Salinity Tolerance of 12 Turfgrasses in Three Germination Media

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Abstraxt. Salinity tolerance of 12 turfgrasses in four groups, creeping bentgrass (Agrostis stolonifera L.), fescues (Festuca spp.), kentucky bluegrass (Poa pratensis L.), and alkaliigrass [Puccinellia distans (Jacq.) Parl.], was evaluated using three germination methods. Seeds were germinated on 1% agar medium, on germination paper, or in a hydroponic system under salinity levels of 0, 5, 10, 15, or 20 g L⁻¹ NaCl. Germination rate and seedling growth of each grass were determined. Salinity reduced the final germination rate (FGR), daily germination rate (DGR), and seedling leaf area (LA) in all tests. On agar medium, no significant difference in salinity tolerance was observed among the four turf groups; however, ‘Turf Blue’ kentucky bluegrass with a corn starch-based coating (coated ‘Turf Blue’) showed a significant higher salinity tolerance than the uncoated one. Using germination paper, creeping bentgrass required the highest salinity level to cause 50% reduction in FGR followed by alkaliigrass, fescues, and kentucky bluegrass. Kentucky bluegrass required the lowest salinity level (9.5 g L⁻¹) to reduce DGR by 50%. With the hydroponic system, alkaliigrass required a salinity level of 26.3 g L⁻¹ to reduce FGR by 50%, the highest among the four groups. Alkaliigrass showed again the highest salinity tolerance with an average of 12.7 g L⁻¹ needed to reduce LA by 50%. Among the grasses, coated ‘Turf Blue’ kentucky bluegrass, ‘Declaration’ creeping bentgrass, and ‘Fults’ alkaliigrass showed the highest salinity tolerance when evaluated on agar medium, on germination paper, or in the hydroponic system, respectively. The present study determined the salinity tolerance of 12 turfgrasses at seed germination and early seedling growth stages and showed that the germination method was a factor affecting the evaluation result and it should be considered in a seed germination test of turfgrass for salinity tolerance.

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Materials and Methods

Seed surface sterilization. Seeds of 12 turfgrasses (Table 1), except coated ‘Turf Blue’ kentucky bluegrass (grass with a corn starch-based coating), were surface-sterilized according to the method of Wang and Zhang (2010). Briefly, seeds were submerged in 95% ethanol for 1 min followed by 2% (v/v) sodium hypochlorite solution for 20 min.
Table 1. Predicted salinity levels (g L⁻¹ NaCl) causing 50% reduction in final germination rate and daily germination rate in kentucky bluegrass, fescues, alkaligrass, and creeping bentgrass on agar medium.

| Group          | PSLF | PSLD |
|----------------|------|------|
| Kentucky bluegrass | 10.3 a | 9.9 a |
| Fescues        | 10.5 a | 9.2 a |
| Alkaligrass    | 12.5 a | 10.0 a |
| Creeping bentgrass | 13.3 a | 10.0 a |

PSLF = the predicted salinity level causing 50% reduction of final germination rate; PSLD = the predicted salinity level causing 50% reduction of daily germination rate.

Seeds were then rinsed three times with sterile deionized/distilled water (ddH₂O) and air-dried in a laminar-flow hood.

Seed germination on agar medium. Surface-sterilized seeds of each grass and coated ‘Turf Blue’ kentucky bluegrass were placed on 1% agar medium (Sigma-Aldrich Co., St. Louis, MO) supplemented with 0, 5, 10, 15, or 20 g L⁻¹ NaCl in 100 × 15-mm petri dishes containing 20 mL of medium with 36 seeds per plate. The EC of the salt solutions was 0.0, 8.4, 15.1, 21.0, and 26.1 dS m⁻¹, respectively, when measured with an EC meter (Model 1054; VWR International LLC, West Chester, PA). The medium was autoclaved at 121 °C and 103 kPa for 25 min before being poured into petri dishes. Dishes containing seeds were sealed with parafilm and placed in an incubator (Model 818; Precision Scientific Inc., Chicago, IL) at 25/15 °C (day/night) under fluorescent light (36 μmol s⁻¹ m⁻²) with an 8/16-h (light/dark) photoperiod (Association of Official Seed Analysts, 2004).

Seed germination on germination paper. Surface-sterilized seeds of each grass and coated ‘Turf Blue’ kentucky bluegrass were placed on germination paper (Anchor Paper Company, St. Paul, MN) in 100 × 15-mm petri dishes with 36 seeds per plate. Before seed plating, the germination paper was moistened with 7 mL of a NaCl solution at 0, 5, 10, 15, or 20 g L⁻¹. Dishes were then sealed with parafilm and placed in the same incubator as for the agar test previously described. After 14 and 26 d, 2 mL of the same salt solution for each treatment was added to the plate to keep the paper moist.

Seed germination in hydroponic system. Non-sterilized seeds were germinated in aerated NaCl solutions at 0, 5, 10, 15, or 20 g L⁻¹ in a growth chamber (Environmental Growth Chambers, Chagrin Falls, OH). Briefly, 12 cells (6-cm diameter) were created on a 30 × 25 × 2-cm foam plate. Two layers of plastic screen were glued on the bottom of the plate. Thirty-six seeds of each grass were placed in one cell. The plate was floated in the appropriate NaCl solution. As seeds germinated, roots penetrated through the plastic screen into the salt solution. The salt solution was refreshed every 4 d with the same concentration. Other conditions were identical to those of the agar medium or germination paper tests.

Data collection and analysis. The number of seeds germinating per plate was recorded three times a week for the agar medium and germination paper tests. Seed germination was defined as an emerged shoot visible under 2× magnification (McCarty and Dudeck, 1993). Final germination rate and DGR were calculated using FGR (%) = 100 × ([∑n]/36) and DGR (%d) = 100 × ([∑(n/D)]/36), respectively, where n was the number of new seeds germinated at each counting and D was the number of days accumulated up to that counting (Wang and Zhang, 2010). In the hydroponic system, only FGR was recorded when the test was ended. Leaves were harvested at the same time and scanned using a photo scanner (V700; Epson America Inc., Long Beach, CA). Leaf area was calculated from the scanned images with WinRhizo (Regent Instruments Inc., Nepean, Canada). All tests were conducted as a randomized complete block design consisting of three replicates of a 12 (grass) × 5 (salinity level) factorial arrangement. To provide an accurate indication of salinity tolerance, FGR was standardized by setting the value of FGR in control (0 g L⁻¹ NaCl) at 100%. Therefore, grass with a higher ratio of FGR under saline condition to FGR in control has greater salinity tolerance (Teolis et al., 2009). Final germination rate and DGR were transformed using arcsine and square root, respectively, to meet the constant variance assumption in the analysis of variance (Wang and Zhang, 2010). All data were subjected to PROC GLM (SAS, 2004). Means were separated with Fisher’s protected least significant difference at P ≤ 0.05. Linear or quadratic regression analysis (PROC GLM) was conducted on nontransformed data to predict salinity levels that cause 50% reduction in FGR, DGR, and LA within each grass. The salinity tolerance was expressed using the predicted salinity level causing 50% reduction of FGR (PSLF), the predicted salinity level causing 50% reduction of DGR (PSLD), and the predicted salinity level causing 50% reduction of LA (PSLL). Results and Discussion

Ranking of salinity tolerance in turfgrasses was similar in transformed and untransformed data in either test (data not shown); thus, data were presented in the original format to avoid difficulty and confusion that might be caused by data transformation.

Effect of salinity on seed germination on agar medium. No significant differences in salt tolerance were observed among four groups when data of PSLF and PSLD within each group were pooled (Table 1). All grasses showed similar salt sensitivity in fescues and alkaligrass. Within kentucky bluegrass, coated ‘Turf Blue’ was the most salt-tolerant, requiring a NaCl level of 15.9 and 17.6 g L⁻¹ to reduce FGR and DGR by 50%, respectively. The salt tolerance of coated ‘Turf Blue’ kentucky bluegrass was also the highest of all grasses tested. In creeping bentgrass, the saltiness levels causing 50% reduction in FGR and DGR were 15.4 and 10.9 g L⁻¹ in ‘Declaration’, which were 27.3% and 17.4% higher than those in ‘L-93’ (Table 1). This result is in agreement with the finding of Wang and Zhang (2010), in which they predicted salinity levels of 16.7 and 11.0 g L⁻¹ to cause 50% reduction in FGR and DGR in ‘Declaration’ and 11.0 and 8.2 g L⁻¹ in ‘L-93’ on agar medium.

Effect of salinity on seed germination on germination paper. In the germination paper experiment, creeping bentgrass showed the highest salt tolerance with a PSLF of 18.7 g L⁻¹ followed by alkaligrass, fescues, and kentucky bluegrass (Table 2). Furthermore, ‘Declaration’ creeping bentgrass was the most salt-tolerant across all grasses used in this test. The predicted salt levels of kentucky bluegrass causing 50% reduction in both FGR and DGR were significantly lower than other groups, indicating that kentucky bluegrass was very salt-sensitive when germination paper was used. Unlike the agar medium test, coated ‘Turf Blue’ kentucky bluegrass did not show high salt tolerance compared with other grasses of kentucky bluegrass. ‘Bewitched’ had the highest PSLD in kentucky bluegrass followed by ‘Divia’ and coated and uncoated ‘Turf Blue’ kentucky bluegrass; however, all grasses in the species of kentucky bluegrass showed similar PSLF. Salinity tolerance of the fescues was reduced in the following order: ‘Marco Polo’ sheep fescue (F. ovina L.) ≥ ‘SR8650’ tall fescue ≥ ‘Davinci’ tall fescue ≥ ‘Smirna’ red fescue. No difference in salt tolerance was noticed in the grasses of alkaligrass.

Effect of salinity on seed germination in a hydroponic system. Final germination rate and LA were used to represent salinity tolerance in the hydroponic test. Alkaligrass exhibited the highest salinity tolerance with an average of 26.3 and 12.7 g L⁻¹ NaCl for PSLF and PSLD, respectively (Table 3). Final germination rate was 56% and 37% for ‘Fults’ and ‘Salty’ alkaligrass, respectively, at the
highest stress level evaluated in the present study (NaCl = 20 g L⁻¹); thus, PSLFs for both alkali grass cultivars were above 20 g L⁻¹ (Table 3). ‘Fults’ alkali grass had the highest salt tolerance of all grasses, in which up to 31.7 and 13.1 g L⁻¹ NaCl were needed to reduce the FGR and LA by 50%. The differences in salt tolerance among kentucky bluegrass, fescues, and creeping bentgrass were not significant. Moreover, no significant difference in salt tolerance occurred among grasses in these three groups, except that ‘Marco Polo’ sheep fescue required a lower salinity level to reduce LA by 50% than the other fescues (Table 3).

Responsiveness of final germination rate, daily germination rate, and leaf area to salt stress. Final germination rate, DGR, and LA decreased as the salinity level increased in all data when media were pooled across grasses (Table 4). The decrease of FGR was slower than that of DGR or LA as salinity level increased. For example, FGR was either unaffected or reduced less than 5% when the salinity level increased from 0 to 5 g L⁻¹. In contrast, DGR was reduced by 15% and 20% when germinated on germination paper and on agar medium, respectively, as the salinity condition increased to the same level. Leaf area was reduced to 93.7 cm² at 5 g L⁻¹ NaCl, 39% lower than that at 0 g L⁻¹. Furthermore, PSLD and PSLL in all grasses were lower than PSLF in all tests, except that PSLD of coated ‘Turf Blue’ kentucky bluegrass was 17.6 g L⁻¹, 10.7% higher than PSLF on the agar medium (Table 1). Thus, DGR and LA seemed more sensitive to salt stress than FGR. Similar findings were reported by Camerato and Martin (2004), Marcar (1987), and Wang and Zhang (2010).

The coating of coated ‘Turf Blue’ kentucky bluegrass is a corn starch-based product that helps hold water. In the present study, FGR of coated ‘Turf Blue’ kentucky bluegrass would be reduced by 50% at a salt level of 15.9 g L⁻¹ on agar medium, 47.4% higher than that of uncoated ‘Turf Blue’, ‘Diva’, and ‘Bewitched’ kentucky bluegrass (Table 1). Furthermore, coated ‘Turf Blue’ kentucky bluegrass had the highest DGR in kentucky bluegrass, even under the highest salinity condition (20 g L⁻¹) on agar medium (Fig. 1). Such a trend was not observed when seeds were germinated on the germination paper or in the hydroponic system (Tables 2 and 3; Fig. 1). It may be that the coating is easily washed off by salt solutions on the germination paper and in the hydroponic tests and thereby losing its function of holding water. However, the gelling agent agar not only provides a support site for seed germination, but also is a buffer system that can buffer sudden changes in temperature, moisture, and pH. This function of the agar medium is similar to the soil system; therefore, coating seeds may be an effective method to increase salinity tolerance of seeds during their germination in the soil.

Camberato and Martin (2004) suggested that seeds might be exposed to higher saline levels as a result of evaporation as the experiment progresses when they were germinated on germination paper. In the present study, dew accumulation was observed inside the petri dishes; thus, saline levels were increased. It is unclear about the extent of salinity increase during the study. However, because all grasses were exposed to identical conditions, relative salinity tolerance in the grasses was still comparable using germination methods. For example, ‘Fults’ and ‘Salty’ alkali grass and creeping bentgrass showed high salinity tolerance, whereas ‘Diva’, ‘Bewitched’, and uncoated ‘Turf Blue’ kentucky bluegrass had low salt tolerance. However, some exceptions were also observed such as variation of salt tolerance of coated ‘Turf Blue’ kentucky bluegrass. Similarly, ‘Declaration’ creeping bentgrass showed superior salt tolerance on the agar medium and germination paper tests but moderate tolerance in the hydroponic test. ‘Marco Polo’ sheep fescue was ranked salt-tolerant in the germination paper test but medium on the agar medium test and sensitive in the hydroponic test. It has been reported that different plants

### Table 2. Predicted salinity levels (g L⁻¹ NaCl) causing 50% reduction in final germination rate and daily germination rate in kentucky bluegrass, fescues, alkali grass, and creeping bentgrass on germination paper.

| Group                   | PSLF (%) | PSLD (%) | Grass                | PSLF (%) | PSLD (%) |
|-------------------------|----------|----------|----------------------|----------|----------|
| Kentucky bluegrass      | 10.4 a   | 9.5 b    | Coated Turf Blue     | 10.0 Aef | 9.2 Bcd  |
| Uncoated Turf Blue      | 9.9 Af   | 9.2 Cd   | Diva                 | 10.8 Ad-f| 9.8 ABe  |
| Bewitched               | 11.1 Ad-f| 9.8 Acd  | Smirna red fescue    | 11.6 Cd-f| 9.8 Ccd  |
| SR8650 tall fescue      | 16.0 Abbc| 11.9 AAbb| Davinci tall fescue  | 14.1 BCc-e| 10.6 BCb-d|
| Fescues                 | 15.2 b   | 11.1 a   | Marco Polo sheep fescue | 19.2 Aab | 12.0 Abc |
| Davinci red fescue      | 15.2 Aab | 12.0 Aab | SR8650 tall fescue   | 14.7 Abc | 9.4 Aa-d |
| SR8650 fescue           | 14.7 Abc | 9.4 Aa-d | Davinci tall fescue  | 13.8 Abc | 10.8 Aa-c |
| Davinci long fescue     | 15.2 Abc | 11.9 Aa-c| Davinci tall fescue  | 13.8 Abc | 10.8 Aa-c |
| Creeping bentgrass      | 18.7 a   | 12.3 a   | Declaration          | 23.0 Aa  | 14.7 Aa  |
| Declaration             | 14.4 Bcd | 9.9 Bcd  | Declaration          | 23.0 Aa  | 14.7 Aa  |

*PSLF = the predicted salinity level causing 50% reduction of final germination rate; PSLD = the predicted salinity level causing 50% reduction of daily germination rate.

### Table 3. Predicted salinity levels (g L⁻¹ NaCl) causing 50% reduction in final germination rate and leaf area in kentucky bluegrass, fescues, alkali grass, and creeping bentgrass in a hydroponic system.

| Group                   | PSLF (%) | PSLD (%) | Grass                | PSLF (%) | PSLD (%) |
|-------------------------|----------|----------|----------------------|----------|----------|
| Kentucky bluegrass      | 10.0 b   | 7.0 b    | Coated Turf Blue     | 10.0 Abc | 7.7 Acd  |
| Uncoated Turf Blue      | 9.9 Abc  | 7.9 Acd  | Diva                 | 9.5 Ac   | 5.8 Ad   |
| Bewitched               | 10.8 Abc | 6.7 Acd  | Smirna red fescue    | 9.6 Ac   | 5.7 Bd   |
| SR8650 tall fescue      | 15.2 Aab | 9.7 Aa-d | SR8650 tall fescue   | 14.7 Abc | 9.4 Aa-d |
| Davinci tall fescue     | 13.8 Abc | 10.8 Aa-c| Davinci tall fescue  | 13.8 Abc | 10.8 Aa-c|
| Fescues                 | 13.3 b   | 8.9 b    | Marco polo sheep fescue | 9.6 Ac   | 5.7 Bd   |
| Smirna red fescue       | 15.2 Abc | 9.7 Aa-d | SR8650 tall fescue   | 14.7 Abc | 9.4 Aa-d |
| Davinci tall fescue     | 13.8 Abc | 10.8 Aa-c| Davinci tall fescue  | 13.8 Abc | 10.8 Aa-c|
| Alkali grass            | 26.3 a   | 12.7 a   | Salty                | 20.9 Aab | 12.2 Aab |
| Davinci red fescue      | 15.2 Abc | 9.7 Aa-d | SR8650 tall fescue   | 14.7 Abc | 9.4 Aa-d |
| Davinci tall fescue     | 13.8 Abc | 10.8 Aa-c| Davinci tall fescue  | 13.8 Abc | 10.8 Aa-c|
| Creeping bentgrass      | 11.2 b   | 9.6 b    | Declaration          | 12.6 Abc | 10.4 Aa-c|
| Declaration             | L-93     | 9.8 Ab-d | Declaration          | L-93     | 9.8 Ab-d |

*PSLF = the predicted salinity level causing 50% reduction of final germination rate; PSLD = the predicted salinity level causing 50% reduction of leaf area.

### Table 4. Effect of salinity on final germination rate (FGR, %), daily germination rate (DGR, %/d), and leaf area (LA, cm²) in three germination media across grasses.

| NaCl (g L⁻¹) | Agar medium | Germination paper | Hydroponic system |
|--------------|-------------|-------------------|------------------|
|              | FGR (%)     | DGR (%)/d         | FGR (%)          |
| 0            | 100.0 a     | 12.4 a            | 100.0 a          |
| 5            | 96.5a       | 10.0 b            | 101.7 a          |
| 10           | 64.7 b      | 5.6 d             | 88.9 b           |
| 15           | 27.4 c      | 2.1 d             | 51.8 c           |
| 20           | 6.8 d       | 0.7 e             | 18.5 d           |

*Mean followed by the same letter in each column are not significantly different at P ≤ 0.05.
respond to salt stress differently. Some plants are only affected by the osmotic stress induced by salinity, whereas other plants are influenced by both imbalance of osmotic potential and ion toxicity in the salt solution (Dodd and Donovan, 1999). ‘Marco Polo’ sheep fescue and ‘Declaration’ creeping bentgrass might be such genotypes that are very sensitive to ionic toxicity, therefore showing reduced salinity tolerance in the hydroponic system compared with the other methods. Overall, this study shows that the seed germination method should be taken into consideration when evaluating salinity tolerance in turfgrass.

Agar medium, germination paper, and hydroponic system are commonly used as germination media for a quick screening of salinity tolerance in plants under a controlled environment. Autoclave facility and trained personnel are needed for medium preparation and seed sterilization when agar medium and germination paper methods are used. Furthermore, some seeds such as buffalograss are very difficult to sterilize; therefore, contamination is always a concern. Salt accumulation on the germination paper may change the salinity stress level constantly. For the hydroponic method, more management (i.e., changing solutions) is needed. Agar medium functions more similar to a soil system, yet hydroponic system has the least limitation for seedling growth. All three methods require certain facilities (transfer hood and incubator), equipment (autoclave and aeration pump), and adequate space. Researchers and practitioners have to choose a proper germination method based on their facility and purpose with understanding of the influence of different germination methods on salinity tolerance.

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