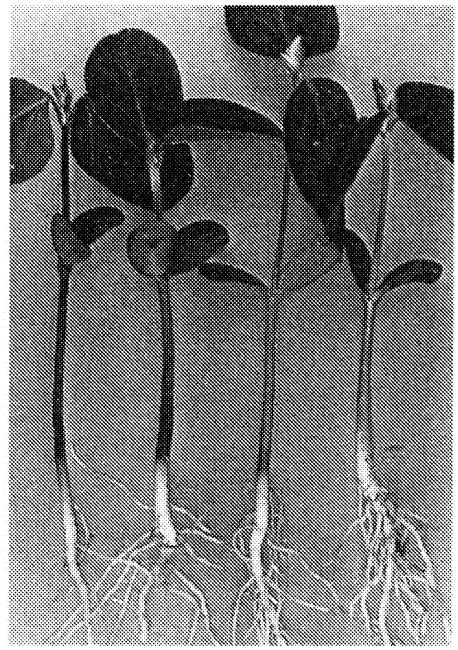

Figure 15.10. Soybean hypocotyl pigmentation patterns: Dark purple, intermediate purple, bronze, and green (left to right; from AOSA Cultivar Purity Testing Handbook, 1991).

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.

For many years, the fluorescence test of ryegrass has been used with great success to help distinguish between two species – annual or Italian (*Lolium multiflorum*) and perennial or English (*Lolium perenne*) ryegrass. The past usefulness of this test was due to the fact that seedling roots of all known annual ryegrass varieties exhibited a positive response, while seedling roots of perennial varieties were nonfluorescent. It may be otherwise impossible to distinguish between annual and perennial ryegrass seeds, although the presence of an awn usually indicates annual ryegrass. However, this characteristic is not dependable, since the awn is quite fragile and may be detached by handling. The accuracy of the fluorescence test has been good enough in the past to use as a measurement of the percentage of the two species when found together in mixtures. Today, however, it is believed that crossing between annual and perennial species in the field has made the fluorescence test of seedlings less meaningful and a search for new tests to distinguish between annual and perennial ryegrass is underway.

Unlike the fluorescence test of oats, which depends on the fluorescence of the seed coat under ultraviolet light, the test for ryegrass is conducted on five- to ten-day-old seedlings grown on white filter paper. The fluorescent substance has been isolated and designated as *annuoline*. It appears as an exudate from the ryegrass roots that are in contact with the paper medium.

Chemical Assays. The ideal genetic purity test would utilize the exposure of a seed to some chemical that would clearly reveal its varietal identity in comparison with related varieties. Unfortunately, such a test does not exist. Several easily performed chemical assays have been developed for use in genetic purity testing. For example, the phenol test (Elekes 1980) is dependent upon a flavenoid reaction in the seed pericarp and has been used successfully for distinguishing wheat and bluegrass varieties. The test is performed by placing the seeds on a paper medium moistened with approximately 1% carbolic acid (phenol solution) for about four hours. Tests are evaluated according to the darkness of staining that occurs; the seeds of some bluegrasses stain in the embryo area and can be distinguished from nonstaining varieties, while in wheat the entire pericarp is observed for the degree of staining. Wheat varieties can be categorized according to whether they stain very dark, medium dark, very light, or remain unstained (Figure 15.11).

Another very useful chemical test for distinguishing soybean varieties is the peroxidase test that separates varieties into two groups based on the presence or absence (Figure 15.12) of the enzyme in the seed coat (Buttery and Buzzell 1968). The test is conducted by removing the seed coat from the seed and placing it into a test tube to which is added 10 drops of a 0.5% guaiacol solution for 10 minutes. After that period, one drop of a 0.1% H_2O_2 is added to the test. A negative test will be indicated by a colorless solution.

Other useful chemical tests include the hydrochloric acid test for oats, sodium hydroxide test for wheat, and potassium hydroxide test for red rice and sorghum. The procedures are provided in the AOSA Cultivar Purity Testing Handbook (AOSA 1991).

Chromosome Counts. During the 1960s, many new tetraploid grass and legume varieties were released in the United States. The double chromosome complement of these varieties compared to their diploid counterparts has provided seed analysts with a built-in genetic purity test by merely counting the number of chromosomes in seedling root tips (Figure 15.13; Will, et al. 1967). Like many other genetic purity tests, chromosome counts cannot be used to distinguish between different varieties with the same chromosome number. However, they are useful in detecting contamination, especially diploid contamination of tetraploid varieties. The tests are a valuable aid in monitoring the genetic purity of certain varieties of certified tetraploid grasses and legumes.

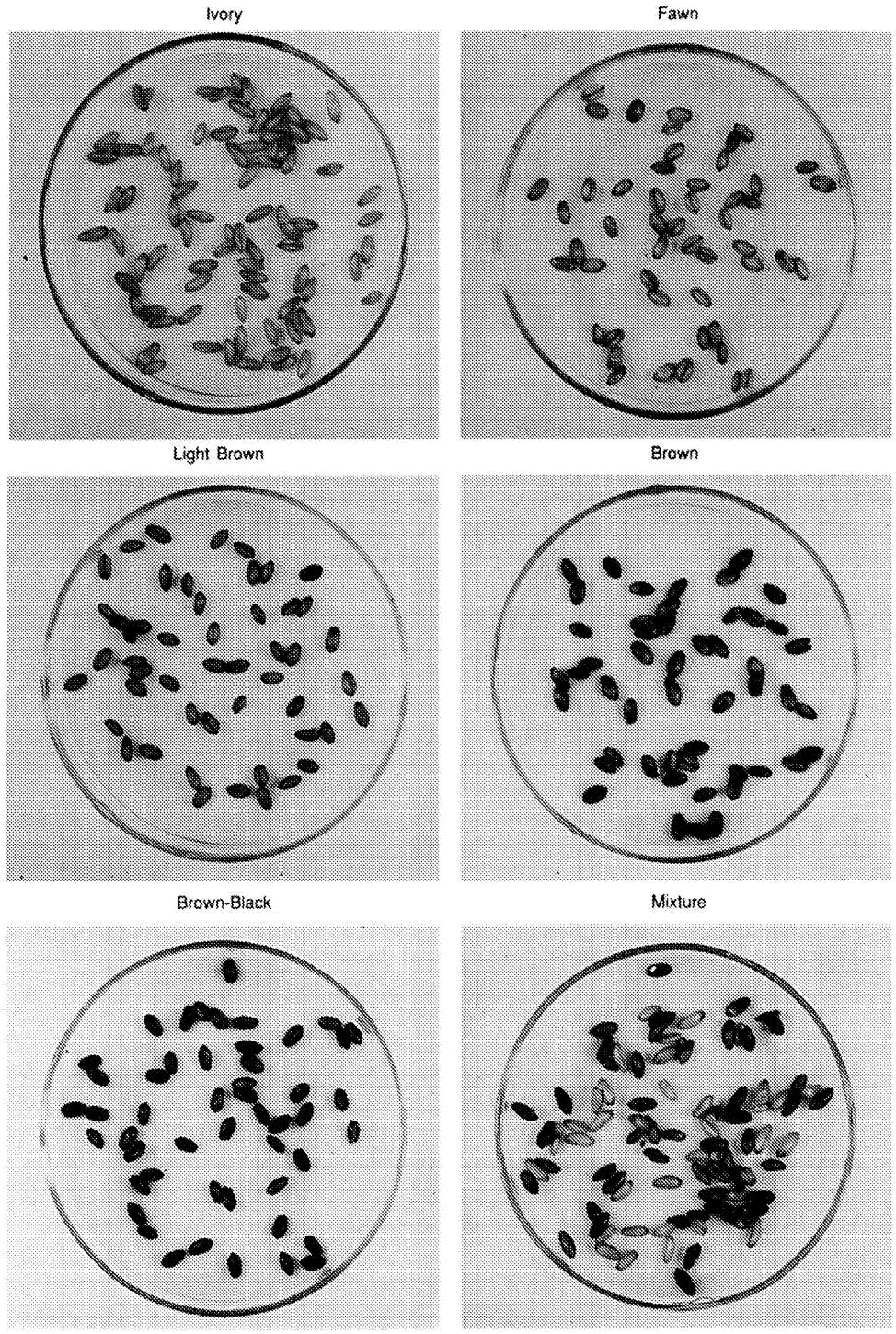

Figure 15.11. The phenol test for wheat: Examples of the five different color categories (From AOSA Cultivar Purity Testing Handbook 1991).

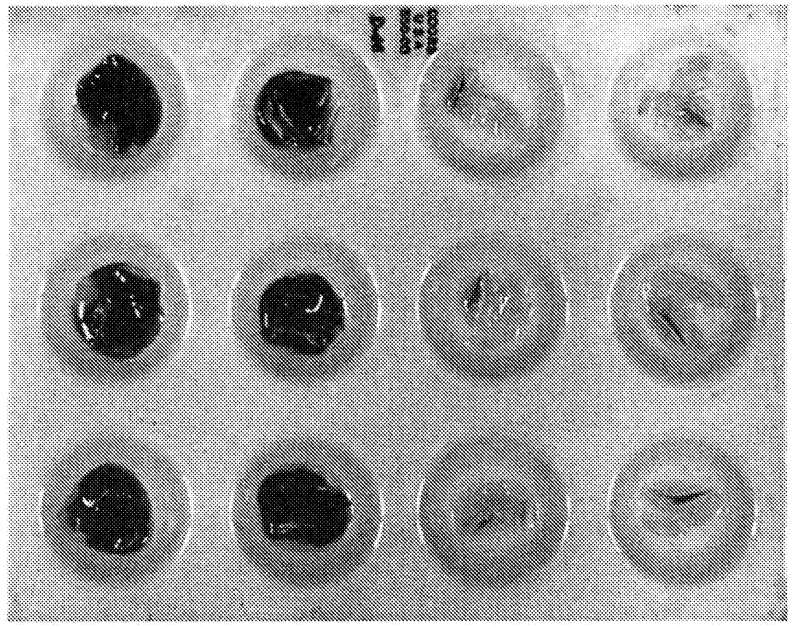

Figure 15.12. The peroxidase test for soybean. Peroxidase positive (left two rows), peroxidase negative (right two rows) (from AOSA Cultivar Purity Testing Handbook, 1991).

The use of chromosome count tests is more limited for higher polyploid species, such as wheat and bluegrasses, because of their difficulty associated with counting the chromosomes and the complexity of their polyploidy.

Chromatographic Methods. Both thin-layer and paper chromatography have been used with varying success in genetic purity testing. Readily observable differences have been reported in thin-layer chromatographic bands among both ryegrass and soybean varieties, and, to a lesser extent, in oat varieties. Thin-layer chromatographic methods have also been used to distinguish soft white from durum wheat and between closely related members of the genus *Trifolium*. Paper chromatography has been used to aid in genetic purity testing of *Brassica* and other species (Payne 1986).

In routine genetic purity testing, the usefulness of chromatographic techniques is limited, primarily because it is difficult to use, a long time is required for the test, and also because different varieties do not always have observable differences in chromatographic bands. This latter problem can sometimes be overcome by refinement of the technique, for example, by use of different absorbents (thin layer), developers, and ultraviolet light to help distinguish differences on the chromatograph.

Herbicide Tolerance. The first herbicide tolerance test was a seed soak method for Sulfonylurea tolerant soybean (STS) seeds (Sebastion and Chaleff 1987). However, following the introduction of Roundup Ready™ soybean seeds in the 1990s, an increased emphasis on detection of those soybean seeds that possessed the trait was essential. For example, should a farmer believe that Roundup Ready soybean seed had been planted, but were not, subsequent

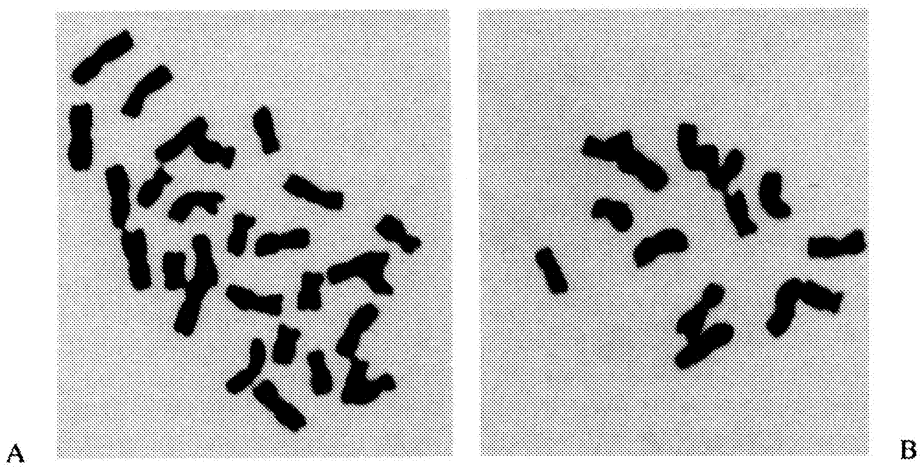

Figure 15.13. Metaphase chromosomes of ryegrass: (A) diploid ryegrass, with 14 chromosomes, and (B) tetraploid ryegrass, with 28 chromosomes (From Will et al. 1967).

spraying of the nonselective herbicide would result in a complete crop failure. Seed technologists quickly identified three approaches to successfully determine herbicide tolerance. These included presoak, substrate imbibition and seedling spray tests.

Presoak. This method allows the seeds to soak in a solution of the herbicide for a predetermined interval. The seeds are then planted and germinated under normal conditions and susceptibility to the herbicide determined. The advantage of the presoak method is that there is less contamination of facilities, equipment, and waste with the herbicide.

Substrate Imbibition. Grote (1992) developed the original substrate imbibition test for testing imidazolinone tolerant corn and Gutormson (1999) published a 13-step method for testing Roundup Ready corn. In this method, the germination medium/substrate is soaked with the herbicide followed by placing the seeds on the moistened medium. This approach allows the seeds to be exposed to the herbicide throughout the duration of the test. The herbicide concentrations used are usually less than those used in the field since the non-trait seeds/seedlings must emerge to express non-trait symptoms in their growth and anatomy. The advantages of the substrate imbibition test are the automation of the method, ease of including a check sample with each replicate, and less steps to plant. Important disadvantages include the requirement for dedicated equipment to avoid herbicide toxicity to other seedlings and environmental concerns regarding the disposal of media containing the herbicides.

Seedling Spray. Seedling spray methods involve growing seedlings in a laboratory or greenhouse and spraying the normal seedlings with the herbicide solution. After several days, the susceptibility or tolerance to the herbicide can be determined (Figure 15.14). Advantages of this test are that it relates well to field conditions and seed quality is of a concern since only emerged seedlings are sprayed. Disadvantages include the increased cost of a laboratory/greenhouse test and the additional time required for seedling development.