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Variation in seed dormancy in *Echinochloa* and the development of a standard protocol for germination testing

D.A. KOVACH, M.P. WIDRLECHNER AND D.M. BRENNER

USDA – Agricultural Research Service, North Central Regional Plant Introduction Station, Iowa State University, Department of Agronomy, Ames, IA 50011-1170 USA (E-mail: david.kovach@ars.usda.gov)

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Summary

The North Central Regional Plant Introduction Station maintains more than 300 accessions of *Echinochloa* representing 15 species from a diverse cross-section of nations and growing conditions from around the world. With such a diverse collection, no single germination-testing protocol was adequate for accurately assessing their viability. By manipulating light conditions, we determined that some accessions were light-requiring and others were dark-requiring. However, no pattern was found for this response based on taxonomy or improvement status. Most accessions tested showed optimal germination when tests were conducted between 25 to 30°C, but both positive and negative photoblastic responses were sometimes expressed, even at lower temperatures. A sequential treatment of darkness followed by light revealed that skotodormancy (dormancy caused by darkness) was being induced in light-requiring seeds. Similarly, a sequential treatment with light followed by darkness revealed that photodormancy (dormancy caused by light) was being induced in dark-requiring seeds. Thus, without prior knowledge of the light requirements of a particular accession, we conclude that a side-by-side germination test where two replicates receive periodic light (12 hours at 30°C / 12 hours dark at 20°C) and the other two are tested in darkness (12 hours at 30°C / 12 hours at 20°C) is best for *Echinochloa*. This approach is recommended for germplasm centers, seed-testing laboratories, and others working with genetically and geographically diverse *Echinochloa* seed lots.

Introduction

Echinochloa is a cosmopolitan genus comprised of 20 to 25 species of panicoid grasses (Poaceae: Panicoideae). Although considered a weed in many agricultural systems, it is also valued by wildlife managers as a food source for waterfowl (Ahn *et al.*, 2004), and in some regions is used as a grain for human consumption (Partohardjono and Jansen, 1996; Global Facilitation Unit, 2009). According to de Wet *et al.* (1983), *Echinochloa* is widely distributed in the warmer parts of the world, with two species that have been domesticated for grain production. *Echinochloa esculenta* (A. Braun) H. Scholz is grown as a relic grain crop of local importance in Asia, and was probably domesticated in Japan or adjacent warm-temperate eastern Asia (IPK, 2009). *Echinochloa frumentacea* Link is cultivated as a cereal crop in the Indian subcontinent and was previously in Egypt (IPK, 2009).

The North Central Regional Plant Introduction Station (NCRPIS) conserves diverse populations of *Echinochloa* as part of the United States Department of Agriculture's National Plant Germplasm System. The NCRPIS maintains more than 300 *Echinochloa* accessions representing 15 species from more than 30 countries. As can be seen from table 1, the NCRPIS collection represents a diverse cross-section of nations and growing conditions.

Table 1. Areas (Taxonomic Databases Working Group, 2009) and countries of the NCRPIS *Echinochloa* collection.

Areas	Countries
Africa	Egypt, Eritrea, Ethiopia, Kenya, Mali, Nigeria, South Africa, Zambia, Zimbabwe
Asia-Temperate	Afghanistan, China, Iran, Israel, Japan, Russian Federation, Turkey
Asia-Tropical	India, Pakistan
Australasia	Australia
Europe	Belgium, Former Serbia and Montenegro, France, Germany, Hungary, Italy, Poland, Portugal, Slovenia, Ukraine
Northern America	United States
Southern America	Argentina, Brazil

Echinochloa testing methods are included in protocols of both the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA), but the substrates prescribed by these two organizations differ. The AOSA (2006; as well as recent 2009 rules) recommends placing seeds between blotters or in paper toweling, which excludes light, while the ISTA (2005) recommends placing seeds on top of blotter paper, which exposes them to light. The photocontrol of dormancy is so complex and varied (Bewley and Black, 1982) that it cannot be assumed that one germination-testing protocol will meet the needs of a genus of such widespread distribution, containing both weedy and domesticated types.

Seeds of *Echinochloa* have been stored at the NCRPIS since 1951. In order to monitor the viability of these seeds, germination tests are regularly conducted on all lots designated for distribution. Past germination tests of various *Echinochloa* species have shown variable responses, sometimes with large differences for the same seed lot. Testing protocols employed for this genus at the NCRPIS have also not always been consistent over time, as different curators and analysts used methods prescribed by either the AOSA or ISTA. In retrospect, no single approach was consistently superior. With the wide diversity of *Echinochloa* accessions at the NCRPIS, research was conducted to develop a protocol incorporating multiple germination-testing methods to ensure more accurate results for each accession.

Methods

A. Twelve-accession germination test

Twelve accessions of five species of *Echinochloa* were tested under the temperature-light conditions recommended by AOSA (2006) and ISTA (2005). Ten of those accessions were selected because of suspected high levels of dormancy in specific seed lots based on previous test results at our station, and two accessions were included as positive controls, because of their previously high level of germination (table 2, experiment A). For each accession under each set of conditions, 50 seeds in three replicates were placed on wetted germination blotter paper (Anchor Paper Company, St. Paul, MN) in 13 × 13.5 × 3.5 cm plastic germination boxes for a 21-day germination test. In the dark treatment (AOSA, 2006), each box was wrapped in aluminum foil to exclude light. In the light treatment (ISTA, 2005), each box was left unwrapped. Three Hoffman Manufacturing Company (Albany, Oregon, USA) SG-8 model germinators, retrofitted with Omron E5CJ controllers (purchased from Hoffman), were set at 30°C with cool-white fluorescent lighting (12 hours) alternating with 20°C in darkness (12 hours), with each germinator housing a replication. Each germinator had 6 15-watt fluorescent tubes illuminating a space approximately 56 cm wide × 61 cm deep × 61 cm tall.

Table 2. Accessions used in the germination tests showing previous germination tests results by NCRPIS personnel.

Accession	Lot[1]	*Echinochloa* species	Country of origin	Previous test results			Experiment[2]
				% Normal	% Dormant	Year tested	
Ames 14521	1996	*E. crus-galli*	United States	23	76	2005	A, C
PI 196214	1993	*E. colona*	India	13	86	2005	A
PI 197854	1990	*E. crus-galli*	Argentina	21	78	2005	A
PI 292598	1983	*E. colona*	Israel	37	60	2005	B, C
PI 383642	1990	*E. crus-galli*	Turkey	0	98	2005	A, B
PI 420160	1990	*E. crus-galli*	France	9	90	2005	A
PI 441986	1990	*E. turneriana* (Domin) Black	Australia	10	87	2005	A, B, C, D
PI 477956	1990	*E. walteri* (Pursh) Heller	United States	24	70	2005	A, B, C, D
PI 649325	2005	*E. crus-galli*	Hungary	12	76	2006	A, D
PI 649326	2005	*E. crus-galli*	Poland	13	86	2006	A, D
PI 649328	1998	*E. crus-galli*	Former Serbia & Montenegro	93	6	2005	A[3]
PI 649333	2005	*E. crus-galli*	Portugal	13	58	2006	A
PI 649349	1984	*E. frumentacea*	India	100	0	2005	A[3]

[1] Year of lot indicates the year in which the seedlot was regenerated. [2] A = Lot used in twelve-accession test; B = Lot used in three-temperature test; C = Lot used in five-temperature test; D = Lot used in test of recommended protocol. [3] Positive control.

B. Three-temperature germination test

Four accessions representing different species of *Echinochloa*, with moderately high to high levels (60 to 98%) of suspected seed dormancy based on earlier test results, were utilized for this experiment (table 2, experiment B). Fifty seeds in each of three replicates were placed on wetted germination blotter paper in wrapped and unwrapped plastic germination boxes, as described above, and placed in three Hoffman SG-8 model germinators, retrofitted with Omron E5CJ controllers, set at three different light-temperature regimens: 1) 30°C with cool-white fluorescent lighting (12 hours) alternating with 25°C in darkness (12 hours); 2) 20°C with cool-white fluorescent lighting (12 hours) alternating with 15°C in darkness (12 hours); and 3) 15°C with cool-white fluorescent lighting (12 hours) alternating with 10°C in darkness (12 hours). Condition 1 (30/25°C) germination tests were completed in 21 days. Condition 2 (20/15°C) germination tests were conducted for 35 days (extended for lower temperatures), after which all boxes were unwrapped and germinator conditions were changed to condition 1 settings for 14 days. Condition 3 (15/10°C) germination tests were conducted for 49 days (extended longer for lower temperatures); as with condition 2, samples were exposed to the condition 1 regimen for 14 days. In the following results and discussion, the low-temperature phase of conditions 2 and 3 will be referred to as the *initial* phase and the resulting germination values as the *initial germination*.

Because the main difference between the AOSA and ISTA methods is the use of darkness or periodic lighting, a reduced amplitude temperature-shift of 5°C was chosen for these experiments in order to focus more on the effects of light and darkness in relation to the chosen temperature regimens rather than on temperature shifts. The temperature shift was not eliminated completely, however, because some temperature shift may be needed to express the light effects (Bewley and Black, 1982). Two lower temperature regimens were included to determine if cooler conditions would affect germination response to light or darkness. When the lower temperatures showed a reduced germination level (especially with the dark-treated seeds), samples were then transferred to condition 1 lighted treatments. The results prompted a follow-up study where a more complete range of temperature regimens was tested, as described below.

C. Five-temperature germination test

Four accessions representing different species of *Echinochloa* with levels of suspected seed dormancy above 60%, based upon past seed germination laboratory tests, were utilized for this experiment (table 2, experiment C). As in the three-temperature experiment, we used PI 292598, PI 441986, and PI 477956. However, PI 383642 was not used because of its unresponsiveness to all initial treatments in the three-temperature experiment, and was replaced with Ames 14521. Fifty seeds in each of two replicates were placed on wetted, white filter-paper disks in 100 mm (dia) × 15 mm (ht) plastic petri dishes. Ten treatments consisted of all combinations of five temperature regimens with light and darkness. The petri dishes were placed on five 'temperature channels' on a Seed Processing Holland BV thermal-gradient table (Model 5008.00.00.A5, Enkhuizen, The Netherlands). Space restrictions limited the test to two replicates. Alternating temperature regimens (12 hour/12 hour) consisted of 30/25, 25/20, 20/15, 15/10 and 10/5°C. The thermal-gradient

table had both clear and opaque covers to provide the light and darkness. A light barrier between the covers and the table top (to exclude light penetration from the clear covers) was provided by placing rolled carpet padding and a black plastic sheet between the two areas. Above the table were four 58-watt cool-white fluorescent lights to provide 12 hours of light per daily cycle during the higher temperature phase. Scoring of dark-treated seeds was done by using green 'safe' lighting, created by securing green and blue-green acetate sheets (Roscolene #874 and #877, Rosco Laboratories Inc., Stamford, CT) in front of 22-watt cool white, 20 cm diameter circular fluorescent bulbs. These germination tests were initially conducted for 21 days. Afterwards, only clear covers were utilized, and the thermal-gradient table was set so all temperature channels received the 25/20°C regimen (25°C + light, 12 hours / 20°C + dark, 12 hours). This follow-up test continued another 21 days.

D. Test of recommended germination protocol

Based on the previous experimental results, four accessions of *Echinochloa* were utilized for this experiment (table 2, experiment D). Two accessions (PI 477956 and PI 441986) are light-requiring for germination, and two accessions (PI 649325 and PI 649326) are dark-requiring. There were four treatments for each accession: 1) 2 weeks in periodic light; 2) 1 week with periodic light, followed by 1 week in darkness; 3) 1 week in darkness, followed by 1 week in periodic light; and 4) 2 weeks in darkness. For each accession under each set of conditions, 50 seeds in three replicates were placed on wetted germination blotter paper in 13 × 13.5 × 3.5 cm plastic germination boxes. For the dark-treatment phase, each box was wrapped in aluminum foil to exclude light. In the light-treatment phase, each box was left unwrapped. Three Hoffman germinators, described above, were set at 30°C with cool-white fluorescent lighting (12 hours) alternating with 20°C in darkness (12 hours) with each germinator housing a replication. The germination test lasted 14 days, with germination scored on days 7 and 14, coinciding with lighting changes for treatments 2 and 3 and the end of the test.

Statistical analysis

Results were analyzed by using the Analysis of Variance, General Linear Model and Regression Procedures with Statistical Analysis Software, version 9.2 (SAS®, 2008). Germination values were evaluated based on the Least Significant Difference and Duncan's Multiple Range tests. The initial phase of the five-temperature germination test was evaluated with quadratic regression to examine relationships between temperature regimens and germination responses.

Results and discussion

A. Twelve-accession germination test

Of the 12 *Echinochloa* accessions, six responded positively to light in the initial germination tests (figure 1, left). In the figure, they are shown ordered from left to right, with the highest light-germination percentages starting on the left. Some accessions showed only

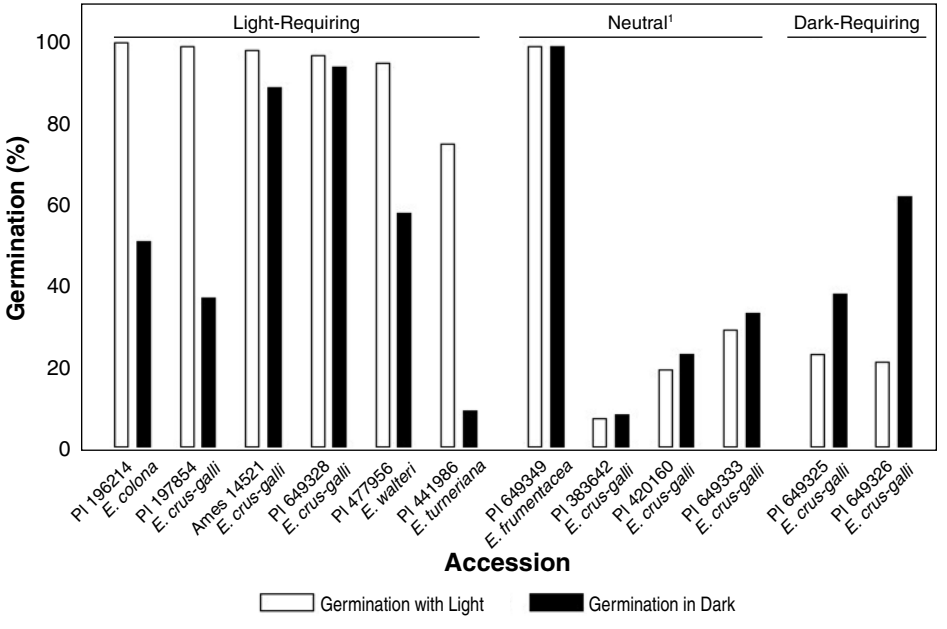

Figure 1. Germination response of 12 *Echinochloa* accessions at 30°C (12 hours) / 20°C (12 hours), grouped by response to light treatments. X-axis lists accession number and species. White bars = light treated (during 30°C cycle). Black bars = continuously dark. [1]nsd between light and dark treatments; all other paired comparisons were significant at $\alpha \leq .05$.

moderate differences in germination between light and darkness (e.g. PI 649328), while others doubled (PI 196214) or quadrupled (PI 441986) in germination in response to periodic light when compared to darkness. Even though there was a significant difference between the light and dark-treated seeds of Ames 14521, the dark germination was higher than expected in this experiment. NCRPIS test results from 2005 (table 2) and those shown in figure 3A displayed much lower germination percentages for dark-germinated samples of this accession.

Two additional accessions (both *Echinochloa crus-galli* (L.) P. Beauv.) showed a negative response to light (figure 1, right), and are shown in order of increasing germination when tested in darkness. However, three of the light-requiring accessions (PI 197854, Ames 14521, and PI 649328) are also *E. crus-galli*. Thus, light requirements for germination cannot be determined solely based on taxonomic identity. Information on the improvement status (wild or cultivated) was not available for all accessions. Of the accessions with known improvement status, three of the light-requiring accessions (Ames 14521, PI 649328, and PI 477956) were wild, as were both of the dark-requiring accessions.

The four remaining accessions showed no significant difference ($\alpha > .05$) between the light and dark treatments (middle of figure 1). These, however, are difficult to classify. For example, PI 649349, a cultivated type from India, germinated at 99% under both light and

dark conditions. This was one of the accessions chosen as a non-dormant control based on NCRPIS germination tests conducted in 2005 (table 2). PI 649349 either lacks innate seed dormancy, as might be expected in a domesticate, or it may have lost dormancy during more than 20 years of storage, and, thus, no conclusion can be drawn regarding positive or negative photoblastic effects. At the other end of the spectrum, PI 383642, collected from an upland rice field in Turkey, did not germinate well under either light or dark treatments. However, in the three-temperature germination test, under the proper conditions more than 80% of these seeds germinated (figure 2B). Dormancy-breaking for this accession required more than light or dark treatments alone.

In relation to the established germination protocols, the ISTA (2005) rules that recommend placing seeds on top of the paper (thus allowing exposure to light) were best for six of the 12 accessions tested here. In contrast, two accessions germinated better following the AOSA (2006) rules that recommend placing seeds between blotters or in paper toweling, thus substantially reducing light exposure.

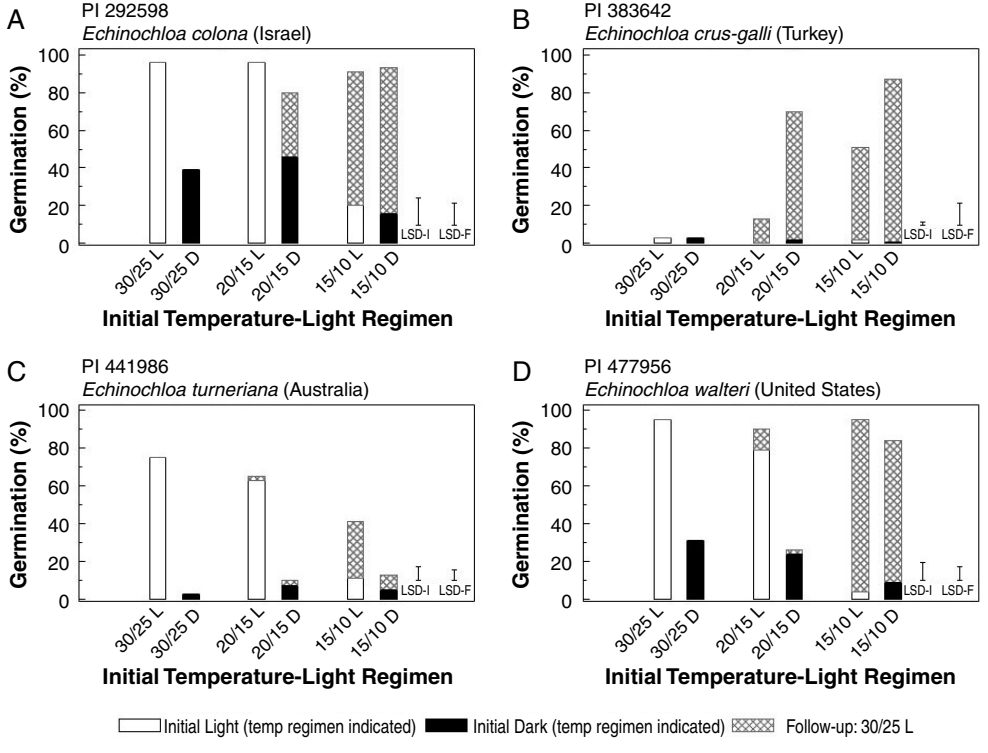

Figure 2. Three-temperature germination test results. Temperature regimens indicated on the x-axis. L = 12 hours light/24-hour cycle; D = constant dark. White bars = initial germination with light. Black bars = initial germination in the dark. Shaded bars = follow-up germination at 30/25°C with light. LSD = Least Significant Difference (α = .05) light versus dark, across temperatures. LSD-I = initial germination; LSD-F = follow-up germination.

B. Three-temperature germination test

Four accessions were exposed to three temperatures regimens of 30/25, 20/15, and 15/10°C. It was our initial hypothesis that running the experiment longer, but at lower temperatures, would eliminate the need for light or dark requirements to break seed dormancy or the use of a long cold-stratification treatment at 5°C, a general practice used on wild-type seeds when germination time is not a factor. But as seen in figure 2, light- or dark-treated seeds did not germinate well at lower temperatures (white and black portions of bars). Subsequent transfer to a 30/25°C regimen with light released substantial seed dormancy (figure 2, A, B, and D, shaded portions of bars). These results show the unpredictable role played by light in the release of seed dormancy at these lower temperatures. PI 383642 (figure 2B) responded better to cool-temperature treatments when first subjected to darkness than when its seeds were only exposed to periodic light. In contrast, PI 441986 (figure 2C) responded better to cool-temperature treatments when exposed to periodic light. Although light-requiring during the initial germination test, PI 292598 and PI 477956 (figure 2 A and D, respectively) failed to display either a light or dark requirement during the cooler-temperature treatments.

Both positive and negative photoblastic responses were sometimes expressed even at the lower temperatures. This should be remembered when conducting short-duration cold, moist treatments to break dormancy, as noted by Romero *et al.* (2005) who reported significant differences in germination when *Echinacea* seeds were subjected to light and darkness during cold, moist pretreatments.

C. Five-temperature germination test

The initial germination for each of the four accessions tested was greater with periodic light (figure 3, white bars) than in darkness (figure 3, black bars) for the 30/25 to 15/10°C temperatures ($\alpha < .001$). In three of the four accessions, initial germination results were highest at 30/25°C. Only PI 292598 (figure 3B) had a higher initial germination at 25/20°C.

Germination was affected by temperature (figure 3). Relationships between temperature regimen and germination could be explained by quadratic regression for all four accessions tested with periodic light ($\alpha < .001$), and for two of the accessions (PI 292598 and PI 477956) tested in darkness ($\alpha < .05$). These regression lines, superimposed on bar graphs in figure 3, suggest that maximal germination should occur at temperatures below the 30/25°C regimen for three of the accessions tested (figure 3 A, B, and C). The regression line for PI 477956 suggests that maximal germination would occur at a temperature above 30/25°C. In contrast to this temperature response, Chauhan and Johnson (2009) showed that germination of *Echinochloa colona* (L.) Link was influenced by light (43% germination in light versus 4% germination in dark), but not by alternating temperatures of 35/25, 30/20, or 25/15°C. This difference may result from the relatively narrow range of temperature conditions or may reflect an accession-specific condition. In contrast, Jursík *et al.* (2004) found that samples of *E. crus-galli* varied greatly in response to temperature. Overall germination was zero at 10°C, optimal in the 20 to 25°C range, and dropped off slightly at 30°C. Germination response to light also depended on temperature (Jursík *et al.*, 2004). At 10°C, there was no germination with light, but slightly above

25% in darkness (a negative photoblastic response). At 20°C, germination was about 94% with light, but only about 44% in the dark (a positive photoblastic response). At 30°C, germination was in the 85 to 95% range, with results statistically the same under light and dark conditions (a neutral photoblastic response). Based on the findings of Chauhan and Johnson (2009) and Jursík *et al.* (2004), together with our own results, we can only conclude that *Echinochloa* seed germination behaviour is complex.

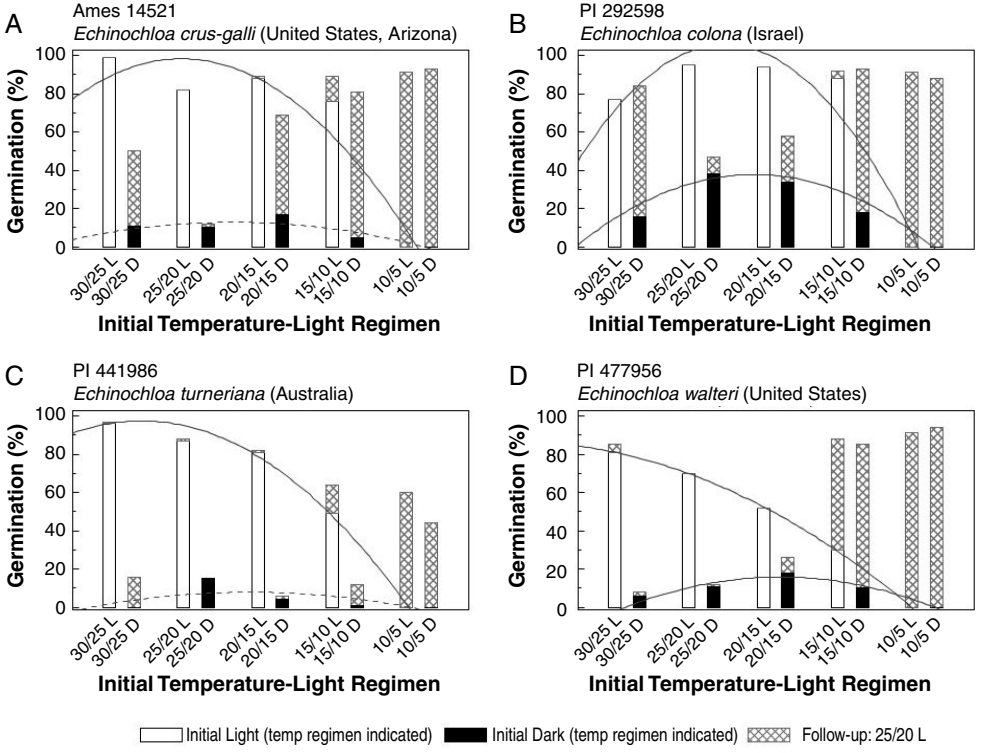

Figure 3. Five-temperature germination test results. Temperature regimens indicated on the x-axis. L = 12 hours light/24-hour cycle; D = constant dark. White bars = initial germination with light. Black bars = initial germination in dark. Shaded bars = follow-up germination at 25/20°C with light. Regression lines oriented to match bar charts. Tcmax = Calculated temperature at which maximum germination is predicted by the regression line. Dashed line = nsd. Solid lines significant at $\alpha \leq .05$.

Regression lines:

A. light: $y = -0.39x^2 + 19.6x - 148$ ($R^2 = 0.87$), Tcmax = 25°C
dark: $y = -0.07x^2 + 3.6x - 29$ ($R^2 = 0.53$), nsd

B. light: $y = -0.62x^2 + 28.0x - 211$ ($R^2 = 0.91$), Tcmax = 23°C
dark: $y = -0.26x^2 + 11.6x - 92$ ($R^2 = 0.79$), Tcmax = 22°C

C. light: $y = -0.30x^2 + 16.7x - 135$ ($R^2 = 0.98$), Tcmax = 28°C
dark: $y = -0.06x^2 + 3.0x - 26$ ($R^2 = 0.34$), nsd

D. light: $y = -0.12x^2 + 8.8x - 76$ ($R^2 = 0.96$), Tcmax = 37°C
dark: $y = -0.14x^2 + 5.9x - 45$ ($R^2 = 0.78$), Tcmax = 21°C

Initial germination percentages in darkness were low for all four accessions tested, and only PI 292598 and PI 477956 (figure 3 B and D) showed significant differences in relation to temperature. Regression estimates (Tc = temperature calculated) for the higher alternating temperature at which maximum germination (Tcmax) is predicted to occur are lower for dark-treated seeds than are the Tcmax values for light-treated seeds. This phenomenon is highly expressed in PI 477956 (figure 3D) where Tcmax drops from 37 to 21°C. Thus, efforts to assess seed viability by using a single germination protocol could be hampered if only the dark treatment is utilized with an upper temperature regimen of 30°C.

Follow-up germination values at 25/20°C with periodic light after the initial treatments of the five-temperature test are graphically represented by the shaded portions of bars in figure 3. In most cases, the follow-up light treatment of these initially dark-treated seeds did not equal the initial, light-treated results under the 30/25, 25/20, or 20/15°C temperature regimens, suggesting that skotodormancy (Evenari, 1956), a secondary dormancy induced in light-requiring seeds by darkness, was expressed at these highest three temperatures. The exception was PI 292598 (figure 3B, 30/25°C). Though not conclusive, it is interesting that in all four accessions the amount of follow-up germination was least at 25/20°C and increased at either higher or lower temperatures. This temperature represents an average for the recommended 20/30°C (low temperature given first in AOSA and ISTA Rules) temperature for AOSA (2006) and ISTA (2005) and is close to the constant 25°C recommended by ISTA (2005). Thus, skotodormancy may express itself greatest at the temperatures recommended by the seed-testing rules.

At the lowest temperature regimen (10/5°C), no germination occurred in the first 21 days, with or without periodic light. But with the raised temperature regimen in the follow-up test, three of the four accessions germinated above 80% (figure 3 A, B, and D), with the fourth, PI 441986 (figure 3C), responding at ca. 50%, indicating that these *Echinochloa* accessions should respond to cold, moist pre-treatments when a light treatment alone cannot release seed dormancy.

Based on our results at temperatures recommended by AOSA and ISTA, a combination of darkness followed by periodic light may not fully counteract skotodormancy imposed during the initial dark interval.

D. Test of recommended germination protocol

Some accessions of *Echinochloa* clearly require light for germination, and others, darkness. Since a sequential treatment of darkness followed by periodic light was not optimal for light-requiring seeds, we conducted side-by-side treatments of light-treated for two replications and dark-treated for the other two replications of a standard germination test. Also tested were two sequential light-condition treatments consisting of light-treated followed by dark-treated germination and dark-treated followed by light-treated germination. The two-week light treated treatment was the best regimen for the light-requiring accessions (figure 4 A and B), and the two-week dark treatment was the best regimen for the dark-requiring accessions (figure 4 C and D). Since it is unknown a priori whether an accession is light- or dark-requiring, this necessitates the testing of both conditions, with the recommendation of two replicates for each condition in order

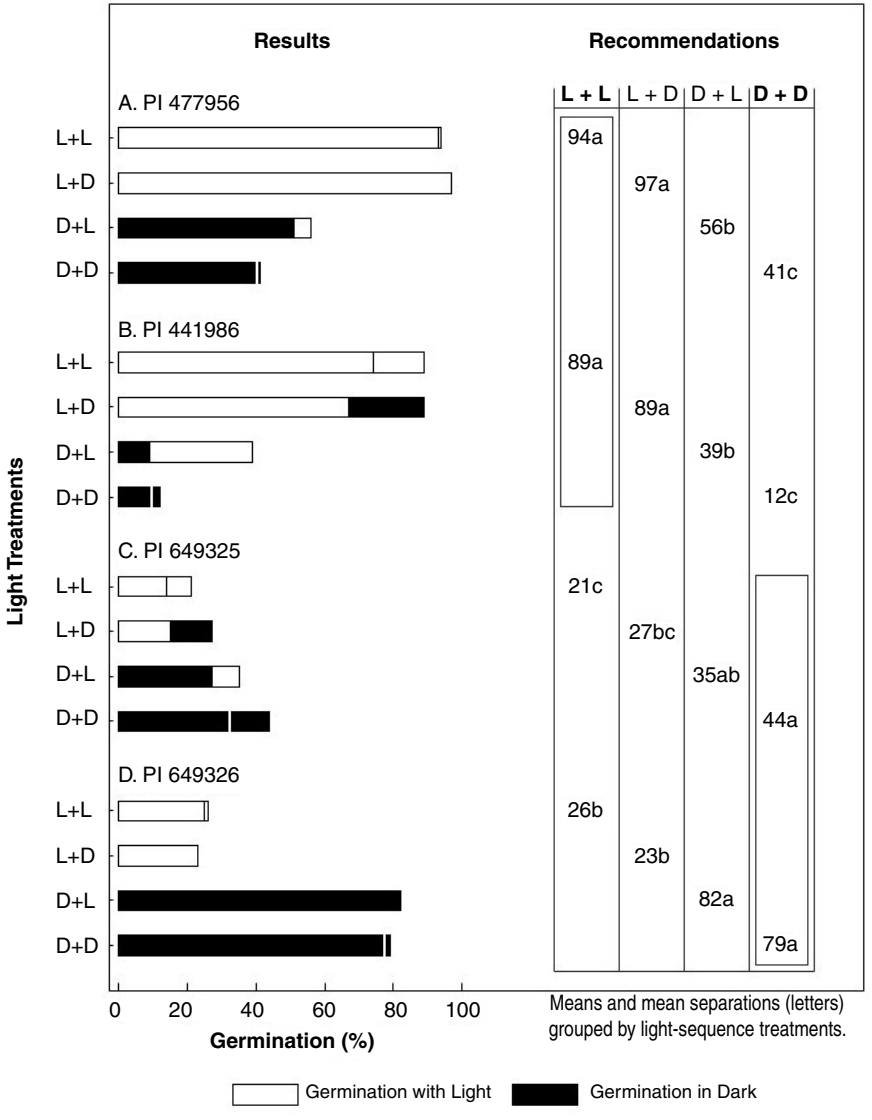

Figure 4. Protocol test results. Light-sequence treatments indicated on the y-axis. L = 12 hours light at 30°C / 12 hours dark at 20°C for 1 week. D = 12 hours dark at 30°C / 12 hours dark at 20°C for 1 week. Germination results are grouped by accession on the left side. The first week's results are on the left of each bar and the second week's results are on the right of each bar; bars with no divider indicate no second week germination. Means and mean separations for each accession are grouped by light-sequence on the right side. Level of significance: $\alpha \leq .05$. Rectangles in the L+L and D+D columns indicate the recommended 'side-by-side' germination test protocol.

to reduce the total number of seeds needed for the test. The disadvantage of splitting the test this way is the reduction of replication. Thus, the idea of a sequential test of one light condition followed by the other was worth evaluating, for then there would be four replicates with the same condition on which to base the evaluation. However, earlier tests (figure 3) showed that a dark-treatment followed by a light-treatment was ineffective in accurately assessing the viability of light-requiring seeds. What remained to be determined was whether a sequential treatment of periodic light followed by darkness would be effective in assessing the viability of both light- and dark-requiring seeds. The results were negative. Although the light-followed-by-dark treatment was effective for light-requiring seeds (figure 4 A and B), it was ineffective for dark-requiring seeds (figure 4 C and D). Also, although the dark-followed-by-light treatment was effective for dark-requiring seeds, it was not effective for light-requiring seeds, as previously shown (figure 3). **Thus, a sequential light-condition test in either order would not be effective in assessing accessions of *Echinochloa* without prior knowledge of the accession's particular light-condition requirements. A side-by-side test of both light and dark treatments, therefore, is recommended.**

Deeper dormancy issues

Nearly complete germination was typically not observed in most seed lots tested (figure 1 and 2B). ISTA (2005) and AOSA (2006) suggested methods, such as scarification (mechanical or acid), hand-removing the lemma and palea along with puncturing the seed coat, long-duration wet, cold pre-treatments, water-soaking or long-term pre-washing, or TZ staining, for determining actual seed viability, but these are labor intensive, accession-specific, or greatly increase test duration. For a seed-testing laboratory or a germplasm-storage facility, where simple manipulation of light and temperature regimens alone does not produce consistently optimal results, alternate methods of seed-dormancy release would be highly desirable. Thus, we will continue to investigate this topic based on studies of diverse *Echinochloa* species and accessions.

Recommendation

Since many *Echinochloa* accessions require light, but others require darkness, for optimal germination, a side-by-side approach is recommended. During the germination test, two replicates should have the seed placed on top of the germination blotter to be exposed to white fluorescent light during the warm cycle of a 20/30°C (12 hours: 12 hours) temperature regimen. Another two replicates should be excluded from light exposure by either placing seeds between and near the centre of two dark, opaque blotter papers or on top of blotter paper but with the germination box carefully wrapped in aluminum foil. Official seed testing laboratories should use 100 seed per replicate. Germplasm centers, where seed quantities are at a premium, could use 50 seed per replicate.

Seeds that do not respond to either a light or dark treatment should be given a follow-up test using a 5°C moist, cold treatment for two months (based on experiment B, condition 3; figure 2, 15/10°C), followed by a 20/25°C light regimen, even though this is a lengthy procedure. Alternate follow-up tests are available in ISTA (2006) and AOSA (2005) rules as noted above.

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