Potassium Nitrate (KNO_3). Seed germination in many species, such as turf grasses, can be stimulated by using a dilute solution (0.1 % to 1.0%) of potassium nitrate as moisture for the germination test. Like prechilling, the use of KNO_3, is a valuable aid in germination of those species benefited by it and has become a routine procedure in the germination testing of many species.

SPECIAL TESTS FOR SEED QUALITY

Although purity, germination, and noxious weed evaluations are routinely performed on almost every seed sample submitted to the laboratory, many additional tests also reflect seed quality. Such special tests are usually performed only when requested; however, they may be done routinely for certain species or for law enforcement or certification samples. These special tests have been developed as byproducts of routine testing procedures in the seed technicians' attempts to learn more about the quality of seed lots. Today, most modern, well-equipped seed laboratories have the capability of conducting such tests.

Genetic Purity Testing

Changes are rapidly occurring in agriculture, many of these at the level of the seed industry. The ability to develop new varieties that differ in all but a single or several genes places an even greater burden on genetic purity testing. It seems certain that seed products developed from molecular biology will become increasingly common because they benefit numerous people. For example, farmers will obtain higher crop yields from improved insect, weed and disease control. Because these controls are obtained without chemical use, less concern will exist about environmental pollution. Farmers will also benefit from lower input costs for pest/weed control and will likely obtain premiums for seeds with selective output traits. Seed companies also will benefit from increased seed premiums that will enhance seed margins. Those companies that are the research and development leaders will likely enjoy a market share advantage from being the first to offer these new products. Finally, gene providers will obtain additional income from per acre technology fees and, in some cases, increased herbicide market share for companies selling seeds of herbicide tolerant varieties.

The continued development of new and improved varieties is the cornerstone of increases in crop yield and agricultural productivity. By definition, a variety of a cultivated crop differs from other varieties of the same species in one or more specific characteristic(s). Such characteristics as maturity, lodging resistance, disease resistance, plant height, and market quality make varieties distinct from one another. More recently, advances in molecular biology have led to the release of new varieties that may differ in as little as one gene for a specific trait such as herbicide tolerance or insect resistance. Farmers and growers are vitally interested in the selection of the variety best suited to their particular field/greenhouse conditions because they recognize that this single decision can have a marked effect on their yields and profit. Genetic purity testing is so important that it has been the subject of a recent book (Wrigley 1995), several reviews (McDonald 1995; 1998; Smith and Register 1998; Cooke 1995; 1998) and specific genetic purity testing protocols have been outlined in the Seed Technologist Training Manual (Society of Commercial Seed Technologists, 2001) and the Cultivar Purity Testing Handbook (AOSA 1991).

When new varieties are developed by plant breeders, a limited amount of seed is increased to quantities sufficient to supply larger grower needs. As this seed is increased, it must be

monitored to ensure that the genetic purity of the breeder seed is not compromised. Two principal concerns exist in maintaining genetic purity. First, the genetic composition of the variety initially developed by the breeder must be the same as that marketed to the grower after several regenerations of seed increase. Second, for hybrid seed crops, the success of hybridization must be ensured by minimizing the percentage of selfing and outcrossing.

In the early days of seed testing, varietal tests, when conducted, were relatively simple for two basic reasons: (1) there were fewer varieties, and (2) there were usually greater differences among varieties. Because of the success of modern plant breeding, the resulting variety explosion, and the appearance of many closely related varieties, seed analysts have been obliged to find newer, more sophisticated ways of distinguishing among varieties in the seed laboratory. Within varieties, genetic purity testing is important so that (Smith and Register, 1998): (1) intellectual property protection through Plant Variety Protection or Utility Patents can be obtained and then subsequently maintained, (2) varieties can be created with uniform appearance and agronomic performance that meet the demands of farmers, processors and consumers, (3) varieties with stable genetic identities can be created so that plant performance can be as predictable as possible given unpredictable environmental fluctuations, and (4) breeders can more completely and precisely characterize and measure genetic diversity so that genetic resources can be thoroughly evaluated in terms of plant performance and effectively utilized for the creation of improved varieties.

Recognizing the importance of these new markets and new agricultural products, seed technology will necessarily be at the forefront of ensuring the genetic purity of these seed products. Moreover, the increasing value of seeds in the future portends that high quality seeds will be paramount to avoid litigation concerning inaccurate identification of varieties. So, seed technologists are challenged to develop an array of more sophisticated genetic purity tests. In some cases, this may be a rather simple process such as imbibing seeds/seedlings in an herbicide to determine their tolerance to the compound. In most cases, however, when only a single gene is modified, more powerful genetic purity tests may be required. Some of these may include the use of immunoassays to detect the proteins produced by the inserted genes. Other approaches may employ the use of electrophoresis on starch or polyacrylamide gels to separate an array of specific proteins/enzymes. Even more sophisticated approaches include newer DNA-based technologies that use the polymerase chain reaction to allow even more discrimination and faster identification of varieties. At the moment, this area is rapidly changing and it is difficult to anticipate which of these tests will provide the seed technologist the greatest benefit in genetic purity testing.

Criteria for a Genetic Purity Test

The ideal genetic purity test must meet four criteria (Payne 1986). First, results must be easy to reproduce, not only within a laboratory, but also among different laboratories. Second, it should be technically uncomplicated so seed technologists can be successfully trained to conduct the test in a minimal amount of time. Third, it should require only a short time to complete. Finally, it should be inexpensive to conduct.

The basic objective of a genetic purity test is to test for the occurrence of traits that help identify a particular variety when grown in different environmental conditions and generations. Thus, it is assumed that these characteristics are environmentally stable and will not change from one generation to the next. The following approaches represent some of the most promising genetic purity tests currently conducted by the seed industry.

Types of Genetic Purity Tests

Field Testing. Traditionally, breeders, seed companies and certification agencies determine genetic purity using physical traits expressed by the seedling and/or mature plant. However, the success of field tests is limited because environmental stress often masks or alters specific seedling and plant anatomical/morphological features. For field testing to be successful, genetic purity tests must be grown (1) in areas where the crop is well adapted, (2) under the best possible cultural practices, and (3) during the proper growing season. Otherwise, the development of the crop may be altered to such an extent that accurate genetic purity results become uncertain. Field testing is also expensive: requiring equipment, planting and harvesting personnel, land use, and training of competent technicians for detecting specific plant traits. Most important, the number of morphological traits available today is limited and many supplemental laboratory tests have been developed and, in some cases, have completely replaced greenhouse and field testing.

Morphological. Distinguishing morphological features has been a major component of genetic purity testing in the laboratory. Morphological studies of the seed, seedling, and mature crop are used.

Seed. This is the simplest type of genetic purity test and probably the earliest used. Such characteristics as seed size, hilum color (Figure 15.9), seed shape, shape of rachilla, lemma and palea characteristics and presence or absence of awns are often evaluated. Although still useful, visual observation of the seed is usually not reliable for positive genetic purity testing and should be used only in conjunction with other tests. For example, Chippewa 64 soybean can easily be distinguished from soybean varieties that do not have a black hilum. However, more sophisticated techniques must be used to distinguish it from other varieties that also have black hila. Seed size is also a useful index of variety; however, it is so variable and so environmentally influenced that it must be used only with extreme caution.

Seedling. Many useful genetic purity tests can be performed on seedlings. Such characteristics as hypocotyl color (Figure 15.10), leaf coloration pattern, vernation (folded

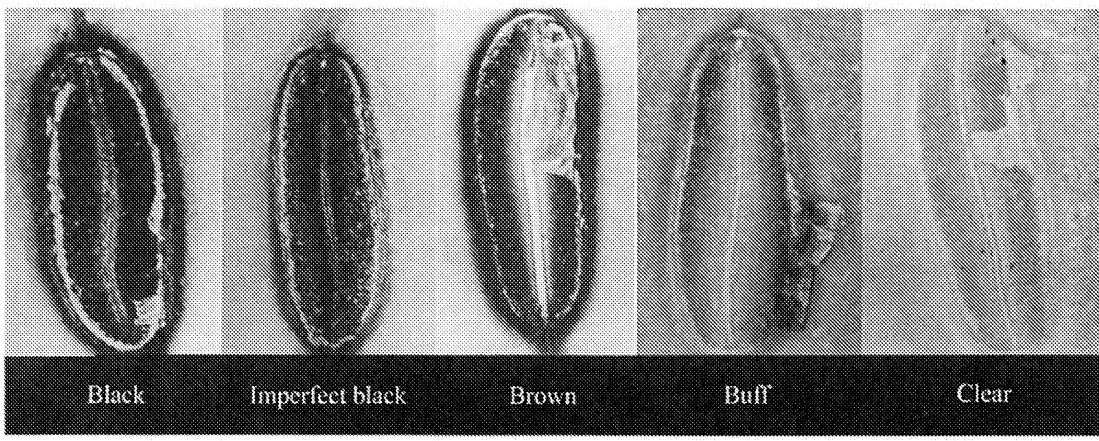

Figure 15.9. Five different hilum colors of soybean seeds. Differences can be observed, even in this black and white illustration, but are much more distinct when shown in color.

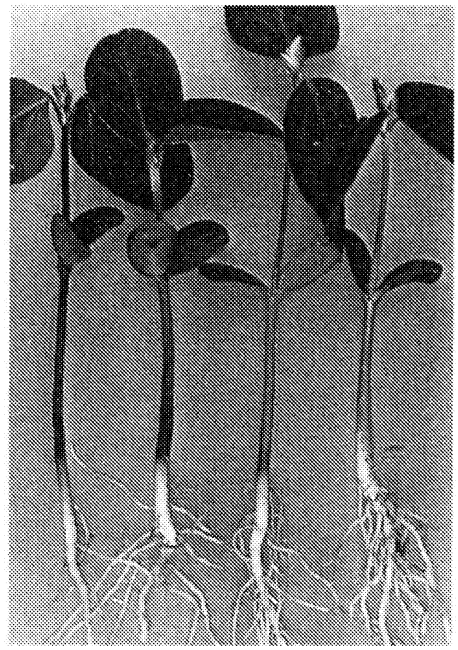

Figure 15.10. Soybean hypocotyl pigmentation patterns: Dark purple, intermediate purple, bronze, and green (left to right; from AOSA Cultivar Purity Testing Handbook, 1991).

or rolled) pattern of the leaf, length of internodes (dwarf vs. normal), pubescence and leaf shape are often examined. Such tests are useful because they may yield more information than do observations of the ungerminated seed and do not require as much time as field grow-out tests.

Crop. Traditionally, distinctions in flower color, stem pubescence color, leaf shape, photoperiodic responses, disease resistance, maturity date, and growth habit are genetic purity traits examined in the greenhouse or field. Greenhouse tests are usually performed in conjunction with seed and seedling tests to substantiate decisions made earlier. Growing plants in the greenhouse, however, involves many of the undesirable characteristics of field testing, e.g., space, time and expense.

Other types of genetic purity tests conducted in the laboratory include ultraviolet light tests, chemical assays, chromosome counts, chromatographic methods, herbicide tolerance, ELISA, electrophoresis of proteins/enzymes, and polymerase chain reaction technologies.

Ultraviolet Light Tests. Response under ultraviolet light has been used for both seed and seedling variety tests with varying success. The lemma, palea, and glumes of certain oat varieties contain substances that fluoresce when exposed to ultraviolet light. The ultraviolet light test, however, has limited usefulness, because many oat varieties show the same response – either fluorescence or nonfluorescence; therefore, the test is useful only when two varieties with opposite responses are being compared.