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Genetics and Breeding for Modified Fatty Acid Profile in Soybean Seed Oil

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Abstract

Soybean [*Glycine max* (L.) Merr.] oil is versatile and used in many products. Modifying the fatty acid profile would make soy oil more functional in food and other products. The ideal oil with the most end uses would have saturates (palmitic + stearic acids) reduced from 15 to < 7%, oleic acid increased from 23 to > 55%, and linolenic acid reduced from 8 to < 3%. Reduced palmitic acid (16:0) is conditioned by three or more recessive alleles at the *Fap* locus. QTLs for reduced palmitic acid have mapped to linkage groups (LGs) A1, A2, B2, H, J, and L. Genes at the *Fad* locus control oleic acid content (18:1). Six QTLs (R^2 = 4-25%) for increased 18:1 in N00-3350 (50 to 60% 18:1) explained four to 25% of the phenotypic variation. M23, a Japanese mutant line with 40 to 50% 18:1 is controlled by a single recessive gene, *ol*. A candidate gene for FAD2-1A can be used in marker-assisted breeding for high 18:1 from M23. Low linolenic acid (18:3) is desirable in soy oil to reduce hydrogenation and *trans*-fat accumulation. Three independent recessive genes affecting omega-3 fatty acid desaturase enzyme activity are responsible for the lower 18:3 content in soybeans. Linolenic acid can be reduced from 8 to about 4, 2 ,and 1% from copies of one, two, or three genes, respectively. Using a candidate gene approach perfect markers for three microsomal omega-3 desaturase genes have been characterized and can readily be used in for marker assisted selection in breeding for low 18:3.

Key words: fatty acid, oil, soybean breeding

Introduction

Soybean oil makes up nearly 60% of the world's oil seed production and is by far the world's dominant vegetable oil (http://www.soystats.com). Five fatty acids make up nearly the entire oil portion of soybean seed. Soybean oil averages 12% palmitic acid (16:0), 4% stearic acid (18:0), 23% oleic acid (18:1), 53% linolenic acid (18:2), and 8% linolenic acid (18:3). The 16:0 and 18:0 fractions are saturated fatty acids and constitute 15% of the soybean oil. The remainder of the oil (about 85%) is made up of unsaturated fatty acids or 18:1, 18:2, and 18:3. Transgenic, induced mutations, natural mutations, and combining two or more genes for enhanced oil traits have been the approaches used in breeding for improved oil content. Several genes have been discovered that affect fatty acid levels in soybean that enable breeders to combine genes to tailor novel fatty acid profiles desired for various end uses. Research since the 1970s has led to a greater understanding of how to genetically alter fatty acids of soybean oil. Since consumer and end-user preferences for soybean oil are changing, breeding goals to modify fatty acid composition in soybean oil are needed to improve uses in industrial, food, and other products (Wilson 2004).

Soybean research priorities have been set, with guidance from consumers and end-users, to initially target fatty acid profiles that have the highest probability to facilitate expanded use of soybean oil in edible and industrial applications in the USA (Wilson 2004). The most visible of these programs was "The Better Bean Initiative" launched in 2000 by the United Soybean Board. This program's aim is to add value to the oil and protein seed components by genetically changing objectionable characteristics. These deliberations have focused on three different oil phenotypes (Table 1). Modification of saturates, oleic acid, and linolenic acid through breeding and biotechnology are being emphasized to develop desired fatty acid phenotypes. It is impractical to commercially develop all oil phenotypes. Perhaps the most desired phenotype for soybean oil is < 7% saturates (16:0 + 18:0), > 55% 18:1, and < 3% 18:3 because of its multiple

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Table 1. Goals for redesigning soybean oil composition for specific food and industrial applications (Wilson 2004).

Fatty acid	Normal oil	Desired composition for specific use		
		Frying	Baking	Industrial
		% Crude soybean oil		
Saturated (16:0 + 18:0)	15	7	42	11
Oleic (18:1)	23	60	19	12
Linoleic (18:2)	53	31	37	55
Linolenic (18:3)	9	2	2	22

uses in many edible and industrial applications (Wilson 2004).

Health effects and improved cold flow of bio-diesel have stimulated research to develop soybean with lower saturated fats. Diets high in saturated fats are associated with high cholesterol and increased heart disease. Lower saturate foods are in demand to lower risks for these health issues. The U.S. Food and Drug Administration's requirement for a food to be labeled "low in saturated fat" is < 1 g of saturated fat per serving. This means that the fatty acid composition of vegetable oil should contain < 7% total saturated fat to make such a claim. Lowering soybean from 15% saturated fat to 7% or less will make soybean oil more attractive to food manufacturers and health-conscious consumers and will improve cold flow of bio-fuels made from soybean oil (Wilson 2004). On the other hand, high saturate soybean oil would make hydrogenation less necessary and is in demand for making margarines and shortenings without *trans*-fats for the evolving health conscious society.

Increased oleic acid (18:1) in soybean oil is important because of health benefits and increased oxidative stability. A diet in which fat consumption is high in 18:1 is associated with reduced cholesterol, arteriosclerosis, and heart disease (Grundy 1986; Wardlaw and Snook 1990; Chang and Huang 1998). High oleic acid also increases oxidative stability and extends the utility of soybean oil at high cooking temperatures. It also will significantly increase soybean oil use in pharmaceuticals, cosmetics, and industrial products such as lubricants and bio-diesel. To improve acceptance of soy-diesel, a high 18:1 level combined with lower saturates (16:0 + 18:0) will improve ignition and cold flow in cooler climates (Wilson 2004).

Soybean oil contains a high concentration of the polyunsaturated linoleic (18:2) and linolenic (18:3) acids. These fatty acids have a high number of double bonds which are susceptible to oxidation resulting in reduced shelf life, low stability at high cooking temperatures, and off-flavors. Oxidation of linolenic acid with three double bonds is the most important factor contributing to the poor functionality of soybean oil. To improve oxidative stability and undesirable taste, soy oil is hydrogenated to reduce double bonds which are sites of oxidative attack and subsequent off-flavor development (Yadav 1996; Liu 1999). Partial hydrogenation of soybean oil increases oxidative stability but leads to the formation of *trans*-fats. Demand from oil seed processors for a lower cost alternative to catalytic hydrogenation for producing oil products with desired flavor and functionality led to research to breed soybeans containing lower linolenic acid (Liu 1999). More recently, health concerns and labeling laws that require listing the amount of *trans*-fatty acids in foods have prompted food companies to seek alternative oils to replace hydrogenated soybean oil to insure that their products contain low levels of *trans*-fats. Thus, emphasis on soybean oil with 3% linolenic acid or less has become a high priority (Yadav 1996; Wilson 2004).

In most Asian countries, soybeans are usually consumed directly as tofu, sprouts, soybean paste, and health supplements etc. As essential fatty acids, linoleic, and linolenic acids are desirable components of soybean oil. Both linoleic acid (w-6) and linolenic acid (w-3) are potential precursors of EPA (20:5) and DHA (22:6), which in diets can have multiple positive health benefits including reduction of cardiovascular disease and improved cognitive function (Brouwer et al. 2004; Gebauer et al. 2006; Connor 2000). Studies suggested that adjusting the intake ratio of omega-6 to omega-3 fatty acids may enhance overall health (Abel et al. 2004). Thus, for health benefits elevated 18:3 genotypes are desirable in food grade soybeans. Also, oils low in saturates and high in polyunsaturated fatty acids, linoleic, and linolenic acids, would have applications for replacing drying oils such as tung and linseed oil in oil-based paints and coatings (Wilson 2004).

Because of the demand for healthier, more functional vegetable oils, greater emphasis is being placed on modifying the fatty acid profile in soybean seed. A review and update on breeding and molecular mapping for modification of saturates (palmitic and stearic acids), oleic acid, linoleic acid, and linolenic acid in soybean oil is presented herein.

Soybean oil quantity

Typically soybean seeds contain about 20% oil on a dry weight basis (Wilson 2004).

Crude oil contains various glycerolipids (primarily phospholipids, diacylglycerol, and triacylglycerol). Triacylglycerol is the main component of the oil. Phospholipids such as phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol have structural functions in cell membranes and may be metabolically involved in triacylglycerol synthesis. Each glycerolipid class is composed of molecular species formed by various combinations of the five fatty acids, 16:0, 18:0, 18:1, 18:2, and 18:3 or polar groups that are esterified at the sn-1, sn-2, and sn-3 stereospecific positions of the glycerol molecule (Wilson 2004).

The increased demand for renewable fuels such as bio-diesel has increased the emphasis on breeding soybeans with higher oil content. Genetic sources are very important in improving soybean oil content. There is a wide range (8-25%) in oil content among accessions in the USDA soybean (*G. max*) germplasm collection (USDA, ARS 2007). However, genes affecting oil content and oil biosynthesis can be affected by the environment such as temperature and rainfall. Also, oil and protein content in

Table 2. QTL locations, markers, significance, and parental source associated with palmitic, (16:0), stearic (18::0), oleic (18:1), linoleic (18:2), and linolenic (18:3) fatty acids in soybean oil.

Fatty acid	LG	Map position (cM)	Marker	R^2 (%)	Parent1	Parent2	References
16:0	A1	3.5	Satt684	33	Cook	N87-2122-4	Li et al. (2002)
16:0	A2	125.4	Satt133	12	N87-984-16	TN93-99	Panthee et al. (2006)
16:0	B2	38.0	A343_1	24	A81-365022	PI468916	Diers and Shoemaker (1992)
16:0	B2	53.5	A018_1	19	A81-365022	PI468916	Diers and Shoemaker (1992)
16:0	B2	-	$UBC122_{-1500}$	10	RG10	OX948	Reinprecht et al. (2006)
16:0	D1b	75.7	Satt537	19	N87-984-16	TN93-99	Panthee et al. (2006)
16:0	D2	-	A_537 A_611	-	C1727	PI479750	Nickell et al. (1994)
16:0	D2	57.5	Sat_092	11	RG10	OX948	Reinprecht et al. (2006)
16:0	J	68.3	K375_1	18	A81-365022	PI468916	Diers and Shoemaker (1992)
16:0	L	89.1	-	13	Essex	Williams	Hyten et al. (2004)
16:0	M	67.0	Satt175	9	Cook	N87-2122-4	Li et al. (2002)
16:0	N	-	$UBC444_{-2300}$	13	RG10	OX948	Reinprecht et al. (2006)
18:0	B2	55.2	Satt168	18	N87-984-16	TN93-99	Panthee et al. (2006)
18:0	B2	72.8-75.3	Satt070 Satt474 Satt556	61	Dare	FAM94-41	Spencer et al. (2003)
18:0	C2	121.3	-	13	Essex	Williams	Hyten et al. (2004)
18:0	F	27.1	Sat_090	10	RG10	OX948	Reinprecht et al. (2006)
18:0	G	76.7	Satt288	10-19	RG10	OX948	Reinprecht et al. (2006)
18:0	J	83.3	A233_1	19	A81-365022	PI468916	Diers and Shoemaker (1992)
18:0	J	12.3	Satt249	11	N87-984-16	TN93-99	Panthee et al. (2006)
18:0	L	58.2	-	16	Essex	Williams	Hyten et al. (2004)
18:1	A1	92.3	A104_1	26	A81-365022	PI468916	Diers and Shoemaker (1992)
18:1	A1	92.6	A170_1	23	A81-365022	PI468916	Diers and Shoemaker (1992)
18:1	A1	102.3	A082_1	28	A81-365022	PI468916	Diers and Shoemaker (1992)
18:1	A1	96.0	Satt211	4	G99-G725	N00-3350	Monteros et al. (2008)
18:1	B2	-	A619_1	19	A81-365022	PI468916	Diers and Shoemaker (1992)
18:1	D2	79.2	Satt389	6	G99-G725	N00-3350	Monteros et al. (2008)
18:1	E	11.0	A242_2	20	A81-365022	PI468916	Diers and Shoemaker (1992)
18:1	E	13.6	Pb	21	A81-365022	PI468916	Diers and Shoemaker (1992)
18:1	E	45.4	Satt263	10	N87-984-16	TN93-99	Panthee et al. (2006)
18:1	G	0.0	Satt163	10	RG10	OX948	Reinprecht et al. (2006)
18:1	G	76.7	Satt288	10	RG10	OX948	Reinprecht et al. (2006)
18:1	G	43.4	Satt394	13	G99-G725	N00-3350	Monteros et al. (2008)
18:1	G	96.6	Satt191	7	G99-G725	N00-3350	Monteros et al. (2008)
18:1	L	82.5	-	35	Essex	Williams	Hyten et al. (2004)
18:1	L	30.9	Satt418	9	G99-G725	N00-3350	Monteros et al. (2008)
18:1	L	71.4	Satt561	.25	G99-G725	N00-3350	Monteros et al. (2008)
18:2	A1	92.3	A104_1	33	A81-365022	PI468916	Diers and Shoemaker (1992)
18:2	A1	92.6	A170_1	30	A81-365022	PI468916	Diers and Shoemaker (1992)
18:2	A1	102.3	A082_1	38	A81-365022	PI468916	Diers and Shoemaker (1992)
18:2	B1	58.9	A118_1	20	A81-365022	PI468916	Diers and Shoemaker (1992)
18:2	B2	-	Fad3i6	70-75	RG10	OX948	Reinprecht et al. (2006)
18:2	E	11.0	A242_2	21	A81-365022	PI468916	Diers and Shoemaker (1992)
18:2	E	13.6	Pb	20	A81-365022	PI468916	Diers and Shoemaker (1992)
18:2	E	44.8	Satt185	13	N87-984-16	TN93-99	Panthee et al. (2006)
18:2	F	93.7	-	10	Essex	Williams	Hyten et al. (2004)
18:2	L	74.5	-	50	Essex	Williams	Hyten et al. (2004)
18:3	B2	-	pB194-1 pB124	85	C1640	PI479750	Brummer et al. (1995)
18:3	B2	87.5	Satt534 Fad3i6	72-78	RG10	OX948	Reinprecht et al. (2006)
18:3	E	6.3	SAC7_1	31	A81-365022	PI468916	Diers and Shoemaker (1992)
18:3	E	11.0	A242_2	23	A81-365022	PI468916	Diers and Shoemaker (1992)
18:3	E	28.3	K229_1	20	A81-365022	PI468916	Diers and Shoemaker (1992)
18:3	E	30.9	A454_1	22	A81-365022	PI468916	Diers and Shoemaker (1992)
18:3	E	34.6	A203_1	22	A81-365022	PI468916	Diers and Shoemaker (1992)
18:3	E	45.4	Satt263	12	N87-984-16	TN93-99	Panthee et al. (2006)
18:3	G	21.9	Satt235	22	N87-984-16	TN93-99	Panthee et al. (2006)
18:3	K	-	A065_3	20	A81-365022	PI468916	Diers and Shoemaker (1992)
18:3	L	36.7	A023_1	26	A81-365022	PI468916	Diers and Shoemaker (1992)
18:3	L	50.6	-	13	Essex	Williams	Hyten et al. (2004)
18:3	L	82.5	-	24	Essex	Williams	Hyten et al. (2004)

[†]The linkage group (LG), and map position (cM) assignation of marker is based on the integrated soybean genetic linkage map, Gmcomposite2003, reported in SOYBASE (http://soybase.org).

soybean seed are negatively correlated. Thus, it will be difficult to develop soybeans with very high oil content and protein content at 40% on a dry weight basis.

Soybean breeders are interested in increasing soybean oil content using major oil loci for marker-assisted selection (MAS). Soybeans with stable oil content across environments will be developed by combining confirmed QTLs. Recent genetic maps show 68 QTLs affecting soybean oil content have been found on all LGs except linkage group N. These QTL have been reported in the Breeder's Toolbox in Soybase (http://www.soybase.org).

Palmitic acid

Palmitic acid is the predominant saturated fatty acid in soybean oil. Generally common soybean cultivars contain about 12% palmitic acid. Because of health risks associated with the cholesterogenic properties of saturated fatty acids, reduced levels of palmitic acid is a goal in breeding for improved soybean oil quality.

Reduced palmitic acid soybean lines have been developed by chemical mutagenesis, recurrent selection, and hybridization (Erickson et al. 1988; Burton et al. 1994; Bubeck et al. 1989; Wilcox and Cavins 1990). At least three recessive genes at the *Fap* locus are responsible for palmitic acid content in soy oil (Wilson 2004; Pantalone et al. 2004). Soybean N79-2077-12, with about 6% palmitic acid, has a recessive fap_{nc} allele and was developed from recurrent selection (Burton et al. 1994). Low palmitic acid germplasm lines C1726 and A22 were derived from mutagenesis of Century and A1937, respectively. C1726, with about 8% palmitic acid, has the recessive *fap1* allele (Bubeck et al. 1989; Wilcox and Cavins 1990). A22, with 6.8% palmitic acid, has the recessive *fap3* allele (Fehr et al. 1991a). Palmitic acid content of F_2 progeny from a cross between A22 x C1726 with the homozygous genotype *fap1 fap1 fap3 fap3* and line A18 (Fehr et al. 1991a) had 4.0% 16:0 compared with 7.1% and 8.0%, respectively for A22 and C1726.

Four recessive alleles have been identified that increased palmitic acid levels. Mutant C1727 (*fap2* allele with 17%), A21 (*fap2b* allele with 20%), and A24 (*fap4* with 18%) were developed by chemical mutagenesis. Various combinations of these alleles can increase palmitic acid content to 35% of crude oil (Fehr et al. 1991b).

Some agronomic traits were affected in genotypes with altered palmitic acid genes reported above. Soybean yield was significantly less in genotypes with both the reduced and elevated levels of 16:0 (Rebetzke et al. 1998; Ndzana et al. 1994; Hayes et al. 2002; Wilcox and Cavins 1990). In comparison to related lines with normal 16:0 levels, high-palmitic acid $BC_1F_{2:4}$ lines averaged across each of three different populations typically had shorter height, smaller seed size, higher protein, lower oil, and reduced oleic and linoleic acids (Hayes et al. 2002). These associations suggest that pleiotropy and/or linkage drag could hinder efforts to develop competitive cultivars with altered palmitic acid phenotypes (Pantalone et al. 2004).

Molecular markers closely associated with palmitic acid alleles have been discovered (Table 2). The *fap2* allele which conditions elevated 16:0 in C1727 was mapped to linkage group D2 (Nickell et al. 1994). An elevated palmitic acid soybean line containing the *fap2* mutation was characterized with a candidate gene approach. The underlying mutation in a *KAS II* gene responsible for the elevated palmitic acid phenotype was discovered and led to the development of a perfect molecular marker assay (Aghoram et al. 2006).

The major low palmitic acid allele fap_{nc} (R^2 =31-38%) in NS79-2077-12 mapped near Satt684 on LG A1 (Li et al. 2002). A low 16:0 soybean line with the fap_{nc} allele was characterized and found to contain a deletion in one of the four *FATB* genes catalyzing a thioesterase function in the fatty acid pathway (Cardinal et al. 2007). Again, a molecular marker assay was described that is specific for the mutant allele and could be used in a breeding program.

QTL associated with reduced palmitic acid in C1726 mapped to LGs B2 and H using SSR markers in a BC_1F_2 population from a cross Cook x C1726. The lowest 16:0 contents were observed where the QTL on LG B2 was homozygous for the allele from normal soybean Cook, and the QTL on LG H was homozygous for the C1726 allele (Pantalone et al. 2004).

Several QTLs associated with palmitic acid content were mapped in populations where the parents had normal palmitic acid content. Diers and Shoemaker (1992) used an F2 derived population of A81-356022 (*G. max*) x PI468916 (*G. soja*) to map the RFLP markers associated with 16:0 levels and found three markers: pA-343a (R^2 = 24%) and pA-18 (R^2 = 19%) on LG B2, and pK-375 on LG J. The low palmitic acid alleles at the LG B2 QTL came from *G. soja*, whereas the one at the LG J QTL was from the *G. max* line. A major QTL for reduced 16:0 from an Essex x Williams population was found in an 89.1 cM region on LG L (R^2 = 13%) and was derived from Williams (Hyten et al. 2004). Recently, two QTLs which reduce palmitic acid, Satt133 on LG A2 (R^2 = 12%) located approximately 7 cM upstream from a QTL affecting oil concentration (Brummer et al. 1997) and Satt537 on LG D1b (R^2 = 20%) were detected from mapping population N87-984-16 x TN93-99 in which both parents had normal fatty acid profiles (Panthee et al. 2006).

Stearic acid

Stearic acid (18:0) content in soybean averages about 4% of the crude oil. Soybean oil higher in the saturated fats 16:0 and 18:0 has improved functional properties for certain food applications, such as solid fats, margarines, and shortenings, and confectionery uses. Genotypes high in stearic acid are preferred because unlike palmitic acid, 18:0 has been shown to be neutral in raising blood serum cholesterol which is associated with heart disease (Yadav 1996). Soybean genotypes with elevated stearic acid have been developed by chemical or X-ray mutagenesis and from natural mutations. However, high 18:0 genotypes developed from these techniques appear to be associated with poor yield potential (Hayes et al. 2002) so development of productive high stearic cultivars may be difficult.

Six elevated stearic acid germplasm lines were reported to carry homozygous recessive alleles. A6 (fas^a) with 30% (Hammond and Fehr 1983b), FA41545 (fas^b) with 15% (Graef et al. 1985), A81-606085 (*fas*) with 19% (Graef et al. 1985), KK-2 (st_1) with 6% (Rahman et al. 1997), M25 (st_2) with 19% (Rahman et al. 1997), and FAM94-41 (fas_{nc}) with 9% (Pantalone

et al. 2002) stearic acid were reported as genetically altered genotypes. It is known that *fas*^{a}, *fas*^{b}, and *fas* are allelic (Graef et al. 1985) and that *fas*^{a} and *fas*$_{mc}$ are allelic and different mutations in the same gene (Pantalone et al. 2004). Rahman et al. (1997) reported that the single recessive genes *st*$_1$ and *st*$_2$ for elevated 18:0 are non-allelic. The *st*$_1$*st*$_1$*st*$_2$*st*$_2$ genotype had 30% 18:0 content, but failed to grow after germination. It is unknown whether *st*$_1$ or *st*$_2$ are allelic to *fas*^{a}, *fas*^{b}, or *fas*.

Molecular markers closely associated with stearic acid alleles have been discovered (Table 2). A QTL associated with high stearic acid mapped to LG J from a mapping population, A81-356022 x PI468916 (Diers and Shoemaker 1992; Pantalone et al. 2004). A gene derived from FAM94-41 for high 18:0 has been mapped to LG B2. Three SSRs, Satt070, Satt474, and Satt556, were highly significant ($P < 0.0001$) with the major QTL near Satt474 having an $R^2 > 61\%$ (Spencer et al. 2003). Hyten et al. (2004) also reported two QTLs on LG C2 and LG L associated with stearic acid from an Essex x Williams population. Panthee et al. (2006) detected two markers, Satt168 (LG B2; $R^2 = 18\%$) and Satt249 (LG J; $R^2 = 12\%$) associated with stearic acid. They reported the gene near Satt249 on LG J from N87-984-16 is a novel allele which gave higher concentrations of stearic acid.

Oleic acid

Soybeans typically contain about 23% oleic acid (18:1). Increasing 18:1 from 55 to 60% in combination with low 18:3 would have edible and industrial applications with high oxidative stability. This oil would have increased stability at high cooking temperatures and would reduce the need for hydrogenation reducing *trans*-fats. High oleic oil could be used in the manufacture of soy-diesel, lubricants, and hydraulic oils. Genes at the *Fad* locus have been shown to be responsible for increasing levels of oleic acid (Wilson 2004). Other studies showed high 18:1 in soybean oil was quantitatively inherited (Burton et al. 1983; Hawkins et al. 1983). The germplasm line N78-2245 was perhaps the first soybean developed with higher levels (51%) of oleic acid by recurrent selection (Wilson et al. 1981). N98-4445A, a mid-oleic soybean accession with an oleic acid concentration of 40 to 70% depending on the environmental growing conditions, was developed from combining several oleic acid genes from a three way cross, N94-2473 x (N93-2007-4 x N92-3907) (Burton et al. 2006).

Molecular markers associated with oleic acid alleles have been disclosed (Table 2). Six QTLs have been mapped and confirmed for high 18:1 in line N00-3350 a derivative of N98-4445A on LGs A1 ($R^2 = 4\%$) at Satt211, D2 ($R^2 = 6\%$) at Satt389, G ($R^2 = 13\%$) at Satt394, G ($R^2 = 7\%$) at Satt191, L ($R^2 = 9\%$) at Satt418, and L ($R^2 = 25\%$) at Satt561 (Monteros et al. 2008).

Rahman et al. (1994) developed M23 with 46% oleic acid content from irradiating seeds of Bay soybean. The increase in oleic acid content in M23 was controlled by a single partially recessive gene, designated as *ol* (Takagi and Rahman 1996). Another allele *ol*^{a} at the same *Ol* locus was found in mutant M11 that contains about 38% oleic acid (Rahman et al. 1996b).

A recent study revealed that the mutation of the *ol* gene in M23 was the result of a deletion at the *Fad2-1a* locus (Sandhu et al. 2007). A PCR-based DNA marker for the high oleic genotype has made it easy to use marker-assisted selection (MAS) to select genotypes with higher oleic acid (Alt et al. 2005a). Because oleic acid content is significantly influenced by the environment in which the seed is produced (Oliva et al. 2006), marker-assisted selection for this trait should increase breeding efficiency.

Combining the M23 gene with genes from other mid-oleic acid sources has resulted in transgressive segregation for higher oleic acid content. Transgressive segregation among lines in the population of N98-4445A x M23 showed oleic acid levels can be increased to > 70% (Alt et al. 2005b).

Although N98-4445A with > 50% oleic acid has been developed, yield potential was less than a cultivar with seed oil containing a normal fatty acid profile (Burton et al. 2006). Similar to lines high or low in saturates, yield drag may be a significant factor in the development of productive non-genetically modified lines with high oleic acid content.

Low heritability and limited variation have contributed to the slow development of high 18:1 cultivars by conventional breeding (Liu 1999). However, transgenic approaches to increase oleic acid content in soybean oil show great promise. Genes for ω-6 and ω-3 desaturases have been cloned in soybean (Lui 1999). This enabled scientists at E. I. du Pont de Nemours and Company (DuPont) to suppress a ω-6 desaturase gene which resulted in a transgenic soybean with about 80% oleic acid content. Genotypes with this transgene have shown excellent stability for 18:1 content across a range of growing conditions. Knowlton et al. (1996) reported on a transgenic soybean with very high levels of oleic (85.6%), low levels of linoleic (1.6%) and linolenic (2.2%) acids. This transgene has shown no negative effect on grain yield and levels of 18:1 were very stable across growing environments. On the other hand, high oleic lines derived from non-GMO sources such as N98-4445A and M23 have yielded less compared to commercial cultivars of similar maturity and 18:1 levels are influenced by growing environments (Oliva et al. 2006).

QTLs for oleic acid content have been mapped to several positions in the soybean linkage group. Diers and Shoemaker (1992) detected three QTLs associated with variation for 18:1 levels. These QTL were close to RFLP markers pA-82 ($R^2 = 28\%$) on LG A1, pA-619($R^2 = 19\%$) on LG B2, and linked to the *pb* locus that determines the sharpness of pubescence ($R^2 = 21\%$) on LG E (Pantalone et al. 2004). A large QTL ($R^2 = 35\%$) was mapped on the position of 82.5 cM on LG L (Hyten et al. 2004) and a single molecular marker related to oleic acid content, Satt263, was mapped on LG E ($R^2 = 10\%$) (Panthee et al. 2006).

Linoleic acid

Linoleic acid (18:2) in soybean seeds is produced primarily by desaturation of oleic acid in phosphatidylcholine on the endoplasmatic reticulum (Ohlrogge and Browse 1995). Linoleic acid is a predominant fatty acid which is typically about 53% in soybean oil (Wilson 2004). The genetic control of linoleic acid has scarcely been studied, perhaps due to the lack of its need for modification. The accumulation of linoleic acid is the result of a balance of omega-6 and omega-3 fatty acid desaturase enzyme activity.

Molecular markers have been used to map the genes for linoleic acid (Table 2). Five RFLP markers; three on LG A1, one on LG B2, and one on LG E, and one morphological marker (*pb*^{c}) were found in a mapping population (Diers and Shoemaker 1992). Two large QTLs were mapped on the position of 93.7 cM (R^2 = 10%) on LG F and of 74.5 cM (R^2 = 51%) on LG L (Hyten et al. 2004). A single molecular marker, Satt185, was mapped on LG E (R^2 = 14%). Markers Satt185 and Satt263 (oleic acid marker) map within 0.6 cM distance of each other suggesting that QTL detected at that locus may be influencing an enzyme involved in the desaturation process from oleic acid to linoleic acid (Panthee et al. 2006).

Linolenic acid

Generally soybean oil has 8-10% linolenic acid (Wilson 2004). Linolenic acid (18:3) has been identified as an unstable component of soybean oil (Liu and White 1992). One of the most important goals of oil quality breeding in soybean has been to reduce its linolenic acid content for oxidative stability and flavor to reduce the need for hydrogenation. Several linolenic acid altered genotypes were developed by recurrent selection, mutagenesis, and germplasm screening.

Through several cycles of recurrent selection, N79-2245 soybean with 51% oleic acid and 4.2% linolenic acid was developed (Wilson et al. 1981). At least three independent genetic loci are associated with seed linolenic acid levels, with mutant alleles identified at *fan*, *fan2*, *fan3*, and *fanx*. Also, multiple alleles at the *fan* locus have been reported. Mutant C1640 (*fan*, 3.4% 18:3) (Wilcox et al. 1984; Wilcox and Cavins 1985), A5 (*fan*, 4% 18:3) (Hammond and Fehr 1983a; Rennie and Tanner 1991), A23 (*fan2*, 5.6% 18:3) (Fehr et al. 1992), KL-8 (*fanx*) and M-5 (*fan*) (Rahman et al. 1996a), M-24 (*fanx*^{a}) (Rahman et al. 1998), and RG10 (*fan-b*, < 2.5%) (Stojsin et al. 1998) were developed by mutagenesis. The *fan* alleles were also found in the USDA Soybean Germplasm Collection. PI123440 and PI361088B contained natural mutations at the *Fan* locus that have been shown to be either allelic or identical to the original *fan* allele in C1640 (Howell et al. 1972; Rennie et al. 1988; Rennie and Tanner 1989a). Evidence of two loci (*fanfan2*) were found in A16 and A17 (Fehr et al. 1992), MOLL (*fanfanx*) (Rahman and Takagi 1997), and LOLL (*fanfanx*^{a}) (Rahman et al. 2001). A29, a very low (1%) linolenic acid line, was reported (Ross et al. 2000). A29 was developed by combining three independent mutations; *fan* from line A5; *fan2* from A23, and *fan3* from a mutagenized derivative of line A89-144003 (Ross et al. 2000).

The molecular genetic basis for the low linolenic acid trait in soybeans has been discovered based on utilizing both gene information from experiments done in Arabidopsis and mutagenized soybean lines identified with the low linolenic acid trait. In Arabidopsis, it was discovered that a single gene, *FAD3*, controlled the conversion of linoleic acid precursors into linolenic acid precursors in the seed oil (Yadav et al. 1993). The soybean genome was shown to have at least three versions of the *FAD3* gene (Bilyeu et al. 2003; Anai et al. 2005). By sequencing and characterizing the *FAD3* alleles in soybean lines with low linolenic acid, corresponding mutations could be found in three of the genes that affected linolenic acid content (Bilyeu et al. 2003; Anai et al. 2005; Bilyeu et al. 2005; Bilyeu et al. 2006; Chappell and Bilyeu 2006; Chappell and Bilyeu 2007). Because the sequence changes identified in the alleles defined the mutations, perfect molecular markers were designed to specifically select the desired alleles (Bilyeu et al. 2005; Bilyeu et al. 2006; Chappell and Bilyeu 2006; Chappell and Bilyeu 2007). Selection by breeder-friendly perfect molecular markers can be an efficient system for soybean improvement, particularly if a backcross strategy is employed (Beuselinck et al. 2006; Bilyeu et al. 2006).

Molecular markers closely associated with linolenic acid alleles have been found (Table 2). The *Fan* locus has been mapped to LG B2 of soybean (Brummer et al. 1995). Four RFLP markers were found using a population from the cross C1640 x PI479750 and were successfully anchored to markers mapping the *Linolen1-2* QTL on LG B2 (Diers and Shoemaker 1992; Brummer et al. 1995). In another study, a DNA fragment missing from the omega-3 desaturase gene in soybean line A5 was mapped to the same region of LG B2 as the *Fan* locus, suggesting that a deletion in this gene was responsible for the reduction in 18:3 in A5 (Byrum et al. 1997; Pantalone et al. 2004).

A study identified and characterized three soybean microsomal omega-3 fatty acid desaturase genes, *GmFAD3A*, *GmFAD3B*, and *GmFAD3C* and determined that the deletion of the *GmFAD3A* gene is responsible for the reduced 18:3 in line A5 (Bilyeu et al. 2003). A point mutation was detected in the *GmFAD3A* gene in mutant C1640 at nucleotide 798 of the coding sequence. A guanine residue was changed to an adenine residue in the C1640 allele resulting in a change from a tryptophan codon to a premature stop codon. A molecular marker assay based on a PCR reaction was developed to distinguish between wild type Williams 82 and low 18:3 line C1640 alleles (Chappell and Bilyeu 2006).

Two point mutations, one in *GmFAD3A* and the other in *GmFAD3C*, were detected in low linolenic acid line CX1512-44. They contributed unequally, but additively to the linolenic acid content (Bilyeu et al. 2005). QTLs related to linolenic acid were

mapped in two different studies using normal linolenic acid soybean populations. Hyten et al. (2004) found major QTLs positioned at 50.6 cM (R^2 = 14%), and 82.5 cM (R^2 = 25%) on LG L and Panthee et al. (2006) also found molecular markers, Satt263 (LG E, R^2 = 14%) and Satt236 (LG G, R^2 = 23%). A major QTL that accounted for 78% of the phenotypic variability for low linolenic acid content derived from RG10 soybean genotypes was found on LG B2 (Satt534 and Fad3i6) (Reinprecht et al. 2006).

Mutations were discovered in all three *GmFAD3*, omega-3 fatty acid desaturase genes in the soybean lines A29 and suggested that combinations of mutant alleles at the three *GmFAD3* loci allowed the development of new germplasm containing 1% linolenic acid in the seed oil along with SNP-based molecular markers that can be used in a backcross breeding strategy (Bilyeu et al. 2006).

There have been few studies in breeding and mapping for high linolenic acid. High levels of linolenic acid soybean accessions are available in USDA soybean germplasm. Eleven *Glycine max* accessions with over 15% 18:3 and 20 *G. soja* accessions with over 20% 18:3 have been reported in the USDA soybean germplasm collection (USDA, ARS 2007). Genetic regulation of linolenic acid concentration in wild soybean suggested that the high-linolenic trait in wild soybean genotypes was determined by a set of desaturase alleles that were different from corresponding alleles in *G. max* (Pantalone et al. 1997). Introgression of these alleles from *G. soja* to *G. max* may lead to the production of high linolenic acid soybean oil for various applications such as omega-3 fatty acid soy-foods and industrial products.

Environmental effect on oil and fatty acid concentration in soybean

Genotype x environmental interactions which influence the oil concentration and fatty acid profile of soybean oil have been addressed in many studies. Studies have indicated that temperature plays an important role in the synthesis of oil and fatty acids. In general, higher temperature increases oil content in soybean seed (Wilson 2004). Soybeans grown under high average temperatures have reduced linoleic acid and linolenic acid, and increased oleic acid content; however, contents of saturated fatty acids were changed little by environment (Howell and Collins 1957; Wolf et al. 1982; Dornbos and Mullen 1992; Rennie and Tanner 1989b; Wilson 2004; Hou et al. 2006).

Instability of oleic acid and linolenic acids under various temperature regimes is a concern. Mid-oleic acid and low linolenic acid genotypes with genetically altered fatty acids developed by mutagenesis were more stable across environments than genotypes with altered fatty acid genotypes developed from conventional breeding techniques (Wilcox and Cavins 1992; Schnebly and Fehr 1993; Primomo et al. 2002; Oliva et al. 2006). The higher the average linolenic acid content of a genotype, the greater the instability it showed across various growing conditions (Oliva et al. 2006). The 1% linolenic acid genotype IA 3017 was very stable and the highest linolenic acid genotypes were the least stable for 18:3 across 10 growing environments. Thus, selecting for the lowest linolenic acid content should produce the most stable genotypes for 18:3.

Oleic acid was influenced significantly by temperature during the final 30 days of the reproductive period (Oliva et al. 2006). The highest 18:1 genotypes were the least stable across growing environments. In this study, N98-4445, an early group IV, had 55 to 60% 18:1 when grown in the southern US states North Carolina and Mississippi, and 39 to 45% when grown at Columbia, MO in the central US. The reduction in 18:1 as growing region moved from south to north was because of lower average temperatures during seed development. On the other hand, M23 was more stable across various environments than N98-4445A in the same study. Thus, genes for elevated oleic acid from different sources may be less influenced by growing conditions. A transgene for high 18:1 was reported to be very stable across growing environments (Knowlton et al. 1996), but to date, it has not been approved for use in commercially grown soybeans. Unless non-transgenic sources for 18:1 concentration can be found that are less influenced by temperature, special consideration will have to be given to production in specific regions using cultural practices and earlier maturity groups to produce desired levels of oleic and linolenic acids in soy oil (Shannon and Sleper 2004). To insure desired 18:1 and 18:3 levels, cultivars will need to be produced in warmer regions like the southern US or during the warmest periods of the summer in cooler areas. This may require adding high 18:1 and low 18:3 traits to earlier maturing cultivars within a region. Planting early cultivars at early planting dates would increase the chance that temperatures are warmest during reproductive growth giving the highest probability of producing oil with high 18:1 and low 18:3. On the contrary, high oleic acid is negatively correlated with the polyunsaturated fatty acids 18:2 and 18:3. Thus, if high polyunsaturated fats are desired, cooler temperatures, later planting, and later maturities would appear to favor this phenotype.

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