DONNA KAREN WOOD HARRIS
Genetic Improvement of Soybean Seed Traits and Resistance to Bud Blight and Root-knot Nematodes
(Under the Direction of H. ROGER BOERMA)

The selection of F_2-derived soybean lines from a population of Benning (42% protein) x Danbaekkong (52% protein) for divergent seed protein content based on a major protein QTL identified one line, G98SF-114 that produced 48% protein and seed yield equal to Benning. An attempt to confirm previously identified QTL for seed protein, seed oil, and seed weight with SSR markers in an F_2-derived population of Young x PI416937 resulted in three of the nine QTL (33%) being confirmed. A major QTL for bud blight (caused by tobacco ringspot virus) was confirmed in the F_2-derived Young x PI416937 population. Evaluation of recently obtained Chinese soybean germplasm lines for resistance to *Meloidogyne arenaria* (MA) and *M. incognita* (MI) (two species of root-knot nematode) found two lines resistant to galling for each of the species (PI594427C and PI594403 for MA and PI594753A and PI594775A for MI), and PI594427C and PI594403 with resistance to MA egg production.

INDEX WORDS: Soybean, *Glycine max*, Benning, Danbaekkong, SSR, QTL, *Meloidogyne incognita*, *Meloidogyne arenaria*, Chinese Plant Introductions, Nematode Resistance, Young, PI416937, Bud Blight, Confirmation, Seed Oil, Seed Protein, Seed Weight

GENETIC IMPROVEMENT OF SOYBEAN SEED TRAITS AND RESISTANCE TO BUD BLIGHT AND ROOT-KNOT NEMATODES

by

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CHAPTER 1

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] belongs to the legume family, and is native to Asia. Today, soybean is a leading global source of protein and oil, and accounts for 54% of the world's oilseed production. The USA produces 47% of the global soybean production, and is the world's leading producer, growing 30 million hectares (74.5 million acres) in 2000. The USA harvested 71.9 million metric tons of soybean in 2000 (Soyatech, 2001).

The soybean is grown in over 29 states, with the value of the U.S. soybean crop at $12.45 billion in 1999 on an average price paid to the U.S. farmer of $181 per metric ton ($4.93 per bushel) (Soyatech, 2001). Europe is the principal market for the U.S. whole soybean export, and Japan is the largest single country customer (Biotechnology Industry Organization, 1999).

Soybean seed not only serve as an important source of nutrition in animal feed, but also for human consumption. It is an important source of dietary fiber and contains a relatively high level of protein and oil. Soybean oil is used extensively in the food industry in products such as cooking oil and salad dressing. Like the protein found in animal products, soybean protein is "complete" in that it contains all eight essential amino acids needed for human health. Soybean is the only vegetable food that contains the protein makeup which is considered to be nutritionally complete (Biotechnology Industry Organization, 1999).

Soybean has been an important protein source in the Orient for millions of people for over 5,000 years. Current production is about 455,000 metric tons of soy protein products for human consumption per year in the USA, or about 1.8 to 2.3 kg per person. Textured soy protein is being used in many places to successfully replace proteins of the meat in recipes, and therefore not only reduce the percent of animal fat in the human diet, but also lower costs. Soybean protein is both economical and ecologically sound. In comparing land efficiency based on a 70 grams of protein per person allowance per day, one hectare of farm land produces 583 days of milk protein, or 190 days of beef protein. In comparison, one hectare of farm land produces 1,302 days of wheat protein and 5,493 days of soybean protein. This clearly shows that using protein source from wheat, and even more so, soybean is a much more efficient use of farm land in terms of protein production. This is especially important for countries in the Orient, such as China and Japan where the available agricultural land per person is 0.13 and 0.05 hectares, respectively (Steinweg, 1999).

With soybean being an important source of protein in the world in both animal feed and human consumption, it is an important trait in breeding programs. Therefore, one objective of this research was to evaluate F_2-derived lines from a population of Benning (conventional seed protein content) x Danbaekkong (elevated seed protein content) for divergent seed protein content based on a major protein QTL. A second objective was to confirm previously identified QTL for seed protein, seed oil, and seed weight in an F_2-derived population of Young x PI416937 using SSR markers that based on the public consensus soybean linkage map were located near the RFLP markers found in the original study to be associated with the QTL.

With the USA being the leading producer of soybean in the world, breeding programs in the USA are also concerned with integrating resistance to pests into their soybean cultivars. The other objectives of this research were to confirm a major QTL in the F_2-derived Young x PI416937 population for bud blight caused by tobacco ringspot virus and to evaluate recently obtained Chinese soybean germplasm lines for resistance to two species of root-knot nematodes.

CHAPTER 2

LITERATURE REVIEW

Root-knot Nematode

Nematodes are roundworms that either live in aquatic or terrestrial environments, or as parasites of plants or animals. Nematodes belong to the kingdom Animalia, and all the plant parasitic nematodes belong to the phylum Nematoda. Annual world wide losses caused by nematodes on life-sustaining crops is about 11%, and on most other economically important crops (such as fruits, vegetables, and non-edible field crops) is about 14%. This accounts for a total annual loss of over $80 billion every year (Agrios, 1997).

Meloidogyne species or root-knot nematodes are plant-parasites of most crop plants. Root-knot nematodes occur all over the world, but are found most often and in the greatest numbers in warm to hot climates with short, mild winters. They attack over 2,000 species of plants, which includes almost all of the cultivated plants, and root-knot nematodes alone reduce world crop production by approximately 5% (Agrios, 1997).

Root-knot nematodes damage plants by feeding on the roots. The feeding activity and the accompanying galling of the roots deprives the plant of nutrients and water as well as causes disfigurement of many root crops. When older plants become infected by the nematodes, yield losses may or may not be great. However, when susceptible plants

are infected at the seedling stage, losses are heavy and can even devastate the crop (Agrios. 1997).

The majority of root-knot nematodes are found in the root zone from 5 to 25 cm below the surface of the soil. The life cycle is approximately 25 days when the temperature remains around 27° C. (Agrios, 1997). Their life cycle begins when the female nematode lays approximately 500 to 1000 eggs in a gelatinous matrix. The first- and second-stage juveniles develop within the egg. The second-stage juvenile hatches from the egg and serves as the infective stage of the nematode, being the only stage that migrates in the soil free from the host tissue (Hussey, 1995). These juveniles do not find roots by random movement, but they are attracted to the host plant by chemical cues emanating from roots (Green, 1971; Prot, 1980). When the second-stage juvenile reaches a susceptible host, it penetrates the root tissue directly behind the root cap and moves intercellularly in the cortex to the region of cell differentiation (Godfrey and Oliveira, 1932; Linford, 1937; Linford, 1942). The feeding of the juveniles on susceptible plants causes pronounced morphological and physiological changes to the parasitized tissue. In particular, an elaborate permanent feeding site, called giant-cells, develops (Bird, 1962). Formation of giant-cells is essential for a successful host-parasite relationship and juveniles will fail to develop without this host response. They must feed without killing the host cells. The tissues that are preferred for development of the giant-cells are phloem parenchyma (Byrne et al., 1977; Christie, 1936; Krusberg and Nielsen, 1958). Once these stelar tissues are found, the second-stage juvenile will feed on five to six cells, modifying the cells into elaborate nutritive giant-cells from which the juvenile will obtain nourishment for development and reproduction (Hussey, 1985). Next, the

nematode goes through a second and third molt giving rise to the third and fourth juvenile stages. The fourth juvenile stage can be distinguished as either male or female. Finally, the male goes through one last molt and emerges as a vermiform adult which becomes free-living in the soil. The female goes through the last molt and then becomes a pear-shaped adult. The female produces eggs in the gelatinous matrix whether fertilized by a male or not (Eisenback, 1985).

Two of the more common root-knot nematode species include *Meloidogyne arenaria* (MA) and *Meloidogyne incognita* (MI). Both of these *Meloidogyne* species are readily found in the southeastern USA. In 1994, soybean yield loss averaged across 16 southern states due to root-knot nematodes was 1.6% and ranged from a trace amount to 7.0% (Pratt and Wrather, 1998).

Control of these nematodes can be a problem. Because of the cancellation of important fumigant nematicides including DBCP (1,2-dibromo-3-chloropropane) and EDB (ethylene dibromide) in past years, plant resistance has become an increasingly important component in nematode management in the Southeast as well as other areas of the USA. Resistance in this case is a term used to describe the capability of a host to "resist" or suppress nematode reproduction as well as development. A cultivar that is partially resistant would support an intermediate level of nematode reproduction, while a highly resistant cultivar would support very little nematode reproduction. An example of the value of using resistant cultivars can be shown by the use of the *Heterodera glycines* resistant soybean cultivar 'Forrest' which cost approximately $1 million to develop and prevented about $401 million dollars in 1980 in soybean yield losses (Bradley and Duffy, 1982).

Screening for resistant lines is best done in a greenhouse environment for a variety of reasons. For one thing, testing can be done throughout the year, but more importantly it can eliminate the non-uniformity of nematode infestations that occur in the field. In the greenhouse, sterilized soil can be used as well as cultured nematodes for the inocula. This eliminates non-uniformity of inoculum and the introduction of other contaminating organisms (Boerma and Hussey, 1992).

In the case of root-knot nematode resistance, the lack of genetic variation for resistance among some plant species is the limiting factor (Fassuliotis, 1985). Fehr (1991) recommends looking for resistance in a step wise manner: i) elite cultivars, ii) elite breeding lines that soon may be a cultivar, iii) acceptable breeding lines with superiority for one or a few characters, and iv) plant introductions of the cultivated species. If the search within the crop species is not successful, then wild relatives could be screened (Boerma and Hussy, 1992).

Previously, Luzzi et al. (1987) conducted an experiment where soybean genotypes in the USDA Soybean Germplasm Collection from Maturity Groups V, VI, VII, and VIII were screened for resistance to both MI and MA. The screening was done in the greenhouse and was based on root galling and nematode reproduction. Five levels of screening were employed using different levels of inoculum (10,000 eggs per plant being the highest level) and number of replications. Results showed that Plant Introduction 96354 (PI96354) was highly resistant to MI and PI200538 highly resistant to MA. Other resistant lines that were found included, Amredo, PI408088, and PI417444 for MI and PI230977 for MA. All resistant lines found for the MI showed lower gall indices, fewer eggs per gram of root, and fewer eggs per root system than Forrest which was the

resistant check. Also, the two resistant lines found for the MA showed these same characteristics compared to the resistant check 'Jackson'. These plant introductions (PIs) can be used in a soybean breeding program to increase genetic diversity of MI and MA resistance and in developing cultivars with high levels of resistance to these species of root-knot nematode (Luzzi et al., 1987).

Currently, elite soybean cultivars do not possess the level of resistance to MI and MA identified in the highly resistant PIs by Luzzi et al., 1987. Hussey et al.(1991) evaluated the level of MI and MA resistance in 139 soybean cultivars from Maturity Groups V to VIII. None of the 139 cultivars was considered to be highly resistant or equivalent in MI resistance to PI96354 or MA resistance to PI200538. Twenty-four percent of the cultivars tested were found to be moderately resistant to MA, and 23% to MI.

Inheritance of resistance to MI in soybean was determined by Luzzi et al. (1994) in crosses of a susceptible cultivar, 'Bossier', with two resistant plant introductions PI96354 and PI417444. The F_1, F_2, and/or F_3 generations were screened for resistance to gall formation in the greenhouse. The heritability of resistance in these PIs was found to range from 0.73 to 0.92. In another study, Luzzi et al. (1994) identified a major gene (*Rmi*) for resistance to MI. This single additive gene for resistance to galling was identified in the cultivar, Forrest. Luzzi et al. (1995) determined inheritance of resistance to MA in soybean using crosses of the susceptible 'CNS' and the three resistant genotypes Jackson, PI200538, and PI230977. For each cross, the F_1, F_2, and/or F_3 populations were screened in a greenhouse for resistance to gall formation. Heritability for resistance to MA was found to range from 0.74 to 0.83.

The soybean germplasm line G93-9009 was selected to combine a high level of resistance to MI with improved agronomic characteristics compared to PI96354. The level of MI resistance in G93-9009 was similar to PI96354, but G93-9009 showed higher seed yield (Luzzi et al., 1996a). Also, the germplasm line G93-9106 was selected for its high level of resistance to MA. G93-9106 has a similar level of MA resistance as PI200538, but has the higher seed yield (Luzzi et al., 1996b).

Tamulonis et al. (1997) identified the genomic location of the resistance genes for MI and MA. For MI, PI96354 was crossed with Bossier, a highly susceptible cultivar, and 110 F_2 plants were produced and genotyped with 121 restriction fragment length polymorphism (RFLP) markers. Two quantitative trait loci (QTL) conditioning number of MI galls were identified which mapped to Linkage Group O (LG O) and LG G on the soybean RFLP map. Together, the two QTL explained 45% of the variation in MI gall formation (Tamulonis et al., 1997b; Li et al., 2001).

For MA, the F_2 population from the cross of PI200538 and the susceptible cultivar CNS was genotyped with 130 RFLPs. Two QTLs conferring resistance to MA gall formation were identified on LG F and LG E. The two QTL accounted for 51% of the variation in gall number (Tamulonis et al., 1997a).

This DNA marker information is being used to incorporate these genes for MI and MA resistance into elite cultivars. Germplasm lines, G93-9009 and G93-9106 were used as sources of resistance. The genes are presently being backcrossed into the cultivars ‘Boggs’, ‘Benning’, and ‘Prichard’ using a combination of phenotypic screening and SSR (short-sequence repeats) markers to identify plants with the resistance genes, as well as to identify plants with the maximum recurrent parent’s genome (Boerma and Mian, 1998).

The objectives of developing soybean cultivars with higher levels of MI and MA resistance and multi-species resistance are very important to increase soybean productivity in the southern USA (Boerma and Hussey, 1991; Boerma and Mian, 1998). Although several PIs have been found to possess high resistance to these two species of root-knot nematode, these PIs also possess several undesirable agronomic characteristics (Luzzi et al., 1987). Examples of these characteristics include, disease and lodging susceptibility, unacceptable seed coat color, and low productivity. Therefore, identification of additional plant introductions with resistance to MI and MA could prove beneficial if these lines possess different resistance genes from those already identified in PI96354 and PI200538.

Six hundred and eight germplasm lines from the southern provinces of the People's Republic of China (PRC) were recently obtained and made available to researchers by Dr. R.L. Nelson (curator of USDA Soybean Germplasm Collection, Urbana, IL) with the support of the Illinois Soybean Program Operating Board and Iowa Soybean Promotion Board. These lines are the first to ever become available to U.S. scientists from many of these provinces in PCR.

These Chinese soybean germplasm originated from latitudes equal to that of Atlanta GA and farther south. However, these accessions range from Maturity Group II to Maturity Group IX due to the fact that soybean in this part of PCR is planted in spring, summer, fall, and winter. Because these germplasm lines have been selected for adaptation over hundreds of years to a wide variety of cropping situations, this germplasm has the potential to possess diversity not previously available in the USA (personal communication, Dr. R.L. Nelson, USDA-ARS, Urbana, IL).

Use of DNA Markers in Soybean Breeding

The ability to use DNA markers to identify plant genes or QTL has played an important role in revolutionizing the science of plant breeding and plant genetics in recent years. DNA markers may allow more effective trait selection by selecting at the molecular instead of the phenotypic level. Because molecular markers are generally phenotypically neutral, many markers can be studied simultaneously without masking the plant's phenotype or interfering with each other.

The use of DNA markers is a relatively new tool for plant breeders, and breeders who have incorporated them into their programs soon realize how DNA markers can compliment the traditional methods of plant breeding (Mohan et al., 1997). Through the use of these markers, the time required to produce an improved crop cultivar can be reduced. They make it possible to accelerate the transfer of desirable genes from germplasm lines or to introgress new genes from related wild species into superior cultivars. While the basic requirements of successful crop improvement have remained constant, the new molecular technologies have provided more powerful and precise ways to help accomplish breeding goals.

The first step in the use of DNA markers for any species is to construct a genetic map with markers distributed at regular intervals across the genome. This has been accomplished in many organisms including many crop species. Among the crop species with a robust DNA marker map is soybean (Cregan et al., 1999).

The selection of a DNA marker system depends on project objectives, population structure, the genomic diversity of the species, marker system availability, time required for analysis, and the cost per unit of information (Staub et al., 1996). In the late 1980s, soybean restriction fragment length polymorphism (RFLP) markers were developed. In the early 1990s the random amplified polymorphic DNA (RAPD) markers were applied in soybean research. In 1999, primers for over 600 short sequence repeat (SSR) markers were reported (Cregan et al., 1999).

Soybean SSR markers were found to be highly allelic even among elite soybean cultivars (Boerma and Mian, 1999). The SSR marker's higher polymorphic index among adapted germplasm (>50%) compared to RFLP markers (<30%) make them more efficient and cost effective for breeding applications. Each pair of SSR primers can produce an unambiguous DNA product that maps to a specific location in the soybean genome. The SSR information can therefore be used across soybean populations which makes them useful for marker-assisted selection (MAS) of different soybean traits. Due to its many advantages over other marker systems, SSR markers are preferred in most soybean breeding programs (Boerma and Mian, 1999).

Most traits of agricultural importance are controlled by several genes. The term quantitative trait loci or QTL, refers to loci containing alleles that differentially effect the expression of a continuously distributed phenotypic trait. Yield is one example of these polygenic traits (Strauss et al., 1992). In the past few years, QTL have been identified for many different traits. Examples of such traits in soybean include; pod dehiscence, plant maturity, oil content, protein content, seed weight, specific leaf weight, leaf size, water use efficiency, aluminum tolerance, plant height, and lodging.

Using DNA markers researchers are able to dissect quantitative traits into a set of discrete loci. The contribution of alleles from each parent at a particular locus can be determined separately from the other loci affecting the trait. This allows for pyramiding the desirable genes for a complex trait into an adapted genetic background (Boerma and Mian, 1999).

As of 8 May 2000, SoyBase (http://genome.cornell.edu/cgi-bin/WebAce/webace?db=soybase) contained 434 soybean QTL that had been reported in the scientific literature. Using semi-conservative criteria for QTL identification, Boerma (2000) estimated that 243 of the 434 QTL reported in SoyBase were potentially independent QTL. This approximate two-fold difference was due to the QTL accounting methods used. In SoyBase, a QTL is indicated at any marker or interval significantly associated with the trait regardless of the degree of linkage of the markers. Boerma (2000) reports a single QTL from a group of linked markers that were significantly associated with the trait and did not include major genes identified by classical genetic approaches. Of the 243 independent QTL, 137 QTL were reported to condition 10% or more of the phenotypic variation in the trait. Compared to the total number of QTL listed for a trait, these QTL have a higher probability of being verifiable in the original population or in another population in which the parents contain these alleles at the QTL. This assumption was based on the population sizes and marker saturation of the current studies (Beavis, 1998).

One possible limitation with the QTL reported in the literature is the lack of research that confirms their existence. Research to confirm existing QTL has shown mixed results. For example, a QTL mapping study by Diers et al. (1992) showed that

none of the identified QTL conditioning iron deficiency chlorosis were effective in divergent selection for the trait among additional lines from the same population. On the other hand, Mudge et al. (1997) found that a single SSR marker on Linkage Group G was 95% accurate in predicting resistance in soybean to the soybean cyst nematode, *Heterodera glycines*. The accuracy of prediction of resistance/susceptibility phenotype was improved to 98% when two SSR markers flanking the resistance QTL were used. The use of SSR markers flanking this QTL to identify soybean lines with resistance to soybean cyst nematode is one of the first applications of MAS in both public and commercial soybean breeding.

Researchers evaluating qualitative soybean traits are expected to include a hypothesis generation and a confirmation generation prior to assignment of a gene symbol (Soybean Genetics Committee, 1997). However, for QTL mapping studies, this confirmation is generally not required in any species (Boerma and Mian, 1999). Considering the mixed results shown in some QTL mapping studies, it seems prudent to perform verification studies before QTL are used in marker assisted breeding.

There are a number of important QTL mapping populations that have been created in soybean. These include the recombinant inbred line population of Minsoy x Noir 1 (Mansur et al., 1996), the F_2-derived line population from the interspecific cross A81-356022 x PI468916 (Keim et al., 1990), the F_2-derived line population of PI97100 x Coker 237 (Lee et al., 1996c), and the F_4-derived line population of Young x PI416937 (YxPI) (Mian et al., 1996a). The YxPI population consists of 120 F_4-derived lines that were developed by single-seed-descent from 120 individual F_2 plants. Quantitative trait loci conditioning a number of soybean traits identified and reported in the YxPI

population, but not yet confirmed include: pod dehiscence, maturity, oil, protein, seed weight, specific leaf weight, leaf size, water use efficiency, leaf ash, plant height, lodging, and bud blight resistance. Each of these QTL mapping studies was conducted using the RFLP marker system (Bailey et al., 1997; Bianchi-Hall et al., 2000; Lee et al., 1996 a,b,c; Mian et al., 1996a,b; Mian et al., 1998).

Lee et al. (1996) reported a number of RFLP markers associated with seed protein and oil content in the YxPI population. The experiment was conducted at three field locations; Plains GA Windblow NC, and Plymouth NC. Thirteen RFLP markers were associated with seed protein, seven of which were independent QTL (significant ($P < 0.01$) markers on different linkage groups or at least 50 cM from another significant marker). The total amount of phenotypic variation explained by these seven markers was 70% and the heritability for seed protein was 83%. Of the seven independent markers, five were significant ($P < 0.05$) in all three locations.

For seed oil content, six markers were detected, four of which represented independent QTL. Together these four independent markers explained 34% of the phenotypic variation for oil content. The heritability for oil content in this population was 89%. Therefore, the majority of the genotypic variation was not explained by these four markers. This suggests that either the identified RFLP markers were not closely linked to the QTL for seed oil, or there are still undetected QTL in unmapped regions of the genome in this population. All four of the markers were significant ($P < 0.05$) in each of the three locations, suggesting that these QTL for seed oil were environmentally stable across locations (Lee et al., 1996).

Seed weight is a trait that plays an important role in the acceptance of a soybean cultivar for different food applications. Mian et al. (1996) evaluated RFLP markers associated with seed weight in the YxPI population. The experiment was conducted at the same three locations that protein and oil data were collected. A total of seven independent RFLP markers were found to be associated with seed weight. Six out of these seven were identified in all three environments. Together, the seven markers explained 73% of the phenotypic variation. The heritability of seed weight in this population was 90%. Therefore, the majority of the phenotypic variation could be explained by the seven RFLP markers (Mian et al., 1996).

Tobacco ringspot virus is a member of the nepovirus group of plant viruses. The virus can cause disease in various plant species, but one of the most severe is bud blight of soybean. Bud blight can reduce soybean yields by 25 to 100% (Demski and Kuhn, 1999). Losses are greatest from this disease when a large amount of infected seed is sown in the field or when young plants are infected. Early symptoms of the virus include stunted growth and curvature of the terminal bud (apical meristem) to form a hook. Later, the buds on the soybean plant will become brown and necrotic, and the pods are usually underdeveloped or aborted. Root and nodule growth are also significantly reduced (Demski and Kuhn, 1999).

Fasoula et al. (1996) identified restriction fragment length polymorphism (RFLP) markers that were associated with bud blight resistance in the YxPI population. In this study, Young was resistant and PI416937 was susceptible to bud blight. A major QTL accounting for 82% of the phenotypic variation in bud blight incidence was mapped on LG F near RFLP marker K644. Two minor QTL were found on LG G and R and

accounted for 8 and 12 % of the variation, respectively. Young contributed the resistance allele for the major QTL on LG F, while PI416937 contributed the resistance alleles for the two minor QTL.

Recently the 'Benning' x 'Danbaekkong' mapping population was developed at the University of Georgia. This population was created for mapping QTL conditioning traits associated with tofu and soymilk quality. Danbaekkong is a MG IV cultivar developed in South Korea for soyfood use (Kim et al., 1996). It possesses a seed protein content of approximately 51% compared to 42% for the MG VII cultivar Benning. Benning is a highly productive, multiple pest resistant cultivar adapted to the southeastern USA (Boerma et al., 1997). The mapping population consists of 180 F_2-derived lines. In 1998 these lines were grown at the Plant Sciences Farm near Athens GA.

An initial SSR map of this population was created by collecting data on approximately 90 equally spaced SSR markers. The 180 lines ranged in protein content from 41 to 51%. The SSR data were associated with the protein data across the entire population by use of analysis of variance procedures. This analysis identified five QTL conditioning protein content. These QTL were positioned on LG A1 (conditioning 8% of the phenotypic variation in protein content), LG A2 (9%), LG E (11%), LG I (49%), and LG K (10%). The major protein QTL on LG I was mapped within 2 cM of Satt239. The lines that were homozygous for the Danbaekkong allele at Satt239 averaged 3.3% higher protein (46.8 vs. 43.5% protein) than the lines homozygous for the Benning allele.

The value of grain-type soybean is determined by the individual component values of the its protein and oil. The protein present in soybean seed is of greater value

than the oil component. With the expanded production of palm, sunflower, and canola oil , the protein component of the soybean seed is expected to become proportionately more valued. Although protein content of soybean seed generally has a high heritability (Burton, 1987), a major difficulty in soybean breeding programs selecting for increased protein has been the existence of a negative correlation between percent protein and seed yield (Burton, 1985). Genotypic correlations between seed protein content and seed yield have generally ranged from -0.74 to 0.54. The identification of a major QTL for protein content provided an opportunity to directly select lines with superior protein and high seed yield.

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CHAPTER 3

AGRONOMIC PERFORMANCE OF SOYBEAN LINES SELECTED FOR ALTERNATIVE ALLELES AT A MAJOR PROTEIN QTL[1]

[1] Harris, D.K., T. Wang, M.A.R. Mian, E.D. Wood, and H.R. Boerma. 2002. To be submitted to Crop Science.

Abstract

A F_2-derived soybean [*Glycine max* (L.) Merr.] population of 'Benning' x 'Danbaekkong' was developed to map quantitative trait loci (QTL) conditioning traits associated with tofu and soymilk quality. Benning is a productive, multiple pest resistant cultivar adapted to the southeastern USA (Maturity Group VII) with approximately 420 g kg^{-1} seed protein content. Danbaekkong is a Maturity Group IV cultivar developed in South Korea for use in human foods. It has a seed protein content of approximately 510 g kg^{-1}. Discovery of a major protein QTL conditioning 49% of the phenotypic variation on Linkage Group I near Satt239 allowed selection of F_2-derived lines from the Benning x Danbaekkong population with divergent protein content for evaluation of their agronomic performance. Twenty-eight $F_{2:4}$ lines (a total of 14 with elevated seed protein content and 14 with conventional seed protein content) along with four entries each of Benning and Danbaekkong were evaluated in replicated field plots at two locations in 1999. A total of 12 lines [six of the highest yielding $F_{2:5}$ elevated protein lines homozygous for the Danbaekkong allele at Satt239 (D/D-Satt239) and six of the highest yielding $F_{2:5}$ conventional protein lines homozygous for the Benning allele at Satt239 (B/B-Satt239)] were selected from the original 28 lines for testing in 2000 along with three entries each of Benning and Danbaekkong. Averaged across both years, five of the six elevated protein F_2-derived lines were the lowest yielding lines. One D/D-Satt239 F_2-derived line, G98SF-114, averaged 485 g kg^{-1} protein and was equal in yield and maturity to Benning.

Introduction

The value of grain-type soybean is determined by the individual component values of the seed protein and seed oil. The protein that is present in soybean seed is of greater value than the oil component. Soybean seed is a major protein source for animal feed and in the human diet in many Asian countries. With the expanded production of palm (*Elaeis guineensis*), sunflower (*Helianthus annuus)*, and canola (*Brassica napus)* oil, the protein component of the soybean seed is expected to become proportionately more valued.

U.S. soybean cultivars average 410 g kg^{-1} protein and 210 g kg^{-1} of oil (Leffel and Rhodes, 1993). Soybean germplasm exist with greater than 500 g kg^{-1} protein. Protein content of the soybean seed generally has a high heritability (Burton, 1987). However, a major difficulty in soybean breeding programs selecting for increased protein has been the existence of a negative correlation between percent protein and seed yield (Burton, 1985). A review of six studies that each included multiple populations found a mean genotypic correlation of –0.18 and a range of –0.74 to 0.54 (Burton, 1987).

Diers et al. (1992) used F_2-derived lines from a cross between a *G. max* experimental line (A81-356022) and a *G. soja* Sieb. and Zucc. plant introduction (PI 468916) to map two major QTL for protein and oil concentration with RFLP markers. The QTL mapped to linkage group (LG) K and LG A which have subsequently been reassigned to LG I and LG E, respectively (Cregan et al., 1999; Shoemaker and Specht, 1995). The QTL on LG I explained 42% of the variation in seed protein content, and was associated with an increase of 24 g kg^{-1} in protein concentration when a line was homozygous for PI 468916 alleles. The QTL on LG E explained 24% of the variation in

seed protein content, and lines homozygous for PI 468916 alleles averaged 17 g kg^{-1} higher protein content. Brummer et al. (1997) also mapped a QTL associated with protein concentration on LG I in the same region reported by Diers et al. (1992) using a population developed from the cross of M82-806 x HHP. M82-806 was derived from a cross of [('Evans' x M62-345) x {('Lincoln' x 'Hawkeye') x 'Harosoy'}], and HHP is a breeding line that is 25% *G. soja* Siebold and Zucc. (Brummer et al., 1997).

Sebolt et al. (2000) evaluated the two protein QTL alleles reported by Diers et al. (1992) by backcrossing the *G. soja* alleles into a *G. max* background. The *G. soja* alleles at the QTL on LG I significantly increased protein concentration, while the *G. soja* alleles at the QTL on LG E did not. A second objective of their study was to evaluate the effect of the *G. soja* protein QTL allele on LG I in three additional *G. max* genetic backgrounds. They found that in two of the three crosses the *G. soja* protein QTL allele was associated with an increase in protein concentration.

In a recent study we mapped protein QTL in a population of 180 $F_{2:3}$-derived lines created by crossing Benning and Danbaekkong (Harris et al., 2000). Benning is a Maturity Group (MG) VII, highly productive, multiple pest resistant cultivar adapted to the southeastern USA with a seed protein content of approximately 42% (Boerma et al., 1997). Danbaekkong is a MG IV cultivar developed in South Korea for tofu and soybean milk production and has a seed protein content of approximately 51% (Kim et al., 1996). The 180 F_2-derived lines were grown in replicated field plots for 2 yr. This study identified five QTL conditioning protein content. These QTL were positioned on LG A1 (conditioning 8% of the phenotypic variation in protein content), LG A2 (9%), LG E (11%), LG I (49%), and LG K (10%). At the protein QTL on LG A1, A2, E, and I, the

Danbaekkong allele increased protein content. The Benning allele increased protein for the QTL on LG K. The major QTL on LG I was mapped within 2 cM of Satt239 in the same genomic region identified in previous studies (Diers et al., 1992; Brummer et al., 1997). The lines that were homozygous for the Danbaekkong allele at Satt239 averaged 33 g kg^{-1} higher protein (468 vs. 435 g kg^{-1}) than the lines homozygous for the Benning allele.

This F_2-derived population of 180 lines provided an opportunity to select lines with similar maturity and divergent protein content. The objective of this study was to evaluate the agronomic performance of F_2-derived lines selected from the Benning x Danbaekkong population for divergent seed protein content based on a major protein QTL.

Materials and Methods

Based on a 1998 replicated field experiment conducted at Athens, GA, twenty-eight MG VII, $F_{2:4}$ lines ranging approximately 2 wk in maturity were selected for this experiment. Fourteen of these lines were selected with elevated protein content (470 to 515 g kg^{-1}) and 14 with conventional protein content (415 to 433 g kg^{-1}) (Table 1). Thirteen of the elevated protein lines were homozygous for the Danbaekkong allele (D/D-Satt239), and 13 of the conventional protein lines were homozygous for the Benning allele at Satt239 (B/B-Satt239). G98SF-4, a line with a protein content of 470 g kg^{-1}, was homozygous for the Benning allele at Satt239 while S98SF-30 with protein content of 433 g kg^{-1}, was homozygous for the Danbaekkong allele at this protein QTL. Both of these lines were included as possible lines with a cross-over in the region of the

Satt239-protein QTL. These 28 lines along with four entries each of Benning and Danbaekkong (total of 36 entries) were grown at the Univ. of Georgia Southwest Branch Experiment Station at Plains, GA and Univ. of Georgia Plant Sciences Farm near Athens, GA in 1999. An experimental unit (plot) consisted of two-rows that were 7 m in length with 76-cm between rows. Plots were sown at a rate of 27 seeds m^{-1} row. The experimental design was a randomized complete block with three replications. At maturity, all plots were end-trimmed to 3.66-m rows in length, and were harvested with a plot combine.

Data were collected for maturity, plant height, lodging, seed yield, seed weight, and protein and oil content of the seed. Protein and oil content of seed from each plot were determined by near infrared transmittance on whole beans and data reported on a moisture-free basis at the USDA Northern Regional Research Center at Peoria, IL. Maturity date was recorded on each plot when 95% of the plants in a plot reached their mature pod color (R8 stage of development; Fehr et al, 1971). Plant height was measured as distance from the ground to the terminal node. Lodging was recorded as a score of one to five with five indicating that all plants in a plot were lying prostrate, and one indicating all plants were standing erect. Both lodging and plant height data were collected at maturity. Data for seed yield are reported on a 130 g kg^{-1} moisture basis. Data for seed weight were determined by weighing 100 seeds from each plot. All data were analyzed by analysis of variance procedures using Agrobase™ (Agronomix Software Inc., Manitoba, Canada). Locations and replications were considered as random effects and genotypes as a fixed effect.

Based on the 1999 field data, six of the highest yielding D/D-Satt239 elevated $F_{2:5}$ protein lines and six of the highest yielding B/B-Satt239 conventional $F_{2:5}$ protein lines were selected from the original 28 lines for evaluation in 2000. These lines were grown along with three entries each of Benning and Danbaekkong (total of 18 entries) at the same two locations as the 1999 experiment. An experimental unit (plot) consisted of four-rows that were 7 m in length with 76 cm between rows. Plots were sown at a rate of 27 seeds m^{-1} row. The experimental design was a randomized complete block with three replications. At maturity, plots were end-trimmed to 3.66 m in length, and the two middle rows of each plot were harvested with a plot combine. Data were collected for maturity, plant height, lodging, seed yield, seed weight, and protein and oil content of the seed. Data for all traits were collected as described for the 1999 experiment.

All data were analyzed by analysis of variance procedures using Agrobase™ (Agronomix Software Inc., Manitoba, Canada). For each year, genotypes were considered as a fixed effect and locations and replications as random effects. For the 12 lines and parents that were included across both years, analysis of variance was conducted considering replications, locations, and years as random effects and genotypes as a fixed effect.

Results and Discussion

In 1999, the mean seed protein content of the 13 conventional B/B-Satt239 protein lines was 442 g kg^{-1} and the 13 elevated D/D-Satt239 protein lines averaged 54 g kg^{-1} higher seed protein content (496 g kg^{-1}) (Table 2). The range in protein content of the 13 elevated D/D-Satt239 and 13 conventional B/B-Satt239 protein lines did not

overlap. The mean protein content of the 13 conventional B/B-Satt239 protein lines was not significantly ($P < 0.05$) different from Benning, and the mean of the 13 elevated D/D-Satt239 protein lines was not different than Danbaekkong. G98SF-4 which was selected for elevated seed protein content in 1998 but was homozygous for the Benning allele at Satt239 had a mean seed protein content of 460 g kg^{-1}, while G98SF-30, which was selected for conventional seed protein content but homozygous for the Danbaekkong allele at Satt239, had a mean seed protein content of 474 g kg^{-1}. It appears that rather than a cross-over between Satt239 and the protein QTL in both lines, that the protein content based on one location in 1998 was not well characterized.

The 13 conventional B/B-Satt239 protein lines averaged 272 kg ha^{-1} higher seed yield than the 13 elevated D/D-Satt239 protein lines. The yield range of the conventional B/B-Satt239 protein lines did overlap with the elevated D/D-Satt239 protein lines. Although the previous selection for high and low protein could cause a potential bias, the phenotypic correlation between protein and seed yield was $r = -0.47$. The mean of the 13 conventional B/B-Satt239 protein lines was not significantly ($P < 0.05$) different from Benning in seed yield, and the mean 13 elevated D/D-Satt239 protein lines was not different from the seed yield of Danbaekkong. Mean maturity for both groups of lines was equal to that of Benning.

Averaged across the two years, the seed yield for the 12 lines ranged from 2,466 to 2,939 kg ha^{-1}, while seed protein ranged from 435 g kg^{-1} to 486 g kg^{-1}(Table 3). Benning averaged 594 kg ha^{-1} higher yield, 96 g kg^{-1} lower protein content, 32 g kg^{-1} higher oil content, and 12 mg $seed^{-1}$ greater seed weight than Danbaekkong. Six of the seven highest yielding F_2-derived lines were from the conventional B/B-Satt239 protein

group of selected lines. This result is consistent with the negative genotypic correlation between seed yield and seed protein content (Burton, 1987). G98SF-114 which was from the elevated D/D-Satt239 protein group averaged 2,835 kg ha^{-1} seed yield and 485 g kg^{-1} seed protein across the four environments. G98SF-114 was similar in plant height, lodging, seed quality, seed weight, seed yield, and maturity to Benning, the high yield, conventional protein parent. However, it's seed averaged 74 g kg^{-1} higher protein than Benning.

For 11 of the 12 lines grown over four environments, elevated protein content was associated with low seed yield and conventional protein content with high seed yield. G98SF-114 had an elevated seed protein content, while maintaining a seed yield equal to that of Benning. Furthermore, the protein QTL on LG A1, E, I, and K identified by markers Satt385, Satt231, Satt239, and Satt441 respectively, are fixed in G98SF-114 for the alleles for increased protein (Table 4). However, at the protein QTL identified by marker Satt429 on LG A2 is not fixed in G98SF-114. Therefore, additional single plant selection from G98SF-114 may identify lines with increased seed protein content. Inbred lines selected from G98SF-114 have the possibility of being equal in protein content to Danbaekkong (approximately 51%).

The identification of a major protein QTL on LG I, provided an opportunity to evaluate the performance of agronomic traits of F_2-derived lines selected for divergent seed protein content based on the alleles at this QTL. From the 28 F_2-derived lines selected from the original population of 180 lines, one line, G98SF-114 was identified with approximately 480 g kg^{-1} protein and a seed yield and maturity equal to the highly productive, multiple pest resistant Benning. G98SF-114 provides germplasm for use as a

parent in breeding programs attempting to combine superior yield and protein content. This line also merits additional evaluation to determine the basis for it's performance. It is possible that a repulsion phase linkage between the protein QTL on LG I and an unidentified yield QTL has been broken or that the Danbaekkong allele at the protein QTL on LG I does not reduce yield in the Benning genetic background. The availability of G98SF-114 allows evaluation of these and other possible alternative explanations. The identification of this line would suggest that it is possible with the help of DNA markers to break the negative association of protein content and seed yield.

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Table 3.1. Seed protein and oil content and maturity of 28 F_2-derived lines selected for divergent protein content from the 1998 replicated field experiment.

Lines	Satt239	Seed protein	Seed oil	Maturity
	alleles‡	----------g kg^{-1}----------		date
Elevated protein lines				
G98SF-57	D/D	515	158	10/27
G98SF-116	D/D	496	181	10/27
G98SF-118	D/D	491	176	10/27
G98SF-16	D/D	490	166	10/27
G98SF-17	D/D	488	169	10/27
G98SF-111	D/D	487	175	10/13
G98SF-114	D/D	485	175	10/27
G98SF-46	D/D	483	180	10/27
G98SF-5	D/D	480	168	10/30
G98SF-3	D/D	479	169	10/23
G98SF-90	D/D	479	180	10/27
G98SF-115	D/D	478	175	10/27
G98SF-2	D/D	478	175	10/20
G98SF-4†	B/B	470	164	10/30
Conventional protein lines				
G98SF-113	B/B	432	196	10/27
G98SF-23	B/B	431	202	10/27
G98SF-98	B/B	431	192	10/27
G98SF-84	B/B	430	203	10/27
G98SF-136	B/B	428	203	10/27
G98SF-129	B/B	428	199	10/27
G98SF-141	B/B	427	198	10/27
G98SF-67	B/B	425	195	10/27
G98SF-86	B/B	424	204	10/27
G98SF-128	B/B	421	202	10/27
G98SF-102	B/B	420	202	10/27
G98SF-97	B/B	419	200	10/27
G98SF-9	B/B	415	203	10/27
G98SF-30†	D/D	433	197	10/27
Parents				
Benning	B/B	395	211	10/27
Danbaekkong	D/D	502	166	10/9

† G98SF-4 and G98SF-30 are possible cross-over types in the Satt239 protein QTL genomic region.
‡ B/B = homozygous for Benning allele, D/D = homozygous for Danbaekkong allele.

Table 3.2. Mean seed and plant traits of selected B/B-Satt239 conventional and D/D-Satt239 elevated protein F_2-derived lines at two locations in 1999.

		Seed								Plant					
		yield		protein		oil		weight		lodging		maturity		height	
	Entries	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
	no.	-----kg ha^{-1}-----		-----g kg^{-1}-----		-----g kg^{-1}-----		---mg seed^{-1}---		----rating‡----		------date------		------cm------	
F_2-derived lines															
Conventional B/B-Satt239	13	2,535a†	2,117-2,769	442a	428-452	189a	184-198	148a	130-171	1.8a	1.5-2.7	10/25a	10/18-10/30	88a	81–94
Elevated D/D-Satt239	13	2,263b	1,828-2,722	496b	486-518	165b	154-173	133b	120-158	2.0a	1.3-2.5	10/25a	10/17-11/08	87a	76-94
Parents															
Benning	4	2,715a	2,601-2,843	420a	419-423	197a	196-199	153a	151-155	2.0a	1.8-2.2	10/26a	10/25-10/27	98a	97-98
Danbaekkong	4	2,155b	2,029-2,285	515b	505-520	163b	160-166	142b	139-144	1.5a	1.3-1.5	9/27b	9/26-9/27	70b	69-70

† Means of F_2-derived lines or means of parents followed by the same letter do not differ based on an LSD (0.05).

‡ Rating: 1 = all plants upright to 5 = all plants prostrate.

Table 3.3. Mean performance of six conventional B/B-Satt239 protein and six elevated D/D-Satt239 protein F_2-derived lines and their parents at two locations in 1999 and 2000.

		Seed					Plant		
Genotype	Satt239	yield	protein	oil	weight	quality	lodging	maturity	height
	alleles‡	Kg ha^{-1}	----------g kg^{-1}---------		mg seed^{-1}	----------rating----------		date	cm
F_2-derived lines†									
G98SF-129	B/B	2,939	442	193	148	1.7	1.9	10/24	84
G98SF-84	B/B	2,899	441	191	148	1.4	1.7	10/25	87
G98SF-113	B/B	2,858	440	191	136	1.5	1.8	10/21	80
G98SF-114	D/D	2,835	485	174	138	1.5	1.9	10/22	89
G98SF-23	B/B	2,834	441	193	165	1.5	1.6	10/24	80
G98SF-86	B/B	2,801	435	193	128	1.4	1.8	10/26	88
G98SF-141	B/B	2,745	435	191	134	1.3	2.1	10/25	87
G98SF-90	D/D	2,632	484	168	124	1.3	1.9	10/24	84
G98SF-118	D/D	2,618	496	166	127	1.5	1.9	10/21	81
G98SF-116	D/D	2,616	492	175	153	1.5	1.6	10/23	85
G98SF-3	D/D	2,592	485	166	119	1.4	1.5	10/21	71
G98SF-16	D/D	2,466	486	162	119	1.4	2.0	10/22	92
LSD(0.05)		349	11	5	16	0.3	0.4	5	2
Parents									
Benning		2,899a§	411a	201a	149a	1.3a	1.9a	10/23a	93a
Danbaekkong		2,305b	507b	169b	137b	1.8b	1.5b	9/29b	58b

† F_2-derived lines are ranked by yield in descending order.
‡ B/B = homozygous for Benning allele; D/D = homozygous for Danbaekkong allele.
§ Means of parents followed by a different letter differ based on an LSD(0.05).

Table 3.4. Marker genotypes of six conventional B/B-Satt239 and six elevated D/D-Satt239 protein F_2-derived lines at four protein QTL.

Genotype	LG A1 Satt385	LG A2 Satt429	LG E Satt231	LG K Satt441
	----------alleles†----------			
Conventional B/B-Satt239 protein				
G98SF-129	D/D	D/D	B/D	B/D
G98SF-84	B/D	B/D	B/D	B/B
G98SF-113	B/D	D/D	B/D	B/D
G98SF-23	B/B†	B/D	-	B/B
G98SF-86	B/D	B/B	B/D	D/D
G98SF-141	B/D	B/D	B/B	B/D
Elevated D/D-Satt239 protein				
G98SF-90	D/D	B/D	D/D	B/B
G98SF-114	D/D	B/D	D/D	B/B
G98SF-118	B/D	B/D	D/D	B/B
G98SF-116	D/D	B/D	B/D	B/B
G98SF-3	D/D	D/D	D/D	B/B
G98SF-16	B/D	B/D	B/D	D/D

† B = Benning allele, D = Danbaekkong allele

CHAPTER 4

CONFIRMATION OF A SOYBEAN QTL CONDITIONING RESISTANCE TO BUD BLIGHT[1]

[1]Harris, D.K., V.A. Fasoula, D.V. Phillips, and H.R. Boerma. 2002.

Abstract

Bud blight, caused by tobacco ringspot virus, can significantly reduce the seed yield and seed quality of soybean [*Glycine max* (L.) Merr.]. A F_2-derived soybean population developed from the cross of 'Young' (resistant) x PI416937 (susceptible) was used to confirm a previously reported QTL on Linkage Group (LG) F near RFLP marker K644 conditioning resistance to bud blight and determine the effectiveness of marker-based selection for bud blight incidence. The population of 180 $F_{2:3}$ lines and 10 entries of each parent were planted in six-plant hills in a randomized complete block design with three replications at the Univ. of Georgia Plant Sciences Farm near Athens, GA in 1995. The soybean plants were naturally infected with tobacco ringspot virus and were scored at maturity for the percent of barren plants with delayed leaf drop and green stems in each plot. Seventeen simple sequence repeat (SSR) markers on LG F within 66 cM of the proposed location of the QTL were tested for polymorphism, and five were found to be polymorphic. The SSR data for the 180 $F_{2:3}$ lines were collected for the five polymorphic markers. Interval mapping was conducted with Mapmaker Exp 3.0/QTLl 1.1b and composite interval mapping with QTL Cartographer Windows Version (version 1.21) to confirm associations between the five markers on LG F and the QTL conditioning resistance to bud blight. Results confirmed a major QTL conditioning resistance to bud blight on LG F located in the 14 cM-interval between Satt114 and Satt510. The most likely position of this QTL was 4 cM from Satt510. The Satt510 marker is within 2 to 3 cM of RFLP marker K644. The QTL for bud blight accounted for 89% of the phenotypic variation in bud blight resistance which approached 100% of the genotypic variation in this population. In this population, lines that were homozygous for the Young band at

Satt510 averaged 15.3% bud blight incidence while lines homozygous for the PI416937 band averaged 95.1%.

Introduction

Tobacco ringspot virus is a member of the nepovirus group of plant viruses. The virus can cause disease in various plant species, but one of the most severe is bud blight of soybean. Bud blight can reduce soybean yields by 25 to 100% (Demski and Kuhn, 1999). Losses are greatest from this disease when a large amount of infected seed is sown in the field or when young plants are infected. Early symptoms of the virus include stunted growth and curvature of the terminal bud (apical meristem) to form a hook. Later, the buds on the soybean plant will become brown and necrotic, and the pods are usually underdeveloped or aborted. Root and nodule growth are also significantly reduced (Demski and Kuhn, 1999).

Fasoula et al. (1996) identified restriction fragment length polymorphism (RFLP) markers that were associated with bud blight resistance in a population of F_4-dervied lines from the cross 'Young' x PI416937 (Burton et al., 1987; Sloane et al., 1990). In this study, Young was resistant and PI416937 was susceptible to bud blight. The mapping population consisted of 120 F_4-derived lines. A major quantitative trait loci (QTL) accounting for 82% of the phenotypic variation in bud blight incidence was mapped on LG F near RFLP marker K644. Two minor QTL were found on LG G and R and accounted for 8 and 12 % of the variation, respectively. Young contributed the resistance allele for the major QTL on LG F, while PI416937 contributed the resistance alleles for the two minor QTL.

The objectives of this study were to confirm the genomic location of the major bud blight QTL on LG F using simple sequence repeat (SSR) markers and determine the

effectiveness of marker-based selection for bud blight incidence in a F_2-derived population of 180 lines developed from the cross of Young x PI416937.

Materials and Methods

A population of 180 F_2-derived ($F_{2:3}$) lines from the cross of Young x PI416937 was developed. The initial cross was made in 1993. The F_1 generation was grown from December 1993 to April 1994 in a greenhouse in Athens, GA. The F_2 generation was grown at the Univ. of Georgia Plant Sciences Farm near Athens, GA in 1994. At maturity, 180 F_2 plants were individually harvested to create F_2-derived lines. In 1995, the 180 F_2-derived lines were planted on 5 June at the Univ. of Georgia Plant Sciences Farm in a randomized complete block design with three replications. Ten entries of each parent were randomized within each replication for a total of 200 entries (180 F_2-derived lines, 10 entries of Young, and 10 entries of PI416937). The experiment was planted in hill plots with 12 seeds per plot. Three weeks after planting, each plot was thinned to six plants per plot. Leaf tissue for DNA extraction was collected from at least 12 plants of each line at the V3 stage of development (Fehr et al., 1971).

Plants were naturally infected with tobacco ringspot virus. The presence of the virus was verified by sampling leaves from the experiment and sending them to Agdia® Inc. in Elkhart, IN for verification of the virus through an ELISA test. At maturity, when 95% of the plants in a plot reached their mature pod color (R8 stage of development, Fehr et al., 1971), the plants were scored for bud blight development. The plots were scored for the percent of barren plants with delayed leaf drop and green stems in each plot. Data

were analyzed by analysis of variance using Agrobase™ software (Agronomix Software Inc, Manitoba, Canada).

In the laboratory, DNA was extracted from leaf tissue using the modified CTAB procedure (Keim et al., 1988). It was then quantified with the UV/VIS Spectrometer (Pekin Elmer) and diluted to 20 ng/µl. DNA bulks consisting of 10 F_2-derived lines without bud blight symptoms and 10 F_2-derived lines with bud blight symptoms were created (Michelmore et al., 1991). A total of four bulks were created, two susceptible (S1 and S2) where each line had a rating of 100% of plants with bud blight symptoms, and two resistant (R1 and R2) where lines ranged in rating from 0 to 5.7% of plants with symptoms.

Seventeen simple sequence repeat (SSR) markers developed by Dr. P.B. Cregan (USDA-ARS, Beltsville, MD) were tested on LG F in a 66 cM region surrounding RFLP marker K644. Fluorescence dye-labeled primers were synthesized (PE-ABI, Foster City, CA) using phosphoramidite chemistry. PCR reactions were prepared using the protocol by Diwan and Cregan (1997). The reactions were performed in a dual 384-well and 96-well GeneAmp® PCR System 9700 or a 384-well ABI 877 robotic thermal cycler (PE-ABI, Foster City, CA). The cycling consisted of 1 min at 95°C, followed by 32 cycles of 25 s for denaturation at 94°C, 25 s of annealing at 46°C, and 25 s of extension at 68°C. At the end of the cycling procedure, the reaction mixtures were held at 4°C. Electrophoresis was run on an ABI-Prism 377 DNA Sequencer (PE-ABI, Foster City, CA) with 12-cm plates at 750 V for 2 h. Lanes were loaded on a 4.8% acrylamide:bisacrylamide (19:1) gel with KLOEHN micro-syringes (Kloehn, Ltd., Las

Vegas, NV). Genescan® (version 3.0) was used to analyze DNA fragments, and then they were scored with Genotyper® (version 2.1).

Five of the 17 SSR markers were polymorphic for Young and PI416937. The parental DNA and the four bulks were evaluated for their DNA fragment (s) at each polymorphic marker. The population of 180 $F_{2:3}$ lines was then evaluated with the five polymorphic markers.

Interval mapping was conducted with the computer program Mapmaker Exp 3.0/QTL 1.1b (Lander et al., 1987; Lincoln et al., 1992) to determine associations between SSR markers on LG F and bud blight incidence. A minimum LOD (Likelihood of odds) of 3.0 and maximum distance of 37.2 cM was used for testing linkages among markers. A minimum LOD score of 2.0 was used to declare the presence of a QTL. QTL Cartographer Windows Version (version 1.21) was also used to confirm the QTL (Basten et al., 1994; Wang et al., 1999). The marker linkage map was imported to QTL Cartographer using the output files from MAPMAKER/EXP. Zmapqtl Model 6 and a forward regression method were used for composite interval mapping. In the Zmapqtl Model 6, two-control markers and a 10-cM window size were selected.

Results and Discussion

The F_2-derived lines ranged from 0 to 100% of plants infected with bud blight. Of the 180 F_2-derived lines, 36 had 100% incidence of bud blight (6 plants/hill x 3 reps = 18 plants) and 13 of the lines had a 0% incidence. PI416937, the susceptible parent, averaged 98.2% incidence and the resistant parent, Young, averaged a 5.8% incidence (Fig. 1). The range of incidence among the 10 entries of Young was 0 to 19%, and

PI416937 ranged from 93.3 to 100% incidence. From bulk segregant analysis, the singular DNA fragment produced from the resistant bulks (R1 and R2) from each of the five polymorphic SSR markers on LG F (Satt114, Satt510, Sat120, Satt335, and Satt362) were the same as the fragment size produced by the resistant parent Young. In addition, the two susceptible bulks produced a singular band which was the same size as the susceptible parent, PI416937 for all five SSR markers.

Mapmaker/QTL analysis mapped the QTL conditioning bud blight on LG F in the 13.9-cM interval between Satt114 and Satt510 (Fig. 2). The most likely QTL location was 4 cM from Satt510. On the USDA/Iowa State Univ. soybean linkage map, Satt510 is located 2.3 cM from RFLP marker K644 (Cregan et al., 1999). This RFLP marker identified the major QTL conditioning bud blight in the earlier study (Fasoula et al., 1996a). The percent of plants with bud blight symptoms averaged 15.3% for the lines homozygous for the Young fragment at Satt510, 95.1% for lines homozygous for the PI416937 band, and 62.6% for the heterogeneous lines. From these results, it would appear that the bud blight QTL acts in an additive manner. The LOD score for the presence of a QTL conditioning bud blight at this location in the 13.9-cM region between Satt510 and Satt114 was 50. The QTL at the most likely site accounted for 89% of the phenotypic variation in bud blight resistance. With the calculated variance component heritability of 91% for bud blight in the present study, this QTL on LG F approached 100% of the genotypic variation in this population. The peak position identified by composite interval mapping with QTL Cartographer was similar to that identified by Mapmaker/QTL.

The accuracy of using Satt510 for marker-assisted selection in this population for determining bud blight incidence was evaluated (Fig. 3). The range of bud blight incidence for the lines homozygous for the Young band at Satt510 and homozygous for the PI416937 band did overlap. F_2-drived lines homozygous for the PI416937 band possessed at least 50% of their plants with bud blight. One line homozygous for the Young band contained 75% of its plants with bud blight. These results suggest selection based on SSR marker Satt510 would effectively eliminate highly susceptible lines, but lines homozygous for the resistant parent band at Satt510 would require phenotypic evaluation with the virus to eliminate the remaining susceptible lines.

These results confirm a major QTL conditioning bud blight resistance located on LG F in the 13.9 cM region between Satt510 and Satt114. Results also indicate that SSR marker Satt510 can be used in marker assisted selection of bud blight resistance in this population to eliminate lines that are susceptible to bud blight. Any lines that are homozygous for the resistant parent band at Satt510 would require additional selection by phenotypic evaluation with the tobacco ringspot virus to identify homogeneous bud blight resistant lines.

Acknowledgement

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Figure 4.1. Distribution of bud blight incidence for 180 F_2-derived soybean lines from the cross of Young x PI416937.

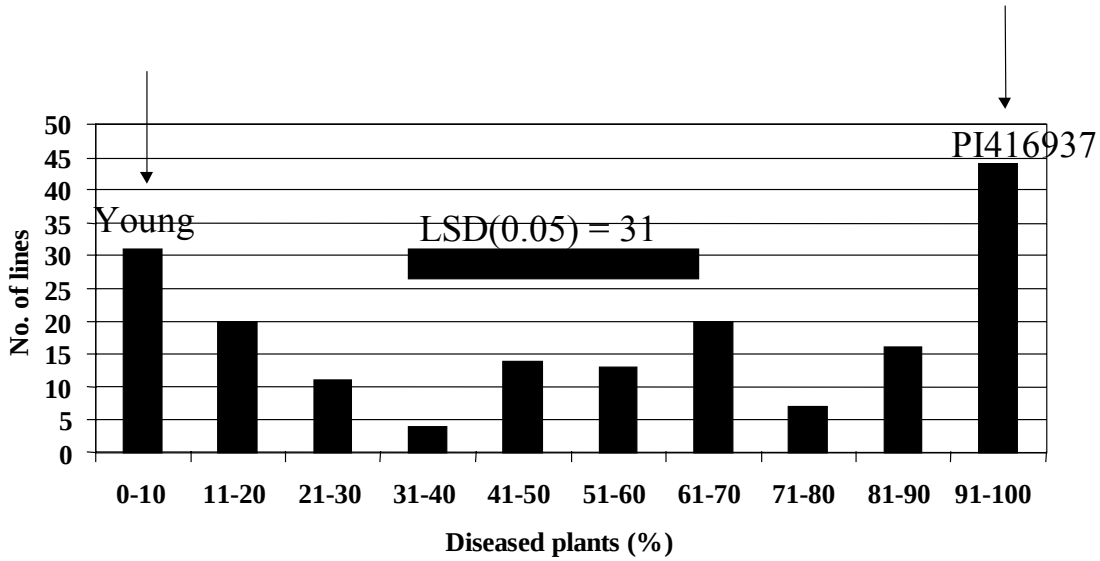


Figure 4.2. QTL likelihood plot for tobacco ringspot virus on Linkage Group F for the Young x PI416937 F_2-derived soybean population based on Mapmaker/QTL analysis (* = most likely QTL location). Location of K644 is approximated based on Cregan et al. (1999) and Narvel et al. (2002).

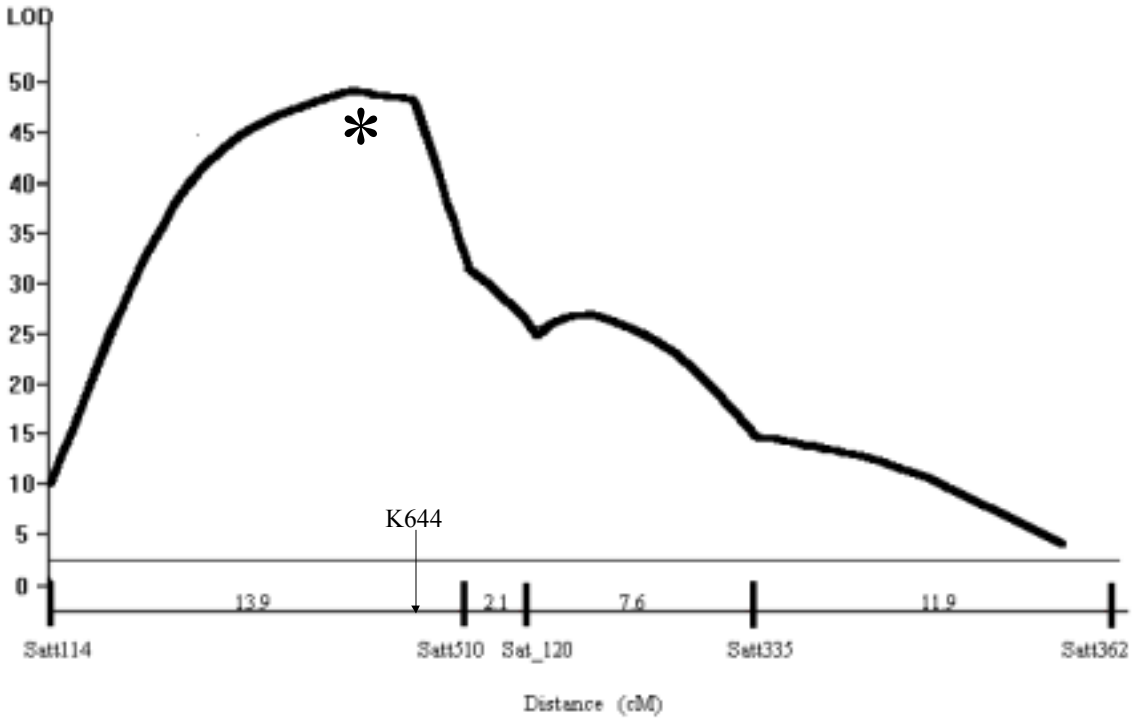
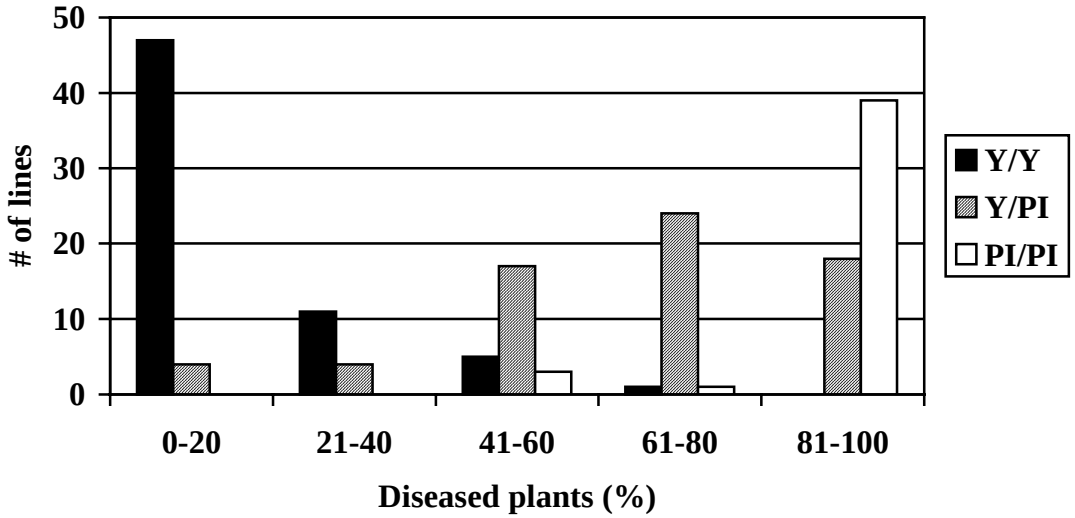


Figure 4.3. Mean incidence of bud blight in 180 F_2-derived soybean lines homozygous for the Young (Y/Y) band, homozygous for the PI416937 (PI/PI) band, or heterozygous (Y/PI) for bands at Satt510.



CHAPTER 5

NEW SOURCES OF SOYBEAN GERMPLASM WITH RESISTANCE TO TWO SPECIES OF ROOT-KNOT NEMATODE[1]

[1]Harris, D.K., H.R. Boerma, R.S. Hussey, and S.L. Finnerty. 2002. To be submitted to Crop Science.

Abstract

Root-knot nematodes (*Meloidogyne* spp.) can significantly reduce yield in soybean [*Glycine max* (L.) Merr.] in the southern USA. The identification of soybean germplasm with unique genes for resistance to root-knot nematodes would allow development of cultivars with higher levels of resistance and thus reduce yield loss in nematode-infested fields. The objective of this study was to evaluate 608 recently imported soybean plant introductions (PIs) from the southern provinces of the People's Republic of China (PRC) for resistance to *M. incognita* (MI) and *M. arenaria* (MA). After three sequential levels of greenhouse screening for MI and MA, with an inoculum of 3000 nematode eggs per plant in Level I and 5000 eggs in Levels II and III, seven resistant PIs were identified for MI and seven for MA. These lines were evaluated in a fourth screening level with an inoculum of 10,000 eggs per plant. The Level IV screening for MI gall number identified PI594753A and PI594775A with resistance equal to the highly resistant check PI96354. PI594470C, PI594538A, and PI594596 produced more galls than PI96354, but produced fewer galls than 'Haskell', the resistant check. PI594401B produced an equal number of galls as Haskell. The number of MI eggs produced on PI594753A and PI594775A roots 56 days after MI inoculation was equal to the susceptible 'Bossier'. The Level IV MA screening for MA gall number identified PI594427C and PI594403 with the same number of galls as the resistant check Haskell, but more galls than the highly resistant PI200538. The number of MA eggs produced by PI594427C was equal to the highly resistant check PI200538. PI594403 produced fewer eggs per plant than PI200538 and an equal number of eggs as the resistant check Haskell. These new sources of MA resistance may possess unique genes for resistance and allow

the development of soybean cultivars with higher levels of resistance to root-knot nematode than presently are available.

Introduction

The genus *Meloidogyne* or root-knot nematode is a plant parasite of almost every crop species. Two of the most common root-knot nematode species include *Meloidogyne arenaria* (MA) and *Meloidogyne incognita* (MI), and these species are commonly found in the southeastern USA. In 1994, the soybean yield loss attributed to root-knot nematodes across 16 southern states averaged 1.6% and ranged from a trace amount to 7.0% (Pratt and Wrather, 1998).

Crop management to reduce losses from these nematodes can be difficult. Because of the cancellation of important fumigant nematicides including DBCP (1,2-dibromo-3-chloropropane) and EDB (ethylene dibromide) in past years, plant resistance has become an increasingly important component in nematode management in the Southeast as well as other areas of the USA (Boerma and Hussey, 1992).

The development of root-knot resistant soybean cultivars requires an effective protocol to screen soybean lines for their level of nematode resistance. Screening soybean lines for resistance is best done in a greenhouse environment so that testing can be done throughout the year, and most importantly, to reduce the non-uniformity of nematode infestations that often occur in field (Boerma and Hussey, 1992). In the greenhouse, test plants can be grown in sterilized soil and cultured nematodes used for the inoculum. This eliminates non-uniformity of inoculum and the introduction of other contaminating organisms.

Luzzi et al. (1987) evaluated 2370 Maturity Group V, VI, VII, and VIII soybean lines in the USDA-ARS Germplasm Collection for resistance to both MI and MA. The screening was done in the greenhouse and resistance was characterized based

on the amount of root gall formation and nematode reproduction. Five levels of screening were conducted using progressively increasing levels of inoculum (ranging from 3000 to 10000 eggs per plant) and number of replications (1 to 10). Plant Introduction (PI) 96354 was found to possess a high level of resistance to MI and PI200538 to MA. PI963534 showed lower MI gall number, fewer MI eggs per gram of root, and fewer MI eggs per root system than the resistant check 'Forrest'. The MA-resistant PI200538 expressed these same resistance characteristics compared to the resistant check 'Jackson'.

Currently, elite soybean cultivars do not possess the PI96354 level of resistance to MI or the PI200538 resistance to MA. Hussey et al. (1991) evaluated the level of MI and MA resistance in 139 soybean cultivars from Maturity Groups V to VIII. None of the 139 cultivars were considered to be highly resistant to MI (PI96354 level) or MA (PI200538 level). Twenty-four percent of the cultivars tested were found to be moderately resistant to MA and 23% to MI.

Inheritance of soybean resistance to MI was determined in crosses of a susceptible cultivar, Bossier, with two resistant plant introductions, PI96354 and PI417444 (Luzzi et al., 1994a). The F_1, F_2, and/or F_3 generations were screened for resistance to gall formation in the greenhouse. The heritability of resistance in these PIs was found to range from 0.73 to 0.92. In another study, Luzzi et al. (1994b) identified a major gene (*Rmi*) for resistance to MI. This single additive gene for resistance to galling was identified in the cultivar, Forrest.

Luzzi et al. (1995) determined inheritance of resistance to MA in soybean using crosses of the susceptible 'CNS' and the three resistant genotypes Jackson, PI200538, and PI230977. For each cross, the F_1, F_2, and/or F_3 populations were screened in a greenhouse for resistance to gall formation. Heritability for soybean resistance to MA was found to range from 0.74 to 0.83.

The goal of developing soybean cultivars with higher levels of MI and MA resistance and multi-species resistance is very important in the southeastern USA (Boerma and Hussey, 1992; Boerma and Mian, 1998). Although PI96354 and PI200538 possess a high level of resistance to MI and MA, respectively, the resistance in both of these PIs is conditioned by one major and one minor QTL (Tamulonis et al., 1997a; 1997b; Li et al., 2001). Therefore, identification of additional sources of resistance to MI and MA could prove beneficial if these lines possess unique resistance genes from those already found in PI96354 and PI200538.

Six hundred and eight germplasm lines from the southern provinces of the People's Republic of China (PRC) were recently received in the USA and made available to researchers by Dr. R.L. Nelson (curator of USDA-ARS Soybean Germplasm Collection Urbana, IL). These lines are the first to become available to U.S. scientists from many of these provinces in PRC. They were collected in PRC from latitudes of 34° N or less. However, these accessions range from Maturity Group II to Maturity Group IX due to the wide range of planting dates for soybean (spring, summer, fall, and winter) in this region. Because these germplasm lines have been selected for a wide range of adaptation over hundreds of years, they have the potential to possess diversity not previously available in the USA (personal communication, Dr. R.L. Nelson, USDA-ARS,

Urbana, IL). The objective of this research was to identify additional sources of MI and MA resistance in these recently obtained Chinese soybean germplasm lines.

Materials and Methods

A greenhouse screening assay was used to evaluate the 608 germplasm lines for MI and MA resistance. The screening assay evaluated the amount of gall formation on soybean roots in four screening levels. Each screening level was designed to systematically eliminate lines with susceptibility (high gall formation) or low levels of resistance to MI and MA gall formation. At each progressive level, the number of replications and/or amount of inoculum was increased (Table 1). A randomized complete block experimental design was used for Levels II, III, and IV.

For all screening levels, the PIs were individually planted into 20.6-cm Ray Leach Single Cell Cone-tainers®. The cones contained approximately 15.6 cm^2 of methyl bromide-fumigated Pacolet sandy loam soil (73% sand, 16% silt, and 11% clay). Three seeds of each soybean genotype were placed in a cone and covered with approximately 2.5 cm of methyl bromide-fumigated sand. Cones were placed in every other row of a Ray Leach 98-cone tray which allowed 45 Chinese PIs and four checks (49 cones/tray) in a 7x7 design. Resistant and susceptible checks were planted in each experiment to determine their degree of gall development and to develop a gall index. The checks for the MI screening were: PI96354 (highly resistant), 'Haskell' (resistant), 'GaSoy17' (susceptible), and 'Bossier' (susceptible). The checks for the MA screening included: PI200538 (highly resistant), Haskell (resistant), GaSoy17 (susceptible), and 'CNS' (susceptible).

Approximately 7 to 10 days after planting, the plants were thinned to one plant per cone and then inoculated with the appropriate number of nematode eggs. The MI and MA inoculum was produced on 'Marion' tomato. The nematode eggs were collected according to the procedure described by Hussy and Barker (1973). The number of eggs per milliliter was adjusted so that the desired inoculum density was applied in a volume of 3 to 5 ml (water + nematode eggs). The inoculum was placed at a soil depth of 2 to 3 cm with an ARTEK® Systems Corporation digital dispensing pump. Each plant was fertilized weekly with 6 mg N, 3 mg P, and 5 mg K. Supplemental light was provided by 400-watt Multivapor® metal halide lamps (Westinghouse Electric Corp., Lamp Division, Bloomfield, NJ 07003) which were suspended 1.4 m above the greenhouse bench. The plants were irrigated twice a day by a mechanical overhead irrigation system with water that was heated to 26°C±3°.

For Level I, II, III, and IV each experiment was terminated approximately 30 days after inoculation when galls had developed on the susceptible checks. Soybean roots were removed from the cones, roots were excised, washed free of soil, and evaluated for gall number. In Level I any PI with 10 galls or less was advanced to Level II. In Level II and III, a scale of 1 (few galls) to 5 (many galls) was used. The scale was established for each experiment based on the number of galls on the various checks. In Level IV the number of galls were counted on each plant in order to more accurately quantify the degree of resistance. Data for galling were analyzed by analysis of variance using Agrobase™ (Agronomix software Inc., Manitoba, Canada).

Two resistant Chinese PIs for MA and two PIs for MI with the highest level of galling resistance were evaluated for nematode reproduction (number of eggs produced

on the soybean root system). Each PI along with resistant and susceptible checks were individually planted into 0.5-L Styrofoam cups using three seed per cup. Each cup was filled with a similar type of soil used for the previous MI and MA screening. Seven to 10 days after planting, each cup was thinned to one plant and inoculated with 5000 nematode eggs. The source of inoculum was from Marion tomato. Fertilizer application and supplemental lighting were the same as previously described. Plants were watered manually twice each day.

Thirty to 70 days after inoculation, when brown egg masses developed on the susceptible check, the experiment was terminated and roots were excised and washed. The eggs were extracted from each root system by cutting the roots into approximately 2.5-cm sections and mechanically shaking them on an Eberbach shaker (Eberbach Crop, Ann Arbor, MI) for 3 min in 300 ml of a 0.5% NaOCl solution in 0.5-L polypropylene jars. The number of eggs collected from each root system was determined by counting the number of eggs in an aliquot of each suspension. Egg data were transformed by a $\log_{10}(x+1)$conversion prior to analysis. Egg data were analyzed by analysis of variance procedures using Agrobase™ (Agronomix Software Inc., Manitoba, Canada).

Results

Meloidogyne arenaria

From the 608 PIs grown in Level I, those with 10 galls or less were advanced to Level II. These PIs produced less than 20% of the galls produced on the susceptible checks GaSoy17 and Bossier (Table 2). In addition, those PIs that did not germinate or produce a seedling were retested in Level II. Of the 116 PIs advanced to Level II, 65 had

less galls than Haskell, and 64 less galls than PI200538, and 48 did not germinate in Level I.

In Level II, PIs with a gall index score of 1 (0 to 15 galls) or 2 (16 to 30 galls) were retained for evaluation in Level III. These PIs produced approximately the same number or less galls than the resistant check Haskell (Table 2). From the 116 PIs in Level II, 23 (20%) were advanced to Level III. Of the 23 PIs evaluated in Level III, 16 produced 30 galls or less, and the other seven produced 5 galls or less. In Level III, PI200538 averaged 7 galls per plant (Table 2). The seven PIs which produced five galls or less were advanced to Level IV.

In Level IV five PIs averaged 30 galls or less and two PIs had 20 galls or less. PI594427C and PI594403 averaged 11 and 14 galls per plant, respectively (Table 3). In the Level IV screening, CNS, Haskell, and PI200538 averaged 57, 17, and 8 galls per plant, respectively. PI594427C was as resistant to MA gall formation as the highly resistant check PI200538 and PI594403 was as resistant as the resistant check Haskell.

PI594427C and PI594403 were evaluated in a MA reproduction experiment. In this experiment, CNS, Haskell, and PI200538 averaged 136,000, 72,000, and 102,000 eggs per plant, respectively (Table 3). PI594427C produced approximately 87,000 eggs per plant and was equal in resistance to PI200538 (highly resistant check). PI594403 produced approximately 62,000 eggs per plant. Therefore, PI594403 produced less eggs per plant than the highly resistant check PI200538 and was equal in resistance to the resistant check Haskell.

Meloidogyne incognita

In the Level I test, PIs with 10 galls or less, or those that did not germinate were advanced to Level II. From the 608 PIs in Level I, 71 (12%) were advanced to Level II (Table 1). Of the 71 PIs, 13 had less galls than Haskell, 10 had less than PI96354, and 37 did not emerge when planted in Level I (Table 2).

In Level II, PIs with a gall index of 1 (0 to 4 galls) or 2 (5 to 8 galls) were advanced to Level III. These PIs produced less than 20% of the galls as the susceptible check GaSoy17 and less than 15% of the galls as the susceptible Bossier (Table 2). Level II eliminated 54 MI susceptible PIs (from 71 in Level II to 17 in Level III). Of the 17 selected PIs, seven averaged 8 galls or less, and the other 9 averaged 4 galls or less.

In Level III, PIs with a gall index of 1 (0 to 5 galls) or 2 (6 to 10 galls) were selected. From 17 PIs in Level III, seven (41%) were advanced to Level IV. Of these seven PIs, six produced 10 galls or less and one averaged 5 galls or less.

In Level IV, PI594775A and PI594753A, did not possess any galls on their roots (Table 4). Bossier averaged 87 galls per plant, Haskell averaged 4 galls, and PI96354 did not produce any galls. Three other of the Chinese PIs, PI594470C, PI594538A, and PI594596, each averaged less than two galls per plant which was less than Haskell (resistant check), but more than PI96354 (highly resistant check).

PI594753A and PI594775A were evaluated for MI reproduction (Table 4). In the MI reproduction experiment, Bossier, Haskell, and PI96354 averaged 10,900, 4,000, and 400 eggs per plant, respectively. The Chinese accession, PI594753A produced approximately 12,000 eggs per plant and PI594775A produced 16,000 eggs per plant.

Both of these PIs produced an equal number of eggs per plant as the susceptible Bossier and significantly more eggs per plant as the resistant checks Haskell and PI96354.

Discussion

Based on MA gall formation, PI594427C was as resistant as the highly resistant PI200538 and PI594403 was equal in resistance to Haskell. In the MA reproduction experiment, PI594427C was equal in resistance to the highly resistant check PI200538. PI594403 produced fewer eggs per plant than PI200538 and was equal in resistance to Haskell. In the MA reproduction experiment, Haskell had significantly fewer eggs per plant than the highly resistant check PI200538. These results are not unexpected when compared with the results of Luzzi et al. (1987) where 'Jackson' the resistant check produced 45,400 eggs per plant compared to 29,000 eggs for PI200538. This difference in egg production (45,400-29,000 = 16,400) was barely greater than the LSD(0.05) value of 15,400.

Based on MI gall number, PI594775A and PI594753A were both equal in resistance to the highly resistant check PI96354. However, based on MI egg production, both of these Chinese PIs produced an equal number of eggs as Bossier, the susceptible check. Further experimentation is required to investigate the genetic basis for the MI gall resistance in these two PIs. The independence of resistance to gall formation and egg production in soybean has not been previously reported. Shepherd (1979) found that on certain cotton (*Gossypium hirsutum*) accessions gall index and egg counts were independent of each other indicating the need for selection for both low gall formation and nematode reproduction in order to develop a highly resistant cotton cultivar. The

results in this experiment also illustrate the importance of quantifying both MI gall and reproduction resistance when searching exotic germplasm for root-knot nematode resistance. Given the limited value of PIs with high reproductive capacity such as PI594775A and PI594753A, it is important to determine the egg production for the three other PIs (PI594470C, PI594538A, and PI594596) that produced fewer galls than Haskell, but more than PI96354.

Our results and previous studies suggest that PI96354 has much higher resistance to MI than PI200538 has to MA (Luzzi et al., 1997; Table 2, 3, and 4). These results indicate the importance of the MA resistance identified in PI594427C and PI594403. These PIs need further study to determine if they possess unique MA resistance genes. Tamulonis et al. (1997a) found two QTLs in PI200538 for MA that accounted for 51% of the variation in gall number. Li et al. (2001) confirmed two QTLs found originally by Tamulonis et al. (1997b) for MI that accounted for 45% of the variation in gall number. Additional sources of MA resistance, such as PI594427C and PI594403, are important if they contain different QTLs or different alleles at existing QTL than those already discovered. This would allow the incorporation of higher levels of MA resistance especially into elite soybean cultivars.

Acknowledgement

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Table 5.1. Number of replications, inoculum densities, and soybean plant introductions screened in each screening level.

Level	Replications	Inoculum density	-----Plant Introductions evaluated----- *arenaria*	*incognita*
	no.	eggs/plant	----------no.----------	
I	1	3000	608	608
II	2	5000	116	71
III	4	5000	23	17
IV	10	10000	7	7

Table 5.2. Number of *M. arenaria* and *M. incognita* galls produced on the soybean checks in the Level I, II, and III screening experiments.

	M. arenaria†				M. incognita‡			
Screening Level	CNS	GaSoy17	Haskell	PI200538	Bossier	GaSoy17	Haskell	PI96354
	---------- galls plant^{-1} ----------							
Level I	53	54	9	8	78	40	3	2
Level II	96	61	27	16	59	45	1	0
Level III	75	80	20	7	89	53	3	0

† CNS = susceptible, GaSoy17 = susceptible, Haskell = resistant, and PI200538 = highly resistant to *M. arenaria*.
‡ Bossier = susceptible, GaSoy17 = susceptible, Haskell = resistant, and PI96354 = highly resistant to *M. incognita*.

Table 5.3. *M. arenaria* gall production on Chinese plant introductions and checks evaluated in Level IV and egg production on selected Chinese plant introductions and checks.

Line	Nematode galls	Nematode eggs
	no. plant^{-1} ± SE	no. x 10^3 ± SE
†PI200538	7.6 ± 2.8	102.4 ± 14.8
†Haskell	16.9 ± 2.8	71.5 ± 8.0
†CNS	56.5 ± 4.9	136.4 ± 15.4
PI594427C	11.3 ± 2.6	87.1 ± 8.4
PI594403	14.1 ± 2.0	62.0 ± 11.3
PI594812	27.4 ± 8.1	-
PI594463B	33.1 ± 3.9	-
PI594442B	41.1 ± 7.2	-
PI594759C	45.3 ± 5.4	-
PI594651	47.9 ± 7.9	-

† PI200538 = highly resistant check, Haskell = resistant check, CNS = highly susceptible check

Table 5.4. *M. incognita* gall production on seven Chinese plant introductions and checks evaluated in Level IV and egg production on selected Chinese plant introductions and checks.

Line	Nematode galls	Nematode eggs
	no. plant^{-1} ± SE	no. x 10^3 ± SE
†PI96354	0.1 ± 0.1	0.4 ± 0.1
†Haskell	3.7 ± 0.8	3.6 ± 1.3
†Bossier	87.3 ± 8.5	10.9 ± 2.2
PI594753A	0.0 ± 0.0	12.1 ± 2.3
PI594775A	0.0 ± 0.0	15.9 ± 3.4
PI594470C	0.8 ± 0.5	-
PI594538A	1.5 ± 0.7	-
PI594596	1.6 ± 0.8	-
PI594401B	2.0 ± 1.0	-
PI594523	86.0 ± 9.8	-

† PI96354 = highly resistant check, Haskell = resistant check, Bossier = highly susceptible check

CHAPTER 6

CONFIRMATION OF SOYBEAN QUANTITATIVE TRAIT LOCI FOR SEED TRAITS[1]

[1]Harris, D.K., V.A. Fasoula, H.R. Boerma. 2002. To be submitted to Crop Science.

Abstract

Marker assisted selection has the potential to improve the efficiency of selection for quantitative traits in soybean, *Glycine max* (L.) Merr. Most quantitative trait loci (QTL) reported in the literature have not been confirmed in a population that is independent from the original mapping study. The objective of this study was to utilize SSR (simple sequence repeat) markers in the genomic region of RFLP markers previously reported (in a F_4-derived Young x PI416937 population) to be associated with QTL for seed protein, seed oil, and seed weight to verify the presence of the QTL in a newly created F_2-derived population. In 1996, 176 F_2-derived lines were grown at the Univ. of Georgia Plant Sciences Farm near Athens, GA and the Univ. of Georgia Southwest Brach Experiment Station near Plains, GA. Lines were randomly assigned to four tests with 44 lines in each test along with three entries of Young and three of PI416937 (total of 50 entries in per test). Each test was planted in a randomized complete block with two replications. QTL were evaluated for protein content (K*pro*-1, C1*pro*-1 and N*pro*-1), oil content (D2/R*oil*-1, L*oil*-1, and J*oil*-1), and seed weight (G*sdw*-1, E*sdw*-1, and C1*sdw*-1). Polymorphic SSR markers in the region of the QTL were selected. The mean seed protein, seed oil, and seed weight of F_2-derived lines across locations and replications were compared for the lines homozygous for the Young SSR band and the lines homozygous for the PI416937 SSR band at each SSR. Three of the nine QTL (33%) reported in the original RFLP mapping study were confirmed using SSR markers in this study. L*oil*-1, G*sdw*-1, and E*sdw*-1 were the QTL confirmed. Although the amount of variation explained by each of the three protein QTL exceeded 10% of the

variation in protein content in the original mapping population, none of the three QTL were confirmed.

Introduction

The ability to use DNA markers to identify plant genes or quantitative trait loci (QTL) has played an important role in revolutionizing the science of plant breeding and plant genetics. However, one possible limitation with the QTL reported in the literature is the lack of research that confirms their existence. Research to confirm reported QTL has shown mixed results. For example, a QTL mapping study by Diers et al. (1992) showed that none of the identified QTL conditioning iron deficiency chlorosis in soybean were effective in divergent selection for the trait among additional lines from the same population. On the other hand, Mudge et al. (1997) found selection based on a single SSR marker on Linkage Group G was 95% accurate in predicting soybean resistance to the soybean cyst nematode, *Heterodera glycines*. The accuracy of prediction of resistance/susceptibility phenotype was improved to 98% when two SSR markers flanking the reported resistance QTL were used. The use of SSR markers flanking this QTL to identify soybean lines with resistance to soybean cyst nematode was one of the first applications of marker-assisted selection (MAS) in both public and commercial soybean breeding. Fasoula and Boerma (1997), evaluated and confirmed 50% of the QTL for seed traits in a soybean population of PI97100 x Coker237 that were previously reported in a different population of the same cross by Mian et al. 1996b and Lee et al. 1996b.

Researchers evaluating qualitative soybean traits are expected to include a hypothesis generation and a confirmation generation prior to assignment of a gene symbol (Soybean Genetics Committee, 1997). However, for QTL mapping studies this confirmation step is generally not required in any crop (Boerma and Mian, 1999).

Considering the mixed results shown in some QTL mapping studies, it would seem prudent to perform a verification study prior to use of MAS for QTL in a plant breeding program.

There are a number of important QTL mapping populations that have been created in soybean. These include the recombinant inbred line population of Minsoy x Noir 1 (Mansur et al., 1996), the F_2-derived line population from the interspecific cross A81-356022 x PI468916 (Keim et al., 1990), the F_2-derived line population of PI97100 x Coker 237 (Lee et al., 1996c), and the F_4-derived line population of Young x PI416937 (YxPI) (Mian et al., 1996a). The YxPI population consists of 120 F_4-derived lines that were developed by single-seed-descent from 120 individual F_2 plants. Quantitative trait loci conditioning a number of soybean traits identified and reported in the YxPI population, but not yet confirmed include: pod dehiscence, maturity, seed oil and protein content, seed weight, specific leaf weight, leaf size, water use efficiency, leaf ash, plant height, and lodging. Each of these QTL mapping studies was conducted using RFLP markers (Bailey et al., 1997; Bianchi-Hall et al., 2000; Lee et al., 1996 a,b,c; Mian et al., 1996a,b; Mian et al., 1998).

Lee et al. (1996b) reported a number of RFLP markers associated with seed protein and oil content in the YxPI population. The experiment was conducted at three field locations; Plains GA, Windblow NC, and Plymouth NC. Thirteen RFLP markers were associated with seed protein, seven of which were independent QTL [significant ($P < 0.01$) markers on different linkage groups or at least 50 cM from another significant marker]. The total amount of phenotypic variation explained by these seven markers was

70% and the heritability for seed protein was 83%. Of the seven independent markers, five were significant ($P < 0.05$) in all three locations.

For seed oil content, six markers were detected, four of which represented independent QTL. Together these four independent markers explained 34% of the phenotypic variation for oil content. The heritability for oil content in this population was 89%. Therefore, the majority of the genotypic variation was not explained by these four markers. This suggested that either the identified RFLP markers were not closely linked to the QTL for seed oil, or there are still undetected QTL in unmapped regions of the genome in this population. All four of the markers were significant ($P < 0.05$) in each of the three locations, suggesting that these QTL for seed oil were environmentally stable across locations (Lee et al., 1996b).

Seed weight is a trait that plays an important role in the acceptance of a soybean cultivar for different food applications and is a component of seed yield. Mian et al. (1996b) evaluated RFLP markers associated with seed weight in the YxPI population. The seed weight data were collected at the same three locations as protein and oil in this population (see above). A total of seven independent RFLP markers were found to be associated with seed weight. Six out of these seven were identified in all three environments. Together, the seven markers explained 73% of the phenotypic variation. The heritability of seed weight in this population was 90%. Therefore, the majority of the phenotypic variation could be explained by the seven RFLP markers (Mian et al., 1996b).

The objective of this study was to utilize SSR markers that based on the public consensus soybean linkage map were in the genomic region of RFLP markers previously

reported to be associated with QTL for seed protein, seed oil, and seed weight to verify the presence of the QTL in a newly created F_2-derived population from the cross Young x PI416937.

Materials and Methods

An independently created population (independent of the original mapping population used to detect the QTL for protein, oil, and seed weight) of Young x PI416937 was developed. This F_2-derived population consist of 180 lines. The cross of Young x PI416937 was made during the summer of 1993. The F_1 generation was grown from December 1993 to April 1994 in a greenhouse in Athens, GA, and the F_2 generation was grown at the Univ. of Georgia Plant Sciences Farm near Athens, GA in 1994. At maturity, the 180 randomly selected plants were individually harvested to create F_2-derived lines. In 1995, the 180 $F_{2:3}$ lines were grown for a seed increase at the Univ. of Georgia Plant Sciences Farm. Leaf tissue for DNA extraction was collected from at least 12 plants of each line at the V3 stage of development (Fehr et al., 1971).

In 1996, the F_2-derived lines of the Young x PI416937 population were grown at the Univ. of Georgia Plant Sciences Farm and the Univ. of Georgia Southwest Branch Experiment Station near Plains GA. These lines were randomly assigned to four tests of 44 lines for a total of 176 F_2-derived lines (four of the original 180 F_2-derived lines were not included). In addition to the 44 F_2-derived lines, each test included three entries each of Young and PI416937 (total of 50 entries per test). Each test was planted in a randomized complete block design with two replications. The experimental unit for each entry was two 4-m rows spaced 76 cm apart at Athens and two 7-m rows spaced 76 cm

apart at Plains. At maturity each plot was harvested. A 50-g seed sample from each plot was sent to the USDA-ARS National Center for Agricultural Utilization Research at Peoria IL for seed composition analysis. An 18 to 20 g sample of seed was analyzed for protein and oil composition with a Model 1255 Infratec NIR food and feed grain analyzer. The protein and oil values were converted to a moisture-free basis. Seed weight for each plot was determined based on a 100-seed sample.

The following QTL were evaluated for protein content (K*pro-1*, C1*pro-1*, and N*pro-1*), oil content (D2/R*oil-1*, L*oil-1*, and J*oil-1*), and seed weight (G*sdw-1*, E*sdw-1*, and C1*sdw-1*) (Table 1). The QTL were designated as follows: K*pro-1*, the K indicates Linkage Group K, *pro* indicates a QTL for protein, and the "1" indicates the first QTL on LG-K. QTL for oil were designated *oil* and seed weight as *sdw*. Linkage groups were reported according to their designations by Cregan et al.(1999). Polymorphic SSR markers developed by Dr. P.B. Cregan (USDA-ARS, Beltsville, MD) in the region of the reported QTL were selected. The RFLP map by Mian et al. (1996a) and the SSR map by Cregan et al. (1999) were used to visually find the most likely location of SSRs near the reported QTL (Fig. 1).

DNA was extracted from leaf tissue using the modified CTAB procedure (Keim et al., 1988). It was quantified with the UV/VIS Spectrometer (Pekin Elmer) and diluted to 20 ng/µl. Fluorescence dye-labeled primers were synthesized (PE-ABI, Foster City, CA) using phosphoramidite chemistry. PCR reactions were prepared using the protocol by Diwan and Cregan (1997). Reactions were performed in a 384-well ABI877 robotic thermal cycler or a dual 384-well and 96-well GeneAmp® PCR System 9700 (PE-ABI, Foster City, CA). The cycling consisted of 1 min at 95°C, 32 cycles of 25 s for

denaturation at 94°C, 25 s of annealing at 46°C, and 25 s of extension at 68°C. Reaction mixtures were held at the end of the cycling procedure at 4°C.

Electrophoresis was run on an ABI-Prism 377 DNA Sequencer (PE-ABI, Foster City, CA) with 12-cm plates at 750 v for 2 h. Lanes were loaded on a 4.8% acrylamide:bisacrylamide (19:1) gel with KLOEHN micro syringes (Kloehn, Ltd., Las Vegas, NV). Genescan® (version 3.0) was used to analyze DNA fragments, and they were scored with Genotyper® (version 2.1). Data for the polymorphic SSR markers were collected for the 176 F_2-derived lines. Single factor analysis of variance was run for each marker using the General Linear Model (GLM) (SAS Institute, 1989). The mean seed protein, seed oil, and seed weight of F_2-derived lines across locations and replications were compared for the lines homozygous for the Young SSR band and the lines homozygous for the PI416937 SSR band near each QTL. Previously reported QTL were assumed to be confirmed if the means of these two groups were significantly different ($P \leq 0.05$) and the parental alleles produced a similar effect as in the original mapping study.

Results and Discussion

Seed protein content

Seed protein of Young ranged from 422 g kg^{-1} in Test 4 to 440 g kg^{-1} in Tests 2 and 3 in while PI416937 ranged from 465 g kg^{-1} in Test 2 to 468 g kg^{-1} in Test 1 (Table 2). The mean seed protein content of Young and PI416937 across all four tests differed by 33 g kg^{-1} in seed protein content. The seed protein content of the progeny ranged from 419 g kg^{-1} in Test 1 to 480 g kg^{-1} in Test 2. The seed protein QTL, K*pro*-1, N*pro*-1, and

C1*pro*-1, each explained greater than 10% of the phenotypic variation in the original mapping study (Table 1; Lee et al., 1996b). The three QTL were also detected across all three locations (Plains GA, Windblow NC, and Plymouth NC). For each protein QTL the allele for increased protein content was contributed by PI416937.

The polymorphic SSR markers used to select for the K*pro*-1 QTL were Satt441 and Satt559 (see Fig. 1 for approximate location to RFLP marker A199). SSR markers Sat084 and Satt530 were chosen to select N*pro*-1. For C1*pro*-1, Satt338 and Satt180 were the polymorphic markers used. When testing the 176 F_2-derived lines with the SSR markers for K*pro*-1, N*pro*-1 and C1*pro*-1, none of the three QTL were confirmed (Table 3). For K*pro*-1 the P-values for Satt441 and Satt559 were 0.8789 and 0.8933, respectively. At the N*pro*-1 QTL the P-values for Satt084 and Satt530 were 0.5998 and 0.9178 respectively, and for C1*pro*-1, the p-values for Satt338 and Satt180 were 0.1247 and 0.6191, respectively.

Seed oil content

Seed oil in the parent Young ranged from 211 g kg^{-1} in Tests 2 and 4 to 215 g kg^{-1} in Test 1 (Table 2). PI416937 ranged in seed oil from 187 g kg^{-1} in Tests 2 and 3 to 189 g kg^{-1} in Test 4. Averaged across all four tests, PI416937 differed from Young in seed oil content by 24 g kg^{-1}. The F_2-derived lines ranged from 184 g kg^{-1} in Test 2 to 216 g kg^{-1} in Test 3. The three seed oil QTL evaluated in this study, D2/R*oil*-1, L*oil*-1, and J*oil*-1, were all putative independent QTLs, and were detected at all three of the locations in the original mapping study (Table 1; Lee et al., 1996b). The percent of phenotypic variation explained for seed oil was 12.9% for D2/R*oil*-1, 7.4% for L*oil*-1, and 7.0% for J*oil*-1

(Table 2). The allele for increased oil content was contributed by Young at D2/R*oil*-1 and J*oil*-1 and by PI416937 for L*oil*-1.

SSR markers Satt311 and Satt208 near D2/R*oil*-1, Satt398 and Satt313 near L*oil*-1, and Satt380 and Satt244 near J*oil*-1 were selected to evaluate the three QTL conditioning seed oil content (Fig. 1). The SSR markers near D2/Roil-1 and Joil-1 were not effective in identifying progeny lines with significant ($P < 0.05$) differences in seed oil content (Table 3). However, L*oil*-1 was confirmed using both markers Satt398 and Satt313. Satt398 explained 8% of the phenotypic variation in oil content and Satt313 explained 7%. The variation explained by L*oil*-1 in this study was similar to the results from the earlier study by Lee et al. (1996b) (Table 1 and 3). As in the original study, the positive allele was contributed by PI416937.

Seed weight

The seed weight of Young ranged from 161 mg seed^{-1} in Test 1 to 169 mg seed^{-1} in Test 4, while PI416937 ranged from 185 mg seed^{-1} in Test 1 to 200 mg seed^{-1} in Test 4 (Table 2). Averaged across the four tests, Young and PI416937 differed by 28 mg seed^{-1} in seed weight. Individual F_2-derived lines ranged from 131 mg seed^{-1} in Test 1 to 234 mg seed^{-1} in Test 4. G*sdw*-1, E*sdw*-1, and C1*sdw*-1 were putative independent QTLs and all three were detected across all locations (Mian et al., 1996b). The R^2 values reported by Mian et al. (1996b) were 22% for G*sdw*-1, 14% for E*sdw*-1, and 10% for C1*sdw*-1 (Table 2). The positive allele was contributed by PI416937 for all three QTL.

Selection for G*sdw*-1, E*sdw*-1, and C1*sdw*-1 was based on SSR markers Satt303, Satt263, and Satt396, respectively (Fig. 1). When testing the progeny with these markers, results confirmed the presence of G*sdw*-1 and E*sdw*-1 (Table 3). For both G*sdw*-1 and

E*sdw*-1 the PI416937 allele increased seed weight. In this study, the Gsdw-1 QTL explained 8% of the variation in seed weight which is somewhat less than the 22% reported by Mian et al. (1996b) (Tables 1 and 3). E*sdw*-1 accounted for 18% of the variation in seed weight in this current study which is similar to the 14% reported by Mian et al. (1996b). The QTL at C1sdw-1 was not confirmed by Satt396 (P = 0.182).

Of the nine previously reported QTL evaluated in this study, three (33%) of these were confirmed by using SSR markers in this study. L*oil*-1, G*sdw*-1, and E*sdw*-1 were the confirmed QTL. Although the R^2 for L*oil*-1 was only 7.4% in the earlier study, it was still confirmed in this study by two SSR markers surrounding the original RFLP marker (Lee et al., 1996b). The R^2 values reported in 1996 for seed protein were all over 10% (Table 2), however none of these three QTL were confirmed.

It is important to note that the SSR markers used in this study were selected based on their alignment with the reported RFLP markers based on the consensus soybean linkage map (Cregan et al., 1999). Therefore, it is unknown if our results would have been different if the original RFLP markers had been used. The limitation of the approach is the actual location of the QTL versus the SSR markers we used in this study. Also, there is the possibility that the RFLP markers that identified QTL in the earlier study were false positives (Type II errors). However, most of the RFLP markers were highly significant and all were identified in all three locations. It is clear that our results strongly support the recommendation of Boerma and Mian (1999) that prior to the use of MAS in plant breeding programs, all QTL should be confirmed.

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Table 6.1. The RFLP markers that initially identified QTL for seed protein, seed oil, and seed weight and the in the 120 F_4-derived lines of Young x PI416937.

Trait	QTL	R^2(%)	Allelic mean		RFLP
			Young	PI416937	
			-------g kg^{-1}-------		
Seed protein†	K*pro*-1	13.8	442	449	A199
	N*pro*-1	11.2	443	448	A071
	C1*pro*-1	12.5	443	449	gac197
			-------g kg^{-1}-------		
Seed oil†	D2/R*oil*-1	12.9	194	191	cr142
	L*oil*-1	7.4	191	193	A023
	J*oil*-1	7.0	194	191	B122
			-----mg $seed^{-1}$-----		
Seed weight‡	G*sdw*-1	22.0	167	187	B031
	E*sdw*-1	14.0	170	184	Blt49
	C1*sdw*-1	10.0	172	185	A059

† Protein and oil QTLs identified by Lee et al. (1996).
‡ Seed weight QTLs identified by Mian et al. (1996).

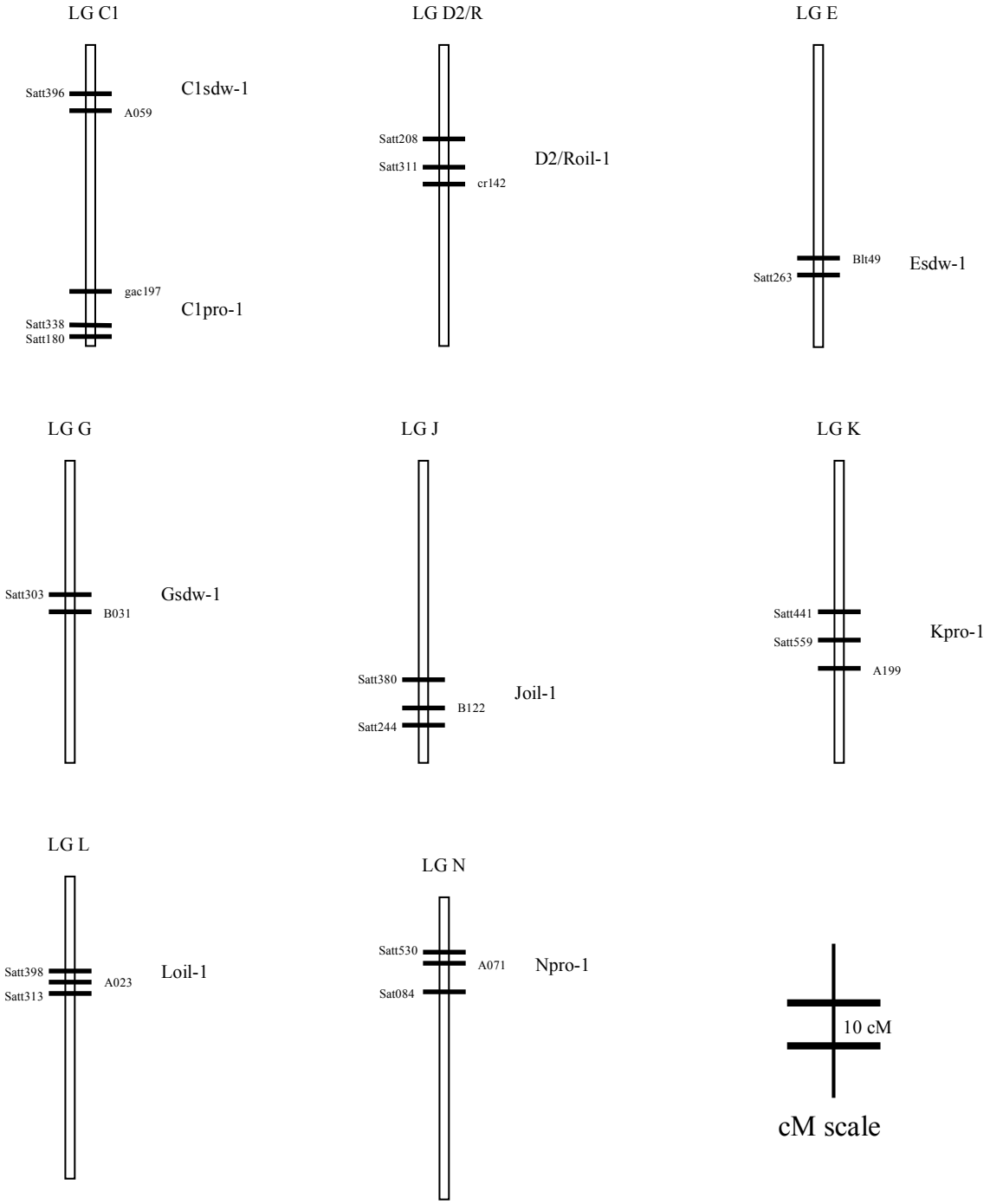
Table 6.2. Mean and range of lines and their parents in four tests for soybean seed protein, seed oil, and seed weight.

	Seed protein			Seed oil			Seed weight		
	Young	PI416937	Progeny range	Young	PI416937	Progeny range	Young	PI416937	Progeny range
	----------g kg^{-1}----------			----------g kg^{-1}----------			----------mg seed^{-1}----------		
Test 1	435	468	419-476	215	188	192-211	161	185	131-217
Test 2	440	465	428-480	211	187	184-214	162	193	140-213
Test 3	440	467	429-476	213	187	187-216	166	192	135-204
Test 4	422	467	392-473	211	189	190-209	169	200	140-234
Mean	434	467	-	212	188	-	165	193	-

Table 6.3. Significance based on single-factor analysis of variance, coefficient of determination, and allelic means for SSR markers used to confirm the presence of QTL for seed protein, seed oil, and seed weight.

				Allelic mean	
Trait	SSR marker	P-value	R^2(%)	Young	PI416937
				--------g kg^{-1}--------	
Seed protein					
K*pro*-1	Satt441	0.8789	-	449	449
	Satt559	0.8933	-	449	447
N*pro*-1	Satt530	0.5998	-	448	448
	Satt084	0.9178	-	450	450
C1*pro*-1	Satt338	0.1247	-	451	448
	Satt180	0.6191	-	447	451
				--------g kg^{-1}--------	
Seed oil					
D2/R*oil*-1	Satt208	0.6129	-	200	200
	Satt311	0.3477	-	204	201
L*oil*-1	Satt398	0.0025	8	199	204
	Satt313	0.0049	7	199	203
J*oil*-1	Satt380	0.9845	-	201	202
	Satt244	0.5161	-	201	201
				-----mg $seed^{-1}$-----	
Seed weight					
G*sdw*-1	Satt303	0.0016	8	167	181
E*sdw*-1	Satt263	0.0001	18	167	187
C1*sdw*-1	Satt396	0.1812	-	171	178

Figure 6.1. Relative location of SSR markers to the previously reported RFLP markers (based on the public consensus linkage map) associated with QTL for seed protein, seed oil, and seed weight (Mian et al., 1996; Cregan et al., 1999).



CHAPTER 7

SUMMARY

Protein QTL were mapped in a population of 180 F_2-derived soybean [*Glycine max* (L.) Merr.] lines created by crossing Benning and Danbaekkong. Benning is a Maturity Group VII, productive, multiple pest resistant cultivar adapted to the southeastern USA with approximately 420 g kg^{-1} seed protein content. Danbaekkong is a Maturity Group IV cultivar developed in South Korea for use in soybean milk and tofu products, and it contains a seed protein content of approximately 510 g kg^{-1}. The identification of a major protein QTL on LG I, provided an opportunity to evaluate the performance of agronomic traits of F_2-derived lines selected for divergent seed protein content based on the alleles at this QTL. Twenty-eight lines (a total of 14 with elevated seed protein content and 14 with conventional seed protein content) along with four entries of Benning and four of Danbaekkong were evaluated in replicated field plots at two locations in 1999. In 2000, a total of 12 lines [six of the highest yielding F2-derived elevated protein lines homozygous for Danbaekkong at Satt239 (D/D-Satt239) and six of the highest yielding F_2-derived conventional protein lines homozygous for the Benning allele at Satt239 (B/B-Satt239)] were selected from the original 28 lines along with three entries of each parent. Five of the six elevated protein F_2-derived lines were lowest yielding lines when averaged across both years. One line, G98SF-114 was identified with approximately 480 g kg^{-1} protein and a seed yield and maturity equal to Benning.

The identification of this line would suggest that it is possible to break the negative association of protein content and seed yield.

Bud blight, caused by tobacco ringspot virus can reduce soybean yields by 25 to 100%. A F_2-derived soybean population developed from the cross of Young (resistant) x PI416937 (susceptible) was used to confirm a previously reported QTL on LG F near RFLP marker K644 conditioning resistance to bud blight and determine the effectiveness of marker-based selection for bud blight incidence. The SSR data for the 180 $F_{2:3}$ lines were collected for five polymorphic markers surrounding the region of the RFLP marker K644. Results confirmed a major QTL conditioning resistance to bud blight on LG F located in the 14 cM-interval between Satt114 and Satt510. The most likely position of this QTL was 4 cM from Satt510. The Satt510 marker is within 2 to 3 cM of RFLP marker K644. The QTL for bud blight accounted for 89% of the phenotypic variation in bud blight resistance which approached 100% of the genotypic variation in this population. In this population, lines that were homozygous for the Young band at Satt510 averaged 15.3% bud blight incidence while lines homozygous for the PI416937 band averaged 95.1%.

Soybean plant introductions (PIs) from the southern provinces of China were evaluated for resistance to *M. incognita* (MI) and *M. arenaria* (MA). After four levels of screening were completed for MI gall number, PI594753A and PI594775A were identified with resistance equal to the highly resistant check PI96354. PI594470C, PI594538A, and PI594596 had more galls than PI96354, but produced fewer galls than Haskell, the resistant check. PI594401B produced an equal number of galls as Haskell. The number of MI eggs produced on PI594753A and PI594775A roots 56 days after MI

infestation were equal to the highly susceptible 'Bossier'. After four levels of screening for MA gall number, PI594427C and PI594403 were identified with the same number of galls as the resistant check Haskell, but more galls than the highly resistant PI200538. The number of MA eggs produced by PI594427C was equal to the highly resistant check PI200538, and PI594403 produced less eggs per plant than PI200538. PI594403 produced an equal number of eggs per plant as the resistant check Haskell. These new sources of resistance may contain different QTLs that those already discovered in the highly resistant checks (PI96354 for MI and PI200538 for MA). If PI594427C and PI594403 are shown to possess unique QTL for MA resistance, they can be used in the development of soybean cultivars with a higher level of MA resistance than current cultivars.

A F_2-derived population of Young x PI416937 was developed. The objective of this study was to utilize SSR markers in the genomic region of RFLP markers previously reported (in an F_4-derived Young x PI416937 population) to be associated with QTL for seed protein, seed oil, and seed weight to verify the presence of the QTL in the newly created F_2-derived population. QTL were evaluated for protein content (K*pro*-1, C1*pro*-1 and N*pro*-1), oil content (D2/R*oil*-1, L*oil*-1, and J*oil*-1), and seed weight (G*sdw*-1, E*sdw*-1, and C1*sdw*-1). Polymorphic SSR markers in the region of the QTL were selected. Previously reported QTL were confirmed if the means of SSR markers homozygous for Young and PI416937 were significantly different ($P \leq 0.05$) and the positive allele was contributed by the same parent as in the original mapping study. Results found that 33% (three of the nine) of the QTL reported in the original RFLP mapping study were confirmed using SSR markers in this study. L*oil*-1 D*sdw*-1, and E*sdw*-1 were confirmed

and the magnitude of their effect and the direction of the effect for the parental alleles were similar in this and the original mapping study.