds., pp. 194–203, AOCS Press, Champaign, Ill, USA, 2004.
- [15] T. Kinoshita, S. M. Rahman, T. Anai, and Y. Takagi, "Inter-locus relationship between genes controlling palmitic acid contents in soybean mutants," *Breeding Science*, vol. 48, no. 4, pp. 377–381, 1998.
- [16] S. R. Schnebly, W. R. Fehr, G. A. Welke, E. G. Hammond, and D. N. Duvick, "Inheritance of reduced and elevated palmitate in mutant lines of soybean," *Crop Science*, vol. 34, no. 4, pp. 829–835, 1994.
- [17] D. Stojisin, G. R. Ablett, B. M. Luzzi, and J. W. Tanner, "Use of gene substitution values to quantify partial dominance in low palmitic acid soybean," *Crop Science*, vol. 38, no. 6, pp. 1437–1441, 1998.
- [18] J. R. Wilcox, J. W. Burton, G. J. Rebetzke, and R. F. Wilson, "Transgressive segregation for palmitic acid in seed oil of soybean," *Crop Science*, vol. 34, no. 5, pp. 1248–1250, 1994.
- [19] V. S. Primomo, D. E. Falk, G. R. Ablett, J. W. Tanner, and I. Rajcan, "Inheritance and interaction of low palmitic and low linolenic soybean," *Crop Science*, vol. 42, no. 1, pp. 31–36, 2002.
- [20] A. J. Cardinal, J. W. Burton, A. M. Camacho-Roger, J. H. Yang, R. F. Wilson, and R. E. Dewey, "Molecular analysis of soybean lines with low palmitic acid content in the seed oil," *Crop Science*, vol. 47, no. 1, pp. 304–310, 2007.
- [21] J. M. Narvel, W. R. Fehr, J. Ininda et al., "Inheritance of elevated palmitate in soybean seed oil," *Crop Science*, vol. 40, no. 3, pp. 635–639, 2000.
- [22] D. L. Stoltzfus, W. R. Fehr, G. A. Welke, E. G. Hammond, and S. R. Cianzio, "A *fap5* allele for elevated palmitate in soybean," *Crop Science*, vol. 40, no. 3, pp. 647–650, 2000.
- [23] D. L. Stoltzfus, W. R. Fehr, G. A. Welke, E. G. Hammond, and S. R. Cianzio, "A *fap7* allele for elevated palmitate in soybean," *Crop Science*, vol. 40, no. 6, pp. 1538–1542, 2000.

- [24] V. R. Pantalone, R. F. Wilson, W. P. Novitzky, and J. W. Burton, "Genetic regulation of elevated stearic acid concentration in soybean oil," *Journal of the American Oil Chemists' Society*, vol. 79, no. 6, pp. 549–553, 2002.
- [25] S. M. Rahman, Y. Takagi, and T. Kinoshita, "Genetic control of high stearic acid content in seed oil of two soybean mutants," *Theoretical and Applied Genetics*, vol. 95, no. 5-6, pp. 772–776, 1997.
- [26] J. L. Alt, W. R. Fehr, G. A. Welke, and J. G. Shannon, "Transgressive segregation for oleate content in three soybean populations," *Crop Science*, vol. 45, no. 5, pp. 2005–2007, 2005.
- [27] M. J. Monteros, J. W. Burton, and H. R. Boerma, "Molecular mapping and confirmation of QTLs associated with oleic acid content in N00-3350 soybean," *Crop Science*, vol. 48, no. 6, pp. 2223–2234, 2008.
- [28] E. Bachlava, R. E. Dewey, J. Auclair, S. Wang, J. W. Burton, and A. J. Cardinal, "Mapping genes encoding microsomal ω -6 desaturase enzymes and their cosegregation with QTL affecting oleate content in soybean," *Crop Science*, vol. 48, no. 2, pp. 640–650, 2008.
- [29] T. Buhr, S. Sato, F. Ebrahim et al., "Ribozyme termination of RNA transcripts down-regulate seed fatty acid genes in transgenic soybean," *Plant Journal*, vol. 30, no. 2, pp. 155–163, 2002.
- [30] E. P. Heppard, A. J. Kinney, K. L. Stecca, and G. H. Miao, "Developmental and growth temperature regulation of two different microsomal ω -6 desaturase genes in soybeans," *Plant Physiology*, vol. 110, no. 1, pp. 311–319, 1996.
- [31] D. Stojšin, B. M. Luzzi, G. R. Ablett, and J. W. Tanner, "Inheritance of low linolenic acid level in the soybean line RG10," *Crop Science*, vol. 38, no. 6, pp. 1441–1444, 1998.
- [32] Y. Reinprecht, S. Y. Luk-Labey, J. Larsen et al., "Molecular basis of the low linolenic acid trait in soybean EMS mutant line RG10," *Plant Breeding*, vol. 128, no. 3, pp. 253–258, 2009.
- [33] T. Flores, O. Karpova, X. Su et al., "Silencing of GmFAD3 gene by siRNA leads to low α -linolenic acids (18:3) of fad3-mutant phenotype in soybean [*Glycine max* (Merr.)]," *Transgenic Research*, vol. 17, no. 5, pp. 839–850, 2008.
- [34] S. M. Rahman, Y. Takagi, and S. Towata, "Inheritance of high linolenic acid content in the soybean mutant line B739," *Breeding Science*, vol. 44, no. 3, pp. 267–270, 1994.