To the Graduate Council:

I am submitting herewith a thesis written by Yamika Nitá Stokes entitled "Quantitative Trait Loci and Heritability Analysis of TN93-99 x PI 416937 F_6 Derived Soybean Progeny." I have examined the final paper 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 and Soil Science.

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Quantitative Trait Loci and Heritability Analysis of TN93-99 x PI 416937 F_6 Derived Soybean Progeny

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DEDICATION

This thesis is dedicated to all of my family and friends for supporting me throughout my college career. I would also like to thank Dr. Harbans Bhardwaj for giving me the inspiration to attend graduate school. My sisters, Delphia Stokes and Alisa Davis, and my brothers Tyvan Stokes and David Salters have been a great motivation to me. Finally, I'd like to thank my fiancé, Curtis Phillips for believing in me when I sometimes didn't believe in myself. Thank you all.

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ABSTRACT

Soybean is now an essential and prominent source of vegetable protein and oil with a multitude of uses in human food and animal feeds. There are numerous industrial applications for the various components of this versatile legume. In the United States, soybean is considered a major grain crop and can be found in almost any food. Soy is also used in numerous industrial products. Most of the U.S. soybean production occurs throughout the Corn Belt to the Mississippi Delta.

One of the most important aspects of plant breeding is the improvement of the quality of crops for human and animal consumption. The use of molecular markers to detect improved traits created by crossing two cultivars is efficient due to its speediness and relative ease of manipulation. Knowing the location or loci on the chromosomes will make future crosses and trait detection easier for plant breeders.

This research intended to (1) use simple sequence repeat (SSR) markers to identify quantitative trait loci (QTL) for plant maturity, plant height, plant lodging, seed size, seed yield, seed oil concentration, and seed protein concentration in an F_6 population from a cross between the soybean lines TN93-99 and PI 416937 and (2) conduct a field test on F_6 pure lines to assess heritability for each quantitative trait.

TN93-99 and PI 416937 were polymorphic for 26% of the total simple sequence repeat markers screened. In the progeny screening for single F_6 plants grown at Knoxville Plant Science Farm in 2000, only 1% of markers displayed significant QTL for plant height [Satt409 (MLG A2) and Satt442 (MLG H)], seed size [Satt583, Satt444, and Satt430 (all on MLG B1)], seed oil concentration [Satt373 (MLG L)], and seed protein

concentration [Satt373 (MLG L)]. QTL analyses were conducted on replicated $F_{6:8}$ plots grown at Knoxville and Springfield, TN, and in a combined analysis. Several major QTL (R^2>10%) and minor QTL (R^2<10%) were discovered for the combined analysis. For plant maturity, the major QTL were Satt547 on MLG J (R^2=10%), Satt331 on MLG O (R^2=16%), and Satt581 on MLG O (R^2=19%). The minor QTL for plant maturity were Satt272 (R^2=7%), and Satt556 (R^2=7%) both on MLG B2, and Satt495 on MLG L (R^2=7%). The plant height major QTL were Satt200 (R^2=12%), and Satt225 (R^2= 10%) on MLG A1, Satt172 on MLG D1b+W (R^2=11%), and Satt581 on MLG O (R^2= 12%). The three minor plant height QTL were Satt272 (R^2=7%) on MLG B2, Satt367 (R^2=9%) on MLG I, and Satt574 (R^2=9%) on MLG J. There was no major plant lodging QTL discovered for the combined analysis. Minor plant lodging QTL were Satt442 (R^2=6%) on MLG H, and Satt495 (R^2=8%) on MLG L. Seed size major QTL were Satt200 (R^2= 11%) and Satt599 (R^2= 16%), both on MLG A1. There were no minor QTL for seed size in the combined analysis. Seed yield major QTL were Satt545 (R^2=16%) and Satt599 (R^2= 20%) with both markers on MLG A1, Satt272 (R^2=10%), and Satt556 (R^2=12%) on MLG B2. There was a minor QTL for seed yield at Satt373 (R^2=9%) on MLG L. Major QTL for seed oil concentration were Satt030 (R^2=21%) and Satt146 (R^2=17%) on MLG F, and Satt215 (R^2=12%) and Satt547 (R^2=10%) on MLG J. A minor seed oil concentration QTL was found at Satt419 (R^2=8%) on MLG I. Major QTL for seed protein concentration were Satt373 on MLG L (R^2=10%), and Satt345 on MLG O (R^2= 13%). There were no minor QTL for seed protein concentration in the combined analysis.

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I. INTRODUCTION

The purpose of plant breeding is to improve the quality of plants. Breeding techniques have been widely used since the beginning of civilization. Mankind began selecting crops that had desirable traits or features, thus utilizing the skill of plant selection. Even the hunter/gatherer selected seeds from certain crops and planted from those chosen.

Gregor Mendel, James Watson, and Francis Crick are three of the most world-renowned geneticists. Mendel's work discovered the inheritance of characters in plants. His hybrid experiments in peas [*Pisum sativum* L.] led to the formulation of the laws of segregation and independent assortment. Mendel's principles have provided the basis for plant genetics (Russell, 1996).

The foundation for modern molecular genetics has been inspired by the research of Watson and Crick, which led to the discovery of the structure of DNA (deoxyribonucleic acid) (Russell, 1996). The DNA molecule takes the shape of a double helix, an elegantly simple structure that resembles a gently twisted ladder. The rails of the ladder are made of alternating units of phosphate and the sugar, deoxyribose; the rungs are each composed of a pair of nitrogen-containing nucleotides.

Soybean [*Glycine max* (L.) Merr.] research was uncommon in the United States until the mid 1900s. Martin G. Weiss along with Jackson L. Carter, of the U.S. Regional Soybean Laboratory at Urbana, Illinois, initiated the development of a comprehensive soybean germplasm collection in the late 1940s. Dr. Richard L. Bernard had been the curator of the northern soybean germplasm collection at USDA-ARS Urbana-

Champaign, IL since 1954, and Dr. Randy Nelson is the current curator. Edgar Hartwig was the curator of the southern soybean germplasm collection, located in Stoneville, Mississippi for over 40 years and the current curator is Dr. Robert Paris. Currently there are about 13,000 strains of soybeans in the USDA germplasm registry, (Hymowitz, 1990).

Soybean were first grown as a crop in China 5000 years ago (www.asa-europe.org, verified July 2, 2002). Soybean belongs to the *Leguminosae* family of plants. The seeds grow in pods that develop in clusters with each pod usually containing two or three seeds. Soybean manufacturers use the cultivated species to make food and industrial products.

Glycine max (L.) Merr. is the cultivated species from which the cross for this thesis research was derived, TN93-99 x PI 416937. Both parental cultivars were chosen because of their differences in protein and oil content. The crossing of the two should create new progeny with a range in agronomic and seed quality traits. The traits that were studied in this experiment were plant maturity, plant height, plant lodging, seed size, seed yield, seed oil concentration, and seed protein concentration.

TN93-99 is an F_4 derived line from the cross, 'Hutcheson' x [(TN85-55 x 'TN 5-85')]. TN85-55 is a selection from the cross, TN77-46 x 'Fayette'. TN77-46 is a selection from the cross 'Forrest' x 'Mitchell'. TN93-99 was selected based on agronomic traits and superior yield. TN93-99 is most similar in its characteristics to the variety 'Hutcheson'. TN93-99 matures one day later than Hutcheson. TN93-99 has white flowers, gray pubescence, tan pod wall, and a determinate growth habit. The seeds are yellow with smooth seed coats and brown hila. The seed size is approximately 140

mg seed^{-1}. Seed yield for TN93-99 in the USDA tests was 3192 kg ha^{-1} compared to 'Hutcheson' with a yield of 2990 kg ha^{-1} for those tests. Average protein concentration for this cultivar is approximately 410 g kg^{-1}. TN93-99 has good lodging and shattering resistance. TN93-99 has been released as a germplasm and is currently registered in the national seed storage germplasm bank in Fort Collins, CO [Pantalone et al., (in press (a))].

The means for TN93-99 and 'Hutcheson' were 2598.0 kg ha^{-1} and 2508.4 kg ha^{-1}, respectively in the 1998-2000 Tennessee State Variety Tests. Yield for TN93-99 and 'Hutcheson' was also compared for six other states (VA, MD, AR, MS, NC, and SC) with means of 3231.8 4 kg ha^{-1} and 3070.6 4 kg ha^{-1}, respectively (Pantalone, pers. comm.). Thus, TN93-99 is a high yielding germplasm that should contribute valuable agronomic alleles in this study.

Pedigree information for PI 416937 is accessible through the U.S. Germplasm Information Network (http://www.ars-grin.gov, verified July 1, 2002). The native Japanese name of PI 416937 is "*Houjaku Kuwazu*," and it is a pure line cultivar. The National Institutes of Agriculture of Japan donated the seeds to the United States Department of Agriculture in 1974. PI 416937 has purple flowers, gray pubescence, yellow seed color, tan pod wall, and a determinate growth pattern. The seed size is approximately 170 mg seed^{-1}. Seed protein concentration for this line is approximately 430 g kg^{-1}. PI 416937 has been used frequently as a breeding parent in the USDA Southern Uniform Tests from 1993- 2002 (Appendix A). Eleven lines carrying a 50% parentage of PI 416937 have been evaluated in the USDA testing program during that period. Five of those progeny (N90- 7199, N90- 7202, N91- 8005, N91-8006, and N93-

1264) were used in further crosses by breeders producing 78 new lines, which were tested in recent USDA Southern Regional Tests. Thus, knowledge of allelic contributions of PI 416937 through QTL research has potential impact on current soybean breeding populations in Southern USA.

PI 416937 has been in aluminum tolerance and drought resistance research (Villagarcia et al., (2001), Bianchi-Hall et al., (2000), Foy et al., (1993), and Goldman et al., (1989) have used PI 416937 in their aluminum tolerance or drought resistant studies. This plant introduction has been proven beneficial for agronomic and stress tolerance improvement, making it a valuable parental germplasm for molecular genetic plant breeding research.

The objectives of this research were to (1) use simple sequence repeat (SSR) markers to identify quantitative trait loci (QTL) for plant maturity, plant height, plant lodging, seed size, seed yield, seed oil concentration, and seed protein concentration in an F_6 population from a cross between the soybean lines TN93-99 and PI 416937 and (2) conduct a field test on F_6 pure lines to assess heritability for each quantitative trait.

II. LITERATURE REVIEW

A. Restriction Fragment Length Polymorphism (RFLP) Mapping of Quantitative Trait Loci (QTL)

This literature review will first discuss soybean QTL mapping by the RFLP technique, then review soybean QTL mapping by the SSR technique, and conclude the review with a discussion on soybean heritability and genetic variance.

There is now increased research that can be found on identifying quantitative traits in the soybean genome. One reason for the availability of information is that QTL can now be located with the aid of DNA markers. Marker usage is a new form of technology that is currently creating a faster means of determining variation among genes at given loci (Shoemaker, 1994).

Restriction fragment length polymorphisms are variations in the DNA. Restriction enzymes are used to cut the DNA in specific patterns. An advantage of working with RFLPs is that the sequence need not be known. All that is needed is a genomic clone that can be used to detect the polymorphism. The polymorphisms result from differing fragment lengths for the same sequential base. A QTL is a region on the chromosome that affects a trait. Many traits are usually affected by more than one gene. QTL mapping is becoming an integral part in the selection of quantitative characters in crop breeding programs (Lee et al., 1996a). Molecular markers can aid in the identification of marker-QTL associations in different environments and populations.

According to Shoemaker (1994), QTL research was conducted to isolate and map the genes in soybean that alter composition. The techniques and germplasm they

developed will help soybean breeders improve end-use value. Researchers have been able to determine the locations of genes on chromosomes, but the basic genetic control of these traits is not known. New cultivars of soybeans with varied compositions can be designed using this technology.

QTL research was done to identify associations in different environments and populations between 'Young' x PI 416937 and PI97100 x 'Coker 237.' Lee et al. (1996a) reported that these two populations were evaluated with RFLP markers to identify additional QTL related to seed protein and oil. One hundred- twenty F_4 derived lines were scored for segregation at 155 RFLP loci and were grown and evaluated at Plains, GA, and Windblow, NC for seed protein and oil in 1994 (Lee et al., 1996a). One hundred-eleven F_2 derived lines and the parents, PI97100 x 'Coker 237,' were evaluated for segregation at 153 RFLP loci. The two locations where phenotypic data for seed protein and oil were obtained were Athens, GA and Blackville, SC. Single-factor analysis of variance (ANOVA) was used to statistically extrapolate data for the Young x PI 416937 population. Five of seven independent markers associated with seed protein, and all four independent markers associated with seed oil in the combined analysis over locations were detected at all three locations. Single-factor ANOVA and interval mapping were used to detect QTL for the PI97100 x Coker 237 population. Three of four seed protein markers and two of three seed oil markers were detected at both locations (Lee et al., 1996a).

The results for both populations revealed the consistency of QTL across locations, which might be due to the high heritability and the relatively few QTL with large effects conditioning these traits. Interval mapping for the PI97100 x Coker 237

population indicated that seed protein and oil content differed in the Athens, GA and Blackville, SC locations. Lee et al. (1996a) stated that these differences might result from the power of QTL mapping being dependent on the level of saturation of the genetic map. There was increased seed protein associated with decreased seed oil in the PI97100 x Coker 237 population (r= -0.61). Various common markers ($P \leq 0.05$) on molecular linkage groups (MLG) E, G, H, K, and UNK2 were identified for both seed protein and oil. In both populations, one QTL on MLG E was associated with seed protein (Lee et al., 1996a).

Another RFLP QTL research project was conducted by Mian (1997) to detect genotypes that differ in chlorimuron ethyl sensitivity (CS). In earlier research, two independent marker loci were linked to two QTL controlling CS in a soybean population derived from a cross of PI97100 (sensitive to chlorimuron ethyl) x Coker 237 (tolerant to chlorimuron ethyl). The main objective for that study was to quantify the association of the two marker loci with seed yield and related traits in the given soybean population after chlorimuron ethyl was applied. One hundred-eleven F_2 derived lines and the parents, PI97100 x Coker 237, were grown in Athens, GA and Blackville, SC where phenotypic data for seed yield and other traits were obtained. Replicated plots were used and the plants were grown in 1994 and 1995. The results were that the two CS marker loci explained as much as 50% of the genetic variation in seed yield and seed number m^{-2}. There were no associations found for seed weight, plant height, lodging, seed protein, and seed oil. There were also no epistastic interactions between the two marker loci for any trait. A strong positive correlation for seed yield (r= 0.74 to 0.94) with seed

number m^{-2}, and the effect of chlorimuron ethyl on seed yield was due mainly to its effect on seed number m^{-2} rather than seed weight (Mian, 1997).

B. Simple Sequence Repeat (SSR) Mapping of QTL

Akkaya, Bhagwat, and Cregan (1992) conducted research to ascertain the presence and degree of simple sequence repeat (SSR) DNA length polymorphism in soybean. A Genbank search revealed no $(CA)_n$ or $(GT)_n$ SSRs with *n* greater than eight in soybean. However, five $(AT)_n$ and one $(ATT)_n$ SSRs with *n* ranging from 14 to 27 were detected (Akkaya et al., 1992). The PCR amplification method used primers that flanked the six SSR loci from 43 homozygous soybean genotypes.

At three loci, amplification produced one PCR product per genotype and revealed 6, 7, and 8 product length variants (alleles) at the three loci, respectively. F_1 hybrids between parents carrying different alleles produced two PCR products identical to the two parents. Codominant segregation of alleles among F_2 progeny was demonstrated at each locus. A soybean DNA library was screened for the presence of $(CA/GT)_n$ SSRs. Sequencing of positive clones revealed that the longest such SSR was $(CA)_9$. Thus, $(CA)_n$ SSRs with *n* of 15 or more are apparently much less common in soybean than in the human genome. In contrast to humans, $(CA)_n$ SSRs will probably not provide an abundant source of genetic markers in soybean. However, the apparent abundance of long $(AT)_n$ sequences should allow this SSR to serve as a source of highly polymorphic genetic markers in soybean (Akkaya et al.,1992).

According to Rongwen et al., (1995) conventional morphological and pigmentation traits, as well as disease resistance, have been used to distinguish the uniqueness of new soybean cultivars for purposes of plant variety protection. Increasing numbers of cultivars and a finite number of conventional characters may be associated with traits not establishing uniqueness (Rongwen et al., 1995). The main purpose of their work was to provide an initial evaluation of microsatellite (SSR) DNA markers to develop unique DNA profiles of soybean genotypes.

Tandemly repeated 205 basepair DNA core sequences make up microsatellites. Some examples of these sequences are $(AT)_n$ / $(TA)_n$ and $(ATT)_n$/$(TAA)_n$. PCR primers are set to amplify a given DNA basepair sequence, which makes viewing polymorphism easier. The term "*n*" is the variation in number of tandem repeats and results in PCR product length differences. SSR alleles preset at three $(AT)_n$ / $(TA)_n$ and four $(ATT)_n$/$(TAA)_n$ loci were found in each of 96 diverse soybean genotypes. Eleven to 26 alleles were found at each of the seven loci. Identical SSR allelic profiles were found on two genotypes and they had similar pedigrees (Rongwen et al., 1995). All 96 genotypes had an average of 0.97 for gene diversity and the 26 North American cultivars had a score of 0.74. These measurements are much higher than those obtained using RFLP markers. SSR markers provide an excellent complement to the RFLP markers that had been previously used by researchers to characterize soybean genotypes.

In 1999, Cregan et al. published their study of an integrated genetic linkage map of the soybean genome by the use of SSR markers. Over the past ten years, molecular genetic maps were primarily based on RFLP markers. "Parental surveys have shown that most RFLP loci have only two known alleles (Cregan et al., 1999)." However, RFLP

probes typically hybridize and map to more than one position in the genome because the soybean is an ancient polyploid. SSR markers are single locus markers with multiple alleles, and the polymorphic potential is dependent on the number of alleles and their frequencies. "Single locus markers provide an unambiguous means of defining linkage group homology across mapping populations (Cregan et al., 1999)." Their research successfully developed and mapped a large set of SSR markers. Six hundred and six SSR loci were mapped in one or more of three populations; the USDA/Iowa State *G. max* x *G. soja* F_2 population, the University of Utah Minsoy x Noir 1 recombinant inbred population, and the University of Nebraska Clark x Harasoy F_2 population. Each SSR marker was mapped to a single locus in the genome. Because SSR loci were segregating in two or all three populations, it was relatively simple to align the 20+ linkage groups derived from each of the three populations into a consensus set of 20 homologous linkage groups presumed to correspond to the 20 pairs of soybean chromosomes.

Orf et al. (1999) conducted research to determine linkage in recombinant inbred soybean *Glycine max* (L.) Merr. populations. RFLP and SSR markers were used in that experiment. Interval mapping was used to identify QTL. Plant height, lodging, date of flowering, reproductive period, maturity, yield, seed weight, seed oil, seed protein, leaf length, and leaf width traits were analyzed in that study.

Chapman (2000) summarizes the utility of SSRs: a) microsatellite markers are highly polymorphic and codominant, b) PCR generated microsatellites allow unambiguous separation of alleles, allowing for a precise calculation of allele frequencies, c) microsatellites are abundant and evenly dispersed throughout the genome d) microsatellites are probably selectively neutral, e) microsatellites are fast and easy to

detect, using only nanograms of DNA, f) the results are highly reproducible, and g) information is easily exchangeable between laboratories.

In 2000, Sebolt et al. used RFLP and SSR markers to identify polymorphisms for the backcross parents, A81- 356022 x an F_2–derived line of A81- 356022 X PI 468916, and their progeny. The main goal of that study was to increase seed protein content and test two *G. soja* QTL alleles in the backcrossing population. There was a significant QTL for the *G. soja* allele found on MLG I.

Research was conducted on plant introductions by Burnham et al., 2002. They compared the genetic diversity present among soybean PIs resistant to the fungal pathogen, *Phytophthora sojae*, in relation to cultivars and breeding lines that represent U.S. breeding germplasm. They sought to develop guidelines for the genetic study and practical use of the resistant resources in applied breeding. A total of ninety-three accessions from South Korea, China, and Japan and 15 genotypes from the USA were evaluated for 52 simple sequence repeat (SSR) primer pairs. Nei's distance estimates were computed by SSR data. Various analyses demonstrated that accessions from South Korea are genetically different from U.S. germplasm, which indicated that the South Korean germplasm may contain alleles not present in U.S. cultivars, such as new alleles for *P. soja* resistance (Burnham et al., 2002).

C. Heritability and Genetic Variance

Heritability may be defined as the phenotypic variation among individuals that is due to genetic differences among them. Broad-sense heritability is estimated from the

ratio of the total genetic variance to the phenotypic variance. Narrow-sense heritability is estimated from the ratio of the additive portion of the genetic variance to the phenotypic variance (Fehr,1991).

Heritability can be pointed out in two contexts: 1) the context of the trait being determined by the genotype of the individuals being observed, and 2) the context of the trait being passed from parents to their progeny (Nyquist, 1991). The first context is known as broad sense heritability (H) or the genotypic variance divided by phenotypic variance. This gives a proportion of the phenotypic variance that is genetic in origin. The second context is narrow sense heritability (h^2) or the additive variance divided by phenotypic variance. The portion of the phenotypic variance that is due to variance in breeding values among the individuals reflects narrow sense heritability, which provides an estimate of the transmissible portion of the genetic variance from parent to offspring (Nyquist, 1991).

Several soybean researchers have conducted heritability studies. For example, Pantalone et al. (1996) conducted heritability research on soybean root fibrosity and genotypic correlations with agronomic and seed quality traits. Seed protein generally has a negative genetic correlation with seed yield, which creates an obstacle in selecting for both traits. However, the population developed by Pantalone et al. (1996) was unusual because it did not exhibit a negative association between seed protein and yield. PI 416937 was chosen for their experiment because of its extensive fibrous root surface area and drought tolerance. Since the southern region of the United States is prone to drought, high yielding lines derived from the plant introduction may be a wise choice for production in that area. Heritability estimates for root score were low: 0.39 on an entry-

mean basis from testing in two years and two locations, and 0.24 from a realized heritability estimate. The genotypic correlation between root score and seed protein concentration was positive ($r_g = 0.42$); thus, the results were somewhat encouraging, and suggested that PI 416937 is a valuable germplasm resource for developing high protein, high yielding breeding lines (Pantalone et al., 1996). Breeders who are currently utilizing PI 416937-derived lines in their development programs would benefit from new molecular genetic knowledge of QTL governing agronomic and seed quality traits.

III. METHODOLOGY

A. Field Breeding Study

A cross between TN93-99 and PI 416937 was made in 1998 in Knoxville, TN. The F_1 plants were sent to Costa Rica, where they were able to grow during the winter of 1998-1999. In April 1999, the F_2 population plants was grown in Costa Rica, and a single pod was harvested from each plant for generation advancement by single seed decent (SSD). In late summer 1999, $F_{2:3}$ plants were harvested in the same manner as the F_2 population. This SSD procedure was followed for the $F_{3:4}$ plants in the late fall of 1999, and the $F_{4:5}$ plants in spring 2000. The $F_{5:6}$ seeds were sent to Knoxville, TN, where they were planted in hill plots (1 seed per hill, at 1 m spacing between hills) at the University of Tennessee, Knoxville Plant Experiment in 2000. Single $F_{5:6}$ plants were threshed separately, and their $F_{6:7}$ seeds were bagged and shipped to Costa Rica to increase seed as $F_{6:7}$ plant rows in the winter nursery. Those rows were each bulk harvested, separately, and their seed was sent back to Knoxville and planted in replicated trials in spring 2001.

Two environments were tested in 2001: Knoxville Experiment Station in Knoxville, TN and Highland Rim Experiment Station in Springfield, TN. A randomized block design was used at both locations. There were three replications of 99 $F_{6:8}$ Recombinant Inbred Lines (RIL) at Knoxville, TN; however, sufficient seed was available to plant only 78 of those RIL in two replications at Springfield, TN. In addition, the lines TN93-99 and '5601T' were included as high yielding checks at both locations as relative agronomic performance gauges. Phenotypes were evaluated for

maturity when 95% of the pods attained their final color. Plant lodging was recorded using a score of 1 (all plants in a row standing erect) through 5 (all plants prostrate). The $F_{6:8}$ plots were harvested in fall 2001 after endtrimming rows to 4.9m. Other phenotypic traits measured in the study were plant height, seed yield, seed size, seed protein concentration, and seed oil concentration. A 20 to 25 gram seed sample, per plot, was sent to the USDA, ARS Northern Regional Research Center in Peoria, Illinois To measure oil and protein concentration of the seed via near-infrared reflectance.

B. DNA Isolation and Characterization

DNA was extracted and purified using the DNeasy Plant Mini Kit, also known as Qiagen Kit (Quigen, Hilden, Germany)(Appendix B). The standard protocol for DNA extraction was followed. Newly developing leaves were collected from the individual F_6 plants and stored at -20°C until used for DNA extraction.

Each extracted DNA sample was quantified using a Hoefer- Dynaquant 200 flourometer. A standard concentration of 100 ng/µL was used to calibrate the flourometer. Two microliters of each sample were added to a cuvette containing 2 ml of 1X TNE (100mM Tris, 10mM EDTA, 2 M NaCl) and Hoescht dye (Appendix C). Each sample was then diluted to achieve a working stock concentration of 40ng/µl.

A PCR master mix was made for each primer before amplification (Appendix C). The master mix for each sample contained 7.4µl dd H_2O, 1µl PCR buffer (10X), 1µl nucleotide mixture (2mM)(Pharmacia, Piscataway, NJ), 0.5µl Klentaq solution (0.5u/µl)(AB Peptides, Inc, St. Louis, MO), 2µl of DNA (20ng/µl), and 0.5 µl forward

and reverse SSR Primer (20 μM). Then, 10 μl of the master mix was added to each reaction tube and spun for 30 seconds. Next, the tubes were placed in a Hybaid MBS thermocycler to amplify the DNA via polymerase chain reaction (PCR) for 35 cycles. The cycling consisted of denaturing, annealing and elongation. Denaturation is the first cycle and occurs for 30 s at 93°C to activate the enzyme and denature the DNA. Annealing takes place as the temperature decreases from 93°C to 48°C for 30 s, and elongation occurs at 72°C for one minute. These temperatures were repeated for 35 cycles.

After the PCR amplifications were completed, a 3 μl (6X) loading buffer (Appendix C) was added to each tube. Each 6% polyacrylamide gel consisted of 28.5ml of 40% bis-acrylamide solution + 160.2ml of 0.60X TBE Buffer + 1.33ml of 10% APS solution + 66.5 μl TEMED (Appendix C). In addition, 50 μl of (10mg/mL) ethidium bromide (Appendix C) was added to the bottom reservoir of 0.5X TBE buffer. Any bubbles found at the bottom of the plates were removed with a pipette. The top reservoir also contained 0.5X TBE Buffer with no ethidium bromide added. Ten microliters of PCR product was added to each well on the gel with a 100kb ladder or two empty spaces placed in the middle lanes. Once all the lanes were loaded, the top lid and clips were placed on the gel plates for support. The electrophoresis apparatus was run at 300 volts (constant voltage) for three hours to allow DNA to migrate. A small fan was used to keep the glass plates cool during the running period. DNA migration banding patterns were visualized under ultra-violet light.

C. Statistical Analyses

Those DNA bands characteristic of TN93-99 at a specific SSR locus for each individual RIL received a score of 1; those bands characteristic of PI 416937 received a score of 3 at a specific SSR locus; heterozygous lines received a score of 2. All bands that were undetectable received a score of 4. An analysis of variance program was used in SAS (SAS Institute, 1998, Cary, NC) to determine the least square means of the data for each of the two testing environments. Those LS Means were then utilized in a single-factor ANOVA program to detect QTL for agronomic and seed quality traits based on DNA band class means. The critical decision significance value to declare a QTL as significant was adjusted to alpha= 0.05/k, where k= the number of polymorphic markers analysed for that specific linkage group. The number of markers varied from 1- 7 for the various linkage groups (Figure 1).

The Proc Corr procedure for SAS was used to estimate correlations among all seven traits. Correlations were considered significant if the p- value was less than 0.05. For $r \geq 0.80$, the correlation was considered to be strong; for $0.40 \leq r \leq 0.79$ the correlation was considered to be moderate, and for $r < 0.39$ the correlation was considered to be weak.

Heritability values were estimated using the Proc Mixed procedure of SAS to derive variance component estimates. Those variance components were then utilized to estimate heritabilities on an entry mean basis at each location, and in a combined analysis. For the individual location data (Knoxville and Springfield) the following equation was used for heritability: $h^2 = \sigma^2_G / \sigma^2_P$, where $P = (\sigma^2_G + \sigma^2/r)$. In this instance,

A1	A2	B1	B2	C1
Satt382	Satt207	Satt197	Satt083	Satt578
Satt042	Satt187	Satt430	Satt272	Satt399
Satt545	Satt424	Satt444	Satt556	Satt190
Satt200	Satt158	Satt583		Satt476
Satt225	Satt409			Satt180
Satt599				Satt396
C2	D1a+Q	D1b+W	D2	E
Satt170	Satt184	Satt216	Satt135	Satt212
Satt489	Satt532	Satt266	Satt002	
Satt079	Satt383	Satt095	Satt389	
Satt307	Satt408	Satt537	Satt413	
Satt371	Satt129	Satt172	Satt386	
	Satt507			
	Satt147			
F	G	H	I	J
Satt146	Satt235	Satt568	Satt419	Satt547
Satt030	Satt324	Satt353	Satt367	Satt215
Satt145	Satt138	Satt442		
Satt252	Satt199	Satt253		
Satt554	Satt472			
Satt425				
K	L	M	N	O
Satt137	Satt006	Satt494	Satt530	Satt445
Satt326	Satt373	Satt346	Satt125	Satt188
Satt475	Satt495		Satt387	Satt345
Satt196			Satt022	Satt581
Satt178			Satt410	Satt331
			Satt255	

Figure 1. Eighty- seven polymorphic markers, listed by molecular linkage group, screened with DNA of 99 F_6 individuals of TN93-99 x PI 416937.

the genetic variance is divided by the phenotypic variance. The phenotypic variance is equal to the genetic variance plus the residual variance divided by the number of reps per location. Heritability estimates were also determined for the combined analysis. The following equation was used to obtain those estimates: $h^2 = \sigma^2_G / \sigma^2_P$, where $P= (\sigma^2_G + \sigma^2_{GE}/ e + \sigma^2/(avgr*e))$. In this case, the genetic variance is divided by the phenotypic variance, but the phenotypic variance is derived with information over environments. For the combined analysis, the phenotypic variance is equal to genetic variance plus the variance of the genotype x location divided by the number of locations plus the residual variance divided by the average of the total number of replications times the number of locations.

A QTL was considered to have a major effect for a trait if its $R^2 \geq 10$. A QTL was considered to have a minor effect for a trait if its $R^2 < 10$. An LS Means statement was added so that the PI 416937 effect could be determined. The effect was estimated for QTL as the difference between trait means for the DNA band marker class 3 (PI 416937 allele type) minus trait means for marker class 1 (TN93-99 allele type) and was reported in SI units as the effect of PI 416937 allele substitution for the trait at the specific SSR locus.

Soybase (http://129.186.26.94, verified July 1, 2002) was utilized to systematically search each of the 20 soybean composite linkage group maps in order to identify previously reported QTL or other QTL related to those discovered in this study. All QTL reported on Soybase within a 10 cM range, upstream or downstream, of the SSR marker locus, which identified QTL in this study, were cited in the Results and Discussion.

IV. RESULTS AND DISCUSSION

In the combined analysis, RIL 2286 was the highest yielding (2975 kg ha^{-1}) (Table 1), which is important because it produced yields comparable to the next highest yielding line, 5601T (2760 kg ha^{-1}), an exceptionally productive, newly released cultivar (Pantalone et al., (in press (b)).

Several of the very lowest yielding lines had high protein concentration (Table 2). However, the correlation between yield and protein was weak (r= -0.17). In fact, there were no strong correlations (r>80) found for the combined location data (Table 3). Protein and oil had the highest correlation (r= -0.76) of all comparisons, which suggests that protein is produced at the expense of oil. Height and maturity and height and lodging were had moderate correlations. All other significant correlations were weak.

The R^2 values for the fit of the model were high to moderately high, and the coefficient of variability (CV) values were moderate to exceptionally low in the analysis of variance combined over locations (data not shown). For example, in the combined analysis high R^2 values with low CV values were found for plant maturity (R^2 = 0.95, CV= 1.4%), plant height (R^2 = 0.87, CV= 9.4%), seed size (R^2 = 0.93, CV= 3.6%), seed oil concentration (R^2 = 0.91, CV= 1.6%), and seed protein concentration (R^2 = 0.90, CV= 1.5%). Seed yield, often one of the most difficult traits to measure in field research had a moderately high R^2 (0.80) and reasonable CV (16.1%) in the combined analysis. These values are important because they provide credibility that QTL detected in this research are representative of the traits measured.

Table 1. The ten highest seed yield values for the combined analysis of TN93-99 x PI 416937 F_6 derived progeny in 2001.

Genotype	Maturity[a]	Lodging[b]	Height[c]	Seed Size[d]	Seed Yield[e]	Oil[f]	Protein[g]
TxP_2286	128.1	1.5	68.6	144.0	2948	188.4	358.4
5601T	121.1	1.6	81.3	127.1	2760	186.1	363.6
TxP_2270	121.4	1.8	62.9	132.6	2738	188.7	371.8
TN93-99	123.3	1.3	73.2	137.0	2728	195.7	352.0
TxP_2386	127.8	1.8	60.1	141.8	2684	177.2	367.3
TxP_2233	126.1	1.3	67.5	153.5	2665	189.4	351.8
TxP_2296	128.8	1.3	60.1	182.3	2657	183.9	371.0
TxP_2440	123.3	1.8	55.2	157.8	2647	180.9	359.2
TxP_2355	126.3	1.9	58.9	154.1	2581	179.1	363.2
TxP_2319	126.3	1.5	63.3	159.3	2555	175.4	384.3

[a] Maturity given in (total days of planting to maturity)
[b] (1-5 scale, where 1= erect and 5=prostrate)
[c] (cm)
[d] (mg $seed^{-1}$)
[e] (g kg^{-1})
[f] (g kg^{-1})
[g] (kg ha^{-1})

Table. 2. The ten lowest seed yield values for the combined analysis of TN93-99 x PI 416937 F_6 derived progeny in 2001.

Genotype	Maturity[a]	Lodging[b]	Height[c]	Seed Size[d]	Seed Yield[e]	Oil[f]	Protein[g]
TxP_2217	119.6	1.3	55.2	140.7	1653	187.2	367.8
TxP_2359	121.5	1.5	44.7	149.8	1653	182.1	370.8
TxP_2294	120.8	1.9	61.0	160.5	1640	182.4	362.1
TxP_2211	131.0	1.5	68.4	129.8	1634	177.4	369.7
TxP_2474	120.9	1.8	50.8	147.8	1563	180.5	373.2
TxP_2475	118.8	1.7	57.4	137.1	1492	178.6	381.6
TxP_2380	120.8	1.6	55.9	122.9	1360	168.1	391.3
TxP_2222	114.3	1.4	57.2	163.3	1352	182.2	381.3
TxP_2244	121.7	1.5	59.9	167.9	1329	177.5	388.5
TxP_2384	110.1	1.3	36.6	147.6	1167	177.6	385.8

[a] Maturity given in (total days of planting to maturity)
[b] (1-5 scale, where 1= erect and 5=prostrate)
[c] (cm)
[d] (mg $seed^{-1}$)
[e] (g kg^{-1})
[f] (g kg^{-1})
[g] (kg ha^{-1})

Table 3. Phenotypic correlation of agronomic and seed quality traits for combined analysis pf TN93-99 x PI 416937 F_6 RIL population.

	MAT[a]	LOD[b]	HT_CM[c]	SS_mg[d]	OIL[e]	PRO[f]	KG_HA[g]
MAT[a]		0.10 0.045	0.42 <0.0001	-0.04 0.456	-0.29 <0.0001	-0.06 0.228	0.33 <0.0001
LOD[b]			0.47 <0.0001	0.18 0.0001	0.02 0.737	-0.18 0.0004	-0.03 0.560
HT_CM[c]				0.19 <0.0001	0.01 0.864	-0.19 0.0001	0.24 <0.0001
SS_mg[d]					0.14 0.005	-0.04 0.410	0.15 0.008
OIL[e]						-0.76 <0.0001	0.11 0.049
PRO[f]							-0.17 0.003

[a] MAT= Maturity given in (total days of planting to maturity)
[b] LOD= Lodging (1-5 scale, where 1= erect and 5=prostrate)
[c] HT_CM= Height in (cm)
[d] SS_mg= Seed size in (mg seed^{-1})
[e] OIL= Oil in (g kg^{-1})
[f] PRO= Protein in (g kg^{-1})
[g] KG_HA= Yield in (kg ha^{-1})
[h] For each data cell, Line 1 represents the r-value and Line 2 represents the p-value.

The ability to detect QTL is in part, related to the heritability of the trait. Our data showed moderate to high heritability for all traits, facilitating the ability to detect QTL.

Heritability estimates on an entry- mean basis, were high for plant maturity (0.98), plant height (0.87), seed size (0.96), seed yield (0.83), seed oil concentration (0.96), and seed protein concentration (0.96) at the Knoxville, TN location (Table 4). Heritability estimates at the Springfield, TN location were lower for every trait, yet remained high for plant maturity (0.90), plant height (0.80), seed size (0.91), and seed yield (0.83) (Table 4). Moderately high heritabilities were found for seed yield (0.73) and for seed protein concentration (0.74) at Springfield, TN and moderate heritabilities were found for plant lodging (0.67 and 0.65, respectively) at Knoxville and Springfield,

Table 4. Mean, standard deviation, range, and heritability for agronomic and seed quality traits in a TN93-99 x PI 416937 F_6 RIL population grown in 2001 at a) Knoxville, TN.

Agronomic Traits	Mean	Standard Deviation	Range	h^2
Plant maturity (total days)	122.5	5.9	109-139	0.98
Plant height (cm)	52.9	8.0	31.3-71.1	0.87
Plant lodging[a]	1.3	0.3	1-2	0.67
Seed size (mg seed^{-1})	145.0	14.1	112.3-185.3	0.96
Seed yield (kg ha^{-1})	2179.0	513.0	1066.1-3398.3	0.83
Seed oil concentration (g kg^{-1})	180.9	7.0	160.7-198	0.96
Seed protein concentration (g kg^{-1})	374.3	11.7	349.7-408	0.96

[a](1-5 scale, where 1= erect and 5=prostrate)

Table 4. Continued at b)Springfield, TN.

Agronomic Traits	Mean	Standard Deviation	Range	h^2
Plant maturity (total days)	122.2	5.7	108-134.5	0.90
Plant height (cm)	65.0	10.0	41.9-87.6	0.80
Plant lodging[a]	2.2	0.4	1.5-3.5	0.65
Seed size (mg seed^{-1})	156.7	15.2	131-200.5	0.91
Seed yield (kg ha^{-1})	2011.6	475.6	1060.2-2934.9	0.73
Seed oil concentration (g kg^{-1})	182.2	7.2	166.5-201	0.83
Seed protein concentration (g kg^{-1})	367.7	12.0	340-391.5	0.74

[a](1-5 scale, where 1= erect and 5=prostrate)

Table 4. Continued at c) combined location analysis.

Agronomic Traits	Mean	Standard Deviation	Range	h^2
Plant maturity (total days)	122.2	5.5	110-136.1	0.88
Plant height (cm)	59.1	8.5	36.6-79.4	0.84
Plant lodging[a]	1.7	0.3	1.3-2.8	0.63
Seed size (mg $seed^{-1}$)	150.2	13.7	122.9-192.9	0.85
Seed yield (kg ha^{-1})	2117.7	398.5	1167.3-2947.5	0.48
Seed oil concentration (g kg^{-1})	181.8	6.4	163.6-199.5	0.80
Seed protein concentration (g kg^{-1})	371.1	10.3	344.8-393.6	0.83

[a](1-5 scale, where 1= erect and 5=prostrate)

TN. These moderate to high estimates of heritability are a reflection of attention to detail in controlling the plot-to-plot error variance of all measured traits from the field research project.

There were 128 polymorphisms initially identified in the parental screening out of 497 total available SSR markers analyzed. Some of those markers were later found to be not polymorphic or difficult to differentiate band lengths. Eighty- seven polymorphic markers were screened with DNA from 99 F_6 derived progeny.

QTL were first analyzed based on data from single F_6 plants grown in 2000. Only four of the 20 MLG displayed significant QTL based on single plant data. There was one marker significant for protein, one for oil, three for seed size, and two for height (Table 5). A QTL (Satt373) associated with both protein and oil QTL was discovered on linkage

Table 5. QTL associated with agronomic and seed quality for F_6 derived single plant progeny of TN93-99 x PI 416937 grown in Knoxville, TN in 2000.

Marker	MLG [a]	Trait	R^2 (%)	Marker Additive p-value	Effect of PI 416937 allele
Satt409	A2	Plant Height	11	<0.01	-2.6 cm
Satt583	B1	Seed Size	7	<0.05	-1.7 mg seed^{-1}
Satt444	B1	Seed Size	12	<0.05	-2.0 mg seed^{-1}
Satt430	B1	Seed Size	12	<0.05	-1.8 mg seed^{-1}
Satt442	H	Plant Height	10	<0.05	-2.5 cm
Satt373	L	Protein	9	<0.05	1.2 g kg^{-1}
Satt373	L	Oil	10	<0.05	-0.6 g kg^{-1}

[a]MLG= Molecular Linkage Group

group L. Height QTL were discovered on linkage groups A2 (Satt409) and H (Satt442). Linkage group B1 had three QTL for seed size (Satt583, Satt444, and Satt430).

QTL analyses were further conducted on 2001 replicated plot data at Knoxville and Springfield, TN, and in a combined location analysis for 2001. Eleven of the 20 MLG displayed significant QTL for this study. There were ten maturity, nine height, four lodging, five seed size, ten yield, eight oil, and six protein QTL discovered. The critical decision significance value for declaring a markers as a significant QTL was adjusted to alpha= 0.05/k, where k= the number of markers analyzed for that specific linkage group. This adjustment enabled us to be conservative in identifying representative QTL.

The linkage groups identified containing maturity QTL were A1, B1, B2, F, I, J, L, and O. Markers Satt331 and Satt581 (MLG O) were detected as major maturity QTL at each location and in the combined analysis (Table 6). Satt545 (MLG A1) and Satt547

Table 6. QTL associated with plant maturity[a] from $F_{6:8}$ progeny of TN93-99 x PI 416937 grown at Knoxville and Springfield, TN in 2001.

Marker	MLG[b]	Location	R^2 (%)	Marker Additive p-value	Effect of PI allele[a]
Satt545	A1	Springfield,TN	12	<0.01	-4.1
Satt197	B1	Knoxville,TN	6	<0.05	2.8
Satt272	B2	Combined	7	<0.05	-2.9
Satt556	B2	Springfield,TN	9	<0.05	-3.3
		Combined	7		-3.0
Satt030	F	Knoxville,TN	9	<0.01	3.8
Satt367	I	Knoxville,TN	6	<0.05	3.0
Satt547	J	Knoxville,TN	7	<0.05	-3.3
		Springfield,TN	10		-3.8
		Combined	10		-2.9
Satt495	L	Knoxville,TN	5	<0.05	2.7
		Springfield,TN	7		2.7
		Combined	7		2.8
Satt331	O	Knoxville,TN	11	<0.01	-3.9
		Springfield,TN	18		-4.7
		Combined	16		-4.2
Satt581	O	Knoxville,TN	12	<0.01	-4.3
		Springfield,TN	20		-4.9
		Combined	19		-4.8

[a] Effect of PI 416937 allele given in total days(days from planting to physiological maturity)

[b] MLG= Molecular Linkage Group

(MLG J) were major QTL at the Springfield, TN location. The PI 416937 effect for the major QTL was negative in each case indicating that PI 416937 allele substitution at those loci would reduce the duration of plant maturity.

The nine height QTL were found on MLG A1, B2, D1b+W, I , J, and O. The major QTL for height were Satt200 (MLG A1), Satt225 (MLG A1), Satt599 (MLG A1), Satt172 (MLG D1b+W), Satt331 (MLG O), and Satt581 (MLG O) (Table 7). All of these QTL were found for the Springfield location and/or in the combined analysis only. PI 416937 expressed negative effects for all major height QTL indicating that substituting a PI 416937 allele at these loci would reduce plant height.

Table 7. QTL associated with plant height[a] from $F_{6:8}$ progeny of TN93-99 x PI 416937 grown at Knoxville and Springfield, TN in 2001.

Marker	MLG[b]	Location	R^2 (%)	Marker Additive p-value	Effect of PI allele[a]
Satt200	A1	Springfield,TN	11	<0.01	-6.8
		Combined	12		-5.7
Satt225	A1	Springfield,TN	11	<0.01	-6.6
		Combined	10		-5.3
Satt599	A1	Springfield,TN	13	<0.01	-6.8
Satt272	B2	Combined	7	<0.05	-4.7
Satt172	D1b+W	Springfield,TN	15	<0.01	-7.6
		Combined	11		-6.1
Satt367	I	Knoxville,TN	9	<0.05	4.9
		Combined	9		5.3
Satt547	J	Knoxville,TN	5	<0.05	-3.8
		Springfield,TN	7		-5.5
		Combined	9		-5.1
Satt331	O	Springfield,TN	11	<0.01	-6.7
Satt581	O	Springfield,TN	14	<0.01	-7.8
		Combined	12		-6.2

[a] Effect of PI 416937 allele given in cm
[b] MLG= Molecular Linkage Group

Satt367 (MLG I) was the only QTL that showed a positive effect for PI 416937. Another QTL on MLG I for plant height was discovered by Sebolt et al. (2001). In their study, a cross was made between for the backcross parents, A81- 356022 x an F_2–derived line of A81- 356022 X PI 468916. The *Glycine soja* alleles found in that study contributed to increased plant height that was greater than that of *Glycine max* lines. Another height QTL was detected by Lark et al. (1995) and was located within 10 cM of the height QTL (Satt172) that we found on MLG D1b+W.

Molecular linkage groups F, H and L displayed QTL for plant lodging were identified at the Springfield, TN location and in the combined analysis. The major QTL for plant lodging was Satt425 located on MLG F (Table 8). Minor QTL for plant lodging were found on MLG H and L in our study. There were two previously discovered QTL for lodging found within 10cM of Satt495 on MLG L. The first was discovered through a cross between Young x PI 416937 (Lee et al., 1996b). In their study, the lodging locus was on marker A169_1 on MLG L.

Table 8. QTL associated with plant lodging[a] from $F_{6:8}$ progeny of TN93-99 x PI 416937 grown at Knoxville and Springfield, TN in 2001.

Marker	MLG[b]	Location	R^2 (%)	Marker Additive p-value	Effect of PI allele[a]
Satt425	F	Springfield,TN	14	<0.01	0.3
Satt253	H	Springfield,TN	9	<0.05	0.2
Satt442	H	Combined	6	<0.05	-0.2
Satt495	L	Springfield,TN	8	<0.05	0.2
		Combined	8		0.2

[a] Lodging score (1-5 scale, where 1= erect, 5= prostrate)
[b] MLG= Molecular Linkage Group

The second marker was discovered by (Specht, 2001) as Satt232 on MLG L.

There were five QTL for seed size in our study on MLG A1 (3 total), B1, and M. Satt200 and Satt599 were major QTL with Satt599 giving the highest R^2 for any trait (R^2 = 26%) (Table 9). PI 416937 effects were negative for the trait meaning that substituting a PI 416937 allele at these loci should reduce seed size.

There were a total of 10 QTL for seed yield, located on MLG A1, B2, I, J, L, M, and O. MLG A1, B2, and L each had two QTL. Linkage groups I, J, M, O each had one QTL. Major QTL for yield were discovered as Satt545 (MLG A1), Satt599 (MLG A1), Satt272 (MLG B2), Satt556 (MLG B2), Satt419 (MLG I), and Satt581 (MLG O) (Table 10). Satt373 (MLG L) and Satt346 (MLG M) had positive PI 416937 effects. The remaining yield QTL showed negative effects for PI 416937 allele indicating that favorable yield would result from substituting a TN93-99 allele at those loci. Sebolt et al. (2000) also found a QTL on LG I for yield.

Eight oil QTL were found on linkage groups A1, F, H, I, and J. Two each were discovered on linkage groups F, I, and J. The major QTL for oil were Satt030 (MLG F), Satt367 (MLG I), Satt146 (MLG F), Satt215 (MLG J), and Satt547 (MLG J) (Table 11). Satt174 (MLG A1), an oil QTL discovered by Orf et al. (1999), was within 10cM of the oil QTL we found on MLG A1.

Table 9. QTL associated with seed size[a] from $F_{6:8}$ progeny of TN93-99 x PI 416937 grown at Knoxville and Springfield, TN in 2001.

Marker	MLG[b]	Location	R^2 (%)	Marker Additive p-value	Effect of PI allele[a]
Satt200	A1	Knoxville,TN	16	<0.01	-11.4
		Combined	11		-9.1
Satt225	A1	Knoxville,TN	9	<0.01	-9.0
Satt599	A1	Knoxville,TN	26	<0.01	-14.3
		Combined	16		-11.0
Satt583	B1	Knoxville,TN	8	<0.05	-8.5
Satt346	M	Knoxville,TN	7	<0.05	-14.3

[a] Effect of PI 416937 allele given in mg $seed^{-1}$

[b] MLG= Molecular Linkage Group

Table 10. QTL associated with seed yield[a] from $F_{6:8}$ progeny of TN93-99 x PI 416937 grown at Knoxville and Springfield, TN in 2001.

Marker	MLG[b]	Location	R^2 (%)	Marker Additive p-value	Effect of PI allele[a]
Satt545	A1	Knoxville,TN	12	<0.01	-358.8
		Combined	16		-323.8
Satt599	A1	Knoxville,TN	16	<0.01	-431.7
		Combined	20		-383.8
Satt272	B2	Knoxville,TN	5	<0.05	-228.5
		Combined	10		-256.8
Satt556	B2	Knoxville,TN	10	<0.05	-308.0
		Combined	12		-267.1
Satt419	I	Springfield,TN	12	<0.05	-343.0
Satt215	J	Knoxville,TN	7	<0.05	-292.6
Satt006	L	Knoxville,TN	6	<0.05	-231.4
Satt373	L	Combined	9	<0.05	235.8
Satt346	M	Knoxville,TN	5	<0.05	222.8
Satt581	O	Knoxville,TN	10	<0.01	-302.1

[a] Effect of PI 416937 allele given in kg ha^{-1}

[b] MLG= Molecular Linkage Group

Table 11. QTL associated with seed oil[a] from $F_{6:8}$ progeny of TN93-99 x PI 416937 grown at Knoxville and Springfield, TN in 2001.

Marker	MLG[b]	Location	R^2 (%)	Marker Additive p-value	Effect of PI allele[a]
Satt225	A1	Knoxville, TN	9	<0.01	-4.2
Satt030	F	Knoxville,TN	8	<0.01	-3.9
		Springfield,TN	16		-5.5
		Combined	21		-5.4
Satt146	F	Combined	17	<0.01	4.8
Satt353	H	Knoxville,TN	7	<0.05	3.6
Satt367	I	Springfield,TN	12	<0.05	-4.8
Satt419	I	Knoxville,TN	6	<0.05	-3.2
		Springfield,TN	9		-4.1
		Combined	8		-3.4
Satt215	J	Springfield,TN	9	<0.05	-4.4
		Combined	12		-4.5
Satt547	J	Springfield,TN	8	<0.05	3.9
		Combined	10		3.8

[a] Effect of PI 416937 allele given in g kg^{-1}

[b] MLG= Molecular Linkage Group

Linkage groups A1, B2, H, I, L, and O displayed significant QTL for protein. The major QTL for this trait were Satt545 (MLG A1), Satt353 (MLG H), Satt373 (MLG L), and Satt345 (MLG O)(Table 12). A previously discovered protein QTL on LG I is Satt127. Sebolt et al. (2000) made this discovery and it is within 10cM of the protein QTL, Satt367, the QTL that we discovered on MLG I. Our research adds to the literature base and suggests that there is likely a genomic region governing oil and protein synthesis in this area of MLG I.

Table 12. QTL associated with seed protein[a] from $F_{6:8}$ progeny of TN93-99 x PI 416937 grown at Knoxville and Springfield, TN in 2001.

Marker	MLG	Location	R^2 (%)	Marker Additive p-value	Effect of PI allele
Satt545	A1	Knoxville,TN	10	<0.01	7.0
Satt083	B2	Knoxville,TN	6	<0.05	-5.6
Satt353	H	Knoxville,TN	11	<0.05	-7.7
Satt367	I	Springfield, TN	8	<0.05	6.7
Satt373	L	Combined	10	<0.05	-5.7
Satt345	O	Knoxville,TN	15	<0.01	9.1
		Combined	13		6.9

[a] Effect of PI 416937 allele given in g kg^{-1}

[b] MLG= Molecular Linkage Group

V. CONCLUSION

TN93-99 and PI 416937 were polymorphic for 26% of the total simple sequence repeat markers screened. In the progeny screening for single F_6 plants grown at Knoxville Plant Science Farm in 2000, only 1% of markers displayed significant QTL for plant height [Satt409 (MLG A2) and Satt442 (MLG H)], seed size [Satt583, Satt444, and Satt430 (all on MLG B1)], seed oil concentration [Satt373 (MLG L)], and seed protein concentration [Satt373 (MLG L)]. QTL analyses were conducted on replicated $F_{6:8}$ plots grown at Knoxville and Springfield, TN, and in a combined analysis. Several major QTL (R^2 >10%) and minor QTL (R^2 <10%) were discovered for the combined analysis. For plant maturity, the major QTL were Satt547 on MLG J (R^2 =10%), Satt331 on MLG O (R^2 =16%), and Satt581 on MLG O (R^2 =19%). The minor QTL for plant maturity were Satt272 (R^2 =7%), and Satt556 (R^2 =7%) both on MLG B2, and Satt495 on MLG L (R^2 =7%). The plant height major QTL were Satt200 (R^2 =12%), and Satt225 (R^2 = 10%) on MLG A1, Satt172 on MLG D1b+W (R^2 =11%), and Satt581 on MLG O (R^2 = 12%). The three minor plant height QTL were Satt272 (R^2 =7%) on MLG B2, Satt367 (R^2 =9%) on MLG I, and Satt574 (R^2 =9%) on MLG J. There was no major plant lodging QTL discovered for the combined analysis. The minor plant lodging QTL were Satt442 (R^2 =6%) on MLG H, and Satt495 (R^2 =8%) on MLG L. Seed size major QTL were Satt200 (R^2 = 11%) and Satt599 (R^2 = 16%), both on MLG A1. There were no minor QTL for seed size in the combined analysis. Seed yield major QTL were Satt545 (R^2 =16%) and Satt599 (R^2 = 20%) with both markers on MLG A1, Satt272 (R^2 =10%), and Satt556 (R^2 =12%) on MLG B2. There was a minor QTL for seed yield at Satt373 (R^2 =9%) on MLG

L. Major QTL for seed oil concentration were Satt030 (R^2=21%) and Satt146 (R^2=17%) on MLG F, and Satt215 (R^2=12%) and Satt547 (R^2=10%) on MLG J. A minor seed oil concentration QTL was found at Satt419 (R^2=8%) on MLG I. Major QTL for seed protein concentration were Satt373 on MLG L (R^2=10%), and Satt345 on MLG O (R^2= 13%). There were no minor QTL for seed protein concentration in the combined analysis.

The QTL discovered in this study will provide soybean breeders with valuable knowledge to pursue marker assisted selection strategies for improving soybean agronomic and seed quality traits.

Heritability estimates were high for many of these traits, which indicated that genetic control is important. Our research suggests that agronomic and seed quality traits may be successfully modified through marker assisted selection programs. Utilizing molecular marker crop improvement tools will increase the value of agronomic and seed quality traits. Additional research will make it easier for plant breeders to achieve genetic gains.

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Appendix A.

PI 416937 derived progeny in Southern Uniform Testing Program for the ten-year period 1993- 2002.

Year	Test	Line	Pedigree	PI 416937 Contribution (%)
1993	UP VII	N91- 8006	N77- 114 x PI 416937	50
1994	UP VI	N91- 8005	N77- 114 x PI 416937	50
1994	UP VII	N90- 7199	N77- 114 x PI 416937	50
1994	UP VII	N90- 7202	N77- 114 x PI 416937	50
1995	UP VIB	N91- 8005	N77- 114 x PI 416937	50
1995	UP VIB	N93- 1128	Brim x PI 416937	50
1995	UP VIB	N93- 1228	Brim x PI 416937	50
1995	UP VIB	VS94- 18	York x PI 416937	50
1995	UT VII	N90- 7199	N77- 114 x PI 416937	50
1996	UP VIB	VS94- 26	Bay x PI 416937	50
1996	UP VIB	VS94- 17	York x PI 416937	50
1996	UP VIB	VS94- 22	York x PI 416937	50
1996	UT VII	N90- 7199	N77- 114 x PI 416937	50
1997	UT VII	N90- 7199	N77- 114 x PI 416937	50
1998	UP VB	N96- 7157	Holladay x N91- 8006	25
1998	UP VB	N93- 1264	Brim x PI 416937	50
1998	UP VB	N96- 7211	Holladay x N91- 8006	25
1998	UP VI	N96- 7083	N90- 7199 x N90- 7241	25
1998	UP VI	N96- 6800	N90- 7202 x N90- 7199	50
1998	UP VI	N96- 7165	Holladay x N91- 8006	25
1998	UP VI	N96- 6809	N90- 7202 x N90- 7199	50
1998	UP VII	N96- 6751	N90- 7202 x N90- 7199	50
1998	UP VII	N96- 7164	Holladay x N91- 8006	25
1998	UP VII	N96- 6767	N90- 7202 x N90- 7199	50
1998	UP VII	N96- 7036	N90- 7199 x N90- 7241	25
1998	UP VIII	N96- 7031	N90- 7199 x N90- 7241	25
1998	UP VIII	N96- 6924	N90- 7216 x N90- 7202	25
1998	UP VIII	N96- 6940	N90- 7216 x N90- 7202	25
1998	UP VIII	N96- 7058	N90- 7199 x N90- 7241	25
1998	UP VIII	N96- 6894	N90- 7216 x N90- 7202	25
1998	UP VIII	N96- 7082	N90- 7199 x N90- 7241	25
1998	UP VIII	N96- 7095	N90- 7199 x N90- 7241	25
1998	UP VIII	N96- 6997	N90- 7202 x N90- 7241	25
1998	UP VIII	N96- 7025	N90- 7199 x N90- 7241	25
1998	UP VIII	N96- 7028	N90- 7199 x N90- 7241	25
1998	UP VIII	N96- 7063	N90- 7199 x N90- 7241	25

1998	UP VIII	N96- 6992	N90- 7202 x N90- 7241	25
1999	UT V	N96- 7157	Holladay x N91- 8006	25
1999	UT V	N96- 7211	Holladay x N91- 8006	25
1999	UT VI	N96- 6800	N90- 7202 x N90- 7199	50
1999	UP VI	N96- 6783	N91- 7202 x N90- 7199	25
1999	UP VI	N97- 9791	N90- 7199 x N91- 7254	25
1999	UP VI	N97- 9812	N90- 7199 x N91- 7254	25
1999	UP VI	N93- 1264	Brim x PI416937	50
1999	UP VI	N96- 7161	Holladay x N91- 8006	25
1999	UP VI	N97- 9839	N90- 7199 x N91- 7254	25
1999	UT VII	N96- 6767	N90- 7202 x N90- 7199	50
1999	UT VII	N96- 6809	N90- 7202 x N90- 7199	50
1999	UT VII	N96- 7083	N90- 7199 x N90- 7241	25
1999	UP VII	N96- 7018	N90- 7199 x N90- 7241	25
1999	UP VII	N96- 7164	Holladay x N91- 8006	25
1999	UP VII	N97- 10227	N91- 7202 x N91- 8005	25
1999	UP VII	N97- 6425	N92- 7005 x N91- 8005	25
1999	UP VII	N97- 6450	N92- 7005 x N91- 8005	25
1999	UP VII	N97- 9756	N90- 7199 x N91- 7254	25
1999	UT VIII	N96- 7031	N90- 7199 x N90- 7241	25
1999	UT VIII	N96- 7082	N90- 7199 x N90- 7241	25
1999	UT VIII	N96- 7095	N90- 7199 x N90- 7241	25
1999	UP VIII	N96- 6752	N91- 7202 x N90- 7199	25
1999	UP VIII	N97- 10074	N90- 7199 x N91- 8005	50
1999	UP VIII	N97- 9747	N90- 7199 x N91- 7254	25
1999	UP VIII	N97- 9765	N90- 7199 x N91- 7254	25
1999	UP VIII	N97- 9782	N90- 7199 x N91- 7254	25
1999	UP VIII	N97- 9783	N90- 7199 x N91- 7254	25
1999	UP VIII	N97- 9788	N90- 7199 x N91- 7254	25
1999	UP VIII	N97- 10226	N91- 7202 x N91- 8005	25
1999	UP VIII	N97- 10258	N91- 7202 x N91- 8005	25
2000	UP V	N97- 10023	N90- 7199 x N91- 8005	50
2000	UP V	N98- 7892	N90- 7199 x NTCPR92- 40	25
2000	UP V	N98- 8445	N91- 8005 x Cook	25
2000	UP V	N98- 8523	N91- 8005 x Cook	25
2000	UT VI	N96- 6783	N91- 7202 x N90- 7199	25
2000	UT VI	N96- 6800	N90- 7202 x N90- 7199	50
2000	UT VI	N97- 9812	N90- 7199 x N91- 7254	25
2000	UP VI	N97- 9729	N90- 7199 x N91- 7254	25
2000	UP VI	N97- 9944	N90- 7199 x N91- 8005	50
2000	UP VI	N98- 7915	N90- 7199 x NTCPR92- 40	25
2000	UP VI	N98- 8560	N91- 8005 x Cook	25
2000	UP VI	N98- 650	N93- 1264 x N93- 1047	25

2000	UT VII	N96- 6767	N90- 7202 x N90- 7199	50
2000	UT VII	N96- 6809	N90- 7202 x N90- 7199	50
2000	UT VII	N96- 7018	N90- 7199 x N90- 7241	25
2000	UP VII	N97- 6450	N92- 7005 x N91- 8005	25
2000	UP VII	N97- 9599	N90- 7199 x Cook	25
2000	UP VII	N97- 9626	N90- 7199 x Cook	25
2000	UP VII	N97- 9631	N90- 7199 x Cook	25
2000	UP VII	N97- 9658	N90- 7199 x Cook	25
2000	UP VII	N97- 9827	N90- 7199 x N91- 7254	25
2000	UP VII	N98- 8443	N91- 8005 x Cook	25
2000	UT VIII	N96- 6752	N91- 7202 x N90- 7199	25
2000	UT VIII	N96- 7031	N90- 7199 x N90- 7241	25
2000	UT VIII	N97- 9788	N90- 7199 x N91- 7254	25
2000	UP VIII	N97- 10074	N90- 7199 x N91- 8005	50
2000	UP VIII	N97- 6439	N92- 7005 x N91- 8005	25
2000	UP VIII	N97- 9607	N90- 7199 x Cook	25
2000	UP VIII	N97- 9612	N90- 7199 x Cook	25
2000	UP VIII	N97- 9636	N90- 7199 x Cook	25
2000	UP VIII	N97- 9782	N90- 7199 x N91- 7254	25
2000	UP VIII	N97- 9783	N90- 7199 x N91- 7254	25
2001	UP V	K1529	Hutcheson x N90- 7199	25
2001	UP V	N99- 701	N93- 1264 x D91- 4759	25
2001	UT VI	N97- 9812	N90- 7199 x N91- 7254	25
2001	UT VI	N97- 9944	N90- 7199 x N91- 8005	50
2001	UP VI	N96- 6755	N90- 7202 x N90- 7199	50
2001	UP VI	N99- 8114	N90- 7199 x Graham	25
2001	UP VI	N99- 8119	N90- 7199 x Graham	25
2001	UP VI	N99- 813	N93- 1264 x Holladay	25
2001	UT VII	N96- 6809	N90- 7202 x N90- 7199	50
2001	UT VII	N97- 9599	N90- 7199 x Cook	25
2001	UT VII	N97- 9658	N90- 7199 x Cook	25
2001	UT VII	N97- 9827	N90- 7199 x N91- 7254	25
2001	UP VII	N97- 9569	N90- 7199 x Cook	25
2001	UP VII	N97- 9693	N90- 7199 x Cook	25
2001	UP VII	N97- 9641	N90- 7199 x Cook	25
2001	UP VII	N98- 7965	N90- 7199 x NTCPR93- 283	25
2001	UP VII	N99- 729	N93- 1264 x D91- 4759	25
2001	UP VII	N99- 732	N93- 1264 x D91- 4759	25
2001	UT VIII	N96- 6752	N91- 7202 x N90- 7199	25
2001	UT VIII	N97- 10074	N90- 7199 x N91- 8005	50
2001	UT VIII	N97- 9612	N90- 7199 x Cook	25
2001	UT VIII	N97- 9636	N90- 7199 x Cook	25
2001	UT VIII	N97- 9783	N90- 7199 x N91- 7254	25

2001	UP VIII	N97- 9595	N90- 7199 x Cook	25
2001	UP VIII	N97- 9677	N90- 7199 x Cook	25
2001	UP VIII	N97- 9636	N90- 7199 x Cook	25
2001	UP VIII	N98- 7961	N90- 7199 x NTCPR93- 283	25
2002	UP V	N99- 8118	N90- 7199 x Graham	25
2002	UP V	N99- 8141	N90- 7199 x Graham	25
2002	UT VI	N96- 6755	N90- 7202 x N90- 7199	50
2002	UT VI	N97- 9812	N90- 7199 x N91- 7254	25
2002	UT VI	N99- 8119	N90- 7199 x Graham	25
2002	UP VI	N99- 8126	N90- 7199 x Graham	25
2002	UP VI	N99- 8137	N90- 7199 x Graham	25
2002	UP VI	N99- 8150	N90- 7199 x Graham	25
2002	UT VII	N97- 9599	N90- 7199 x Cook	25
2002	UT VII	N97- 9658	N90- 7199 x Cook	25
2002	UT VII	N97- 9693	N90- 7199 x Cook	25
2002	UP VII	N90- 7199	N77- 114 x PI 416937	50
2002	UP VII	N96- 6809	N90- 7202 x N90- 7199	50
2002	UT VIII	N96- 6752	N91- 7202 x N90- 7199	25
2002	UT VIII	N97- 10074	N90- 7199 x N91- 8005	50
2002	UT VIII	N97- 9612	N90- 7199 x Cook	25
2002	UT VIII	N97- 9636	N90- 7199 x Cook	25
2002	UT VIII	N97- 9677	N90- 7199 x Cook	25
2002	UT VIII	N98- 7961	N90- 7199 x NTCPR93- 283	25

Appendix B.

A standard protocol for DNA extraction was followed (Dneasy plant mini handbook):

1. Plant tissue was ground to a fine powder using liquid nitrogen and a mortar and pestle. The solution was transferred to an appropriately sized tube without allowing the mixture to thaw.

2. 400µL of Buffer AP1 and 4µL of RNase A stock solution (100mg/ml) were added to a maximum of 100mg of ground plant tissue and vortexed vigorously.

3. The tube was incubated in a 65°C waterbath for 30-120 minutes, inverted several times to ensure mixing.

4. One hundred and thirty microliters of Buffer AP2 were added to the lysate, mixed, and incubated for 5 minutes on ice.

5. The tube was centrifuged at 14,000 rpm for five minutes.

6. The lysate was applied to the QIAshredder spin column (lilac colored) sitting in a 2 mL collection tube and centrifuges for 2 minutes at 14, 000 rpm.

7. The flow thru fraction, which contained the DNA, from step 6 was transferred to a new tube without disturbing the cell- debris pellet.

8. One half the volume of Buffer AP3 and one whole volume of ethanol (96- 100%) were added to the cleared lysate and mixed gently by pipetting to avoid shearing (use a cut pipette tip).

9. Next, 650 µL of the mixture from step 8 was applied to a DNeasy mini spin column sitting in a 2 mL collection tube. Then, the column was centrifuged for 1 minute at about 8000 rpm. The flow through was discarded the collection tube was reused (DNA is now bound to the filter).

10. Step 9 was repeated with the remaining sample. Flow through and collection tube were discarded.

11. The DNeasy column was placed in a new 2 mL collection tube with 500 µL of Buffer AW added and centrifuged for 1 minute at about 8000 rpm. Flow- through was discarded and the collection tube was reused in step 12.

12. Five hundred microliters of Buffer AW was added to DNeasy column and centrifuged for 2 minutes at 14, 000 rpm to dry the column membrane (DNA is still bound to the column).

13. The DNeasy column was transferred to a 2 mL microfuge tube and 100 µL of preheated (65°C) Buffer AE was directly pipetted into the column. Next, the column was incubated for 5 minutes at room temperature and then centrifuged for 1 minute at about 8000 rpm to elute (Elution 1).

14. Step 13 was repeated once as described to form a second elution (Elution 2).

Appendix C.

Stock solutions

1X TNE- (100mM Tris, 10mM EDTA, 2 M NaCl)

PCR Master Mix -(7.4µl dd H_2O, 1µl PCR buffer (10X), 1µl nucleotide mixture (2mM)(Pharmacia, Piscataway, NJ), 1.5µl Klentaq solution (0.5u/µl)(AB Peptides, Inc, St. Louis, MO), 2µl of DNA(20ng/µl), and 1µM forward and reverse SSR Primer)

6X Loading Buffer- 0.25% bromophenol blue, 15% Ficoll (Type 400; Pharmacia) in water

6% polyacrylamide gel- 28.5ml of 40% bis-acrylamide solution + 160.2ml of 0.60X TBE Buffer + 1.33ml of 10% APS solution + 66.5µl TEMED

(10mg/mL) ethidium bromide - 1g ethidium bromide in 100ml water.

VITA

Yamika Nitá Stokes was born on May 31, 1978 in Newport News, VA, where she was raised. She attended grade school at Dunbar-Erwin Elementary and Hidenwood Elementary School. She also attended Huntington Middle School and graduated from Ferguson High School with an advanced diploma in 1996. She attended Virginia State University in Petersburg, VA and received a B.S. in Agriculture in 2000. Yamika enrolled at the University of Tennessee and received a M.S. in Plant and Soil Science in 2002.