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Breeding low palmitic low linolenic soybeans

Carolyn Meyer Cherrak

To the Graduate Council:

I am submitting herewith a thesis written by Carolyn Meyer Cherrak entitled "Breeding low palmitic low linolenic soybeans." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant, Soil and Environmental Sciences.

Vincent R. Pantalone, Major Professor

We have read this thesis and recommend its acceptance:

Dennis R. West, Sharon L. Melton, John R. Mount

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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John R. Mount, Professor Food Science and Technology

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Interim Vice Provost and
Dean of The Graduate School

BREEDING LOW PALMITIC LOW LINOLENIC SOYBEANS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Carolyn Meyer Cherrak
May 2001

DEDICATION

This thesis I dedicate

To my husband

Djamel Eddine Cherrak

who provides me with unlimited love, support and encouragement

To my parents

Dennis William Meyer and Martha Sue Braddock Meyer

who have dedicated their lives in raising and caring for me

To my sister

Susan Denise Meyer Peterson

who has pushed me to be more courageous

And to my daughter

Miriam Leila Cherrak

who was born during my study for this degree and fills me with a great source of inspiration

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ABSTRACT

Commercial soybean oil currently does not meet the demands of health conscious consumers because it includes 10-12% palmitic acid, a saturated fatty acid and forms trans fatty acids upon hydrogenation. Linolenic acid has less oxidative stability and is responsible for the development of undesirable flavors. Therefore, a cross was made between N97-3708-13, a low palmitic, low linolenic line and Anand, a high yielding cultivar. The goal of the cross was to produce a population between a normal soybean cultivar and a low palmitic, low linolenic fatty acid soybean line. Moreover, to estimate heritabilities of fatty acid traits and to correlate fatty acid and agronomic traits in order to guide breeders who wish to improve soybean oil for human consumption. From the progeny, two-hundred F_2 single plants were grown in a honeycomb design during the first year (1999) and in the second year (2000) $F_{2:4}$ two row plots were grown in a randomized block design with three replications of 128 genotypes. In addition, a population of F_4 single plants were grown in $F_{2:4}$ rows. Fatty acid composition was determined by gas chromatography. Some high yielding progeny were developed, with the highest ranking at 4116.5 kg ha $^{-1}$. Some modified fatty acid lines were developed including the lowest at 4.2% and 4.3% of palmitic and linolenic respectively. Smaller seed size was correlated with lower palmitic acid. Palmitic acid and linolenic acid had high heritabilities of 0.65 and 0.73 respectively. Breeders utilizing low palmitic, low linolenic germplasm in crosses with normal elite lines can select F_2 individuals with the lowest levels of these traits with

expectation of recovering pure line progenies that retain these modified fatty acid traits.

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1. GENERAL INTRODUCTION

The seed of soybean [Glycine max (L.) Merr.] is a composed of approximately 40% protein, 20% lipids, 35% carbohydrates which includes cellulose, hemicellulose, and sugars on a dry weight basis (Hui, 1996). Soybeans provide humans with a multitude of uses including pharmaceuticals, nueutraceuticals, industrial products, food products, and animal feed.

From a nutritious aspect, many food products such as tofu, soymilk, tempeh, flour, concentrates, isolates, textured protein, and oil can be derived from soybean (Liu, 1997). Soybean accounts for about 30% of the world's market for vegetable oil (Carter et al., 1991). In the United States 84% of fat in margarine and 65% of fat in shortenings is soybean oil (Dupont et al., 1991).

The average fatty acid composition of commercial soybean oil is 11% palmitic, 4% stearic, 24% oleic, 54% linoleic and 7% linolenic (Hui, 1996). The naming of these fatty acids can be represented by an abbreviation, systematic name, common name or symbol (table 1).

The first number of the abbreviation represents the number of carbons found in the compound and the second number tells how many double bonds are in the compound's structure. For example, 16:0 means there are 16 carbons in the fatty acid chain and no double bonds, however, 18:1 has 18 carbons and one double bond. The letter n, means omega and this shows the position of

Table 1. Nomenclature of the common fatty acids found in soybean.

Abbreviation	Systematic name	Common name	Symbol
16:0	Hexadecanoic	Palmitic	P
18:0	Octadecanoic	Stearic	St
18:1 (n-9)	9-Octadecenoic	Oleic	O
18:2 (n-6)	9,12-Octadecadienoic	Linoleic	L
18:3 (n-3)	9,12,15-Octadecatrienoic	Linolenic	Ln

Adapted from Fennema (1996)

the double bond when counting the carbons starting from the methyl end (CH_3) of the fatty acid chain (table 2).

Oleic, 18:1 (n-9), means the double bond is located on the ninth carbon from the methyl end linoleic, 18:2 (n-6), means the first double bond is on the sixth carbon. In the systematic name *anoic* means no double bonds are present as in the case of 16:0 hexadecanoic and 18:0 octadecanoic. When *enoic* is found in the systematic name at least one double bond is present. When a number is at the beginning of the systematic name, the position of the double bond is known by counting the carbons from the carboxyl end (COOH). For example, the chemical structure of the 18:2 fatty acid 9,12-Octadecadienoic in table 2 shows that the two double bonds are found at the ninth and twelfth carbons (Fennema, 1996).

Fatty acids can be referred to as saturated or unsaturated fats. A saturated fat such as palmitic (16:0) and stearic (18:0) have no double bonds and an unsaturated fat such as oleic (18:1), linoleic (18:2) and linolenic (18:3) have double bonds. The more double bonds found in the fatty acid chain the more unsaturated the fat. Oleic (18:1) is considered a monounsaturated fatty acid, and 18:2 and 18:3 are considered polyunsaturated fatty acids. Linolenic (18:3) is the most unsaturated fat as it contains three double bonds in its structure.

The saturated fatty acid, palmitic acid is a major health concern as it has been correlated to cardiovascular disease. The National Cholesterol Education Program conducted 30 years of research with the results indicating that saturated

Table 2. The chemical structure of five natural fatty acids found in soybeans.

Fatty Acid	Chemical Structure Methyl (omega) end — Carboxyl end
Palmitic	16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Stearic	18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Oleic	18 17 16 15 14 13 12 11 10 **9** 8 7 6 5 4 3 2 1 $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH=CHCH_2CH_2CH_2CH_2CH_2CH_2\ CH_2COOH$ 1 2 3 4 5 6 7 8 **9** 10 11 12 13 14 15 16 17 18
Linoleic	18 17 16 15 14 13 **12** 11 10 **9** 8 7 6 5 4 3 2 1 $CH_3CH_2CH_2CH_2CH_2CH=CHCH_2CH=CHCH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ 1 2 3 4 5 **6** 7 8 9 10 11 12 13 14 15 16 17 18
Linolenic	18 17 16 **15** 14 13 **12** 11 10 **9** 8 7 6 5 4 3 2 1 $CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ 1 2 **3** 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Adapted from Fennema (1996)

fatty acids such as palmitic acid are the most potent dietary component responsible for elevating plasma cholesterol. Dupont et al. (1991) suggested keeping saturated fatty acids in the human daily diet below 10% of total calories. In addition the FDA allows labeling low saturated foods with less than 7% of saturated fat per serving size (pers. comm. Pantalone, 2000).

Health conscious consumers currently favor a lower saturated fat diet. Nevertheless, saturated fat is an important component in the oxidative stability and nutritional quality of lipid containing foods (Hildebrand, 1992). This creates a challenge for the food processing industry to find efficient ways to reduce saturated fats in food products.

Linolenic acid, is an essential unsaturated fatty acid; however, too much in soybean oil is responsible for the development of an off-flavor problem known as flavor reversion (Fennema, 1996). This is caused by autoxidation, a catalytic process leading to free radical chain reactions resulting in polymerization of undesirable odors, and flavors and oxidative products such as hydroperoxides and cyclic peroxides (Hiroaki et al., 1995 and Crapiste et al., 1999).

Hydrogenation is a standard technique used to saturate the double bonds of the linolenic acid with hydrogen. This is done in order to discourage autoxidation and to promote oxidative stability (Dupont et al., 1991). Hydrogenation is actively used today for products such as soybean oil. Although this process enables the food industry to convert liquid oils into semisolid fats used for shortenings and margarine and improves the oxidative stability, there is some concern about possible health effects of the formation of by-products.

During hydrogenation or partial hydrogenation some of the double bonds may be relocated and/or transformed from the usual *cis* to the *trans* configuration. This can form trans fatty acids during hydrogenation or a complex mixture of reactive products in partial hydrogenation. These trans fatty acids constitute 20-40% of some margarines and shortenings (Fennema, 1996).

The safety of these trans fatty acids is controversial because their physiological properties, metabolism and long-term effects on health are not well understood (Fennema, 1996). Carter et al. (1998) noted that hydrogenation of soybean oil results in trans fatty acids, which may not be beneficial to health. Shen et al. (1997) report that trans fatty acids increase total and low-density lipoprotein cholesterol levels and decrease high-density (good) lipoprotein cholesterol. Moreover, 80% of trans fatty acids of the human diet are from hydrogenated vegetable oil with the average consumption of a person being 6.5g/day to12g/day.

Li et al. (1999) reported that the trans content of edible oils might be regulated soon as it has been associated with arteriosclerosis and heart disease. Indeed the Food and Drug Administration (FDA) is currently looking at possible health effects of trans fatty acids, and is considering requiring labeling the content of trans fatty acids in food products. Mandatory trans labeling may come in 2002 (Anon., 2000). In order to be "trans fat free" the food must contain less than 0.5 g of trans fat and less than 0.5 g of saturated fat per serving, which rounds down to zero. Moreover, a food can not be labeled as low in saturated fat, low in cholesterol, lean, or extra lean unless there is less than 0.5 g of trans

fat and less than 1.0 g of saturated fat per serving. Also, a nutrition claim such as calcium-rich cannot be stated if more than 4 g of combined saturated and trans fat are present in a serving of the food product such as milk. But under these conditions, a nutrient claim such as low sodium could exist if attention was brought to the amount of saturated fat such as butter or margarine. The FDA is discussing a proposal to include trans fat with saturated fat in the Nutrition Facts panel on packaged foods (Anon., 2001).

Alternative methods to hydrogenation are being investigated such as interesterification in attempts to produce healthier soybean oil. Interesterification is a process involving the rearrangement of fatty acids randomly among the triacylglycerol molecules of the fat (Fennema, 1996). This process improves the consistency of fats. Kok et al. (1999) looked at interesterifying a highly saturated soybean oil to produce a trans-free margarine. Recently, plant breeders have targeted the development of a low palmitic, low linolenic soybean. The low palmitic trait will promote healthier, less saturated oil, and the low linolenic trait will improve the oxidative stability of the oil and may enable the production of a lower trans-fatty acid vegetable oil (Carter et al., 1998).

Dare is a standard soybean cultivar whereas N97-3708-13 is a modified fatty acid line, bred for superior agronomic performance. The N97-3708-13 soybean line was developed with low palmitic acid (4%) and low linolenic acid (3.4%), a combination of traits, which has never existed until now. Since this is a new combination of traits, it is important to document how these characters influence other important soybean traits. The purpose of this thesis research

study is i) to determine heritability estimates for palmitic and linolenic acids and ii) to determine correlations between these two fatty acids and yield, protein, and oil concentration. This information will provide breeders with the knowledge necessary to combine desirable traits and meet the processor goals outlined in page 16.

Soybean germplasm, N97-3708-13, a sister line to Satelite has been developed with low palmitic and low linolenic fatty acids. Table 3 compares a commercial soybean cultivar, "Dare", to N97-3708-13.

Table 3. Fatty acid composition of selected germplasm.

Germplasm	Fatty Acid (%)				
	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Dare[a]	12.3	2.9	21.3	56.1	7.4
N97-3708-13[b]	4.0	3.3	20.7	68.6	3.4

[a] Standard soybean (Wilson, 1986)
[b] Developed at USDA–ARS in Raleigh, NC, by Dr. J. W. Burton.

2. LITERATURE REVIEW

2.1 FOOD PROCESSING QUALITY

Gas chromatography is a common method used for the analysis of fatty acid composition in seed oils (Hammond, 1991). The preparation method he described involves first crushing the seed and extracting part of the lipid with hexane. In unpublished research conducted with W.R. Fehr, they found that oil obtained from finely ground seed and crushed seed, when compared, showed that the fatty acid composition of partly extracted seeds was representative of the whole oil. They stated that the extraction time can be long and suggested that the seed be left overnight with no problems with the analysis. They described that soybean oil may be trans-esterfied to methyl esters by different methods. Sodium methoxide solutions are said to be efficient and inexpensive catalysts but are very sensitive to moisture or free fatty acids in the sample. Methoanolic solutions of boron trifluoride, hydrogen chloride, or sulfuric acid can esterify free fatty acids and are not as sensitive to moisture. Hammond (1991) stated that the gas chromatographic separation can be done using packed or capillary columns but leaned toward the rapid analyses and superior separations achieved with capillary columns with sophisticated injection systems. The best stationary phases for these analyses at the time of this article was 50% cyanopropyl-50% cyanomethylpolysiloxane phases. The advantages include temperature stability and polarity, which allows a good separation of the unsaturated fatty acids. The author also suggested that isothermal operation is possible but temperature programming may be desirable. The use of an automatic sampler can perform

hundreds of injections with good reproducibility and allows a full use of the instrument.

In addition to the method described by Hammond (1991), the American Oil Chemist Society (AOCS) Official Methods handbook describes the Ce 2-66 Official Method, Preparation of Methyl Esters of Fatty Acids. The definition states that the method provides a means for preparing methyl esters of long-chain fatty acids for further analysis by Gas Liquid Chromatography, as in methods such as C3 1d-91 (n-3 and n-6 fatty acids by capillary Gas Chromatography). The scope states that the method is applicable to common fats, oils and fatty acids. The procedure involves adding 0.5N methanolic sodium hydroxide to the oil sample then boiling; next add BF_3 and shake before adding heptane with a small amount of anhydrous sodium sulfate, or the oil sample can be centrifuged before adding heptane and methanolic KOH, shaking centrifuging and injecting the upper layer.

Since normal soybean oil contains about 11% palmitic and 7% linolenic acid there has been much research done to reduce these fatty acids of soybean. Part of the science of altering soybean fatty acids requires testing to see how well the oil produced responds in food processing. Testing involves looking at the oil stability when exposed to high heat during frying as well as the effects on flavor, odor, and texture.

Mounts et al. (1988) conducted an experiment comparing three soybean oils with altered linolenic acid concentrations of 3.3%, 4.2% and 4.8% and a normal soybean cultivar with 7.7% linolenic oil. Soybean oil stability was measured via peroxide values. Peroxides are the main initial products of

autoxidation and are measured as a way of interpreting lipid oxidation (Fennema, 1996). After storage at 60°C and 190°C, no significant differences were observed in flavor stability. The normal linolenic commercial oils exhibited 'fishy' odors whereas the low linolenic oils did not have undesirable odors.

Liu and White (1992) studied high-temperature stability of soybean oils with altered fatty acid compositions. When the linolenic acid was decreased, there was an improvement in both stability and flavor quality.

Hui-Rong et al. (1992) compared canola oil [*Brassica napus* and *Brassica rapa* (*campestris*)] to six different soybean oils each varying in fatty acid compositions developed from plant breeding. Canola typically has a fatty acid profile of 3.5% palmitic, 1.5% stearic, 60.1% oleic, 20.1% linoleic, and 9.6% linolenic (Hui, 1996). It was found during deep fat frying that all the soybean oils had a common flavor, odor and texture. The normal soybean oil was not stable during heating and frying and exhibited a fishy odor. Hydrogenated soybean oil was found to have low flavor quality but improved stability. The unhydrogenated soybean oil had better flavor. The low linolenic varieties [A17 (1.5%), A16 (1.9%), A87-191039 (1.8%)] were more stable than the commercial cultivars Hardin and BSR101 when compared to the canola oil.

Warner and Mounts (1994) compared 6.2% standard linolenic oil and 3.7% low linolenic oil with 0.4% modified hydrogenated linolenic soybean oil. It was found that after heating, the modified soybean oil did not exhibit the fishy odor normally found in standard soybean oil. Also, with the low linolenic oil, free

fatty acids, polar components and foam heights were significantly less with significantly better flavor quality.

Mounts et al. (1994) looked at soybeans created by Hammond and Fehr by induced mutation breeding and hybridization. They compared three low linolenic lines of 1.7%, 1.9% and 2.5% to the commercial cultivar, Hardin with 6.5% linolenic acid. The low linolenic oils had improved flavor stability in accelerated storage tests. In addition, flavor quality of potatoes fried in the low linolenic oils was significantly better than when fried in standard soybean oil. There were only 0.3% of free fatty acids after 15 hours of frying showing no significant difference in the total volatile content. The low linolenic oils were particularly beneficial for improved oil quality during cooking and frying. Also, the flavor quality was enhanced, lacking the common fishy odor found in foods fried in normal soybean oil.

In another study by Mounts et al. (1994) three low linolenic soybean lines N83-375 (5.5%), N89-2009 (2.9%) and N85-2176 (1.9%) were compared to two hydrogenated soybean oils (HSBO). They found that the low linolenic oils had significantly lower odor intensity scores when compared to the HSBO. The very low linolenic line N85-2176 had significantly better flavor quality. Mounts et al. (1994) indicated that oils with low linolenic produced by plant breeders are potential alternatives to hydrogenated frying oils. Naturally low linolenic soybean oils have yet to be fully tested for their attributes.

Melton et al. (1994) studied the stability of fried food flavor (oil physical properties, volatile and nonvolatile decomposition products). They analyzed

degradation through free fatty acids (FFA), food oil sensor (FOS), and total polar compounds (TPC) of two soybean oils [soybean A with an iodine value (IV) of 94 and soybean B with an IV of 112] and one palm olein. The iodine value is the number of grams of iodine that will react with the double bonds in 100g fat where the average iodine value of unhydrogenated soybean oil is 125-135 (Liu, 1997). French fries were fried over a four-day period. The FFA, FOS and TPC increased significantly in all oils with TPC and FFA higher in the palm olein. Flavor of french fries was more desirable in soybean oil as compared to one day of palm olein. More research is needed to determine when to discard specific frying oils.

Warner and Knowlton (1997) looked at frying quality and oxidative stability of high-oleic corn oils. The genetically modified high oleic oil had lower total polar compounds than normal corn oil and sunflower oil. In addition, it had good flavor, quality and stability after 60 days of storage and performed well in high-temperature frying. The high oleic sunflower, safflower and canola oil improved frying stability.

Shen et al. (1997) analyzed the oxidative stability of soybean oils that contained high plamitic and low linolenic fatty acids. Analyses of the peroxide values revealed an increase in oxidative stability for the modified oil compared to normal soybean oil. In addition, solidification temperatures were higher than normal. This is noteworthy because it has been previously shown that soybean oil is less stable at high temperatures than oils with saturated fat. Also, oxidative stability can be improved by changing fatty acid composition. Plant breeding can

be used to decrease the polyunsaturated fatty acids such as linolenic acid and increase the content of saturated fatty acids such as palmitic, which can enhance the stability of the soybean oil. This does not involve the process of hydrogenation that leads to the undesirable production of trans fatty acids. However, elevation of saturated fatty acids could themselves impose health risks. Therefore, soybean oils bred for high palmitic and low linolenic fatty acids may have greater oxidative stabilities than traditional soybean oils. It remains to be determined how low palmitic, low linolenic soybean will perform for oxidative stability and other oil processing characteristics.

2.2 BREEDING LOW PALMITIC , LOW LINOLENIC SOYBEANS

To support the food processing industry, soybean breeders are trying to develop desirable, healthy soybean oil with altered fatty acid composition. American soybean processors have developed the breeding goals outlined in table 4.

These goals stem from two main factors: one being the demand from the food industry to make a lower saturated fat soybean oil without trans-isomers, the other being that naturally low linolenic oils have improved oxidative stability and flavor characteristics, which are superior to hydrogenated soybean oil (Carter et al., 1998). The development of a new cultivar may meet the demands of the industry.

Table 4. Breeding goals of soybean expressed as concentration of protein, oil, and fatty acids (palmitic, oleic, and linolenic).

Composition	Percentage
Protein	> 43
Oil	20 to 22
Fatty acids	
low palmitic	< 4
high oleic	30 to 60
low linolenic	< 4

(Carter et al., 1998)

2.3 HERITABILITY

Soybeans producing the combination of low linolenic and low palmitic fatty acids have never existed before. Therefore, much research is needed on this type of soybean. Heritability estimations are important measures, which guide plant breeders in developing populations for genetic improvement. The exact meaning of the term heritability may be confusing as there is not just one definition. Jacquard (1983) states there are three definitions of heritability and it is important to distinguish among them to avoid misinterpretations. Bell (1977) described these three definitions as three stages in the evolution of the word heritability. Beginning in 1832 or before, heritability was used to denote the capability of inheriting characteristics. Around the end of the 1800's, the word was seen interpreted as environmental fluctuations distinct from genotypic differences. Then starting in 1936, heritability was used to express a ratio of additive genetic variance to the total phenotypic variance within a population. Hanson (1963) has generalized the meaning of heritability as one of the number of ways of gathering statistics to obtain genetic information. He argued that the definition of heritability should be flexible in order to include both the animal and plant geneticists' approach. The modes of reproduction differ between plant and animals. He defined heritability in terms of what he called the selection unit. He stated that plant geneticists consider the concepts of heritability and selection advance as complementary. Lush has defined heritability as the fraction of the observed variance, which was caused by the difference in heredity (Nyquist, 1991).

Heritability helps plant breeders find answers to questions regarding genetic variability, testing genetic populations, breeding procedures, cultivar and trait manipulations (Burton, 1987). Plant breeders more specifically look at the concept of heritability as the relative importance of genetic and nongenetic factors in the expression of phenotypic differences among genotypes in a population (Fehr, 1987). Heritability may also be described as the proportion of the observed variation in a progeny that is inherited (Poehlman et al., 1995). Selection is more effective when genetic variation in relation to environmental variation is high than when it is low (Poehlman et al, 1995).

Most heritability estimates are made by evaluating a set of lines in one or more environments and then from analysis of variance, estimate genotypic and phenotypic variance (Burton, 1987). Heritability can be determined by the ratio of genotypic variance, σ^2_G to the phenotypic variance (σ^2_{PH}): $h^2 = \sigma^2_G/\sigma^2_{PH}$. The genotypic variance is the variation caused by genetic differences among individuals. These differences are expressed as additive (σ^2_A), dominance (σ^2_D) and epistatic (σ^2_I) variances. The phenotypic variance is calculated from the observed variations in the quantitative character. These differences are expressed as genotypic variance (σ^2_G), environmental variance (σ^2_E), and genotype x environment, (σ^2_{GE}). The σ^2_{GE} is made up of the following: genotype x location (σ^2_{GL}), genotype x year (σ^2_{GY}), and genotype x location x year (σ^2_{GLY}) (Poehlman et al., 1995).

Heritability can be expressed as broad-sense or narrow-sense. Broad-sense heritability is the ratio of the variance of the genotypic values in the

population to the phenotypic variance: $\sigma^2_G/\sigma^2_{PH} = \sigma^2_G/(\sigma^2_G+\sigma^2_{GE}+\sigma^2_E)$ and is that proportion of the phenotypic variance that is determined by the genotype. Narrow-sense heritability is the relative importance of additive effects in determining the phenotypic variance and is the ratio: $\sigma^2_A/\ \sigma^2_{PH} = \sigma^2_A/(\sigma^2_G+\sigma^2_{GE}+\sigma^2_E)$ and it expresses the extent to which the phenotypes are determined by the genes transmitted from the parents (Nyquist, 1991). The environmental variables cause variation in an experiment and the analysis of variance provides a simple statistical procedure for measuring the relative importance. Table 5 below outlined by McIntosh (1993) illustrates this analysis. Genotypes are considered as a random effect, representing a random sample of potential genotypes from the population.

Frey and Horner (1957) looked at heritability in standard units. They compared heritability percents using two regression methods: conventional and standard units. They concluded that both methods proved to be in agreement with actual and predicted gains when looking at the date of heading in oatgrass.

Rebetzke et al. (1996) studied the phenotypic variation for saturated fatty acid content in soybean. This work was initiated because of an increased concern over high saturates in human diets, which have encouraged plant breeders to develop soybean lines to produce oil with reduced saturated fatty acid concentration. They were questioning if the development of soybean lines with low or high saturated fatty acid content could be accomplished through evaluation and selection in a few environments contrasting for temperature. They found that selection of a superior genotype for saturated fatty acid

Table 5. An analysis of variance for a randomized complete block design with three replications over multiple environments.

Source of variation	Df	MS	EMS
Environment	e-1	M_1	$\sigma^2 + r\,\sigma^2_{GE} + g\,\sigma^2_{R(E)} + rg\,\sigma^2_E$
Rep (Env)	e(r-1)	M_2	$\sigma^2 + g\,\sigma^2_{R(E)}$
Genotype	g-1	M_3	$\sigma^2 + r\,\sigma^2_{GE} + re\,\sigma^2_G$
Gen X Env	(g-1)(e-1)	M_4	$\sigma^2 + r\,\sigma^2_{GE}$
Error	e(r-1)(g-1)	M_5	σ^2

composition might not correlate well from one environment to another. It appears that genetic systems conditioning palmitic and stearic acids are independent. Separate breeding strategies need to be adopted in order to make simultaneous improvement for the two saturated fatty acid traits. The results showed significant differences among environments for fatty acid contents. This suggests that the environment from which soybean oil is collected will influence its proportion of saturated fatty acid.

Narvel et al. (2000) looked at inheritance of elevated palmitate in soybean seed oil. The objective was to determine genetic control of the elevated palmitate content in A25. No maternal effect or dominance was found for palmitate content between F_1 and parent crosses. The phenotypic analysis in the elevated palmitate in A25 appeared to be controlled by an allele fap 6. Increasing the palmitate improves oxidative stability, decreasing the need for hydrogenation in shortening and margarine.

Rebetzke et al. (1997) looked at genotypic variation for fatty acid content in selected *Glycine max* x *Glycine soja* populations. The reason for this study stemmed from a need for soy oil to have an increased concentration of saturated, monounsaturated, or polyunsaturated fatty acids. They found genotypic differences for oil quality were significant among populations and families within populations. High narrow-sense heritability estimates for palmitic and linolenic acid contents suggested that individual F_2 plants could be selected for either trait. Moreover, wild soybean crosses may be a useful source of genes to extend genotypic variation for linolenic and total polyunsaturated fatty acid contents.

While heritability estimates provide breeders with valuable information on population improvement, it is important to determine what effect selection on one trait will have on other important traits. If for example, breeders decide to reduce palmitic and linolenic acids, what response will this have on the plant's productive yield capacity, or its ability to synthesize protein or oil?

Pantalone et al. (1997) looked at the relationship between seed mass and linolenic acid in progeny of crosses between cultivated and wild soybean. A strong negative association was found between the seed mass and linolenic acid. It was suggested that soybean breeding could be used to develop a cultivar by back crossing an elite line to a *G. soja* plant introduction with more than 70% polyunsaturated fatty acid concentration to develop a new highly polyunsaturated line, which could be utilized by oleochemicals industry.

Dolde et al. (1999) looked at tocopherols in breeding lines and effects of planting location, fatty acid composition, and temperature during development. The objectives were to determine tocopherol concentration in seeds of selected germplasm and to investigate the effects of genetic background, planting location, and temperature on tocopherol quantity and quality of four native types of tocopherols quantified from soybean using normal phase high performance liquid chromatography with ultraviolet detection. The total tocopherol concentration in soybean ranges from 1205 to 2195μg g^{-1}. The tocopherol concentration correlated to linolenic acid with modified and unmodified fatty acid concentration. Breeding for altered fatty acid composition of the 12 soybean lines was shown to have a negative impact by planting location, breeding line,

and tocopherol concentration. Temperature may affect the relationship between tocopherol and unsaturated fatty acid. Low linolenic acid has been associated with low tocopherol concentration. It was suggested to maintain the genetics and altering the environment to study the relationship between linolenic acid and tocopherol concentration.

Pe′rez-Vich et al. (2000) looked at the genetic relationship between loci controlling the high stearic and the high oleic acid traits in sunflower. The objectives were to determine the genetic relationships between the high stearic (HAOL-9; *Ol* alleles) and the high oleic (CAS-3; E*s1, Es2* alleles) traits and to combine both characters. The F_1, F_2, BC_1F_1, both parents, and F_3 seeds were analyzed by gas liquid chromatography. It was concluded that neither trait was independently inherited. It was suggested that there is a genetic linkage between the alleles *Es2* and *Ol.*

Ross et al. (2000) studied agronomic and seed traits of 1%-Linolenate soybean genotypes. The objective was to compare (<20g/kg)1% linolenic to 2% linolenic acid lines (>20g/kg). The results stated there were no major differences between 1 and 2% linolenic acid lines and it should be possible to develop acceptable cultivars with <20g/kg linolenate with desired maturity, lodging and plant height without impeding seed weight, protein or oil content. Moreover, soybean oil with reduced linolenic acid has superior oxidative stability, which could decrease the need to hydrogenate the conventional soybean oil.

Wilcox et al. (1994) stated that if palmitic acid were reduced it would increase the value of soybean oil. Therefore, Burton et al. (1994) developed a

low palmitic soybean, N79-2077-12 and two progeny lines, N87-2122-4 and N90-2013 with low palmitic genes. Erickson et al. (1998) identified soybean mutants with low palmitic and Wilcox and Cavins released a line, C1726, in 1990. Fehr et al. (1991) identified the mutant, A1937NMU-173, that had 6.8% palmitic acid.

Pszczola (2000) reported that the researchers of USDA-ARS, Raleigh, N.C. developed a soybean, which contains half the concentration of linolenic acid of commercial varieties. Soybean oil known as "Soyola" has been produced from these soybeans that were grown using conventional breeding methods and does not require hydrogenation. Soyola is suitable for the frying and salad oil industries and is currently being grown in the southern U.S.

Research remains to be conducted on germplasm development, which brings together both low palmitic and low linolenic fatty acids. Check cultivars for this thesis research project includes 'Hutcheson' (Buss et al., 1988) a normal soybean cultivar, widely grown throughout the Mid-south and Southern United States; and N97-3525 and TN99-376, new soybean lines recently developed for their modified fatty acid composition. N97-3525 and TN99-376 exhibit low palmitic (4%) and low linolenic (4%) acid concentrations. This new combination of fatty acids has never existed previously. Moreover, N97-3525 and TN99-376 are remarkably agronomic for a modified fatty acid line. N97-3525 was evaluated for yield and other characteristics in the 1999 Southern Regional Testing program over nine locations. N97-3525 is likely to become the first low palmitic, low linolenic soybean cultivar to be released for production as 'Satelite" (pers. comm. Wilson).

3. MATERIALS AND METHODS

3.1 FIELD EXPERIMENT

In the summer of 1998, a cross was made between two soybean plants at the Central Crops Research Station of North Carolina State University in Raleigh, NC. A North Carolina experimental line, N97-3708-13, used as the female was fertilized by pollen of the experimental line, S94-1956, now known as the cultivar, 'Anand'. The N97-3708-13 parent has the characteristics of a low percentage of both palmitic and linolenic fatty acids as it comes from the cross Soyola x [BRIM (2) x N88-431 (2) x (N90-2013 x C1726)]. Soyola has the low linolenic trait and C1726 has the low palmitic fatty acid trait. N97-3708-13 is of maturity group (MG) VI (6.0). Anand is a high yield cultivar of MG V (5.7). It has purple flowers and tawny pubescence. The seeds of Anand are yellow with black hila and the plants are resistant to races 2, 3, 5, and 14 of the soybean cyst nematode (*Heterodera glycines*) and moderately resistant to stem canker (*Diaporthe phaseolorum*). Anand was developed in 1999 in Missouri and its parents are Holladay and Hartwig. The goal of our cross is to produce a population between a normal and a low linolenic and low palmitic fatty acid line in order to estimate heritabilities of fatty acid traits, and correlate fatty acid traits and agronomic traits, in order to guide breeders who wish to improve soybean oil for human consumption.

In the fall of 1998, the resulting F_1 seed was harvested and sent to the USDA-ARS winter nursery in Puerto Rico allowing F_2 seed to be planted the following spring in Tennessee.

The F_2 seeds were planted on 09 June 1999 at Knoxville Experiment Station in Knoxville, TN. Two hundred F_2 single plants were grown in a honeycomb design at the Knoxville Plant Science Farm. According to Janick (1995), the honeycomb design has been developed by Fasoulas between the years of 1973 and 1993. This design gets its name because of the hexagonal arrangement of single plant hill plots in the field. It is desirable as the design allocates entries under comparable growing conditions by effectively sampling for environmental variation. This allows error related to spatial heterogenity to be reduced and allows for stability of performance early in the breeding program (Janick, 1995). Figure 1 depicts a generalized diagram of a honeycomb design (Janick, 1995). The position of the single plants is represented by black dots. The lower case d represents the interplot distance in length units, in our case it was 30 in or 76.2 cm. The row length was calculated with the formula $\mathbf{d\sqrt{3}/2}$= 30 in (1.7321)/2= 26 in (76.2 cm). The side of the hexagon used to offset every other row was calculated with the formula $\mathbf{d\sqrt{3}/2 \times 2/3}$= 30 in (1.7321)/2(.6667)= 17.3 in (43.8 cm). The plot area is represented by the hexagon and can be calculated with the formula $\mathbf{d^2\sqrt{3}/2}$= (900 in^2)(1.7321)/2= 779.4 in^2 (1978.7 cm^2). The seeds were planted by hand and 30 in (76.2 cm) rows were measured leaving 34.5 in (87.6 cm) spacing between each plant with every other row offset by 17.3 in (43.8 cm). Three seeds were placed per hill to ensure germination and upon growth cut back to only one seedling. As the seedlings developed into the F_2 plants, the following traits were

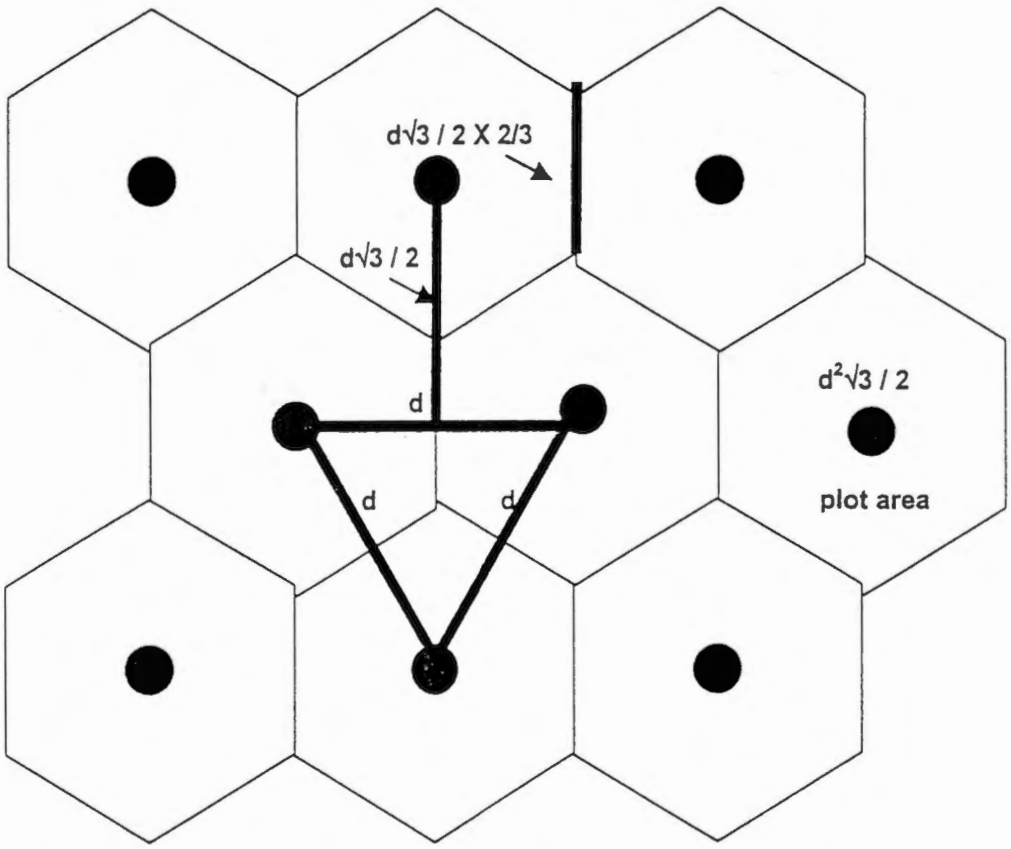

Figure 1. Honeycomb design. (Janick, 1995)

measured and recorded for each hill recorded for each hill plot, maturity date, plant height, 100 seed weight, protein and oil concentration and fatty acid composition. Flower color was recorded as purple or white. Pubescence color was recorded as gray or tawny and was recorded on a mature plant. Maturity was observed when 95% of the plant had lost its leaves and pods had reached their mature color. Maturity was checked twice a week and maturity date recorded within three days back dating, calling that day, or within three days by calling ahead. The plant height was measured from the soil surface to the apex of the main stem and reported in centimeters. In the fall of 1999, each F_2 plant was harvested individually using a single plant thrasher. Total and 100 seed weights were recorded in grams and two sub samples were taken for protein and oil and for fatty acid analyses. The remainder of the $F_{2:3}$ seeds were placed into an envelope and sent to a winter nursery in Costa Rica. Approximately twenty-five grams of the $F_{2:3}$ seeds were packaged and sent to the National Center for Agricultural Utilization Research, Peoria, Illinois for analysis of protein and oil using a Model 1255 Infratec Near Infrared Resonance (NIR) food and feed analyzer. In addition, *ca.* 10 seeds were kept at the University of Tennessee for measurement of fatty acid concentration (see section 4.2).

Meanwhile, in Costa Rica in the fall of 1999, each envelope containing the $F_{2:3}$ seed from each F_2 plant grown in Knoxville was planted as $F_{2:3}$ rows in order to increase the amount of seeds. In the winter of 2000 the $F_{2:3}$ rows were harvested in bulk and the $F_{2:4}$ seeds from each individual row were placed in their respective labeled bag and shipped to Knoxville. This provided a sufficient

quantity of $F_{2:4}$ seeds to test in a replicated, multiple environment field experiment in 2000.

The second year of the project (2000) was initiated by planting a randomized complete block design with three replications at three different locations representative of East, Middle, and West Tennessee. The seeds were planted early to mid-June at the following locations: the Knoxville Experiment Station in Knoxville,TN (KES), Highland Rim Experiment Station (HRES) in Springfield, TN, and Ames Plantation (AMES) in Grand Junction, TN. The plot entries were composed of 128 genotypes including the two parents, N97-3708-13 and Anand as well as five checks, 'Hutcheson', N97-3525, TN99-368, TN99-370, TN99-376, and one filler, TN4-86. Seeds were sown at *ca.* 8 seeds per foot (0.30 meter) in a row. At KES and HRES the seeds were planted 20 ft (6.10 m) in length with 30 in (76.2 cm) between rows. At AMES the seeds were planted 30 ft (9.1 m) in length with 30 in (76.2 cm) between rows. Two-row plots were established at both KES and HRES. One-row plots were established at AMES.

The following traits were recorded or measured on the $F_{2:4}$ rows: flower color, flower date, pubescence color, maturity date, plant height, lodging, 100 seed weight, moisture, yield, protein and oil concentration, and fatty acid composition. Flower color was recorded as purple, white or segregating rows when at least 80% of the plants within a row were in full bloom. The time of flower color was recorded in Julian dates as day of the year. Pubescence color was recorded as tawny, gray, or segregating and when at least 95% of the plants were mature. The time of maturity was recorded in days it took to mature. Plant height was

measured from the surface of the soil to the apex of an average plant within the row and was reported in centimeters. Lodging was given a score (1-5), ranging from 1 being erect to 5 being prostrate. Hundred seed weight was recorded in grams. Plot moisture and yield were measured at harvest time after passing through a combine. Protein and oil concentration, as well as fatty acid composition were recorded in the same manner as previously mentioned for F_2 single plants.

Before harvesting the end of the rows were hand trimmed to normalize their lengths. At KPSF the 20ft (6.1 m) rows were trimmed to 16ft (4.9 m), at HRES (due to poor germination, resulting from a 15 cm rain the day after planting) each row was meticulously trimmed to 16 ft (4.9 m), 12 ft (3.7 m), 8 ft (2.5 m) or 4 ft (1.2 m), and at AMES the 30 ft (9.1 m) rows were trimmed to 26 in (7.9 m) in length. Data had to be dropped from HRES and AMES. At HRES prior to recording yield or sampling for seed quality traits, harvested plot bags were discarded inadvertently by experiment station personnel. At AMES an exceptional early severe frost (08 Oct 2000, 27°C), interrupted seed fill, making it impossible to mechanically harvest plots.

Parent-offspring regression was analyzed using data from single F_2 plants and $F_{2:4}$ rows. Parent-offspring regressions were calculated on seed size, protein, oil, palmitic acid, oleic acid, and linolenic acid (section 4.2). This was done in order to estimate heritability. Nyquist (1991) indicated that in self-fertilizing species, parent-offspring regression is commonly used to estimate heritabilities. A common estimator has been the regression of family means in

one environment on the individuals (F_2) in the previous year in the same location. In parent-offspring regression, a model $y = \beta_o + \beta_1 X$ is used where β_1 is the slope that represents heritability.

3.2 FATTY ACID CONCENTRATION

The system currently used in our laboratory has many of the same principles as previously mentioned by Hammond in section 2.1 Food Processing Quality. Soybean seeds from either a single F_2 plant or $F_{2:4}$ row representing a single genotype were used for analysis. Five seeds were placed between two pieces of thick metal and lightly cracked by gently hitting one side of the metal with a hammer. Each sample of the crushed seed was transferred to a test tube using a funnel. One ml of the extraction solvent mixture, chloroform:hexane:methanol (8:5:2, v/v/v), was then added to the test tube containing the cracked seed and closed with a stopper. The test tubes were left for at least 4hrs. After the extraction, 100µl of the oil sample was placed in a 1.5ml autosampler vial. Then 75µl of methylating reagent (a solution mixture of 50ml of sodium methoxide, 20ml of petroleum ether, and 10ml of ethyl ether, and 1ml of hexane) was added to the vial before capping. The triacylglycerols and phospholipids are saponified and the fatty acids are liberated and methyl groups are attached to form fatty acid methyl esters (FAME). This was done to prevent esterification of the individual fatty acids with each other and to increase volatility of the FAME. The samples were analyzed for composition of the following five fatty acids: palmitic, stearic, oleic, linoleic, and linolenic using a Hewlett Packard HP 6890 series Gas Chromatograph (GC) System equipped with a model 7683 auto sampler, and

7673 flame ionization detector, and an immobilized 30m X 0.53mm inner diameter, J&W 125-2332 capillary column with 0.5μm fused stationary phase. Operation conditions were as follows: carrier, Helium (20mL/min); 20:1 (v/v) split injection; injection temperature, 250^{O}C; detector temperature, 275^{O}C; and column temperature, 230 ^{O}C. The RM-1 standard suitable for measuring soybean oil was used to determine the relative fatty acid concentration of the experimental samples. The standard came in 100mg including 6 mg palmitate, 3 mg stearate, 35 mg oleate, 50 mg linolenate, and 3 mg linolenate. The standard was diluted to 5 mg/mL solution using hexane and 0.5 ml was placed into a 1.5 mL GC autosampler vial. The HP chemstation was set to establish and save in memory a standard curve for each of the five following fatty acids: palmitic, stearic, oleic, linoleic, and linolenic. Two standards were run with every 99 samples to make sure the correct fatty acid concentration of the standards were being read correctly. The peak area was determined by running samples through the GC. As they eluted the retention time allowed the correct standard curve to be retrieved from the HP chemstation file. The fatty acids were eluted after the solvent peak as follows: palmitic, stearic, oleic, linoleic, and linolenic. The response factor was derived from the ratio peak area/concentration of the standard for each fatty acid standard. Then the relative fatty acid concentrations for each of the five fatty acids were determined for each of the unknown samples and expressed as percentages.

4. RESULTS AND DISCUSSION

4.1 F_2 POPULATION

The F_2 population of the cross between N97-3708-13 x Anand was established as single plants planted in a honeycomb design. A random population was verified in the F_2 single plants by calculating a chi-squared analysis (χ^2) on the single genes, for flower color, pubescence color and the combination (two genes) of flower color and pubescence color (Appendix D). As expected, a 3:1 monohybrid (single gene) and 9:3:3:1 (two gene) model was found for the F_2 population. The genetic concept of chance deviation is based on the following assumptions: each allele is dominant or recessive, segregation is operative, independent assortment occurs, and fertilization is random (Klug and Cummings, 2000).

Chi-squared analysis was calculated for palmitic acid as a two-gene model. There were 14 plants observed with double-recessive phenotypes with an average mean of 3.5% palmitic acid. The other 165 plants had a mean of 8.3% for palmitic acid. This fit a chi-squared with 1/16 having the homozygous double recessive and 15/16 including the dominant allele. The chi-squared calculated was 0.85, which was lower than the chi-squared from the table with 1 degree of freedom, 3.84. The presence of modifying genes influence the biochemical pathways of enzymes governing fatty acid synthesis, hence distributions typically show continuous variation rather then discrete phenotypic classes. Therefore, the double recessive is the only directly observable phenotype for palmitic acid.

A chi-squared analysis was calculated for a one-gene model of linolenic acid. There were 48 individual plants with a recessive mean of 4.0% for linolenic acid. There were 131 individuals with a mean of 7.5% for linolenic acid. The chi-squared calculated was 2.7, which is lower than the 3.84 table value for 1 df. Therefore ¼ were homozygous recessive and ¾ had an expression of the dominant gene. A single recessive gene governing low linolenic and two recessive genes governing low palmitic is consistent with the pedigree of the modified fatty acid parent, N97-3708-13 (pers. comm., Burton).

Table 6 shows the mean with standard error and minimum and maximum range for the F_2 plant traits maturity (Julian days), height (cm), seed yield per plant (g), seed size (g), protein and oil concentrations (%). The maturity ranged from day 268 to day 307 with a mean of day 299. This corresponds to a late maturity group (MG) V. Seed size had a mean of 16.3 g 100^{-1}. Seed size is thought to be associated with polyunsaturated oils. According to Pantalone et al. (1997) in a *G. max* X *G. soja* population, the smaller the seed mass the more highly polyunsaturated the oil. However, in our population derived from two *G. max* lines, there was no significant association between seed size and linolenic acid. This enabled us to select for low-linolenic plants from across any desired seed size class. The mean protein and oil concentrations were 41.3% and 19.7%

Table 6. The mean with standard error and minimum and maximum range for the agronomic traits of maturity, height, seed yield per plant, seed size, protein and oil on a dry matter basis of the N97-3708-13 x Anand F_2 soybean population.

Trait	Mean	Standard error	Range	
			Minimum	*Maximum*
Maturity (days to mat)	138	± 0.4	107	147
Height (cm)	48	± 0.4	18	91
Seed yield per plant (g)	72.3	± 2.96	6.6	211.7
Seed size (g)	16.3	± 0.15	11.8	22.5
Protein (%)	41.3	± 0.11	37.6	45.4
Oil (%)	19.7	± 0.07	16.7	22.9

respectively, values that are typical for production soybeans. Table 7 shows the mean with standard error and minimum and maximum range for the fatty acids: palmitic, stearic, oleic, linoleic, and linolenic. The mean for the palmitic and linolenic acids of this F_2 population fell within or slightly lower than the normal range of 8% to12% typically observed for soybean. Both palmitic and linolenic showed individuals as low as 4.1% representing about 50% reduction in the normal levels of these two fatty acids. The oleic content had a mean of 22% with a high range of 27%. Future breeding efforts to further elevate the oleic acid would be desirable because a high oleic content has been shown to reduce serum cholesterol levels and has good oxidative stability (Liu, 1997).

A normal distribution was observed for the agronomic traits of plant height, single plant seed yield, seed protein concentration and oil concentration for this random F_2 population. This is illustrated in figure 2 with a frequency distribution for the oil concentration, which ranged from *ca.* 16% to 23% with the majority between 19% and 21%, as is typical for soybean.

4.2 $F_{2:4}$ POPULATION

The $F_{2:4}$ progeny were established as two-row plots 4.9 meters in length. Table 8 shows the mean with the standard error and minimum and maximum range for the agronomic traits of maturity (Julian days), height (cm), lodging (1-5 scale), yield (Kg ha $^{-1}$), seed size (g 100 $^{-1}$), protein and oil concentration (%) of the N97-3708-13 x Anand $F_{2:4}$ population. Lodging had an unexpected high mean of 2.7. Production cultivars typically show lodging scores 2.0 or lower.

Table 7. The mean with standard error and minimum and maximum range for the fatty acids: palmitic, stearic, oleic, linoleic, and linolenic of the N97-3708-13 x Anand F_2 soybean population.

Trait	Mean	Standard error	Range	
			Minimum	*Maximum*
Palmitic acid (%)	8.0	± 0.15	4.1	12.9
Stearic acid (%)	4.1	± 0.05	2.7	6.1
Oleic acid (%)	22.0	± 0.17	17.1	27.2
Linoleic acid (%)	59.1	± 0.19	51.0	66.7
Linolenic acid (%)	6.6	± 0.11	4.1	11.3

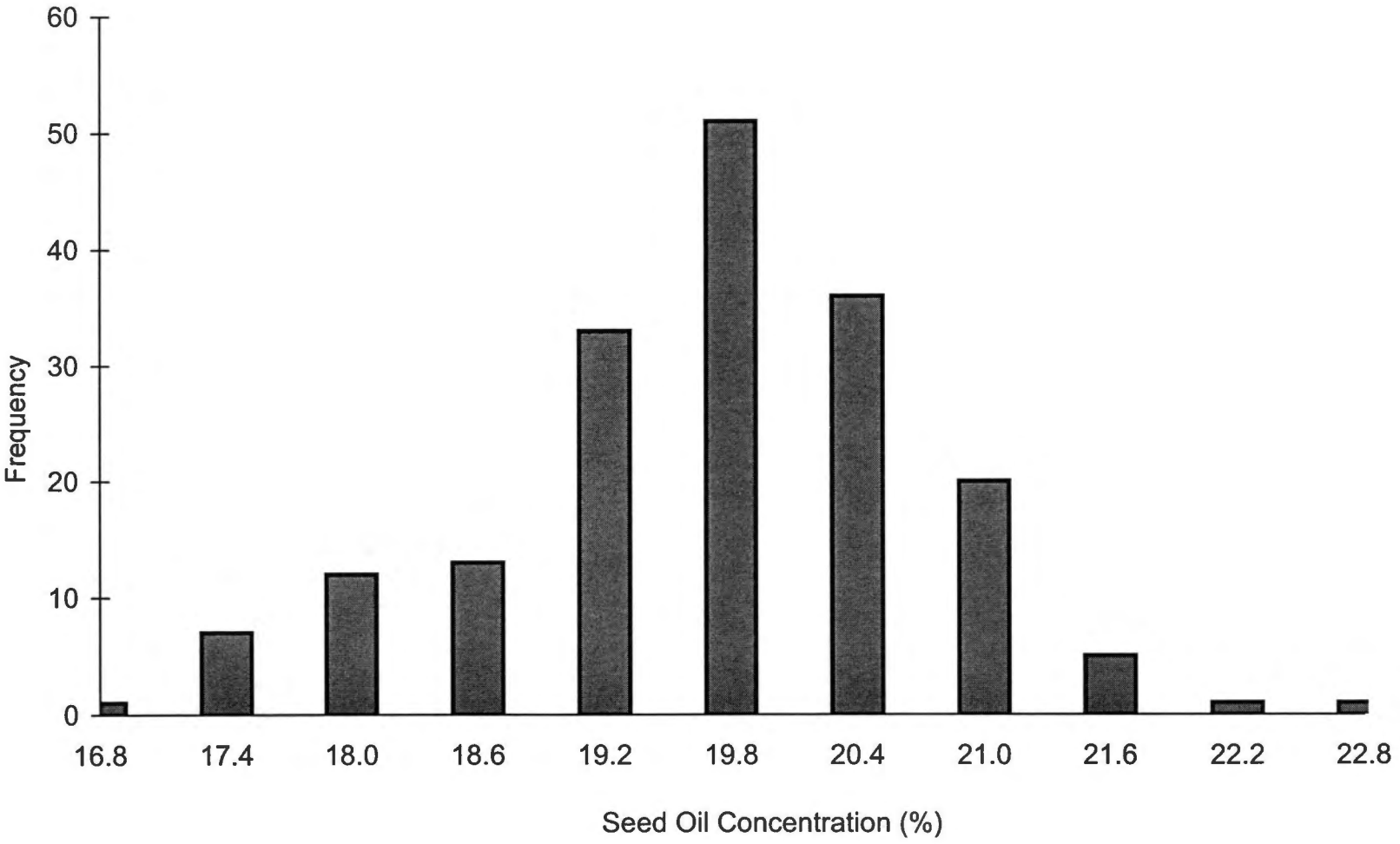

Figure 2. Frequency Distribution for Seed Oil Concentration of N97-3708-13 x Anand F_2 Population

Table 8. The mean with standard error and minimum and maximum range for the agronomic traits of maturity, height, lodging, yield, seed size, protein and oil on a dry weight basis of the individual genotypic observations in the N97-3708-13 x Anand $F_{2:4}$ soybean population.

Trait	Mean	Standard error	Range	
			Minimum	*Maximum*
Maturity (days to maturity)	132	±0.5	120	142
Height (cm)	98	± 0.1	64	122
Lodging[a]	2.7	± 0.10	2.0	5.0
Yield (Kg ha $^{-1}$)	2734.0	± 32.27	705.5	4764.0
Seed size (g)	15.4	± 0.09	11.0	19.8
Protein (%)	38.8	± 0.95	31.3	41.7
Oil (%)	20.8	± 0.06	18.2	23.0

[a] Lodging was based on a score of 1 to 5, with 1= all plants erect, and 5= all plants prostrate.

Yield had a mean of 2734.0 kg ha $^{-1}$ with the highest progeny ranking 4764.0 kg ha $^{-1}$ representing highly productive soybean lines. Ross et al. (2000) studied the agronomic and seed traits of 1% and 2% linolenate soybean genotypes. After evaluating the top 27 high and low plants for linolenate concentration the authors concluded that there was a lack of major agronomic differences between 1% and 2% lines (compared to checks) and that it should be possible to develop acceptable low linolenic cultivars. In our study estimates of the $F_{2:4}$ progeny demonstrate high yield potential that equals or exceeds that of production soybean cultivars and supports the conclusion made by Ross et al. (2000) that it may be possible to develop acceptable low linolenic cultivars. Agronomic traits (with the exception of total yield per plant) can be compared to the agronomic traits of the F_2 population. The means did not differ greatly except for plant height, which were 48 cm for the single plants and 98 cm for the whole rows. For maturity the $F_{2:4}$ mean was 4 days earlier, the seed size was 0.9 g smaller, the protein was 2.5% lower, and the oil was 1.1% larger.

Table 9 lists the mean with standard error and minimum and maximum range for the fatty acids: palmitic, stearic, oleic, linoleic, and linolenic of the N97-3708-13 x Anand $F_{2:4}$ population. The mean difference was 0.7% higher in the $F_{2:4}$ for palmitic, 0.4% smaller for stearic acid, 1.0% smaller for oleic acid, 0.6% lower for linoleic acid, and 0.3% lower for linolenic acid as compared to the F_4 population (table 7). The consistency of F_2 versus $F_{2:4}$ means suggests that early generation selection may be possible. This concept will be discussed in more depth later in this paper.

Table 9. The mean with standard error and minimum and maximum range for the fatty acids: palmitic, stearic, oleic, linoleic, and linolenic of the individual genotypic observations in the N97-3708-13 x Anand $F_{2:4}$ soybean population.

Trait	Mean	Standard error	Range	
			Minimum	*Maximum*
Palmitic acid (%)	8.7	± 0.16	3.9	12.6
Stearic acid (%)	3.7	± 0.03	2.8	5.2
Oleic acid (%)	21.0	± 0.15	17.8	28.2
Linoleic acid (%)	59.7	± 0.19	51.1	65.2
Linolenic acid (%)	6.9	± 0.13	4.2	10.6

Table 10 shows the mean ± standard error for the parents and checks in the $F_{2:4}$ population for the agronomic traits: maturity (days to maturity), height (cm), lodging (1-5), yield (Kg ha $^{-1}$), seed size (g), protein and oil on a dry matter basis. Table 11 shows the mean ± standard error for the fatty acid traits (total relative concentration,%): palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. It is noteworthy that the low-palmitic, low-linolenic parent N97-3708-13 produced yield equivalent to that of the parent Anand, which is a high yielding new (1999) variety that ranked second highest for yield in the 2000 Tennessee State Variety test (Graves et al., 2001). It is further noteworthy that the new University of Tennessee low-palmitic, low-linolenic germplasm, TN99-376 produced high yields, again supporting the concept that highly productive low saturated fat, low linolenic cultivars may be able to be developed for Tennessee producers. Moreover, there were some progeny that yielded higher than the check, Hutcheson. In addition, there were genotypes that were lower in palmitic and linolenic acid than the parents and checks, a very encouraging observation for applied breeders. However, before applied cultivar development can continue, breeders need to know how modified fatty acids will affect other important traits.

Table 12 shows the correlation coefficients of maturity, height, lodging, yield, seed size, protein, oil, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. The traits of most interest to this project are palmitic acid and linolenic acid.

Table 10. The mean ± standard error of the $F_{2:4}$ soybean population of N97-3708-13 x Anand of the parents and checks for the agronomic traits: maturity (mat) in days to maturity, height (Ht) in cm, lodging (Lod), yield (Yld) in kg ha $^{-1}$, seed size (SS) in g, protein (Pro) and oil in % on a dry weight basis.

Genotype	Agronomic traits						
	Mat ± 3.0 (days to mat.)	Ht ± 7.16 (cm)	*Lod ± 0.31	Yld ± 115.52 (kg ha $^{-1}$)	SS ± 0.45 (g)	**Pro ± 0.63	Oil ± 0.58
Parents							
Anand	284	55.0	2.3	2472.6	13.7	37.1	22.1
N97-3708-13	303	100.8	3.7	2481.6	16.6	39.9	19.2
Checks							
Hutcheson	284	72.8	2.3	2983.2	14.0	38.9	21.9
N97-3525	296	99.9	3.7	2436.8	14.9	39.2	18.9
TN99-368	294	95.7	3.3	2387.5	16.1	41.3	18.8
TN99-370	300	94.0	2.7	2311.3	15.8	39.8	18.7
TN99-376	289	102.4	3.0	2763.7	15.5	40.1	19.5

* Lodging was based on a score of 1 to 5, with 1 = all plants erect and 5 = all plants prostrate

** Protein and oil are expressed in percent on a dry matter basis

Table 11. The mean ± standard error of the $F_{2:4}$ soybean population of N97-3708-13 x Anand of the parents and checks for the fatty acid traits: palmitic acid (16:0) in %, stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3).

Genotype	*Fatty acid traits				
	16:0 ± 1.35	18:0 ± 0.14	18:1 ± 0.98	18:2 ± 1.31	18:3 ± 0.71
Parents					
Anand	12.2	3.5	20.1	55.5	8.6
N97-3708-13	4.9	3.9	21.4	64.3	5.5
Checks					
Hutcheson	11.3	3.9	18.4	57.4	9.0
N97-3525	4.5	4.2	24.2	62.0	5.1
TN99-368	4.3	4.2	24.5	62.0	5.0
TN99-370	4.2	4.2	23.4	63.2	5.0
TN99-376	5.0	3.8	24.5	61.7	5.0

* All fatty acids are expressed in percentages based on total relative concentration of individual samples

Table 12. Correlation Coefficients comparing maturity (Mat), height (Ht), lodging (Lod), yield (Yld), seed size (SS), protein (Pro), oil, palmitic acid (16:0), oleic acid (18:1), and linolenic acid (18:3) of $F_{2:4}$ rows in 2000 at Knoxville,TN.

	Ht	Lod	Yld	SS	Pro	Oil	16:0	18:0	18:1	18:2	18:3
Mat	0.20**	-0.04	0.20**	0.16**	-0.25**	-0.30**	0.06	0.17*	-0.42**	0.28**	-0.05
Ht		0.09	0.10	0.04	-0.12*	-0.05	0.20*	0.11*	-0.12*	-0.03	-0.08
Lod			0.12*	0.13*	0.12*	-0.14	-0.05	-0.04	-0.01	0.08	-0.05
Yld				0.19*	0.11*	-0.09	0.12*	0.02	-0.11*	-0.02	-0.00
SS					0.17**	-0.09	-0.12*	0.11*	0.09	0.01	0.01
Pro						-0.19*	0.02	0.16*	0.23**	-0.16*	-0.10*
Oil							0.13*	-0.11*	0.13*	-0.21**	0.05
16:0								0.28**	-0.26**	-0.56**	-0.09
18:0									0.21**	-0.21**	-0.53**
18:1										-0.44**	-0.26**
18:2											-0.29**

Note: *ca.* 350 observations for each trait

*,** significant ($P \leq 0.05$ and $P \leq 0.01$, respectively)

Palmitic acid was significantly positively correlated to height, yield, and oil (r; 0.20, 0.12, and 0.14 respectively). As the height and yield increases there is an increase in the amount of palmitic acid. Unfortunately, as the amount of palmitic acid decreases, so does the oil concentration. Rebetzke et al. (1998) reported the opposite; that seed oil was significantly greater among the reduced plamitic lines. In our study there was a significant negative correlation (r, -0.12) between palmitic acid and seed size. The greater the seed size the lower the palmitic acid. This is encouraging because larger seed was also correlated with greater yield (r, 0.12) and suggests that development of high yielding low palmitic lines is possible. However, there was not a significant relation found between palmitic acid and maturity, lodging, or protein. Rebetzke et al (1998) reported that genetic modifiers conditioning palmitic acid content seem independent of genes controlling seed yield, suggesting that selection for reduced 16:0 content among lines homozygous for the low palmitic gene may be achieved without reduction in seed yield. Moreover, Rebetzke et al. (1998) reported that there was no significant association between palmitic acid and protein, an observation that we confirmed in our $F_{2:4}$ population.

There was a significant negative correlation between linolenic acid and and protein (r; -0.10). As the linolenic acid decreases there is a favorable increase in the amount of protein. This contrasts Rebetzke et al. (1998), which states there was no significant relation between linolenic content and protein. In our study there was a significant negative correlation between linolenic acid and stearic acid, oleic acid, and linoleic acid (r; -0.53, -0.26, -0.29 respectively). As

stearic acid, oleic acid, and linoleic acids increase there is a decrease in the amount of linolenic acid. Selection for a high level of oleic acid tends to reduce the level of linolenic acid and improves the flavor and stability of the oil (Liu, 1997). There was no significant association found between linolenic acid and height, yield, seed size, oil or palmitic acid.

Another trait of interest to breeders is oleic acid. There was a significant positive association between oleic acid and protein and stearic acid (r; 0.25, 0.21 respectively). As the oleic acid increases so does the amount of protein and stearic acid. There was a significant negative association between oleic acid and maturity and palmitic acid (r; -0.44, -0.26 respectively). Therefore, the amount of oleic acid increases with greater maturity and with reduced palmitic acid. This compares to the report by Rebetzke et al. (1998) that oleic and linolenic acid contents were significantly greater ($p<0.01$) for reduced palmitic lines. Moreover, Rebetzke et al. (1998) reported a significant negative correlation ($p<0.05$) between oleic and palmitic acids. In our study there was a significant positive correlation between oleic acid and oil concentration (r; 0.13). The amount of oleic acid increased with increasing oil concentration. Unfortunately, there was a significant negative correlation between oleic acid and height and yield (r; -0.12 and –0.11 respectively). The amount of oleic acid increased with shorter plants and lower yields. Oleic acid was not significantly affected by lodging or seed size.

Yield was found to be significantly positively associated to seed size. The larger the seed size the higher the yield. Pantalone et al. (1997) found a strong

negative association between linolenic content and seed mass in *G. max.* x *G. soja.* In our $F_{2:4}$ population they were not significantly correlated. Pantalone et al. (1997) also found a positive correlation between oil and seed size, but in our $F_{2:4}$ population oil and seed size showed a negative correlation. Burton (1987) reported that seed yield and protein typically show a negative genetic correlation whereas in our $F_{2:4}$ population there was no correlation.

There was a significant negative association between oil and protein (r; -0.19). If oil content is increased there will be a decrease in protein content. An negative relationship between oil and protein content was also reported by Poehlman and Sleper (1995).

The parent-offspring regressions was analyzed using data from single F_2 plants and $F_{2:4}$ rows. The F_2 population is indicated on the x axis and the $F_{2:4}$ generation is indicated on the Y axis for different characteristics of the soybean (figures 3 through 8). All the traits showed a positive slope, as the trait increased in the F_2 population it also increased in the $F_{2:4}$ generation indicating a genetic control of expression for each trait. However, each slope varied, representing the degree of heritability for the trait.

Seed size had a low heritability of 0.19 with a low r^2 value of 13%. Protein had a heritability of 0.24, which was explained by an r^2 of 10%. Oil had a heritability of 0.29 with the $F_{2:4}$ generation being explained by a r^2 of 22%. Palmitic acid had the second highest heritability of the calculated traits. It had a heritability of 0.65 and the $F_{2:4}$ generation was explained by 67%. This compares to Rebetzke et al. (1997), which reported high narrow sense heritability for

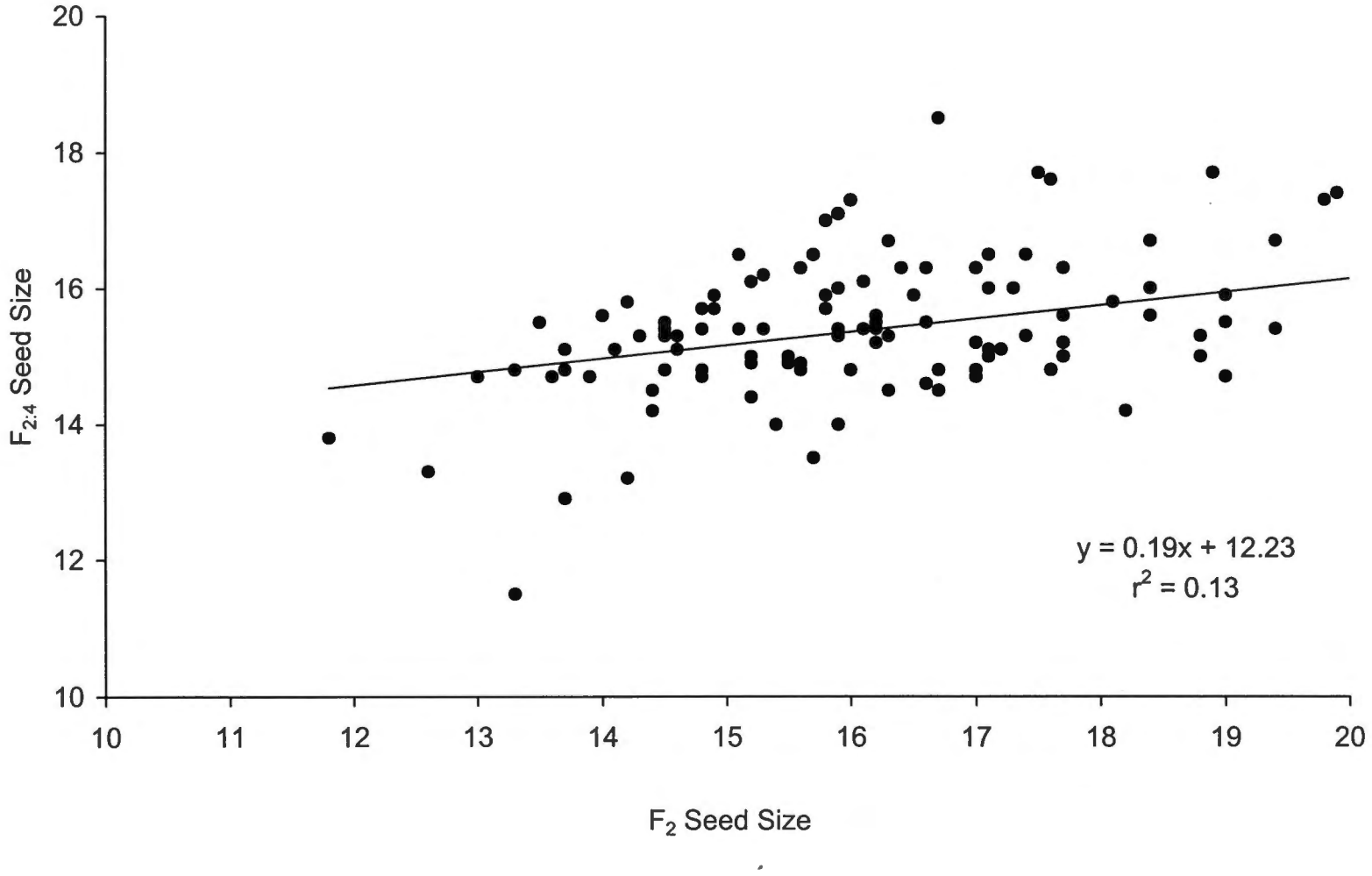

Figure 3. Linear regression of F_2 single plant seed size versus $F_{2:4}$ line seed size

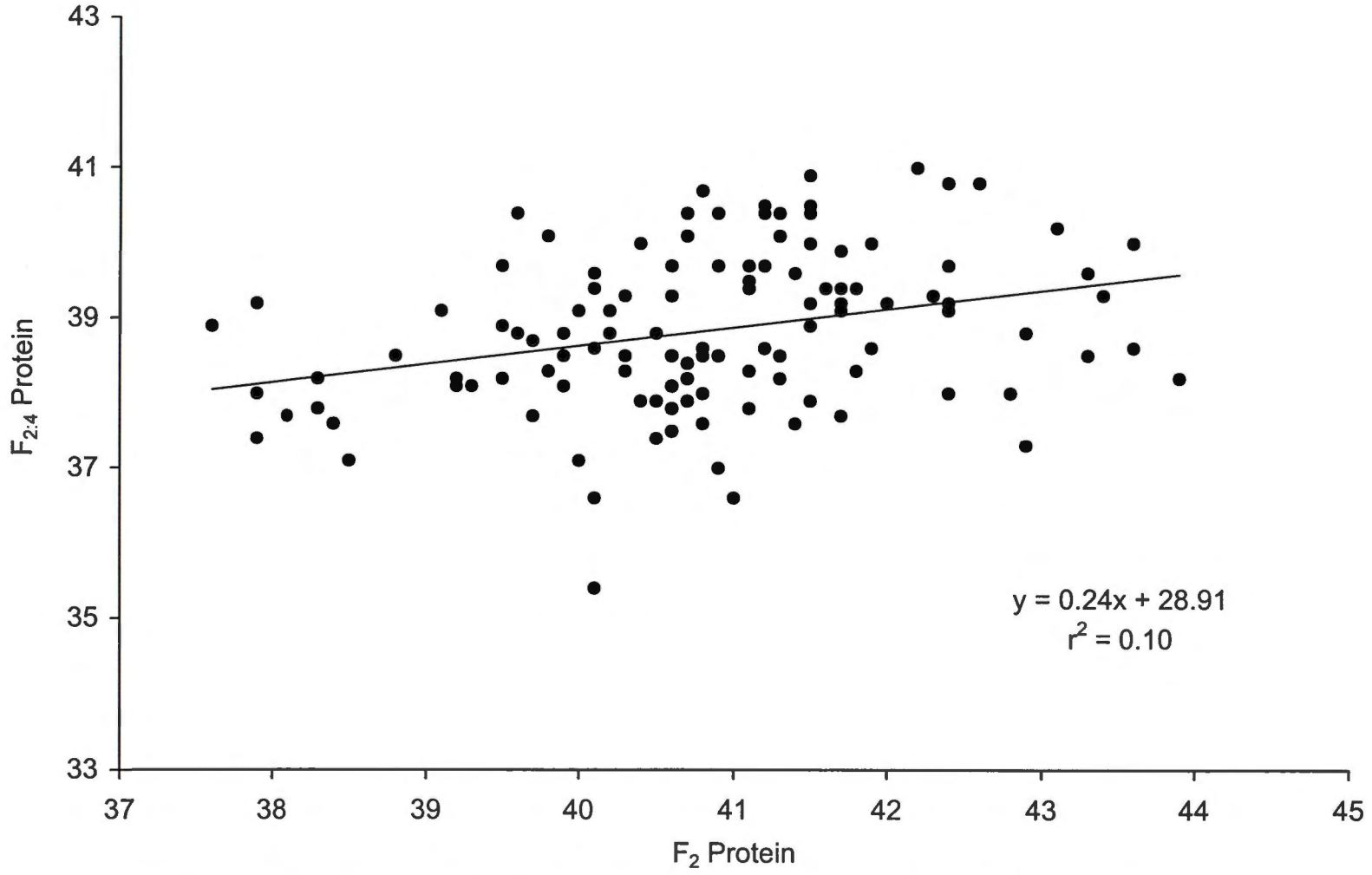

Figure 4. Linear regression of F_2 single plant protein versus $F_{2:4}$ line protein concentration

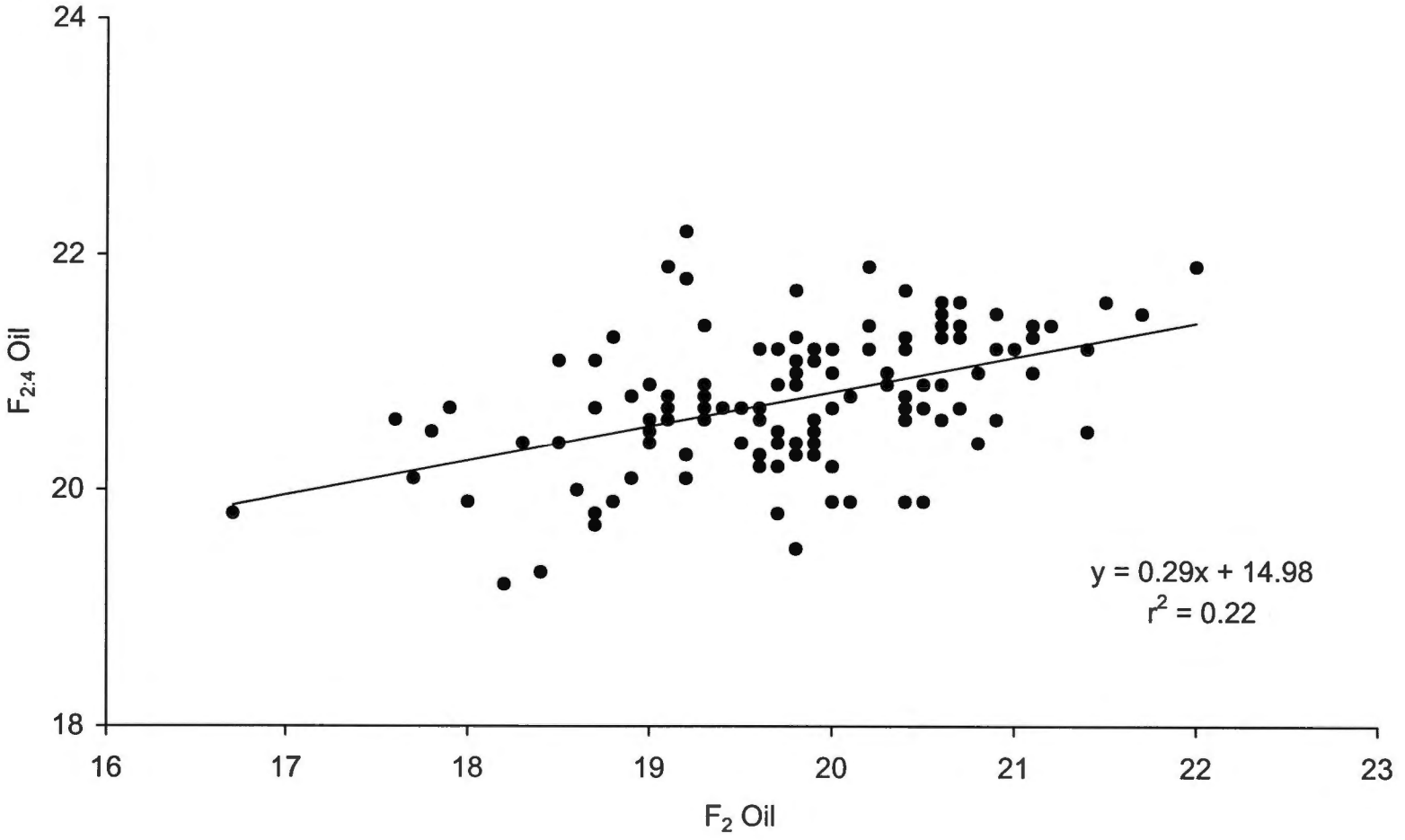

Figure 5. Linear regression of F_2 single plant oil versus $F_{2:4}$ line oil concentration

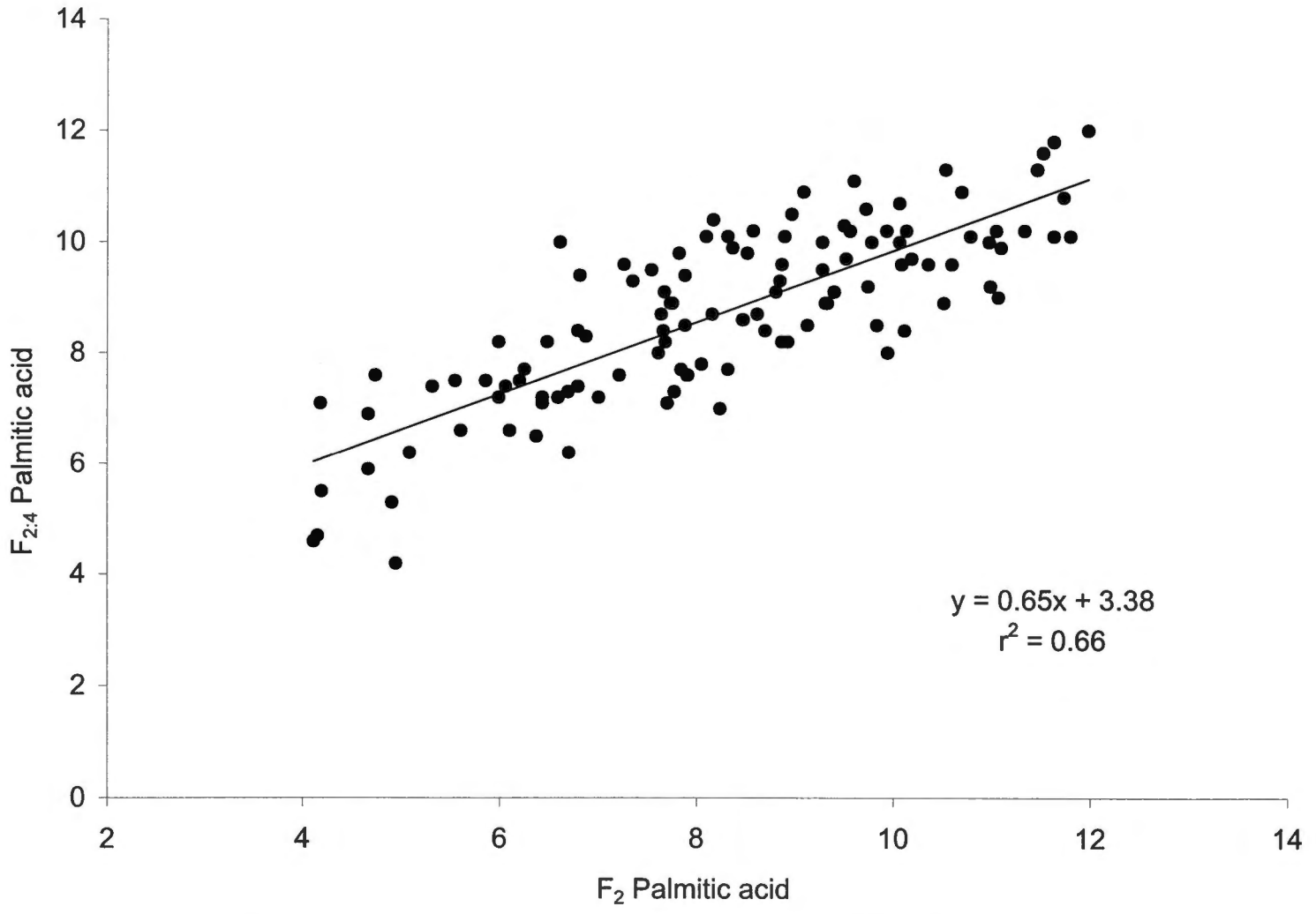

Figure 6. Linear regression of F_2 single plant palmitic acid versus $F_{2:4}$ line palmitic acid

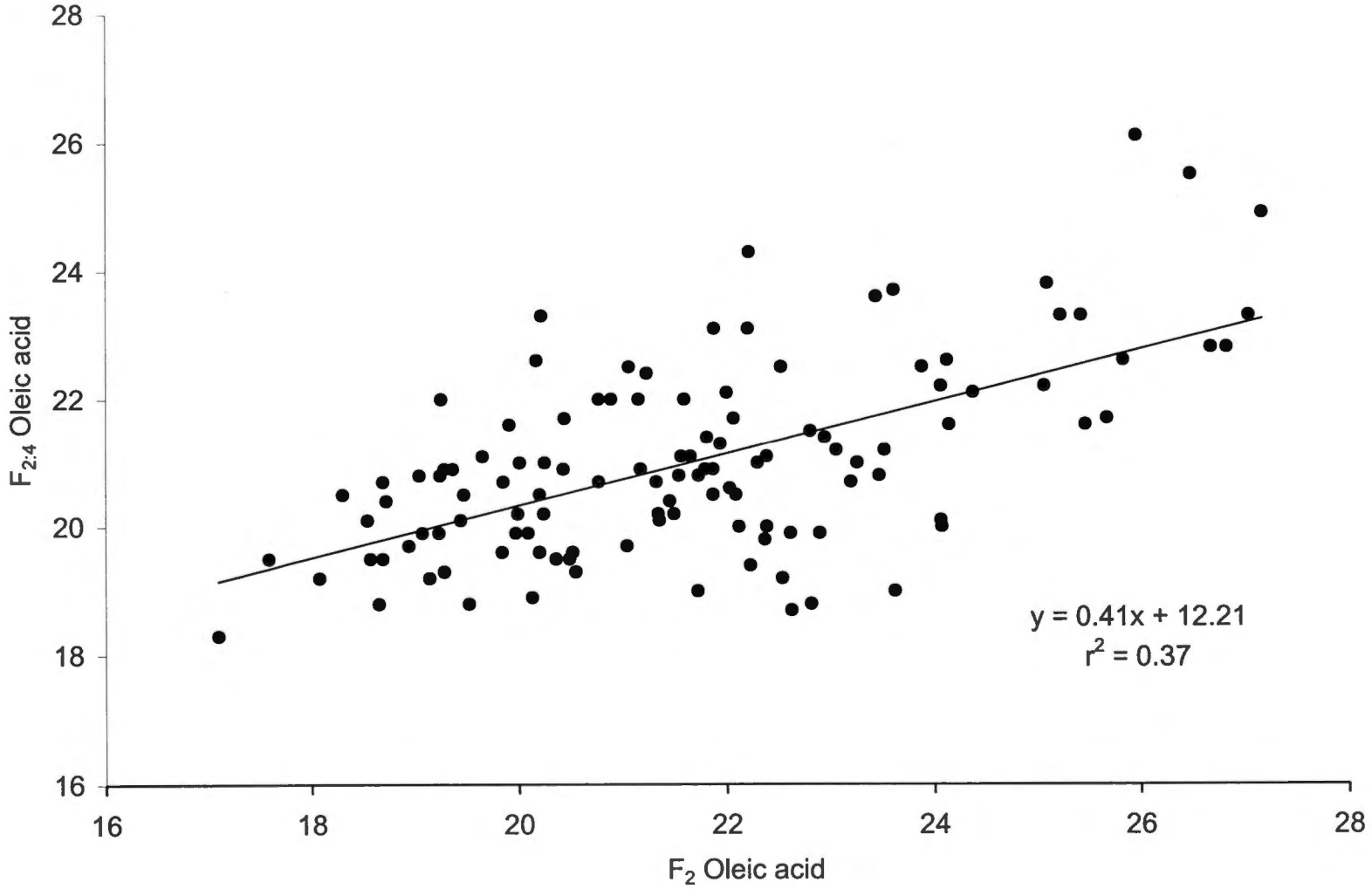

Figure 7. Linear regression of F_2 single plant oleic acid versus $F_{2:4}$ line oleic acid

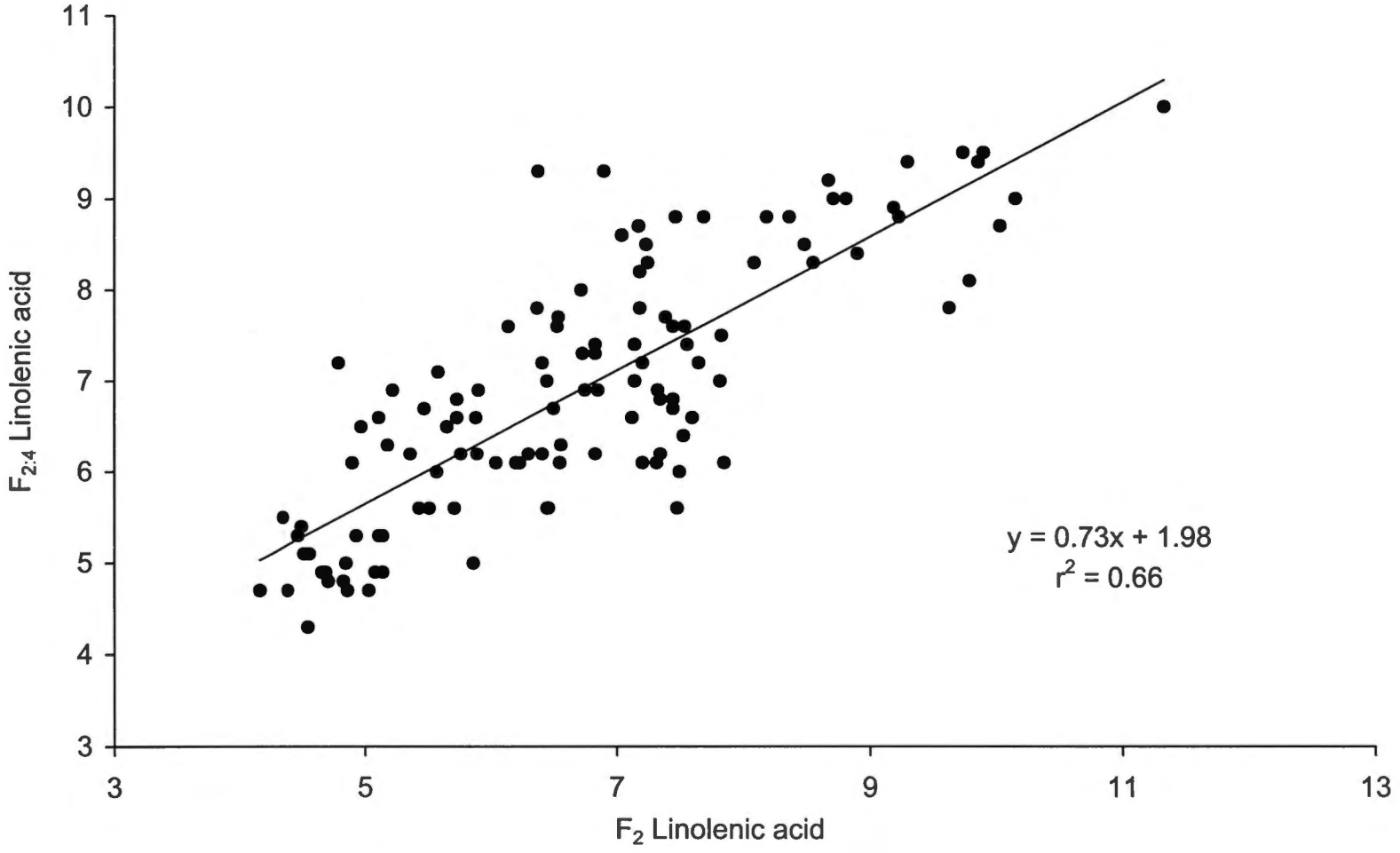

Figure 8. Linear regression of F_2 single plant linolenic acid versus $F_{2:4}$ line linolenic acid

palmitic (0.67 to 0.98). In our study oleic acid had a heritability of 0.41 and an r^2 value of 37%. This was in the same range as reported by Rebetzke et al. (1997) where estimated heritability for oleic was 0.36 to 0.66. In our study linolenic acid had the highest heritability of all the measured traits of 0.73 with an r^2 of 67%. We agree with the conclusion of Rebetzke et al. (1997) that selection for elite palmitic or linolenic acids could be accomplished in early generation by identifying superior F_2 plants.

To further explore this idea, we examined the fatty acids of single-plant seeds of the F_4 population of single plants within a small sample of seven $F_{2:4}$ rows. A high heritability was shown through linear regression among the F_2 single-plant population and the mean among F_4 single plant within the $F_{2:4}$ row for both palmitic acid and linolenic acid with an r^2 of 0.78 and 0.75 respectively (Figures 9 and 10). This supports our findings about the heritability of the palmitic acid and linolenic acid association between the F_2 single-plant population and a limited bulk seed sample from the $F_{2:4}$ rows discarded above. This suggests that recombinant plants carrying the traits of interest can be selected in the early breeding generations allowing the rapid genetic transfer of fatty acid traits.

Tables 13 and 14 show the genotypes corresponding to the F_2 single-plant with the amount of palmitic acid and linolenic acid and the F_4 single-plant population mean, minimum, and maximum range of palmitic acid and linolenic acid within the seven $F_{2:4}$ rows. When comparing minimum range of the palmitic acid and linolenic acid contents of the F_2 single plants to the F_4 single plants, an

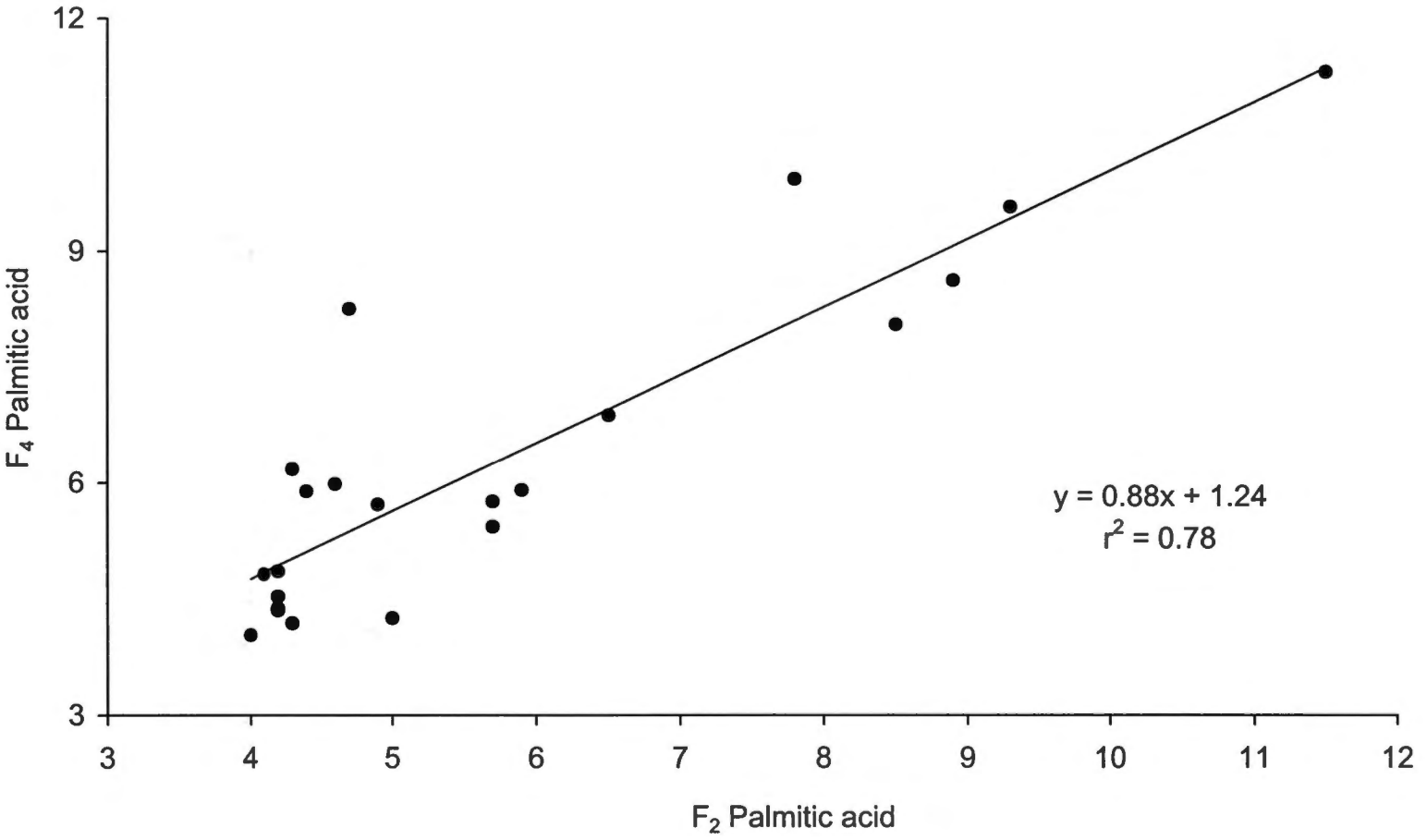

Figure 9. Linear regression of palmitic acid for F_2 single plant versus F_4 single plant within $F_{2:4}$ rows

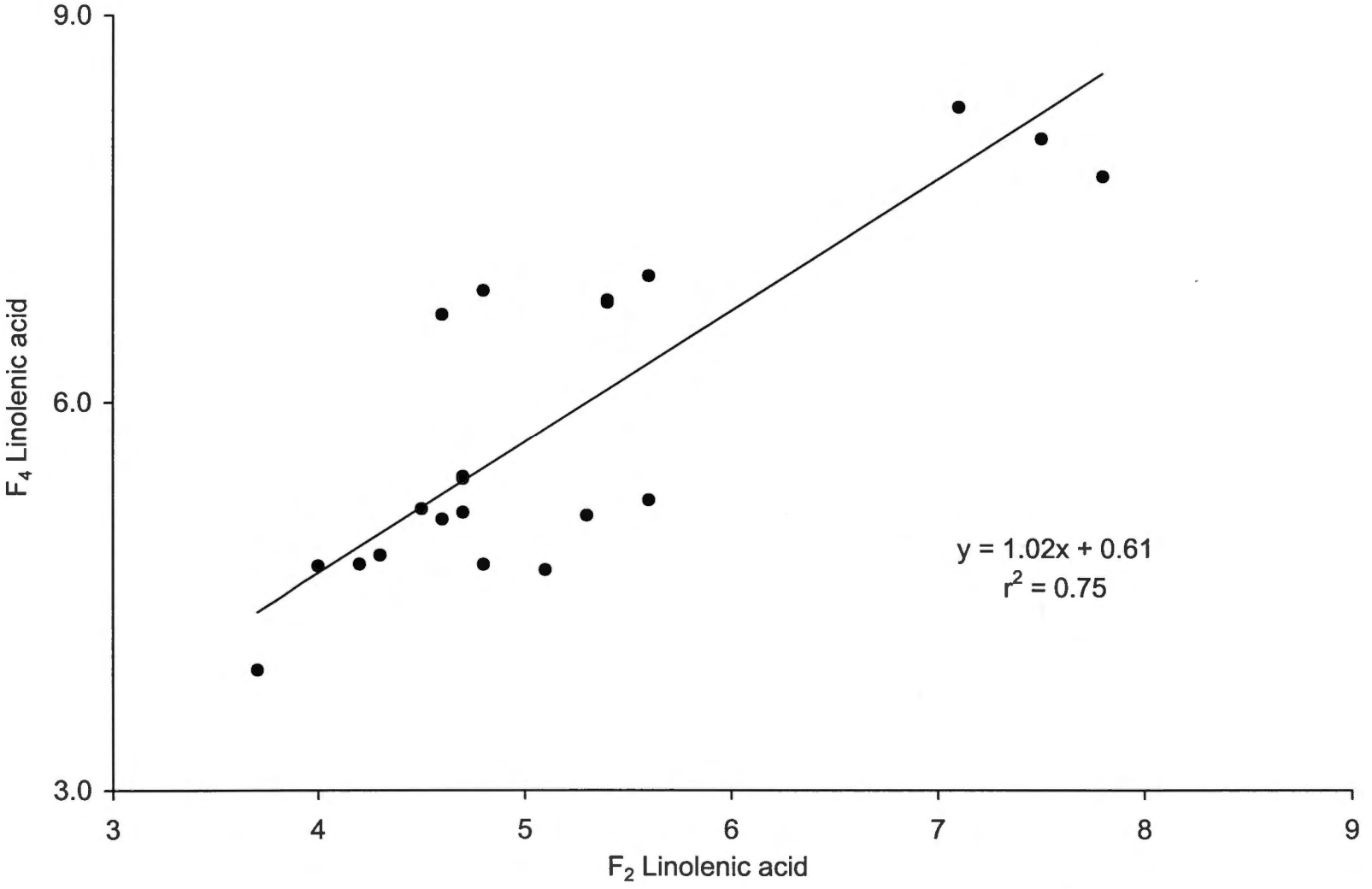

Figure 10. Linear regression of linolenic acid for F_2 single plant versus F_4 single plant within $F_{2:4}$ rows

Table 13. Mean, minimum and maximum range of palmitic acid (%) palmitic acid concentration (%) for single F_4 plants within $F_{2:4}$ lines, and corresponding level of palmitic acid of F_2 plant from which they were derived.

	F_2	F_4		
Genotype		Minimum	Mean	Maximum
504	4.1	3.9	4.8	7.9
464	4.2	3.9	4.4	5.9
502	4.2	3.7	4.9	8.8
558	4.2	3.6	4.5	9.2
589	4.9	4.3	5.7	9.3
546	8.5	4.2	8.0	11.6
444	8.9	4.3	8.6	12.5

Table 14. Mean, minimum and maximum range of linolenic acid concentration (%) for single F_4 plants within $F_{2:4}$ lines, and corresponding level of linolenic acid of the F_2 single plant from which the F_4 single plants were derived.

	F_2	F_4		
Genotype		Minimum	Mean	Maximum
444	4.2	3.9	4.7	8.9
546	4.5	4.4	5.2	6.7
589	4.8	3.7	6.9	9.4
464	5.1	3.9	4.7	5.8
558	5.6	3.9	7.0	9.9
502	7.1	5.2	8.3	11.2
504	7.8	4.8	7.7	9.9

even lower content is reported for both the palmitic acid and linolenic acid. For example, genotypes 546 and 444 have an F_2 value for palmitic of 8.5% and 8.9% respectively, which is about normal for soybean. However, the F_4 single-plant values are 4.2% for palmitic acid and 4.3% for linolenic acid. Similarly, genotype 464 has an F_2 value of 4.2% for palmitic acid and 5.1% for linolenic acid with an F_4 minimum value of 3.9% palmitic acid and 3.9% for linolenic acid. This shows the importance of carrying forward bulk rows in order to create good sources of germplasm. If selections are made solely upon the F_2 plant value, or if single-seed descent populations are formed, valuable sources of germplasm could be lost. Breeders utilizing low-palmitic, low-linolenic germplasm in crosses with normal elite lines can select the F_2 individuals with the lowest levels of these traits with reasonable expectation of recovering pure-line progenies that retain the traits. For example, the F_2 plant with the lowest palmitic acid (4.0%) produced all low-palmitic F_4 single plant progenies (3.8% to 4.3% palmitic). Similarly, the F_2 plant with the lowest linolenic acid (3.7%) produced all low-linolenic F_4 single plant progenies (3.7 to 4.4% linolenic). Moderately low palmitic F_2 plant selections produced F_4 single plant progenies ranging from low to normal palmitic acid, and moderately low linolenic F_2 plant selections produced F_4 single plant progenies ranging from low to normal linolenic acid. This suggests that breeders working with limited population sizes may still be able to identify low recombinants in later stages of selection, and illustrates the importance of deriving pure lines in order to maintain the stability of the fatty acid traits.

Tables 15, 16, and 17 show the best ten percent of high yielding, low palmitic acid, and low linolenic acid of the 128 genotypes of the $F_{2:4}$ population. Genotype 450 was the highest yielder at 4116.5 kg ha^{-1}(table 16). Genotype 442 had the lowest palmitic acid at 4.2% (table 16), and genotype 555 had the lowest linolenic content at 4.3% (table 17). In addition, this low linolenic genotype 555 was the third highest in yield (3565.5 kg ha^{-1}). The genotype 464 has a combination of both low linolenic (4.9%) low palmitic (4.7%) with a yield of 2387.5 kg ha^{-1} comparable in yield productivity to the parental cultivars (table 10). Genotype 575 also combines both high yield 2983.2 kg ha^{-1} and low linolenic acid at 4.9%. The presence of these low palmitic or low linolenic lines with favorable yielding capabilities demonstrates the practical development of modified fatty acid lines suitable for production agriculture. More research is needed to effectively combine low palmitic with low linolenic fatty acids and high yields and to evaluate such new lines for suitability for food processing applications.

Table 15. The top ten percent of high yielding $F_{2:4}$ lines of N97-3708-13 x Anand soybean.

Genotype	Mean yield (kg ha $^{-1}$)
450	4116.5
516	3565.5
555	3547.6
406	3504.0
592	3502.8
565	3490.5
490	3426.7
530	3393.1
421	3357.3
445	3316.9
418	3283.4
402	3265.4

Table 16. The top ten percent of low palmitic $F_{2:4}$ lines of N97-3708-13 x Anand soybean.

Genotype	Mean palmitic acid (%)
442	4.2
504	4.6
464	4.7
589	5.3
451	5.4
558	5.5
481	5.9
441	6.2
574	6.2
401	6.5
522	6.6
409	6.6

Table 17. The top ten percent of low linolenic $F_{2:4}$ lines of N97-3708-13 x Anand soybean.

Genotype	Mean linolenic acid (%)
555	4.3
561	4.7
403	4.7
514	4.7
444	4.7
401	4.8
448	4.8
464	4.9
520	4.9
534	4.9
481	4.9
575	4.9

5. CONCLUSION

There is a growing demand from health conscious individuals to consume oil with less saturated fat and trans fatty acids as studies have shown these fats to contribute to heart disease. Moreover, the FDA has written a proposal to implement the labeling of trans fatty acids. Currently, soybean oil is being used in the world's market for vegetable oil and for margarine and shortenings. However, the commercial soybean oil of today does not meet optimal health guidelines, which has prompted soybean breeders to develop a low palmitic, low linolenic, high yielding soybean for oil production. Soybeans are one of the major cash crops for Tennessee as certain soybean varieties are well adapted to this area. In our study, a low palmitic, low linolenic soybean line, N97-3708-13 was successfully crossed with a high yielding cultivar, Anand. Correlation of agronomic traits between the F_2 single plants and $F_{2:4}$ lines revealed positive findings such as a smaller seed size was significantly associated with reduced palmitic acid and higher yield. The F_2 single plant population contained germplasm with low palmitic acid and low linolenic acid. The parent-offspring regression of palmitic acid and linolenic acid of the F_2 and $F_{2:4}$ lines had high heritabilities, which is encouraging for plant breeders as it indicates that selection during early generations for these traits should be effective. Lastly, when comparing the F_2 single plants to the F_4 single plants within $F_{2:4}$ rows, a greater range in values was found in the F_4 generation. Soybean breeders should be able to use this variability to create a healthier soybean that could be used to meet consumer's demand. Additional research is needed to evaluate the

properties of these new soybean oils for acceptance and use by the food industry.

6. FUTURE RESEARCH

An F_2 generation allows plenty of opportunity for future breeding projects due to its genetic variability. Some superior genotypes for the desired genetic traits of low palmitic, low linolenic, and high yield have been found in the $F_{2:4}$ rows and $F_{2:4}$ single plants grown in this experiment. However, a genotype with all the combined traits does not yet exist. The $F_{4:5}$ seed needs to be grown in order to capture other possible genotypes with one or all of these desired traits. In addition, the $F_{4:5}$ seed needs to be planted in multiple environments to evaluate genotypic correlations, which would help understand the association of genotypes across different environments. Future germplasm or present germplasm available could be crossed together or with other elite lines and then inbred to produce a more desirable pure line.

The oil processing characteristics need to be studied on both the crude and refined, bleached, deodorized oil of new soybean lines with desired traits. This will require a large amount of oil for food quality assessment testing. Specific proposed characteristics to be measured from crude oil are listed below. Values in parentheses indicate acceptable commercial processing specification. The future soybean oil will be determined to be acceptable for oil processing if it meets or favorably exceeds minimum specifications, or meets or favorably is less than maximum specifications as listed following, in comparison with normal, commercial soybean oil.

1. Lovibond color (3.0 red and 1.0 yellow)
2. Free fatty acid content (1.0% maximum)
3. Neutral oil loss (7.5 maximum)

4. Iodine value (124-139)
5. Calcium content (100 ppm)
6. Magnesium content (100 ppm)
7. Chlorophyll pigments (1000 ppm)
8. Flash point (250°F)
9. Moisture and volatiles (0.5%)
10. Tocopherols
11. Fe, Cu, Zn, and Ni
12. Unsaponifiable matter
13. Fatty acid composition

Specific characteristics to be measured from RBD oil include:

1. Oxidative stability index
2. Refractive index
3. Cold test
4. Carbon number fingerprint
5. Chlorophyll pigments

Another challenge is to find a way to get the oil extracted at a reasonable cost, while mimicking industrial qualifications that would include not only extracting the oil, but also separating out the protein and carbohydrates for industrial use and meeting very specific requirements.

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APPENDICES

APPENDIX A

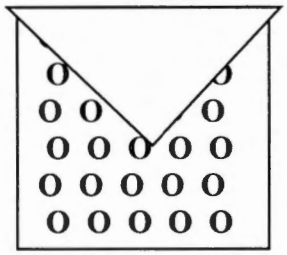
Overview of population development of low palmitic low linolenic soybean

1998 Summer N97-3708-13 X Anand

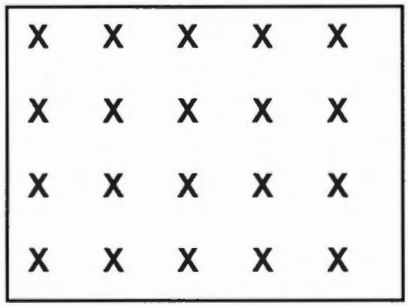
1998 Fall F_1 seeds harvested from cross

1998-99 Winter F_1 seeds planted in Puerto Rico winter nursery and grown as F_1 single plants

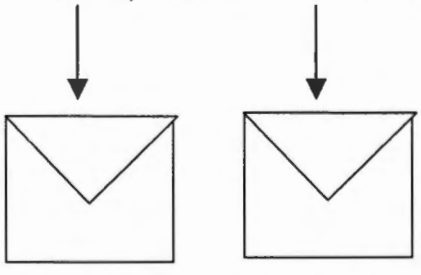
1999 Spring F_2 seeds from F_1 single plant collected and sent to Knoxville in an envelope



1999 Summer F_2 single plants grown in Knoxville;
1/4 AA 2/4 Aa 1/4 aa genotypic ratio for single gene trait



1999 Fall F_2 plants harvested individually and the $F_{2:3}$ seeds placed in an envelope and sent to winter nursery in Costa Rica



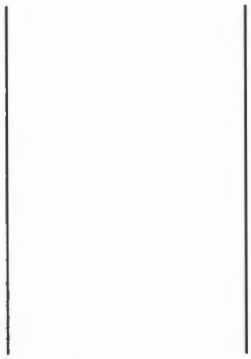
$F_{2:3}$ seeds $F_{2:3}$ seeds

APPENDIX A continued

1999/2000 Winter

Each envelope with $F_{2:3}$ seeds was planted in Costa Rica as $F_{2:3}$ rows for increasing amount of seeds;
1/4 AA 2/4 Aa 1/4 aa genotypic ratio among the $F_{2:3}$ rows;
1/4 AA 2/4 Aa 1/4 aa genotypic ratio within the $F_{2:3}$ rows

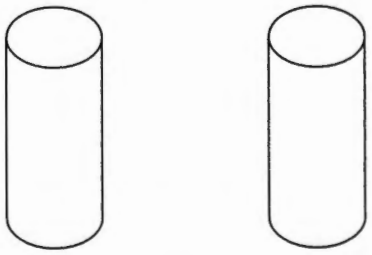
$F_{2:3}$ row $F_{2:3}$ row



2000 Spring

$F_{2:3}$ rows bulk harvested and $F_{2:4}$ seed placed in individual bags and sent to Knoxville

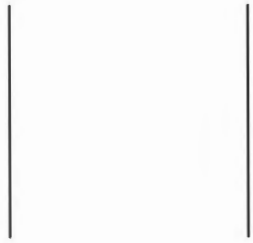
$F_{2:4}$ bag of seed $F_{2:4}$ bag of seed



2000 Summer

$F_{2:4}$ seed planted into $F_{2:4}$ rows at Knoxville Plant Science Farm
1/4 AA 2/4 Aa 1/4 aa genotypic ratio among the $F_{2:4}$ rows;
3/8 AA 2/8 Aa 3/8 aa genotypic ratio within the $F_{2:4}$ rows for single gene trait

$F_{2:4}$ row $F_{2:4}$ row



APPENDIX B

Yield data calculations for $F_{2:4}$ population.

In order to record yield in the desired bushels per acre, a conversion factor for each location was calculated using plot area. For the KPSF location the plot area was determined by multiplying two 16 ft rows by 15 in of width on either side of the rows (2.5 ft) which, equals 2(16 ft)(2.5 ft)= 80 ft^2. For the AMES location the plot area was determined by multiplying one 30 ft row by 15 in of width on either side of the row (2.5 ft) which, equals 1(30 ft)(2.5 ft)= 75 ft^2. These plot areas were then used in the following calculation to determine the conversion factor for bushels per acre for each location. For the KPSF location (43560 ft^2 /acre) (bushel/60lbs) (lbs/80 ft^2)= 9.075 bushel/acre. For AMES (43560 ft^2/acre) (bushel/60lbs) (lbs/75 ft^2)= 9.68 bushel/acre. Then the conversion factor for each location was used in the following formula, which adjusted for 13% moisture and considered each plots individual weight when calculating the yield in bushels/acre (bu/a):

Weight of plot (lbs) X (100 - moisture / 87) X conversion factor of location

Note AMES yield data was first converted from kg to lbs before entering into the formula. Finally bu/a was converted to Kg/hectare by multiplying the bu/a by a conversion factor of 67.19.

APPENDIX C

Chi-squared analysis of flower color for the F_2 population.

Purple versus white flower color (3:1 ratio)		
Observed numbers (O)	Expected numbers (E)	$(O-E)^2 / E$
130	(181)(3/4) = 136	0.2647
51	(181)(1/4) = 45	0.8000
Total 181	181	χ^2 calculated = 1.0647
		χ^2, 1df, 0.05 = 3.84

APPENDIX C continued

Chi-squared analysis of pubescence color for the F_2 population.

Tawny versus gray pubescence color (3:1 ratio)		
Observed numbers (O)	Expected numbers (E)	$(O-E)^2 / E$
135	(181)(3/4) = 136	0.0074
46	(181)(1/4) = 45	0.0222
Total 181	181	χ^2 calculated= 0.0296
		$\chi^2$1df, 0.05 = 3.84

APPENDIX C continued

Chi-squared analysis of two traits (flower color and pubescence color) for the F_2 population.

Purple or white flower color versus tawny or gray pubescence color (9:3:3:1 ratio)		
Observations	Expected numbers (E)	$(O-E)^2 / E$
100	(181)(9/16) = 102	0.0098
30	(181)(3/16) = 34	0.1176
35	(181)(3/16) = 34	0.0294
16	(181)(1/16) = 45	0.1176
Total 181	181	χ^2 calculated= 2.43
		χ^2,3df, 0.05 = 7.82

VITA

I was born in Greeneville, Tennessee on 27 January 1970. I attended Eastview Elementary, Greeneville Middle School, and graduated Greeneville High School in 1988. I then attended the University of Tennessee in Knoxville, TN and received a bachelor of science from the department of Food Science and Technology in 1992. In order to pursue my interests in education and international agriculture development, I traveled to Lesotho, Southern Africa on a two-year assignment as a Peace Corps education volunteer. My primary responsibility was instructing the classes of agriculture and biology for Ntlana-tsana High School in the village of Khanyane. I also had the opportunity to work with many developing aid organizations along with the local government and village to implement a water supply system and build extra classrooms for our school. Upon my return to the US I worked in Greeneville, TN at the agricultural experiment station until I moved back to Knoxville to pursue a master of science in plant and soil sciences with an emphasis on food science and technology. I was married to Djamel-Eddine Cherrak on 27 July 1999 and I gave birth to our first daughter, Miriam Leila Cherrak on 18 July 2000.