EVALUATION OF NARROW AND BROAD LEAFLET ISOLINES OF SOYBEANS

by

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Chapter I

INTRODUCTION

Isolines developed by backcrossing have been commonly used to estimate the influence of a morphological or physiological trait on yield and other characters. The leaflet shape in soybean, Glycine max (L.) Merrill, has been one of the traits investigated with this technique to compare the performances of the broad or "normal" leaflet type and the narrow leaflet one in terms of seed yield and several other characters. The interest in the narrow leaflet type arises from its association with high number of seeds per pod and the distinct characteristics of its canopy. In previously reported studies, isolines were developed by backcrossing the narrow leaflet gene into broad leaflet cultivars (Arora, 1966; Hicks et al., 1969; Jeffers and Shibles, 1969; Hartwig and Edwards, 1970; Egli et al., 1970; Hiebsch et al., 1976; Waranyuwat, 1976; Jain and Singh, 1978).

This approach was criticized by Cooper (1975) who pointed out that the genetic background which complements the normal type may not complement the contrasting trait. He proposed that in order to evaluate the true worth of a specific morphological or physiological trait, we must evaluate each trait within a genetic background which maximizes that trait's potential value for increasing soybean yields.

The objective of this study was to evaluate the effects of leaflet type on seed yield and other agronomic and miscellaneous traits by using narrow and broad leaflet isolines. The goal of the breeding procedure employed was to develop pairs of isolines whose genetic backgrounds were a random recombination of the narrow and broad leaflet genotypes of their parents. This procedure permits comparisons of the leaflet types in a broader range of genetic backgrounds than is possible through use of the backcross method.

Chapter II

REVIEW OF LITERATURE

Most soybean cultivars are characterized as having plants with broad or ovate leaflets. However, a few of the cultivars in the U. S. (Payne, 1976) and a few from eastern Asia (Bernard and Weiss, 1973) are reported to have plants with a very distinct long, narrow, or lanceolate leaflet.

The first report on the inheritance of leaflet shape in soybean was by Takahashi and Fukuyama in 1919 (cited by Woodworth, 1932). Studying the hybrids between the normal (broad) and narrow types, they obtained in the F_2 generation a ratio of 1 broad:2 intermediate:1 narrow. Woodworth (1932) arrived at essentially similar results except that the F_2 generation segregated in a ratio of 3 broad:1 narrow. However, there were a few F_2 plants that seemed to be intermediate in leaf shape between the twc parents. He suggested the symbols Na and na for this character pair. Domingo (1945) observed that the NaNa and Nana genotypes exhibit normal leaflets and the nana genotype exhibits narrow leaflets. This observation substantiated earlier reports that narrow leaflet is monogenic recessive. As was summarized by Weiss (1970), the na gene transforms the normally ovate soybean leaflet to a lanceolate leaflet. The heterozygote is somewhat intermediate but more nearly resembles the NaNa ovate shape. Phenotypic distinction of Nana and NaNa is difficult among some plants and particularly in certain crosses.

The relationship of the narrow leaflet trait with a higher number of seeds per pod was observed by several authors. Takahashi (1934) assumed that each trait was due to a different gene and postulated a linkage between the genes r (narrow leaflet) and f (three-seeded pods, F being two-seeded), with about 10% crossing over. Domingo (1945) made the same assumption and asserted that the narrow leaflet gene (na) is closely linked with the gene for high number of seeds per pod, with approxiamtely 9% recombination. Johnson and Bernard (1963) called attention to the lack of proof of the existence of two gene pairs and postulated that the leaf and pod characteristics probably are pleiotropic effects of the same gene. This postulation was confirmed by Weiss (1970), who concluded in his studies that (1) the narrow leaflet gene, na exerts a major pleiotropic effect in stimulating the occurrence of four-seeded pods; (2) expression of the four-seeded character is favorably influenced by vigor of the plant; and (3) minor modifying genes may contribute to the frequency of four-seeded pods. Therefore, as was indicated by Bernard and Weiss (1973), Takahashi's genes r and f and Domingo's na and high seeds per pod genes are all considered to be the same gene, now symbolized as <u>ln</u>

An oval leaflet trait was also found by Domingo (1945), who proposed the symbols <u>O</u> and <u>o</u> for the gene pair controlling the normal and oval terminal leaflet, respectively. He reported this locus to be epistatic to the <u>Na</u>/<u>na</u> locus, resulting in the following genotypes: <u>Na-O-</u>, normal; <u>Na-oo</u>, oval; <u>nanaO-</u> and <u>nanaoo</u>, narrow. He also indicated the <u>o</u> gene to be linked with the gene for low number of seeds per pod, <u>lo</u>, with approximately 8% crossing over. <u>Lo</u> is considered intermediate in seeds per pod. Johnson and Bernard (1963) pointed out that Domingo did not consider whether the traits were due to the same gene. According to Weiss (1970), although these traits were not included in his studies, Domingo's report makes it seem likely that the oval leaflet gene has a pleiotropic effect in inducing few seeds per pod.

The fact that the narrow leaflet gene (<u>ln</u>) has a pleiotropic effect in stimulating high number of seeds per pod, plus theoretical considerations dealing with the canopy architecture, encouraged several plant breeders to introduce this trait into commercial cultivars in order to attempt to increase yield (American Society of Agronomy, 1969, 1971).

Sakamoto and Shaw (1967) suggested that an increase in yield could possibly be achieved by selecting cultivars whose natural inclination leads to deeper penetration of useful solar energy to a greater number of leaves. Shaw and Weber (1967) determined that a greater light penetration, resulting in a greater amount of plant canopy having light intensity above the leaf compensation point, generally resulted in greater yields. Actually, Johnston et al. (1969), working with 'Amsoy' and 'Wayne' cultivars, and Schou et al. (1978), working with 'Beeson', reported an average increase in seed yield per plant of 17 and 48%, respectively, when supplemental light was added to the plants. Bauer (1970) arrived at similar results by mechanical alteration of the canopy shape. Ogren (1975) summarized the information from other crops and concluded that modification of soybean canopy architecture might increase photosynthetic assimilation of CO_2 by permitting deeper penetration of sunlight into the canopy, therefore allowing a more even distribution of incident energy.

Arora (1966) found that narrow leaflet, oval leaflet, and five-leaflet isolines yielded less than the normal recurrent parents ('Harosoy' and 'Clark'), but the depression in yields was not great and not significant in general. He also found that the narrow leaflet trait was associated with a higher number of pods with three or more seeds, and concluded that the changing of number of seeds per pod had little effect on seed yield. Hicks et al. (1969), also growing Harosoy and Clark normal and narrow leaflet isolines, obtained no difference in yield between these two leaflet types, but the narrow leaflet lines had fewer pods per plant and more four-seeded pods than the other types. Hartwig and Edwards (1970) developed narrow leaflet isolines using a line closely related and very similar to the cultivar 'Lee' as the recurrent parent. These isolines had either a higher or a similar number of seeds per pod than the broad leaflet recurrent parent and had similar seed yields. Waranyuwat (1976) failed to confirm the hypothesis that narrow leaflet soybeans would yield higher than the normal leaflet type. His study used nearly isogenic lines of the Harosoy and Clark cultivars with both determinate and indeterminate stem types. The narrow leaflet characteristic appeared to be of no benefit in both cultivars and both termination types as compared to the normal leaflet shape. Jain and Singh (1978) observed no significant differences between narrow and normal leaflet isolines for yield and yield components in both the backgrounds (Harosoy and Clark) with which they worked.

Hicks et al. (1969) determined that more light penetrated into the canopies of the narrow leaflet type than the normal one. Egli et al. (1970) speculated that the higher apparent photosynthesis (AP) rate on a leaf area basis of the Harosoy narrow leaflet line may have been the result of better distribution of light throughout the canopy. Also, the amount of leaf area actively phtosynthesizing may have been the same for both types but, in the case of the narrow leaflet type, the amount would represent a higher percentage of the total leaf area thus giving a higher rate on a leaf area basis. Nevertheless, the lack of differences in the AP rate on a ground area basis between Harosoy normal and Harosoy narrow leaflet lines supports the data reported by Hicks et al. (1969) showing no differences in yield between the two isolines. On the other hand, Jeffers and Shibles (1969) estimated higher photosynthesis per unit of ground area in Harosoy narrow leaflet than in 'Harosoy-63', although they did not evaluate yield. Su et al. (1971) proposed that a small leaflet, non-branching type, or a narrow leaflet, medium branching type might be better in solar radiation penetration and yield than branching cultivars having a broad leaflet, especially when grown in close spacings. Hiebsch et al. (1976), growing 'Clark 63' and 'SRF 400' (two near isogenic lines) at various combinations of population and row spacing, concluded that leaflet type did not significantly affect photosynthesis or yield.

The influence of the narrow leaflet trait on other plant characteristics was also investigated. Arora (1966) observed that the narrow leaflet lines were about two days earlier and were taller than their recurrent parents. The narrow leaflet Clark also exhibited a significant increase in lodging. Waranyuwat (1976) also found that, in general, the narrow leaflet gene caused earlier maturity in both cultivars and termination types and decreased plant height in the determinate isolines. However, the narrow leaflet trait tended to decrease height and lodging in indeterminate Harosoy and increase height and lodging in indeterminate Clark isolines.

Arora (1966) found that the narrow leaflet trait generally caused an increase in oil percentage, though not always accompanied by a corresponding decrease in protein percentage. On the other hand, Hicks et al. (1969) obtained no differences in oil percentage between the leaflet types, but protein percentage was lower in the narrow leaflet type than in the normal one. Hartwig and Edwards (1970) revealed that a narrow leaflet isoline, also having a higher number of seeds per pod, was similar in oil and protein percentage to its recurrent parent. Similar results were also observed by Jain and Singh (1978).

In summary, the association of the narrow leaflet gene (*ln*) with high number of seeds per pod appears to be clearly demonstrated, but the expectation of further improvement in seed yield was not supported by the available literature. The side effects of this gene on agronomic characteristics and seed composition were not consistent over environments and genetic backgrounds.

Chapter III

MATERIALS AND METHODS

Field experiments of this study were conducted at the Eastern Virginia Research Station, Warsaw, Virginia, on a Sassafras sandy loam soil (Typic Hapludult, fine sandy, silicious, mesic) during 1976, 1977, and 1978.

Derivation of Isolines

A brief outline of the approach utilized to develop narrow and broad leaflet soybean isolines is as follows:

(a) In 1970, five crosses were made between five broad leaflet genotypes adapted to Virginia and a narrow leaflet line (D64-4731) from Mississippi (Table 1). The F_1 generation was grown in the greenhouse at Blacksburg, Virginia, during the following winter. The succeeding generations were advanced under field conditions at Warsaw, Virginia.

(b) In the F_4 generation of crosses 1 to 4, a range of five to ten broad leaflet single plants per cross were selected, and the progeny of each one was planted in a row the next year. In cross 5, this procedure was delayed until F_5.

Table 1. Pairs of isolines obtained from five crosses.

Cross number	Parentage	Generation composited F_6	F_7
		Pair number	
1	V67-1370 X D64-4731†	1	-
2	V67-1250 X D64-4731	2 to 7	11
3	V69-862 X D64-4731	8 & 9	-
4	D64-4731 X V66-318	10	12 to 14
5	D64-4731 X 'Hood'	-	15 & 16
Total number of pairs		10	6

† Narrow leaflet parent.

(c) In the F_5 generation, five to ten narrow and broad leaflet plants were individually selected within each segregating row and their progenies were grown in rows the next year. The non-segregating rows of both leaflet types were discarded.

(d) In the F_6 generation, a narrow leaflet progeny row and a non-segregating broad leaflet one, which traced to the same F_4 heterozygous single plant, were selected and considered a pair of isolines. The criterion employed to select and to pair rows, was to look for rows which were similar in height, maturity, etc., but which differed in leaflet shape. Selection for characteristics other than leaflet shape was merely to avoid obvious mismatches and should have minimal effect since the number of lines to choose from in each case was very small. In this way, ten pairs of isolines were obtained in F_6 (Table 1). Each selected row was harvested in bulk in order to get enough seed to start the evaluation in conventional plots in F_7 (1976).

(e) Within some F_6 segregating rows, once again narrow and broad leaflet plants were selected. Following the same procedure described previously, six additional pairs of isolines were obtained in F_7 (Table 1). The evaluation of these isolines started in F_8 (1977). The F_7 segregating rows were discarded.

In general, these isolines were of group V maturity and had the determinate growth type.

Field Procedures

Ten pairs of isolines were evaluated in field plots in 1976. Six additional pairs were included in 1977 and 1978. Planting dates were May 27, May 19, and May 30, respectively. Each entry was replicated three times using a split-plot design with pairs as main plots and leaflet types as sub-plots. Plots consisted of three rows, 6.10 m long, spaced 91 cm apart. Seeding rate was about 30 seeds per meter of row. Conventional cultural practices were applied to these experiments.

Data Collection

Data on maturity, plant height, lodging, seed size, and yield were collected from all plots in each year. Plots were considered mature when the pods were brown and most of the leaves had dropped, and maturity was expressed as days after August 31. Height was measured at maturity, and expressed as the average length of plants in centimeters from the ground to the extremity of the main stem. Lodging was rated on a 1 to 5 scale, with 1 being almost all plants erect and 5 all plants prostrate. Seed size was determined as the weight in grams of 100 seeds. Yield was estimated by harvesting a 5.03 m length from the mid-section of the center row of each plot. Actual seed weight was recorded after the seed of all entries had a uniform moisture content and was expressed in kilograms per hectare.

Plant samples were taken from six pairs of isolines in 1978, all of them from cross 2 (Table 1). Six plants per plot were sampled from a border row twice during the growing season - before flowering and at maturity.

(a) Plants sampled near flowering were separated into leaves and stems. Ten leaflets were randomly chosen and 50 discs were removed with a cork borer, comprising a total area of approximately .48 dm^2. The leaves, stems, and discs were dried and weighed. Leaf weight per plant (g) was obtained by expressing the leaf weight of the six plant sample on a per plant basis. Leaf area per plant (dm^2) was derived by relating the 50 disc weight and area to the leaf weight per plant. Specific leaf weight (SLW) was computed by expressing the 50 disc weight on a unit of leaf area basis (g/dm^2). Leaf:stem ratio is the result of dividing leaf weight by stem weight.

(b) Plants sampled at harvest were separated into stems and pods. Stems were weighed, pods were counted and threshed, and seeds were counted and weighed. Data were recorded for seed weight per plant (g), number of seeds per plant, number of pods per plant, and number of seeds per pod. Seed size was computed as before. Seed:stem ratio is the result of dividing seed weight by stem weight. Oil and protein percentages were determined with an infrared reflectance analyzer.

Statistical Analyses

The results were analyzed by standard split-plot analysis of variance. The L.S.D. test was used to compare leaflet type means within pairs of isolines (Steel and Torrie, 1960). Correlation coefficients among some traits were calculated using individual plot data. Combined analyses over years were not performed because the variances were not homogeneous for all traits.

Chapter IV

RESULTS AND DISCUSSION

YIELD

The average effect of the leaflet type on yield was non-significant in 1976 and 1977, when 10 and 16 pairs, respectively, of narrow and broad leaflet isolines were evaluated (Tables 2, 3, 4, and 5). In 1978, when these same 16 pairs were tested, the broad leaflet type significantly (P = .05) outyielded the narrow leaflet type (Tables 3 and 6). The differences among pairs of isolines were highly significant (P = .01) only in 1977, but the pair x isoline interaction was not significant in any year (Table 2), showing that the effects of isoline leaflet shape are consistent over pairs.

The existence of a significant difference in yield between the leaflet types in 1978 while there was no significant difference in 1976 or 1977 is some indication that different environments might favor one type over the other. However, a valid statistical test of the year x leaflet type interaction is not possible, as mentioned earlier.

These results indicate that the narrow leaflet isolines on the average are similar to the broad leaflet ones in

Table 2. Mean squares from the analyses of variance for yield and agronomic traits of narrow and braod leaflet isolines.

Year	Source	d.f.	Mean squares				
			Yield (kg/ha)	Maturity (days after 8/31)	Height (cm)	Lodging (1 to 5)	Seed size (g/100 seeds)
1976	Pair (P)	9	91507.43	75.18 **	101.74 **	.0053	10.30 **
	Error (a)	18	38266.77	1.71	22.86	.0025	.65
	Isoline (I)	1	9866.07	26.67 **	34.84 **	.0082	25.35 **
	P x I	9	23642.81	2.96 **	5.21	.0034	1.35 **
	Error (b)	20	13746.66	.73	3.33	.0028	.15
1977	Pair (P)	15	232610.91 **	20.96 **	310.95 **	.1720 **	12.17 **
	Error (a)	30	37571.61	1.21	15.45	.0172	.78
	Isoline (I)	1	3771.33	.04	130.11 **	.8067 **	20.44 **
	P x I	15	40692.87	.29	7.38	.0716 **	.34
	Error (b)	32	27192.90	.36	7.19	.0147	.32
1978	Pair (P)	15	83110.21	169.87 **	395.77 **	.6457 **	8.63 **
	Error (a)	30	67548.75	4.09	36.24	.0870	.42
	Isoline (I)	1	40541.04 *	66.67 **	210.75 **	.0104	15.20 **
	P x I	15	18240.22	15.27 **	39.43 *	.1282 **	.67 **
	Error (b)	32	9645.73	3.48	17.27	.0483	.13

*,** Significant difference at 5 and 1% levels of probability, respectively.

Table 3. Mean perfomances for yield and agronomic traits of narrow (N) and broad (B) leaflet isolines.

Year	Yield (kg/ha)		Maturity (days after 8/31)		Height (cm)		Lodging (1 to 5)		Seed size (g/100 seeds)	
	N	B	N	B	N	B	N	B	N	B
1976	2265	2239	48.4 **	49.7	65.2 **	66.7	1.01	1.04	15.3 **	16.6
1977	2620	2632	53.8	53.9	65.0 **	67.4	1.07 **	1.25	17.4 **	18.3
1978	2431 *	2473	43.2 **	44.9	78.0 **	81.0	1.48	1.46	13.2 **	14.0
Average†	2439	2488	48.5	49.5	69.4	71.7	1.19	1.25	15.3	16.3

*,** Significant difference between narrow and broad leaflet isolines at 5 and 1% levels of probability respectively.

† Combined analyses over years were not performed.

Table 4. Comparisons of means for yield and agronomic traits of narrow (N) and broad (B) leaflet isolines in 1976.

Pair number	Yields (kg/ha) N	Yields (kg/ha) B	Maturity (days after 8/31) N	Maturity (days after 8/31) B	Height (cm) N	Height (cm) B	Lodging (1 to 5) N	Lodging (1 to 5) B	Seed size (g/100 seeds) N	Seed size (g/100 seeds) B
1	2351 **	2058	55.0	55.3	66.9	66.0	1.00	1.00	17.7 **	18.9
2	2463	2463	49.0	50.3	62.7	61.8	1.06	1.00	16.5	17.1
3	2469	2325	48.7	48.3	65.2	66.0	1.00	1.00	14.6 **	16.1
4	2269	2233	47.7 **	49.7	63.5 *	66.9	1.00	1.07	14.7 **	16.7
5	2275	2298	47.3 **	49.3	63.5 *	67.7	1.03 *	1.13	15.9 **	17.7
6	2367	2302	48.3 **	52.3	62.7	63.5	1.00	1.03	16.2 **	17.8
7	2094	2058	47.7	48.0	58.4	59.3	1.00	1.00	15.0 **	16.4
8	2131	2282	49.3	49.3	72.0	73.7	1.03	1.10	14.2 *	14.9
9	2062	2111	50.0 **	53.0	68.6	69.4	1.00	1.03	16.1	15.5
10	2170	2265	40.7	41.3	68.6 *	72.8	1.00	1.00	12.3 **	15.2
Average	2265	2239	48.4 **	49.7	65.2 **	66.7	1.01	1.04	15.3 **	16.6

*,** Significant difference between narrow and broad leaflet isolines at 5 and 1% levels of probability, respectively.

Table 5. Comparisons of means for yield and agronomic traits of narrow (N) and broad (B) leaflet isolines in 1977.

Pair number	Yield (kg/ha)		Maturity (days after 8/31)		Height (cm)		Lodging (1 to 5)		Seed size (g/100 seeds)	
	N	B	N	B	N	B	N	B	N	B
1	2522	2722	56.7	57.3	62.7	64.3	1.00	0.00	19.1 **	20.5
2	2706	2667	54.0	54.0	60.1	61.8	1.00	1.10	18.6	19.2
3	2897	2709	54.0	54.0	61.0	61.8	1.00	1.10	16.6	17.5
4	2749	2841	54.0	54.7	61.8	61.8	1.00 **	1.43	16.5 **	17.9
5	2690	2542	54.0	54.0	61.8 *	67.7	1.10 **	1.80	17.4	18.1
6	2722	2834	54.0	54.3	63.5	65.2	1.00	1.2-	18.8	18.5
7	2887 *	2535	54.0	54.0	63.5 *	69.4	1.20	1.30	16.4 *	17.5
8	2584	2492	54.0	54.0	62.7	63.5	1.00	1.00	17.6 *	18.7
9	2476	2532	54.0	52.2	63.5 *	68.6	1.00	1.10	18.0	18.2
10	2134	2173	49.3	48.7	77.0	77.0	1.00 **	1.47	15.4 **	16.7
11	2690	2640	54.0	54.0	61.0	65.2	1.00	1.00	16.1 **	17.4
12	2265	2325	52.3	51.7	72.8 *	77.9	1.20 **	1.63	17.1	17.7
13	2676	2667	54.0	54.3	60.1	59.3	1.00	1.00	18.0 *	19.2
14	2292 *	2657	51.3	52.0	75.4	76.2	1.27	1.27	15.8	16.5
15	2745	2851	56.0	56.0	54.2	55.9	1.00	1.00	16.2 **	17.6
16	2877	2926	55.7	55.7	79.6	82.1	1.30 **	1.60	21.2	22.0
Average	2620	2632	53.8	53.9	65.0 **	67.4	1.07 **	1.25	17.4 **	18.3

*,** Significant difference between narrow and broad leaflet isolines at 5 and 1% levels of probability, respectively.

Table 6. Comparisons of means for yield and agronomic traits of narrow (N) and broad (B) leaflet isolines in 1978.

Pair number	Yield (kg/ha)		Maturity (days after 8/31)		Height (cm)		Lodging (1 to 5)		Seed size (g/100 seeds)	
	N	B	N	B	N	B	N	B	N	B
1	2269	2265	50.7	51.3	82.1	86.4	1.37	1.40	15.4 **	16.4
2	2644	2676	44.3	44.3	72.0	66.9	1.30	1.33	13.6	13.6
3	2525	2519	44.0	45.3	72.8	74.5	1.13	1.10	11.9 **	13.1
4	2436	2519	44.3	45.7	69.4	75.4	1.23	1.37	12.0 **	13.0
5	2509	2512	44.7	46.0	77.0	76.2	1.63	1.57	13.5 **	14.9
6	2479	2525	44.7	46.7	71.1	76.2	1.27	1.60	14.2	14.0
7	2568	2443	44.0	45.7	74.5	73.7	1.27	1.33	12.8 **	13.8
8	2045 **	2354	47.0	47.3	79.6 *	87.2	1.23	1.47	12.4 *	13.0
9	2351 **	2591	46.0	47.3	81.3 *	89.7	1.20	1.40	12.5 *	13.2
10	2344	2404	30.3 **	37.0	80.4 *	88.9	1.53	1.37	11.6 **	13.9
11	2528	2653	45.7	46.3	81.3	79.6	1.63	1.40	12.6 *	13.3
12	2453	2446	32.3	31.3	82.1	84.7	3.07 **	2.17	13.2 **	14.3
13	2558	2466	46.0	45.7	81.3	75.4	1.47	1.37	13.8 *	13.2
14	2463	2492	30.7 **	42.3	87.2 **	98.2	1.53	1.53	12.4	12.9
15	2334	2292	48.3	47.7	65.2	64.3	1.47	1.27	12.4 **	13.6
16	2397	2400	48.3	48.0	90.6 *	98.2	1.37	1.70	16.2 **	17.1
Average	2431 *	2473	43.2 **	44.9	78.0 **	81.0	1.48	1.46	13.2 **	14.0

*,** Significant difference between narrow and broad leaflet isolines at 5 and 1% levels of probability respectively.

terms of seed yield. As was outlined previously, the genetic backgrounds are random recombinations of the genotypes of the narrow and broad leaflet parents. In other words, the purpose of the breeding procedure employed in this study was not to favor in any way either leaflet type by providing it with a better genetic "envircnment". It could be argued that perhaps none of these random genetic backgrounds actually permitted the expression of the potential value of the ln gene for increasing yield. Indeed, the narrow leaflet parent (D64-4731) used in all five crosses is not one of the original types, but was obtained through several crosses involving twc broad leaflet cultivars (Lee and Clark), and the narrow leaflet source, T109. The pedigree of D64-4731 is Lee 2*//Clark 2*/T109. Therefore, it might be hypothesized that a portion of the genes complementary to ln were lost due to segregaton. Consequently, in spite of the breeding procedure employed in this study, the narrow leaflet gene might be in disadvantageous or non-complementary backgrounds, which in turn would not allow the evaluation of its true worth (Cooper, 1975). On the other hand, preliminary results seemed to indicate that the narrow leaflet type, T109 (introduced from Korea), does not have a good yield potential and probably is not well adapted (Bernard and

Creemens, 1969). What is clear from these results is the fact that the simple procedure of incorporating the <u>ln</u> gene into any genotype does not necessarily assure by itself an improvement in seed yield. It can further be suggested that the leaflet type should not be a criterion <u>per se</u> in selecting lines or choosing a cultivar. Only a careful evaulation of yield compared to adapted check cultivars can ultimately determine the real value of any line.

In general, these findings are supported by those previously reported by Arora (1966), Hicks et al. (1969), Hartwig and Edwards (1970), Hiebsch et al. (1976), Waranyuwat (1976), and Jain and Singh (1978), though in all these cases the backcross method was used to develop isolines.

<u>AGRONOMIC TRAITS</u>

Differences in maturity date between narrow and broad leaflet isolines were highly significant in 1976 and 1978, but not in 1977 (Table 2). The weather conditions during the growing season of 1977 prevented a clear expression of this trait, making the data less reliable. The narrow leaflet isolines were earlier each year, but the greatest average difference was only 1.7 days (Tables 3, 4, 5, and 6). A few of the within-pair comparisons were significant in 1976 and 1978 but the pairs were not consistent over years. These data agree with those reported earlier by Arora (1966) and Waranyuwat (1976).

Highly significant differences in plant height between the leaflet shape isolines were observed in all three years (Table 2). The narrow leaflet isolines were consistently 1.5 to 3.0 cm shorter (Tables 3, 4, 5, and 6). Waranyuwat (1976) arrived at similar results in both termination types of Harosoy isolines and in the determinate type of Clark isolines. The significant pair x isoline interaction in 1978 appears to be due to several pairs in which the narrow leaflet isoline was taller than the broad leaflet one, although none of these within-pair differences was significant.

Tables 3, 4, 5, and 6 show that the average difference in lodging score between leaflet types was very small in all three years. Only in 1977 was this difference highly significant (Table 2), with the narrow leaflet type having a slightly lower lodging score than the broad leaflet one (Tables 3 and 5).

According to several authors, lateness, tallness, and high yield seem to be associated (Anand and Torrie, 1963; Johnson and Bernard, 1963; Kwon and Torrie, 1964). This association could explain the 1978 yield results, in which the largest differences in maturity and height were also observed, favoring the broad leaflet isolines (Tables 3 and 6). The differences in maturity, height and lodging by themselves, in spite of being statistically significant in several cases, do not appear to indicate any practical advantage of one leaflet type over the other. The interactions between pairs and isoline types were also statistically significant in several instances, though they were not consistent over years (Table 2). Also, the interaction mean square is considerably smaller than the isoline mean square in most cases. Consequently, in terms of breeding, these interactions do not appear to be of any practical consequence.

YIELD COMPONENTS

Table 7 shows the results of the statistical analyses of yield components sampled in six pairs in 1978. It was assumed that the plots did not differ in terms of plant population; therefore, no attempt was made to sample the number of plants per unit of area. Yield data from the whole plots of these six pairs were also analyzed.

Averaged over the six pairs, the broad and narrow leaflet isolines did not differ in yield as measured by whole plots

Table 7. Mean squares from the analyses of variance for yield and yield components of six narrow and broad leaflet isoline pairs in 1978.

		Mean squares					
Source	d.f.	Yield (kg/ha)	Seed wt/plant (g)	Seeds/plant	Seed size (g/100 seeds)	Pods/plant	Seeds/pod
Pair (P)	5	25670.05	8.01	304.87	2.99 *	71.39	.0207
Error (a)	10	53180.39	9.59	469.29	.82	82.97	.0223
Isoline (I)	1	327.03	5.29	14.06	3.46 **	35.34	.0693
P x I	5	7715.23	.40	50.02	.64	6.38	.0066
Error (b)	12	6427.11	2.87	161.71	.34	37.14	.0166

*,** Significant difference at 5 and 1% levels of probability, respectively.

or the sampled plants (Tables 7 and 8). In general, the sampled plants showed the same yield rank as the yield of the whole plots, strengthening the assumpticn of uniform stands.

In order to analyze these data further, yield per plant can be divided into number of seeds per plant and seed size. The leaflet shape did not affect the number of seeds per plant but it had a highly significant effect on seed size. Table 8 shows that the broad leaflet type had bigger seeds than the narrow leaflet one, these results being consistent with those indicated in Tables 4, 5, and 6. These data suggest that the advantage of the broad leaflet strains in terms of seed size, while being statistically significant, was not great enough to produce a significant superiority in yield. The number of seeds per plant can be separated into number of pods per plant and number of seeds per pod (Table 8). The leaflet types did not differ significantly in number of pods per plant although the broad leaflet lines were somewhat higher. The narrow leaflet isolines were significantly higher in number of seeds per pod, though at the 10% level of probability. This coincides with the higher number of three and four-seeded pods associated with the narrow leaflet trait. Thus the higher number of seeds per pod of the narrow leaflet isolines appears to be

Table 8. Comparisons of means for yield components of six narrow (N) and broad (B) leaflet isoline pairs in 1978.

Pair number	Yield (kg/ha)		Seed wt/plant (g)		Seeds/plant		Seed size (g/100 seeds)			Pods/plant		Seeds/pod	
	N	B	N	B	N	B	N		B	N	B	N	B
2	2644	2676	12.7	14.1	94.0	102.4	13.5		13.7	39.8	45.2	2.4	2.3
3	2525	2519	10.5	11.3	87.6	90.1	12.0		12.6	37.4	38.6	2.3	2.3
4	2436	2519	11.4	12.0	93.3	86.1	12.1	**	14.0	38.0	37.6	2.5	2.3
5	2509	2512	13.3	14.1	95.8	97.3	13.9		14.5	38.5	39.5	2.5	2.5
6	2479	2528	11.2	12.2	81.6	87.4	13.7		14.0	33.0	36.5	2.5	2.4
7	2568	2443	13.4	13.2	105.5	102.1	12.8		13.0	43.6	44.9	2.4	2.3
Avg.	2527	2533	12.1	12.8	93.0	94.2	13.0	**	13.6	38.4	40.4	2.4	2.3

*,** Significant difference between narrow and braod leaflet isolines at 1% level of probability.

counteracted by a lower number of pods per plant. No interactions between pairs and isoline types were observed for yield and its components.

Although these results refer to only six pairs tested in only one year, they suggest a plausible explanation for the general absence of a significant effect of the leaflet shape on yield. With regard to the 1978 whole test (16 pairs), the yield advantage of the broad leaflet isolines, in spite of being significant, was less than 2%. Averaged over three years the difference is considerably less than 1% (Table 3).

Arora (1966), Hicks et al. (1969), and Hartwig and Edwards (1970) also detected differences in some yield components but a significant difference in yield was not observed in any of the studies. As Cooper (1975) commented, there tends to be a strong compensatory relationship between the yield components which results in little net gain from selection for any one trait.

In summary, these results suggest that the influence of the _Ln_/_ln_ locus on the yield components is negligible from the point of view of their further consequence on yield. The pleiotropic effect of the leaflet shape on the number of seed per pod is not strongly expressed in these data.

The differences in seed size between brcad and narrow leaflet isolines were consistent over the three years of testing, with the broad leaflet ones being bigger, though the range of differences from .8 to 1.3 g per 100 seeds does not suggest any practical implication (Tables 3, 4, 5, and 6). The pair x isoline interactions were highly significant in two of the years, but appear to be of little consequence since the within-pair differences were not consistent over years (Tables 4, 5, and 6).

SEED COMPOSITION

Tables 9 and 10 indicate that the average effect of the leaflet shape on oil and protein percentage was non-significant. The respective pair x isoline interactions were not significant either. Once again these data refer to only six pairs evaluated in 1978; consequently, they suggest rather than prove a tendency. Hicks et al. (1969) reported no differences in oil percentage between broad and narrow leaflet isolines, whereas Hartwig and Edwards (1970), and Jain and Singh (1978) found no differences in either oil or protein percentage between the same kind of isolines. Apparently, the seed composition does not offer any reason to select either leaflet type over the other.

Table 9. Mean squares from the analyses of variance for seed composition of six narrow and broad leaflet isoline pairs in 1978.

Source	d.f.	Mean squares Oil (%)	Protein (%)
Pair (P)	5	.3167	.8724
Error (a)	10	.2700	.6509
Isoline (I)	1	.0011	.1111
P x I	5	.2544	.7124
Effor (b)	12	.1639	.5747

Table 10. Comparisons of means for seed composition of six narrow (N) and broad (B) leaflet isoline pairs in 1978.

Pair number	Oil %		Protein %	
	N	B	N	B
2	22.2	21.7	38.8	39.2
3	21.4	21.5	38.9	38.3
4	21.5	21.4	38.7	39.2
5	22.0	21.6	38.4	38.9
6	21.1	21.6	40.1	39.3
7	21.5	21.9	39.2	38.4
Average	21.6	21.6	39.0	38.9

MISCELLANEOUS TRAITS

Several other traits were measured in an attempt to further characterize these two sorts of isolines. Data were collected in 1978 from the same six pairs mentioned previously.

Leaf area per plant varied significantly with the leaflet shape, the narrow type having less area than the broad one (Tables 11 and 12). Jain and Singh (1978) arrived at similar conclusions and they speculated that the reduction in leaf size may be desirable from an agronomic standpoint as it would be possible to increase plant population per unit area. If it is assumed that there were no differences in plant population, leaf area per plant might be considered as an expression of leaf area index (LAI). From this point of view, these results would coincide with those revealed by Hicks et al. (1969), who found the normal type surpassing the LAI of the narrow leaflet one. The leaves of the two sorts of isolines did not differ significantly in specific leaf weight (SLW), although the narrow leaflet isolines tended to be slightly higher (Tables 11 and 12). It appears that the higher SLW of the narrow leaflet isolines might compensate for their smaller leaf area. Indeed, Egli et al. (1970) reported a higher apparent photosynthesis rate on a leaf area basis in Harosoy narrow leaflet compared to the

Table 11. Mean squares from the analyses of variance for miscellaneous traits of six narrow and broad leaflet isoline pairs in 1978.

Source	d.f.	Mean squares: Leaf area/plant (dm^2)	SLW (g/dm^2)	Leaf wt/plant (g)	Leaf:stem ratio	Seed:stem ratio
Pair (P)	5	22.40	.0006	2.07	.0109	.0297
Error (a)	10	19.01	.0005	2.72	.0215	.0647
Isoline (I)	1	84.74 *	.0038	5.50 *	.3280 **	.1237 **
P x I	5	5.93	.0005	.86	.0564	.0155 *
Error (b)	12	9.15	.0009	.72	.0331	.0056

*,** Significant difference at 5 and 1% levels of probability, respectively.

Table 12. Comparisons of means for miscellaneous traits of six narrow (N) and broad (B) leaflet isoline pairs in 1978.

Pair number	Leaf area/plant (dm^2) N		B	SLW (g/dm^2) N	B	Leaf wt/plant (g) N		B	Leaf:stem ratio N		B	Seed:stem ratio N		B
2	19.2		22.4	.35	.35	6.68		7.86	2.54		2.46	1.68		1.56
3	17.1		16.5	.37	.35	6.45		5.82	2.37		2.51	1.53		1.51
4	14.0		16.5	.39	.36	5.29		5.98	2.36		2.51	1.71		1.59
5	15.8		20.9	.37	.33	5.79		6.96	2.31	*	2.68	1.79	**	1.49
6	14.5		18.6	.38	.34	5.44		6.21	2.29	*	2.74	1.55		1.54
7	14.6		18.7	.35	.35	5.14	*	6.67	2.48		2.61	1.77		1.65
Average	15.9	*	18.9	.37	.35	5.80	*	6.58	2.39	**	2.58	1.67	**	1.56

*,** Significant difference between narrow and broad leaflet isolines at 5 and 1% levels of probability, respectively.

normal cultivar. The significantly greater dry matter in the leaves of the broad leaflet plants (Tables 11 and 12) did not appear to be related to yield potential, as is indicated by the fact that there were actually no differences in yield between leaflet types in the sampled pairs (Table 7). In 1978, several cultivars and lines were tested at Blacksburg, including pairs 7 and 10 from this study (G. R. Buss, unpublished data). Leaf area per plant, leaf weight per plant, and SLW were recorded on four sampling dates during the growing season. Similar results were obtained in each sampling date; leaf area and leaf weight per plant were higher in the broad leaflet isolines, and SLW tended to be a bit higher in the narrow leaflet ones. Thus the higher SLW of the narrow leaflet lines appears to be real, but difficult to prove statistically.

The ratio of leaf weight to stem weight was significantly higher in the broad leaflet isolines, indicating that a larger proportion of photosynthesis products was diverted to leaf rather than to stem formation during the vegetative stage of growth (Tables 11 and 12). A high leaf to stem ratio might be considered advantageous, but it is not evident from these data.

The narrow leaflet isolines exhibited significantly higher seed weight to stem ratio, suggesting that proportionately more photosynthate was converted to seed than to stems (Tables 11 and 12). Seed to stem ratio was the only miscellaneous trait showing a significant pair x isoline interaction, which was the result of the advantage of the narrow leaflet strains varying in magnitude among pairs of isolines.

The differences in these traits are, in some sense, an expression of "superiority" or "inferiority" in certain physiological processes, though the absence of difference in yield seems to be the consequence of a kind of balance among them. The lack of relationship of yield with any of these traits is shown by the low correlation coefficients observed between them and seed weight per plant (Table 13). As Ogren and Rinne (1973) concluded, if photosynthetic productivity can be increased, photosynthate utilization and distribution will have to be tailored to efficiently use the increased photosynthate supply.

Table 13. Correlation coefficients between seed weight per plant and several miscellaneous traits obtained from six pairs of isolines in 1978.

Trait correlated with seed weight/plant	Correlation coefficient (r)
Leaf area/plant (dm^2)	.31
Specific leaf weight (g/dm^2)	-.14
Leaf weight/plant (g)	.29
Leaf:stem ratio	.29
Seed:stem ratio	.41 *

* Significant at 5% level of probability.

Chapter V

SUMMARY AND CONCLUSIONS

Narrow and broad leaflet isolines were used to investigate the effect of leaflet shape in soybean. Crosses were made between five broad leaflet genotypes adapted to Virginia and a narrow leaflet line from Mississippi (D64-4731). Narrow and broad leaflet plants were selected within the progeny of heterozygous broad leaflet F_4 and F_5 single plants. In the next generation, a narrow leaflet progeny row and a non-segregating broad one, which traced to the same F_4 and F_5 single plant, were selected and considered a pair of isolines. Therefore, the genetic background of each pair was a random recombination of the narrow and broad leaflet genotypes of the parents. All isolines were about group V maturity and had the determinate growth type.

Ten pairs of isolines were evaluated in field plots in 1976 and these same ten pairs plus six additional ones were evaluated in 1977 and 1978. A split-plot design was used with pairs as main plot and leaflet type as sub-plots.

Data were collected on seed yield, maturity, plant height, lodging, and seed size from all plots in each year. In 1978, plants were sampled near flowering and at maturity from six pairs of isolines. Data recorded from samples taken near flowering included leaf area per plant, specific leaf weight (SLW), leaf weight per plant, and leaf:stem ratio. From the samples taken at maturity, data for yield components, seed composition, and seed:stem ratio were obtained.

No significant differences in seed yield were observed between narrow and broad leaflet isolines in 1976 and 1977. In 1978 the broad leaflet types performed significantly better, though their yield advantage was less than 2%. The yield difference over three years was less than 1%. The lack of a significant pair x isoline interaction in any year indicates that the relative yields of narrow and broad leaflet isolines are similar regardless of genetic background. These results revealed that the narrow leaflet isolines are similar in seed yield compared to the broad leaflet ones. There was some indication that undefined environmental factors might favor one leaflet type over another in certain years.

The narrow leaflet isolines were significantly earlier than the broad leaflet ones in 1976 and 1978, but not in 1977. They also were significantly shorter in all three years. Only in 1977 was the difference in lodging significant, the narrow leaflet type having slightly less lodging. But the magnitude of the differences in these agronomic traits, in spite of being statistically significant in several cases, does not imply any advantage of one leaflet type over the other from a practical point of view. The pair x isoline interactions were significant in several instances, though not consistent over years.

In six pairs sampled in 1978, the effects of the leaflet shape on number of seeds per plant and number of pods per plant were non-significant. The narrow leaflet isolines showed a tendency for a higher number of seeds per pod, significant at the 10% level. The narrow leaflet isolines had significantly smaller seeds, as was found in each year of testing. Nonetheless, there were no significant differences in yield between leaflet types, either measured by whole plots or the sampled plants. It appears that, even though the Ln/ln locus has a significant effect on some yield components, there is a compensation by other components to produce a negligible effect on yield.

Broad and narrow leaflet isolines of these six pairs did not differ in terms of oil and protein percentage.

Data on several miscellaneous traits were measured in 1978 on the same six pairs. The narrow leaflet isolines exhibited significantly less leaf area per plant, less leaf weight per plant, and lower leaf:stem ratio than the broad leaflet ones. They did not differ significantly in SLW, although the narrow leaflet lines tended to have higher SLW. The narrow leaflet strains showed higher seed:stem ratios. All these miscellaneous traits showed low correlation coefficients with yield per plant.

The results of these experiments suggest some further research. One planting date was used each year, and the same seeding rate and row spacing were used throughout this study. The interactions of the leaflet shape with these and other cultural practices appear to merit investigation.

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EVALUATION OF NARROW AND BROAD LEAFLET ISOLINES OF SOYBEANS

by

Francisco A. Mandl

(ABSTRACT)

This study was conducted to evaluate the effect of leaflet shape on yield and other characters of soybeans, *Glycine max*. (L.) Merrill, by using narrow and broad leaflet isolines. The breeding procedure employed developed pairs of isolines whose genetic backgrounds were a random recombination of the narrow and broad leaflet genotypes of their parents.

Results obtained from 1976 to 1978 indicated that the narrow leaflet isolines on the average are similar to the broad leaflet ones in seed yield. The narrow leaflet isolines were earlier, but the average difference was no more than 1.7 days. They also were consistently 1.5 to 3.0 cm shorter in all three years. The difference in lodging between leaflet types was always very small. The differences in these agronomic traits, though significant in several instances, do not imply any practical advantage of either leaflet type.

Data from six pairs of isolines sampled in 1978 showed that leaflet shape had a significant effect on seed size,

but not on seeds per plant, pods per plant, and seeds per pod. Narrow and broad leaflet isolines showed similar oil and protein contents.

The narrow leaflet isolines of the same six pairs exhibited less leaf area per plant, less leaf weight per plant, lower leaf:stem ratio, and higher seed:stem ratio than the broad leaflet ones. The difference in specific leaf weight was non-significant. None of these traits was associated with yield per plant.