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# System For Crop Variety Identification

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# SYSTEMS FOR CROP VARIETY IDENTIFICATION

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Varietal identification is the cornerstone of pure seed production and certification because it:

- Indicates yield potential.
- Provides information on optimum crop establishment such as date of planting, row width, plant type, etc.
- Relates maturity and harvesting information.
- Identifies disease resistance.
- Suggests particular fertility regimes.

These parameters are essential components of any successful farming operation. A farmer recognizes that he must have assurance that the variety of seed purchased is the variety of seed desired. It was for this reason that the certification process was established. Farmers realize that certified seed has been inspected for trueness-to-type and can be confident of the quality of seed purchased.

In 1970, the United States Congress passed the Plant Variety Protection Act which had an immediate impact on the seed industry. The purpose of the Plant Variety Protection Act is "To encourage the development of novel varieties of sexually reproduced plants and to make them available to the public, providing protection available to those who breed, develop, or discover them, and thereby promoting progress in agriculture in the public interest." Clearly, this significant preamble was designed to encourage private seed companies to initiate their own breeding programs--a role which to that point in time had been fulfilled by public university and government breeders. For the first time, a private seed company which released a new variety could have it protected from competitor infringement for 18 years. To obtain Plant Variety Protection, however, the protected variety must meet the following three criteria:

(1) Novelty - Clearly distinct from all other varieties.

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(2) Stability - Sexually reproducible while retaining its essential and distinct characteristics.

(3) Uniformity - Variants are describable, predictable and commercially acceptable.

Recognizing the intended objective of the Plant Variety Protection Act to promote plant breeding programs within the private sector, how sucessfull has it been? One measure is to compare the number of companies with soybean breeding programs in 1970 as well as to identify the number and quality of breeders actively developing new soybean varieties today (Table 1).

There can be little question that the Plant Variety Protection Act has modified seed industry research programs to include a strong commitment to breeding. But this change in research personnel still fails to assess whether these efforts in breeding have been reflected in the release of new varieties.

Table 2 demonstrates that 230 new soybean varieties have been issued PVP certificates from 1971 to 1981! In addition to this remarkable increase in soybean germplasm, the trend is clearly apparent that many of these varieties were developed and protected during the last five years. It seems reasonable to assume that there will be a continuing increase in the development of new soybean varieties by the private seed sector in the foreseeable future.

However, this proliferation of newly developed soybean varieties has made taxonomic characterization of varieties via traditional field evaluation of morphological features increasingly inadequate.

Because variety identification plays an integral role in seed certification, the development of laboratory tests which provide improved differentiation has become necessary. Laboratory procedures furnish several additional characteristics useful for genetic purity determination and offer the promise of rapid and and inexpensive analyses for future use in variety identification.

At present, in order to determine if seed is genetically pure, a certification agency employs inspectors to make field observations of the morphological characteristics of crops grown for seed. However, field testing possesses several undesirable characteristics:

- The crop must be grown in areas where the variety is well adapted, under the best cultural practices, and during the proper growing season.

- The variety must be judged for 'trueness-to-type' at precise times.

Table 1. Effect of PVP on private soybean breeding programs.

| Year | Number of Companies | Number of Breeders | Breeders With Ph.D. |
|---|---|---|---|
| 1970 | 6 | 6 | 4 |
| 1983 | 28 | 60 | 29 |

Table 2. Soybean VP certificates issued from 1971 to 1981.

| Year | Certificates | Year | Certificates |
|---|---|---|---|
| 1971 | 9 | 1977 | 20 |
| 1972 | 8 | 1978 | 20 |
| 1973 | 13 | 1979 | 35 |
| 1974 | 15 | 1980 | 37 |
| 1975 | 11 | 1981 | 49 |
| 1976 | 13 | | |
| | | TOTAL | 230 |

- An individual who possesses a thorough knowledge of the variety is required for identification.

- Field testing generally requires at least six months for variety determination in order that all characteristics are expressed during a growing season.

- Field testing is expensive, requiring equipment, planting and harvesting personnel in addition to inspectors and land use.

- The number of morphological characteristics useful in variety characterization is no longer adequate for identification of all varieties.

Current soybean variety identification techniques are, for these reasons, inadequate. As a result, the development of laboratory tests to differentiate varieties has recently been emphasized (Payne, 1979; McDonald, 1979; Wagner and McDonald, 1981). Laboratory techniques offer the promise of being more rapid and less expensive than field testing. Further, analysis time is flexible and numerous additional traits useful in taxonomic characterizations are available.

The objective of this study was to examine and develop rapid, uncomplicated, inexpensive and repeatable means of differentiating soybean varieties which can be applied to seed certification and breeding programs. Much of the research presented in this study was initially reported by Wagner and McDonald (1982).

## Materials and Methods

### Samples included in the Tests

Soybean seeds harvested in 1977 and 1978 was requested from institutional growers or from seed companies. Thirty varieties from the 1977 harvest were received by February, 1978. The seed level for each variety, i.e., breeder, foundation, registered or certified, is provided in Table 3. Each of the varieties was tested in the field and the laboratory.

Twenty-nine 1978 varieties were received by April, 1979. Twenty-three of the 30 1977 varieties were included, bringing the total number of varieties examined to 36. Table 3 provides the seed level for these, each of which was tested in the field and laboratory.

All seed samples were stored at room temperature in the laboratory until used.

Table 3. Level of seed (C-certified, R-registered, F-foundation) from the 1977 and 1978 harvests.

| Cultivar | Seed Level | Year(s) of Harvest | Cultivar | Seed Level | Year(s) of Harvest |
|---|---|---|---|---|---|
| Agripro 20 | F | 1977 | Sloan | F | 1978 |
| Agripro 25 | F | 1977 | S 1244 | F/F | 1977/1978 |
| Agripro 26 | F | 1977 | S 1346 | F/F | 1977/1978 |
| Amsoy 71 | F/F | 1977/1978 | S 1474 | F/F | 1977/1978 |
| Beeson | F/F | 1977/1978 | S 1492 | F/F | 1977/1978 |
| Calland | F/F | 1977/1978 | S 1578 | F/F | 1977/1978 |
| Cumberland | F | 1978 | SRF 150-P | F/R | 1977/1978 |
| Elf | F | 1978 | SRF 200 | F/F | 1977/1978 |
| FFR 111 | F | 1977 | SRF 307-P | F/R | 1977/1978 |
| FFR 223 | F/C | 1977/1978 | SRF 350 | F/F | 1977/1978 |
| FFR 224 | F/C | 1977/1978 | SRF 400 | F/R | 1977/1978 |
| FFR 335 | F/C | 1977/1978 | SRF 450 | F/F | 1977/1978 |
| FFR 337 | C | 1978 | Vickery | F | 1978 |
| FFR 444 | F/C | 1977/1978 | Wayne | F/F | 1977/1978 |
| Matsoy | C | 1977 | Wells | F/F | 1977/1978 |
| Mitchell | F/C | 1977/1978 | Williams | F/F | 1977/1978 |
| Oakland | F | 1978 | Woodworth | F/f | 1977/1978 |
| P-61-22 | F | 1977 | | | |
| Rockford | F | 1977 | | | |

Field Tests

In order to verify that the varieties were labelled and identified correctly, field tests were performed for seeds received in both years. The field tests for the 1977 and 1978 varieties were planted on June 2, 1978 and June 5, 1979, respectively. One 3 m long row of each variety was planted. Seeds were planted at a rate of six seeds per 30 cm. Border rows were alternated with the rows of varieties, and two border rows were planted around the perimeter of the plot. Three morphological characteristics were visually observed in the field (stem pubescence color, leaf shape, and flower color) and compared to those outlined by the Ohio Seed Improvement Association (OSIA, 1977-80).

Laboratory Tests

1. Hilum color. A minimum of 10 seeds from each variety were examined and placed in one of five hilum color categories: clear, buff, brown, imperfect black and black (the imperfect black hilum is bordered by a brown line, distinguishing it form the completely black hilum).

2. Hypocotyl color. In order to determine hypocotyl color, a minimum of 10 seeds of each variety were placed in a watered medium composed of 1/3 sand, 1/3 soil, and 1/3 vermiculite, and allowed to germinate under fluorescent lighting (450 $Em^{-2}s^{-1}$). After seven to 10 days, hypocotyl color was examined and the varieties were placed in one of two categories, green or purple hypocotyl.

3. Peroxidase test. The method of Buttery and Buzzel (1968) was used to analyze peroxidase content. Seed coats were removed from a minimum of ten seeds from each variety with a razor blade. Each coat was then placed in an individual test tube. Ten drops of 0.5% (v/v) guaiacol were added to each tube. After 10 minutes, one drop of 0.1% (v/v) hydrogen peroxide was added to each tube. Cultivars were placed into one of two groups based on the formation (positive) or absence (negative) of a reddish brown color.

4. Electrophoresis. The polyacrylamide gel electrophoresis apparatus used in this study included a Buchler Instrument chamber which held 18 electrophoresis tubes and had a total buffer capacity of 1.71. The power supply was ISCO model 493.

(a) Gel preparation. For each electrophoretic run, 18 cylindrical glass tubes 7.5 cm long with an inner diameter of 5 mm were thoroughly washed, dried, inserted into serological stoppers and placed into a gel stand. Forty ml of 7.5% acrylaminde lower gel solution were prepared by mixing one part A to one part C to two parts fresh F (see appendix). The F solution was always added last. Using

a disposable pipette, the gel solution was added to the 6 cm mark on the tubes, making sure that no air bubbles were trapped in the tubes. One drop of distilled water was then added in order to deter formation of a meniscus on the gel's upper surface. The appearance of a sharp boundary line approximately 2 mm below the top of the gel 10 minutes to one hour later indicated that the gel had polymerized. If polymerization did not occur within one hour, the gels were placed in a convection oven set at 34C for one hour.

After lower gel polymerization was complete, the liquid on top of the gel was removed with a flick of the wrist and the tubes were placed back into the holder. Forty ml of 2.5% acrylamide upper gel solution were prepared by combining one part of B, two parts of D, one part of E and four parts of distilled water (see appendix). Six mm of this solution were pipetted onto the top of the lower gel, and one drop of distilled water was added to prevent meniscus formation. The gels, in the stand, were then placed under a flourescent lamp for 30 minutes, allowing the upper gel to photopolymerize. When ready, the upper gel appeared opaque. Prior to placing the tubes in the electrophoresis apparatus, the liquid on top of the gel was removed with a flick of the wrist. The gels were then used within three hours. If not, they were placed in a plastic bag and refrigerated overnight.

The tubes containing gels were placed in the grommets in the upper buffer chamber of the elctrophoresis apparatus. Enough tris-glycine buffer (solution G--see appendix) was added to the lower plastic dish so that the tubes made contact with it. The upper buffer chamber was replaced on the stand, and enough tris-glycine buffer was added to easily cover the tops of the tubes. Any air bubbles in the tops of the tubes were removed with a pipette. With a small syringe, 0.05 ml of a concentrated protein extract in a buffer specific for he protein being analyzed was added to the top of each of the gels in the apparatus. The syringe was rinsed with distilled water between sample application in order to avoid contamination. After all the samples were applied, a drop of tracking dye (0.001% (w/v) bromophenol blue, 10% (w/v) in sucrose was pipetted onto the top of each gel. Because it is a highly charged, small molecule, the tracking dye migrated faster than the proteins in the sample, and therefore, monitored the progress of electrophoresis.

The electrodes were then attached, positive polarity to the lower dish, negative to the upper, and the power supply was turned on to 60 mA constant current. This allowed approximately 3.3 mA to run through each of the 18 gels.

After approximately 65 minutes, the tracking dye reached the bottom of the gel tubes. The power supply was turned off and the electrodes detached. The tubes were removed form the apparatus. The gels were excised, or rimmed, by inserting a hypodermic needle attached to a water filled syringe between the gel and the glass tube.

The needle was held steady as the tubes were rotated, causing the gel to be detached from the glass tube. The gels were then placed into small glass test tubes and were stained according to the particular protein being analyzed. In cases where the stain was permanent, the gels were analyzed spectrophotometrically, measuring absorbance at an appropriate wavelength. In this manner, spectrophotometric scans, which represented the banding patterns, were achieved.

Zymograms, pictorial representations of banding patterns, were drawn for ephemeral isozyme stains which did not allow spectrophotometric analysis.

RF values ($RF = \frac{\text{distance traveled by protein}}{\text{distance traveled by tracking dye}}$) were also calculated for protein bands of interest. Gels were photographed immediately after staining was complete.

Two isozyme systems present in the unimbibed soybean seed were analyzed: B-amylase and urease.

(b) Preparation of the seed protein samples.

(1) B-amylase. An individual sample was prepared by grinding three unimbibed seeds of each variety into a fine powder with mortar and pestile set in ice. The powder was then mixed with 6.0 ml of buffer solution which consisted of an equal mixture of 0.1 M sodium acetate and 0.1 N acetic acid made of 10% (w/v) in sucrose (pH 5.0) and maintained at 5C (Larsen, 1967). The addition of sucrose ensures that the sample will fall to the top of the gel when applied. The mortar and pestle were rinsed between each exraction in order to avoid sample contamination. The homogenates were placed into centrifuge tubes. If centrifugation was not applied immediately, the tubes were placed in ice. Each of the samples was centrifuged at 20,200 x G at 5C for 10 minutes. The centrifuged samples were then placed in ice until ready for application to the polyacrylamide gel. All samples were prepared fresh daily. A minimum of 15 seeds of each variety were analyzed for B-amylase.

(2) Urease. An individual sample was prepared by grinding three unimbibed seeds of each variety with a mortar and pestle set in ice. The powder was then mixed with 6.0 ml of distilled water made 10% (w/v) in sucrose, and maintained at approximately 5C (Buttery and Buzzel, 1971). The soybean material and extractant solution were combined with the pestle. Samples were centrifuged as described previously. Again, all samples were prepared daily. A minimum of 15 seeds of each variety were analyzed for urease.

(c) Staining procedures.

(1) B-amylase (general protein). After removal of the glass tubes, each gel was soaked for one to eight hours at room temperature in 8 ml of staining solution composed of 0.1 g Coomassie Brilliant Blue in 10 ml of ethanol and combined with 250 ml of 12% (w/v) trichloroacetic acid (Bashuk and Zillman, 1978). After the dark blue bands were resolved, the gels were placed in distilled water. Spectrophotometric scans were made at a wavelength of 540 nm.

(2) Urease. Each gel was soaked for 10 minutes at room temperature in 8 ml of staining solution consisting of 25 mg cresol red dissolved in 90 ml of 0.2 m Na acetate buffer (45 ml 0.2 M acetic acid + 45 ml 0.2 M Na acetate), and 60 ml 7% (v/v) acetic acid (Buttery and Buzzell, 1971). The gels were then quickly transferred to 8 ml of a solution consisting of 1.6 g urea, 0.1 g $Na_2$ EDTA, and 25 mg cresol red dissolved in 150 ml distilled water. Within five to 20 minutes, bright purple red bands were resolved. The banding pattern for urease was recorded immediately after resolution as the solution quickly caused the entire gel to stain. Due to the ephemeral nature of the stain, spectophotometric scans were not made.

## Results

### Field Tests

Results of the field tests were consistent with variety characteristics outlined by the Ohio Seed Improvement Association, with two exceptions. 'Agripro 20' which was listed as possessing a brown hilum, was placed in the buff category in this study, as well as in another (Payne, 1979). In addition, 'SRF 307-P', listed as having a buff hilum, was placed in the brown grouping (ICIA, 1978).

### Laboratory Tests

1. Hilum color. Hilum color determination categorized the Ohio soybean varieties as indicated in Table 4. The 36 soybean varieties were subdivided into five groups. The largest group (black) contained 14 varieties. Results for the same variety from the two different growing seasons were identical.

2. Hypocotyl color. Hypocotyl color determination categorized the 36 Ohio soybean varieties as indicated in Table 5. A total of 27 varieties were classified as purple and nine varieties possessed green hypocotyls. A correlation between the seedling hypocotyl color and the flower color was shown to exist. Cultivars possessing green hypocotyls produced white flowers while varieties having purple hypocotyle produced purple flowers. Results for the same variety from the two different growing seasons were identical. The genetic linkage

Table 4. Differentiation of 36 soybean varieties certified in Ohio based on five hilum color categories (number in parenthesis indicates the total for each group).

| Hilum Color | | | | |
|---|---|---|---|---|
| Clear (9) | Buff (2) | Brown (5) | Imperfect Black (6) | Black (14) |
| Amsoy 71 | Agripo 20 | Mitchell | Agripo 25 | Calland |
| FFR 111 | S 1492 | Sloan | Agripo 26 | Elf |
| FFR 223 | | S 1474 | Beeson | FFR 224 |
| Matsoy | | S 1578 | Cumberland | FFR 335 |
| P-61-22 | | SRF 307-P | Rockford | FFR 337 |
| S 1346 | | | Wells | Oakland |
| SRF 150 | | | | S 1244 |
| SRF 200 | | | | SRF 350 |
| Vickery | | | | SRF 400 |
| | | | | SRF 450 |
| | | | | Wayne |
| | | | | Williams |
| | | | | Woodworth |
| | | | | FFR 444 |

Table 5. Differentiation of 36 soybean cultivars certified in Ohio based on hypocotyl color and seed coat peroxidase reaction (number in parenthesis indicates the total for that group).

| Hypocotyl Color | | Seed Coat Peroxidase | |
|---|---|---|---|
| Purple (27) | Green (9) | Positive (16) | Negative (20) |
| Agripro 20 | FFR 335 | Agripro 20 | Agripro 25 |
| Agripro 25 | FFR 337 | Amsoy 71 | Agripro 26 |
| Agripro 26 | Sloan | Cumberland | Beeson |
| Amsoy 71 | S 1492 | FFR 111 | Calland |
| Beeson | SRF 307-P | FFR 223 | Elf |
| Calland | SRF 350 | Matsoy | FFR 224 |
| Cumberland | Wayne | Mitchell | FFR 335 |
| Elf | Williams | P-61-22 | FFR 337 |
| FFR 111 | Woodsworth | Rockford | Oakland |
| FFR 223 | | S 1244 | Sloan |
| FFR 224 | | S 1578 | S 1346 |
| Matsoy | | SRF 150 | S 1474 |
| Mitchell | | SRF 200 | S 1492 |
| Oakland | | SRF 450 | SRF 307-P |
| P-61-11 | | Vickery | SRF 350 |
| Rockford | | Williams | SRF 400 |
| S 1244 | | | Wayne |
| S 1346 | | | Wells |
| S 1474 | | | Woodworth |
| S 1578 | | | FFR 444 |
| SRF 150-P | | | |
| SRF 200 | | | |
| SRF 400 | | | |
| SRF 450 | | | |
| Vickery | | | |
| Wells | | | |
| FFR 444 | | | |

between imperfect black hila and purple hypocotyl color, demonstrated in past work (Bernard and Weiss, 1973), was substantiated.

3. Peroxidase test. The peroxidase test grouped the 36 varieties into two categories as indicated in Table 5. Sixteen varieties yielded a positive peroxidase reaction; 20 varieties demonstrated a negative reaction. Results for the same variety from the two different growing seasons were the same.

4. Electrophoresis.

(a) B-amylase. Two patterns resulted when gels were stained for general protein. One exhibited a fast moving B-amylase band (RF = 0.51), the other a slow moving band (Rf = 0.46), as indicated by Figure 1.

The 36 varieties were categorized as possessing the fast or slow moving B-amylase band as indicated in Table 6. Twenty-eight varieties possessed a fast moving, B band; eight varieties demonstrated a slow moving, A band. Banding patterns within the same variety did not differ among growing seasons.

(b) Urease. Two isozyme banding patterns resulted when gels were stained for urease. One exhibited two bands (Rf = 0.11, 0.30); the other, one band (Rf = 0.30), as indicated by Figure 2. Spectorphotometric scans were attempted but did not succeed due to the ephemeral nature of the urease stain.

The 36 varieties were categorized as possessing the two or one band(s), as indicated in Table 6. Three varieties exhibited either banding pattern, i.e., seed urease varied. Since the seed used was deemed pure, these varieties apparently possessed the genetic ability to produce either urease banding pattern. Seventeen varieties possessed two bands; 16 varieties demonstrated one band. Banding patterns did not differ within the same variety from different growing seasons.

Summary of results. Combining the data from the five tests successful in differentiating soybean varieties in this study culminated in the separation depicted in Figure 3. Of the original 36 varieties examined, 15 were identified using the five tests in this identification system. Further, 22 groupings were established with no grouping possessing more than six varieties.

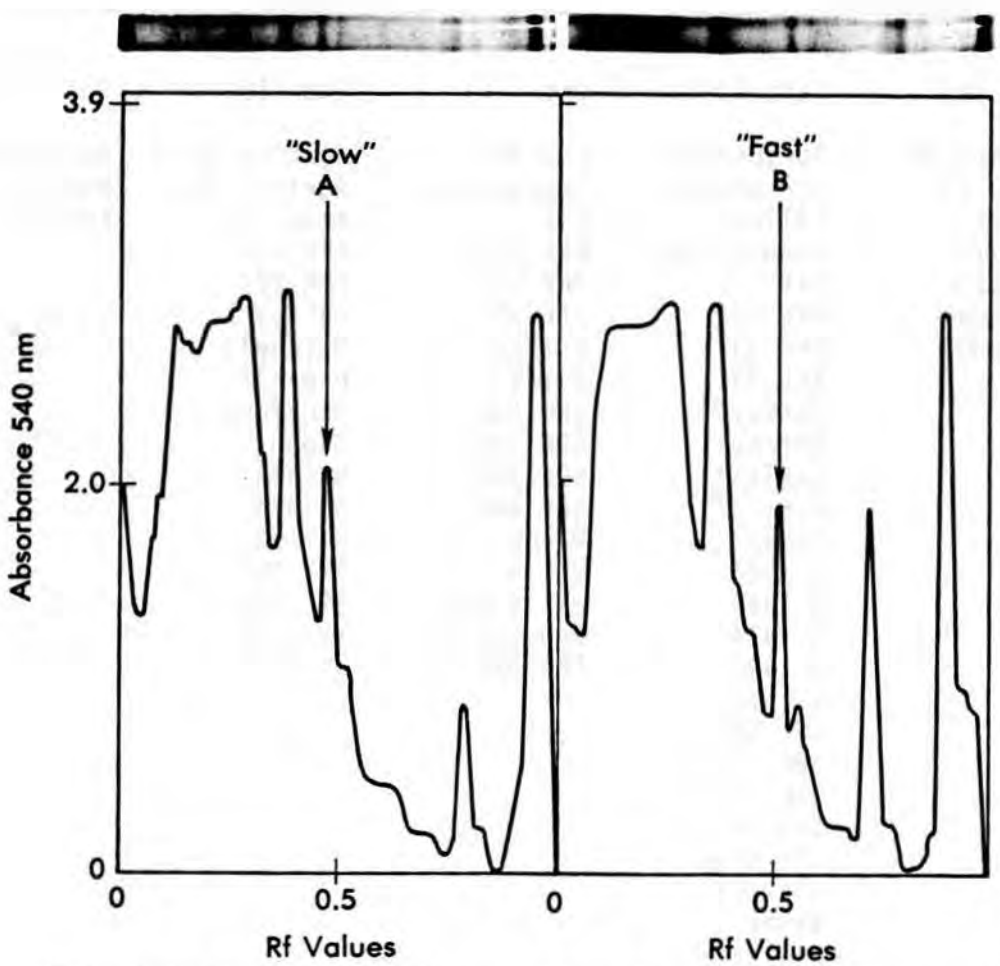


FIG. 1.—Spectrophotometric scans and photographs of the two B-amylase banding patterns of soybean cultivars, slow (A) and fast (B).

Table 6. Differentiation of 36 soybean cultivars in Ohio based on electrophoretic analysis of B-amylase and urease, extracted from the unimbibed seed (number in parenthesis indicates the total for that group).

| B-Amylase | | Urease | | |
|---|---|---|---|---|
| Slow (8) | Fast (28) | One (17) | Two (16) | Both (3) |
| Agripro 20 | Agripro 25 | Calland | Agripro 20 | Agripro 26 |
| Amsoy 71 | Agripro 26 | Cumberland | Agripro 25 | Beeson |
| Beeson | Calland | Elf | Amsoy 71 | FFR 335 |
| FFR 111 | Cumberland | FFR 224 | FFR 111 | |
| FFR 223 | Elf | FFR 337 | FFR 223 | |
| Rockford | fFR 224 | Oakland | Matsoy | |
| SRF 200 | FFR 335 | S 1244 | Mitchell | |
| Wells | FFR 337 | S 1492 | P-61-22 | |
| | Matsoy | SRF 150 | Rockford | |
| | Mitchell | SRF 307-P | Sloan | |
| | Oakland | SRF 350 | S 1346 | |
| | P-61-22 | SRF 400 | S 1474 | |
| | Sloan | Wayne | S 1578 | |
| | S 1244 | Wells | SRF 200 | |
| | S 1346 | Williams | SRF 450 | |
| | S 1474 | Woodworth | Vickery | |
| | S 1492 | FFR 444 | | |
| | S 1578 | | | |
| | SRF 150 | | | |
| | SRF 307-P | | | |
| | SRF 350 | | | |
| | SRF 400 | | | |
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| | Vickery | | | |
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| | Woodworth | | | |
| | FFR 444 | | | |

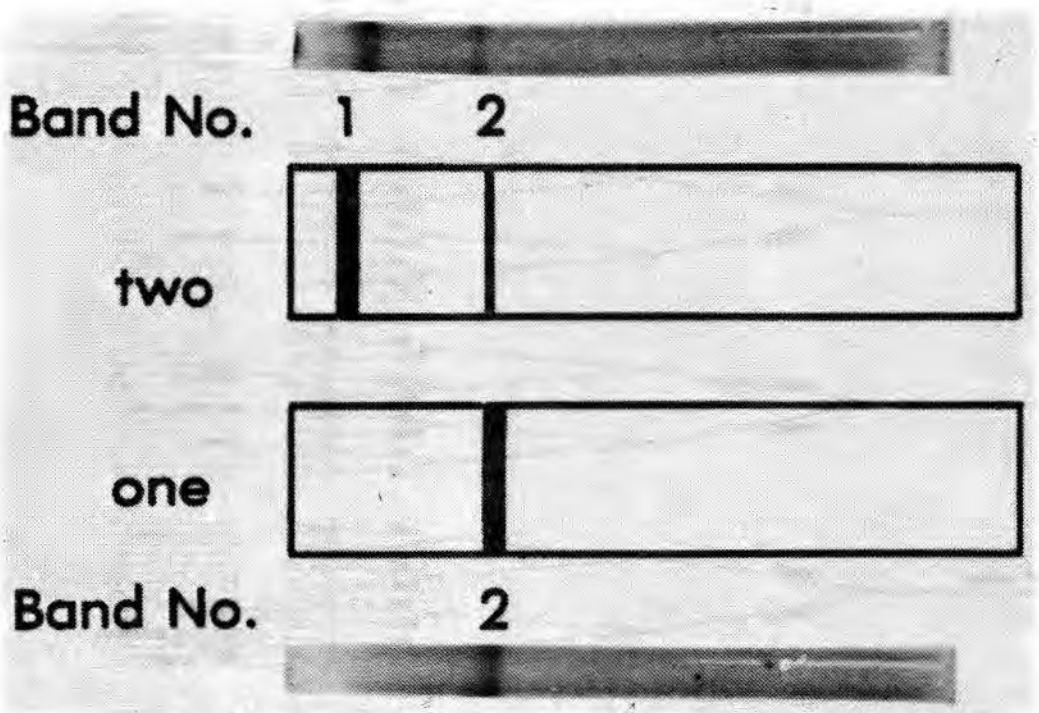


**FIG. 2.—Zymograms and photographs of the two urease banding patterns of soybean cultivars, two and one.**

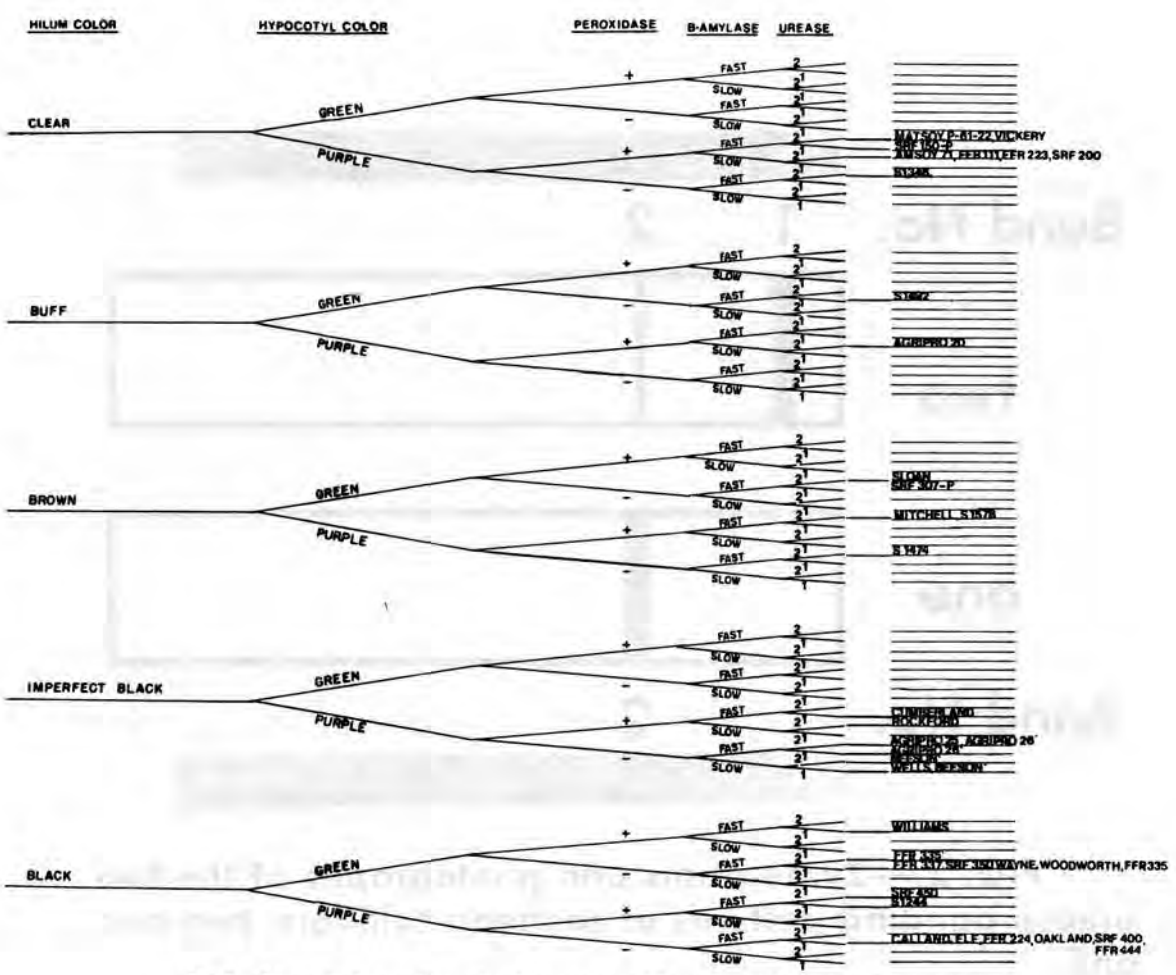


FIG. 3 —Schematic diagram illustrating the separation of 36 soybean cultivars via laboratory tests.

# Discussion

## Laboratory tests

A test useful for variety identification should possess several characteristics. It should be relatively uncomplicated, quick, consistent and inexpensive to perform. The test should also allow immediate observation of 'off-types'. Finally, if possible, the feature under examination should be possessed by individual plants or plant parts. Since it is unlikely that any single test will completely separate all varieties, several tests were examined in this study. Unimbibed seeds were used for all electrophoretic protein determinations because they are relatively stable physiologically and, therefore, were expected to provide repeatable results under standardized conditions. Further, in general, tests which yielded qualitative results, e.g., type of isozyme present, type of pigment present, etc., were employed, because qualitative data are less subject to such factors as seed vigor, storage, etc., than quantitative parameters. Finally, testing seeds of the same variety from different years allowed a comparison of results from different seed lots, addressing the question of repeatability between growing seasons.

The hilum color test was useful, but possessed several undesirable characteristics. Treated seed masked the hilum such that categorization was difficult. In addition, the difference between a buff and a brown hilum was minimal and differentiating the two was difficult. However, untreated, healthy seed, and experience, the hilum color test is a valuable technique for distinguishing varities. Hilum color is reportedly controlled by the alleles of four genes, some of which are pleiotropic (Bernard and Weiss, 1973).

The hypocotyl color test, although requiring a minimum of seven days to perform, proved to be a reliable method for separating soybean varieties. However, the separation resulted in only two groupings, with a large majority of varieties falling into the purple hypocotyl category (Table 5). Still, because of the association between flower color and hypocotyl color, this test is much more rapid than field observations. It has been reported that hypocotyl pigmentation is a result of the pleiotropic effect of one gene (Benard and Weiss, 1973).

The peroxidase test emerged as a useful assay for variety identification. Results obtained were consistent, but only when great care was taken to remove and test only the seed coat and none of the cotyledonary tissue. The latter yields a positive peroxidase test regardless of the seed coat reaction. Inheritance of peroxidase activity has been shown to be monogenically controlled (Buttery and Buzzell, 1969).

Electrophoresis proved to be an excellent means of characterizing variations in protein content among soybean varieties. The relatively low equipment cost, swiftness of analysis, ease of operation and ability to analyze simple seed protein samples repeatably contributed to the feasibility of its use for variety identification. Variations among varieties were detected for two isozyme systems, B-amylase and urease. In past work (Bernard and Weiss, 1973; Larsen and Caldwell, 1968), the B-amylase band was shown to be controlled by two codominant alleles at a single locus. Further, varieties with black, brown or buff hila exhibit the slow B-amylase band, while varieties with clear or imperfect black hila possess either the slow or fast B-amylase band (Payne, 1979).

The banding pattern achieved for soybean seed urease labelled "two" in this study differed from that reported originally by Buttery and Buzzell (1971). The former study resulted in two banding patterns, one with a fast moving band, another possessing a band of slower mobility. The urease banding pattern was shown to be monogenically controlled, the fast migrating band dominant over the slow moving band. In the present study, two bands were resolved for varieties deemed by Buttery and Buzzell as possessing the slow moving band. Similar results have been reported in other studies (Payne, 1979). It is possible that the slow moving band may dissociate due to factors such as the buffer employed, stability of the molecule, etc., producing a faster moving artifact (Buttery and Buzzell, 1971).

The separation of the varieties using the laboratory tests demonstrated in this study shows considerable improvement over the separation of the same varieties achieved via field testing. As indicated in Figure 4, the use of hilum color, flower color, leaf shape and stem pubescence color, the four most commonly employed characteristics in field testing, separates the 36 varieties in 13 groups. Only six varieties are isolated exclusively. The largest number of varieties remaining in any one group is six. Laboratory testing is, therefore, a more effective means of differentiating varieties in terms of the number of varieties which may be exclusively identified.

## Feasibility of Laboratory Testing Procedures

Laboratory tests offer several important advantages over field testing in terms of effectiveness of identification, distinct and readily observable characteristics, and use of space. In addition to the electrophoretic analyses employed in this study, other electorphoretic tests have been reported as useful in soybean variety differentiation (Gorman and Kiang, 1977; Payne and Koszykowski, 1978). although experimentation has generally not been performed with soybean seeds, chromatographic (Buttery and Buzzell, 1973; Stewart, Asen, Massier and Norris, 1980) and serological (Esposito, Ulrich and Burrell, 1966) procedures have demonstrated taxonomic utility within

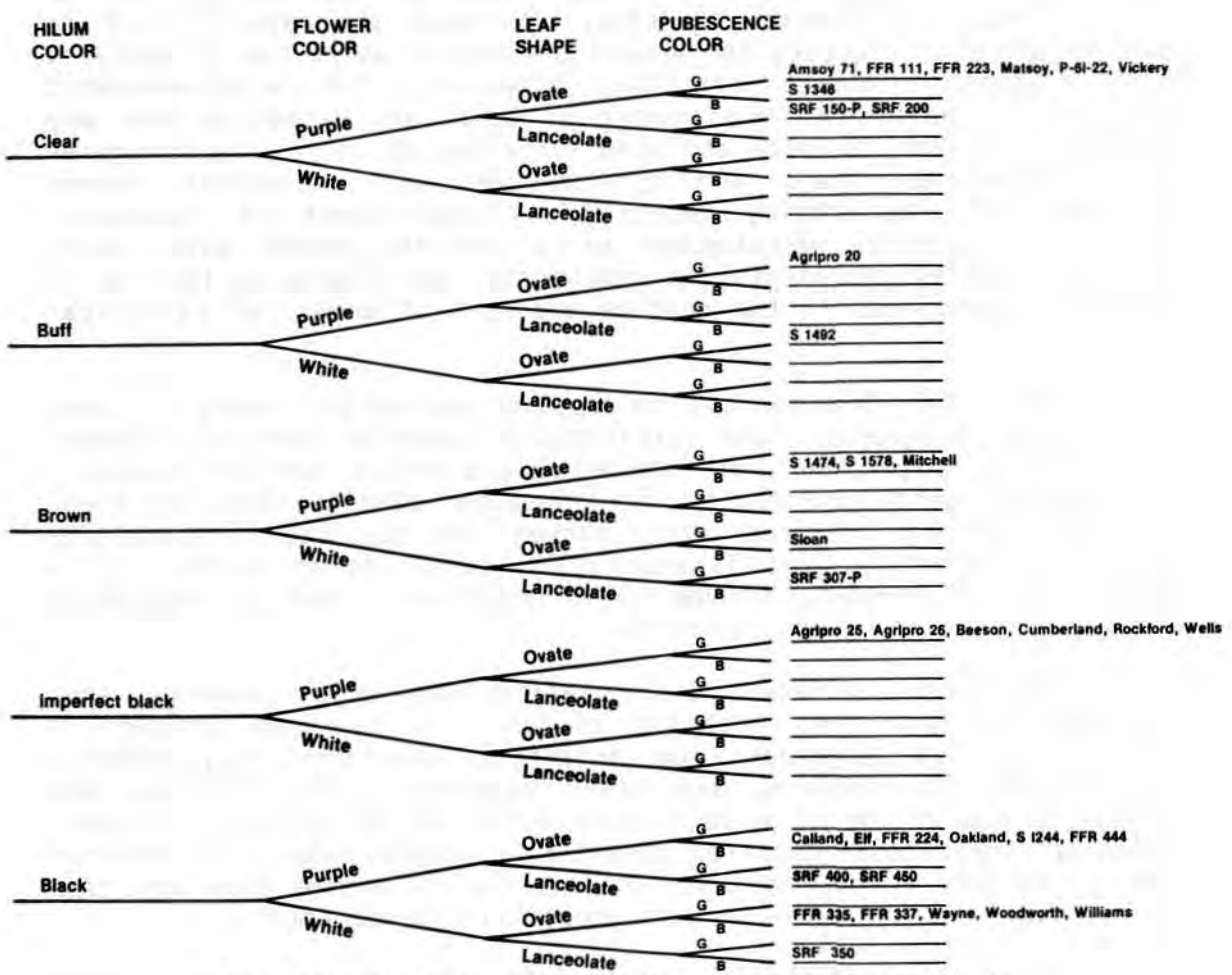


FIG. 4.—Schematic diagram illustrating the separation of 35 soybean cultivars via field tests.

several other crop species. Cytological (Davis and Heywood, 1965; Will, Kronsted and Tekrony, 1967; Solbrig, 1968) and ultrastructural (Chuang and Heckard, 1972; Krause, 1978; Newell and Hymowitz, 1978) methods also offer great potential for reliable intraspecific identification.

There are, admittedly, many unanswered questions regarding the use of laboratory tests for seed certification. The laboratory testing format must be feasible in terms of time, cost and logistical considerations. Assuming identical sample size, laboratory testing procedures cannot presently compete in terms of cost and time with the field sequential sampling method developed by the Association of Official Seed Certification Agencies. Although the material cost is low for laboratory testing the number of samples which can be analyzed in a reasonable amount of time, especially for electrophoretic analysis, is small. A large number of tests and excessive time are required. However, as more and more varieties of increasing homogeneity are developed, field testing procedures will undoubtedly become less adequate, encouraging technological improvement of laboratory tests. In addition, development of a sampling method which would require a smaller representative sample size would enhance the use of laboratory techniques in the routine analysis of seeds for certification.

Laboratory testing can be applied to other facets of seed certification programs. Some certification agencies currently augment initial field inspection with laboratory procedures such as hypocotyl color and the peroxidase tests. Certification agencies also may field test seed from the previous year's harvest for purposes of establishing variety purity. This is especially useful in determining if a certain lot of breeder, foundation or registered seed is adequately pure for further seed multiplication.

Laboratory variety identification not only benefits seed certification, but crop breeding as well. The crop breeder is interested in developing new crop varieties. Laboratory tests offer a quick method of screening new plant genotypes, and detecting any changes in the genome of a seed stock as it is multiplied or stored. Similarly, these tests offer an essentially endless source of information to variety review boards, which serve to ensure that new crop varieties are unique in one or more genetically based traits.

There are, admittedly, constraints upon the total replacement of field testing by laboratory testing for variety identification. However, based upon the results reported in this study and others, laboratory measures may serve as a viable supplement to present seed testing programs, and are likely to play an increasingly important role in the future.

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## Appendix

Procedures for preparing 7.5% acrylamide lower-gel, pH 8.9; 2.5% acrylamide upper gel, pH 6.9; and tris glycine buffer, Ph 8.3.

## Solutions

| Lower Gel | | Upper Gel | |
|---|---|---|---|
| A. IM HCl | 24.00 ml | B. IM $H_3PO$ | 25.60 ml |
| Tris | 18.20 g | Tris | 05.70 g |
| TEMED* | 00.23 ml | TEMED* | 00.46 ml |
| Water to 100 ml (pH 8.9) | | Water to 100 ml (pH 6.9) | |
| C. Acrylamide | 30.00 g | D. Acrylamide | 10.00 g |
| Biasacrylamide | 00.80 g | Bisacrylamide | 02.50 g |
| Water to 100 ml | | Water to 100 ml | |
| F. Ammonium persulfate | 00.14 g | E. Ribofavin | 4.00 mg |
| Water to 100 ml | | Water to 100 ml | |
| G. Buffer (ph 8.3) | | | |
| Tris | 03.00 g | | |
| Glycine | 14.40 g | | |
| Water to 100 ml | | | |

*TEMED is N, N, N', N' tetramethylethylenediamine.

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