Electrophoretic Differences in Seed Proteins Among Varieties of Soybean, *Glycine max* (L.) Merrill[1]

Arnold L. Larsen[2]

ABSTRACT

Seed proteins of 61 soybean varieties were analyzed by disc electrophoresis. The stained proteins in the polyacrylamide gels revealed two components that separated the varieties into two major groups. Component "A" was present in 13 varieties, and component "B" was present in 48 varieties. In no instance were A and B observed in a single variety. Repeated tests with seed from various environments produced no exceptions to the varietal grouping with respect to these two proteins.

Additional index words: **genetics, protein extraction, disc-electrophoresis, polyacrylamide, hilum color.**

THIS study was initiated to determine if differences in seed proteins exist which might be used to supplement morphological characters for identifying soybean varieties.

Stahmann (6), in a review of plant proteins, discussed uses, properties, and techniques of studying the proteins of soybean seed. Briggs and Mann (1) demonstrated the analytical advantages of electrophoresis in characterizing the proteins of soybeans. They found that preparations called "glycinin" were mixtures of components whose composition ratios were not identical among the various preparations reported.

Hilty and Schmitthenner (3) used starch-gel and acrylamide-gel electrophoresis to compare leaf proteins prepared from 'Harosoy' and 'Harosoy 63' soybean varieties; separation of proteins was most complete with acrylamide-gel electrophoresis. However, no differences were noted in the protein composition between the two varieties.

Using DEAE-cellulose column chromatography to study the protein content of five varieties of soybeans, Knox, et al. (4) found that protein patterns of Harosoy, 'Lindarin' and 'Hawkeye' were somewhat similar, whereas the patterns for the whey proteins of 'Adams' and 'L59g-3R' differed radically. They further concluded that the Adams variety contained whey proteins not found in other varieties. Morrison[3] found that paper-strip and starch-gel electrophoresis were not effective in establishing differences among the five varieties and that DEAE-cellulose chromatography was a better analytical tool for this purpose.

Schwartz (5), using starch-gel electrophoresis on mutant enzymes extracted from maize seeds, detected several distinctive types of enzymes that occurred singly and in combination in various inbred lines and their hybrids. Tombs (7) used both agar-gel and acrylamide-gel electrophoresis to identify five classes of peanut seed containing varying ratios of arachin A and B; these classes were apparently variations among seeds within a single seed lot.

[1] Contribution from the Market Quality Research Division, ARS, USDA. Received Dec. 21, 1966.

[2] Research Botanist, FCAP, Seed Research Laboratory, Beltsville, Maryland.

[3] Morrison, Joseph Louis. 1962. Column chromatography of soybean whey proteins. Ph.D. Thesis. Lafayette, Ind., Library, Purdue University.

MATERIALS AND METHODS

Seed samples of 64 soybean varieties representing diverse locations and several years, were collected from the entire soybean-production area of the United States and Canada.

A 5-g seed sample of each variety was ground finely in a small grinder-mixer and then mixed with 20 ml of 0.1 N acetate buffer[4] (pH4.8). After an overnight extraction at 4 C, the gruel was centrifuged at 30,000 ×g and O C for 30 minutes, the pellet discarded and the supernatant adjusted to pH 8.0 with 0.1 N KOH. The solution was refrigerated for 1 hour and then centrifuged at 15,000 ×g and O C for 15 minutes. The final supernatant was a light-yellow, clear solution containing approximately 10 to 13 mg protein per ml.

Electrophoretic analyses were conducted on the prepared protein as described by Davis (2), except that the "spacer" and "sample" gels were not used. The electrophoretic cell (Fig. 1) was constructed to accommodate eight glass tubes. Before attachement, the tubes were stoppered at their lower ends and filled with the acrylamide solution. After polymerization, the stoppers were removed and the tubes positioned into the cell at the lower side of the upper buffer pan. TRIS-glycine buffer (pH 8.3) was poured into the upper and lower pans so that both exposed ends of the polyacrylamide gel contacted the buffer. Fifty microliters of a mixture containing 4 parts protein solution and 1 part 2 M sucrose solution were layered carefully between the top surface of the gel and the buffer solution. Electrodes from a DC power supply where then attached to the cell, the positive electrode to the lower pole. The protein migrated toward the positive pole in an electrical field of 5 milliamps per tube.

After 1 hour, the gels were removed from the tubes and the migrated proteins were stained overnight with aniline blue-black. Following staining, the unfixed dye was removed by electrophoresis.

RESULTS

The banding patterns were highly reproducible following standardization of the protein extraction and electrophoretic procedures. The pattern was uniform within each variety and was not affected by lot differences, year produced, location grown, or electrophoretic run (Fig. 2). In each gel, about 21 bands were visible. The general intensity of staining and spacial arrangement of the bands were similar for the proteins of all varieties.

The main difference found among soybean varieties was the presence of two proteins which separated

[4] The extractant was an equal mixture of 0.1 N soduim acetate and 0.1 N acetic acid.

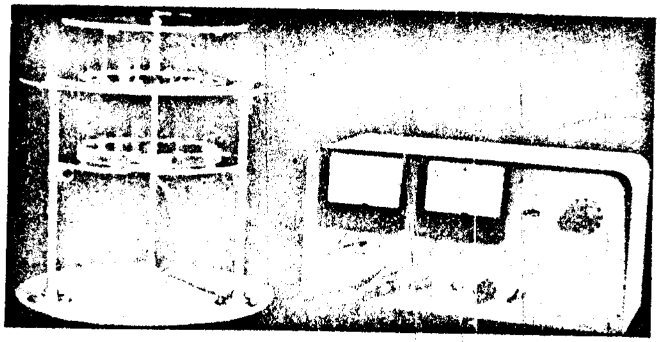

Figure 1. Electrophoresis cell and power supply.

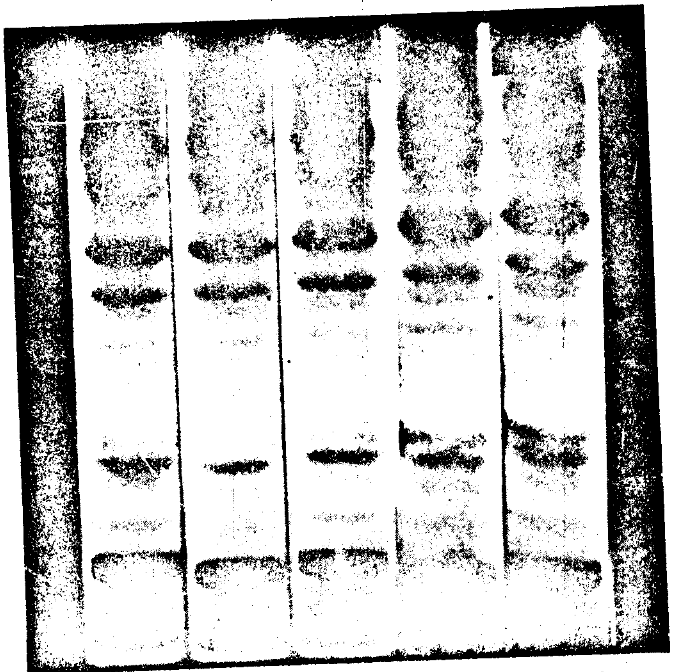

Figure 2. Polyacrylamide gels illustrating uniformity in protein-banding patterns. Gels represent 5 different seed lots of Lindarin 63 soybeans.

Figure 3. Banding patterns of seed protein from 6 soybean varieties. From left to right: Acme, Comet and Harosoy 63 display the A protein whereas Hawkeye, Ross and Shelby have the B protein.

the varieties into two groups. These two proteins are designated as A and B (Fig. 3). Of the 61 varieties classified, 13 contained only the A protein and 48 contained only the B protein (Table 1). All varieties with black and imperfect-black hilums possessed the B protein. Among the varieties tested which had buff or brown hilums, only Lindarin and Lindarin 63 had the A protein. Approximately two-thirds of the varieties with yellow hilums had the A protein and the remainder the B. None of the varieties had both proteins. Electrophoretic analysis of proteins extracted from seeds with seed coats (including hilums) removed, showed that the A and B proteins were not derived from the hilum.

DISCUSSION

The consistent association of either the A or B protein with a variety, regardless of environmental influences, suggests that the protein might be a stable entity in that variety's genotype.

Because plant breeders have not been known to select for either of these proteins, random distribution throughout the genotypes would be expected. Among the varieties tested, however, those with the B protein outnumber those with the A protein about four to one. The uneven distribution of proteins A and B among the varieties might be attributed to the predominance of the B protein in the original parents from which the varieties used in this study were derived. Alternatively, the proteins might be linked genetically to an agronomic trait. The possible association of protein types with hilum color needs further study before a linkage relationship is proposed.

Grouping of varieties by DEAE-cellulose column chromatography, as used by Knox et al. (4) and by Morrison[3], does not fit the grouping illustrated in Table 1 as determined by disc electrophoresis. Furthermore, the number of protein components the procedure was able to detect differed among varieties and, in each case, the number was considerably less than the 21 protein components found for all the varieties by acrylamide-gel electrophoresis.

Morrison's electrophoretic procedure did not detect the A and B proteins, possibly because the initial protein extraction was at a neutral pH. Preliminary studies, done before establishing the procedure described herein, indicated a poor electrophoretic resolution of proteins extracted at pH 7.0 with either water or saline solutions. With neutral extractions, a substance which interfered with protein migration during electrophoresis (probably a portion of the lipids) remained suspended in the supernatant. However, an attempt to remove the lipids from the soybean meal with hexane and other lipid solvents before extracting the proteins at pH 7.0, did not remove the interfering substance. The substance was not present when the initial extraction of proteins was made with a solvent of pH 4.8.

Table 1. Association of soybean varieties and hilum color with A and B proteins.

A Protein		B Protein					
Buff	Yellow	Black		Buff		Brown	Yellow
Lindarin	Acme	Bienville	Kent	A-100	Hardee	Hill	Bombay
Lindarin 63	Amsoy	Bragg	Lee	Adams	Hood	Imperfect Black	Capital
Gray	Comet	Chippewa	Lincoln	Adelphia	Jackson	Hawkeye	Delmar
Hardome	Crest	Chippewa 64	Norchief	Bethal	JEW 46	Hawkeye 63	Traverse
Henry	Harosoy	Clark	Rebel	Blackhawk	JEW 101	Ogden	
	Harosoy 63	Clark 63	Ross	Coker 102	Merit	Pickett	
	Mandarin	Flambeau	Semmes	Dare	Renville	Scott	
	Monroe	Ford	Shelby	Davis	Stuart		
	Portage	Grant	Wayne	Dorman	Wabash		
				Hampton			

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Relationship of Winter Habit and Malting Quality in Spring × Winter Barley Crosses[1]

D. H. Smith, Jr.[2]

ABSTRACT

Twelve crosses of winter × spring barley were made using hardy winter varieties and high-malting-quality spring lines as parents. F_3 rows of the progenies of these crosses were classified as spring, winter, and intermediate, based on heading date. Bulks were made within crosses of each growth habit class and malted in micromalting equipment. Quality evaluation of the bulk malts show that malt quality is independent of growth habit. Winter growth habit proved independent of any association detrimental to malting quality.

Additional Index Words: micromalting of multiline bulks, cold water extraction, early planting to accomplish vernalization.

THE degree of independence of factors associated with malting quality and growth habit were investigated to determine whether there was any real barrier to the production of a winter barley with a spring type quality pattern. Malting characteristics of seed produced on plants in three growth habit categories (spring, intermediate, and winter), from winter × spring crosses and grown under the same field conditions, were used to give some answers to the problem. Spring and winter characteristics have been defined by Wiebe and Reid (3). "Intermediate" is used herein to describe a spring type which requires a longer period to heading than its spring parent.

Malting quality is a complex of a number of chemical and morphological traits, varietal and, therefore, heritable. But the inheritance is mainly quantitative. Dickson and Grafius (Personal communication) have found a high correlation between progeny mean malting characteristics and midparent values in spring barleys.

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[2] Research Geneticist, Crops Research Division, ARS, USDA (formerly Assistant Instructor, Department of Crop Science, Michigan State University). Research supported by Malting Barley Improvement Association, Milwaukee, Wis.

MATERIALS AND METHODS

Twelve crosses were made using spring and winter barleys as parents (Table 1).

Spring barley parents were selected for their superior malting qualities, shown by quality evaluations made at the U. S. Department of Agriculture Barley and Malt Laboratory in Madison, Wis. Winter parents were picked for their agronomic characteristics, particularly their winter habit.

Progenies of these crosses were grown in the greenhouse for two generations. Seed from each F_2 plant was then planted in a single hill in the field in the early spring before normal planting dates for spring cereals, when the soil was thawed only to a depth of about 2.5 cm (1 inch). This early planting gave a temperature cold enough to vernalize the winter types in the population. Each hill was harvested and threshed individually. Such a procedure made it possible to obtain seed for malting from the three types, winter, intermediate, and spring, grown in the same environment.

Growth habit was determined in a separate planting made in May. Heading notes were taken and the rows in each cross were classified: spring, intermediate, and winter. Spring habit included all rows heading at the same time as or before the spring parent of the cross. Intermediate included all rows that headed later than the spring parent, and winter included all rows that failed to head. Rows of segregating plants were not included in the three groups.

The seed from the early planting was bulked in three categories for each cross by taking an equal amount of seed from

Table 1. Parentage of winter × spring barley crosses made in 1959 and 1960 at East Lansing.

Cross number	Winter parent	Spring parent
59 301	Dicktoo CI 5529	(Moore × Anoldium) × Montcalm
59 302	Dicktoo CI 5529	(Moore × Anoldium) × Montcalm
59 303	Dicktoo CI 5529	Moore $\times^2$ Montcalm
59 304	Dicktoo CI 5529	Liberty × Kindred
59 305	Dicktoo CI 5529	Moore $\times^2$ Montcalm
59 306	Dicktoo CI 5529	Moore $\times^2$ Montcalm
59 307	Dicktoo CI 5529	Moore × (Kindred × Bay)
59 308	Dicktoo CI 5529	Moore × (Kindred × Bay)
60 301	Dicktoo CI 5529	Montcalm CI 7149
60 302	Hudson CI 8067	Kindred CI 6969
60 303	Dicktoo CI 5529	Kindred CI 6969
60 304	Hudson CI 8067	Trail CI 9538

Table 2. The number of F_3 lines within growth categories of 12 winter × spring barley crosses.

Cross number	Habit category			
	Spring	Intermediate	Winter	Segregating
59 301	15	59	25	21
59 302	10	57	12	11
59 303	28	58	10	10
59 304	47	54	11	13
59 305	11	18	0	13
59 306	19	26	5	35
59 307	3	19	16	4
59 308	38	36	18	15
60 301	8	10	11	2
60 302	56	73	13	20
60 303	87	50	7	33
60 304	46	72	12	25