Inheritance of the Gene(s) Controlling Leaflet Shape in Soybean

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(ABSTRACT)

Many soybean [*Glycine max* (L.) Merrill] cultivars have narrow leaflet shape but it is not known if all of these lines derive this trait from the *ln* gene or another locus. This project was conducted to determine the inheritance of the narrow leaflet trait in several soybean genotypes and wild [*Glycine soja* Sieb. et Zucc.] accessions, and also to determine the allelism of the genes for this trait in the selected lines. The parents, F_1, F_2 and $F_{2:3}$ generations were grown at Kentland Research Farm near Blacksburg, VA or in the greenhouse. The F_2 and $F_{2:3}$ generations (where available) were observed for segregation in leaflet shape. The populations were scored as having either broad or narrow leaflets using visual classification and leaf measurements when necessary. 'Camp' was crossed with broad leaflet parent 'Essex' to study the inheritance of the narrow leaflet trait in Camp. Observation of the F_2 and $F_{2:3}$ generations lead to the conclusion that a single recessive gene controls leaflet shape in Camp. Narrow leaf parents 'SRF 400' and Camp were crossed with lines having the *ln* gene (T41, S56, and D64-4731). None of the crosses among Camp, T41, SRF 400, S56 and D64-4731 segregated for leaflet shape in the F_2 generation leading to the conclusion that they all have the *ln* allele at the same locus controlling lanceolate leaflet shape. T313, a line containing a gene for narrow rugose leaflets (*lnr*), was crossed with Camp to study allelism between the *lnr* and *ln* genes. Segregation for leaflet shape was observed in the F_2 and $F_{2:3}$ generations allowing the conclusion that the *lnr* gene controlling the narrow rugose leaflet trait in T313 is at a locus independent from the *ln* gene. A deficiency of narrow rugose plants was observed in all of the populations with T313 as a parent, and was theorized as being caused by selection against *lnr* gametes. After adjustment for the *lnr* deficiency, the F_2 data appeared to fit a 9 broad : 3 narrow : 4 narrow rugose ratio. Three *G. soja* lines were crossed to broad and narrow leaflet parents and the F_2 generations were examined to determine the inheritance of the very narrow leaf phenotype. The results indicate that there are one or two recessive genes controlling narrow leaflet shape in the *G. soja* accessions, which are not allelic to the *ln* gene. Since these populations were not advanced to the F_3 generation, definite conclusions cannot be drawn about the genetics of the very narrow leaf phenotype.

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Introduction

Soybean, *Glycine max* (L.) Merrill, is an annual leguminous species that is cultivated mainly for its seed. It originated from northeastern China about 4,000 years ago and is now grown worldwide. The main soybean producers include the United States, Brazil, China, Argentina, Indonesia, and Russia (Hildebrand et al., 1986). Soybean is used in a variety of different industries, providing products for human consumption, livestock feed and industrial purposes. Liu (1997) stated that soybeans were traditionally used for consumer food products such as tofu, soymilk, soy sprouts and natto in the Far East and crushed for oil and meal in the West. Soybeans are a major source of protein and oil for both the human population and various livestock. Soybean seed consists of 40% protein and 20% oil and is therefore a major source of protein and oil for commercial products. It is used as a grain legume to produce a high protein animal feed, and "40% of the world's edible vegetable oil comes from soybeans" (Hildebrand et al., 1986). More than half of the annual harvested soybean crop is crushed to produce oil because it is used so extensively in commercial products such as cooking oils, margarine, processed foods, and industrial uses as well (Christou et al., 1990). Soybean is an essential commercial crop, it is the second largest cash crop, after corn, in the United States today (Moore and Collins, 1989). The world market for food-grade soybeans is currently estimated at one million metric tons and continues to grow with the constant innovation of new commercial soyfoods (Liu, 1997).

Specialty soyfood products require soybeans with specific morphological and quality traits. Natto, a popular soyfood in Japan, is made from small-seeded soybeans (Liu,

1997). Mandl and Buss (1981) reported a difference in seed weight among broad and narrow leaflet soybean isolines; narrow leaflet plants consistently had smaller seeds than broad leaflet plants. Gaining a better understanding of the inheritance of the narrow leaflet trait may benefit breeding efforts for specialty small-seeded soybean markets.

The morphological trait of interest to this study is leaflet shape of soybeans. Leaflet shape can be classified into two categories, ovate (normal) and lanceolate (narrow). Soybean cultivars more commonly have ovate leaflets, although the narrow leaflet trait is found in some Asian cultivars and in many wild [*Glycine soja* Sieb. et Zucc] types. Beversdorf (1993) stated that *G. max* and *G. soja*. represent the cultivated and wild counterparts, respectively, of a single species within the sub-genus. He also reported that *G. max* and *G. soja* were highly cross compatible. Some accessions of *G. soja* exhibit very narrow leaflets.

Genetic studies are conducted to determine if a trait is heritable, how many genes are involved and the possible genetic relationships among genes from different sources with similar phenotypes (Liu, 1997). Liu defines heritability as the proportion of the total variance that is due to genetic variation while the remainder of the total is due to environmental variation. This concept applies primarily to traits in which the environment plays a significant role in determining the phenotype. Because leaflet shape has thus far been attributed to a single gene, it is considered a qualitative trait and is not influenced considerably by the environment. Once the inheritance of the gene(s) controlling a certain trait is understood, this information can be used to further enhance

the crop in terms of yield improvement, pest resistance, or many other vital characteristics. Identifying the gene(s) controlling leaflet shape in soybeans and their inheritance will expand the understanding of the genetics of this crop and facilitate the use of this knowledge in future research. Such genes may be of interest to soybean breeders for incorporation into new cultivars. Leaf shape is an important characteristic that needs to be evaluated for the efficient collection and preservation of soybean genetic resources (Oide and Ninomiya, 2000). In order for the different soybean varieties to be classified, the different leaflet shape categories need to be defined and understood. Correct leaf shape evaluation is essential to record and preserve important genetic resources (Oide and Ninomiya, 2000).

Poehlman and Sleper (1995) defined morphological markers as visible characteristics used to construct a detailed genetic map of an organism; observing these morphological features helps identify the locations of specific genes on a chromosome. Understanding the genetic map of a crop species is important to a plant breeder because it provides essential information about linkage and recombination of desirable genes (Poehlman and Sleper, 1995). A few qualitative traits are used as morphological markers in soybean, the most common being flower color, hilum color, pubescence color and pod color. Leaflet shape can also be used as a morphological marker to verify the success of cross-pollination or used for cultivar identification. In the past, research has been conducted to study the inheritance of this trait, but most efforts focused on leaflet shape in correlation with other traits such as the number of seeds per pod. Several narrow leaf accessions are available, but little has been done to determine the allelism of the genes responsible for

the trait. This experiment will add to the known information and enhance understanding of the genetics of leaflet shape trait in soybean. The objectives of this project are to determine the inheritance of the narrow-leaflet trait in several soybean genotypes and *G. soja* accessions and to determine allelism of genes for the narrow-leaflet trait in the selected sources.

Literature Review

There have been a few previous research efforts designed to study the genetics of leaflet shape in soybean. Most of the articles on this topic attribute the trait to a single gene, with the ovate phenotype being the dominant genotype and lanceolate the recessive one.

One of the first published reports on the inheritance of soybean leaflet shape was conducted by Domingo (1945), in which he also studied the correlation between leaflet shape and the number of seeds per pod. He used T173 as his lanceolate leaflet line, which carries the narrow leaflet gene from its PI 88351 parent. Domingo reported that narrow leaflet plants tend to have more seeds per pod than broad leaflet plants. He distinguished between ovate and lanceolate leaflet shape by taking measurements from the terminal leaflets. Domingo found that narrow leaflet plants produced only narrow leaflet plants and his F_2 and F_3 data strongly supported a 3:1 ratio of ovate to lanceolate leaflet plants, allowing him to conclude that a single dominant gene controls leaflet shape. More specifically, homozygous recessive plants (*nana*) have narrow leaflets and homozygous dominant and heterozygous plants (*Na__*) have broad leaflets.

Dorchester (1945) studied morphological characteristics of soybean that could be used for varietal identification. One of these traits was leaflet shape; he studied the shape of both the unifoliolate and the trifoliolate leaves of soybean. He was mainly studying ovate leaflet plants but found that some varieties had unifoliolate leaves and trifoliate leaflets with elongated features, described as distinctly greater in length than width. He concluded that these differences in leaf shape could be used to distinguish varieties from

one another, especially those having similar seed characters. Although he identified 'Patoka' and 'Mt. Carmel' as having longer leaflets, they would be considered broad leaflet plants by the standards used in the current experiment. Dorchester determined that leaflet shape could be used as an adequate morphological marker to distinguish soybean cultivars.

Weiss (1970) reported that a single gene (*na*) was responsible for lanceolate leaflets, and plants with lanceolate leaflets produced a significantly higher proportion of four-seeded pods. He used an undocumented narrow leaflet line that he claims was proven identical to the narrow leaflet trait in T173 and therefore, also has the *na* gene. He concluded that the close association of the narrow leaflets and the number of seeds per pod was due to a pleiotropic effect of the *na* gene in stimulating growth of four seeded pods rather than to closely linked genes. In a combined effort, Bernard and Weiss (1973) attributed leaflet shape to a single gene, with the homozygous dominant (*LnLn*) and the recessive (*lnln*) genotypes being broad and narrow-leaved, respectively. The heterozygote (*Lnln*) was intermediate. Bernard and Weiss assigned a new gene symbol, *ln*, to the narrow leaflet trait. They concluded that *na*, the symbol used by Domingo, and *ln* are the same gene that is responsible for lanceolate leaflets and a high number of seeds per pod in soybeans. A new gene symbol was assigned to this trait because a group of loci that control related traits generally have the same initial letter, and the previously assigned symbols did not match the others in the group. In this case, leaf morphology includes the *lf* gene responsible for the number of leaflets per leaf, the *lo* gene controlling oval leaflets and a

low number of seeds per pod, and the *ln* gene for narrow leaflets and a high number of seeds per pod.

Sawada (1988) used cultivars of broad, narrow, and intermediate leaflet shapes to study the inheritance of this trait. In this study, he used ‘Kitakomachi’ as the broad leaflet parent, ‘Isuzu’ as the narrow leaflet parent and ‘Toiku 187’ as the intermediate leaflet parent. He combined the broad and intermediate F_2 phenotypes and reported a perfect fit to a 3:1 ratio. He defined a method of classification using measurements from the central leaflet to categorize plants into different leaflet shape groups. By dividing the length by the width, he obtained a ratio. Leaflets with ratios less than 2.6 were considered broad and those with ratios greater than 2.6 were considered narrow.

Another study conducted by Wilcox and Abney (1991) used seed from the strain C1421, a BC_6 derivative of ‘Adelphia’, and induced mutation by treating them with ethyl methanesulfonate. One M_2 family exhibited very narrow, rugose leaflets. Because the mutant phenotype appeared similar to that caused by a viral infection, the plants were tested for the presence of different viruses. All tests were negative, indicating that the rugose nature of the leaflets was not due to the presence of a virus. They crossed the narrow rugose plants with the normal C1421 plants and obtaining data fitting a 3:1 ratio of normal to rugose leaflet plants in the F_2 population. F_3 progeny rows from individual F_2 plants were evaluated and found to adequately fit a 1:2:1 genotypic ratio for homozygous broad, heterozygous, and homozygous narrow rugose plants. Therefore, they concluded that the narrow rugose phenotype is controlled by a single recessive allele

and assigned *lnr* as the gene symbol. The mutant line containing the *lnr* gene is maintained in the Genetic Type Collection as T313. Amberger et al. (1992) also observed a wrinkled leaf type morphology among somaclonal variants obtained from culture regenerated soybeans. This study was conducted to produce desirable genetic diversity of soybean plants using culture-regenerated plants. They observed plants exhibiting wrinkled and curled leaflets and through inheritance studies reported that this trait segregated irregularly and failed to fit any predicted genetic ratios. They concluded that the study of variant phenotypes produced by tissue culture techniques have potential for contributing to the understanding of the soybean genome.

Mandl and Buss (1981) reported on the agronomic performance of narrow and broad leaflet soybean isolines. They used D64-4731 as the narrow leaflet parent in this study; D64-4731 received the *ln* gene from the T109 parent. T109 is a pure line derived from PI 84631, which is documented in the soybean genetic type collection as having the narrow leaflet gene *ln*. They found similar seed yields for both leaflet types indicating that narrow leaflet shape is neither an advantage nor a disadvantage in commercial cultivars. Differences were detected among different isolines in this experiment for some morphological and agronomic traits; the narrow leaflet varieties tended to be shorter in height, have smaller seeds, and less lodging. Since both types had similar yields, but the narrow leaf lines had smaller seeds, it was implied that they produced a larger number of seeds, by setting more seeds per pod.

You et al. (1995) studied soybean leaflet shape and its correlation to the number of seeds per pod as a potential way to improve seed yield in southern China. He used a near isogenic line derived from a cross with SRF 400 as one of his narrow leaflet parents. He planted narrow and broad leaflet lines at three different population densities. In comparing population densities, he found no significant difference in seed yield or the number of pods per plant, but he did find a significant interaction between leaflet shape and planting density of soybean plants. He reported that narrow leaflet lines were favorable to yield at high population densities and broad leaflet varieties were more favorable to yield at lower population densities. The average yield of the narrow leaflet lines was highest (2561 kg/ha) at the highest population density (31.2 plants/m^2) used in the experiment, while the broad leaflet plants yielded highest (2504 kg/ha) at the lowest population density (18.7 plants/m^2). He concluded that narrow leaflet plants could be used to improve canopy architecture and enhance the number of seeds per pod of commonly used cultivars in southern China to increase seed yields. Wells et al. (1993) also reported that narrow leaflet shape in soybean provides an opportunity to substantially alter canopy architecture. They studied how two morphological traits, brachytic stems and lanceolate leaflet shape could be used to genetically manipulate canopy structure in order to alter light environments and increase plant productivity. Although they failed to show a significant improvement in performance using brachytic stems and narrow leaflets, increasing productivity by genetically altering canopy structure still has potential for future investigation. The concepts of canopy architecture and planting density are some of the possible applications associated with narrow leaflet plants that could be used in a soybean breeding program to improve upon existing cultivars.

Suh et al. (2000) conducted a genetic study to explore the possibility of optimizing leaf area and leaf shape to improve the photosynthetic rate and ultimately the yield in soybeans. They used Camp as one of the cultivars in determining gene action and heritability of leaf and reproductive traits. Cultivars with lanceolate leaflets and smaller leaf area have better light distribution through the canopy and higher photosynthetic rates than those with larger, oval leaves. They found that most of the progenies exhibited more lanceolate than oval leaf types. Because the leaf shape values (ratio of leaf length to leaf width) observed in the progeny were not greater than the midparents and their parent values, it was determined that leaf shape is affected by additive gene interactions. Also, leaf shape was more affected by additive gene action than leaf area, therefore allowing gene effects for leaf shape to be efficiently transferred to new populations. They concluded that the predominance of additive effects for leaf shape and leaf area could be used in breeding programs for genetic gain and to enhance the photosynthetic rate of soybean cultivars.

Materials and Methods

In this experiment, ten different soybean genotypes were used as parents in a combination of crosses to study the inheritance of the narrow leaflet phenotype. All of the parents have lanceolate leaflets except for 'Williams 82', which has broad leaflets. PI 507729, PI 522235 and PI 578357 are *Glycine soja* accessions originating from Russia that exhibit extremely narrow leaflets (Figure 1). The narrow leaflet strain S56, also referred to as T109 in the soybean genetic type collection, is the *ln* source for another narrow leaflet strain used in this experiment, D64-4731. T41 also has the *ln* gene (Bernard and Weiss, 1973) but it is not known from which source this gene came from because the pedigree of this strain is unknown. It has not been determined if the other two narrow leaflet cultivars used in this experiment, Camp and SRF 400, have the *ln* gene or another locus controlling the narrow leaflet trait. T313 was included as a parent because of its unique rugose narrow leaflet phenotype (Wilcox and Abney, 1991), illustrated in Figure 2. Williams 82, Camp, D64-4731 and SRF 400 were from collections maintained by the breeding program at Virginia Tech and the remainder were obtained from the USDA Soybean Germplasm Collection (University of Illinois). The parents used in the study and information regarding pedigree and source of each cultivar are listed in Table 1. All of the parents and progeny in this experiment were grown at Kentland Research Farm near Blacksburg, VA or in the greenhouse. All of the seeds exhibiting hard seed coats were scarified prior to planting. All populations were planted in the field with a research plot planter with 76.2 cm spacing between rows, except for the F_1 plants which were planted by hand.

A crossing block was planted at Kentland Research Farm, with four staggered planting dates in order to synchronize flowering. In the greenhouse, where the photoperiod was adjusted to 14 h with artificial lighting, two planting dates were used. Morphological markers such as flower color, pubescence color and pod color were used to differentiate crosses from selfs in the F_1 and succeeding generations.

The first crosses were made in the summer of 1998 in the crossing block at Kentland Research Farm. F_1 plants were grown along with the parental cultivars to compare leaflet shape in the greenhouse in winters of 1998 - 1999 and 1999 - 2000. Additional crosses were made the first winter in the greenhouse. Plants that were identified as true crosses were harvested and F_2 populations were planted in the field the following summer. Crossing was continued in the field in summer 1999 to complete the planned series of proposed crosses, especially those with the *G. soja* parents.

In the summer of 1998 the F_2 population of 'Essex' x Camp was grown at Kentland Research Farm. Four-row plots with 40 seeds in each row were planted from each of the three F_1 plants. The row length for F_2 field plots was 6.08 m. Only the two inside rows of each population were observed, about 55-60 plants were labeled and scored for leaflet shape. Essex is a broad leaflet cultivar with the pedigree 'Lee' x S55-7075. This population was studied to determine the inheritance of narrow leaflets in Camp. Single rows of 48 $F_{2:3}$ families were grown and evaluated in 1999 to confirm the F_2 data. The row length for the $F_{2:3}$ progeny rows was 3.04 m and there were about 50 seeds planted in

each row. The plants were visually classified as either narrow or broad leaflet or measured when classification was difficult in both the F_2 and F_3 generations.

Narrow leaflet varieties having the *ln* gene were crossed with other narrow leaflet varieties of unknown genotypes for allelism tests. Specifically, Camp and SRF 400 were crossed with sources of the *ln* gene (D64-4731, T41, and S-56). The F_2 and $F_{2:3}$ generations were characterized for segregation of leaflet shape. T313 was also crossed with Camp to study allelic relationships of the *ln* and *lnr* genes. The three *G. soja* PIs were crossed with Williams 82 to determine the inheritance of their narrow leaflet trait. They were also crossed with the other narrow leaf parents to determine the allelism of the genes controlling narrow leaflets.

The *G. soja* accessions used in the crosses have very narrow leaflets with lateral leaflets almost three times as long as they were wide and the terminal leaflets were four to five times longer than wide. This vast difference in leaflet shape was helpful in detecting true crosses made with the PIs because the selfs were quickly eliminated if the F_1 plants did not resemble the *G. soja* parent. The *G. soja* accession was always the male parent, and therefore if the F_1 plant had similar morphological traits as the female parent it was considered to be a self.

Data on leaflet shape were first obtained by visually scoring individual plants as either narrow or broad leaflet. When classifying progeny from crosses with T313, a narrow rugose category was used in addition to the broad and narrow classes. The leaflets of each

plant then were measured to alleviate difficulty of visual classification of segregates into the different leaf shape categories. Taking leaf measurements provided better guidelines for scoring of plants for leaflet shape than visual classification. Each leaflet in a trifoliolate leaf was measured lengthwise and widthwise in millimeters. The length was then divided by the width to obtain the length to width ratio (L/W). Once the leaflet shape data were converted to ratios, the L/W ratios were analyzed to compare different plants and different leaflets on the plants. This provided an index to use in classifying the plants into either the broad or narrow leaflet categories in cases where visual classification was difficult. Statistical analysis defined the indices for each leaflet shape category. Broad leaflet plants had L/W ratios of less than or equal to 1.5 and narrow leaflet plants had L/W ratios of greater than 2.0. By observing measurement data from different narrow leaflet parental cultivars it was determined that the L/W ratio of the terminal leaflet was consistently higher compared to the lateral leaflet ratios. Therefore, the terminal leaflet was used exclusively to classify the progeny, instead of measuring all three leaflets.

In 1998 and 1999 the three *G. soja* lines were crossed with Williams 82 and Camp to study the inheritance of the very narrow phenotypes exhibited by the PIs. F_2 progeny derived from crosses with the *G. soja* segregated into an array of different leaflet shapes. The simple narrow and broad leaflet classification system used for other populations did not adequately represent all of the many intermediate types observed. Templates were used to sort the plants into different leaf shape categories. Each template represented a specific leaflet L/W range. The templates were created in the shape of a leaflet with the dimensions of the larger ratio in the range and each had a diagram of the narrower ratio

drawn inside it. Based on a sampling of the parents and the segregating populations, six leaflet shape categories with L/W ranges as follows were established: 1.5-2, 2-2.5, 2.5-3, 3-3.5, 3.5-4, and >4. A terminal leaflet of each plant was matched to the best fitting template and the ratio was recorded. Using the templates and only the terminal leaflet allowed a more efficient method of classifying plants than taking actual measurements from the leaves and then calculating ratios.

Each of the F_2 plants from crosses with T313 was given an individual plant number so data from the F_2 generation could be compared to the F_3 generation. F_2 plants grown in 1998 and 1999 were harvested individually and grown as $F_{2:3}$ progeny rows the following summer.

Chi-square tests were run on the data from the F_2 and F_3 populations that were segregating for leaflet shape to determine goodness of fit to expected ratios for simple Mendelian inheritance.

Results and Discussion

By observing many different soybean plants, it was determined that all three of the leaflets in a trifoliolate leaf typically are not the same size. The leaflet measurement data from this study indicate that all of the leaflets of ovate plants tend to have similar L/W ratios and that ratios do not differ much between cultivars, but the terminal leaflet of most of the lanceolate parents is narrower than the lateral leaflets (Table 2). Similar to the methods employed by Domingo (1945) and Sawada (1988), it was decided to use only terminal leaflets to classify plants, in order to obtain consistent data and to maximize differences between broad and narrow leaflet plants. Narrow leaflet plants generally have leaflets that are more than twice as long as they are wide, while broad leaflet plants tend to have L/W ratios of around 1.5. These figures provided a good reference for scoring the progeny for either ovate or lanceolate leaflet shape. It is also evident that a large difference is present in the L/W ratio among the narrow leaf cultivars, and that the range among cultivars appears to be greater for the terminal leaflets than the lateral leaflets.

Crosses among Camp, T41, SRF 400, S56 and D64-4731 did not segregate in the F_2 generation for leaflet shape (Table 3). Each cross segregated for other traits such as pubescence color or flower color indicating that they were true crosses. From this, it was concluded that these lines have alleles at the same locus controlling lanceolate leaflet shape. It is documented in the Genetic Type Collection that the T41 cultivar has the *ln* gene responsible for narrow leaflet shape in soybeans (Bernard and Weiss, 1973). Therefore, it was concluded that Camp, SRF 400, D64-4731, and S56 also have the *ln* gene.

The Essex x Camp cross segregated for leaflet shape (Table 3). The F_2 and $F_{2:3}$ data taken from three populations proved analogous with results obtained by Domingo (1945), Bernard and Weiss (1973), and Sawada (1988) in which narrow leaflet shape is controlled by a single recessive gene. Out of 172 F_2 plants, 127 had broad leaflets and 45 had narrow leaflets fitting a 3:1 ratio (X^2=0.12, P < 0.95). The F_2 genotypes, as determined from the $F_{2:3}$ data, fit a 1:2:1 ratio of all broad-leaved rows : rows segregating for broad and narrow : all narrow-leaved rows (X^2=0.33, P = 0.8-0.95) and confirmed that Camp has a single recessive gene for narrow leaflet shape.

The T313 x Williams 82 cross segregated for leaflet shape in the F_2 generation. The F_2 progeny exhibited two different leaf types, broad leaflet plants like Williams 82 and narrow rugose leaflet plants like T313. The data were tested against a 3:1 ratio of broad to narrow rugose plants, but a poor fit was observed because of the low proportion of narrow rugose plants (Table 4). Although the data failed to fit the 3:1 ratio for simple inheritance of a single gene, the narrow rugose trait was still considered to be controlled by a single recessive gene. This is because the previous study by Wilcox and Abney (1991) reported that the *lnr* gene was responsible for narrow rugose leaflet shape.

Crosses of T313 with narrow leaflet lines Camp and SRF 400 also segregated for leaflet shape (Table 3). The F_1 plants of Camp x T313 (Figure 3) and T313 x SRF 400 exhibited ovate leaflets. Since all of these parents have narrow leaflets, the occurrence of a new phenotype in the F_1 and segregating generations indicates that different genes are responsible for leaf shape in these cultivars. The F_2 progeny in both populations

segregated into three different leaf classifications: broad, like the F_1 plants, narrow, like Camp and SRF 400, and narrow rugose, like T313. Figure 4 shows the segregation in leaflet shape of the Camp x T313 F_2 population grown at Kentland Research Farm the summer of 1999. Occurrence of the broad leaflet phenotype in the F_1 and F_2 generations, indicates that *ln* and *lnr* are not at the same locus. A genetic model was proposed for the Camp x T313 cross in which two genes, one from Camp (*ln*) and one from T313 (*lnr*), segregate independently to produce the three different phenotypes observed in the F_2 generation (Figure 5). The homozygous recessive *lnrlnr* gene is assumed to be epistatic over the *Ln/ln* gene. The model proposes that the F_2 should segregate in a 9:3:4 ratio of broad : narrow : narrow rugose leaflet plants. The F_2 data for both Camp x T313 and T313 x SRF 400 were tested to fit this ratio and exhibited differing results (Table 5). Fifteen populations were observed, nine from Camp x T313 and six from T313 x SRF 400. Seven of these populations fit the 9:3:4 ratio with P values greater than 0.05, but only one population from the Camp x T313 cross showed a good fit with a P value greater than 0.2. The combined Camp x T313 and T313 x SRF 400 did not fit the expected ratio ($P<0.01$), therefore it was assumed that these populations do not follow the proposed genetic model. The Camp x T313 F_2 data did not test well for homogeneity of the combined populations ($P<0.01$), primarily because some populations were a good fit to the 9:3:4 ratio while others showed highly significant deviations from it.

The $F_{2:3}$ generation from three Camp x T313 families were grown in the summer of 2000 at Kentland Research Farm. The progeny segregated for broad, narrow and narrow rugose leaflet plants. The F_2 plants were assigned genotypes based on the segregation observed

in their respective $F_{2:3}$ families. The resulting data were then tested against the 9:3:4 genotypic ratio predicted by the model (Table 6). As with the F_2 phenotypic data, the F_2 genotypic data did not fit the expected ratio. The genotypic data did confirm that the narrow leaflet trait is controlled by more than one gene in these cultivars. The number of genes and gene action was not determined.

In all of the populations studied in this experiment involving T313 as a parent, the observed number of narrow rugose plants in segregating families was always noticeably lower than expected. Alternative genetic models such as linkage or multiple genes could explain the observations. Going on the assumption that the original two-gene model was correct, an analysis of the segregation of the individual genes was conducted in the Camp x T313 cross. Segregation for the *Ln/ln* gene among the F_2 plants gave a good fit to the expected 1:2:1 ratio, but segregation for the *Lnr/lnr* gene gave a poor fit to this ratio (Table 7). The poor fit is due to a deficiency of narrow rugose and heterozygous F_2 plants. The previous genetic study of T313, by Wilcox and Abney (1991) however, did not report a deficiency of narrow rugose plants. A possible explanation for the deficiency observed in this experiment, is some kind of differential survival of the gametes or embryos possessing this gene. Narrow rugose plants were frequently less vigorous than other plants, but if low survival of narrow rugose F_2 plants were the sole cause of the deficiency, one would not expect a deficiency of heterozygotes. Lower than expected frequency of all genotypes containing *lnr* might seem to favor the existence of a lower survival rate of *lnr* gametes.

Following the assumption that *lnr* gametes had a functional frequency less than the normal 0.5, new frequencies were calculated using the observed numbers of each of the three F_2 genotypes for the *Lnr*/*lnr* gene in the Camp x T313 population. The observed allelic frequencies in the F_2 population were calculated to be 0.63 *Lnr* and 0.37 *lnr*.

Using the calculated gametic frequencies of the *Lnr/lnr* gene along with the normal frequencies of the *Ln/ln* gene, adjusted expected ratios were calculated for all of the T313 crosses. The adjusted ratios were then compared to the segregations observed. The F_2 progeny of T313 x Williams 82 provided a good fit to the adjusted ratio of 6.3:1 for simple inheritance of a single gene (Table 8). The adjusted dihybrid ratio of 4.7:1.6:1 greatly improved the fit of the Camp x T313 (P=0.5-0.7) and T313 x SRF 400 (P=0.3-0.5) F_2 populations (Tables 9 and 5), although the P value for the homogeneity test for the Camp x T313 cross was less than 0.01 (Table 9). However, upon closer inspection of the individual Camp x T313 populations, it appears that only two are responsible for the poor fit. Interestingly, the family showing the poorest fit, F_2-477, provided one of the best fits to the 9:3:4 ratio. The other family that showed a poor fit to the adjusted ratio had an even poorer fit to 9:3:4 (Table 5). Even though the Camp x T313 population still shows significant heterogeneity, the fact that the adjusted ratio provided an acceptable overall fit for all three crosses supports the theory that a form of gametic selection is occurring. Similarly, the F_2 genotypic data from Camp x T313 were tested against the adjusted ratio of 1 all broad leaflet plants : 2 segregating for broad and narrow leaflet plants : 1 all narrow leaflet plants : 2 segregating for broad and narrow rugose plants : 4 segregating for broad, narrow and narrow rugose leaflet plants : 2 segregating for narrow and narrow

rugose leaflet plants : 4 all narrow rugose leaflet plants. A better fit was observed for the adjusted ratio than to the original ratio (Tables 10 and 6). Although the P value is less than 0.05, it is greater than 0.01 and not low enough to totally reject the hypothesis.

In analyzing the data from the $F_{2:3}$ progeny of the Camp x T313 cross, it was observed that 20 of the $F_{2:3}$ progeny rows from narrow rugose F_2 plants contained a few plants of other leaf types, generally in frequencies too low to be explained easily by genetic segregation. The proposed model states that $F_{2:3}$ progeny derived from F_2 narrow rugose plants are homozygous recessive and thus their progeny should not exhibit broad or narrow leaflet types. One possible explanation for the appearance of other leaf types is outcrossing of the F_2 plants with surrounding plants of different genotypes. The calculated outcrossing frequency in the $F_{2:3}$ population was 19.7 %. That frequency was higher than the 0.5 to 1% outcrossing normally observed in soybeans (Weber and Hanson 1961). High rates of outcrossing could be due to flower deformation or low viability of pollen. Pollen from both narrow rugose plants and non-rugose plants was observed under the microscope (100X) but no visible differences were noted.

The proposed genetic model for Camp x T313 denotes that four types of segregation can be observed in the $F_{2:3}$ generation (Figure 5). Broad leaved F_2 plants can have $F_{2:3}$ families segregating 3 broad : 1 narrow, 3 broad : 1 narrow rugose or 9 broad : 3 narrow : 4 narrow rugose. Narrow leaf F_2 plants can have $F_{2:3}$ families segregating 3 narrow : 1 narrow rugose. The ratios were recalculated using the adjusted gametic frequencies, producing new ratios of 7.2:1 broad : narrow rugose, 7.2:1 narrow : narrow rugose, and

4.7:1.6:1 broad : narrow : narrow rugose. The 3 broad : 1 narrow ratio did not need to be adjusted because those F_2 plants are homozygous *Lnr* and are therefore unaffected by irregular segregation of the *lnr* gene. All of the $F_{2:3}$ families segregating for leaflet shape were tested against the proposed ratios using both unadjusted and adjusted frequencies (Table 11). Once again it is evident that there is a deficiency in narrow rugose plants and much better fits are obtained with the adjusted ratios. The rows segregating for all three of the leaflet types still had a rather poor fit to the ratio (P=0.01-0.05) but the X^2 value is much improved from the original calculation. It is evident that the deficiency remains in the narrow rugose category, which could have been influenced by poor emergence of weak plants or premature death due to environmental conditions, although no observations were made of either phenomenon.

A cross between Williams 82 and PI 522235 produced viney F_1 plants with a average terminal leaflet L/W ratio of 2.3 which is slightly lower than the midparent value. Plants in the F_2 generation exhibited both broad and narrow leaflets. Williams 82, the broad leaflet parent, was measured using the leaflet shape templates to have a ratio range of 1.5-2.0. PI 522235, the narrow leaflet parent, had a length by width ratio of greater than 4.0. These cultivars represent the two extremes of the ratio ranges on the templates used in measuring the progeny (Table 12). The F_2 plants fit a 3:1 phenotypic ratio (X^2=0.843, P=0.3-0.5) for a single recessive gene controlling the very narrow phenotype of PI 522235. The data were tested against a 1:2:1 ratio in which the homozygous dominant had L/W ratios of 1.5-2.0, the heterozygous with L/W ratios 2.0-2.5, and homozygous recessive with L/W ratios >2.5 (Table 13) and showed a good fit (X^2=1.450, P=0.3-0.5).

Therefore, the tentative conclusion is that a single gene is responsible for the extremely narrow leaflet shape in PI 522235, but this population must be carried out to the F_3 generation in order to verify this.

Camp was crossed with PI 522235 in 1999 and the F_1 plants were grown in the greenhouse the winter of 1999. The F_2 generation, grown at Whitethorne Farm in the summer of 2000, segregated for broad and narrow leaflet plants (Figure 6). Camp, a narrow leaflet line, had a L/W ratio of 2.5-3.0 using the templates and PI 522235 was greater than 4.0. The occurrence of plants in the progeny with L/W ratios that exceed the parental ranges (L/W 1.5-2.5) indicates that the Camp gene (*ln*) is probably not allelic to the gene controlling narrow leaflet shape in PI 522235. A genetic model for Camp x PI 522235 can be proposed with two genes responsible for leaflet shape, the *ln* gene from Camp and the n_1 gene from PI 522235 (Figure 7). The F_2 data were tested against a 9:4:3 ratio for plants with L/W ranges of 1.5-2.5 : plants with L/W ranges of 2.5-3.0 : plants with L/W ranges of >3.0 (Table 13). The data showed a good fit to the proposed ratio (X^2=0.859, P=0.5-0.7), supporting the two locus theory. While the model appears to explain the observations, a definite conclusion about the inheritance of the genes controlling this trait requires further evaluation of this population, at least to the F_3 generation.

Williams 82 was crossed to PI 507729, which has a ratio of 3.5-4.0 using the templates. The F_1 plant for this cross exhibited intermediate type leaflets with a L/W ratio of 1.7. The F_2 phenotypic data for Williams 82 x PI 507729 fit a 3:1 ratio (X^2=2.0, P=0.05-0.2)

for a single recessive allele controlling narrow leaflet shape in PI 507729. The population segregated 1:2:1 (X^2=3.361, P=0.05-0.2) for plants with L/W ranges of 1.5-2.0 : plants with L/W ranges of 2.0-2.5 : plants with L/W ranges of >2.5 (Table 14). The data support the theory that a single recessive gene is responsible for the very narrow leaflet shape in PI 507729.

Camp was crossed to PI 507729 in 1999 and the F_2 generation segregated for leaflet shape in the 2000 field tests. Since the F_2 data for Williams 82 x PI 507729 fit the expected segregation ratio for a single gene, similar to the Williams 82 x PI 522235 cross, it was assumed that this population (Camp x PI 507729) would segregate for two genes, like the Camp x PI 522235 progeny. The data were tested against a 9:4:3 ratio but failed to fit that model (Table 14). However, they do fit a 15:1 ratio if the dividing line for the classes is moved to a L/W ratio of 4 (X^2=0.926, P=0.3-0.5). This change could be rationalized by assuming that the most narrow leaflet type is expressed only in plants containing the recessive gene from both parents and that the PI 507729 gene interacts with the Camp gene in a different way from the PI 522235 gene. Further study of the F_3 generation will be necessary to determine the inheritance of the PI 507729 gene.

PI 578357, with a L/W ratio of 3.5-4.0 using the templates, was also crossed with Williams 82. The F_1 plants had intermediate leaf types with a L/W ratio of 1.7. The F_2 data were tested against a 1:2:1 genetic ratio but were an extremely poor fit (Table 15). Alternatively, if the data are classified so that leaflets as narrow as PI 578357 are compared with the remainder and tested against the 15:1 ratio, the fit is better but still

with a P value of less than 0.01 (Table 15). However, if the data are reclassified so that L/W ratios greater than 3.0 are considered narrow, they provide a good fit to the 15:1 ratio (X^2=0.998, P=0.5-0.7). Obviously, there is no clear-cut genetic interpretation for the data and further research is needed.

In this experiment no segregation was observed in the F_2 generation when Camp was crossed with four other narrow leaflet lines (T41, SRF 400, D64-4731 and S56). Therefore, it was concluded that all of these cultivars have the *ln* gene controlling the narrow leaflet trait. Data from F_2 and F_3 generations of Camp x T313 and T313 x SRF 400 indicate that the gene controlling the narrow rugose leaflet trait in T313, *lnr*, is at a locus independent from the *ln* gene. A deficiency of narrow rugose as well as heterozygous plants was observed in the F_2 and F_3 generations of the populations with T313 as a parent. Although this phenomenon was not observed in the previous genetic study of T313 (Wilcox and Abney, 1991), it was theorized in this study that the deficiency was caused by differential survival of *lnr* gametes. The adjustment of expected ratios for apparent differential survival of gametes provided improved fits for all of the data from populations involving T313 as a parent. Inheritance of the narrow leaflet trait in PI 522235, PI 507729, and PI 578357 appears to be controlled by one or two recessive genes, but further study is needed to come to a definitive conclusion. However, since F_2 plants in the crosses of Camp with PI 507729 and PI 522235 were observed with leaflets broader than either parent, it can be concluded that the gene(s) in the PIs are not at the *ln* locus.

Figure 1A. *Glycine soja* accession PI 507729 used as one of the parents in crosses.

Figure 1B. A comparison of the leaflet size and shape of *Glycine soja* accession PI 578357 (left) and *Glycine max* cultivar Camp (right).

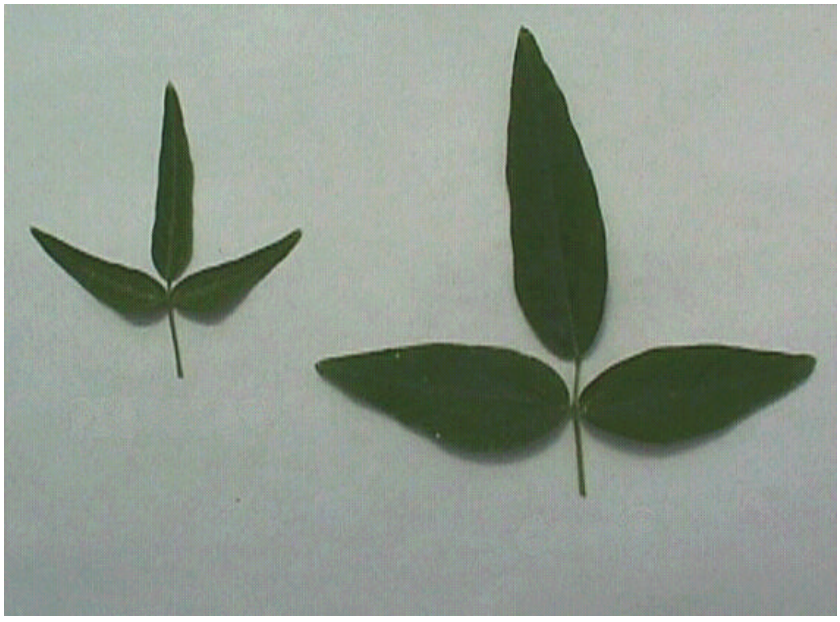


Figure 2. Soybean line T313, exhibiting narrow, rugose leaflets, growing in the field at Blacksburg, VA.

Figure 3. The broad leaflet F_1 soybean plant from the Camp x T313 cross, growing in the greenhouse.

Figure 4. Camp x T313 F_2 population segregating for broad, narrow and narrow rugose leaflets growing in the field at Blacksburg, VA.

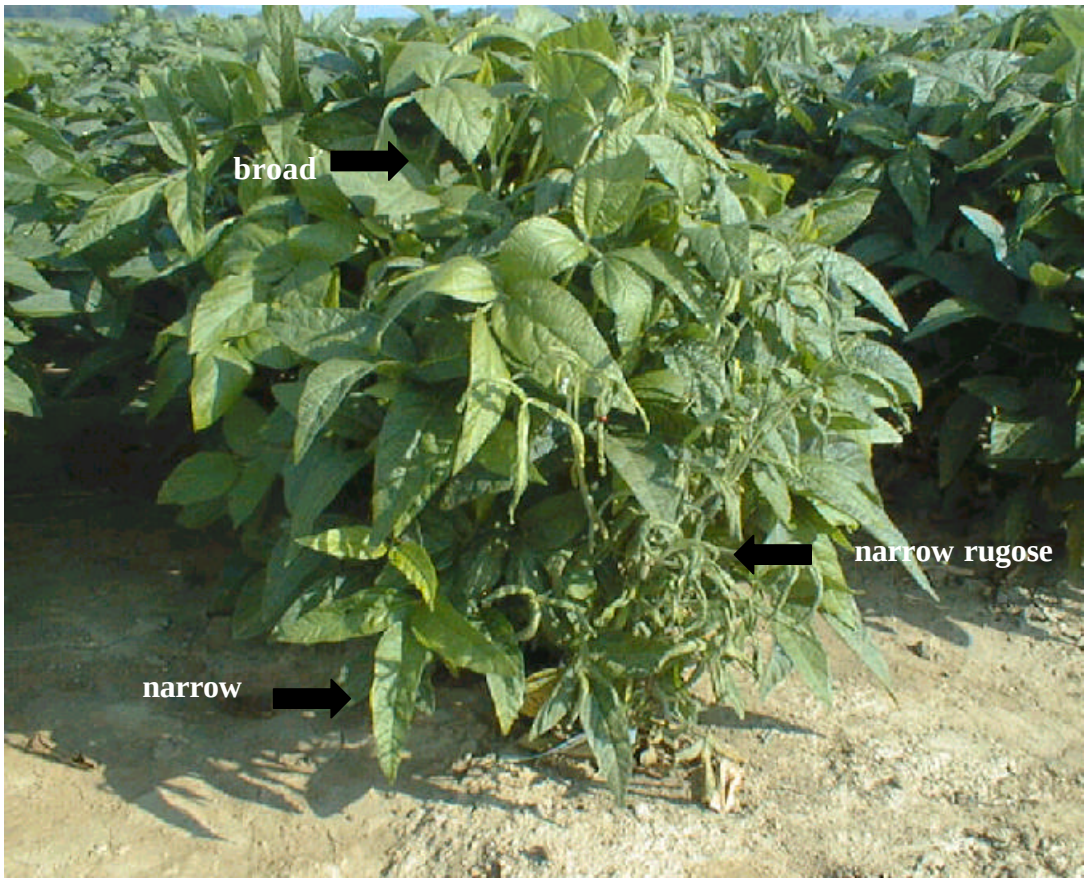
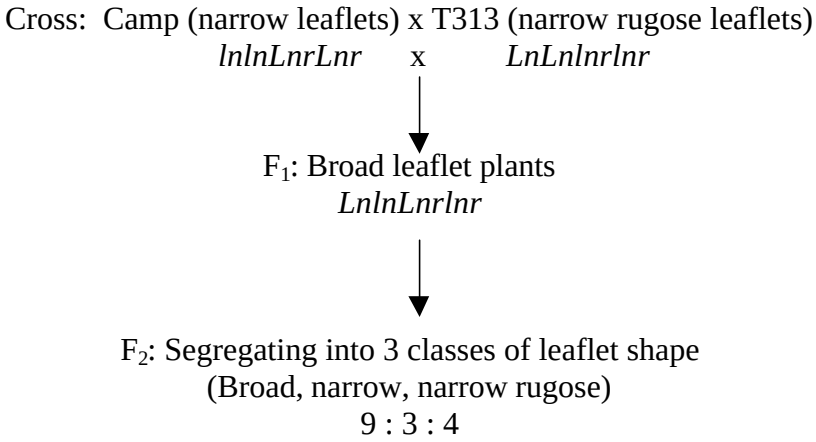


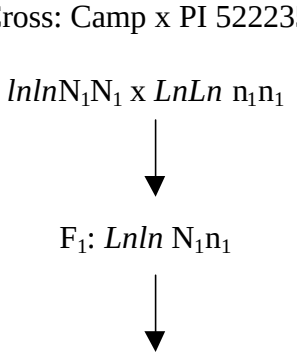
Figure 5. The proposed 2-locus genetic model for leaflet shape in Camp x T313 soybean population.



Generation	Genotype	Ratio	Phenotype	$F_{2:3}$ Phenotype
F_1	*LnlnLnrlnr*		Broad	
F_2	*LnLnLnrLnr*	1	Broad	All Broad
	LnlnLnrLnr	2	Broad	Broad/Narrow (3:1)
	lnlnLnrLnr	1	Narrow	All Narrow
	LnLnLnrlnr	2	Broad	Broad/Narrow rug. (3:1)
	LnlnLnrlnr	4	Broad	Broad/Narrow/Narrow rugose (9:3:4)
	lnlnLnrlnr	2	Narrow	Narrow/Narrow rugose (3:1)
	_ _ lnrlnr	4	Narrow rug.	All Narrow rugose

Figure 6. F_2 progeny of Camp x PI 522235 segregating for narrow (left) and broad (right) leaflet shape, growing in the field at Blacksburg, VA.

Figure 7. The proposed 2-locus genetic model for leaflet shape in Camp x PI 522235 soybean population.



Generation	Genotype	Ratio		Phenotype (L/W Ratio)	
F_1	*Lnln* N_1n_1				
F_2	*LnLn* N_1N_1	1		1.5-2.0	
	LnLn N_1n_1	2		2.0-2.5	
	Lnln N_1N_1	2	9	2.0-2.5	1.5-2.5
	Lnln N_1n_1	4		2.0-2.5	
	LnLn n_1n_1	1		2.5-3.0	
	Lnln n_1n_1	2	4	2.5-3.0	2.5-3.0
	lnln N_1N_1	1		2.5-3.0	
	lnln N_1n_1	2	3	>3.0	>3.0
	lnln n_1n_1	1		>3.5	

Table 1. Parent sources, pedigree information and morphological information used in note taking and verifying soybean crosses.

Genotype	Pedigree	Origin	Flower color	Pubescence color	Pod color	Maturity group	Stem type
Williams 82	Williams *7 / Kingwa	Illinois	white	tawny	tan	III	indeterminate
Camp	Essex / Unknown *G. soja*	Virginia	purple	gray	tan	V	determinate
D64-4731	Lee *2 // Clark *2 / T109	Mississippi	purple	tawny	tan	V	determinate
SRF 400	Clark 63 *7 / D61-5141	Illinois	purple	tawny	brown	I	indeterminate
T313	EMS mutant C1421	Indiana	white	gray	tan	III	indeterminate
T41	Unknown	Illinois	white	tawny	brown	IV	determinate
S56	T109	South Korea	white	tawny	brown	III	determinate
PI 507729	*G. soja*	Russia	purple	tawny	black	000	indeterminate
PI 522235	*G. soja*	Russia	purple	tawny	black	II	indeterminate
PI 578357	*G. soja*	Russia	purple	tawny	black	000	indeterminate

Table 2. Average length by width (L/W) ratios and standard deviations taken from mature trifoliolate leaflets of different parental soybean genotypes grown in Blacksburg, VA in 1998 and 1999.

			Terminal leaflet		Lateral leaflets	
Year	Name	Leaf shape	L/W ratio	n	L/W ratio	n
1998	Essex	ovate	1.5±0.03	9	1.5±0.1	16
1999	Williams 82	ovate	1.4±0.2	10	1.4±0.1	19
1998	Camp	lanceolate	2.6±0.4	9	2.2±0.1	16
1999	Camp	lanceolate	2.8±0.3	10	2.3±0.2	20
1999	D64-4731	lanceolate	2.5±0.2	5	2.2±0.1	10
1999	SRF 400	lanceolate	2.8±0.2	5	2.4+0.1	10
1999	T41	lanceolate	3.1±0.3	5	2.5+0.1	10
1999	T313	lanceolate	3.1±0.3	5	2.4+0.1	10
1999	PI 507729	lanceolate	4.6±0.6	5	3.0±0.3	10
1999	PI 522235	lanceolate	5.6±0.3	5	3.4±0.1	10
1999	PI 578357	lanceolate	4.2±1.1	5	2.9±0.1	10

Table 3. Morphological data recorded from the soybean F_2 populations expected to segregate for leaflet shape at Kentland Research Farm.

A. F_2 populations that did not segregate for leaf type.

Year	Cross	Leaf type	Flower color	Pubescence color	Stem type	Plants observed
1999	Camp x D64-4731	narrow	purple	segregating		260
1999	Camp x SRF 400	narrow	purple	segregating		260
1999	Camp x S-56	narrow	segregating	segregating		260
1999	Camp x T41	narrow	purple	segregating		130
1999	SRF 400 x D64-4731	narrow	purple	tawny	segregating	260
2000	T41 x SRF 400	narrow	segregating	tawny		200
2000	T41 x D64-4731	narrow	segregating	tawny		200

B. F_2 populations that segregated for leaf type.

Year	Cross	Leaf type	Flower color	Pubescence color	Plants observed
1998	Essex x Camp	segregating	purple	gray	172
1999	Camp x T313	segregating	purple	gray	192
2000	Camp x T313	segregating	purple	gray	333
2000	Williams 82 x PI 507729	segregating	segregating	tawny	216
2000	Williams 82 x PI 522235	segregating	segregating	tawny	89
2000	Williams 82 x PI 578357	segregating	segregating	tawny	274
2000	Camp x PI 507729	segregating	purple	segregating	121
2000	Camp x PI 522235	segregating	purple	segregating	305
2000	T313 x SRF 400	segregating	segregating	segregating	368
2000	T313 x Williams 82	segregating	white	segregating	214

Table 4. Segregation for leaf type in a soybean F_2 population derived from a cross of T313 x Williams 82.

2000 F_2 No.[1]	Broad	Narrow rugose	Total	X^2 for 3:1	DF	P
406	30	4	34	3.176	1	0.05-0.2
407	37	10	47	0.348	1	0.5-0.7
408	49	7	56	4.667	1	0.01-0.05
409	33	3	36	5.333	1	0.01-0.05
410	36	5	41	3.585	1	0.05-0.2
				17.109		
Obs.	185	29	214			
Exp.	160.5	53.5	214	14.96	1	<0.01
Heterogeneity				2.149	4	0.7-0.8

[1] The number assigned to the population used in data collection and analysis.

Table 5. Chi square evaluation of a soybean F_2 population segregating for leaf shape derived from Camp x T313 and T313 x SRF 400 in 1999 and 2000.

F_2 No.[1]	Pedigree		Broad	Narrow	Narrow rugose	Total	X^2 for 9:3:4	DF	P
$00F_2$-411	T313 X SRF 400		40	15	11	66	2.613	2	0.2-0.3
$00F_2$-412	T313 X SRF 400		45	12	12	69	2.652	2	0.2-0.3
$00F_2$-413	T313 X SRF 400		34	8	7	49	3.907	2	0.05-0.2
$00F_2$-414	T313 X SRF 400		30	5	6	41	4.789	2	0.05-0.2
$00F_2$-415	T313 X SRF 400		53	18	7	78	10.69	2	<0.01
$00F_2$-416	T313 X SRF 400		44	15	6	65	8.627	2	0.01-0.05
							32.978	12	
Total		Obs.	246	73	49	368			
		Exp.	207	69	92	368	27.678	2	<0.01
	Heterogeneity						5.3	10	0.8-0.95
$00F_2$-417	Camp X T313		28	3	2	33	10.855	2	<0.01
$00F_2$-418	Camp X T313		34	11	5	50	6.009	2	0.01-0.05
$00F_2$-419	Camp X T313		50	14	6	70	10.482	2	<0.01
$00F_2$-420	Camp X T313		40	27	7	74	19.624	2	<0.01
$00F_2$-421	Camp X T313		31	12	11	54	0.823	2	0.5-0.7
$00F_2$-422	Camp X T313		34	11	7	52	3.7	2	0.05-0.2
$99F_2$-476	Camp X T313		43	13	6	62	7.879	2	0.01-0.05
$99F_2$-477	Camp X T313		30	14	21	65	3.437	2	0.05-0.2
$99F_2$-479	Camp X T313		34	21	10	65	9.152	2	0.01-0.05
							71.961	18	
Total		Obs.	324	126	75	525			
		Exp.	295.31	98.44	131.25	525	34.611	2	<0.01
	Heterogeneity						37.35	16	<0.01

[1] The number assigned to the population used in data collection and analysis.

Table 6. Chi square evaluation of leaflet shape segregation in Camp x T313 $F_{2:3}$ soybean progeny.

	Proposed F_2			Rows				
F_2 Phenotype	Genotype	$F_{2:3}$ Phenotype	Ratio	Obs.	Exp.	X^2	DF	
broad	*LnLnLnrLnr*	broad	1	24	11.5	13.587		
broad	*LnlnLnrLnr*	broad/narrow	2	38	23	9.783		
narrow	*lnlnLnrLnr*	narrow	1	22	11.5	9.587		
broad	*LnLnLnrlnr*	broad/rugose	2	18	23	1.087		
broad	*LnlnLnrlnr*	broad/narrow/rugose	4	29	46	6.283		
narrow	*lnlnLnrlnr*	narrow/rugose	2	16	23	2.130		
rugose	_ _ *lnrlnr*	rugose	4	37	46	1.761		
						44.218	6	P<0.01

Table 7. Segregation of individual genes for leaflet shape observed in Camp x T313 F_2 soybean plants as determined from $F_{2:3}$ progeny.

ln:[1]

F_2 Genotype	*LnLn*	*Lnln*	*lnln*	Total	X^2 for 1:2:1	
No. families	42	67	38	147	1.368	P=0.5-0.7

lnr:[2]

F_2 Genotype	*LnrLnr*	*Lnrlnr*	*lnrlnr*	Total	X^2 for 1:2:1	
No. families	84	63	37	184	28.52	P<0.01

[1] *ln* is the gene responsible for narrow leaflet shape in Camp.

[2] *lnr* is the gene responsible for narrow rugose leaflet shape in T313.

Table 8. Chi square evaluation of leaflet shape segregation in T313 x Williams 82 F_2 soybean progeny using the adjusted ratio of 6.3:1 broad : narrow rugose plants to compensate for differential survival of gametes from T313.

2000 F_2 No.[1]	Broad	Narrow rugose	Total	X^2	DF	P
406	30	4	34	0.108	1	0.7-0.8
407	37	10	47	2.284	1	0.05-0.2
408	49	7	56	0.068	1	0.7-0.8
409	33	3	36	0.877	1	0.3-0.5
410	36	5	41	0.079	1	0.7-0.8
				3.416		
Obs.	185	29	214			
Exp.	184.69	29.32	214	0.004	1	0.8-0.95
Heterogeneity				3.413	4	0.3-0.5

[1] The number assigned to the population used in data collection and analysis.

Table 9. Chi square evaluation of leaflet shape segregation in F_2 progeny from Camp x T313 and T313 x SRF 400 soybean crosses using the adjusted ratio of 4.7:1.6:1 broad : narrow : narrow rugose plants to compensate for differential survival of gametes from T313.

F_2 No.[1]	Pedigree		Broad	Narrow	Narrow rugose	Total	X^2	P	DF
$00F_2$-411	T313 X SRF 400		40	15	11	66	0.624	0.7-0.8	2
$00F_2$-412	T313 X SRF 400		45	12	12	69	1.232	0.5-0.7	2
$00F_2$-413	T313 X SRF 400		34	8	7	49	0.802	0.5-0.7	2
$00F_2$-414	T313 X SRF 400		30	5	6	41	2.149	0.3-0.5	2
$00F_2$-415	T313 X SRF 400		53	18	7	78	1.497	0.3-0.5	2
$00F_2$-416	T313 X SRF 400		44	15	6	65	1.120	0.5-0.7	2
							7.424	0.7-0.8	12
Total		Obs.	246	73	49	368			
		Exp.	238.09	79.36	50.55	368	0.820	0.5-0.7	2
	Heterogeneity						6.604	0.7-0.8	10
$00F_2$-417	Camp X T313		28	3	2	33	5.868	0.05-0.2	2
$00F_2$-418	Camp X T313		34	11	5	50	0.596	0.7-0.8	2
$00F_2$-419	Camp X T313		50	14	6	70	1.929	0.3-0.5	2
$00F_2$-420	Camp X T313		40	27	7	74	9.920	<0.01	2
$00F_2$-421	Camp X T313		31	12	11	54	2.185	0.3-0.5	2
$00F_2$-422	Camp X T313		34	11	7	52	0.011	>0.99	2
$99F_2$-476	Camp X T313		43	13	6	62	0.961	0.5-0.7	2
$99F_2$-477	Camp X T313		30	14	21	65	19.774	<0.01	2
$99F_2$-479	Camp X T313		34	21	10	65	5.148	0.05-0.2	2
							46.392	<0.01	18
Total		Obs.	324	126	75	525			
		Exp.	339.66	113.22	72.12	525	2.279	0.3-0.5	2
	Heterogeneity						44.113	<0.01	16

[1] The number assigned to the population used in data collection and analysis.

Table 10. Chi square evaluation of leaflet shape segregation in Camp x T313 $F_{2:3}$ soybean progeny using ratios adjusted for differential survival of *Lnr/lnr* gametes.

F_2 Phenotype	Proposed F_2 Genotype	$F_{2:3}$ Phenotype	Exp. Ratio	No. Rows Obs.	No. Rows Exp.	X^2	
broad	*LnLnLnrLnr*	broad	0.106	24	18.3	1.785	
broad	*LnlnLnrLnr*	broad/narrow	0.212	38	36.5	0.062	
narrow	*lnlnLnrLnr*	narrow	0.106	22	18.3	0.748	
broad	*LnLnLnrlnr*	broad/rugose	0.114	18	21.4	0.540	
broad	*LnlnLnrlnr*	broad/narrow/rugose	0.228	29	42.9	4.504	
narrow	*lnlnLnrlnr*	narrow/rugose	0.114	16	21.4	1.363	
rugose	_ _ *lnrlnr*	rugose	0.124	37	25.2	5.525	
						14.527	P=0.01-0.05

Table 11. Chi square evaluation of $F_{2:3}$ segregation of Camp x T313 soybean progeny combined according to F_2 genotypes and using unadjusted ratios and ratios adjusted for differential survival of gametes from T313.

Proposed F_2 Genotype	Expected Ratio		Leaf Shape Classification				X^2	P
			total	broad	narrow			
LnlnLnrLnr	3 broad :1 narrow	Obs.	1303	1010	293		4.391	0.01-0.05
		Exp.	1303	977.25	325.75			
			total	broad	rugose			
LnLnLnrlnr	3 broad : 1 rugose	Obs.	626	529	98		29.402	<0.001
		Exp.	626	469.5	156.5			
	6.3 broad : 1 rugose	Exp.	626	540.25	85.75		1.983	0.2-0.3
			total	broad	narrow	rugose		
LnlnLnrlnr	9 broad : 3 narrow : 4 rugose	Obs.	955	648	170	135	68.401	<0.01
		Exp.	955	537.19	179.19	238.75		
	4.7 broad : 1.6 narrow : 1 rugose	Exp.	955	617.86	205.96	131.18	7.858	0.01-0.05
			total	narrow	rugose			
lnlnLnrlnr	3 narrow : 1 rugose	Obs.	496	426	70		31.355	<0.01
		Exp.	496	372	124			
	6.3 narrow : 1 rugose	Exp.	496	428.05	67.95		0.072	0.7-0.8

Table 12. Leaf shape scoring data of F_2 soybean progeny from soybean crosses with PI 522235, PI 507729, and PI 578357 using length by width ratio templates.

			Length x Width Ratio Range Classifications					
F_2 No.[1]	Pedigree	Total	1.5-2	2-2.5	2.5-3	3-3.5	3.5-4	>4
$00F_2$-401	Williams 82 x PI 522235	23	5	11	7			
$00F_2$-402	Williams 82 x PI 522235	39	10	20	6	2	1	
$00F_2$-403	Williams 82 x PI 522235	27	3	14	7	3		
	Total	89	18	45	20	5	1	
$00F_2$-570	Camp x PI 507729	121	7	28	31	25	25	5
$00F_2$-571	Camp x PI 522235	74	7	26	22	4	13	2
$00F_2$-572	Camp x PI 522235	91	22	38	18	7	6	
$00F_2$-573	Camp x PI 522235	128	27	52	27	10	11	1
$00F_2$-574	Camp x PI 522235	12	5	2	3	1	1	
	Total	305	61	118	70	22	31	3
$00F_2$-580	Williams 82 x PI 507729	216	44	109	49	9	5	
$00F_2$-581	Williams 82 x PI 578357	150	17	74	48	9	2	
$00F_2$-582	Williams 82 x PI 578357	124	12	69	33	7	3	
	Total	274	29	143	81	16	5	

Parents:

Name	Ratio Range
Williams 82	1.5-2
Camp	2.5-3
T41	2.5-3
PI 507729	3.5-4
PI 522235	>4
PI 578357	3.5-4

[1] The number assigned to the population used in data collection and analysis.

Table 13. Chi square evaluation of leaflet shape segregation in F_2 soybean progeny from Williams 82 x PI 522235 and Camp x PI 522235 crosses.

			Length x Width Ratio			X^2	DF	P
F_2 No.[1]	Pedigree	Total	1.5-2	2-2.5	≥2.5	1:2:1		
$00F_2$-401	Williams 82 x PI 522235	23	5	11	7	0.392	2	0.8-0.95
$00F_2$-402	Williams 82 x PI 522235	39	10	20	9	0.077	2	0.95-0.99
$00F_2$-403	Williams 82 x PI 522235	27	3	14	10	1.565	2	0.05-0.2
						2.034	6	0.8-0.95
	Total	89	18	45	26	1.450	2	0.3-0.5
	Heterogeneity					0.584	4	0.95-0.99
		Total	1.5-2.5	2.5-3	≥3	9:4:3		
$00F_2$-571	Camp x PI 522235	74	33	22	19	4.342	2	0.05-0.2
$00F_2$-572	Camp x PI 522235	91	60	18	13	3.476	2	0.05-0.2
$00F_2$-573	Camp x PI 522235	128	79	27	22	2.388	2	0.3-0.5
$00F_2$-574	Camp x PI 522235	12	5	2	3	0.009	2	0.8-0.95
						10.243	8	0.2-0.3
	Total	305	179	70	56	0.859	2	0.5-0.7
	Heterogeneity					9.384	6	0.05-0.2

[1] The number assigned to the population used in data collection and analysis.

Table 14. Chi square evaluation of leaflet shape segregation in F_2 soybean progeny from Williams 82 x PI 507729 and Camp x PI 507729 crosses.

			Length x Width Ratio			X^2	DF	P
F_2 No.[1]	Pedigree	Total	1.5-2	2-2.5	>2.5	1:2:1		
$00F_2$-580	Williams 82 x PI 507729	216	44	109	63	3.361	2	0.05-0.2
		Total	1.5-2.5	2.5-3	≥3	9:4:3		
$00F_2$-570	Camp x PI 507729	121	35	31	55	62.046	2	<0.01
				1.5-4	≥4	15:1		
				116	5	0.926	1	0.2-0.3

[1] The number assigned to the population used in data collection and analysis.

Table 15. Chi square evaluation of leaflet shape segregation in F_2 soybean progeny from the cross between Williams 82 and PI 578357.

			Length x Width Ratio			X^2	DF	P
F_2 No.[1]	Pedigree	Total	1.5-2	2-2.5	>2.5	1:2:1		
$00F_2$-581	Williams 82 x PI 578357	150	17	74	59	23.547	2	<0.01
$00F_2$-582	Williams 82 x PI 578357	124	12	69	43	17.080	2	<0.01
						40.627	4	<0.01
	Total	274	29	143	102	39.423	2	<0.01
	Heterogeneity					1.204	2	0.5-0.7
			1.5-3.5	3.5-4		15:1		
$00F_2$-581	Williams 82 x PI 578357	150	148	2		6.198	1	0.01-0.05
$00F_2$-582	Williams 82 x PI 578357	124	121	3		3.105	1	0.05-0.2
						9.303	2	<0.01
	Total	274	269	5		9.157	1	<0.01
	Heterogeneity					0.146	1	0.7-0.8
			1.5-3	3-4		15:1		
$00F_2$-581	Williams 82 x PI 578357	150	139	11		0.697	1	0.3-0.5
$00F_2$-582	Williams 82 x PI 578357	124	114	10		0.301	1	0.5-0.7
						0.998	2	0.5-0.7
	Total	274	253	21		0.935	1	0.3-0.5
	Heterogeneity					0.063	1	0.8-0.9

[1] The number assigned to the population used in data collection and analysis.

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Vita

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