Soybean Hypocotyl Growout Test for Varietal Purity

Hypocotyl Color Classification in Soybeans

A Literature Review

Varietal purity in soybeans can be challenging due to the narrow genetic base, along with gene linkage and pleiotropic (one gene influences multiple, seemingly unrelated phenotypic traits) effects. At SoDak Labs, we utilize hilum color, peroxidase reaction, and hypocotyl color to help confirm varietal purity of soybean lots. For the hypocotyl test, 100 seeds are grown at 25C using a sand/peat mix under intense light to promote true hypocotyl color expression in 7-day old seedlings which are classified as either purple or green, the two traditionally recognized categories.

Coined as "Nutriculture", the hypocotyl method utilized in a majority of seed testing facilities is based on Hoagland and Arnon (1950) at the California Agricultural Experiment Station. Their method describes seedling growth in inert soil under continuous light in a controlled environment (25C) and watered with a nutrient solution (Hoagland No 2). Modern potting soil is cautioned against as it contains phosphates that can inhibit anthocyanin production or purple pigment (AOSA Rules for Seed Testing, 2024).

Additionally, strong light intensity is recommended as it is critical for anthocyanin development and low lighting could lead to difficulties in distinguishing pigment differences. However, with the use of continuous light, further variations within the traditional purple and green classifications could be observed. Nittler (1975) found when 13 soybean cultivars were germinated in the dark and then exposed to continuous light (2300-foot candles (fc)) variations in the intensity of purple pigmentation could be observed. Payne and Sundermeyer (1977) also observed variations within 63 lots of 20 cultivars classified as green. When grown under continuous light (3450 fc cool white light), and compared to a Nikerson color fan with a white background, 12 of the 20 cultivars had a bronze band. This bronze band was present at emergence (moderate olive 5Y 5/3) and had intensified by day 4 post emergence to a deep purple (7.5P 3/9) to strong purple (7.5P 5/10). The remaining 8 cultivars were classified as a yellow-green (5GY 7/10). Palmer (1979) also observed a similar bronze to purple pattern in certain cultivars in his research comparing hypocotyl color classification when grown under 3600 fc, 2400 fc, or in the field. Palmer noted the bronze banding was more intense at 3600 fc compared to 2400 fc or in the field. Additionally, Palmer noted that cultivars classified as

FIGURE 1. Hypocotyl color growout test after seven (7) days of growth.

FIGURE 2. Green hypocotyl color soybean seedlings.

FIGURE 3. Purple hypocotyl color soybean seedlings.

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intermediate purple under 3600 fc, were classified as light purple at 2400 fc or in the field.

Since photoperiod length and intensity can influence pigmentation development, to standardize laboratory methods acceptable lightings of 3100 fc from cool white fluorescent tubes or 300 fc from incandescent bulbs were recommended along with 4 classifications: green (all green), bronze (bronze on lower hypocotyl), light purple (majority of purple pigmentation on hypocotyl), dark purple (dark purple on hypocotyl and epicotyl) (Cultivar Purity Handbook, 2024).

HPLC

Soybean hypocotyl color is a morphological trait and a result of certain anthocyanins present that can be observed with the eye as a certain color. Using high pressure liquid chromatographic (HPLC), Peters (1984) observed that the anthocyanins present in both purple and bronze hypocotyl lines were delphinidin, petunidin, and malvidin in a 1:4:38 ratio, respectively. However, the malvidin content in purple lines was six times greater than the bronze hypocotyl lines. The highest anthocyanin activity was 2-3 days post emergence, with activity decreasing in the apical portion of the hypocotyl after day 3. Palmer and Payne (1979) suggested quercetin may be present for cultivar lines designated as bronze. However, it has been noted that malvidin and quercetin are closely related compounds as they are derived from different parts of the same branched biosynthetic pathway, and that bronze hypocotyl lines are truly dilute purple (Peters, 1984).

Genetics

Hypocotyl color is correlated to flower color, as cultivars with W1 allele have purple pigmentation in the flower and seedling hypocotyl and those with w1 allele have green hypocotyls and white flowers. (Payne 1978, Nielsen, 2004)). Payne (1979) found cultivars within the w1 allele genotypes with the T allele has a pleiotropic impact, as tawny (T_Td_) and light tawny (T_tdtd) pubescent plants had bronze hypocotyls but gray (t allele) (ttTd_ or tttdtd) pubescent plants did not. When observing hypocotyl color among the F3 generation of crosses of a green and purple hypocotyl line, it was suggested the pigmentation involves several minor genes and pigment intensity as a marker trait for plant breeding may be impractical (Peters, 1984). Peters also showed the T allele partially substitutes for the W1 locus by producing the same pigment, but in lower amounts.

Based on the literature review, and evaluation of numerous soybean cultivar lines, it is SoDak's practice to classify as either purple or green hypocotyls, unless a Plant Variety Protection (PVP) certificate with variety details can be provided that lists a different hypocotyl color for the soybean line.

FIGURE 4. Bronze variation of green hypocotyl color soybean seedlings.

Citations

AOSA Rules for Seed Testing. 2024. Cultivar Purity Handbook. Chapter 3: Plant Characteristics Tests in the Growth Chamber and Greenhouse.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method of growing plants without soil. Calif.Agric.Exp. Stn. Cir. 347.32 p.

Nittler, L.W. 1975. Research to improve testing soybeans for varietal purity. N.Y.Food Life Sci. 8(4):15-17.

Payne, R.C., and L.F. Morris. 1976. Differentiation of soybean cultivars by seedling pigmentation patterns. J. Seed Technol. 1:1-9.

Payne, R.C., and Sundermeyer, E.W., 1977. Pigmentation difference of soybean cultivars with green hypocotyl color when grown in continuous light. Crop Science. Vol 17. May-June. 479-480.

Payne, R.G., 1978. Some New Tests and Procedures for Determining Variety (Soybeans). AOSA-SCST Cultivar Verification Symposium. Volume 3, Number 2. 61-77

Palmer, R.G., and Payne, R.C., 1979. Genetic Control of hypocotyl pigmentation among white-flowered soybeans grown in continuous light. Crop Science. Vol 19.124-126.

Peters, D.W., Wilcox, J.R., Vorst, J.J., and Nielsen, N.C. 1984. Hypocotyl Pigments in Soybeans. Crop Science. Vol 24. 237-239