The Effect of Glycinebetaine Priming on Seed Germination of Six Turfgrass Species under Drought, Salinity, or Temperature Stress

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Abstract. Glycinebetaine (GB) seed priming enhances stress tolerance in various plants during the germination and seedling growth stage; however, information regarding turfgrass is limited. In this study, GB at 5 to 50 mM was used to prime seeds of six turfgrass species to evaluate the potential of GB priming in enhancing tolerance to drought, salinity, and sub-optimal temperature during germination. Stress tolerance was determined as relative final germination percentage (FGP) and daily germination percentage (DGP), expressed as percentage of germination under stress conditions compared with the control treatment (i.e., unprimed seeds germinated under non-stress condition) for each species. Daily germination percentage was more sensitive to stress than FGP. Perennial ryegrass (Lolium perenne L.) showed high tolerance to drought, salinity, and chilling temperatures (5 and 10 °C below optimal germination temperature) followed by tall fescue (Festuca arundinacea Schreb.) and creeping bentgrass (Agrostis palustris L.), whereas kentucky bluegrass (Poa pratensis L.), bermudagrass [Cynodon dactylon var. dactylon (L.) Pers.], and zoysiagrass (Zosia japonica Steud.) were stress-sensitive. Kentucky bluegrass and bermudagrass showed higher germination at 10 mM GB under temperature stress and drought and temperature stresses, respectively; however, other grasses showed limited responses to seed priming. Our results showed that the efficacy of GB priming is plant-, GB concentration-, and stressor-dependent.

Plant growth and development is affected by various environmental stresses such as drought, salinity, and suboptimal temperatures. During the seed germination and seedling growth stages, plants are the most vulnerable to stresses (Almansouri et al., 2001). Rapid and uniform seed germination will help plants establish a healthy stand. Seed priming has been used to enhance seed germination and seedling growth in various plants (Farooq et al., 2006). During the priming period, seed is hydrated with water (i.e., hydropairing) or other solutions. The embryo is pre-enlarged and the germination rate is enhanced (Austin et al., 1969; Gray and Steckel, 1977). Research shows that seed priming enhances germination in many crops, especially under environmental stress conditions. For instance, Zheng et al. (1994) reported that hydropaired canola (Brassica napus L.) seeds had a higher germination rate and stronger seedling vigor compared with the unprimed seeds under low-temperature conditions. Similar results were observed in hydropaired and KNO₃-primed sunflower (Helianthus annuus L.) under saline and drought conditions (Kaya et al., 2006). Polyethylene glycol 8000 (PEG8000)-primed soybean (Glycine max L.) showed enhanced seed germination and growth under chilling conditions (Li et al., 2010). However, excess priming may interrupt enzyme activity, resulting in poor germination and abnormal growth (Ajouri et al., 2004; Farooq et al., 2006).

Glycinebetaine (GB) is the most abundant osmoprotectant produced in plants in response to dehydration induced by drought, salinity, and suboptimal temperatures (Ashraf and Foolad, 2007; Chen and Murata, 2008). Accumulation of GB under stressful environmental conditions has been well documented in many plants including barley (Hordeum vulgare L.) (Nomura et al., 1995), wheat (Triticum aestivum L.) (Naidu et al., 1991), and sorghum [Sorghum bicolor (L.) Moench] (Weinberg et al., 1984). Non- or low-GB-accumulating plants showed enhanced stress tolerance upon the transformation of genes regulating the GB-synthesis pathway (Ashraf and Foolad, 2007; Sakamoto and Murata, 1999). Furthermore, exogenous applications of GB improved the chilling tolerance in tomato (Solanum lycopersicum L.) (Park et al., 2006) and maize (Zea mays L.) (Farooq et al., 2008). Foliar applications of GB also significantly increased salinity tolerance in creeping bentgrass (Agrostis palustris L.)(CB), kentucky bluegrass (Poa pratensis L.) (KB), and perennial ryegrass (Lolium perenne L.) (PR) (Hu et al., 2012; Yang et al., 2012). Ashraf and Foolad (2007) reported that GB had multiple functions such as adjusting osmotic potential (Ψs), protecting enzyme and membrane integrity, stabilizing enzymes and proteins, and detoxifying reactive oxygen in plants grown under abiotic stress conditions. Recent reports also show that GB may have

Table 1. Final germination percentage and daily germination percentage of creeping bentgrass as affected by salinity and priming treatments.

| Treatment | Final germination percentage | Daily germination percentage |
|-----------|------------------------------|-----------------------------|
| Salinity (dS·m⁻¹) | 98.5 ± 7.5 a' | 98.2 ± 9.4 a |
| 0         | 92.3 ± 12.2 b   | 80.0 ± 14.2 b               |
| 5         | 77.9 ± 12.9 c   | 59.7 ± 11.9 c               |
| 15        | 57.3 ± 17.5 d   | 38.8 ± 13.6 d               |
| 20        | 28.8 ± 13.5 e   | 15.7 ± 7.0 e                |
| Priming treatment | 72.1 ± 13.7 abc | 56.7 ± 16.4 bc |
| Unprimed | 67.5 ± 15.2 bc | 55.5 ± 16.5 bc |
| Water    | 65.5 ± 13.7 c   | 53.9 ± 13.7 c               |
| Glycinebetaine 5 mM | 77.6 ± 12.2 a | 64.6 ± 14.9 a |
| Glycinebetaine 10 mM | 73.2 ± 14.8 ab | 60.0 ± 14.8 ab |
| Glycinebetaine 200 mM | 74.1 ± 15.3 ab | 61.8 ± 15.9 ab |

*This preliminary experiment was conducted in a five (salinity level) × seven (priming treatment) factorial design, arranged in a randomized complete randomized block design with three replicates (petri dishes). Data of unprimed seeds germinated at 0 dS·m⁻¹ (i.e., the control treatment) were standardized as 100% and other data were presented as percentage of the control.

#Means ± standard error following the same letter of different salinity levels or priming treatments were not different at P ≤ 0.05.

NS means nonsignificant differences at P ≤ 0.05.
A role in the salt overly sensitive pathway under salt stress (Ashraf and Foolad, 2007; Chinnusamy et al., 2005). Most of the aforementioned studies focused on the GB effects on mature plants. Studies on GB enhanced plant tolerance to abiotic stresses through seed priming, however, are limited (Farooq et al., 2008; Mahmood et al., 2009; Zhang and Rue, 2012). The objective of this study was to determine if GB seed priming has the potential to improve turfgrass tolerance of drought, salinity, and suboptimal temperatures.

Materials and Methods

Plant materials and seed priming. Six turfgrass species were included in this study: four cool-season grasses, ‘Stonewall’ tall fescue (Festuca arundinacea Schreb.) (TF), ‘L-93’ CB, ‘Kenblue’ KB, and ‘Zoom’ PR, and two warm-season grasses, ‘Zenith’ zoysiagrass (Zoysia japonica Steud.) (ZOY) and ‘Riviera’ bermudagrass [Cynodon dactylon var. dactylon (L.) Pers.] (BER). Seeds were soaked in continuously aerated deionized/distilled water (ddH2O) or 5, 10, and 50 mM of GB for 24 h at 25 ± 2°C following the procedure used by Zhang and Rue (2012). Glycinebetaine concentrations were selected based on the results of a preliminary study in which the highest germination rate was obtained when seeds were primed with 10 mM GB under saline conditions (Table 1). The ratio of seed weight to solution volume was greater than 1:5 to ensure adequate quantity of solution for absorption (Farooq et al., 2006). Seeds were then rinsed three times with distilled water and air-dried to the original weight in a laminar-flow hood (12 h) in the dark. Unprimed seeds were also included in the experiment. Seeds of each grass (unprimed and primed) were surface-sterilized using the methods of Zhang et al. (2011) before the germination test under each stressful environment.

Expt. I: Priming effect on seed germination under drought stress. Forty unprimed or primed seeds of each grass were placed on a germination paper (Anchor Paper Company, St. Paul, MN) in a 100 × 15-mm petri dish. The germination paper was saturated with 10 mL of PEG6000 solution to provide drought condition (Emmerich and Hardegree, 1991). The level of drought stress was determined to

Table 2. Analysis of variance of final germination percentage (FGP) and daily germination rate (DGR) of unprimed and primed seeds of six turfgrass species under drought, salinity, and temperature stress.

| Source of variance | Drought (D) | Salinity (S) | Temperature (T) |
|--------------------|-------------|--------------|-----------------|
| df                 | FGP         | DGR          | FGP             |
|                    |             |              |                 |
| Drought (D)        | 3           | ***          | ***             |
| Grass (G)          | 5           | ***          | ***             |
| Priming (P)        | 4           | NS           | NS              |
| G × D              | 20          | *            | NS              |
| G × P              | 12          | **           | *               |
| G × D × P          | 60          | ***          | ***             |
|                      |             |              |                 |
| Grassy            | 4           | ***          | ***             |
| Grass × Priming    | 5           | ***          | ***             |
| Grass × Temperature | 4           | ***          | ***             |
| Grass × Priming    | 4           | NS           | NS              |
| Grass × D × P      | 4           | NS           | NS              |
| Grass × Priming × D| 4           | NS           | NS              |
| Grass × Priming × P| 4           | NS           | NS              |
| Grass × D × T      | 4           | NS           | NS              |
| Grass × Priming × T| 4           | NS           | NS              |
| Grass × D × T × P  | 4           | NS           | NS              |

* , **, *** represent significant differences at the 0.05, 0.01, and 0.001 P level, respectively. NS represented nonsignificant differences at the 0.05 P level.

FGP = final germination percentage; DGR = daily germination percentage.
be 0.0, –0.4, –0.8, or –1.2 MPa by calculating the $\gamma_s$ of PEP6000 solution following the method of Michel and Kaufmann (1973). Petri dishes were then sealed with parafilm and placed in a culture room at 25 ± 2 °C under fluorescent light (36 μmol·s⁻¹·m⁻²) with a 8/16-h (light/dark) photoperiod. Environmental conditions were monitored with a HOBO Temp/RH/Light data logger (Model U12-012, Onset Corp.) at a 30-min interval during the experiment.

Data collection and analysis. Seed germination, defined as an emerged shoot visible under 2x magnification, was counted and recorded three times weekly for 4 weeks (McCarty and Dudek, 1993) in each experiment. FGP, representing total germination rate over a 4-week period, and DGP, representing germination rate over time, were calculated following the method of Zhang et al. (2011) in which $\text{FGP} (%) = 100 \times \left(\frac{\text{S}}{\text{n}}\right)$ and $\text{DGP} (%) = 100 \times \left(\frac{\text{n} \times \text{D}}{\text{S}}\right)$, respectively, where $\text{n}$ was the number of new seeds germinated at each counting and $\text{D}$ was the number of days accumulated up to that counting.

Expt. I was a six (turfgrass species) × four (seed priming treatment) × four (drought level) factorial design arranged in a randomized complete block design to minimize potential shelf effect (block) in the culture room. Each treatment had three replicates (petri dishes). Expt. II was identical to Expt. I in experimental design, except that five levels of salinity were included. Expt. III was arranged in a split-plot design. Whole plots were incubators (temperature regimes), where the subplot treatments [six (turfgrass species) × five (seed priming treatment) factorial combinations] were applied in separate incubators in a completely randomized design with three replications (petri dishes). The whole-plot treatment (temperature) had to be replicated sequentially in time (twice) rather than concurrently because of limited availability of incubators.

To avoid variations in seed size and seedling vigor in different turfgrass species, data of unprimed seeds germinated under non-stress conditions (i.e., the control treatment) were standardized as 100% for each species and other data were presented as percentage of the control within each species under drought stress. Data of unprimed seeds germinated at 0.0 dS·m⁻¹ were separated with Fisher’s protected least significant difference when a significant difference occurred ($P \leq 0.05$).

Results

Expt. I: Priming effect on seed germination under drought stress. An interaction between turfgrass and priming treatment occurred in FGP (Table 2). Seed priming had no influence on FGP among all grasses except BER, in which BER seeds primed with 10 μm GB had

(36 μmol·s⁻¹·m⁻²) with a 8/16-h (light/dark) photoperiod. The OPT for the cool-season grasses was 25/15 °C (day/night) (Association of Official Seed Analysts, 2004). The OPT for the warm-season grasses was 30/20 °C (day/night), different from that (35/20 °C) suggested by the Association of Official Seed Analysts (2004) to compensate the number of incubators used in the present study. This temperature setting (30/20 °C) was still within the optimal germination conditions for warm-season grasses with temperature ranging from 20 to 35 °C (Aldous, 1999). Conditions in incubators were monitored with HOBO Temp/RH/Light data loggers (Model U12-012, Onset Corp.) at a 30-min interval during the experiment.
ZOY, and TF showed a greater reduction of drought conditions (–0.4 MPa), KB, BER, 95.4% of the control) (Fig. 1A). Under minor CB and BER had the lowest FGP (average = 103.6% of the control), and had the highest FGP, followed by KB, TF, and PR showed a similar trend in DGP in which PR, CB, and ZOY had DGP higher than other grass species tested under low saline conditions, 5 dS m⁻¹. Creeping bentgrass had a similar DGP as TF at 10 dS m⁻¹; however, TF outperformed CB as salinity levels increased to 15 and 20 dS m⁻¹.

Expt. III: Priming effect on seed germination under temperature stress. A turfgrass × priming treatment interaction occurred in FGP and DGP (Table 2). The priming treatment did not affect FGP in CB, BER, and ZOY (Fig. 3A). Kentucky bluegrass seeds primed with GB at 10 mM had a higher FGP than other priming treatments (average = 58.7% of the control). The FGP of TF and PR increased with GB concentrations and seeds primed at 50 mM GB showed a similar level of FGR as the unprimed and hydroprimed seeds. The DGP of KB and BER was improved by 10 mM GB compared with the unprimed seeds (Fig. 3B). Hydroprimed CB seeds had a higher DGP than unprimed seeds, but not different from those primed with GB (average = 98.2% of the control). In contrast, TF primed with 5 and 10 mM GB showed decreased DGP compared with the unprimed seeds. Perennial ryegrass and ZOY seeds did not respond to the priming treatments.

Interactions involving FGP and DGP between grass species and temperature occurred (Table 2). At OPT, grasses showed little variations in FGP (Fig. 4A). Chilling temperatures (OPT-5 °C and OPT-10 °C) had a limited effect on FGP in CB, TF, and PR, whereas it reduced FGP by 46.9% and 88.0% in the other grasses at OPT-5 °C and OPT-10 °C, respectively (Fig. 4A). Kentucky bluegrass had the highest FGP of all the grasses tested at OPT+5 °C, 20% and 41% higher than ZOY and other grasses (average = 95% of the control), respectively. As the temperature increased to OPT+10 °C, differences in FGP further separated among the grasses; however, only KB had a FGP less than 50% of the control.

Tall fescue had a DGP of 82% of the control at OPT, which was significantly lower than other grasses (Fig. 4B). Creeping bentgrass and PR had a similar DGP at OPT-5 °C, followed by TF, and BER, ZOY, and KB had the lowest DGP. A similar trend was observed at OPT-10 °C. As the temperature increased from OPT to OPT+5 °C, DGP of all grasses increased with the highest and lowest increase in KB and TF, respectively.
At OPT+10 °C, DGP of grasses decreased in the following order: ZOY > CB, PR, and BER > TF > KB.

**Discussion**

Plants are more susceptible to stresses during the seed germination and seedling stages (Almansouri et al., 2001). In general, a decrease of FGP indicates reduced seed germination, whereas a decrease of DGP represents delayed germination of seeds. The results of this research showed that DGP is more sensitive to environmental stresses. For example, salinity stress caused an average of 47.3% and 33% decrease in DGP and FGP, respectively, when data were pooled across salinity levels (Fig. 2). Similarly, a greater reduction occurred in DGP compared with FGP under drought and chilling conditions (Figs. 1 and 4). However, a DGP increase of 31% occurred, whereas only a 7% increase was obtained in FGP when seeds germinated at OPT+5 °C (Fig. 4). A higher stress sensitivity of DGP was also reported in previous research (Dai et al., 2009; Zhang et al., 2011, 2012), suggesting that DGP appears to be a more reliable indicator of stress tolerance than FGP in turfgrasses. Using turfgrass species with a high DGP may lead to fewer environmentally related risks during establishment.

Plants experience a wide range of undesirable environmental conditions during their life cycle. Large inter- and intraspecific variations in stress tolerance exist in plants (Fry and Huang, 2004; Marcum, 2007). Cross-tolerance (i.e., plants resistant to one stress are often more resistant to others) may occur if the stressors induce similar damage and their signaling pathways converge (i.e., cross-talk) (Knight and Knight, 2001; Wang et al., 2003). For instance, drought, salinity, and extreme temperatures are manifested primarily as osmotic stress. Plants accumulated compatible solutes (e.g., carbohydrates, amino acids, and GB) under all three stresses to help stabilize proteins and cellular structures, maintain cell turgor, and remove excessive reactive oxygen species (Krasensky and Jonak, 2012). Ranking of relative tolerance of the six turfgrass species evaluated in the present were fairly consistent across drought, salinity, and suboptimal temperatures (except OPT+5 °C), in which PR was a tolerant species followed by TF and CB, and BER, ZOY, and KB were relatively stress-sensitive (Figs. 1, 2, and 4). Kentucky bluegrass had the highest FGP and DGP at OPT+5 °C of all grasses (Fig. 4), in contrast to its low tolerance to drought, salinity, and chilling temperature (OPT+5 °C and OPT+10 °C) (Figs. 1, 2, and 4). It might be because the OPT+5 °C temperature setting (30/20 °C) remains within the optimum temperature range for KB seed germination (16 to 30 °C) (Aldous, 1999) and its high thermal requirement for seed germination was met sooner at this higher temperature than others (Larsen and Bibby, 2005). As the temperature increased to OPT+10 °C, KB had the lowest FGP and DGP of all grasses (Fig. 4). Dai et al. (2009) and Zhang et al. (2011) reported that PR had high salinity tolerance followed by TF and CB, and KB was salinity sensitive during germination, consistent with our findings. Fry and Huang (2004) reported that BER had the best drought tolerance (excellent) followed by ZOY and TF (very good) and KB (good), whereas PR and CB yielded fair drought tolerance. Bermudagrass and ZOY also had excellent tolerance to high temperatures followed by TF (good), CB and KB (medium), and PR (fair) (Beard, 1973). In contrast, CB and KB had excellent and good tolerance to low temperatures, respectively, followed by TF and ZOY (medium), whereas PR and BER were sensitive to low temperatures (Beard, 1973). The discrepancy between this study and other research may be caused by different evaluation stages: seed germination stage in the current study and vegetative growth stage in other research (Beard, 1973; Fry and Huang, 2004). Early reports showed that relative stress tolerance may differ between germination and vegetative growth (Dai et al., 2009; Wang et al., 2011; Zhang et al., 2013). Large intraspecific variations in stress tolerance may also contribute to the difference between the present research and other findings.

Farooq et al. (2008) reported that maize seeds soaked in 50, 100, and 150 mg L⁻¹ GB...
enhanced chilling tolerance during germination and the seedling growth stage with the best results observed at 100 mg L\(^{-1}\), suggesting that GB had the potential of improving stress tolerance through seed priming. Results from Mahmood et al. (2009) showed that GB priming increased drought tolerance in three of five wheat cultivars and seeds primed at 50 mM GB performed better than those primed at 0 and 100 mM. However, GB priming (at 10 and 30 mM) had no influence on salinity tolerance of another two wheat cultivars in the research of Akhter et al. (2009). Such inconsistent results indicate that the efficacy of GB priming in stress enhancement is plant- (species/cultivar) and concentration-dependent. Zhang and Rue (2012) observed that GB (100 mM) priming increased FGP and seedling vigor of PR, KB, CB, and TF under the salinity stress. However, GB showed no influence on their germination under the same stress condition in the present study (Table 2). Low concentration of GB (less than 50 mM) in this study might be one of the causes. In addition, both NaCl and CaCl\(_2\) were used to induce salinity in the present study, but only NaCl was used in the previous research (Zhang and Rue, 2012). The discrepancy between the two studies might also be caused by the different salt mixtures. A similar result was reported by Yu et al. (2013). They found that GB responded differently to NaCl, Na\(_2\)CO\(_3\), Na\(_2\)SO\(_4\), and CaCl\(_2\) because the electrical conductivity, \(\psi_s\), and pH varied in these salt solutions. Furthermore, our results showed that six turfgrass species primed with the same levels of GB responded differently to drought, salinity, and suboptimal temperatures (Table 2; Figs. 1, 2, and 4). Iqbal and Ashraf (2005) suggested that the efficacy of seed priming varies under different stresses, priming agents, and plant species/cultivars. Ajouri et al. (2004) reported that primed barley seeds had a higher germination rate than the unprimed seeds after a 9-week storage at 4 °C under dark. Similar results were also observed by Powell et al. (2000) in which hydropromed improved germination of low vigor cauliflower seeds (B. Oleracea L. var. botrytis) (slow germinating and short shelf life) and such a positive effect of priming maintained up to 12 months. Fry et al. (1993) reported a faster establishment of hydropromed buffalograss [Buchloë dactyloides (Nutt.) Engelm.] compared with unprimed seeds in the field. Rehman et al. (2011) reported that CaCl\(_2\)-primed rice (Oryza sativa L.) seeds had reduced sterile spikelets and low abortive and chalky kernels and increased kernel nutrient content in a field experiment. Winter wheat germination and emergence were enhanced by seed priming under laboratory, greenhouse, and field conditions; however, seed priming showed no benefit for grain yield in the same study (Giri and Schillingler, 2003).

Thus, further research should be conducted to validate the result of this research under field conditions.

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