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Is the Tetrazolium Test a Reliable Alternative to the Gemination Test in Grasses?

V.N. Soares, S.G. Elias* and G.I. Gadotti

Currently, the standard germination test (SGT) is the official test for evaluating seed viability in seed testing laboratories around the world. However, this test takes 3–4 wk to complete for most grass species, including the prechilling treatment period to break dormancy in newly harvested and dormant seed. The tetrazolium test (TZ), however, offers a quick viability determination within 24–48 h, even for dormant seeds. This study was conducted to compare SGT and TZ results for tall fescue (TF; *Festuca arundinacea* Schreb.), annual (ARG) and perennial (PRG) ryegrass (*Lolium multiflorum* L. and *Lolium perene* L., respectively), orchardgrass (OG; *Dactylis glomerata* L.), bentgrass (BG; *Agrostis* spp.), Kentucky bluegrass (KBG; *Poa pratensis* L.), and fine fescues (FF; *Festuca* spp). A total of 3432 samples representing the seven species were evaluated for viability by SGT and TZ during a 5-year period (2009–2013) at the Oregon State University Seed Laboratory (OSUSL). According to the germination tolerance table of AOSA, both SGT and TZ results were within tolerance in 94.2% of TF samples, 95.2% of ARG samples, 90.4% of PRG samples, 93.7% of OG samples, 90.8% of FF samples, 83.9% of BG samples, and 42.4% of KBG samples. For individual out-of-tolerance samples, dormancy was the main reason for higher viability by TZ compared to SGT results. As dormancy is gradually broken over time, SGT results are expected to be within tolerance with TZ results. Based on this study, it can be concluded that the TZ can be used as an alternative viability test to SGT for TF, ARG, PRG, OG, FF, and possibly for BG. However, the higher KBG dormancy levels would cause SGT results to be below TZ results, especially for freshly harvested seeds. Using TZ as a viability test can save 2–4 wk of testing time and deliver faster results to seed producers.

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Tips for Soybean Variety Verification by Hila Color

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Soybean [*Glycine max* (L.) Merr.] hilum color can be a useful tool in variety verification, as it is a varietal characteristic described by a breeder that can be readily observed in a seed sample. Certifying agencies, seed companies and others use soybean hila color not only to verify that a lot matches the variety description, but also to determine if it meets a varietal purity standard. Background information about the growth characteristics of soybeans could help analysts with problematic samples.

Variability in hila color intensity. Some varieties have especially dark or light hila as a characteristic. However, hilum color intensity can also differ from year to year and within the seed lot itself. Environmental factors, varying maturity within the field at harvest, diseases, etc., can cause a range of intensity within a seed lot. For instance, seed with normally black hila harvested while green could have a buff-like, reddish-black, or even gray color. Difficulties in drawing the line between two colors in a sample could be due to variability within the sample.

Linkage. There are several genetic linkages between hilum color and other traits in soybeans. Pubescence (color of the hairs on the plant) is one such trait. Plants with gray pubescence will have imperfect black, buff, yellow or gray hila. Plants with tawny or light tawny pubescence will have black, brown, imperfect yellow or gray hila.

Imperfect yellow. An imperfect yellow hilum has a spot of brown pigment at the micropyle, which may or may not migrate into the hilum. Imperfect yellow may also be described as tan or tan spot. Some breeders do not recognize this color characteristic and will describe their varieties as yellow. Classifying a seed with a yellow hilum as an offtype in a variety which has been described as imperfect yellow would not be recommended, as it may simply be a seed with very little pigment migration.

Mottled seed. Some seed lots may contain mottled seed. Mottles are streaks or blotches of color extending from the hila throughout the seed coat. The color of mottling should be the same as the hila color (or brown in the case of yellow or imperfect yellow varieties). If that is not the case, the seed should be classified as an offtype. **Smith (4)**, in describing the bean pod mottle virus, reported that "Seed mottling results from pigments diffusing from the hilum. It can be a symptom associated with virus-infected plants, including Bean Pod Mottle Virus and Soybean Mosaic Virus. Some seed are entirely black or brown, but bicolored seed are by far more common. Hilum color will determine the color and intensity of seed coat discoloration. Mottled seeds are an issue for grain grading, especially for food grade soybeans. Mottling is associated with poor germination and yield. All mottled seed is not caused by virus, however. Insect feeding and physiological stresses can also cause seed mottle."

Purple seed stain. A disease called 'purple seed stain' can discolor soybeans with a purple stain that emanates from the hila. Purple seed stain (Cercospora

blight) is caused by the fungus *Cercospora kikuchii* (T.Matsumoto & Tomoy.). Seed lots with a high percentage of stained seed may be docked for grain. Germination may also be affected (3).

Black or brown seed. Occasionally, all-black or all-brown seeds will be found in a seed sample. These seeds are the result of mutations or genetic anomalies, and as long as the color corresponds to the hila color of the variety, they are not considered offtypes (2).

Canadian hila colors. Plant breeders in Canada evaluate soybean hila color using a slightly different set of color descriptors. When examining a variety described in Canada, it would be useful to refer to Canadian descriptors to assure correct evaluation (1).

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Using Morphological Seedling Features to Distinguish between Annual and Perennial Ryegrass

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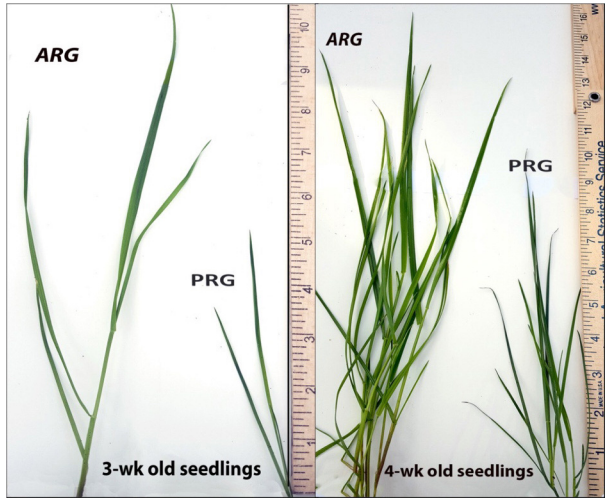
The grow-out test (GOT) is used to differentiate between annual (ARG, *Lolium multiflorum* Lam.) and perennial (PRG, *Lolium perenne* L.) ryegrass. Although this test is reliable, it takes a long time-2 wk for germination and fluorescence tests (FL) and 6 wk in the greenhouse (GH)-for annual types to form heads. The objective of this research was to explore a method to shorten the GOT by modifying the evaluation criterion, basing it on seedling morphology instead of forming heads. Three studies were conducted. In the first, three PRG samples were spiked with different numbers of ARG seeds to measure the accuracy and speed in identifying ARG seedlings in a GH. The second and the

Figure 1. Differences in seedling height, blade width and color between annual (ARG) and perennial (PRG) ryegrass after three and four weeks of growth in a greenhouse.



third studies aimed to determine the precision and speed of detecting ARG seedlings in low and high varietal fluorescence levels (VFL) of turf and forage PRG, respectively. In the first and the second studies (low perennial VFL turf varieties), only fluorescent seedlings, at the end of the germination test, were transplanted into a GH to determine whether they were true annual or perennial types. In the third study (high perennial VFL forage varieties), 400 seeds were planted directly in a GH. In all studies, stem height, leaf width and leaf color were measured every other day for 6 wk. In the first and second studies, it was possible to accurately differentiate between annual and perennial types based on seedling morphology after 3–4 wk (Fig. 1). In the third study, the only criterion to differentiate forage PRG with high VFL (50% or more) from true ARG types was by forming heads, necessitating direct GH planting for 6 wk before evaluation. Using seedling morphology criteria to separate annual from low VFL perennial types could save 2–3 wk compared to the current forming-heads evaluation method, while keeping the same level of accuracy.

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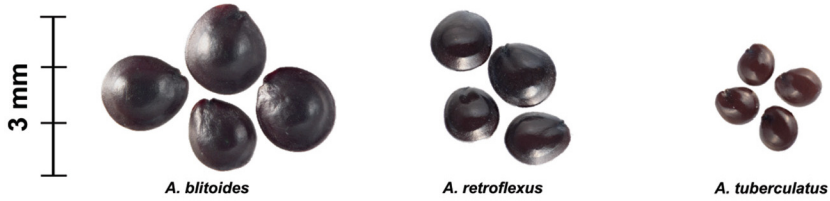
Figure 1. Seed size classification groups of *Amaranthus* species.



Identification of Seeds of *Amaranthus* Species and Species Groups

Jennifer Neudorf, Ruojing Wang*, Angela Salzl and Taran Meyer

Plants of *Amaranthus* species are common in ruderal habitats, and consist mostly of weeds with some edible species. This genus contains serious agricultural pests, such as *Amaranthus tuberculatus* (Moq.) J.D. Saur, a primary noxious weed in the Canadian *Weed Seeds Order*, and *A. palmeri* S. Watson, a noxious weed in a few states in the United States. In Canada and the United States, 38 *Amaranthus* species are reported to occur. Seed morphology is similar among *Amaranthus* species and makes identification challenging. Common and regulated *Amaranthus* species in Canada and the United States were classified into three species groups based on seed size. During examination, consistent features were found to distinguish between species in the large- and small-seeded groups. The medium-seeded group exhibited higher similarity among species and were difficult to consistently identify. Feature observations from the examination of the seeds of select *Amaranthus* species are detailed below. Depicted seeds were selected to show typical features; damaged, deformed or immature seeds may not exhibit all features.

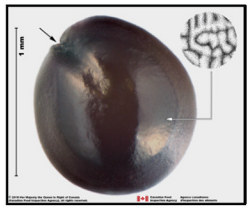
Amaranthus species can be classified into three main groups based on seed size (Fig. 1).

Large-seeded species group (Fig. 2). *Amaranthus blitoides* S. Watson seeds were black in color, round or egg-shaped, appeared inflated in edge view, with a

Figure 2. Large-seeded species group (length up to 1.8 mm).

Amaranthus blitoides *Amaranthus blitum* *Amaranthus muricatus*

Figure 3. Medium-seeded species group (length up to 1.5 mm).



Amaranthus retroflexus

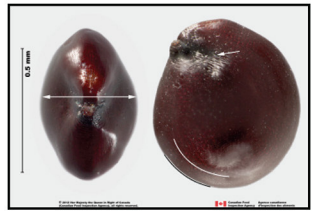
Amaranthus palmeri

faintly textured, dull surface. *Amaranthus blitum* L. seeds were dark red colored, either round or egg-shaped, the hilum was in an open notch, and the surface was glossy and smooth. *Amaranthus muricatus* (Moq.) Hieron. seeds were black, egg-shaped with a strongly textured, dull surface. There were consistent distinguishing features among the seeds in the large-seeded group to identify individual species.

Medium-seeded species group (Fig. 3). Seeds of *A. hybridus* L., *A. powellii* S. Watson and *A. retroflexus* L. had many features in common: generally black, some dark red colored, oval or egg-shaped, a glossy surface with faint grooved reticulations with small, curved interspaces. Sufficient features to distinguish these species could not be found for this study. Seeds of *A. palmeri* had some interesting features: egg-shaped with a strongly angled narrow end and prominent wide end, and reticulations with large angular interspaces. These features may require further study to gauge their consistency and identification strength.

Small-seeded species group (Fig. 4). The seeds of *A. tuberculatus* were oval, egg or teardrop shaped, with a roughened hilum that appeared pinched on the sides.

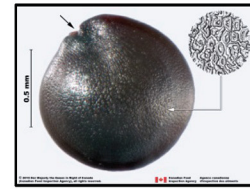
Figure 4. Small-seeded species group (length up to 1.0 mm).



Amaranthus tuberculatus

Amaranthus albus

Amaranthus spinosus



Amaranthus viridis

The surface was glossy, and the rim markings were faint or absent; the seeds of most other *Amaranthus* species had a distinctive rim. *Amaranthus albus* L. seeds were broadly egg-shaped, inflated in edge view, with a notably glossy surface. The seeds of *A. spinosus* L. were round or oval shaped, inflated in edge view with the hilum at one end of the seed in an open notch. The surface was notably glossy with a frosted rim. *Amaranthus viridis* L. seeds were black colored, round, inflated in edge view with an open hilum notch and a dull, strongly textured surface. There were consistent distinguishing features among the seeds in the large seeded group to identify individual species.

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Germination Temperature Comparisons of *Brassica napus*

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The goal of seed testing rules and methods is to promote and ensure testing uniformity. The current germination test methods described in the *International rules for seed testing* (ISTA), *Rules for testing seeds* (AOSA) and *Canadian methods and procedures for testing seed* (Canadian M&P) allow different germination temperatures for testing rapeseeds or canola (*Brassica napus* L. subsp. *napus*). The different rules specify two alternating temperatures: 15–25 °C (Canadian M&P and AOSA) and 20–30°C (ISTA and AOSA), and two constant temperatures: 25 °C (Canadian M&P) and 20 °C (ISTA). This study evaluated the equivalence of each temperature for testing uniformity and potential method harmonization. In an initial study, six commercial seed lots with germination ranging between 85–95% were tested under the Canadian M&P, using 400 seeds per lot, at one laboratory. Normal seedling percentage was significantly different among the four temperatures. Germination at 20 °C consistently produced the highest percentage of normal seedlings among the six seed lots, and 20–30 °C gave the lowest percentage. A referee study to test the four temperatures was then conducted with 30 participating laboratories or analysts in Canada and the United States. Participants were separated into 12 groups for 12 replications based on number of samples they test per year and the experience level they claimed, within which five laboratories were chosen to do all four temperatures. There were significant differences among the four temperatures for percentage germination, and again 20 °C resulted in the highest germination among the four temperatures tested. There was no interaction between temperature and lot among the five laboratories that performed tests under all temperatures. The referee study demonstrated once again that variation among laboratories was the largest variable affecting testing uniformity.

Minimizing laboratory variation and improving testing uniformity could be achieved by, for example, limiting options such as temperatures and media, rule harmonization, and better seedling descriptions.

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