

# **ABSTRACTS**

*from oral and poster presentations  
given at the 2017*

*International Seed Testing Association  
the 107<sup>th</sup>*

*Association of Official Seed Analysts  
and the 94<sup>th</sup>*

*Society of Commercial Seed Technologists  
(ISTA/AOSA/SCST)*

*annual meeting*

*held in Denver, Colorado, on  
June 14–24, 2017*

# Potential for Early Counts of Radicle Emergence and Leakage of Electrolytes as Quick Tests to Predict the Percentage of Normal Seedlings

Alison A. Powell\*, Linda Kerr, Kazim Mavi, Marie-Hélène Wagner and Stan Matthews†

The potential for early counts of radicle emergence (RE) and electrical conductivity (EC) of seed leachates to predict the production of normal seedlings was investigated in 10 seed lots of radish (78–99% normal seedlings) and 9 seed lots of oilseed rape (> 65% normal seedlings). In radish, germination tests (4 × 50 seeds, top of paper) conducted on three occasions in the experimental period (May and December, 2014; September, 2015) revealed little evidence of deterioration. On each occasion, a 48 h RE count predicted the production of normal seedlings (%) with an  $R^2 \geq 0.90$  ( $p \leq 0.001$ ), indicating that over 90% of the variation in normal seedlings was accounted for by RE. Similarly, RE counts on oilseed rape conducted in two laboratories after 3 d (4 × 100 seeds; GEVES, France) or 2 d (2 × 100 seeds; Alexander Harley Seeds, UK) of test initiation gave  $R^2$  values of 0.83 ( $p \leq 0.01$ ) and 0.92 ( $p \leq 0.001$ ), respectively. EC of water-soluble seed leachates for both species (3 replicates of 100 seeds in 40 mL-radish, or 50 mL-oilseed rape) also related to percentage normal seedlings. In radish, EC readings were taken on four occasions during the experimental period. EC readings after 1, 3, 5, 17 and 24 h were highly predictive of normal seedlings ( $R^2 \geq 0.83$ ,  $p < 0.001$ ). An early reading at 3 h gave  $R^2 = 0.93$  and a 17 h reading,  $R^2 = 0.97$ , indicating that early assessment of normal seedlings was possible. A 24 h EC reading also predicted normal seedlings in oilseed rape with  $R^2$  values of 0.88 ( $p \leq 0.001$ ; Harleys) and 0.87 ( $p \leq 0.001$ ; GEVES). EC at 5 h was also significant ( $R^2 = 0.45$ ,  $p < 0.05$ ) and at 17 h, highly significant ( $R^2 = 0.75$ ,  $p < 0.01$ ). Seed lots of oilseed rape that produced over 90% normal seedlings could be identified as having above 75% RE after 2 or 3 d, or an EC at 24 h of  $\leq 120 \mu\text{S cm}^{-1} \text{ g}^{-1}$ , and in radish, lots with  $\geq 80\%$  RE at 48 h or  $\text{EC} \leq 160 \mu\text{S cm}^{-1} \text{ g}^{-1}$  at 17 h had 95% normal germination or above. This data reveals the potential for an early RE count or EC reading to predict germination within 24 to 48 h, and will be discussed on the basis of an aging/repair hypothesis. The potential for RE to predict normal germination is being investigated further by ISTA.

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Alison Powell and Stan Matthews, University of Aberdeen, Aberdeen, UK; Linda Kerr, Alexander Harley Seeds Ltd, Milnathort, UK; Kazim Mavi, University of Mustafa Kemal, Antakya, Turkey; Marie-Hélène Wagner, GEVES, Angers, France. \*Presenter; †Corresponding author (agr791@abdn.ac.uk). Received 30 June 2017.

## Radicle Growth in Phyto-Agar Gels as a Vigor Test for Multiple Species

Riad Baalbaki

Radicle growth, a commonly used parameter in vigor tests, can be a good predictor of seed quality. However, effects of some test factors, such as substrate and moisture, on radicle growth can limit this parameter's predictive efficiency. The use of agar as substrate is particularly suited to measuring radicle growth over time, can reduce variability by eliminating substrate and moisture effects, and allows inclusion of additional evaluation criteria such as 'driving force.' Using high and low vigor samples of lettuce (*Lactuca sativa* L.), broccoli (*Brassica oleracea* var. *botrytis* L.), rice (*Oryza sativa* L.) and tall fescue (*Festuca arundinacea* Schreb.), we tested the hypothesis that variation in radicle development during the early stages of seedling growth, measured under variable agar concentrations, was a good general predictor of seed vigor. Seeds were planted in vertical phyto-agar gels (7.5 cm long and 1.5 cm wide) at three concentrations, 0.65, 0.75 and 0.85% (w/v). Samples were concurrently planted in potting soil in the greenhouse. Experiments were laid out as a RCBD with 4 replications of 50 seeds each. Laboratory data was collected on percentage germination, time to radicle emergence, root dry weight, total root length, root extension growth (maximum depth in agar reached by the radicle), and speed of germination. Root development data was recorded every 4 h using a digital documentation system. Greenhouse data included emergence rate, shoot and root fresh and dry weights (20–30 d from initiation), and speed of emergence. The number of normal, abnormal and dead seeds did not vary with agar concentration ( $p \geq 0.05$ ). Measurement of root extension growth was a better indicator of seed vigor than total root length at different agar concentrations, and was highly correlated with greenhouse emergence results ( $p \geq 0.001$ ). All tested species exhibited the same patterns of root growth in agar at different concentrations. Accordingly, root extension growth appears to be an efficient and generally applicable vigor test. However, a wider selection of species should be tested to verify the universal applicability of this vigor test. Furthermore, additional experiments are needed to determine if moisture availability (osmotic potential) is a confounding factor. Finally, the robustness of this test to slight variations in test conditions, such as changes in depth of planting or volume of agar, should be investigated.

# ***Avena* Species (Oats): Fatuoid Identification and its Importance to the Seed Industry**

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Fatuoids ('false wild oats') are present in certain varieties of cultivated oats from which they genetically derive, but are not always recognized or properly identified during purity and/or noxious-weed seed analyses. This work aimed to bring awareness to the seed industry that fatuoids exist and should be properly identified, in order to increase accuracy and uniformity during purity and noxious-weed seed analyses of cultivated oat species. Fatuoid seeds have often been mistaken for either other crop or *Avena fatua* L. (wild oat) seeds, a noxious-weed seed. The effects of training, studying the morphology of oat seeds, using seed keys, characteristic charts, and having knowledge of the accurate purity component to properly place these derivatives in, will lead to increased experience and skill levels of analysts, as well as higher accuracy and uniformity in the seed testing industry. Previous studies reporting that fatuoids were frequently placed in the wrong purity component due to misidentification demonstrated that more research, comparing fatuoid characteristics to *A. sativa* L. (cultivated oat) and *A. fatua*, and distinguishing them from other crop and weed seeds, was a need in the seed industry. Acquiring knowledge of various cultivars and derivatives, using references that refer to the details of fatuoid oats, and workshops conducted by experienced analysts, would result in a continual increase of accuracy, uniformity, and compliance in the seed testing industry.

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United States Department of Agriculture, Seed Regulatory and Testing Division (Anitra.Walker@ams.usda.gov). Received 27 September 2017.

## **Palmer Amaranth: Identification from ITS DNA Sequencing**

Robert Price\*, Toni Bartling, Joshua Kaste, Patrick Woods,  
Denise Thiede, Deborah Meyer and Farhad Ghavami

Palmer amaranth (*Amaranthus palmeri* S. Watson), a dioecious annual species native to arid areas of Mexico and the southwestern USA, has become a highly invasive weed of agricultural fields in the southeastern and central USA. It is now listed as a prohibited noxious weed seed in Ohio, Minnesota and Iowa, a state noxious weed in Delaware, and a harmful weed not allowable in exports to China. Seeds of *Amaranthus* species that may be encountered as contaminants in commercial seed lots, e.g., of small grains or of species used in conservation plantings, are often very difficult to distinguish reliably by their morphology. Based on preliminary evidence in the literature, we chose to sequence DNA from the ITS region between the nuclear ribosomal RNA genes, to provide an alternative approach for identifying individual seeds of

Palmer amaranth. To validate this approach, we obtained ITS sequences from known vouchered Palmer amaranth populations from northern and southern California, Arizona, Kansas, Illinois, Indiana and Minnesota, and from ten other *Amaranthus* species including apparent close relatives of Palmer amaranth and several weed species widely established in the USA [*A. albus* L.; *A. arenicola* I. M. Johnst.; *A. blitoides* S. Watson; *A. blitum* L.; *A. californicus* (Moq.) S. Watson; *A. deflexus* L.; *A. powellii* S. Watson; *A. retroflexus* L.; *A. spinosus* L.; *A. tuberculatus* (Moq.) J. D. Sauer]. All of these authenticated samples of Palmer amaranth yielded sequences with a highest identity match of 99% or higher to only Palmer amaranth sequences in BLAST searches of the GenBank database, while those authenticated from morphology as other species gave highest matches of 99% or higher only to species or groups of species other than Palmer amaranth. Thus, the method appears effective in identifying individual seeds as Palmer amaranth or “*Amaranthus* sp., not Palmer amaranth,” and in some cases provides strong evidence as to the other tested species. This approach has been used to identify over 1000 individual amaranth seeds submitted from commercial seed samples. SNPs from the ITS region were found to reliably differentiate between Palmer amaranth and the other *Amaranthus* species in our data and the GenBank database. This has allowed the design of specific primers for PCR tests to assess the presence or absence of Palmer amaranth seeds in bulked samples of up to 100 seeds that may include multiple amaranth species.

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## Seed Identification from ITS DNA Sequencing — an Update

Robert Price\*, Patrick Woods and Deborah Meyer

The ITS region between nuclear ribosomal RNA genes was amplified and sequenced from DNA extracted from samples of individual or small numbers of seeds from diverse flowering plant families, including Acanthaceae, Amaranthaceae, Amaryllidaceae, Apiaceae, Asphodelaceae, Asteraceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Malvaceae, Meliaceae, Plantaginaceae, Poaceae, Primulaceae, Ranunculaceae, Rosaceae, and Solanaceae. Amplification and sequencing of the ITS region used the highly conserved primers ITS1 and ITS4 (White et al., 1990), which are of fungal origin, but equivalent angiosperm-specific primers can also be used. Resulting sequences were identified by comparison to sequences in the international GenBank database by BLAST search, and only unique matches of 99–100% identity were treated as probable identifications to individual species. All 41 samples with successful amplification were placed in the correct family

by the sequence analyses, as judged by morphological analyses of the seed or fruit material. Thirty-five of the samples (85%) were identified successfully to a plausible individual genus by the best sequence match. Four samples that were identified only to tribe or family represented large and/or subtropical to tropical groups with limited sequence sampling (Asteraceae tribes Gnaphalieae and Vernoniae, Meliaceae, and Sterculiaceae = Malvaceae s.l.). Two samples were identified to small groupings of genera due to issues of intergeneric hybrid origin [*Elymus repens* (L.) Gould, quackgrass, Poaceae] or ongoing changes in generic circumscription (*Anthemis* L. sp., Asteraceae). Twenty-one of the samples (51%) were successfully identified to a likely individual species or species-pair by the sequence analyses. Identifications were made only to genus in cases where the maximum sequence match was below 99% [e.g., a tropical *Astripomoea* A. Meeuse (Convolvulaceae) and a tropical *Ocimum* L. (Lamiaceae)], and/or multiple species gave the same percentage matches in identity [e.g., in some samples from the large genera *Cestrum* L., (Solanaceae), *Lathyrus* L. and *Vicia* L. (Fabaceae), *Setaria* P. Beauv. (Poaceae), *Potentilla* L. (Rosaceae), and *Ruellia* L. (Acanthaceae)], or the seed, fruit or spikelet morphology suggested that the sample may represent a related species, which might not be represented in the GenBank database. Results suggested that ITS sequencing from individual seeds was a useful approach for identifying seed contaminants that were difficult to identify morphologically due to nonrepresentation in reference collections, atypical appearance, or loss of characters in seed conditioning. The same extraction, amplification and sequencing methodology has also been used successfully to identify unknown samples from fresh or dried leaves, flower or fruit tissue, or dried pollen.

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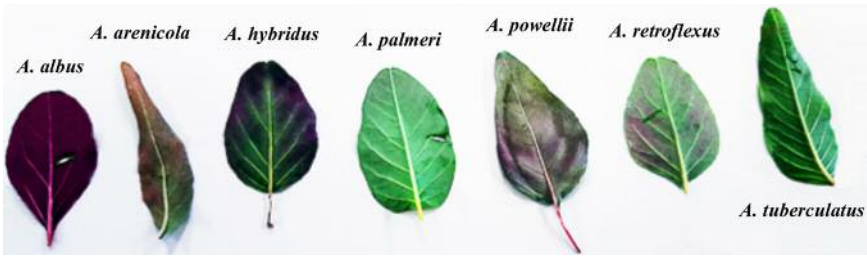
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## Grow-out Test to Distinguish Different Amaranth Species

Sabry G. Elias\* and Yeaching Wu

Amaranth (*Amaranthus* spp.) is an annual weed that grows aggressively, causing significant yield losses in many crops. It is a problematic genus that has spread to at least 28 states of the USA. Morphological similarities among seeds of amaranth made it difficult to identify species accurately, and therefore an alternative test was urgently needed. A study was conducted to identify different species of amaranth based on morphological differences in leaf color, shape, texture, leaf

**FIGURE 1.** Leaf shape and color patterns of 7 amaranth species after 3 wks of growth in the greenhouse.



petiole and stem color. Seven genetically pure amaranth species were acquired from ARS-USDA Plant Introduction Station, Ames, IA, USA: *A. albus* (tumbleweed), *A. arenicola* (sandhills amaranth), *A. hybridus* (smooth pigweed), *A. palmeri* (Palmer amaranth), *A. powellii* (Powell amaranth), *A. retroflexus* (red-root pigweed), and *A. tuberculatus* (waterhemp). Individual seeds of each species were planted in 50-cell greenhouse trays. Trays were watered as needed and fertilized weekly with 1 tbsp of Miracle-Gro® for every gallon of water. Greenhouse temperature was maintained at  $24\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$ . Light of approximately  $232\text{ mmol m}^{-2}\text{ s}^{-1}$  using high pressure sodium lamps was provided daily from 5:00 pm to 8:00 am. Observations of leaf and stem traits were collected weekly. Palmer amaranth (*A. palmeri*) has been recently listed as a noxious weed in Delaware, Minnesota and Ohio. Leaves of *A. palmeri* were hairless, compared to waterhemp which was pubescent on the stems and leaves. Leaves were also diamond-shaped with a whitish vein on the backside (Fig. 1). Leaves alternated and grew symmetrically around the stem, giving Palmer amaranth a rosette appearance. The leaf petiole of *A. palmeri* is longer than the leaf itself. At three weeks, the stem was light purple at the base and green at the top. According to this study, *A. palmeri* could be differentiated from other amaranth species based on leaf and stem characteristics after a 3-week grow-out in a greenhouse. At different stages of plant development, distinctive features can be used to identify different amaranth species under greenhouse or field conditions. Efforts are underway to develop a DNA test to differentiate *A. palmeri* from other amaranth species.

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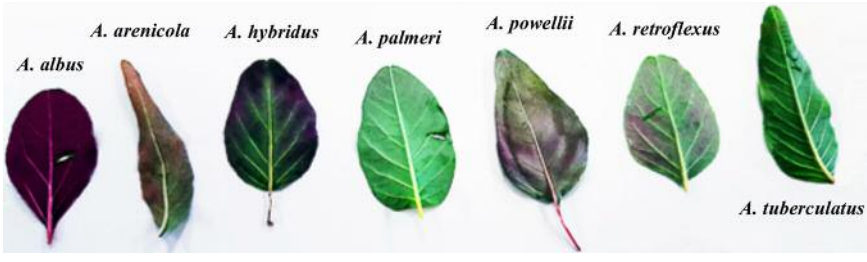
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Amaranth (*Amaranthus* spp.) is an annual weed that grows aggressively, causing significant yield losses in many crops. It is a problematic genus that has spread to at least 28 states of the USA. Morphological similarities among seeds of amaranth made it difficult to identify species accurately, and therefore an alternative test was urgently needed. A study was conducted to identify different species of amaranth based on morphological differences in leaf color, shape, texture, leaf

**FIGURE 1.** Leaf shape and color patterns of 7 amaranth species after 3 wks of growth in the greenhouse.



petiole and stem color. Seven genetically pure amaranth species were acquired from ARS-USDA Plant Introduction Station, Ames, IA, USA: *A. albus* (tumbleweed), *A. arenicola* (sandhills amaranth), *A. hybridus* (smooth pigweed), *A. palmeri* (Palmer amaranth), *A. powellii* (Powell amaranth), *A. retroflexus* (red-root pigweed), and *A. tuberculatus* (waterhemp). Individual seeds of each species were planted in 50-cell greenhouse trays. Trays were watered as needed and fertilized weekly with 1 tbsp of Miracle-Gro® for every gallon of water. Greenhouse temperature was maintained at  $24\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$ . Light of approximately  $232\text{ mmol m}^{-2}\text{ s}^{-1}$  using high pressure sodium lamps was provided daily from 5:00 pm to 8:00 am. Observations of leaf and stem traits were collected weekly. Palmer amaranth (*A. palmeri*) has been recently listed as a noxious weed in Delaware, Minnesota and Ohio. Leaves of *A. palmeri* were hairless, compared to waterhemp which was pubescent on the stems and leaves. Leaves were also diamond-shaped with a whitish vein on the backside (Fig. 1). Leaves alternated and grew symmetrically around the stem, giving Palmer amaranth a rosette appearance. The leaf petiole of *A. palmeri* is longer than the leaf itself. At three weeks, the stem was light purple at the base and green at the top. According to this study, *A. palmeri* could be differentiated from other amaranth species based on leaf and stem characteristics after a 3-week grow-out in a greenhouse. At different stages of plant development, distinctive features can be used to identify different amaranth species under greenhouse or field conditions. Efforts are underway to develop a DNA test to differentiate *A. palmeri* from other amaranth species.

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# Identification of Secondary Noxious Brome Species in the Canadian *Weed Seeds Order* (2016)

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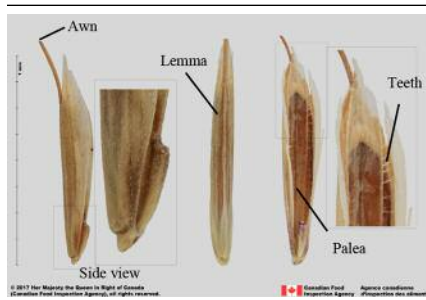
Four brome species, field brome, Japanese brome, cheat and downy brome, are classified as 'secondary noxious' under the Canadian *Weed Seeds Order* (2016). These species have been introduced into Canada from temperate regions of Europe and Asia and from northern Africa. Japanese, brome, cheat and downy brome are widespread in Canada; field brome is found in Ontario. These species may be found as weeds in either domestic or imported seeds. The florets of these secondary noxious brome species could be morphologically similar to brome species not on the *Weed Seeds Order*. Seed morphological features of these brome species (Figs. 1–4) are described below.

**Field brome (*Bromus arvensis* L.):** Floret is long and narrow with a size of 7.0–9.0 mm (L) × 1.0–1.5 mm (W), oval-shaped and tends to have straight sides. Floret base is weakly ridged and ends abruptly in the side view. Lemma is thin and papery with translucent edges, with smooth or short hairs on the upper half. Palea tends to be of similar length as the lemma, with long and thin teeth. Caryopses can be reddish-brown or purplish, and are weakly to strongly curled. Awn is long, arises below the split in the lemma, and is about 6–11 mm.

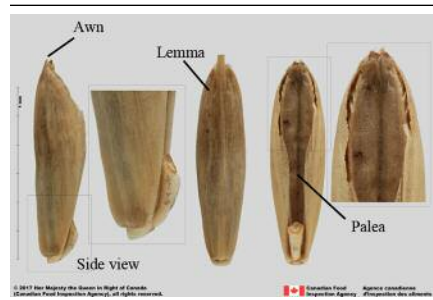
**Cheat (*Bromus secalinus* L.):** Floret is long and wide with a size of 6.5–8.5 mm (L) × 1.8–2.5 mm (W), oval-shaped with curved sides. Floret base is weakly ridged, wide and ends abruptly in the side view. Lemma is thick and smooth with short hairs along the curled edges. Palea is similar in length to both the lemma and caryopsis, with short and thin teeth. Caryopses are reddish-brown and strongly curled. Awn is short, arises from the top of the lemma, and is about 3–6 mm.

**Japanese brome (*Bromus japonicus* Houtt.):** Floret is long and wide with a size of 7.0–9.0 mm (L) × 1.0–2.0 mm (W), oval-shaped with a flared top. Floret base is strongly ridged and humped in the side view. Lemma is papery with translucent edges, with short or long hairs on the upper half. Palea tends to be

**FIGURE 1.**  
Field Brome (*Bromus arvensis*)



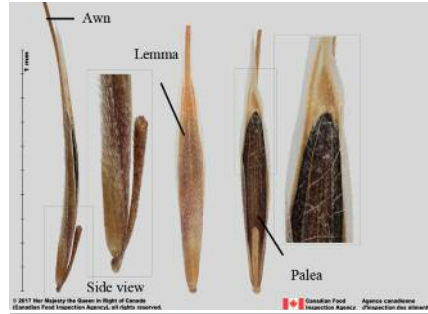
**FIGURE 2.**  
Cheat (*Bromus secalinus*)



**FIGURE 3.**  
Japanese brome (*Bromus japonicus*)



**FIGURE 4.**  
Downy brome (*Bromus tectorum*)



a similar length as the caryopsis, with long and thin teeth. Caryopses can be reddish-brown or purplish, and are flat or weakly curled. Awn is long, arises below the split in the lemma, and is about 8–13 mm.

**Downy brome (*Bromus tectorum* L.):** Floret is very long and narrow with a size of 9.0–12.0 mm (L) × 1.0–2.0 mm (W), oval-shaped and flattened, and appears arched in the side view. Floret base is weakly ridged, narrow and appears notched in the side view. Lemma is papery and hairy with wide translucent edges and top; hairs can be short or long. Palea tends to be as long as, or shorter than, the caryopsis, with very long and thin teeth. Caryopses tend to be purple and lie flat. Awn is very long, appears as part of the lemma, and is about 10–18 mm.

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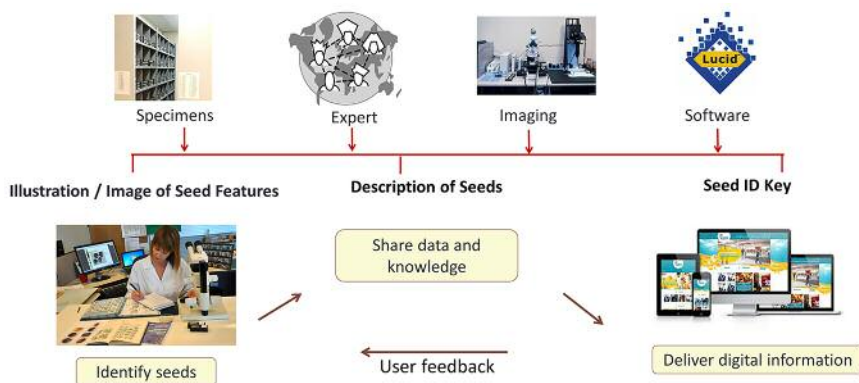
## Development of a Digital Tool for Seed Identification

Ruojing Wang\*, Jennifer Neudorf and Angela Salzl

**Seed identification.** Identifying unknown seeds, especially those of noxious weeds, is a routine and important diagnostic test in seed certification. Seed identification is primarily based on seed morphological features according to taxonomic classification. Digital identification tools allow seed identification features to become accessible virtually, making them an important work tool and reference in a seed laboratory.

**Digital tool development.** The following is a digital tool development flow-chart and required resources:





**Digital tool main components:** a) *Web navigation.* A web-based identification tool provides a complete tool kit that satisfies the end user's needs. In addition to the main components of ID fact sheets, image gallery, interactive key matrix (e.g., Lucid Key), it should also include a home page, user instructions and other resources such as a glossary, literature references, contact and feedback information. b) *Fact sheets.* Fact sheets provide complete identification descriptions and associated morphological features in multimedia: text description of seed features, images or illustrations of seeds such as feature close ups, population variation of seeds, complete shape or color profiles, and species surface or specific feature descriptions. A comparison of similar species is also desirable in various formats, e.g., a comparison chart, descriptions of morphological differences or identification tips, and images or illustrations. c) *Identification key.* The interactive taxonomic key matrix is assisted by computer programs or software (e.g., Lucid Key) to develop identification keys for plant families, genera or species, allowing variable stages of identification. Identification keys can have media assistance, and feature guides and advice for end users. User-friendly seed ID keys should not require special training on the software. A good computer program should also have tolerance for feature selection errors and uncertainties. d) *Image gallery.* The image gallery hosts seed feature illustrations or image collections designed to assist in identification, such as sorting by taxonomic classification according to family and genus; seed feature categories such as seed size, seed color, or seed shape; or other search needs such as key words, scientific or common names. This is a reference tool that can be used for initial identification screening, training, or to familiarize users with seed morphologies.

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