Molecular Markers Associated with Linolenic Acid Content in Soybean

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ABSTRACT: An altered FA profile with decreased linolenic (18:3) acid in soybean germplasm was developed by crossing N97-3708-13, a soybean line with reduced 18:3 (<5.4%) and 'Anand,' a normal soybean cultivar (9.7% 18:3). The resulting recombinant inbred lines are promising because they may promote healthier oil with improved oxidative stability and flavor. The objective of this study was to utilize the population N97-3708-13 × Anand to identify simple sequence repeat (SSR) markers associated with 18:3 content. Two markers, Satt534 and Satt560, which are located approximately 10 cM apart from each other, near the *Fan* locus on linkage group B2, were identified as quantitative trait loci significantly associated with 18:3 content ($P = 0.001$, $R^2 = 0.59$, individually). The SSR markers identified in this study should be useful for implementation of marker-assisted selection for low-18:3 genotypes in soybean breeding programs.

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KEY WORDS: Fatty acid, marker-assisted selection, oil quality, soybean breeding, SSR markers.

Soybean [*Glycine max* (L.) Merr.] is a major source of vegetable oil in the world. Soybean oil typically contains 11, 4, 23, 54, and 8% palmitic (16:0), stearic (18:0), oleic (18:0), linoleic (18:2), and linolenic (18:3) acid, respectively (1,2). Linolenic acid is considered an unstable component because it is easily oxidized and is responsible for the development of an off-flavor and odor of the oil. At present, nutritionists consider that the ideal edible oil should contain about 3% 18:3. In the oil industry, the nutritional quality of edible oil is improved by reducing the 18:3 levels in favor of 18:2. Partial hydrogenation of soybean oil is the process used commercially to lower the amount of 18:3. However, this process also results in the production of *trans*-FA that are considered of concern to human health. The low-18:3 trait in soybean may be incorporated into high-yielding lines through plant breeding (3). Genetic changes that influence soybean FA composition also have been made through chemical or X-ray mutagenesis techniques (4). Conventional breeding techniques have then transferred those traits to more agronomically adapted genetic backgrounds.

The genetic control of 18:3 content in soybean appears somewhat complex. Some studies have reported that the trait is regulated by a single recessive allele designated *fan* (5,6), and other studies have suggested that this trait might be governed by one major gene and several minor ones (7,8). The chemical pathway involved in FA biosynthesis is also complex. The biosynthesis of 18:3 is the result of a desaturation reaction catalyzed by a membrane-associated ω-3 desaturase. Moreover, the pathway in leaf tissue is different from that in reproductive tissues. Byrum *et al.* (9) suggested that the low-18:3 phenotype obtained with the *Fan* locus in soybean (A5) was the result of a mutation in the microsomal ω-3 linoleate desaturase gene, which mapped to linkage group B2, and in the plastid desaturase ω-3 linoleate, which mapped to linkage group G. From the literature, it appears that the heritability of 18:3 can be low: $h^2 = 0.10$ to 0.47 (10), to moderately high: $h^2 = 0.73$ (11), depending on the lines used as parents in the cross. Expression of this phenotype may also be influenced by environmental factors (12). Therefore, identification of molecular markers closely associated with 18:3 should be of interest to facilitate gene transfer to high-yielding genotypes. Although restriction fragment length polymorphism (RFLP) markers associated with 18:3 in soybean have been described (6,13), the verification of more easily used simple sequence repeat (SSR) markers associated with 18:3 would provide tools for enhancing genetic gain for 18:3 in soybean breeding. Quantitative trait loci (QTL) are regions of the genome that govern a particular trait, such as 18:3 content. The objective of this study was to identify specific SSR markers revealing QTL that influence the level of 18:3 content in soybean oil.

MATERIALS AND METHODS

Field experiment. A soybean population was initiated in 1998 from a cross between N97-3708-13, a low-18:3 germplasm, and the cultivar 'Anand,' which has normal oil composition and is high yielding. N97-3708-13 was selected from the cross 'Soyola' × ['Brim' (2) × N88-431 (2) × (N90-2013 × C1726)]. Soyola contributed the low-18:3 trait. A recombinant inbred line (RIL) population was developed to the F_5 single-plant generation, derived *via* single-seed descent from different F_2 individuals. This RIL population was screened for molecular markers associated with seed 18:3 content.

Chemical analysis. Soybean seeds from single F_5 plants were sampled from the RIL population for FA analyses. Each crushed seed was transferred to a test tube with 1.0 mL of chloroform/hexane/methanol (8:5:2, by vol). After 4 h, 100 μL of the oil sample was transferred to a 1.5-mL autosampler

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vial. Then 75 µL of methylating reagent (a mixture of 50 mL sodium methoxide, 20 mL petroleum ether, 10 mL ethyl ether, and 1 mL hexane) was added to the vial before capping. FAME were analyzed using a Hewlett-Packard 6890 series gas chromatograph equipped with a model 7683 auto sampler, FID, and a 30 m × 0.53 mm i.d. J&W (Folsom, CA) 125-2332 capillary column with 0.5 µm fused stationary phase. Operation conditions were as follows: carrier, helium (20 mL/min); 20:1 (vol/vol) split injection; injection temperature, 250°C; detector temperature, 275°C; and column temperature, 230°C. The RM-1 standard (Supelco Co., Bellefonte, PA) was used to calculate the relative FA concentrations of the experimental samples.

Bulk segregant analysis. Single $F_{5:6}$ seeds from 90 RIL were grown in the greenhouse along with the two parents (N97-3708-13 and Anand). DNA was isolated from fresh leaves after 3 wk of growth. The Qiagen Plant Easy DNA Extraction Kit (Qiagen, Hilden, Germany) was used to obtain purified DNA. DNA isolated from the 12 plants with the highest 18:3 content (averaging 11.4%) was then pooled together to form the normal 18:3 content bulk, and DNA from 12 plants with the lowest 18:3 content (averaging 4.2%) was pooled to form the low 18:3 content bulk, according to techniques described by Michelmore *et al.* (14) and Giovannoni *et al.* (15). The two bulks were screened with our current primer collection (780 SSR markers) distributed throughout the 20 molecular linkage groups (MLG). Particular attention was paid to SSR mapped to MLG B2 (16). Polymerase chain reactions (PCR) were carried out in a Hybaid multiblock thermocycler (CLP, San Diego, CA) with the following profile: (i) 94°C for 3 min × 1 cycle; (ii) 94°C for 25 s, 47°C for 25 s, and 72°C for 1 min × 35 cycles; and finally (iii) 72°C for 5 min × 1 cycle. Primers were purchased from SIGMA Genosys (The Woodlands, TX). PCR products were then separated by electrophoresis using a 6% nondenaturing polyacrylamide gel containing ethidium bromide and visualized *via* an UV transilluminator (AlphaImager, Alpha Innotech Corp., San Leandro, CA). Markers showing polymorphisms between the bulks were later used to screen the parents for matching polymorphisms. Markers consistently polymorphic were then selected to score parental alleles among DNA from 90 RIL in the $F_{5:6}$ population, following a procedure similar to that employed by Spencer *et al.* (17).

Data analysis. Only the SSR markers that exhibited banding patterns that were reproducible and consistent between the two parents as well as among the 90 $F_{5:6}$ RIL were considered for statistical analyses. QTL analyses for SSR markers associated with the 18:3 trait were analyzed using SAS v6.0 (18).

RESULTS AND DISCUSSION

This research was designed to identify molecular markers associated with loci controlling 18:3 content in a population of soybean lines with high-yield potential that was segregating for 18:3 content. Soybean germplasms with low 18:3 were developed from chemical or X-ray mutagenesis treatments, and further hybridizations of these mutants were made by several researchers. The *fan* allele was found in the three mutants: C1640, A5, and M-5, and in the introductions: PI 361088B and PI 123440 (19). Later, *fan*2 was found in A-23 (20), *fan*x in IL-8 (19), and finally *fan*x[a] in M-24 (7). Evidence of two loci also have been described by several authors (7,8,20).

In our study we worked with the RIL from the cross Anand × N97-3708-13. The distribution of 18:3 in the inbred lines varied continuously from 4.1 to 13.4% of total lipid and ap-

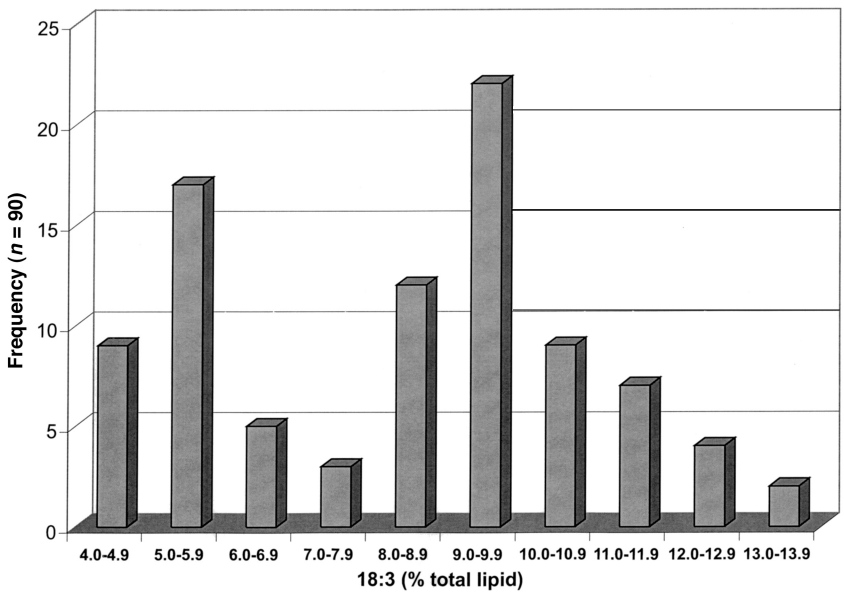

FIG. 1. Frequency distribution of 18:3 concentration of 90 recombinant inbred lines of N97-3708-13 × Anand soybean population. N97-3708-13, 5.4 ± 1.0% 18:3; Anand, 9.7 ± 1.8% 18:3.

peared indicative of a major gene as well as modifier gene effects (Fig. 1).

Seventy-four markers were polymorphic between the two bulks from a total of 780 SSR markers screened, covering all 20 linkage groups of soybean. Polymorphisms were detected at various genomic regions, but only three markers showed consistent polymorphisms between the bulks and the parents (data not shown). Satt512 (non-mapped), Satt534 (LGB2), and Satt560 (LGB2) were identified as putative genomic regions governing 18:3 content (see Fig. 2 as an example for Satt534).

The three putative 18:3 QTL were further tested against the population of 90 RIL segregating for 18:3 content. One of the markers, Satt512, which is not currently mapped on any linkage group, was eliminated because it showed a nonsignificant association with 18:3 ($P > 0.05$) over the population of 90 RIL. Therefore, only two markers, Satt534 and Satt560 on linkage group B2, were identified as QTL, each significant at $P \leq 0.001$. They individually explain about 60% of the variation for 18:3 content (Table 1). Satt534 and Satt560 are both located at the bottom of linkage group B (Fig. 3), where they map approximately 10 cM apart from each other and close to RFLP markers previously identified in the region of the *Fan* locus (6,16).

Recent advances in molecular biology have begun to elucidate the underlying biochemical mechanisms and the genes controlling 18:3 in soybean (8). The development of an integrated molecular map is a major contribution that will enhance genetic gains (16). RFLP markers associated with 18:3 already have been placed on the soybean linkage map (6,12), but modern soybean breeders now utilize SSR markers to expedite selections because of the high level of polymorphism and general ease of use and interpretation of SSR markers. The two SSR markers identified here flank a 10-cM interval containing a major gene governing 18:3 content (Fig. 3).

This knowledge provides breeders with information on incorporating marker-assisted selection to accelerate the development of soybeans that exhibit lower 18:3 concentration. Further studies in other populations will be useful to detect the minor genes that influence 18:3 content in soybean. Additionally, breeders can test over a range of environments and over a range of soybean populations to strengthen the applicability of these results over different genetic backgrounds.

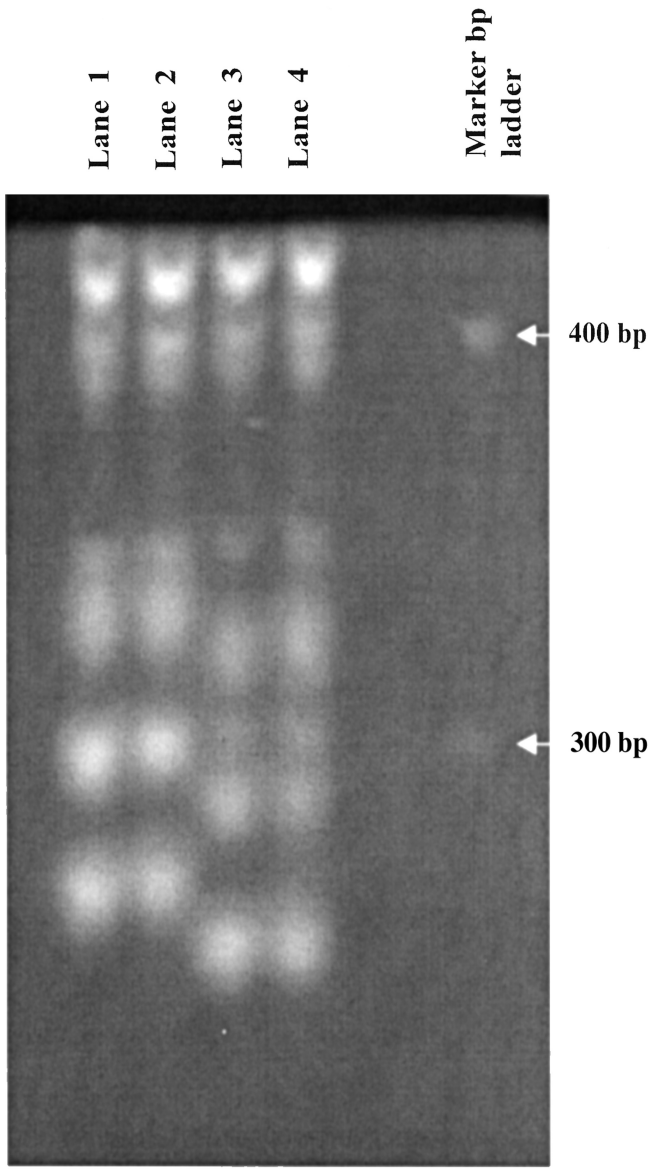

FIG. 2. DNA polymorphisms between soybean parental lines (N97-3708-13 and Anand) and normal-18:3 and low-18:3 DNA bulks with simple sequence repeat (SSR) molecular marker Satt534. Lane 1, Anand; lane 2, normal-18:3 DNA bulk; lane 3, N97-3708-13; lane 4, low-18:3 DNA bulk.

TABLE 1
Markers Significantly Associated with Variation in 18:3 Content in an $F_{5:6}$ Recombinant Inbred Line Population from the Cross N97-3708-13 × Anand

				Mean 18:3 (%) ± SE	
SSR marker	Linkage group	R^2	P	N97/N97 class[a]	Anand/Anand class[b]
Satt534	B2	0.59	0.0001	5.8 ± 1.5	9.8 ± 1.5
Satt560	B2	0.59	0.0001	6.4 ± 1.6	10.1 ± 1.6

[a]N97/N97 = the class of recombinant inbred line (RIL) progeny exhibiting the homozygous N97-3708-13 allele at the simple sequence repeat (SSR) marker locus.
[b]Anand/Anand = the class of RIL progeny exhibiting the homozygous Anand allele at the SSR marker locus.

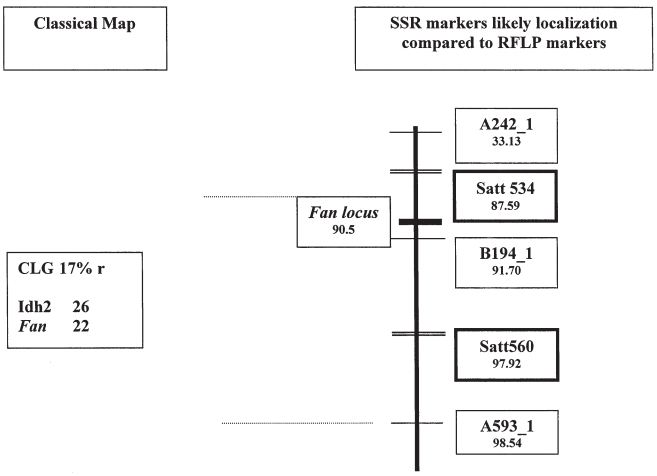

FIG. 3. Likely map position of the *Fan* locus and two SSR markers (Satt534 and Satt560) compared to other markers on linkage group B2. Distances are reported in centiMorgan units. RFLP, restriction fragment length polymorphism; for other abbreviations see Figure 2.

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