AOSA-SCST CULTIVAR VERIFICATION SYMPOSIUM

Hilton Hotel, Lincoln Nebraska

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INTRODUCTORY REMARKS

Dwight W. Lambert,[1] *Chairman*

During the past two decades there has been a great increase in the number of cultivars[2] released to the public. Much of this increase can be attributed to the interest by the private sector of the seed industry in developing proprietary products. Also contributing to the rising number of cultivars in both the private and public sectors of the seed industry are the changes in methods of plant breeding. For years we had only "pure line" cultivars, then came the "hybrids," now we have "synthetic," "strain cross," "multiline" and "clonal" type cultivars, plus a few others.

All of the new cultivars, regardless of how they are produced, leave the seed technologist somewhat confused as to a means of identification. Twenty years ago we had few cultivars of perennial ryegrass; now we have many cultivars of "fineleaved" perennial ryegrass. Also, we had only Kentucky bluegrass, rough bluegrass, annual bluegrass and Canada bluegrass, and now we have many cultivars of Kentucky bluegrass.

We should not complain about the number of cultivars available. To keep abreast of requirements of the consuming public, both in this continent and abroad, we need increased production; and new cultivars are one of the significant ways of accomplishing this. With all of the new cultivars we need to continue to maintain order in the market place and therefore as seed technologists we must increase our ability to make cultivar identifications. It is interesting to note that with the change in the state of the art of plant breeding has also come changes in the state of the art of seed identification.

The symposium on verification of cultivars is timely. It allows a review of work currently being used to identify cultivars as well as a look into the future. The papers that follow present cultivar identification from the viewpoints of a breeder, physiologist, technologist, and regulator. They describe identification procedures in the field and in the laboratory.

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[2]The terms cultivars or cultivar and varieties or variety, respectively, as used throughout the symposium papers are considered to be synonymous.

VARIETAL DETERMINATION MADE IN THE SEED LABORATORY

W. P. Ditmer[1]

A seed law is a "truth in labeling" law whether it is the Federal Seed Act or the Pennsylvania Seed Act. Varietal names are used with many kinds of seed. When varietal names are required to be shown on the label, then we are obligated to determine if the name used is correct. How many of us are making varietal determinations, or are we looking only at the kind of seed and the other components?

Varietal verification can be more difficult than routine purity and germination testing, but it is not impossible. While it takes time, varieties of many kinds of seed can be identified. Numerous types of tests have been developed, but individually they are of little value. For years we have been using numerous procedures that have been recognized by the Association of Official Seed Analysts (AOSA), such as: the various seed keys, fluorescence tests, phenol tests, and growth tests. In addition, we are trying some chemical tests other than the phenol test. Since 1975, we have been working with Dr. Guy McKee of the Crop Ecology and Physiology Laboratory, The Pennsylvania State University, toward developing varietal testing systems. Through a process of elimination, by using various tests, we have been able to determine if the variety is correctly or incorrectly labeled. A system starts with the simpler tests at the purity board, working up to the more sophisticated tests. In 1977, we were fortunate to have a research project entitled "Development and Evaluation of Varietal Purity Testing Systems for Small Grain, Forage and Turf Crops" funded from Harness Racing Research Funds. To date, results are looking promising. This is a 2-year grant which we hope will be extended.

While we are using the multiple test approach to verify the varieties of some kinds, in other kinds individual tests are still used, because that is all that is available. Most of the varietal work in the laboratory has been done only with agricultural seeds and then primarily with members of the *Gramineae* family (cereals and lawn grasses) and some *Leguminosae* family. No work has been done with other plant families.

Let us take a look at what the Pennsylvania Seed Laboratory is now doing to determine varietal identification. This information is given crop by crop rather than by individual tests.

Kentucky bluegrass—As long as I have been with the Pennsylvania State Seed Laboratory, we have been using seed keys for the identification of Kentucky bluegrass. These include keys that were developed by Miss Musil and later by Miss Colbry and Mrs. Wiseman. The key in "Identification of

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Crop and Weed Seeds," Agricultural Handbook 219, covered four varieties. Later, more varieties were introduced and they still could be separated with the seed key. However, with the new varieties that have come along recently, all cannot be separated individually and are divided into groups. In other words, some varieties can be separated from others, and some cannot. For example: 'Baron' can be separated from 'Fylking,' 'Common,' and 'Majestic,' but not from 'Bonnieblue,' 'Sydsport,' or 'Pennstar.' Pennstar can be separated from Bonnieblue but with difficulty. Color, shape, length, width, texture and venation on the lemma are some of the seed characteristics that are used. The keys are valuable tools, but a person must keep working with them. The newer varieties are not written into the existing keys. When a new variety comes on the market, we obtain what we hope are authentic samples. Our analysts study these samples and fit them into existing groups. As the analyst studies these samples, he obtains a personal knowledge and mental image of the variety. The phenol test is used, but we only read two colors. This test groups varieties, but the grouping can at times be different than the grouping by seed characteristics. The growth chamber has been used to evaluate coleoptile color, leaf sheath color and type of growth. This is an area that needs more work. The electrophoresis test has possibilities based on a graduate study, but we have not tried it.

Ryegrass—The fluorescence test is used to separate annual and perennial ryegrass. Sometimes the awns on annual ryegrass are useful for purposes of identification. Seed size can be of some use—the fine textured perennial ryegrasses usually have a smaller seed. We have been working with the phenol test on perennial ryegrass varieties reading two colors—dark or light. The various varieties do not stain all light or all dark, so a variety will have a range of so many light colored seeds. For example: 'Pennfine' has a range of light seeds from 4–18% and 'Manhattan' 20–48%. The tests are repeatable. Many lots of the certified and breeder's class of certified seed were tested to arrive at these ranges. We have determined the range for Pennfine and Manhattan perennial ryegrasses on a bulk basis and not seed for seed. Also, we have been working with coleoptile color, but this area needs some standardization between the work at The Pennsylvania State University and our laboratory. In addition, Dr. McKee's study has shown that electrophoresis can be a useful tool.

Fescue—We have made attempts to separate varieties of red fescue, but seed characteristics are too similar to make an identification with certainty. We cannot separate red fescue from chewings or hard fescue by seed characteristics; but, hard fescue can be separated from red fescue by the use of a 1% ammonia solution sprayed on the roots. After spraying, the hard fescue roots fluoresce green and the red fescue roots fluoresce yellow under the black light. Meadow fescue, tall fescue, and the ryegrasses can be separated by the shape of the seeds.

Barley—There are times when seed characteristics such as shape can be

used to make a separation. In Pennsylvania, shape can be used to determine if a variety is a spring or winter type barley. The spring type is symmetrical in shape and the winter type is 1/3 symmetrical in shape and 2/3 twisted in shape. The pubescence on the awn (if present) or rachilla is useful. The variety 'Pennrad' has long hairs on the rachilla; all other varieties have short hairs. The phenol test can be used to separate some of the spring type varieties. The phenol color is the same for all winter varieties. The aleurone layer has a purple color under the seed coat of most varieties, but 'Barsoy' does not have this color. We have used the growth chamber for a quick heading test to determine if a variety is a spring or winter type. The heading test can also be used for determining if awns are present. Put these tests together in some order, and a partial separation is accomplished. The work that we have done with barley indicates that we, as seed analysts, have overlooked numerous seed characteristics that could be used to separate bulk samples.

Oats—We have probably done more work with the oats than any of the other cereals. The fluorescence tests on seeds is an old and reliable test and the first in our series of tests. Seed shape, size, and color can also be useful. The coleoptile test in the growth chamber is helpful to identify some varieties, but this test needs to be standardized. Chemical tests such as phenol, HCl, HNO_3, and KOH can be useful. We have worked with these and have been successful, but more work needs to be done.

Wheat—In most cases, seed characteristics are not very useful for varietal verification. If a lot is not threshed or cleaned too hard, the brush characteristics could be useful. Also, size and color at times can be useful, although size has been shown to differ significantly among some varieties. Seed treatments and weathering do hinder identification when color is evaluated. The phenol test is an important test for wheat. In Pennsylvania, 'Redcoat,' 'Abe' and 'Arthur 71,' all react the same to the phenol test. However, a NaOH test will separate Redcoat from Abe and Arthur 71. The coleoptile test in the growth chamber will give the same separation. The seed size (length-width) can be used to separate Abe and Arthur 71.

Legumes—The only member of the legume family that we identify as to variety is soybean. We use seed size, shape, color, and hilum color. Attempts have been made to identify varieties of alfalfa, crownvetch and birdsfoot trefoil by making plantings in a growth chamber and observing seedlings and flower color.

Some of these methods for variety verification are time consuming. But many of these tests can be done as part of the routine program, starting at the purity board. Under a systematic approach to verification, you start with the seed, then chemical tests, the germinated seedling from the germination test, the growth chamber, electrophoresis, chromatography and then, where? We think the verification problem can be solved and varieties identified. We are obligated by the law to do it.

VARIETAL FIELD TESTING

Gail Fenderson[1]

Field testing for variety verification, at least in Oklahoma, is carried out mainly for the purpose of seed law enforcement. However, it can be helpful in supplementing laboratory test results particularly when plants are grown to maturity. Greenhouse or environmental growth chamber testing for varietal determination appears to be more adapted to short term tests and usually can be supervised by laboratory personnel. Since we do not have greenhouse and growth chamber facilities readily available to us at the Oklahoma Department of Agriculture, I will limit my remarks to testing for varietal purity in the field.

A varietal field testing program can be a relatively simple operation or very difficult, expensive and time consuming. Official laboratories that must rent land, do their own planting and plot care will find field plantings expensive and time consuming. We have an arrangement with the Oklahoma State University Experiment Station for varietal field testing. The experiment station is paid a fee on a per sample basis for preparing, planting and caring for the field plantings. It furnishes the land, equipment, and personnel to make small plot plantings. Also, check samples are furnished where needed, and experiment station personnel may make readings, in addition to ours, on certain kinds as to heading date, bloom date, height and date of maturity. After reviewing notes that have been taken from the field planting, it is our responsibility to determine if a variety is labeled correctly. The experiment station personnel are willing to cooperate by planting and caring for the plots but do not want to become directly involved with enforcement of the law by making final determinations of the results.

In conducting our field planting program, we have found the following to be most important for its success. (1) The land used must be uniform for texture, fertility, slope and moisture. (2) The plot area should be rotated from year to year or fallowed to prevent volunteer plants. (3) A uniform stand, both within the check plots and within the plots for samples being examined, is essential. When it is necessary to thin the population so individual plants can be counted and identified, all plots should be treated alike. A varying plant population can cause one row to look different than another in growth habit, height, and maturity. It has been noted that a low vigor sample planted under adverse conditions, can produce plants which are later to mature and therefore appear different from the check sample.

In Oklahoma, we make field tests of several kinds of seed. We have found that notes concerning each sample at each stage of growth are most helpful.

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Each kind of agricultural seed when planted has certain stages of growth when traits may be expressed which help differentiate varieties of a particular kind. Stage of maturity is a key trait, best expressed at the flowering and heading stages (Fig. 1). However, stage of maturity notes should be only one of several readings made during the growing season. Also, readings should include phenotypic characteristics, environmental changes and any other supportive data.

In addition to making field tests of those kinds where varietal identification is not possible through seed characteristics, we do some field testing of separations made by analysts in the laboratory. This type of testing can promote confidence in the analyst. Also, it provides support for seed characteristics used to make these separations.

During the past eight years, mislabeling of rye seed as to variety name has been a serious problem. By making two field plantings, both in the spring and fall, of each sample, we were able to provide satisfactory testing of varieties and determine those that were mislabeled as to variety (Fig. 2). Using this field testing program, we showed that many declarations of variety by the grower or shipper were not valid. As a result of the field tests, we were able to promulgate a regulation in Oklahoma providing that rye seed could be labeled as to variety only when the seed was the certified class or higher of certified seed. A special permit for varietal labeling of rye seed

Figure 1. An example of tall off-type oat plants detected in a shorter variety.

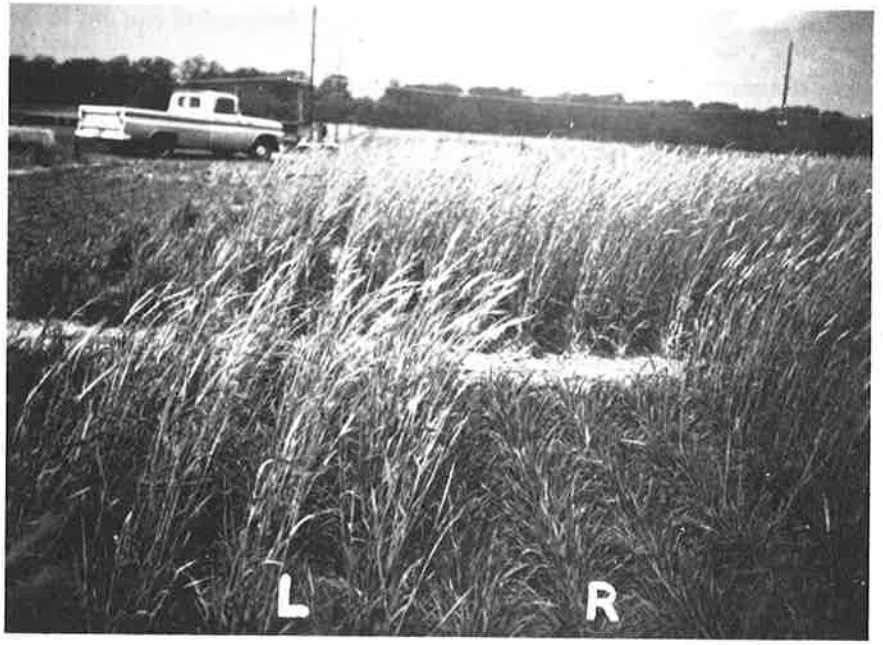

Figure 2. A field planting of rye, showing a check sample of the variety Elbon on the left (L) and a sample mislabeled as to the variety Elbon on the right (R).

can be issued if the seed is no more than one year from the certified class.

Field testing is also carried out on samples of oats, barley and wheat seed. Because cereal grains have a quick, high tillering ability, plant counts need to be made soon after emergence. Also, I have found that notes on plant leaf color and the plants growth habit are best made prior to the "jointing stage." As mentioned previously, some of the samples in field testing are to support laboratory separations that were made by seed characteristics or by other tests such as the infra-red (blacklight) for oat and phenol reaction for wheat seed.

In Oklahoma, field tests for varietal differentiation have been carried out on sorghum varieties longer than any other kind of seed. At first the field tests were used to determine the difference between the "sumac" varieties, millets, and white-seeded sorghums, many of which had the same seed characteristics. Later, the sorghum and sorghum-sudangrass hybrids were included in the field testing program. Field tests on the hybrids showed mainly the offtypes (forage type plants in grain varieties, male sterile plants, or sudangrass and Johnsongrass offtypes or out crosses).

There is some field testing of cowpea seed. Variety names of cowpeas are often descriptive of the variety, for example 'Pinkeye Purple Hull.' Also, in addition to varieties we have different "types" of cowpea such as

"Crowder." Both the descriptive name and type designation can aid in the identification of the plants in the field test.

In summary, field planting of seed for varietal verification can be very exacting. However, you should be aware that seed, soil, climate, and field management can all contribute to the varietal response and should be taken into consideration when making the final verification.

CULTIVAR VERIFICATION TESTING AS AN INTEGRAL PART OF SEED TESTING IN CANADA[1]

A. B. Ednie, N. S. Dhesi, and J. Pauksens[2]

Abstract

Cultivar Verification Testing started in Canada on a small scale in the early 1930's. A full field plot testing program covering the cultivar purity testing of pedigree seed was initiated by Agriculture Canada's Plant Products Division in 1959. The application of laboratory testing methods and the development of laboratory methods was started after 1965 and has led to the development or modification of many routine laboratory test methods. Cultivar purity testing programs have been initiated in the Plant Products Division District Seed Laboratories in order that these laboratories can provide this type of analytical service to its districts enforcement staff and other persons involved in the seed pedigree system.

Additional index words: **cultivar purity testing, seed testing organization.**

Introduction

I would like to thank the Program Committee for providing us with this opportunity to discuss how we in the Plant Products Division of Agriculture Canada are approaching cultivar verification testing.

Cultivar verification testing was started in earnest by the Plant Products Division in 1959 with the creation of a professional position to be responsible for analyzing seed for varietal purity. Prior to this time, field plot tests were carried out in the Maritimes, mainly on vegetable crops and grasses. From time to time samples were also tested at various federal research stations upon request and were examined by plant breeders located at these stations.

Mr. John Pauksens was appointed to the position mentioned above and due to his hard work and perseverence he is now directly in charge of 45 acres of test plots in Ottawa and co-ordinates the testing done on a further 50 acres of plots in Regina, Saskatchewan.

Dr. Dhesi joined John in 1965 as his assistant and, through his efforts in experimenting with laboratory test methods and adapting them to Canadian cultivars, we gradually build up laboratory methods for testing seed samples for trueness to cultivar. Various officers-in-charge of our district laboratories were also dabbling in cultivar verification techniques and using them to deal with specific situations as they arose. By the late 1960's it

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[2]Laboratory Head and Biologists.

became evident that more emphasis had to be placed on laboratory methods because of the unduly long turn-around time involved with field tests because of the way in which laboratory tests could complement field tests in adding additional characteristics that could separate cultivars. The Division then decided that a major effort should be made to incorporate a laboratory cultivar verification testing program into the district seed laboratories.

At present, we have an organization set-up where there are two units of the Seed Biology Laboratory involved in cultivar verification. John Pauksens is in charge of the Cultivar Verification Control Testing Unit whose main function is to carry out the routine testing of official samples using both field plot and laboratory tests. He is overseeing the annual testing of some 7,000 samples. His Unit also maintains the control samples or breeder seed samples of each crop kind licensed in Canada. His Unit also prepares field inspection guides for our Division's field staff and assists in annual training schools held by various districts prior to the initiation of their field inspection program. His Unit also reviews new cultivars prior to licensing so that they are adequately described both for licensing purposes and for field inspection purposes.

Dr. Dhesi is now in charge of the Cultivar Verification Method Development (CVMD) Unit. This Unit is responsible for the development of new routine testing methods that can be easily carried out in the district laboratories and for functional direction regarding the use of these methods in the district laboratories. This Unit also works on the development of specialized biochemical testing techniques and provides a special test service program to the Division's headquarters section responsible for Canada's seed program and to the Division's six districts. The Unit organizes workshops and seed schools aimed at giving basic training to seed analysts from the districts who have been selected to move into this area of seed testing. Dr. Dhesi's Unit also collaborates with J. Pauksens' Unit in carrying out investigations on problem samples sent in from the districts as well as from headquarters.

The major thrust of our testing program in Ottawa is at the early generations of pedigreed seed as post-control testing at this level is a pre-control for the next generation down. The cost benefit is also greatest here as a problem lot removed from the seed multiplication system at an early stage can effect greatest saving in time, dollars and hardship. The major crops grown in Canada which are tested for cultivar purity are listed in Table 1. The district laboratories have the responsibility of developing a testing capability to provide an analytical service to their districts enforcement needs and to the various people and organizations involved in the pedigree seed system. Each district is responsible for developing their own monitoring program and their respective laboratories are preparing themselves to provide the analytical backup for these programs.

Table 1. Crop kinds tested for cultivar purity in Canada and the number of cultivar involved.

Crop Group	Crop Kind	Number of Cultivars
Cereals	Common wheat	38
"	Durum wheat	9
"	Oats	38
"	Barley	46
"	Rye	15
"	Triticale	2
"	Buckwheat	5
"	Corn	343
Field Legumes	Pea	6
"	Field beans	22
"	Faba beans	4
"	Soybean	22
Oil Seeds	Rape	14
	Mustard	4
	Flax	2
	Sunflower	10
Root Crops	Rutabaga	2
Forage Legumes	Alfalfa	36
	Clovers	25
	Trefoil	8
	Sainfoin	1
	Vetch	2
Forage Grasses	Timothy	39
	Bromegrass	11
	Orchardgrass	17
	Fescues	22
	Reed canary	4
	Rye grass	4
	Sudan grass	6
	Sorghum	36
	Millet	2
Lawn Grasses	Bluegrass	21
	Bent grass	10
	Wheat grasses	10
	Wild rye	4
	TOTAL	840

Field Plot Testing

As mentioned previously, field plot tests are located in Ottawa, Ontario (45 acres) and Regina, Saskatchewan (50 acres). Field plot testing follows the general principles described in the International Rules for Seed Testing (3) and the Organization for Economic Co-operation and Development (OECD) standards (1). Space planting is used for post control tests of corn, root crops and sunflower and for investigational purposes on forage crop control samples. All other samples are sown in row plots with a self-propelled seeder. To facilitate the cultural operations a standard plot length of 7 m is used for most of the crops.

Barley, buckwheat, flax, oats, wheat and rape are sown in four row plots. Large seeded field legumes and all forage crops are sown in two row plots. Table 2 outlines details of the plot size, row spacing and number of plants per plot and per sample of various crops.

Detailed notes are taken for plant characteristics through the various stages of growth until maturity. A strict crop rotation program is followed to eliminate the possibility of volunteer contaminant plants.

Laboratory Testing

Laboratory tests based on several established reliable characteristics are now being used. Most of these have been included in the "Methods and Procedures of Seed Testing in Canada" (2). The methods are used routinely for screening out contaminated seed lots. At present, routine laboratory testing is mainly confined to wheat, barley, oats, corn, soybeans, clovers, rapeseed,

Table 2. Design of field plots used for testing cultivar purity.

Crop Type	Plot Specifications							
	Row Length (M)	Rows per plot	Space row to row (cm)	Distance between plots (cm)	Number of repli-cates	Number of plants per plot	Number of plants per sample	Duration of tests (years)
Row seeding								
Small grain field crops	7	4	25	50	2	1500	3000	1
Large seeded field legumes	7	2	30	75	2	150	300	1
Small seeded forage legumes and grasses	7	2	30	75	2	200	400	2
Spaced planting								
Root crops, sunflower	7	2	30	75	8	50	400	1
Corn (maize)	30	1	90	90	2	100	200	1
Forage grasses and legumes (controls)	7	1	90	90	6	10	60	2

ryegrasses, timothy and bird's-foot trefoil. Table 3 gives a brief outline of the tests which are being performed with each of the crops.

The separation of wheat and barley cultivars by kernel characteristics is conducted according to the Handbook on Varietal Identification by Kernel Characteristics by C. H. Owen and M. M. Ainslie (11). The phenol test described by Walls (12) is used routinely for testing wheat samples for cultivar impurities. Recently, we have found that extending the test to 48 hours has enabled us to separate additional cultivars which were indistinguishable after the normal four-hour treatment period. Seed Treatment with thiram

Table 3. Seed and seedling characteristics being used to test for cultivar purity.

Seed or seedling characteristic used

Crop Kinds	Seed					Seedling										
	Morphology	Fluorescence	Aleurone colour	Phenol test	GLC for fatty acids	Coleoptile colour	Coleoptile length	Hypocoty colour	Pubescence	Internode length	Leaf sheath length	Size and type	Forced flowering	Growth regulator response	TLC-phenols for phenolic compound	Electrophoresis for isozymes
Wheat	X			X		X			X					X		
Barley	X		X				X			X	X					X
Oats	X	X				X			X							
Corn																X
Soybeans	X							X							X	
Clovers													X			
Timothy												X				
Ryegrass													X			
B.F. Trefoil					X							X				
Rapeseed																

was also found to affect the phenol test results. Treated seed is now rinsed with methanol prior to testing, to remove seed treatment. Barley samples are routinely checked for purity of aleurone color.

A one percent HCl solution is used to enhance the aleurone color of barley (8). Controlled environment conditions are also used to separate barley cultivars by differential growth rates of coleoptile, first leaf sheath and first internodes (10). Differential growth rate response of cultivars to gibberellic acid (7) is also used for separating wheat cultivars. Anthrocyanin pigmentation on coleoptile and pubescence on leaf and leaf sheath (9) is used to separate cultivars of oats. Soybean cultivars are separated by leaf phenolic compound content (6). The separation of early and late cultivars of red clover is completed using controlled environmental conditions (5). Samples of pedigreed rapeseed are all tested for erucic acid content by the gas-liquid chromatography method developed by Barrette (4).

The CVMD Unit in Ottawa is investigating the use of biochemical methods for the detection of minor differences between cultivars based on chemical and biochemical ingredients of the plant tissues and results appear to be promising.

Gel-electrophoresis has been used successfully in the differentiation of barley and corn cultivars on the basis of isoenzyme difference. Presently the Seed Biology Laboratory, in collaboration with Guelph University, has a project to determine the validity and utility of isoenzyme pattern differences as a tool for identification of corn hybrids, and to develop a testing system based on isozyme assay. One post graduate student is working on this project under an extramural grant from Agriculture Canada. An isozyme laboratory, with all the equipment and facilities, is now being set up in the Seed Biology Laboratory.

Thin-layer chromatography has been a useful tool for the identification of soybean cultivars on the basis of phenolic compound differences, gas-liquid chromatography is already being used as a routine test for the quantitation of certain fatty acids of the rapeseed cultivars. Up to the present, these chemical analysis methods have been used mostly for complaint and investigational samples.

It is difficult to envisage how many of these chemical and biochemical tests will be provided by all the district laboratories in the future. However, they would provide a new challenge for our analysts who wish to take up a new area of expertise in seed testing. As mentioned earlier, our Division is anxious to maintain and increase the development of its ability to carry out laboratory analysis for cultivar purity. The district laboratories are having to organize themselves to meet this demand; however, they must do so within present resource allocation restraints. There are many difficulties to overcome in meeting the goals; however, the problem should provide an interesting challenge to both the officers-in-charge of the laboratories and to analysts wishing to move into new field of seed testing.

The challenge of organization of the laboratories is being met by basically two systems. One organizational structure is to create a separate unit responsible for cultivar verification testing thus adding a third section to the traditional laboratory organization of having purity and germination section. In this situation the section is equipped to carry out seedling and seed tests independently of the other sections with samples being submitted separately to this section. The section may work in conjunction with the purity section in that the purity analyst can screen out samples with obvious problems and refer them to the specific unit.

A second system is to have the already established purity and germination sections take on the added responsibilities of doing cultivar verification testing as well as their normal testing and have a position established to coordinate the work. This system offers the interested purity or germination analyst an opportunity to take on new responsibilities and challenges. At present both systems are being used by our Division's district seed laboratories depending on their staff size and space allocation.

One must be aware that cultivar purity testing in the laboratory provides a new challenge to the seed analyst as the final report on a sample may have entailed a series of tests rather than just one test as is the case for purity and germination. This is demonstrated by (Fig. 1) which details how one can proceed to analyze a sample of winter wheat. Note that two working samples are tested using a different test procedure on each working sample to enable the identification of the presence of seeds of another cultivar(s) in the submitted sample taken from a lot labelled to be of a particular cultivar. All present winter wheat cultivars in Canada can be separated by using a combination of test procedures.

However, this is the exception rather than the rule, therefore, another challenge we must face is the fact that a large number of the cultivars

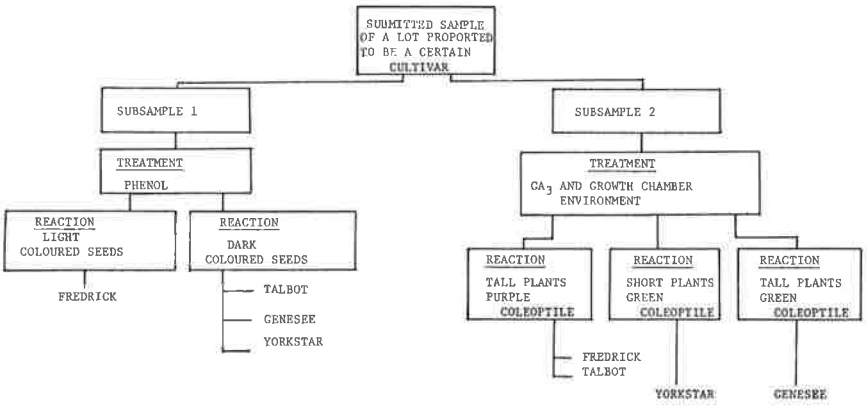

Figure 1. Multiple cultivar purity testing system for detecting cultivar impurities in eastern winter wheat cultivars.

licensed in Canada are closely related genetically making them very difficult to separate. At present we are able, through the use of various tests based on different seed or seedling characteristics, to identify groups of cultivars of similar characteristics. Our method development activity is focused on finding a new test that can be used to separate the members of each group. We are fortunate that the varieties developed in Canada are for the various regions of Canada; therefore, any one laboratory does not have to be able to differentiate all the wheat varieties in Canada. However, the cereals still present major problems in separation. This challenge will be met with the development of expertise by our analysts and their use of their ability acquired during seed testing to observe subtle morphological differences that can be used to differentiate varieties. This phenomenon is already occurring and the frequency of success will increase with our increased effort and experience in cultivar verification testing.

In summary then I have taken this opportunity to outline our Department's organization and efforts with regard to cultivar verification testing. I think seed analysts should look forward to the expansion in this area of testing as a challenge that will provide them with an opportunity to increase the many abilities they now have in a very interesting and new field of work.

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A PLANT BREEDER LOOKS AT THE PROBLEM OF DISTINGUISHING VARIETIES[1]

John W. Schmidt[2]

The degree of varietal purity expected in crop plants depends largely on the form of pollination. However, even in highly self-pollinated crops, such as the small grains, newly-released cultivars may vary considerably in their genetic purity. A cultivar may appear genetically uniform based on morphological criteria yet be quite variable on a physiological basis. The desired purity in a small grain cultivar is influenced by the requirements for seed certification and industrial or food use, and the need for stability in varying environments (drouth, cold, diseases, insects). The actual purity is influenced a great deal by the selection procedures used in developing the cultivar, and these in turn depend to a considerable extent on the philosophy of the breeder. The need to reduce the cost of a plant breeding program, the desire to reduce the time requirement for developing a cultivar and the expected "life time" of the cultivar also have their effect.

In the earlier part of the century, new small grain cultivars were mostly individual plant selections from "land varieties." Such "land varieties" usually could be described as a heterogeneous population of related and homozygous individuals, often quite similar to each other as a result of natural and artificial mass selection. Thus individual head or plant selections could produce a very uniform progeny with a high degree of genetic purity—hence the image of "pure line varieties."

Today most cultivars do not originate as selections from such "land varieties" but from selections within segregating populations arising from hybridization. The simple single cross of yesterday between two relatively homozygous parental lines is today often replaced by three-way and double crosses among parental lines which themselves genetically could retain some heterozygosity and heterogeneity. The variability in such hybrid populations thus depends on the genetic dissimilarity of the parental lines used and, also, on the generation of selection. There is today a trend toward broadening the germplasm by using genetically dissimilar parental stocks and to select in early generations following a cross and then to accept more genetic heterogeneity in the resulting selection. Why would anyone practice early generation selection? Early generation selection may reduce the time needed to develop a new cultivar and thus save both time and money. Further, an early generation selection would be expected to consist of a composite of genotypes and thus may have an increased range of adaptation

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and performance through population buffering (1). The cooperative state-federal small grains breeding program at the University of Nebraska—Lincoln emphasizes early generation selection on the premise that it has been an effective factor in the production of widely-adapted hard red winter wheat cultivars (2, 3). Also, according to Shebeski and Evans (4), if 25 independent favorable gene pairs are segregating in a cross between two self-pollinating parental lines, then in the F_2, 1 plant in 1330 could have all the favorable genes (not homozygous, of course) while in the F_4 only 1 plant in 1.8 million will still have the same chance. Thus, the chances of deriving a selected population with the greatest number of favorable factors is greatest in the F_2 and diminishes rapidly with delayed selection. However, the chances of obtaining a F_2-derived line with any great degree of uniformity are remote. Those chances increase with delayed generation selection. Thus a selection in the F_3 generation is a compromise between having the largest number of favorable genes in a population and a greater probability of passable uniformity. Obviously, here there could be a possible conflict between a breeding philosophy and the more rigid requirements of seed certification and plant variety protection standards. However, breeders are attempting to produce new cultivars as uniform as practical for the easily distinguishing traits.

Variants in newly-released cultivars may arise from a number of sources. These cultivars during their testing period and early increase are present in a nursery containing a larger array of genotypes and outcrossing can occur. While small grain breeders attempt to remove such hybrids from these seed stocks, some escape detection and their delayed appearance in later generations is due to the natural segregation process. Also, some of these variants are contaminants that occur in the process of handling hundreds and thousands of lines in the breeding and testing nurseries. Other variants are due to a chromosome loss or a mutation. Finally, variants, such as those for plant height, are more visible today as plant height is being reduced either by choosing transgressive segregants for shorter height or by incorporating genes for dwarfness. Tall plant height variants are simply more obvious in the resulting dwarf, semi-dwarf or reduced height cultivars than in the older, taller cultivars.

A second point that is often raised by non-breeders concerned with cultivar identification is the lack of distinguishing markers. Within a crop group such as hard red winter wheat there is not a wealth of distinguishing characteristics and many cultivars are closely related. For example, in the 1978 Southern Regional Performance Nursery (Hard Red Winter Wheat) at least 9 of the 23 experimental lines have the 'Centurk' cultivar as one parent. Thus, these lines are bound to have many characteristics in common. Further, in the Hard Red Winter Region today there are 11 cultivars that are either selections or backcross derivatives from 'Scout.'

When we plan the current year's crosses for the Nebraska wheat breeding

program, we do not give high priority to the presence of marker genes. The purpose for the cross determines the parent lines used. The following example may be beneficial:

Goal: Develop a hard red winter wheat cultivar with improved stem rust resistance.

Choice of parents: Centurk and 'Sage', two widely adapted cultivars with different genes for stem rust resistance.

Attributes	*Centurk*	*Sage*
Stem rust resistance	SR 5, 6, 8, 9a, 17	SR 2, 17, 24
Glumes	white	white
Awns	long	long
Beak length	intermediate	short
Coleoptile color	green	green
Kernel type	small, hard, red	large, hard, red
Plant height (1977 SRPN)	88 cm	90 cm
Maturity (days to flowering after January 1)	150 days	149 days
Dough mixing requirement	long	short
Reaction to soil borne mosaic virus	intermediate	very susceptible
Reaction to wheat streak mosaic virus	moderately susceptible	moderately tolerant

Lines derived from the above cross may not have very obvious distinguishing characteristics yet have considerable value as cultivars. The best distinguishing characteristic would be the combined stem rust resistance identifiable specifically only through seedling rust reaction testing. This suggests that the term "marker" needs to be expanded to include certain disease and insect reaction tests. Need for a specific marker would have dictated against the above cross, or if required would have added considerably greater effort and cost.

Finally, there is today considerable emphasis on multiline cultivars. The Iowa State Agricultural Experiment Station has had early and late season multiline oat cultivars for some years now. The international spring wheat program of CIMMYT (International Center for the Improvement of Maize and Wheat) is strongly into multilines now.

What is a multiline? It is a method by which a fairly uniform appearing cultivar is constituted from a set of sublines where each subline may contain a genetic component for a trait different from that of the other sublines. A good example is a multiline wheat cultivar made up of a number of morphologically similar sublines each having a different gene for stem rust resistance—SR5, SR6, SR7, SR8, SR10, SR17, SR24, etc. In essence this is a composite of concurrent backcross lines. Such a population

may be more stable for stem rust resistance than the so-called pure-line cultivar. Obviously, this does not simplify varietal classification.

I would summarize this discussion thus: 1) The pure line theory is still as valid as it ever was, but many small grain breeders are interested in cultivars with some useful heterogeneity, partly as a hedge against genetic vulnerability. 2) Early generation selection, in addition to providing a degree of heterogeneity, reduces the cost and time needed to develop new cultivars. 3) Choice of parent lines used in hybridization programs is dependent on the reason for the cross. Inclusion of marker genes usually would be incidental and if required might add to the cost and effort required to produce the desired end product. 4) Multiline cultivars are commanding more attention. Their use will add complexity to varietal classification.

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SOME NEW TESTS AND PROCEDURES FOR DETERMINING VARIETY (SOYBEANS)

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Seed samples are tested for cultivar purity for several reasons. Cultivar testing by State seed laboratory personnel is usually directed toward determining if a seed sample is correctly labeled as to cultivar and free of off-types. This usually results in testing to determine if the sample or a portion of the sample has one or more characteristics that differ from those of the cultivar recorded on the label. In most cases it is not essential to identify a mislabeled sample as to cultivar. Plant breeders, seed firms and commercial seed testing laboratories, on the other hand, may be interested in actually identifying the sample being tested. Identification of a seed sample as to cultivar is usually a much more difficult task than determining if a sample is correctly labeled.

The most useful type of cultivar testing procedure would be one that is inexpensive, technically uncomplicated, and requires little time to complete. It should also be adapted to testing single seeds or seedlings, thereby allowing for the detection of off-types. Examples of this type of test are the phenol test for wheat seed and the fluorescence test for oat seed and ryegrass seedlings.

The use of taxonomic keys to identify species of naturally occurring and cultivated plants with observations of a series of morphological characteristics is well known. The keys using seed characteristics found in Agriculture Handbooks No. 30 and No. 219 have been valuable as aids in identifying crop species. However, with the increased number of crop cultivars being developed by plant breeders, it is impossible to identify many cultivars by seed characteristics. A logical approach to this problem would be the expansion of the seed key to include seedling characteristics and a series of tests designed to reveal cultivar related biochemical and physiological differences of seeds and seedlings.

Recently there have been several series of tests proposed for identifying wheat cultivars (34, 50). These groups of tests begin with the evaluation of seed characteristics and progress to observations and tests requiring greater expense, technical expertise, and time to complete.

In this paper a series of 13 tests and observations with which it was possible to identify 52 out of 60 commonly grown soybean cultivars are presented and discussed.

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Hilum Color Evaluation

Background: Soybean hilum color is controlled by alleles of four genes (5). Some of these genes are pleiotrophic and also control flower, hypocotyl, and pubescence color (5). Seeds of soybean cultivars are usually classified as having clear, buff, brown, black, or imperfect black hila. The imperfect black hilum consists of a black center surrounded by a brown (20) or buff (5) ring. Occasional mention is made of soybean seeds with gray hila (5, 33).

Procedure: We have classified cultivars as having either clear, buff, brown, black, or imperfect black hila by visual observation and magnification with a hand lens (Tables 1–5). Since single seeds can be evaluated, this procedure is useful for the detection of off-type seeds as well as cultivar identification.

Slight differences in hilum pigmentation may prove useful to further differentiate soybean cultivars placed in the same hilum color class. For example, under magnification pale green pigmentation appears to be present in the center of the hila of 'Essex' soybean seeds while pigmentation in this location appears to be pinkish-buff in seeds of most other cultivars classified as having buff hila (personal communication, Mr. Gail Fenderson).

Criticism: It has been reported that fungal infection may cause discoloration and darkening of the hilum (37). This could result in infected seeds being mistakenly classified as off-types. It is also suspected that the treatment of soybean plants with the fungicide benomyl used in an attempt to improve the quality of seed produced, may result in the darkening of hilum pigmentation.

Occasionally we have had difficulty determining if certain seeds had buff or brown hila, or in other cases, black or imperfect black hila. Problems of this type can be solved by planting the seeds and observing the pubescence color of the resulting seedlings. Seeds with clear, buff or imperfect black hila produce seedlings with gray pubescence while seeds with brown or black hila produce seeds with tawny pubescence (5). In addition, all seeds with imperfect black hila produce seedlings with purple hypocotyl and flower color (5).

Seed Coat Peroxidase Test

Background: Buttery and Buzzell (9) were able to separate soybean cultivars into two groups based on the presence of either high or low seed coat peroxidase activity. A genetic analysis revealed that seed coat peroxidase activity is controlled by a major gene (12). High activity results from the presence of the dominant allele while low activity results from the presence of the homozygous recessive alleles (12).

Procedure: Seed coats were removed from individual seeds and placed in separate test tubes. Seed coats can be removed easily if the seed is first cut in

half through the long axis of the hilum with a razor blade. Since peroxidase activity is detectable in cotyledonary tissue, all fragments of cotyledon adhering to the seed coat should be removed. Ten drops of 0.5% guaiacol were added to each test tube and after 10 min a drop of 0.1% hydrogen peroxide was added. The appearance of a dark reddish-brown color in the solution indicating high peroxidase activity (+) or the absence of this color indicating low peroxidase activity (−) was recorded after 60 sec. Most cultivars were classified as having all seeds that are either peroxidase (+) or peroxidase (−) (Tables 1–5). However, several cultivars are comprised of both peroxidase (+) and peroxidase (−) seeds (Tables 1–5). Since single seeds can be evaluated, the peroxidase test can be used to detect off-type seeds in a seed sample. References: 9, 39.

Criticism: Experiments indicate that the outcome of the seed coat peroxidase test is unaffected by seed quality or seed storage conditions (39). However, when we tested seed coats removed from small, green, immature seeds of peroxidase negative cultivars, high peroxidase activity was detected. Seed coat peroxidase activity decreased as these immature seeds dried until it was undetectable in seed coats of seeds with a moisture content below 20 percent. It appears that high seed moisture may cause an increase in seed coat peroxidase activity of seeds normally testing peroxidase negative. Therefore, seeds with a high moisture content should be dried before they are tested with this procedure.

Hypocotyl Length Test

Background: When germinated in the dark for 10 days at 25 C, large seeds of cultivars such as 'Corsoy' produced seedlings with long hypocotyls while large seeds of cultivars such as 'Amsoy 71' produced seedlings with short hypocotyls (6, 41, 44). Small seeds of all cultivars produced seedlings with long hypocotyls (6, 41, 44). It has been suggested that an inhibitor, present in cotyledonary tissue of certain soybean cultivars, acts to suppress hypocotyl elongation during germination in the dark at 25 C (8). One (17) to several (21) genes, with dominance for long hypocotyls, may be involved in this expression of hypocotyl length.

Procedure: Large and small seeds were separated from each soybean sample. Seeds considered large were held by a screen with 6.7 mm (17/64 in) round holes and seeds considered small passed through a screen with 5.95 mm (15/64 in) round holes. At least 50 seeds of each size from each sample were tested. The seeds were placed along the 65 cm edge of 65 × 30 cm moistened germination towel, 4 cm from the top of the towel and 4 cm apart. After positioning the seeds, the towels were rolled and placed vertically in 15 × 15 × 15 cm plastic containers with closed bottoms and sides (one gallon plastic bottles with tops removed). Six rolled towels and 150 ml water were put in each container. The containers were placed in a darkened germinator at 25 C. At the end of 10 days hypocotyl length was meas-

ured to the nearest 1.0 mm and sample averages were calculated. Seedlings with damaged hypocotyls or broken cotyledons were considered abnormal and not measured. The hypocotyl length of seedlings germinating from small seeds served as a comparative check to insure that the shorter hypocotyl length recorded for large seeds of certain cultivars was real and not due to abnormal germination conditions. Cultivars were recorded as having large seeds that produced seedlings with short, intermediate, or long hypocotyl when compared to seedlings of the small seed check samples (Tables 1–5). References: 7, 41, 44.

Criticism: Since there is some variation in hypocotyl length among seedlings of the same sample, this testing procedure is not well adapted to the detection of off-types. Evidence indicates, if abnormal seedlings are discounted, seed quality (vigor) has little affect on the results of the hypocotyl length test (44). However, difficulties can arise in obtaining an adequate number of seedlings for evaluation from samples with low germination percentages. In addition, deviation from the 25 C temperature tend to reduce the cultivar related differences in hypocotyl length (18).

Seedling Observations and Tests

Growth chamber planting instructions: Seeds, spaced 2.5 cm apart in rows 5.0 cm apart, were planted 2.5 cm deep in 46.0 × 30.5 × 7.5 cm flats filled to a depth of 6.0 cm with quartz sand. Seedlings were watered with Hoaglands Number 1 complete nutrient solution (23) every 4th day and distilled water on other days, unless stated otherwise. The position of the flats in the growth chamber was changed daily to minimize possible microenvironmental effects on the seedlings. The temperature in the growth chamber was maintained constantly at 24.5 ± 1.0 C. Unless stated otherwise, seedlings were exposed to continuous light at 505μ $Em^{-2}S^{1}$ (3200 fc) from incandescent bulbs and cool white fluorescent tubes measured at the surface of the sand in the flats. References: 36, 46.

Each of the four seedling observations that follow can be used to evaluate individual seedlings and therefore has the potential to detect off-type seedlings.

Seedling Pigmentation

Background: Traditionally soybean cultivars have been separated into two groups based upon having seedlings with either green or purple hypocotyl color. It has been observed that seedlings with purple hypocotyl color develop purple flowers while seedlings with green hypocotyl color develop white flowers. This is reportedly due to the pleiotrophic effect of one gene (5). However, recently cultivar related differences in the degree and pattern of pigmentation have been detected in seedlings with both green and purple hypocotyls grown in continuous intense light (36, 46, 48). A genetically

based linkage between pubescence color and certain pigmentation patterns has also been reported (38).

Procedure: Seedlings were evaluated for pigmentation development upon emergence and for the next 20 days. Five distinct pigmentation patterns were observed: green (green pigmentation only); bronze (bronze pigmentation on the lower hypocotyl); light purple (purple pigmentation mostly confined to the hypocotyl); dark purple (dark purple pigmentation on the hypocotyl and epicotyl); and intermediate purple (dark purple hypocotyl with light purple epicotyl). Each cultivar was characterized as having seedlings with one pigmentation pattern (Tables 1–5). References: 46, 48.

Criticism: The extent of pigmentation development in soybean seedlings can be influenced by photoperiod length and intensity and the phosphate content of the growing medium (46). For instance, seedlings of cultivars classified as intermediate purple when grown under continuous illumination of 3600 fc, appeared light purple when grown under lesser illumination (2400 fc) (46) or in the field (35, 46). In addition, daily high phosphate nutrient applications reduced purple pigmentation development significantly (46).

Pubescence Color

Background: Soybean cultivars have been differentiated into two groups based on the presence of either tawny or gray stem pubescence observed on field grown plants. Pubescence color is controlled by two gene pairs (3). One gene pair, with tawny being dominant and gray recessive, controls the presence or absence of brown pigmentation (3). This gene is pleiotrophic, also exerting an influence on hilum color (3, 5). The other gene pair regulates the intensity of the brown pigmentation (3).

Procedure: The color of pubescence on the edge of trifoliate leaflets was observed on seedlings, 20 days after emergence with the aid of 40 X magnification. Seedlings were classified as having either tawny or gray pubescence. One pubescence color was characteristic of all seedlings of a cultivar (Table 1–5). Reference: 47.

Criticism: Our classification of leaflet pubescence color in the laboratory agreed, in every instance, with our field observations of stem pubescence color (35). However, diseased or chlorotic seedlings should not be evaluated for leaflet pubescence color since these abnormalities could lead to a distortion of pubescence pigmentation. All cultivars in Tables 1–5 having seeds with black or brown hila have pubescence that is recognizably tawny. However, it is genetically possible that cultivars may be developed with black or brown hila that will have light tawny or near gray pubescence color. This pubescence color could be misclassified as gray.

Pubescence Angle

Background: Grabe (20) classified primary leaf pubescence as erect or appressed on greenhouse grown seedlings of a number of soybean cultivars.

Recently Bernard (4) indicated that pubescence erectness on the upper leaf surface is under two-gene control. He was able to classify plants resulting from an experimental cross as having erect, semi-erect, or appressed pubescence on the upper leaf surface (4).

Procedure: Pubescence angle was determined by observing the pubescence on the upper surface of fully expanded trifoliate leaflets of plants, 20 days or more after emergence. The leaflets were held parallel to the line of sight and magnified with a hand lens. Observations were confined to pubescence at a distance from the midvein, since pubescence in the midvein region tends to be erect on all samples. Pubescence angle was recorded as erect, intermediate, or appressed (Tables 1–5). Most cultivars were characterized as having seedlings with erect pubescence. Reference: 47.

Criticism: Our experiments indicate that the light intensity to which seedlings are exposed and seedling turgidity may have some effect on pubescence erectness. Variation in these conditions could make the consistent classification of pubescence angle of seedlings with intermediate angle pubescence difficult.

Leaflet Shape

Background: Most soybean cultivars are characterized as having plants with broad or ovate leaflets. However, a few cultivars are reported to have plants with narrow or lanceolate leaflets. The narrow leaflet trait, controlled by one gene pair (5), is associated with a high number of seeds per pod, frequently four (24).

Procedure: Leaflet shape was determined by observing the shape and calculating the length/width ratio of fully expanded trifoliate leaflets on seedlings approximately 20 days after emergence. Cultivars were classified as having seedlings with either lanceolate or ovate leaflets (Tables 1–5). Seedlings of all the SRF (Soybean Research Foundation) cultivars evaluated were classified as having lanceolate leaflets with average length/width ratios of 2.0 or higher (40). Seedlings of all other cultivars had ovate leaflets with average length/width ratios below 1.6 (40). Reference: 40.

Criticism: For all samples, our leaflet shape classification of growth chamber grown seedlings was identical to that recorded for field grown plants prior to flowering (35). However, leaflets that expanded after flowering on field grown plants of certain "ovate leaflet" cultivars with indeterminate growth habit appeared lanceolate (45). Therefore, the growth stage at which leaflet shape is determined can be important.

It cannot be assumed that all cultivars with lanceolate leaflets will be SRF cultivars since there are several cultivars with lanceolate leaflets, such as 'Miles,' being released by agencies other than the Soybean Research Foundation.

Metribuzin Sensitivity

The same seedlings observed for the seedling characteristics previously discussed may also be evaluated for metribuzin sensitivity or photoperiod response.

Background: Metribuzin (4 amino-6-tert-butyl-3 (methylthio)-as-triazine-5(4H) one) is a preemergence or preplant herbicide used to control certain broadleafed weeds in soybean plantings. Plants of several cultivars, such as 'Semmes' and 'Tracy,' have shown signs of metribuzin injury when grown in fields treated with this herbicide (22, 31, 49). It has been reported that the response of soybean cultivars to metribuzin may be controlled by one gene (16), however evidence indicates that several genes may be involved (42). Procedures for evaluating the metribuzin sensitivity of soybean cultivars grown in the field (31, 49) and in hydroponic culture (2) have been published. We have been using a growth chamber procedure to differentiate soybean cultivars by seedling sensitivity to metribuzin (42).

Procedure: Seedlings were grown in the growth chamber under a daily light regime of a 16 hr photoperiod followed by an 8 hr period of darkness. When the unifoliate leaves of the seedlings were fully expanded, 0.30 ppm of metribuzin dissolved in Hoaglands Number 1 complete nutrient solution (23) (adjusted to pH 6.5) was applied directly to the surface of sand in the flats. Five hundred ml of solution was applied to each flat daily until the 10th day of application, when the quantity was increased to 750 ml/flat. Individual plants were evaluated for metribuzin injury daily. The first sign of metribuzin injury was the appearance of brown spots or areas on the foliage. This was followed by desiccation and curling of the leaves, leaf drop and death of the plant. The test was terminated after 38 days of metribuzin application. A certain amount of variation in metribuzin sensitivity among plants of the same cultivar was detected. Therefore, data was reported as the average number of days of metribuzin application required for 75% of the plants of each cultivar to show signs of injury or be killed. Reference: (42).

A continuous gradient of decreasing metribuzin sensitivity among the cultivars tested prevents them from being placed in clearly defined groups by this criteria. For this reason, and since the detailed results are published elsewhere (42), metribuzin sensitivity appears in Tables 1–5 as "sensitive" versus "insensitive" only where it could be critical for the identification of certain cultivars.

Criticism: This testing procedure is useful for differentiating some cultivars. However, since a sample average is needed, it is somewhat limited in its usefulness for detecting off-type seedlings in a sample. In addition, variation in metribuzin concentration, metribuzin-nutrient solution pH, frequency of nutrient application, and photoperiod length can affect test results (42).

***Photoperiod Test* (Flowering)**

Background: Normally, soybean cultivars adapted to southern latitudes require a longer period of time to flower than do cultivars adapted to northern latitudes. However, the length of time needed for southern cultivars to flower can be reduced by growing them under a shorter photoperiod. It has been reported that there are three gene pairs that influence the photoperiod response on flowering in soybeans (5). One of these gene pairs exerts its primary effect when plants are grown under fluorescent lights with incandescent lighting excluded (11, 25).

Procedure: Seedlings, thinned from the original planting density until they were spaced 5 cm apart in rows 5 cm apart, were exposed to daily photoperiods of 10, 13½, 15½ and 18 hrs of fluorescent light followed by appropriate periods of darkness. The number of days from planting required for each plant to produce a flower in which the petals could be observed was recorded. In most cases the tests were terminated 75 days after planting. Data was summarized as the number of days required for half of the plants of each cultivar to flower in each photoperiod treatment. Cases where plants of a cultivar failed to flower before the test was terminated were also noted. Reference: (11, 25).

A continuous gradient of increasing time required for flowering was recorded for the soybean cultivars evaluated (unpublished data). This prevented the cultivars from being placed in clearly defined groups by this criteria. For this reason, and since the detailed results will be published elsewhere, the photoperiod response (early versus late flowering) and critical photoperiod appear in Tables 1–5 only where it could be necessary for the identification of certain cultivars.

Criticism: A small amount of variation in photoperiod response was detected among plants of the same cultivar. Therefore, while this testing procedure is useful for differentiating some cultivars, especially those in different maturity groups, some difficulty may be encountered in detecting off-type plants by photoperiod response.

Protein Electrophoresis

Background: Protein electrophoresis is a technique with which a mixture of proteins can be separated into distinct bands in a gel that has been placed into an electric field. This separation into distinct bands is due to differences in the size (molecular weight) and charge of the proteins involved. After electrophoresis, the protein bands are stained for visual observation with a general protein stain or a stain to indicate the presence of a specific enzyme. The presence of a particular protein in an organism is assumed to be under genetic control. Therefore, samples characterized by different protein bands are considered to differ genetically while samples having the same protein bands may be the same.

General procedure: Proteins were extracted from ground seeds at a rate of 0.1 g of seed/ml of extraction buffer for 1 hr at 4.0 C. The extraction mixture was centrifuged at 27,578 X g for 15 min at 4.0 C. After centrifugation, 100 μl samples of clear protein extract thickened with sucrose were subjected to disc electrophoresis at 4mA/tube for about 1½ hr using 7.0% polyacrylamide gels (7.5 × 0.50 cm). After the protein and enzyme bands were stained, Rf values were calculated to aid in the identification of stained protein or enzyme bands. The Rf value is the distance a band migrates or travels in the gel divided by the total migration distance possible. Reference: 15, 27, 43.

Each of the four electrophoretic procedures that follow can be used to test single seeds and therefore they have the potential to detect off-type seeds in a seed sample.

General Protein

Background: Larsen (27) was able to divide soybean cultivars into two groups based on the presence of either the A or B protein band. These protein bands are controlled by a single gene pair, the alleles of which are codominant (5, 28). The A protein band is thought to have come from 'Mandarin,' an early soybean introduction used in many breeding programs (29).

Procedure: Proteins were extracted in 0.1 N acetate buffer at pH 4.5 (equal amounts 0.1 N sodium acetate and 0.1 N acetic acid) (27). After electrophoresis the gels were stained overnight in 0.2% analine blue-black dissolved in 7.0% acetic acid. Unbound stain was removed in 7.0% acetic acid. The protein bands stained blue. Cultivars were recorded as having either the A or B protein band (Tables 1–5). Reference: 27.

Criticism: With one exception, seeds of all cultivars we have tested with buff, brown or black hila have the B protein band. Cultivars with seeds having clear or imperfect black hila have either the A or B protein band. Therefore, the value of this testing procedure appears limited to testing seeds with clear or imperfect black hila. Results of our tests indicate that the A and B protein band may be the enzyme amylase. The staining technique to detect amylase activity requires a shorter time to complete than the general protein staining procedure.

Urease

Background: Buttery and Buzzell (10) detected two forms of the enzyme urease in electrophoresed soybean seed extracts. One of these, a band located near the top of the gel was designated the "slow" band while the other, a band traveling further in the gel was designated the "fast" band (10). The cultivars they tested were divided into two groups based on the presence of either the "fast" or the "slow" urease band (10). Experimental crosses indicated that the urease isoenzymes were under the control of one gene, with the "fast" band dominant over the "slow" (10).

Procedure: The seed proteins were extracted in distilled water. After electrophoresis the enzymes were stained by soaking the gels in 0.05% cresol red dissolved in 90 ml of 0.2 M acetate buffer (equal amounts of 0.2 M acetic acid and 0.2 M sodium acetate) and 60 ml of 7.0% acetic acid. After soaking for 10 min the gels were transferred to a solution of 1.6% urea, 0.1% Na_2EDTA, and 0.5% cresol red dissolved in distilled water. In 10 to 15 min the urease bands stained red. Most cultivars were classified as having all seeds with either the "fast" band or the "slow" band (Tables 1–5). However, several cultivars were characterized as having some seeds with the "fast" band and other seeds with the "slow" band (Tables 1–5). Reference: 10.

Criticism: It has been reported that when extracted under certain conditions the "slow" band may dissociate into smaller components (10). During electrophoresis these smaller components apparently migrate to a position in the gel similar to the position occupied by the "fast" band (10). Although there was a decrease in staining intensity, we detected no change in urease band position when extracts from poor quality (vigor) seed or seed stored for long periods of time were tested.

Alcohol Dehydrogenase

Background: Up to seven alcohol dehydrogenase bands have been reported in electrophoresed extracts prepared from imbibed soybean cotyledons (19). Three different cultivar specific alcohol dehydrogenase banding patterns were reported for the samples tested (19). Upon critical evaluation, we found two bands that were darkly stained after a short period of staining. The other bands stained faintly after being in the staining solution for a long period of time. Some of these faintly staining bands were absent from electrophoresed extracts of poor quality (vigor) seeds. The other faintly staining bands were artifacts due to the background staining characteristic of prolonged staining with a tetrazolium based stain.

Procedure: Proteins from cotyledons of seeds imbibed on filter paper for 4 hours in the dark at room temperature were extracted in 0.1% SDS (sodium dodecyl sulfate) dissolved in 0.1 N sodium acetate (pH 8.0). After electrophoresis the enzymes were stained by placing the gels in a solution of 10.5 ml of 95% ethanol, 0.0075 g PMS (phenazine methosulfate), 0.0267 g NBT (nitro blue tetrazolium) and 0.045 g NAD dissolved in 150 ml of 0.05 M $Na_2H\ PO_4$ (pH 7.0). The staining gels were kept in the dark at room temperature. After staining for 15 to 30 min the enzyme bands turned blue. The majority of the cultivars tested were characterized as having two bands while the remaining cultivars had one band (Tables 1–5).

Criticism: Only three of the cultivars we tested were characterized as having one alcohol dehydrogenase band. Therefore, while this staining technique would be valuable for identifying seeds of these three cultivars, its general usefulness may be somewhat limited. In addition, care should be taken not to leave the gels in the staining solution for a prolonged period of

time since this could result in the presence of faintly staining enzyme bands that are unstable or artifacts.

Esterase

Background: Five esterase bands have been consistently detected in electrophoresed soybean seed extracts (43). One esterase band (number four, Rf 0.63) was reported to stain darkly for 'Ogden,' 'Kent' and cultivars developed from crosses involving Ogden or Kent (43). This band stained faintly in samples of all other cultivars (43). In addition, another band (number five, Rf 0.81) that stained clearly and with medium intensity in other cultivars, was recorded as diffuse and faint for three cultivars (43).

Procedure: Proteins were extracted in 0.1% SDS (sodium dodecyl sulfate) dissolved in 0.1 N sodium acetate (pH 8.0). The electrophoresed gels were placed in a staining solution containing 75 mg of Fast Blue RR salt, 2.25 ml of substrate solution (20 mg α-naphthyl acetate in 20.0 ml of 50% acetone) and 150 ml of phosphate buffer (0.6 M, pH 6.1). The staining gels were kept in the dark at room temperature for 30 to 60 min. Esterase activity was indicated by the appearance of brown bands. Cultivars were separated into three groups based upon the staining intensity of bands number four (Rf 0.63) and number five (Rf 0.81) (Tables 1–5). Reference: 43.

Criticism: When using differences in the staining intensity of enzyme bands to help differentiate cultivars, care must be taken to assure that these differences in staining intensity are not related to the length of seed storage or seed quality (vigor). We found no evidence of a decrease in staining intensity resulting from poor quality seed or long storage time for the two esterase bands used to aid in cultivar identification (43). However, we did observe variation in staining intensity among seeds of the same cultivar for the remaining three esterase bands (43).

Other Tests

We have evaluated the use of seed characteristics such as size, shape and seed coat bloom (dull versus shiny) for differentiating soybean cultivars. We have found that seed size varies greatly both within and among seedlots of the same cultivar (35). We have also found that seeds of the same cultivar may vary in shape. This may be due to the position of the seed in the seed pod and may be influenced by environmental conditions during the pod filling stage of development. Seeds with dull seed coats that are hand held for observation may pick up oil from the skin and appear shiny. Therefore, while seed size and shape and seed coat bloom can be used to form a general opinion about the identity of a sample, they should not be used in a critical evaluation.

It has also been reported that soybean cultivars differ in characteristics such as the ability to germinate at low temperatures (32) and tolerance to saline conditions (1). When evaluating these testing procedures, we observ-

ed variation in test results among high and low vigor seed lots of the same cultivar. This variation was of sufficient magnitude to mask cultivar differences. Therefore, tests such as these lack reliability due to their reliance on high vigor seeds and would be unsuitable for use in a general testing program.

Check Samples

It is essential that appropriate check samples be tested along with a "test" sample when using any of the procedures previously described. The characteristics or responses of the samples being tested should be compared with those of the check sample. The use of a check sample for comparative purposes will insure that a difference between test results obtained for a "test" sample and those reported for a certain cultivar are not due to a testing procedure malfunction.

We have detected off-types in 15% of the samples (certified or higher class of certified seed) tested. In several cases the entire seed lot was mislabeled. Therefore, care should be taken to obtain authentic check samples from reliable sources.

Conclusions

With the 13 procedures described, we were able to identify 52 of 60 soybean cultivars tested. The remaining eight cultivars were found in four groups of two each. Cultivars in two of the four groups can be separated by different reactions to pathogens. One of these groups is comprised of 'Hood' and 'Hood 75.' Hood is susceptible to *Phytophthora megasperma* Drechs var. *sojae* A. A. Hildeb. while Hood 75 is resistant to races 1, 2 and 3 of this pathogen (14). Several methods for testing soybean seedlings for resistance to races of *Phytophthora* have been reported (26, 30). With a testing procedure of this type Hood could be separated from Hood 75.

Another group of two cultivars is comprised of 'Lee 68' and 'Lee 74.' Lee 74 is resistant to root-knot nematode while Lee 68 is susceptible (13). The other two groups are comprised of 'Wayne' and 'Woodworth' in one group and 'SRF 350' and 'SRF 425' in the other.

In conclusion, it should be possible for seed analysts armed with a "taxonomic key," instructions for a series of tests, and the knowledge of the cultivars grown in their area to identify most soybean cultivars with the aid of several intelligently chosen tests.

Table 1. Key for the identification of soybean cultivars with clear hila.

Cultivar	Single Seed Analysis								Sample Analysis	
	Observation			Electrophoresis						
	Peroxidase	Pigmentation	Leaflet Shape	General Protein	Urease	Esterase .63	Esterase .81	Alcohol Dehydrogenase	Hypocotyl Length	Photoperiod
Mandarin	+	DP	O	A	S	L	M	2	L	E 18
Harosoy 63	+	DP	O	A	S	L	M	2	L	L
Amsoy 71	+	DP	O	A	S	L	M	2	S	
Corsoy	+	DP	O	B	S	L	M	2	L	
Hark	+	DP	O	B	F	L	M	2	S	
SRF 150	+	DP	L	B	F	L	M	2	-	
SRF 200	+	DP	L	A	S	L	M	2	S	
Evans	+	G	O	A	S	L	M	2	L	
Traverse	+	G	O	B	S	L	M	2	L	
Steele	-	DP	O	A	F	L	M	2	I	
Delmar	-	G	O	B	F	L	L	1	L	

The pubescence color is gray and pubescence angle erect for all cultivars.

Table 2. Key for the identification of soybean cultivars with imperfect black hila.

Cultivar	Single Seed Analysis						Sample Analysis	
		Observation	Electrophoresis					
	Peroxidase	Pigmentation	General Protein	Urease	Esterase .63	Esterase .81	Hypocotyl Length	Photoperiod
Wells	-	DP	A	F	L	M	S	E 18
Bonus	-	LP	A	F	L	M	S	L
Beeson	-	LP	A	F,S	D	M	S	
Pickett 71	-	LP	B	F	L	M	L	L 15½
Hawkeye	-	LP	B	F	L	M	L	E
Ogden	-	LP	B	S	D	M	-	
Semmes	+,-	LP*	B	F	L	M	S	

The pubescence color is gray, pubescence angle is erect, leaflet shape is ovate, and there are two alcohol dehydrogenase bands for all cultivars.

Table 3. Key for the identification of soybean cultivars with brown hila.

Cultivar	Single Seed Analysis				Electrophoresis
		Observation			
	Peroxidase	Pigmentation	Leaflet Shape	Pubescence Angle	Urease
Hill	-	B	O	E	F
Coker Hampton 338	-	B	O	I	S
SRF 307P	-	B	L	E	F
S-1474	-	DP	O	E	F,S
Jupiter	+	LP	O	A	F

The pubescence color is tawny, hypocotyl length is long, general protein band is B, esterase band .63 is light, esterase band .81 is medium, and there are two alcohol dehydrogenase bands for all cultivars.

Table 4. Key for the identification of soybean cultivars with buff hila.

Cultivar	Single Seed Analysis								Sample Analysis		
	Observation			Electrophoresis							
	Peroxidase	Pigmentation	Pubescence Angle	General Protein	Urease	ADH	Esterase .63	Esterase .81	Hypocotyl Length	Metribuzin	Photoperiod
Hood	+	LP	I	B	S	2	L	M	L		L
Hood 75	+	LP	I	B	S	2	L	M	L		L 15½
York	+	LP	E	B	S	2	L	M	L		E
Hodgson	+	DP	E	B	F	2	L	M	S		
Agripro 20	+	DP	E	A	S	2	D	M	-		
Davis	+	G	E	B	S	2	L	M	L		
McNair 800	+	G	A	B	S	2	L	M	L		
Cobb	+	G	I	B	S	1	L	M	L		
Dare	-	G	E	B	F,S	2	L	M	L	I	L 15½
Merit	-	G	E	B	F	2	L	M	L	S	E
Coker Hampton 136	-	DP	E	B	S	2	L	M	L		
Coker Hampton 266A	-	DP	I	B	S	2	L	M	L		
Essex	-	LP	E	B	S	1	L	M	L		

The pubescence color is gray and leaflet shape ovate for all cultivars.

Table 5. Key for the identification of soybean cultivars with black hila.

Cultivar	Single Seed Analysis						Sample Analysis		
	Observation			Electrophoresis					
	Peroxidase	Pigmentation	Leaflet Shape	Pubescence Angle	Urease	Esterase .63	Esterase .81	Hypocotyl Length	Metribuzin	Photoperiod
Williams	+	B	O	E	F	L	M	L	I	E L 15½
Forrest	+	B	O	E	F	L	M	L	I	
Swift	+	B	O	E	F	L	L	S		
McNair 600	+	LP	O	E	F	L	M	S		
Kent	+	LP	O	E	S	D	M	I		
SRF 450	+	LP	L	E	S	D	M	I		
Cutler 71	+ -	LP	O	E	S	D	M	S		
Lee 68	-	LP*	O	E	F	L	M	L		E 13½
Lee 74	-	LP*	O	E	F	L	M	L		E
Mack	-	LP	O	E	F	L	M	L		L
Clark 63	-	LP*	O	E	F	L	M	S		L
Chippewa 64	-	LP	O	E	F	L	L	S		E 15½
Calland	-	LP	O	E	F	D	M	L	S	E
Agripro 27	-	LP	O	E	F	D	M	I	S-I	E 18
Columbus	-	LP*	O	E	F	D	M	S	I	L
Ransom	-	LP	O	E	S	L	M	L		E
Hutton	-	LP	O	E	S	L	M	L		L 13½
SRF 400	-	LP	L	E	F	L	M	S		
SRF 350	-	B	L	E	F	L	M	L		
SRF 425	-	B	L	E	F	L	M	L		
Wayne	-	B	O	E	F	L	M	L		
Woodworth	-	B	O	E	F	L	M	-		
Bragg	-	B	O	I	F	L	M	L	I	L
Tracy	-	B	O	I	F	L	M	L	S	E 13½

The pubescence color is tawny, there are two alcohol dehydrogenase bands, and the general protein band is B for all cultivars.

Legend For Tables

Peroxidase : +, positive; −, negative

Pigmentation : DP, dark purple; LP, light purple; LP* light purple—intermediate under strong light; B, bronze, G, green

Leaflet shape : O, ovate; L, lanceolate

Pubescence angle : E, erect; I, intermediate; A, appressed

General protein : A, band A; B, band B

Urease : F, fast band; S, slow band

Esterase : band Rf 0.63—D, dark staining—L light staining; band Rf 0.81—M, medium staining—L, light staining

Alcohol dehydrogenase : 1, one band, 2, two bands

Hypocotyl length : for large seeds; L, long; I, intermediate; S, short

Metribuzin test : I, insensitive; S, sensitive

Photoperiod test : L, late flowering; E, early flowering

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