Abstracts

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Distinguishing Western Wheatgrass (*Pascopyrum smithii*) and Couchgrass (*Elymus repens* subsp. *repens*) Florets

Jennifer Neudorf*, Ruojing Wang and Susan Putz

Western wheatgrass [*Pascopyrum smithii* (Rydb.) Barkworth & D.R.Dewey] is native to North America, and is recognized as a crop kind by the Canadian *Seeds Act and Regulations*. Couchgrass or quackgrass [*Elymus repens* (L.) Gould subsp. *repens*], a native of Eurasia and exotic to North America, is a regulated weed classified as 'Primary noxious' in the Canadian *Seeds Act and Regulations*. Both species are perennials adapted to similar growing areas and conditions in North America. It is important to correctly identify these species to ensure the correct labelling of seed and to minimize the spread of couchgrass, reducing the costs of controlling this noxious weed. These two species are similar and the florets can be difficult to distinguish. Staff at the Canadian National Seed Herbarium have examined these species and identified characteristics that may aid in distinguishing them. The distinguishing features of western wheatgrass and couchgrass seeds include: the shape of the rachilla and the sinus enveloping the base of the rachilla, the shape of the suture (the basal point of attachment), the shape and spacing of the teeth along the edges of the palea, and the features of the callus. Western wheatgrass has a wedge-shaped rachilla, a V-shaped sinus, a triangle shaped suture, and palea teeth that are both long and short and are closely spaced. Western wheatgrass also has a depressed line above the callus with hairs on the callus. Couchgrass has a parallel-sided rachilla, a U-shaped sinus, an oval shaped suture, palea teeth that are thick and widely spaced, and a bump above the callus without hairs. Florets may not present all of the features described due to immaturity, mechanical, chemical or insect damage, or disease. Cultivating expertise in taxonomy and good reference specimens will increase the chances of an accurate identification.

Canadian Food Inspection Agency – National Seed Herbarium, Seed Science and Technology Section, Saskatoon Laboratory, Saskatoon, Saskatchewan, Canada. *Corresponding author (jennifer.neudorf@inspection.gc.ca). Received 30 May 2012.

Comparison of Perennial Ryegrass Purity by Allelic Discrimination, AOSA and USDA Growout, and Fluorescence Tests

Daniel Curry

The objective of this study was two-fold: first, to determine the percent annual ryegrass (*Lolium multiflorum* Lam.) contamination by allelic discrimination (AD) on spiked perennial ryegrass (*Lolium perenne* L.) samples (study one); and second, to compare the AD perennial ryegrass purity results with various methods using the fluorescence test, two growout tests and an extended growout

test (study two). Gulf annual ryegrass seed was placed into eight seed packets, with perennial ryegrass seed added to contaminate the 400 seed samples at levels from 0 to 10%. Nine samples from seed industry lots were split into 5 g sister samples and sent to three laboratories for testing using various methods depending on the laboratory: the AD test, fluorescence test, the AOSA growout test, and the USDA 400-seed growout test. Study one showed that AD test results were very similar to the initial spiking levels. Study two showed the AD test results were within tolerance of the USDA 400-seed growout test in 7 of the 9 samples. The AD and AOSA seed growout tests were within tolerance 5 of 9 times, and the AOSA seed growout test was within tolerance of the USDA 400-seed growout test 9 of 9 times. Fluorescence test results were comparable to the optional growout and genetic test results, which indicated that the more contaminated a perennial ryegrass sample was, the further apart were the results. The AOSA growout test was extended to 70 days in the greenhouse to show that results can vary in the growout test if plants are finaled beyond the recommended time.

Oregon State University Seed Services, Oregon State University, Corvallis, OR (Daniel.Curry@oregonstate.edu). Received 3 June 2012.

Intolerance with Annual-Perennial Ryegrass Testing Using DNA: Results of the STRF Study Across Laboratories

Reed E. Barker* and Judy Barker

Seed analysts and seed control agencies have become intolerant of ryegrass (*Lolium* spp.) tests being out of tolerance. Seedling root fluorescence (SRF) has been used to distinguish annual (*L. multiflorum* Lam.) from perennial (*L. perenne* L.) ryegrass for years. For some samples, however, this test has proven to be highly variable across laboratories doing the testing, with test results being out of tolerance and resulting in stop sales. A ryegrass allelic discrimination (RAD) test that examines variants in DNA sequences closely associated with plant growth types has been developed. When examined across three laboratories, there were differences among the laboratories for some samples when the RAD test was coupled with SRF. Statistical analyses, however, found that when variation associated with SRF alone was removed, there were no significant differences among laboratories in RAD test results. Tolerance tables are based on normal sample-to-sample variation (sampling error) and on variation associated with conducting the test itself (experimental error). To develop accurate tolerance tables for the RAD test, a grant from the Seed Technology Research Foundation (STRF) was provided in order to examine aspects of experimental error. The objective of the STRF study was to examine the actual RAD reaction values and final decisions of growth type prediction measured in four laboratories. Our attempt was to control all other sources of experimental variation in the study. Both among and within laboratory sources of RAD reaction variation were measured by including "blind" duplicate samples.

Reactions were determined on a seedling by seedling basis, rather than by seed lot, because the RAD test is based on genetic determination on an individual seed/plant basis. Variation among duplicate samples within laboratories was trivial and not significant. Only one plant in each of two laboratories was classified differently than its duplicate. Preliminary data analysis indicated that deviations from expected results among laboratories were also insignificant, indicating that standard tolerance tables based on sampling error alone may be sufficient for the RAD test. The RAD test relies on a SRF prescreening to reduce costs. Because of the apparent large experimental variation associated with SRF, however, we suggest that updated tolerance tables based on actual experimental evidence for the SRF test be developed and those tables also be used for the RAD test.

Grass Genomic Testing, Inc. 1615 Sisters Ct. NW, Salem, OR 97304-2089. *Corresponding author (reed@grassdna.com). Received 3 July 2012.

Osmopriming Improves Germination Performance and Stress-Tolerance of 'Bloomsdale' Spinach: Cellular Mechanisms Invoking 'Priming-Memory' and 'Cross-Tolerance'

Rajeev Arora* and Keting Chen

Osmopriming is a pre-sowing treatment that exposes seeds to low external water potentials created by osmotica such as polyethylene glycol (PEG) and salts, thus allowing only partial imbibition. This induces a 'head-start' of germination-related activities, and primed seeds exhibit improved germination. Some evidence indicates that primed seeds have improved stress tolerance, but little is known about the biochemistry of such response. Spinach (*Spinacia oleracea* L.) seeds were used as the model to study the cellular mechanism of osmopriming-induced stress tolerance. First, an optimal priming protocol for 'Bloomsdale' spinach was developed. Osmopriming with −0.6 MPa PEG 8000 at 15 °C for 8 days resulted in most improved germination, (assessed by percentage germination, speed and uniformity), as well as chilling and drought tolerance. Next, we conducted a comprehensive investigation of an antioxidant system, dehydrins (DHN), and aquaporins (AQP) metabolism during priming and post-priming germination under optimum and stress environments. Results indicated that osmopriming facilitated a rapid re-establishment of antioxidant network in the germinating seeds, DHN transiently accumulated during the early stages of osmopriming but re-accumulated in primed germinating seeds (but not in unprimed ones) when exposed to drought, and AQP were transiently up-regulated during osmopriming and the primed-seeds had higher expression of AQP during germination. Taken together, these observations led us to propose a model for the cellular mechanism

(based on a two-pronged strategy) for priming-induced stress tolerance. First, priming sets in motion germination-related activities (e.g., respiration, cell elongation, changes in protein profiles such as AQP, etc.) that facilitate the transition of quiescent dry seeds into a germinating state and lead to improved germination potential. Second, osmopriming imposes abiotic stress on seeds that represses radicle protrusion but stimulates stress-responsive elements (antioxidants, DHN etc.), potential inducers of 'cross-tolerance'. Together, these two strategies constitute a 'priming memory' which can be recruited upon a subsequent stress-exposure, and mediate greater stress-tolerance.

Department of Horticulture, Iowa State University, Ames, IA 50011. *Corresponding author (rarora@iastate.edu). Received 5 July 2012.

Characterization of Genetic Loci Associated with Maize Seed Quality

Jamie Seeman, M. Paul Scott, Nicholas C. Lauter, Hannah Cox and Allen D. Knapp*

Reduced seed quality, influenced by environment and genetics, causes germination problems in maize (*Zea mays* L.). The objective of this study was to identify quantitative trait loci (QTL) associated with seed vigor as defined by saturated cold germination testing in a seed lot of the IBM Syn4 population consisting of 203 recombinant inbred lines. A saturated cold germination test was conducted with cold temperatures ranging from 8 to 16 °C. Germination test scores for each line were used as a trait to identify QTL by composite interval mapping. Significant QTL were identified on all chromosomes, and these QTL each explained 14% or less of the variance. Our design also allowed us to map loci controlling the temperature sensitivity of the germination effect. Significant QTL for this effect were identified on all chromosomes, and none explained more than 6% of the variance. To understand the genetic control of cold germination in greater detail, we also mapped cold germination scores separately for each temperature in the study. This allowed us to dissect the temperature dependence of the QTL identified in the first phase of the study. The majority of the QTL mapped using individual temperature data were significant at only one temperature in the study, and none were significant at more than two temperatures. Our results are consistent with a model in which germination in cold temperature is regulated by many loci with small effects, and these loci function at very specific temperatures. Some of these loci control temperature sensitivity in addition to germination at a specific temperature, and other loci control temperature sensitivity alone. These results underscore the complexity of the genetic control of seedling emergence.

M. Paul Scott and Nicholas C. Lauter, USDA-ARS, Ames, IA; Jamie Seeman, Hannah Cox and Allen D. Knapp, Department of Agronomy, Iowa State University, Ames, IA. *Corresponding author (adknapp@iastate.edu). Received 5 July 2012.

The Use of Trueness to Variety Tolerances in Seed Testing

Sabry Elias* and Riad Baalbaki

Trueness to variety tolerances were established for varietal attributes visible in seeds, seedlings or plants, by growth chamber, greenhouse or field testing. Miles (1963) prescribed these tolerances in the '*Handbook of tolerances and of measures of precision for seed testing*[1]'. No information is available in the Association of Official Seed Analysts rules for seed testing (AOSA, 2011)[2] on when or how to use the trueness to variety tolerances. The objectives of this study were to clarify the principles and applications of these types of tolerances, provide an example of their usage, and examine the validity of the current table of tolerances for fungal endophyte tests (Table 14K) used by AOSA[3]. Trueness to variety tolerances follow the binomial distribution and are appropriate for comparing varietal characteristics of two test results or a labeled value and a second test obtained in the same or different laboratory. The tolerances can be determined for a number of seeds, seedlings or plants ranging from 10 to 1000, regardless of the weight of the seeds. These tolerances are not based on percent by weight, as is the case in purity tolerance tables. These tolerances can be applied to seed lots of a single variety or to individual varieties that can be easily distinguishable in a mixture. Comparing two results of the grow-out test to distinguish between annual and perennial ryegrass is one example of the application of the trueness to variety tolerances. Results of this study suggested that the current tolerance table (14K) in the AOSA rules is erroneously used to calculate tolerances for fungal endophyte. Table 14K is adopted from Table V1, '*Trueness to Variety*' by Miles (1963) and is appropriate for comparing test results of observable, phenotypical varietal traits. No visual variation between infected and non-infected seeds or plants with fungal endophyte can be observed, thus it is not appropriate to use this table for endophyte tolerances. A new tolerance table for comparing two test results of fungal endophyte is needed to replace the current invalid one in the AOSA rules.

S. Elias, Department of Crop and Soil Science, Oregon State University, OR; R. Baalbaki, California Department of Food and Agriculture, Sacramento, CA. *Corresponding author (sabryelias@oregonstate.edu). Received 12 July 2012.

[1] Miles, S.R. 1963. Handbook of tolerances and of measures of precision for seed testing. Proc. Int. Seed Test. Assoc. 28(3): 525–686.

[2] AOSA. 2011. Rules for testing seeds, Vol. 1. Principles and procedures. Assoc. Offic. Seed Anal., Ithaca, NY.

[3] AOSA. 2011. Table 14K–Tolerances for fungal endophyte tests when results are based on 30 to 400 seeds, seedlings or plants in a test. p. 14–31. *In* Rules for testing seeds, Vol. 1. Principles and procedures. Assoc. Offic. Seed Anal., Ithaca, NY.

Effect of Factoring Procedure of Multiple Seed Units on Purity Testing Results of Orchardgrass (*Dactylis glomerata* L.)

Adriel Garay and Sabry Elias*

Factoring is a mathematical procedure to determine the percentage of pure seed present in multiple seed units (MSU) of orchardgrass (OG). It is applied on the MSU that are included in the heavy fraction of the sample after the blowing procedure has been completed. This procedure is used by the Association of Official Seed Analysts (AOSA, 2011)[1], whereas the International Seed Testing Association (ISTA, 2012)[2] treats both single seed units (SSU) and MSU as pure seed units. The objectives of this research were to: 1) measure the extent of the increase in inert matter content caused by applying the factoring procedure within a laboratory; 2) compare the planting value of OG SSU and MSU within the same sample; 3) compare the purity test results across laboratories with and without applying the factoring procedure; and 4) measure the time needed to complete the purity test for each method. The pure seed percentage of 645 OG samples were determined at the Oregon State University Seed Laboratory and were analyzed with and without applying the factoring procedure to determine the magnitude of the increase in inert matter content caused by applying the factoring procedure. Germination of SSU and MSU of 10 random OG samples was compared to determine the planting value of SSU and MSU within each sample. Six OG samples were tested at nine laboratories in a national referee to compare the purity test results across laboratories with and without applying the factoring procedure. Factoring the MSU increased the inert matter content by an average of 2.0% in purity test results of the 645 OG samples. The range of the increase in pure seed content may vary depending on number of MSU in each sample. Multiple seed units found in the heavy fraction of 10 OG samples had planting value similar to the SSU. The average germination capacity of SSU of the 10 samples was 91%, compared to 88% for the MSU found in the same samples. The average increase in purity test results of the six OG samples tested in nine laboratories in the national referee was 1.89% when the non-factoring procedure was applied. Comparable germination test results were found in both methods. Purity testing without factoring saved an average of 43% of the time compared to the current factoring method, as measured by the laboratories that participated in the national referee. The factoring procedure increased the total inert matter reported in a purity test by AOSA and slowed down the testing process compared to a non-factoring procedure. The consistency in purity test results in the non-factoring method was similar to the current factoring method. The non-factoring method did not introduce any change to the germination test results and saved time. The non-fac - toring method simplifies testing by avoiding lengthy separation and calculations. It also achieves harmonization in dealing with MSU of OG with ISTA rules.

Department of Crop and Soil Science, Oregon State University, OR. *Corresponding author (sabryelias @oregonstate.edu). Received 12 July 2012.

[1]AOSA. 2011. Rules for testing seeds, Vol. 1. Principles and procedures. Assoc. Offic. Seed Anal., Ithaca, NY.

[2]ISTA. 2012. International rules for seed testing. Int. Seed Test. Assoc., Bassersdorf, Switzerland.