Agronomic and seed traits of soybean lacking seed lipoxygenase isozymes

by

James Mark Narvel

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Plant Breeding

Major Professor: Walter Fehr

Iowa State University

Ames, Iowa

1997

Graduate College

Iowa State University

This is to certify that the Master's thesis of

James Mark Narvel

has met the requirements of Iowa State University

Signatures have been redacted for privacy

TABLE OF CONTENTS

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] represents an abundant and economical source of highly nutritional and functional vegetable protein (Hildebrand and Kito, 1984). The consumption of soybean-containing foods as an alternative protein source by humans is being encouraged due to their relatively low saturated fat content and lack of cholesterol. Soy foods have also been purported as possessing anticancer properties contributed by certain phytochemicals. Soybeans have been used extensively for the production of traditional soy foods such as tofu and soymilk (Mathews, 1989). Soy flour and soy protein concentrates and isolates are used as ingredients in meat and dairy foods, as well as in baked goods to improve their structure, texture, and nutrient profiles while reducing their overall market cost (Young, 1985). However, the flavors of soy foods are considered objectionable to some consumers (Eskin et al., 1977). Unacceptable flavor is one of the major factors that has limited their wide spread use, predominantly in Western countries, although soybeans are a staple high protein food source in Eastern countries (Eskin et al., 1977).

Soybean seeds contain three lipoxygenase isozymes, designated L-1, L-2, and L-3, that catalyze the hydroperoxidation of polyunsaturated fatty acids containing a double bond system referred to as a cis,cis-1,4-pentatdiene moiety (Axelrod, 1981). Two polyunsaturated fatty acids containing this type of double bond system that occur in soybean seeds are linoleic and linolenic acid (Axelrod, 1981). The polyunsaturated fatty acid hydroperoxides generated from the lipoxygenase reactions and their degradation products

are largely responsible for the off-flavors found in soy foods (Wilson, 1996). The genetic removal of the three seed lipoxygenase isozymes may improve the flavor of soy foods.

Each soybean seed lipoxygenase isozyme is controlled by a single gene that is inherited in Mendelian fashion (Hildebrand, 1996). Absence of each isozyme is recessive to its presence (Hildebrand, 1996). No published data are available on the agronomic and seed characteristics of seed lipoxygenase-free soybean compared with normal soybean. The objective of my study was to determine the influence of genetically eliminating the three seed lipoxygenase isozymes on the performance of soybean for agronomic and seed traits of economic value.

LITERATURE REVIEW

Soybean Seed Lipoxygenase Isozymes

Lipoxygenase is an enzyme that catalyzes as a primary reaction the hydroperoxidation of polyunsaturated fatty acids that contain a cis,cis -1,4-pentadiene moiety (R–CH=CH–CH_2–CH=CH–R') through the addition of oxygen (Axelrod, 1981). Lipoxygenases have been reported in a wide range of organisms and have been identified in the seed, seed coat, pod wall, stem, leaf, hypocotyl, and radicle of soybean (Siedow, 1991). The lipoxygenase isozymes identified and characterized in soybean seeds are designated lipoxygenase-1 (L-1), lipoxygenase-2 (L-2), and lipoxygenase-3 (L-3), hereinafter collectively referred to as L1-3 (Axelrod, 1981). Isozymes are variant forms of an enzyme that are produced by different genetic loci. They catalyze the same principal reaction, but each isozyme has unique biochemical and/or physical properties compared with other forms.

L1-3 are relatively abundant in mature soybean seeds on a protein basis with L-3 accounting for ≈ 2 %, L-1 for ≈ 1.0 %, and L-2 for ≈ 0.5 % of the total seed protein (Hildebrand, 1996). L-2 has the highest reactivity level, L-3 the second highest, and L-1 the least reactive level (Hildebrand, 1996). Therefore, on an activity basis, there are similar amounts of the three isozymes found in the mature seeds of conventional cultivars (Hildebrand, 1996).

The major polyunsaturated fatty acids containing a cis,cis -1,4-pentadiene moiety that occur in soybean seeds are linoleic (18:2) and linolenic (18:3) acid (Axelrod, 1981).

Linoleic acid accounts for ≈ 54 % and linolenic acid accounts for ≈ 8 % of the total fatty acid composition in the seed oil of conventional cultivars (Wilcox, 1984). The majority of all fatty acids in soybean seeds occur in the ester-bound form and only trace amounts occur in the free-form that are negatively charged at physiological pH (Zhaung et al., 1991).

Siedow (1991) summarized the major biochemical properties that differentiate the soybean seed lipoxygenase isozymes. L-1 has a pH optimum of ≈ 9.0. It exhibits a marked preference towards free polyunsaturated fatty acids and shows little activity towards their ester-bound forms. L-2 and L-3 each have a pH optimum between 6.0-7.0. They show moderate activity towards esterified polyunsaturated fatty acids, but are more reactive towards their free-forms.

Hildebrand (1989) characterized the general mechanism of the hydroperoxidation of polyunsaturated fatty acids by each soybean seed lipoxygenase isozyme. The principal substrate of each isozyme is either linoleate or linolenate. The first step in the lipoxygenase pathway is the removal of a hydrogen from the C11 methylene group of the substrate forming a lipoxygenase polyunsaturated fatty acid radical complex. Hydrogen extraction is facilitated by a single iron atom that is bound by each isozyme that functions as the isozyme's catalytic center. Under aerobic conditions, the complex reacts with oxygen forming a polyunsaturated fatty acid peroxy radical. The final step is the conversion of this intermediate to the corresponding polyunsaturated fatty acid hydroperoxide and subsequent release of the isozyme in its oxidized state.

Lipoxygenase-Mediated Off-Flavors in Soy Foods

The polyunsaturated fatty acid hydroperoxides generated from the lipoxygenase reaction both directly and indirectly contribute to the off-flavors of soy foods (Rackis et al., 1979). These compounds may be converted to aldehydes by the enzyme hydroperoxide lyase through hydroxyl group elimination and the aldehydes may be reduced to alcohols by the enzyme alcohol dehydrogenase (Eskin et al., 1977; Hildebrand, 1989). The major compounds responsible for the off-flavors of soy foods, in addition to the polyunsaturated fatty acid hydroperoxides, are *n*-hexanal, *n*-hexanol, *n*-pentanol, and *n*-heptanol (Eskin et al., 1979). The compounds are present in whole soybeans due to the action of lipoxygenase and the other enzymes previously mentioned, or by other mechanisms, and may be further generated by these events during the homogenization of soybeans for food applications (Rackis et al., 1979). The compounds are reactive and can bind covalently to soy proteins resulting in their inclusion in the final food product (Davies et al., 1987). The off-flavors of soy foods have been characterized by some consumers as beany, bitter, painty, grassy, and green-tasting (Wolf, 1975; Rackis et al., 1979).

Several chemical and physical treatments exist for alleviating the flavor problems of soy foods (Wilson, 1996). Thermal inactivation or adjusting the soybean homogenate to a lower pH can reduce or eliminate lipoxygenase activity, but can result in the denaturation and insolubilization of other valuable proteins that may reduce the overall nutritional quality of the food (Davies et al., 1987). In addition, the heat treatment can impart a cooked flavor to foods that may have a more objectionable taste to some consumers than the lipoxygenase-

derived flavors (Wolf, 1975). With any method, there is an added cost that may limit the use of soybeans as a cost-effective ingredient in foods (Wilson, 1996).

Genetics of Soybean Seed Lipoxygenases

Soybean genotypes lacking either L-1, L-2, or L-3 in their mature seeds were identified by biochemical assays used to test for a lack of the lipoxygenase activity of each isozyme or by immunological and electrophoretic methods used to identify the absence of each lipoxygenase protein (Hildebrand and Hymowitz, 1981; Kitamura et al., 1983; Davies and Nielsen, 1986). The genetic basis for the deficiency of each isozyme is a null mutation of each gene that eliminates expression of a functional isozyme (Hildebrand, 1996).

The first soybean genotypes found to be deficient for L-1 were two plant introductions, PI 133226 and PI 408251 (Hildebrand and Hymowitz, 1981). Hildebrand and Hymowitz (1982) found that L-1 was controlled by a single gene inherited in a Mendelian fashion with absence of L-1 recessive to its presence. The symbol *Lx1* was assigned to the allele coding for presence of L-1 and *lx1* to the null allele coding for absence of L-1 in mature seeds. No other naturally occurring *lx1lx1* soybean genotypes have been reported.

Lack of L-3 was identified in 'Wasenatusa' and 'Ichigowase' by Kitamura et al. (1983) and in 'Tohoka No. 74' by Kitamura et al. (1985). Kitamura et al. (1983) evaluated the inheritance of L-3 and determined that it was controlled by a single gene inherited in a Mendelian fashion with absence of L-3 recessive to its presence. The symbol *Lx3* was assigned to the allele coding for presence of L-3 and *lx3* to the null allele coding for absence

of L-3 in mature seeds. No other naturally occuring *lx3lx3* soybean genotypes have been reported.

Davies and Nielsen (1986) identified a soybean genotype designated PI 86023 that lacked L-2. They found that L-2 was controlled by a single gene inherited in a Mendelian fashion with absence of L-2 recessive to its presence. The symbol *Lx2* was assigned to the allele coding for presence of L-2 and the symbol *lx2* to the null allele coding for absence of L-2 in mature seeds. No other naturally occurring *lx2lx2* null soybean genotypes have been reported. They also measured the genetic relationship of *lx2* with *lx1* and *lx3*. They determined that *lx3* is independently inherited and that *lx1* and *lx2* are tightly linked in repulsion (*lx1Lx2/Lx1lx2*), which prevented the development of the genotype *lx1lx1lx2lx2lx3lx3*, hereinfter referred to as the triple-null genotype.

Hajika et al. (1991) used gamma-irradiation to induce a triple-null genotype. They treated 1200 F_2 seeds from the cross between 'Kanto 101' (*Lx1Lx1lx2lx2lx3lx3*) and 'Kanto 102' (*lx1lx1Lx2Lx2lx3lx3*) and identified a single M_3 seed with the triple-null genotype. Gamma-irradiation may have caused genetic recombination between the *Lx1-lx1* and *Lx2-lx2* loci or may induced the conversion of *Lx1* to *lx1* or *Lx2* to *lx2*. Kitamura (1991) developed a triple-null genotype by treating seeds of Kanto 101 (*Lx1Lx1lx2lx2lx3lx3*) with gamma-irradiation, which converted *Lx1* to *lx1*. In the triple-null genotype, the *lx1* and *lx2* null alleles are so tightly linked in coupling (*lx1lx2/Lx1Lx2*) that they are inherited as if they were at the same locus. Therefore, the triple-null trait is inherited for breeding purposes as two-gene-type system with *lx1* and *lx2* independent of *lx3*.

Gene Expression of Soybean Seed Lipoxygenases and the Molecular Bases for Their Deficiencies

Hildebrand et al. (1991) evaluated the expression of L-1, L-2, and L-3 by measuring the levels of their lipoxygenase activity in various soybean seed tissues at different developmental stages. They determined that L-2 and L-3 were expressed during all developmental stages of the soybean axes and cotyledons until seed maturity. L-1 was expressed when the immature seed was ≥ 6-7 mm in length and in all subsequent stages until seed maturity. They suggested that the similar timing of expression of all three isozymes might be the result of the genes being duplications.

The molecular basis for the L-2 null mutation was characterized by Wang et al. (1994) as a spontaneous nucleotide transversion of thymidine (T) to adenine (A) at nucleotide position 1596 in the coding sequence of the L-2 gene structural gene (*Lox2*). The symbol *lox2* was assigned to designate the mutant allele of the L-2 structural gene. The transversion resulted in the substitution of a histidine for a glutamine residue at position 532 in the amino acid sequence of the L-2 protein. The amino acid substitution occurred in the histidine-rich-motif-region of the L-2 protein that participates in the binding of iron to preserve the structure of and impart functionality to the L-2 isozyme. They found that the *lx2*-encoded protein was expressed at the same level as the *Lx2*-encoded protein in immature seeds. However, the protein was inactive and absent in mature seeds. They concluded that the mutation led to a loss of the lipoxygenase activity and the structural stability of L-2, which caused its degradation during seed maturation.

The molecular basis for each of the L-1 and L-3 null mutations was partially characterized by Wang et al. (1994). They determined that soybean genotypes deficient for the L-1 or L-3 isozyme lack detectable messenger RNA (mRNA) transcript levels of their corresponding structural gene. They indicated that the L-1 and L-3 deficiencies may be due to a mutation in the regulatory sequences of each isozyme's structural gene. They suggested that this may prevent the transcription or impair the transcriptional processing of *Lox1* and *Lox3* . Wang et al. (1995) identified two single nucleotide substitutions in the promoter region of *Lox3*. They measured the transient expression of the mutant promoter in tobacco cell culture and detected transcriptional levels lesser than those from the normal promoter. They indicated that the low level of expression from the mutant promoter may be due to its ability to exhibit some functionality in tobacco cells compared with its complete loss of function in maturing soybean seeds. They also suggested that a mutation in the downstream regulatory sequences of *Lox3* may, in conjunction with the previously identified promoter mutations, synergistically account for the total loss in expression of L-3. No further studies have been reported that fully characterize the molecular basis for the L-1 null mutation.

Agronomic and Seed Trait Performance of Lipoxygenase-Null Soybean Lines

Pfeiffer et al. (1992) measured the effect of eliminating L-1 on several agronomic and seed traits. They measured the performance of soybean isolines with and without L-1 derived from the crosses between 'Williams' x PI 133226 (*lx1lx1*) and 'Altoona' x PI 133226. There were no significant differences between the performance of the two types of isolines from either cross for seed yield, lodging, plant height, seed weight, seed protein

content, and seed oil content. The maturity of the *lx1lx1* isolines was significantly delayed, but the mean difference from the *Lx1Lx1* isolines was less than one day.

Kitamura et al. (1987) measured the performance of several lipoxygenases-null types for agronomic and seed traits. Near-isogenic lines lacking L-1, L-2, L-1 and L-3, and L-2 and L-3 were developed by backcrossing lines of each lipoxygenase type to the Japanese cultivar 'Suzuyutaka' that has the normal lipoxygenase genotype. The performance of the lipoxygenase-null lines for seed yield, maturity, plant height, seed weight, seed protein content, and seed oil content was not significantly different from the performance of Suzuyutaka. Kitamura et al. (1995) reported that the triple-null cultivar 'Kyushu 111' developed in Japan has growth, developmental, and seed production characteristics similar to those of conventional cultivars.

Evaluation of the Lipoxygenase Phenotype of Seeds

Development of triple-null soybean cultivars requires testing procedures to identify the absence of each isozyme in seeds. Electrophoretic and immunological methods that have been previously used for this purpose afford accurate measurement, but are relatively expensive and time consuming. Suda et al. (1995) developed three simple and rapid colorimetric assays that selectively identify the absence of each isozyme by testing for lack of their lipoxygenase activity. The substrate for each test is linoleate. The selectiveness of each assay for its isozyme is based on the pH optimum for activity of the isozyme and the strong tendency of L-3 to oxidize β-carotene in the presence of linoleate. The assays can be

used to identify triple-null seeds or triple-null plants by progeny testing to determine its genotype.

The basis for the L-1 and L-2 assays is the detection of linoleate hydroperoxidation in a seed sample through the use of an indicator dye that is bleached when hydroperoxidation occurs. The pH of the L-1 test solution is 9.0 and the indicator dye is methylene blue. If L-1 is present in a seed sample due to the *Lx1*- genotype, it will catalyze the hydroperoxidation of linoleate forming a linoleate hydroperoxide. Methylene blue extracts a hydrogen from the hydroperoxide group resulting in its reduction and subsequent bleaching of its natural blue color (Toyosaki, 1996). If the isozyme is absent in a seed sample (*lx1lx1*), linoleate hydroperoxidation will not occur and methylene blue will not be bleached. The pH of the L-2 test is 6.0 and follows the same principle as the L-1 test.

The pH of the L-3 test solution is 6.6 and involves the use of a yellow β-carotene dye. If L-3 is present in the seed sample due to the *Lx3*- genotype, it will oxidize linoleate and β-carotene, which produces a clear solution. Absence of L-3 in a seed sample (*lx3lx3*) will result in no linoleate or β-carotene oxidation and the solution remains yellow.

MATERIALS AND METHODS

Development of Backcrossed-Derived Lines

F_1 seeds heterozygous for the three seed lipoxygenase loci were obtained from Keisuke Kitamura at the National Agriculture Research Center, Yatabe, Tsubuka, Japan in 1992. The F_1 seeds from the cross 'AGS 129' (*Lx1Lx1Lx2Lx2Lx3Lx3*) with a triple-null line (*lx1lx1lx2lx2lx3lx3*) were designated by the soybean breeding project at Iowa State University as AX9325. AGS 129 is a normal cultivar from Japan. The triple-null parent was developed by Keisuke Kitamura (1991) by treating seeds of the Japanese cultivar 'Kanto 101' (*Lx1Lx1lx2lx2lx3lx3*) with gamma-irradiation. Seven F_1 seeds from AX9325 were planted in November 1992 at the Iowa State University-University of Puerto Rico soybean breeding nursery at Isabela, Puerto Rico and individual F_1 plants were harvested to obtain F_2 seed. Ten F_2 seeds per F_1 plant were split into two portions. One portion, approximately two-thirds of a whole seed that contained the embryonic axis, was kept for planting. The portion not containing the embryonic axis, approximately 1/3 of a whole seed, was sent to the Indiana Crop Improvement Association (Layfayette, IN) for ELISA (enzyme-linked-immunosorbent-assay) analysis to determine the presence or absence of each isozyme. A total of 40 F_2 seeds from AX9325 were analyzed and five F_2 seeds were identified as triple-null.

Two triple-null F_2 plants were crossed to the high seed protein and large-seeded cultivar 'IA2020' of Maturity Group II that is used for making tofu. The crosses were made in May 1993 at Isabela, Puerto Rico. The F_1 seeds from the crosses were collectively

designated AX10087. The seeds were planted in January 1994 at Isabela, Puerto Rico to obtain F_2 seeds. A total of 256 F_2 seeds from two F_1 plants of AX10087 were split and the portion not containing the embryonic axis was sent to the Indiana Crop Improvement Association for ELISA analysis to identify triple-null F_2 seeds. A total of 16 F_2 seeds identified as triple-null were planted in May 1994 near Ames, Iowa at the Agronomy and Agricultural Engineering Research Center of Iowa State University. Only three triple-null F_2 plants flowered early enough to be used for backcrossing to IA2020. The backcrosses of IA2020 (IA2020 x AX10087-2) was designated AX12306, IA2020 (IA2020 x AX10087-4), was AX12307, and IA2020 (IA2020 x AX10087-10) was AX12308. At maturity, seven BC_1F_1 seeds from AX12306, eight from AX12307, and 14 from AX12308 were harvested. The BC_1F_1 seeds were planted in October 1994 at Puerto Rico and each BC_1F_1 plant was harvested individually. The BC_1F_2 seeds from each BC_1F_1 plant were kept as a separate backcross family. A variable number of BC_1F_2 seeds from each backcross family were planted in May 1995 near Ames, Iowa. At maturity, BC_1F_2 plants were classified as early, mid, or late maturing. For this study, BC_1F_2 plants of midseason maturity were used to develop triple-null (*lx1lx1lx2lx2lx3lx3*) and normal lipoxygenase (*Lx1Lx1Lx2Lx2Lx3Lx3*) BC_1F_2 -derived lines for the study.

Triple-null and normal BC_1F_2 plants were identified by evaluating BC_1F_3 seeds from each plant by the colorimetric assay developed by Suda et al. (1995). Four individual BC_1F_3 seeds from each BC_1F_2 plant were tested for the presence or absence of each lipoxygenase isozyme. Four seeds were tested to have a 99% probability of detecting the presence of one of the isozymes from a plant heterozygous at one or all lipoxygenase loci

(Sedcole, 1977). Seven additional seeds from each BC_1F_2 plant that tested positive for an isozyme were evaluated to determine if the BC_1F_2 plant was homozygous normal for all lipoxygenase loci. A total of 11 seeds was used to have a 95% probability of detecting a single seed that lacked one of the isozymes from a heterozygous plant (Sedcole, 1977).

The genetic variability among backcross families for a quantitative trait was expected to be greater than the variability within backcross families. To equally average the genetic background among the two groups of lines, an equal number of triple-null and normal BC_1F_2 -derived lines were developed from each backcross family. Three backcross families from AX12306, three backcross families from AX12307, and seven backcross families from AX12308 were identified as containing at least one triple-null and one normal lipoxygenase BC_1F_2 plant. The number of backcrossed-derived lines of each lipoxygenase type from each backcross family within each backcross population is presented in Table 1. A total of 27 BC_1F_2 -derived triple-null and 27 BC_1F_2 -derived normal lines from 13 different backcross families were evaluated in this study.

The $BC_1F_{2:3}$ lines were planted in December 1995 at Isabela, Puerto Rico to increase their seed. The $BC_1F_{2:4}$ seeds from each line were harvested in bulk. A sample of 10 individual seeds from each line was tested for the three lipoxygenase isozymes using by the colorimetric assay developed by Suda et al. (1995) to confirm their lipoxygenase genotype. A total of 1080 BC_1F_4 seeds from each BC_1F_2 -derived line were used to plant the replicated field tests.

Table 1. Entries in the replicated test grown in Iowa in 1996.

Backcross population-family	No. of lines	
	Triple-null	Normal lipoxygenase
AX12306-2	1	1
AX12306-3	3	3
AX12306-4	4	4
AX12307-3	2	2
AX12307-4	1	1
AX12307-5	2	2
AX12308-1	1	1
AX12308-2	1	1
AX12308-3	5	5
AX12308-7	1	1
AX12308-8	1	1
AX12308-9	3	3
AX12308-10	2	2

Field-Plot Design

The experimental design was a randomized complete-block with two replications at three locations in central Iowa: Ames, Hubbard, and Grand Junction. The set consisted of 60 entries: 27 $BC_1F_{2:4}$ triple-null lines, 27 $BC_1F_{2:4}$ normal lines, the recurrent parent IA2020 and five checks. The checks included 'IA1002', 'IA2010', 'IA2022', 'IA3006', and A92-733016 that were all developed by Iowa State University. IA1002 was a cultivar of Maturity Group I that is used for making tofu and had high seed protein content, large seeds, and lacked the L-2 isozyme. IA2010 was a cultivar of Maturity Group II that is used for making tofu and had high seed protein content, large seeds, and lacked the L-2 isozyme. IA2022 was a conventional high-yielding cultivar of Maturity Group II. IA3006 was a large-seeded cultivar of Maturity Group III that is used for the production of vegetable soybeans or the Japanese fermented product miso. A92-733016 was an elite line of Maturity Group II that had high seed protein content, large seeds, and lacked the L-2 and L-3 isozymes. At each location, the plots were two rows 2.7 m long with 69 cm between rows within the plot and 102 cm between rows of adjacent plots. The seeding rate was 32 seeds m^{-1} of row.

Data Collection

All traits were measured on each plot at all locations and replications. Maturity was measured as days after August 31 when 95 % of the pods had reached their mature color. Lodging and plant height were measured when the plants were mature. Lodging was scored on a scale of 1.0 (all plants erect) to 5.0 (most plants prostrate). Plant height was

determined by measuring the distance in centimeters from the soil surface to the terminal node of the main stem from a representative plant within each plot. Both rows of each plot were harvested with a self-propelled plot combine. The protein, oil, and moisture contents of each plot were determined from a random sample of ≈ 1000 seeds with a Teactor A/B (Hooganas, Sweden) Infratech 1221 near-infrared whole grain analyzer (NIR). The seed yield and the seed protein and seed oil content of each plot was expressed on a 13 % moisture basis. Average seed weight was determined in mg sd^{-1} from a random sample of 200 clean whole seeds.

Fatty Acid Analysis Procedure

The fatty acid composition of each entry was measured by gas chromatography as described by Hammond (1991). Two random 5-seed bulk samples from each entry were crushed between two rectangular aluminum plates (21 x 21 cm) in a hydraulic press under approximately 40 kg cm^{-2} pressure. The crushed samples were covered in approximately 0.1 ml of hexane and stood for ≈ 18 hours to extract the oil from the seeds. A 0.1 ml sample of the extracted oil was transferred to a vial and 0.5 ml of a 1M solution of sodium methoxide in methanol was added. Each sample reacted for ≈ 30 minutes to hydrolyze the fatty ester from their glycerol moiety and to convert each individual fatty ester to the methyl ester form. The samples were gently agitated at 10-minute intervals to maximize methyl ester formation. After the 30-minute period, 0.15 ml of distilled water was added to separate the methyl esters from the sodium methoxide. Approximately 0.1 ml of the methyl ester extract dissolved in hexane was injected into a Hewlett Packard (Avondale, PA) 5890

gas chromatograph fitted with Durabond-23 capillary columns (J & W Scientific, Deerfield, IL). The columns were heated to 200 °C and the fatty esters were separated according to their size and conformational structure. The percentage of the five major fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acid) in each sample was determined by calculating its corrected integrated peak area following flame ionization.

Data Analysis

The data were analyzed as a randomized complete-block design. Locations, replications, and entries within the two lipoxygenase types were considered random effects. An additive model was used for the analysis of variance of individual environments:

$$Y_{ij} = \mu + R_i + G_j + e_{ij},$$

where:

Y_{ij} = the observed value of the j^{th} genotype in the i^{th} replication,

μ = the overall mean,

G_j = the effect of the j^{th} genotype (j = 1 to 54),

and e_{ij} = the random plot error of the j^{th} genotype in the i^{th} replication (i = 1 and 2).

Variation due to entries was partitioned into three components: variation among triple-null lines, variation among normal lines, and the orthogonal comparison of the two lipoxygenase types (Table 2).

Table 2. Form of the analysis of variance and expected mean squares for an individual location.

Sources of variation	Df†	Expected mean squares
Replications (R)	r-1	$\sigma^2_e + g\sigma^2_R$
Entries (G)	g-1	$\sigma^2_e + r\sigma^2_G$
Triple-null (Tn)	tn-1	$\sigma^2_e + r\sigma^2_{Tn}$
Normal (N)	n-1	$\sigma^2_e + r\sigma^2_N$
Tn vs. N	1	$\sigma^2_e + r\theta^2_{Tn\ vs.\ N}$
Error	(r-1) (g-1)	σ^2_e
Total	rg-1	

† r , g, tn, and n represent the number of replications, entries, triple-null lines, and normal lines, respectively.

For the combined analyses across locations, the following additive model was used:

$$Y_{ijk} = \mu + E_i + (R/E)_{ij} + G_k + (GE)_{ik} + e_{ijk}$$

where:

μ = the overall mean,

E_i = the effect of the i^{th} environment (i = 1 to 3),

$(R/E)_{ij}$ = the effect of the j^{th} replication within the i^{th} environment (j = 1 to 2),

G_k = the effect of the k^{th} genotype (k = 1 to 54),

$(GE)_{ik}$ = the effect of the interaction between the i^{th} environment and the k^{th} genotype,

and e_{kij} = the random plot error of the k^{th} genotype in the j^{th} replication in the i^{th} environment.

Variation due to entries was partitioned into the same components as for the analysis of individual locations (Table 3).

Table 3. Form of the analysis of variance and expected mean squares combined over locations.

Sources of variation	Df ‡	Expected mean squares
Environments (E)	e-1	$\sigma^2_e + r\sigma^2_{EG} + g\sigma^2_{R/E} + rg\sigma^2_E$
Replications (R/E)	e(r-1)	$\sigma^2_e + g\sigma^2_{R/E}$
Entries (G)	g-1	$\sigma^2_e + r\sigma^2_{EG} + re\sigma^2_G$
Triple-null (Tn)	tn-1	$\sigma^2_e + r\sigma^2_{ETn} + re\sigma^2_{Tn}$
Normal (N)	n-1	$\sigma^2_e + r\sigma^2_{EN} + re\sigma^2_N$
Tn vs N	1	$\sigma^2_e + r\sigma^2_{E\,(Tn\ vs.\ N)} + re\theta^2_{Tn\ vs.\ N}$
Environments x Entries	(e-1)(g-1)	$\sigma^2_e + r\sigma^2_{EG}$
Env. x Triple-null	(e-1)(tn-1)	$\sigma^2_e + r\sigma^2_{EN}$
Env. x Normal	(e-1)(n-1)	$\sigma^2_e + r\sigma^2_{ETn}$
Env. x (Tn vs N)	(e-1)	$\sigma^2_e + r\sigma^2_{E\,(Tn\ vs.\ N)}$
Pooled error	e(r-1)(g-1)	σ^2_e
Total	erg-1	

‡ e, r, g, tn, and n represent the number of environments, replications, entries, triple-null lines, and normal lines, respectively.

The analysis of variance was performed on the data using the proc ANOVA of the SAS software package (SAS Institute, 1992). Only the triple-null and normal lines were considered in the analysis. The variation among lines and the orthogonal comparisons between triple-null and normal lines were evaluated using F-tests with the pooled entry by environment interaction mean squares as the error term for the combined analysis across locations (Steel and Torrie, 1980). The standard error (SE), least significant difference (LSD), and coefficient of variation (CV) were calculated as described by Steel and Torrie (1980).

Formulas for these calculations were:

$SE = (MSE/n)^{1/2}$ $LSD = t_{\alpha} (2MSE/n)^{1/2}$ $CV = (\sigma^2)^{1/2}/\bar{x}$

where:

t_{α} = value of t at the 0.05 or 0.01 probability levels,

MSE = error mean squares for the analysis of inividual environments; pooled entry by environment interaction mean squares for the combined analysis across locations,

n = number of observations in an entry mean (n = 2 for an individual environment; n = 6 for across environments),

$\bar{x}$ = overall mean,

and σ^2 = experimental error.

RESULTS

There were significant differences ($P < 0.01$) among locations for all traits measured, except for maturity. The mean seed yield of triple-null lines was not different ($P > 0.05$) than the mean of normal lines at the three individual locations and combined across locations (Tables 4, 5, and 6). The variation among triple-null lines for yield was significant at Hubbard, but not for the other two locations. The variation among normal lines for yield was significant at each location. There was a significant genotype by environment interaction for yield of the normal lines, but not for the triple-null lines (Table 5).

The highest yielding triple-null line was similar in performance to the highest yielding normal line. The 10 highest yielding entries included four triple-null and six normal lines. The entries with the highest and lowest mean seed yield were both normal lines (Table 6). The mean yield of the lowest normal line was 2588 kg ha^{-1}, compared with 2849 kg ha^{-1} for the lowest triple-null line. The normal line with the lowest mean yield was consistently low at each of the three locations. It was not clear why the line yielded so poorly. The entry with the second lowest yield was also a normal line, which had a mean of 2728 kg ha^{-1}. The 10 lowest yielding entries included three triple-null and seven normal lines.

The triple-null and normal lines had the same mean time of maturity (Table 6). There were significant differences in maturity among lines within each group at each location (Table 4). The 10 earliest maturing entries included six triple-null and four normal lines. The 10 latest maturing entries included six triple-null and four normal lines.

Table 4. Analyses of variance for agronomic and seed traits of lines at individual locations.

		Mean Squares								
		Seed yield			Maturity			Lodging		
Sources of variation	Df	Ames	Hubbard	Grand Junction	Ames	Hubbard	Grand Junction	Ames	Hubbard	Grand Junction
Replications	1	35425	227701*	20861	8.3	6.3*	1.6	0.59*	0.02	0.01
Entries (G)	53	116629	190763**	139495*	23.6**	10.4**	6.3**	0.43**	0.46*	1.05**
Triple-null (Tn)	26	65964	134245**	114560	22.1**	13.2**	3.9**	0.43**	0.56**	1.31**
Normal (N)	26	171122**	254463**	169436*	25.8**	8.3**	9.3**	1.04**	0.37	0.81*
Tn vs N	1	17125	4021	9352	1.3	1.8	1.1	0.33	0.00	0.04
Error	53	75624	48412	81358	3.3	1.3	0.7	0.14	0.26	0.27
CV (%)		8.1	8.0	9.3	6.4	3.6	3.7	12.1	18.3	20.1

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 4. (Cont.)

Sources of variation	Df	Mean Squares					
		Height			Seed weight		
		Ames	Hubbard	Grand Junction	Ames	Hubbard	Grand Junction
Replications	1	94	197	5	5896**	416**	13962**
Entries (G)	53	101**	115*	230**	735**	459**	837**
Triple-null (Tn)	26	122**	103	230**	940**	485**	897**
Normal (N)	26	82*	103	218**	556**	424**	640**
Tn vs N	1	37	716**	533**	45	685**	4382**
Error	53	47	66	52	220	2821	387
CV (%)		7.2	8.7	7.4	6.5	3.0	7.3

Table 4. (Cont.)

Sources of variation	Df	Mean Squares					
		Protein			Oil		
		Ames	Hubbard	Grand Junction	Ames	Hubbard	Grand Junction
Replications	1	0.7	2.3**	0.7	34.7**	15.7**	2.7**
Entries (G)	53	1.6**	1.7**	0.8	0.5	0.5	0.3*
Triple-null (Tn)	26	2.1**	1.7**	1.0	0.5	0.6	0.3*
Normal (N)	26	1.1*	1.7**	0.6	0.2	0.2	0.2
Tn vs N	1	1.4	0.5	0.3	6.3**	8.2**	1.4**
Error	53	0.6	0.15	0.6	0.5	0.4	0.2
CV (%)		2.0	1.0	2.1	4.2	3.9	2.5

Table 5. Analyses of variance combined over locations for agronomic and seed traits.

		Mean Squares			
Sources of variation	Df	Seed yield	Maturity	Lodging	Height
Environments (E)	2	10905283**	2112.3	6.30**	445**
Replications(R/E)	3	94662	5.4*	0.21	99
Entries (G)	53	199501*	30.6**	0.93**	320**
Triple-null (Tn)	26	120200	30.2**	1.2**	347**
Normal (N)	26	286465*	32**	0.72**	265**
Tn vs N	1	285	4.2	0.06	1042**
E x Entries	106	123693**	4.9**	0.51**	63
E x Tn	52	97284	4.4	0.58**	54
E x N	52	154278**	5.6	0.45**	69
E x (Tn vs N)	2	15107	0.0	0.16	122
Pooled Error	159	68465	1.8	0.22	55
CV (%)		8.5	4.8	16.8	7.8

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 5. (Cont.)

		Mean Squares		
Sources of variation	Df	Seed weight	Protein	Oil
Environments (E)	2	11080**	19.2**	6.5**
Replications (R/E)	3	6758**	1.2	17.7**
Entries (G)	53	1203**	2.0**	0.6**
Triple-null (Tn)	26	1160**	2.5**	0.7**
Normal (N)	26	1264**	1.6	0.3
Tn vs N	1	729	0.6	5.8**
E x Entries	106	414**	1.0**	0.3
E x Tn	52	582**	1.2**	0.4
E x N	52	178	0.9**	0.1
E x (Tn vs N)	2	2192**	0.8	5.0**
Pooled Error	159	187	0.5	0.4
CV (%)		5.8	1.8	3.6

Table 6. Mean performance of triple-null and normal lines across locations for agronomic and seed traits.

	Trait						
					Seed		
Lines	Seed yield	Maturity	Lodging	Plant height	Weight	Protein	Oil
	(kg ha $^{-1}$)	(days)	(score)	(cm)	(mg sd $^{-1}$)	(g kg $^{-1}$)	(g kg $^{-1}$)
Triple-null	3066	28	2.7	94	237	376	166
(Range)	2849 - 3474	24 - 31	2.1 - 3.3	80 - 108	210 - 267	365 - 388	160 - 175
Normal	3067ns[a]	28ns	3.0ns	98**	234ns	377ns	169**
(Range)	2588 - 3486	23 - 34	2.6 - 3.4	84 - 109	190 - 259	364 - 392	164 - 173

** Significant difference between the mean performance of triple-null and normal lines at the 0.01 probability level.

[a] ns = No significant difference between the mean performance of triple-null and normal lines at the 0.05 probability level.

The mean lodging of triple-null lines was not different ($P > 0.05$) from normal lines (Table 6). There were significant differences for lodging score among lines within each group combined across locations (Table 5). The 10 entries that had the best lodging score included six triple-null and four normal lines. The 10 entries that had the poorest lodging score included five triple-null and five normal lines.

The mean plant height of normal lines was significantly taller than the triple-null lines combined across locations, but the difference was only 3 cm greater (Table 6). There were significant differences among lines within each group for plant height combined across locations (Table 5). The 10 entries with the tallest plant height included four triple-null and six normal lines. The 10 entries with the shortest plant height included four triple-null and six normal lines.

The mean seed weight of triple-null lines was not different ($P > 0.05$) from normal lines (Table 6). There were significant differences among lines within each group for seed weight combined across locations (Table 5). The 10 entries with the greatest seed weight included seven triple-null and three normal lines. The 10 entries with the lowest seed weight included four triple-null and six normal lines.

The mean seed protein content of normal lines was not different ($P > 0.05$) from triple-null lines (Table 6). The variation among triple-null lines for protein content combined across locations was significant, but was not significant among normal lines (Table 5). The 10 entries with the highest protein content included five triple-null and five normal lines. The 10 entries with the lowest protein content included seven triple-nulls and three normal lines.

The mean seed oil content of normal lines was 3 g kg^{-1} greater than triple-null lines and the difference was significant combined across locations (Table 6). The variation among triple-null lines for oil content combined across locations was significant, but was not significant among normal lines (Table 5). The entry with the highest and the entry with the lowest oil content were both triple-null lines. The 10 entries with the highest oil content included three triple-null and seven normal lines. The 10 entries with the lowest oil content included nine triple-null and one normal line.

Fatty acid analysis was initially conducted to determine the influence of eliminating the seed lipoxygenase isozymes on the fatty acid composition of soybean seeds. The data indicated that some lines were homogeneous or heterogeneous for elevated stearic acid (18:0) content. The source of the lipoxygenase null alleles used for the development of the triple-null lines evaluated in this study were derived from Kanto 101 that was treated with gamma-irradiation to induce a triple-null genotype (Kitamura, 1991). Induced mutagenesis can produce random genetic changes both in the desired and undesired direction. The mutagenic event must have altered the genetic makeup for stearic acid composition since lines homogeneous or heterogeneous for elevated contents of this fatty acid were observed. The stearic acid content of normal soybean cultivars grown in North America is approximately 40 g kg^{-1} (Wilcox, 1984). Levels for stearic acid content among the lines in this study ranged from approximately 40 g kg^{-1} to 200 g kg^{-1}. Lines with an average stearic acid content of 70 g kg^{-1} or greater were considered to be either homogeneous or heterogeneous for elevated stearic acid content. Thirteen triple-null lines and 15 normal lines were considered to be homogeneous or heterogenous for high stearic acid content, which indicated that the allele(s)

conferring elevated levels of stearic acid were not linked to any of the lipoxygenase-null alleles. Since the frequency of triple-null and normal lines with high stearic acid content was relatively equal, the comparisons between the two types of lines for the other traits measured in this study should not be biased. However, due to differences in stearic acid content among lines, a comparison between triple-null and normal lines for fatty acid composition was not conducted. The mean contents of the five major fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acid) of each entry across locations are indicated in Table 12 (Appendix D).

DISCUSSION

Elimination of the three seed lipoxygenase isozymes did not alter the mean performance of soybean lines for most of the traits that were measured. Although there were significant differences between triple-null and normal lines for several traits, the magnitude of the differences would not hinder the development of acceptable triple-null cultivars. My results agree with previous studies that did not identify any physiological role for the seed lipoxygenase isozymes (Kitamura et al., 1985; Pfieffer et al., 1992).

The mean protein content of triple-null lines was only 1 g kg^{-1} or 0.1 percentage unit less than normal lines. Four triple-null lines had an average protein content equal to or slightly greater than the recurrent parent IA2020. This suggested that elimination of the isozymes caused an increase in the biosynthesis of other seed proteins.

It seems that the seed lipoxygenase isozymes do not have any physiological role in the growth and development of soybean. Siedow (1991) suggested that the isozymes may serve as seed storage proteins. He also suggested that the products of the lipoxygenase reaction may function in response to phytopathogen attack. Pfeiffer et al. (1992) measured the influence of elimination of seed lipoxygenases on susceptibility to seed infection by the pod and stem blight fungus *Phomopsis longicolla*. The seed germination of soybean isolines of 'Century' lacking a single isozyme and isolines lacking a combination of L-1·L-3 and

L-2·L-3 were compared with Century under infested and uninfested conditions. They detected no significant difference in seed germination between the isolines and Century

under any conditions. They did not study the influence of the combined absence of L-1·L-2 or the absence of the three isozymes on susceptibility to *P. longicolla* or to other seed-type pathogens. The evaluation of triple-null soybean lines under disease conditions should be carried out to determine if the absence of the isozymes would increase susceptibility that could negatively affect agronomic or seed trait performance.

APPENDIX A

MEANS OF ENTRIES ACROSS LOCATIONS

Table 7. Mean performance of all entries for agronomic and seed traits across locations.

Entry no.[a]	Seed yield [b]	Maturity [c]	Lodging [d]	Plant height [e]	Seed weight	Protein [f]	Oil [g]
	(kg ha^{-1})	(days)	(score)	(cm)	(mg sd^{-1})	(g kg^{-1})	(g kg^{-1})
612001	2958	31	3.0	108	241	375	164
612002	3474	29	3.3	100	257	386	163
612003	2914	24	2.7	89	267	388	165
612004	3176	24	2.6	81	238	383	163
612005	3002	27	2.8	80	239	382	167
612006	2930	29	2.8	90	229	376	169
612007	3067	25	3.0	81	233	381	167
612008	3018	29	3.0	95	226	380	167
612009	3121	27	2.2	85	234	385	168
612010	3005	29	2.6	90	250	375	168
612011	3145	29	2.4	86	250	370	167
612012	2849	31	2.4	92	258	380	161
612013	2852	31	2.9	88	243	378	162
612014	3203	25	2.8	94	247	380	167
612015	3071	25	2.1	92	226	379	167
612016	3132	28	3.0	98	234	367	168
612017	3072	27	2.7	104	245	365	163
612018	3165	27	2.2	100	251	369	167
612019	2964	24	2.9	96	244	370	160
612020	3179	29	2.9	96	234	368	164
612021	3274	27	2.6	99	214	371	166
612022	2981	31	3.2	107	210	372	168
612023	3266	30	3.2	99	219	378	168
612024	3033	27	2.7	93	227	365	175
612025	2982	27	3.0	97	218	376	171
612026	3015	30	2.5	96	232	380	170
612027	2928	29	2.3	104	229	373	171
612028	2968	30	3.2	102	237	381	167
612029	3203	29	2.8	104	238	375	167
612030	3430	27	2.7	98	259	382	173
612031	3109	24	3.0	96	232	380	172
612032	3026	28	2.8	101	223	392	167
612033	3166	29	2.8	95	242	376	169
612034	3155	26	3.1	99	246	378	167
612035	2867	29	3.2	87	249	378	166
612036	3177	29	2.7	103	239	372	164
612037	3242	30	3.2	90	237	381	165

Table 7. (Cont.)

Entry no.[a]	Seed yield [b]	Maturity [c]	Lodging [d]	Plant height [e]	Seed weight	Protein [f]	Oil [g]
	(kg ha^{-1})	(days)	(score)	(cm)	(mg sd^{-1})	(g kg^{-1})	(g kg^{-1})
612038	2728	29	3.3	92	211	377	167
612039	3312	29	2.6	99	242	375	172
612040	2989	29	3.3	103	239	381	171
612041	3399	23	2.9	84	237	374	170
612042	3047	28	2.8	103	237	378	170
612043	2999	29	2.8	101	235	377	172
612044	3058	25	2.6	86	228	376	171
612045	2831	30	2.6	97	190	376	171
612046	3199	29	3.4	109	213	369	169
612047	2887	29	3.3	104	228	378	168
612048	3032	25	2.9	86	218	364	171
612049	2587	34	3.4	100	222	368	166
612050	3163	28	2.8	97	253	380	170
612051	3203	26	2.6	98	245	377	170
612052	3215	26	3.0	91	231	378	171
612053	2743	31	3.2	104	236	374	170
612054	3125	26	3.0	105	250	379	168
612055	2988	19	2.8	76	216	379	166
612056	3149	23	3.1	86	223	399	164
612057	3290	26	2.3	92	231	383	171
612058	3424	27	2.0	95	169	364	171
612059	3384	29	2.3	81	267	382	164
612060	3116	24	2.5	84	239	377	169
SE [h]	144	0.9	0.3	3.2	8.3	0.4	0.2
LSD [i] $_{0.05}$	285.1	1.8	0.6	6.3	16.4	0.8	0.4
LSD [j] $_{0.01}$	376.8	2.4	0.8	8.4	21.7	1.0	0.5
CV [k] (%)	8.5	4.8	16.8	7.8	5.8	1.8	3.6

[a] Entries 1 -27 are triple-null lines, entries 28 - 54 are normal lines, and entries 55 - 60 are checks (IA1002, IA2010, IA2020, IA2022, IA3006, and A93-733016, respectively).

[b] Yield: expressed on a 13% moisture basis.

[c] Maturity: recorded as days after August 31 when 95% of pods reached their mature color.

[d] Lodging: scored on a scale of 1 (erect) to 5 (prostrate).

[e] Plant height: measured as the distance in centimeters from the soil surface to the terminal bud of the main stem.

[f] Protein: expressed on a 13% moisture basis.

[g] Oil: expressed on a 13% moisture basis.

[h] SE: standard error of the mean

[i] LSD: least significant difference at the 0.05 probability level.

[j] LSD: least significant difference at the 0.01 probability level.

[k] CV: coefficient of variation.

APPENDIX B

MEANS OF TRIPLE-NULL AND NORMAL LINES AT INDIVIDUAL LOCATIONS

Table 8. Mean performance of triple-null and normal lines at individual locations for agronomic and seed traits.

		Location					
		Ames		Hubbard		Grand Junction	
Trait	Lines	Mean	Range	Mean	Range	Mean	Range
Seed yield	Triple-null	3389	2998-3782	2735	2109-3186	3073	2593-3617
(kg ha $^{-1}$)	Normal	3364ns[a]	2404-3741	2747ns	1586-3264	3092ns	2465-3542
Maturity	Triple-null	29	23-34	31	28-36	23	20-26
(days)	Normal	29ns	23-35	32ns	28-36	23ns	19-32
Lodging	Triple-null	2.8	1.5-3.0	2.8	2.0-3.8	2.6	1.0-3.8
(score)	Normal	3.4ns	3.0-4.3	2.8ns	2.0-3.8	2.6ns	1.5-3.8
Height	Triple-null	95	82-107	91	81-109	96	75-116
(cm)	Normal	96ns	84-109	97*	76-111	100*	75-114
Seed weight	Triple-null	228	165-268	244	217-288	238	200-276
(mg sd $^{-1}$)	Normal	227ns	181-255	249*	208-279	225*	179-249
Protein	Triple-null	378	348-394	377	360-390	372	360-387
(g kg $^{-1}$)	Normal	381ns	365-397	378ns	353-396	371ns	360-383
Oil	Triple-null	167	158-178	166	152-176	166	155-174
(g kg $^{-1}$)	Normal	172*	166-176	172*	164-177	164*	156-168

* Significant difference between the mean performance of triple-null and normal lines at the 0.01 probability level.

[a] ns = No significant difference between the mean performance of triple-null and normal lines at the 0.05 probability level.

APPENDIX C

MEANS OF ENTRIES AT INDIVIDUAL LOCATIONS

Table 9. Mean performance of all entries for agronomic and seed traits grown at Ames.

Entry no.[a]	Seed yield [b]	Maturity [c]	Lodging [d]	Plant height [e]	Seed weight	Protein [f]	Oil [g]
	(kg ha^{-1})	(days)	(score)	(cm)	(mg sd^{-1})	(g kg^{-1})	(g kg^{-1})
612001	3442	31	1.5	107	213	372	165
612002	3782	31	2.5	103	246	391	162
612003	3249	23	2.5	98	268	390	161
612004	3579	23	2.5	84	248	394	164
612005	3260	28	2.5	84	225	383	162
612006	3095	30	2.5	92	241	382	166
612007	3234	26	2.8	83	235	393	168
612008	3525	30	2.8	98	213	374	165
612009	3522	28	2.8	89	222	387	168
612010	3367	31	2.8	82	237	380	165
612011	3336	32	2.8	85	249	383	169
612012	2998	33	2.8	92	257	384	166
612013	3287	34	2.8	84	252	389	158
612014	3588	24	2.8	96	248	381	168
612015	3363	23	2.8	91	242	389	170
612016	3455	29	2.8	97	219	348	167
612017	3308	28	2.8	105	218	370	159
612018	3585	27	2.8	100	245	366	169
612019	3269	23	3.0	103	229	377	160
612020	3546	31	3.0	96	165	369	170
612021	3316	28	3.0	92	197	373	163
612022	3256	33	3.0	106	199	374	170
612023	3617	32	3.0	99	223	379	169
612024	3374	26	3.0	91	217	364	178
612025	3261	29	3.0	98	217	374	174
612026	3653	33	3.0	95	218	384	173
612027	3231	30	3.0	107	221	375	174
612028	3436	32	3.0	98	235	385	168
612029	3478	31	3.0	106	233	375	168
612030	3700	25	3.0	92	255	386	174
612031	3482	23	3.0	90	221	391	174
612032	3268	28	3.0	94	237	397	167
612033	3741	31	3.3	92	242	377	169
612034	3371	26	3.3	101	235	377	170
612035	3336	30	3.3	84	239	387	166
612036	3671	32	3.3	97	224	380	170
612037	3570	33	3.3	96	226	392	166
612038	2404	31	3.3	89	188	382	169
612039	3398	31	3.3	100	237	376	176
612040	3342	31	3.5	102	229	378	174

Table 9. (Cont.)

Entry no.[a]	Seed yield [b]	Maturity [c]	Lodging [d]	Plant height [e]	Seed weight	Protein [f]	Oil [g]
	(kg ha^{-1})	(days)	(score)	(cm)	(mg sd^{-1})	(g kg^{-1})	(g kg^{-1})
612041	3700	23	3.5	89	235	378	176
612042	3507	30	3.5	101	230	382	176
612043	3357	30	3.5	101	231	377	175
612044	3317	24	3.5	92	224	380	173
612045	2937	33	3.5	91	181	379	176
612046	3595	32	3.5	109	206	367	174
612047	3327	30	3.5	105	220	388	168
612048	3702	24	3.5	89	218	365	173
612049	2672	34	3.8	96	210	370	171
612050	3324	28	3.8	94	247	388	171
612051	3355	25	3.8	96	229	385	173
612052	3382	26	3.8	85	229	380	175
612053	3312	35	4.0	104	219	376	171
612054	3144	23	4.3	96	250	389	169
612055	2970	20	4.0	75	209	390	170
612056	3597	23	3.3	88	202	410	167
612057	3726	29	2.8	91	233	391	172
612058	4237	29	2.0	101	150	375	175
612059	4418	30	2.8	84	259	386	166
612060	3885	25	3.0	82	224	381	171
SE [h]	194	1.3	0.3	4.9	10.5	0.6	0.7
LSD [i] $_{0.05}$	550	3.6	0.7	13.7	29.7	1.6	1.4
LSD [j] $_{0.01}$	734	4.7	1.0	18.3	39.7	2.1	1.9
CV [k] (%)	8.1	6.4	12.1	7.2	6.5	2.0	4.2

[a] Entries 1 -27 are triple-null lines, entries 28 - 54 are normal lines, and entries 55 - 60 are checks (IA1002, IA2010, IA2020, IA2022, IA3006, and A93-733016, respectively).

[b] Yield: expressed on a 13% moisture basis.

[c] Maturity: recorded as days after August 31 when 95% of pods reached their mature color.

[d] Lodging: scored on a scale of 1 (erect) to 5 (prostrate).

[e] Plant height: measured as the distance in centimeters from the soil surface to the terminal bud of the main stem.

[f] Protein: expressed on a 13% moisture basis.

[g] Oil: expressed on a 13% moisture basis.

[h] SE: standard error of the mean

[i] LSD: least significant difference at the 0.05 probability level.

[j] LSD: least significant difference at the 0.01 probability level.

[k] CV: coefficient of variation.

Table 10. Mean performance of all entries for agronomic and seed traits grown at Hubbard.

Entry no.[a]	Seed yield [b]	Maturity [c]	Lodging [d]	Plant height [e]	Seed weight	Protein [f]	Oil [g]
	(kg ha)	(days)	(score)	(cm)	(mg sd)	(g kg)	(g kg)
612001	2378	36	3.8	106	257	373	162
612002	3025	34	3.5	101	263	390	165
612003	2901	29	2.8	81	266	388	167
612004	2943	28	2.0	81	227	388	162
612005	2952	31	2.5	82	248	387	172
612006	2763	33	3.3	89	217	380	170
612007	2641	28	3.5	82	234	384	167
612008	2446	35	3.5	89	230	386	161
612009	2966	31	2.5	84	246	388	167
612010	2785	32	2.5	96	236	374	167
612011	2797	30	2.5	82	250	366	164
612012	2345	36	3.3	87	261	385	163
612013	2515	35	3.5	90	228	379	162
612014	3186	29	3.0	95	250	378	167
612015	2740	31	2.3	98	237	386	165
612016	2869	31	3.3	91	243	373	164
612017	2800	29	2.0	98	264	360	167
612018	2818	31	2.5	99	247	366	168
612019	2737	28	2.5	89	244	369	152
612020	2834	32	2.3	90	288	364	157
612021	3029	29	2.8	102	233	361	171
612022	2290	35	3.5	100	228	375	172
612023	2989	34	3.0	94	226	382	172
612024	2484	32	2.5	87	241	364	176
612025	2846	29	3.3	86	236	380	173
612026	2109	34	2.0	92	253	382	171
612027	2662	34	2.5	97	245	377	174
612028	2711	34	3.0	98	252	383	170
612029	2774	34	3.0	100	244	382	170
612030	3104	32	3.0	102	279	389	177
612031	2937	29	3.0	99	248	387	177
612032	2726	32	3.0	106	253	396	170
612033	2800	32	3.3	93	258	380	172
612034	2968	30	2.5	94	258	378	170
612035	2181	35	3.0	96	263	378	173
612036	2860	33	2.8	98	255	366	166
612037	2795	33	2.5	76	254	385	173
612038	2943	32	3.0	100	227	379	164
612039	2996	32	2.5	99	252	378	175
612040	2531	32	3.5	101	254	390	174

Table 10. (Cont.)

Entry no.[a]	Seed yield[b]	Maturity[c]	Lodging[d]	Plant height[e]	Seed weight	Protein[f]	Oil[g]
	(kg ha^{-1})	(days)	(score)	(cm)	(mg sd^{-1})	(g kg^{-1})	(g kg^{-1})
612041	3003	28	2.3	87	245	383	170
612042	2798	31	3.0	95	262	381	174
612043	2639	33	2.3	96	250	377	174
612044	2878	29	2.0	89	239	376	173
612045	2438	34	2.8	93	208	381	174
612046	2606	32	3.5	110	233	361	171
612047	2151	34	3.0	98	248	371	170
612048	2652	30	3.0	85	237	353	175
612049	2498	36	3.8	98	231	363	167
612050	2922	32	2.5	97	263	384	174
612051	3094	30	2.3	98	260	377	175
612052	2842	29	2.3	91	241	378	172
612053	2454	35	3.0	97	252	380	173
612054	2874	31	2.5	111	271	386	173
612055	3233	22	3.0	72	226	387	172
612056	3237	28	4.0	86	238	406	167
612057	3453	29	2.3	97	239	386	173
612058	3593	30	2.3	91	163	367	177
612059	3258	33	2.5	78	286	388	166
612060	3305	27	3.0	85	244	377	176
SE[h]	156	0.8	0.4	5.8	5.2	0.3	0.5
LSD[i]$_{0.05}$	440	2.3	1.0	16.3	14.6	0.4	1.3
LSD[j]$_{0.01}$	587	3.0	1.4	21.7	19.5	1.0	1.7
CV[k] (%)	8.0	3.6	18.3	8.7	3.0	1.0	3.9

[a] Entries 1 -27 are triple-null lines, entries 28 - 54 are normal lines, and entries 55 - 60 are checks (IA1002, IA2010, IA2020, IA2022, IA3006, and A93-733016, respectively).

[b] Yield: expressed on a 13% moisture basis.

[c] Maturity: recorded as days after August 31 when 95% of pods reached their mature color.

[d] Lodging: scored on a scale of 1 (erect) to 5 (prostrate).

[e] Plant height: measured as the distance in centimeters from the soil surface to the terminal bud of the main stem.

[f] Protein: expressed on a 13% moisture basis.

[g] Oil: expressed on a 13% moisture basis.

[h] SE: standard error of the mean

[i] LSD: least significant difference at the 0.05 probability level.

[j] LSD: least significant difference at the 0.01 probability level.

[k] CV: coefficient of variation.

Table 11. Mean performance of all entries for agronomic and seed traits grown at Grand Junction.

Entry no.[a]	Seed yield[b]	Maturity[c]	Lodging[d]	Plant height[e]	Seed weight	Protein[f]	Oil[g]
	(kg ha)	(days)	(score)	(cm)	(mg sd)	(g kg)	(g kg)
612001	3055	26	3.8	110	254	380	164
612002	3617	23	3.8	96	262	377	162
612003	2593	21	2.8	88	266	387	166
612004	3007	20	3.3	77	238	367	163
612005	2796	23	3.5	75	245	375	168
612006	2934	23	2.5	88	230	366	170
612007	3326	22	2.8	77	231	365	167
612008	3084	23	2.8	98	235	380	174
612009	2876	23	1.3	81	235	381	169
612010	2863	23	2.5	92	276	373	171
612011	3301	25	2.0	91	250	360	169
612012	3203	23	1.0	97	256	371	155
612013	2753	23	2.5	90	248	366	167
612014	2835	21	2.5	90	243	381	165
612015	3109	21	1.3	87	200	363	166
612016	3071	23	3.0	106	241	381	172
612017	3109	23	3.3	108	254	365	164
612018	3093	23	1.3	100	262	376	164
612019	2887	20	3.3	96	260	364	167
612020	3158	23	3.5	101	251	370	166
612021	3477	23	2.0	103	211	380	164
612022	3397	25	3.0	116	202	366	162
612023	3192	24	3.5	103	208	373	164
612024	3241	23	2.5	100	222	367	170
612025	2839	23	2.8	108	203	375	166
612026	3282	24	2.5	100	226	374	165
612027	2890	23	1.3	107	221	367	165
612028	2756	24	3.5	110	224	375	164
612029	3357	23	2.5	107	237	369	165
612030	3486	23	2.0	101	243	371	168
612031	2907	21	3.0	98	227	361	166
612032	3084	23	2.5	102	179	383	164
612033	2958	23	1.8	100	225	372	166
612034	3126	22	3.5	103	244	379	161
612035	3084	23	3.3	82	245	370	160
612036	3000	23	2.0	114	239	372	157
612037	3361	23	3.8	98	230	365	156
612038	2839	23	3.5	88	219	372	167
612039	3542	23	2.0	99	236	372	166
612040	3094	23	3.0	105	233	374	165

Table 11. (Cont.)

Entry no.[a]	Seed yield [b]	Maturity [c]	Lodging [d]	Plant height [e]	Seed weight	Protein [f]	Oil [g]
	(kg ha^{-1})	(days)	(score)	(cm)	(mg sd^{-1})	(g kg^{-1})	(g kg^{-1})
612041	3494	19	3.0	75	230	360	165
612042	2838	23	2.0	112	220	371	161
612043	3001	23	2.8	106	225	378	168
612044	2980	23	2.3	77	221	374	166
612045	3119	23	1.5	106	181	368	164
612046	3397	24	3.3	108	200	378	163
612047	3182	23	3.3	109	216	374	166
612048	2744	21	2.3	85	200	374	166
612049	2591	32	2.8	105	224	370	161
612050	3243	23	2.0	99	249	368	167
612051	3160	22	1.8	99	246	369	162
612052	3421	24	3.0	97	222	376	166
612053	2465	23	2.5	111	238	367	166
612054	3356	23	2.3	109	230	362	161
612055	3402	16	1.5	84	212	361	155
612056	3294	18	2.0	85	230	380	158
612057	3399	20	2.0	89	222	372	169
612058	3183	23	1.8	94	193	349	162
612059	3204	23	1.8	82	256	373	160
612060	2840	21	1.5	86	248	371	165
SE [h]	202	0.6	0.4	5.1	12.0	0.6	0.3
LSD [i] $_{0.05}$	570	1.7	1.0	14.5	33.9	1.6	0.8
LSD [j] $_{0.01}$	762	2.3	1.4	19.3	45.2	2.1	1.1
CV [k] (%)	9.3	3.7	20.1	7.4	7.3	2.1	2.5

[a] Entries 1 -27 are triple-null lines, entries 28 - 54 are normal lines, and entries 55 - 60 are checks (IA1002, IA2010, IA2020, IA2022, IA3006, and A93-733016, respectively).

[b] Yield: expressed on a 13% moisture basis.

[c] Maturity: recorded as days after August 31 when 95% of pods reached their mature color.

[d] Lodging: scored on a scale of 1 (erect) to 5 (prostrate).

[e] Plant height: measured as the distance in centimeters from the soil surface to the terminal bud of the main stem.

[f] Protein: expressed on a 13% moisture basis.

[g] Oil: expressed on a 13% moisture basis.

[h] SE: standard error of the mean

[i] LSD: least significant difference at the 0.05 probability level.

[j] LSD: least significant difference at the 0.01 probability level.

[k] CV: coefficient of variation.

APPENDIX D

MEAN FATTY ACID COMPOSITION OF ENTRIES ACROSS LOCATIONS

Table 12. Mean fatty acid composition of all entries across locations.

Entry no.a	Fatty acid [a]					
	16:0	18:0	18:1	18:2	18:3	Sat
	g kg -1					
612001	100	80	201	528	91	180
612002	99	62	232	526	81	161
612003	96	60	265	500	79	157
612004	98	49	261	517	75	147
612005	99	70	213	535	83	169
612006	99	80	209	529	84	179
612007	99	58	228	533	82	157
612008	98	44	255	530	74	141
612009	105	42	266	515	72	147
612010	99	79	208	532	82	178
612011	85	151	150	514	100	237
612012	101	40	240	538	81	141
612013	109	44	216	544	88	153
612014	104	44	255	521	76	149
612015	92	43	254	532	79	135
612016	98	75	244	502	81	173
612017	90	138	180	499	94	227
612018	99	75	225	519	83	174
612019	97	86	216	516	86	183
612020	80	174	161	492	94	254
612021	91	172	151	488	98	263
612022	98	94	209	514	85	192
612023	102	40	237	539	82	142
612024	105	52	245	516	83	157
612025	97	50	273	502	78	147
612026	97	38	240	544	80	135
612027	95	86	257	478	93	143
612028	101	42	244	530	84	142
612029	101	39	240	536	85	140
612030	98	56	250	522	75	154
612031	95	65	257	505	78	160
612032	96	43	259	526	76	139
612033	100	63	231	526	81	163
612034	93	86	203	533	86	179
612035	81	175	155	501	89	256
612036	94	150	152	510	95	243
612037	94	118	192	510	87	212

Table 12. (Cont.)

Entry no.[a]	Fatty acid [b]					
	16:0	18:0	18:1	18:2	18:3	Sat
	g kg -1					
612038	92	113	171	533	91	205
612039	104	40	243	536	76	145
612040	102	41	261	523	74	143
612041	104	45	254	521	77	149
612042	97	46	247	534	77	143
612043	95	79	236	510	81	173
612044	91	152	170	494	94	243
612045	98	54	269	517	62	152
612046	102	88	194	524	93	190
612047	93	132	178	508	89	225
612048	90	143	189	494	85	232
612049	89	119	177	522	93	208
612050	106	43	256	522	74	148
612051	104	42	260	517	78	146
612052	102	42	250	528	79	144
612053	100	90	201	526	84	189
612054	97	95	210	516	83	191
612055	104	68	228	518	83	172
612056	102	46	234	536	82	149
612057	97	55	240	532	76	152
612058	104	43	244	530	79	147
612059	106	38	250	521	86	144
612060	103	37	241	538	81	141

[a] Entries 1 -27 are triple-null lines, entries 28 - 54 are normal lines, and entries 55 - 60 are checks (IA1002, IA2010, IA2020, IA2022, IA3006, and A93-733016, respectively).

[b] = 16:0 palmitic, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, and 18:3 = linolenic acid.

REFERENCES

Axelrod, B., T.M. Cheesborough, and S. Laasko. 1981. Lipoxygenase from soybean. Methods Enzym. 71:441-51.

Davies, C.S, and S.S. Nielsen. 1986. Genetic analysis of a null-allele for lipoxygenase-2 in soybean. Crop Sci. 26:460-462.

Davies, C.S., N.C. Nielsen, and S.S. Nielsen. 1987. Flavor improvement of soybean preparations by genetic removal of lipoxygenase-2. J. Am. Oil. Chem. Soc. 64(10):1428-1433.

Eskin, N.A., S. Grossman, and A. Pinsky. 1977. Biochemistry of lipoxygenase in relation to food quality. Crit. Rev. Food Sci. Nutri. 9(1):1-33.

Hajika, M., K. Igita, and K. Kitamura. 1991. A line lacking all three seed lipoxygenase isozymes in soybean induced by gamma-ray irradiation. Jpn. J. Breeding. 41:507-509.

Hammond, E.G. 1985. Stability of soybean oil to oxidation. p. 251-258. *In* R. Shibles (ed.) World soybean research conference III: Proceedings. Westview Press, London.

Hammond, E.G. 1991. Organization of rapid analysis of lipids in many individual plants. *In* H.F. Linskens, and J.F. Jackson (ed.) Modern methods of plant analysis. Volume 12: Essential oils and waxes. p. 321-330. Jackson, Springer-Verlag, New York.

Hildebrand, D.F., and T. Hymowitz. 1981. Soybeans lacking lipoxygenase-1. J. Am. Oil Chem. Soc. 58:583-586.

Hildebrand, D.F., and T. Hymowitz. 1982. Inheritance of lipoxygenase-1 activity in soybean seeds. Crop Sci. 22:851-853.

Hildebrand, D.F., and M. Kito. 1984. Role of lipoxygenase in soybean protein quality. J. Agric. Food Chem. 32:815-819.

Hildebrand, D.F. 1989. Lipoxygenases. Physiol. Plant. 76:249-253.

Hildebrand, D.F., R.T. Versluys, and G.B. Collins. Changes in lipoxygenase isozyme levels during embryo development. Plant Science. 75:1-8.

Hildebrand, D.F. 1996. Genetics of soybean lipoxygenases. p. 33-38. *In* G. Piazza (ed.) Lipoxygenase and lipoxygenase pathway enzymes. American Oil and Chemical Society Press, Champaign, IL.

Kitamura, K., C.S. Davies, N. Kaizuma, and N.C. Nielsen. 1983. Genetic analysis of a null-allele for lipoxygenase-3 in soybean seeds. Crop Sci. 58:583-586.

Kitamura, K., T. Kumangi, and A. Kikuchi. 1985. Inheritance of lipoxygenase-2 and genetic relationship among genes for lipoxygenase-1, -2, and -3 isozymes in soybean seeds. Jpn. J. Breeding. 35: 413-420.

Kitamura, K., A. Kikuchi, and K. Harada. 1987. Performance of near-isogenic lines lacking seed lipoxygenases. Soybean Genet. Newsl. 14:109-112.

Kitamura, K. 1991. Spontaneous and induced mutations of seed storage proteins in soybean (*Glycine max* (L.) Merr.). Gamma Field Symposia. 30: 61-69.

Kitamura, K. 1995. Genetic improvement of nutritional food processing quality in soybean. Japenese Agriculture Review Quarterly. 29:1-8.

Mattews, R.H. 1987. Legumes: Chemistry, technology and human nutrition. p. 99-156. Macel Dekker, Inc. New York, NY.

Pfeiffer, T.W., D.F. Hildebrand, and D.M. TeKrony. 1992. Agronomic peformance of soybean lipoxygenase isolines. Crop Sci. 32:357-362.

Rackis, J.J., D.J. Sessa, and D.H., Honig. 1979. Flavor problems of vegetable food proteins. J. Am. Oil Chem. Soc. 56:262-271.

Sedcole, J.R. 1977. Number of plants necessary to recover a trait. Crop Sci. 17:667-668.

Siedow, J.W. 1991. Plant lipoxygenase: structure and function. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42: 145-188.

Suda, I., M. Hajiki, Y. Nishibi, S. Fururta, and I. Kazunori. 1995. Simple and rapid method for the selective detection of individual lipoxygenase isozymes in soybean seeds. J. Agric. Food Chem. 43:742-747.

Toyosaki, T. 1996. Mechanism of methylene blue bleaching by lipoxygenase. J. Sci. Food Agric. 70:75-78.

Wang, H.W., T. Takano, D. Shibata, K. Kitamura, and G. Takeda. 1994. Molecular basis of a null mutation in soybean lipoxygenase 2: substitution of a glutamine for an iron-ligand histidine. Proc. Natl. Acad. Sci. USA. 91:5828-5832.

Wang, H.W., T. Kato, T. Takano, D. Shibata, K. Kitamura, and G. Takeda. 1995. Two single-base substitutions involved in altering in a paired-base box of AAATAC in the promoter region of lipoxygenase L-3 gene impair the function in tobacco cells. Plant Science. 109:67-73.

Wilcox, J.R. 1984. Breeding soybeans for improved oil quantity and quality. p. 380-386. *In* R. Shibles (ed.) World soybean research conference III: Proceedings. Westview Press, London.

Wilson, L.A. 1996. Comparison of lipoxygenase-null and lipoxygenase containing soybeans for foods. p. 209-222. *In* G. Piazza (ed.) Lipoxygenase and lipoxygenase pathway enzymes. American Oil and Chemical Society Press, Champaign, IL.

Wolf, W.J. 1975. Lipoxygenase and flavor of soybean protein products. J. Agric. Food Chem. 23(2):136-140.

Young, S.L. 1985. Soy protein in processed meats and dairy foods. p. 182-190. *In* R. Shibles (ed.) World soybean research conference III: Proceedings. Westview Press, London.

Zhaung, H., D.F. Hildebrand, R.A. Andersen, and T.R. Hamilton-Kemp. 1991. Effects of polyunsaturated free fatty acids and esterified linoleoyl derivatives on oxygen consumption and C_6 aldehyde formation with soybean seed homogenates. J. Agric. Food Chem. 39(8):1357-1364.

ACKNOWLEDGMENTS

I would like to thank Dr. Walter Fehr and all members of the Soybean Breeding Project for providing a learning environment that exceeded my expectations. I would like to extend my appreciation to my family for their unbridled support during all my endeavors.