Germination of Bahiagrass in Response to Temperature and Scarification

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Abstract. The influence of temperature and various scarification treatments on the physical changes of the lemma and germination of bahiagrass (Paspalum notatum FlUGE) were studied. Mechanically scarified seeds readily germinated on an agar medium. Acid-scarified seeds germinated better than untreated seeds, but not as readily as mechanically scarified seeds. All caryopses excised from nongerminated acid-scarified seeds readily germinated on an agar medium after 17 days. Excised caryopses were placed on a temperature gradient bar corresponding to positions at 15.5°, 21°, 26.5°, 32°, and 37.5°C. All caryopses germinated at 31° to 37.5°C, but germination and seedling growth were best at 32° to 37.5°C. Lipase and acid scarification improved germination when seeds were held at 24°C, but did not enhance germination when seeds were held at 32°C. Scanning electron microscopy revealed that acid scarification removed the cuticular substances of the lemma and the substances in the fissure of the germinating lid, probably facilitating entrance of the water and earlier emergence of the coleorhiza. The data suggest that the lemma and palea are physical barriers in bahiagrass.

Bahiagrass, a member of the Paniceae tribe, is an important grass grown in the southeastern United States. It is widely used for gold courses, lawns, recreational areas, roadsides, and pastures. The plant is grown from seed that characteristically germinate slowly and erratically. The caryopsis is enclosed by the lemma and palea (4). Acid scarification increased bahiagrass germination (3) and removal of the lemma and palea promoted rapid germination of various Paspalum spp. (16). Similar work has been reported on other genera in the Paniceae. Dormancy in Digitaria milanjana and D. pentzii was overcome by removal of the lemma and palea (1). Hacker (5, 6) reported a similar finding in D. milanjana, and suggested that dormancy is an adaptation to the long dry season of such tropical grasses. Post-harvest dormancy has been reported for Panicum maximum (7, 17), P. coloratum (21), P. hirsutum (13), and P. dichotomiflorum (2, 18, 19).

Most workers agree that acid scarification reduced dormancy, but no agreement is made on the mechanism by which it enhances germination. Tischler and Young (21) suggested that acid scarification destroys a germination inhibitor or modifies the lemma and palea to allow inhibitors to diffuse out of the caryopsis of Panicum coloratum. Other reports suggest that dormancy in panacoid grasses may be due to inhibitors in the lemma and palea. Okada (11) showed that soaking dormant, green Panicum maximum seeds in water 1 month after harvest increased germination. In a subsequent study, he concluded that hulling (removal of bracts) from green P. maximum seeds improved germination by removal of inhibitors rather than by increasing oxygen or moisture availability (12). While acid scarification is the method of choice for alleviating seed coat dormancy, aging or after-ripening has been recognized as a natural method for breaking dormancy (22). Harty et al. (7) showed that dormancy in P. maximum largely disappeared between 50 and 300 days of aging in storage, but they still found some dormant seeds at the end of their study.

Little attention has been directed to the influence of temperature on bahiagrass seed germination. Hsu et al. (9) concluded that slow germination and low seedling vigor limit establishment of several perennial warm-season panacoid grasses and that temperature is a major environmental factor influencing both processes. In a comparison of germination of temperate and tropical grasses (including Paspalum notatum), tropical grasses germinated earlier at 35°C than at 25°C or 15°C (23). Ray and Stewart (16) reported that Paspalum dilatatum germinated in four days at 30°C, but required 25 days at 25°C.

The physical characteristics of the lemma influence germination of panacoid grasses, but little work has been done to relate lemma structure to germination. Reeder (14) stated "the caryopsis of panacoid grasses is tightly enclosed by the individual lemma and palea and there must be a means for the radicle of the embryo to emerge to germinate." He further suggested that natural selection would favor those seeds that had a weak spot directly above the position of the radicle. Hitchcock (8)

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observed the indurated lemma in the Paniceae tribe to have a lunate line of thinner texture above the protruding radicle at germination. Roost and Simper (15) refer to the weak area of the usually hard silicated lemma as the germination lid through which emerges the coleorhiza. Thomasson (20) reported a germination lid on fossilized lemmas of *P. elegans*, thus confirming that the germinating lid is not of recent origin. We now report on the influence of temperature and various scarification treatments on germination of bahiagrass.

**Materials and Methods**

*General.* “Seed” is used herein in the generic sense (10) and includes the glumes, lemma, palea, and caryopsis of bahiagrass (4). Freshly harvested ‘Pensacola’ seeds, obtained from a commercial source, were held in an ambient laboratory environment (21° to 24°C) until used for experiments. Seeds were used within 5 months after harvest. Each experiment was repeated after seed had aged 5 to 8 months. Only data obtained from the non-aged seed are presented. All seeds, except where indicated, were handled aseptically. Seeds were surface-sterilized in 5.25% sodium hypochlorite for 20 min and rinsed three times in sterile deionized water. For germination tests, seeds were placed in 10-cm petri plates containing deionized water solidified with 0.7% sterile agar. A seed was considered germinated when the coleorhiza was visible. All data were subjected to analysis of variance or regression analysis. Mean separation, when appropriate, was Duncan’s multiple range test at the 5% level.

**Acid and mechanical scarification.** Seeds that were acid-scarified were placed in 50% *H₂SO₄* for 20 min, rinsed three times in sterile water, and sterilized as above. Other seeds were mechanically scarified. A portion of the distal end of the lemma was excised with a surgical scalpel. Care was exercised to avoid cutting the palea and injuring the caryopsis. The cut provided an entrance for moisture to the caryopsis while maintaining the tight fit and integrity of the lemma and palea. Control seeds were sterilized, but were not mechanically or chemically scarified. There were 10 petri plates each containing eight seeds for each treatment. Plates were placed in an incubator maintained at 28°C. After 17 days, the caryopses were excised from seed that had not germinated. These excised caryopses were placed back on the sterile medium and maintained at 28°C for an additional 6 days. The experiment was analyzed as a randomized block design with a single petri plate used as the experimental unit.
Table 1. Influence of temperature and acid scarification on germination of bahiagrass on agar medium.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Scarification</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
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<tbody>
<tr>
<td>24</td>
<td>None</td>
<td>0.2</td>
<td>1.3</td>
<td>2.3</td>
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<td>24</td>
<td>Acid</td>
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<td>3.3</td>
<td>3.9</td>
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<td>Acid</td>
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<td>3.0</td>
<td>3.8</td>
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Significance

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<td>NS</td>
</tr>
</tbody>
</table>

*Total of eight seeds per petri plate.
NS, **: Nonsignificant or significant at the 0.05% or 0.01% levels, respectively.

Table 2. Influence of temperature and various scarification treatments on percent germination of bahiagrass germinated in peat-vermiculite.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
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<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
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<td>26</td>
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<td>32</td>
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<td>24</td>
<td>Water (37°C)</td>
<td>5.5</td>
<td>28</td>
<td>33</td>
<td>35</td>
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<td>None</td>
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<td>41</td>
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<tr>
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<td>36</td>
<td>37</td>
<td>43</td>
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<td>45</td>
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Significance

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*Based on 1000 seeds
**: Significant at P = 0.05 or 0.01, respectively.

**Lemma and palea extraction.** Lemmas and paleas, separated from 100 seeds, were soaked in 5 ml of water for 72 hr at 24°C. Extract was filtered through a sterile 0.2-μm filter and the filtrate was added to 95 ml of sterile warm liquid agar (0.7%). The agar extract was divided among 10 petri plates. Caryopses were excised aseptically from sterilized seeds and placed either in petri plates containing the agar extract or in petri plates with only agar (control). Bare caryopses were compared to groups of mechanically and acid-scarified seeds that were also placed on petri plates with only agar. Plates were placed in an incubator at 32°C. There were a total of 10 petri plates each containing five caryopses or seeds per treatment. The experiment was analyzed as a randomized block design with a single petri plate used as the experimental unit.

**Temperature.** Caryopses were excised aseptically and placed on 0.7% agar in petri plates. Plates were placed on a temperature gradient bar at positions corresponding to 15.5°, 21°, 26.5°, 32°, and 37.5°C. Temperatures were monitored continuously with an Augus automatic temperature recorder. There were three petri plates each containing five caryopses at each position (temperature). Shoot and root length were measured after 3 and 7 days.

**Acid scarification and temperature.** Seeds were acid scarified and sterilized. Other seeds were also sterilized, but not scarified. Seeds of each type were placed in petri plates containing agar and randomly assigned to incubators maintained at 24° or 32°. Each treatment consisted of five petri plates each containing 10 seeds. The experiment was analyzed as a split-plot design with temperature as the main plot and acid scarification as sub-plots with each petri plate as the experiment unit.

**Lipase, temperature and scarification.** Seeds were scarified by one of four methods. One group of seeds were soaked in lipase (1.5 g liter⁻¹) for 16 hr at 37°C. A control group was soaked in water without lipase for 16 hr at 37°C. A third group was acid-scarified as outlined above. A fourth group was not scarified or held in warm water. Seeds were not handled aseptically and were sown in flats containing peatmoss-vermiculite (1:1, v/v). One-hundred seeds were sown per row, with five rows or replications for each treatment. Flats were placed in incubators maintained at 24° or 32°. In this experiment, a seed was considered germinated when the coleoptile became visible. The experiment was repeated and results from both tests were combined for analysis as a split-plot design with temperature as the main plot and scarification treatments as sub-plots.

**Scanning electron microscopy.** Seed samples were collected and fixed in 5 formalin : 5 acetic acid : 40 water : 50 ethanol, (v/v) (FAA) and held overnight in a refrigerator. The following day, tissues were dehydrated in an ethanol series (60% to 100%) in increments of 10% for 15 min in each concentration. Tissues were rinsed three times in absolute ethanol and critical-point-dried on a Denton DCP-1 dryer with CO₂ as the intermediary fluid. Tissues were mounted on aluminum stubs and sputter-coated with gold in an Eiko IB-2 ion coater. Tissues were stored in a desiccator until viewing. Samples were analyzed in a Hi-

Fig. 5. Scanning electron photomicrographs of the lemma surface of bahiagrass seeds. Glumes have been removed. (A) Not scarified. (B) Acid-scarified. (C) Overall view with lunate fissure of germination lid. (D) Magnified view of fissure with cuticular substances before scarification. (E) After hot water treatment. (F) Emergence of coleorhiza through lemma via the germination lid. Scale bar = 5 μm (A and B); 50 μm (D and E); 500 μm (C and F).
Results and Discussion

Non-scarified seeds germinated slowly at 24°C (Fig. 1). Mechanical scarification of the lemma and palea enhanced germination, but germination was most rapid when caryopses were excised (Figs. 1 and 2). Excised caryopses placed on an agar medium germinated 100% in 3 days. Our findings support those of Burton (3) and Ray and Stewart (16) and suggest that the lemma—palea is a physical barrier to germination. Excised caryopses germinated readily and equally on a medium containing lemma and palea extracts as well as on a medium without extracts (Fig. 2). Bahiagrass apparently does not have water-soluble inhibitors, as do other panacoid grasses (10, 11, 19). Excised bahiagrass caryopses without the lemma—palea do not have an internal dormancy or require an after-ripening period (3). Our seeds were fresh, and excised caryopses readily germinated without any chemical treatment or chilling period.

Temperature had a profound effect on germination and seedling growth (Figs. 3 and 4). Excised caryopses responded cyclically to temperature. Caryopses held at 32° and 37.5° produced longer shoots than those at 26.5° or 21°. Caryopses held at 15.5° for 7 days had no coleoptiles visible; the coleorhizas were swollen, but the primary roots were not visible. At 21°, germination was slow with poor shoot and root growth. These data agree with Yoon et al. (23) and Ray and Stewart (16). Bahiagrass is a warm-season tropical grass and requires elevated temperatures for rapid germination and growth.

Temperature also influenced germination of intact seeds. Seeds held at 32°C germinated faster than those held at 24°C (Tables 1 and 2). Acid scarification aided germination when seeds were held at 24°, but scarification had no effect on germination when seeds were held at 32° (Table 1). Acid-scarified seeds held at 24° had similar levels of germination after 21 days as unscarified seeds held at 32°. This phenomenon was apparent for seeds germinated under sterile conditions (Table 1) and for seeds germinated in a peatmoss-vermiculite medium (Table 2). Lipase increased germination of seed held at 24°, but had no effect at 32°.

Scanning electron microscopy revealed the germination lid located near the proximal end of the lemma (Fig. 5C). Rost and Simpler (15) reported that the germination lid on the floret of bahiagrass is 0.25 × 0.75 mm. They suggested the function of the germination lid was to facilitate emergence of the coleorhiza through the hard silicated lemma during germination. We concur with their observations, since the lunate germination lid of the mature lemma had similar dimensions. There is a fissure delineating the germination lid in the lemma. The fissure is loosely filled with cuticular substances (Fig. 5D). Lipase, 37°C water, and acid scarification altered, by varying degrees, the cuticular surface of the lemma and palea (Fig. 5 A and B). However, the greatest effect of scarification was the removal of substances in the fissure of the germination lid (Fig. 5E), which probably facilitated the entrance of water and eased emergence of the coleorhiza (Fig. 5D).

We conclude that the dormancy of bahiagrass seeds is not due to internal physiology conditions or an inhibitor, but rather to the physical barrier imposed by the lemma and palea. Acid scarification and lipase treatments removed substances found in the fissure of the germinating lid, probably facilitating the entrance of water to the caryopsis, thus enhancing germination.

Bahiagrass germination is temperature-dependent, with a maximum response at ≈35°C.

Literature Cited
