Diverse Responses of Perennial Ryegrass Accessions to Submergence Stress

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SUMMARY: Submergence can severely affect the growth of perennial grasses. The objective of this study was to characterize the responses of perennial ryegrass (*Lolium perenne* L.) accessions to submergence and its recovery following de-submergence. One hundred globally collected perennial ryegrass accessions were submerged for 7 d followed by 7 d of recovery, respectively. The accessions were generally grouped into three types: fast growth with maintenance of color (escape), slow growth with maintenance of color (quiescence), and slow growth with loss of color (susceptible). Diverse responses of perennial ryegrass accessions to submergence are useful in creating more tolerant materials and in further characterizing physiological and molecular mechanisms of submergence tolerance.

Turfgrass management and production is largely influenced by climate variability and weather extremes. The increased frequency of flooding negatively affects grass quality. Grass plants can be exposed to either waterlogged or submerged conditions after a flooding event. Submergence describes the condition in which the whole plant is completely covered by water after a flooding event. Plants can die more quickly under submergence due to a lack of oxygen. Submergence stress either inhibits or enhances plant growth, depending on type of species and survival strategies on growth characteristics under water (Bailey-Serres and Voesenek, 2008). The variable growth and physiological responses of different perennial grass species and ecotypes to submergence stress provide an important basis for identifying molecular mechanisms of submergence tolerance and molecular markers linked to stress tolerance.

Grass species, water depth, light levels, and temperature may all have an impact on plant survival under excess water conditions. A few reports have illustrated the effects of various flooding stresses on perennial grass physiology. Water levels occurring at 15-, 5-, and 1-cm below the soil surface all significantly decreased turf quality, chlorophyll concentration, root water-soluble carbohydrate, and soluble protein concentrations in five creeping bentgrass (*Agrostis palustris* L.) cultivars, but to a lesser extent in tolerant cultivars (Jiang and Wang, 2006). Ten Kentucky bluegrass (*Poa pratensis* L.) cultivars varied largely in shoot dry weight, chlorophyll concentration, and cell membrane leakage under flooding stress occurring at the soil surface (Wang and Jiang, 2007). These results demonstrate the differential responses of perennial grass species and cultivars to various flooding stresses. Further research is needed to explore phenotypic responses of diverse ecotypes within a perennial grass species to submerged conditions and recovery following stress.

ADDITIONAL INDEX WORDS:
Flooding, Growth, Physiology, Tolerance

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Perennial ryegrass is one of the most important cool-season turf grasses. This grass has world-wide distribution, diverse germplasm, diploid genetics, and available genomic resources, thus providing a good model for further study of the genetic basis of flooding tolerance. The objective of this study was to characterize the responses of perennial ryegrass germplasm to submergence and its recovery following de-submergence. The results will be valuable for identifying the molecular mechanisms of submergence tolerance

Materials and Methods

One hundred accessions of perennial ryegrass were initially planted in a greenhouse at Purdue University, West Lafayette, IN, USA. Ninety-nine accessions were finally used for the analysis including 32 wild, 36 cultivated, and 31 uncertain materials from 41 countries. The seeds were sown in plastic pots (4-cm diameter, 9-cm deep) containing a sandy-loam soil with a pH of 6.9 in a greenhouse, and each accession was propagated through multiple times. Plants were watered daily and fertilized once a week with a soluble fertilizer (N- P2O5-K2O, 24-8-16) (Scotts Inc., Marysville, OH, USA) to provide 240 kg N ha^{-1}, 33 kg P ha^{-1}, 132 kg K ha^{-1} and micronutrients. During the growing periods, the average day air temperatures and photosynthetic photon flux density (PPFD) in the greenhouse were 19.3 ± 1.5 ºC and 200 μmol m^{-2} s^{-1}, respectively. Experiment lasted from Oct. 6 to Nov. 28 of 2009.

Submergence stress was imposed by submerging the grass pots in (86 cm length × 38cm width × 30 cm height) plastic containers with tap water. The water level was kept at 5 cm below the top of the container. The control pots were placed in the same size containers without water. The stress treatments began on Nov. 14 of 2009. No nutrients were supplied to the plants during the treatment. The water was not changed but algae were removed if they accumulated. After 7 d of stress, the submerged plants were taken out for recovery for 7 d. Upon recovery, water and nutrients were then applied to both the control and the stressed plants at the rate described previously. To identify growth exclusively achieved under submergence or after recovery, all plants were cut to 5-6 cm prior to stress or before recovery to obtain a uniform height. Plant height in each pot was recorded. During the periods of submergence stress, the average air and water temperatures and PPFD were 19.8 ± 1.0 °C, 17.5 ± 0.8 °C, and 113 μmol m^{-2} s^{-1}. During recovery, the average air temperatures and PPFD were 20.5 ± 0.6 °C and 100 μmol m^{-2} s^{-1}.

At the end of the 7 d treatments, plant height (HT) was measured by recording the longest leaf blade. At 7 d, the leaves corresponding to this HT were cut and the tissues were dried in an oven at 80°C for 3 d. The absolute growth rate (GR) was calculated as dry weight per day during a 7-d period for all treatments. The total water-soluble carbohydrate (WSC) was measured in the selected accessions that represented different types of tolerant and susceptible groups of perennial ryegrass accessions under submergence condition. The WSC was measured using the anthrone method (Koehler, 1952) with some modifications. The WSC was extracted from 20-50 mg of leaf power with 1 mL double distilled water. The extract was shaken for 10 min and centrifuged at 11,000 × g for 10 min, and the supernatant was collected. The extraction was repeated two more times and the supernatant was pooled. A 1 mL of extract was mixed with 7 mL freshly prepared anthrone [200 mg anthrone + 100 mL 72% (w/w) H_2SO_4] and placed in a boiling water bath for 8 min. After cooling, the absorbance at 625 nm was read. The standard curve was made using glucose in a range of 5 to 300 μg mL^{-1}.

The experiment was a split plot design in two treatments (control and submergence) by 100 accessions of factorial arrangements. One accession had been dropped during statistical analysis because too many numbers were missing. Each accession and treatment was replicated three times and three benches represented three replicates for each accession. The pots were completely randomly assigned into five containers within the control or submerged regime for each bench, respectively. During the recovery period, both the control and submerged grasses were also arranged in the same way as described previously. Statistical analysis was performed using SAS (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Large variations in HT and GR of perennial ryegrass were observed under the control and submergence stress. Compared to the pattern of the controls, the overall distribution in HT and GR shifted towards a high frequency of lower values across accessions under submergence stress (Fig. 1). For example, approximately 20 % of the accessions had an HT of 9 to 10 cm under the control condition, while approximately 25 % of the accessions had an HT of 7 to 8 cm under the submergence stress. The decreased leaf elongation in perennial ryegrass was consistent with other reports demonstrated in common reed [(*Phragmites australis* (Cav.) Trin. ex Steud.] seedlings (Mauchamp et al., 2001) and bermudagrass (*Cynodon dactylon* L.) (Tan et al., 2010). After 7 d re-submergence, the overall distribution in HT and GR shifted towards a high frequency of higher values (Fig. 1).

Escape (T1), quiescence tolerance (T2), and susceptible types (ST) of perennial ryegrass accessions were generally identified under the submergence conditions. An increased 0.37 cm of HT was observed for T1 under stress, indicating submergence-induced elongation. T1 had higher HT and GR than T2 and ST under stress (Fig. 2). Compared to the control, a 49 % reduction in GR was observed in ST (Fig. 2). The plants with an escape strategy respond to submergence by enhanced shoot elongation. This escape strategy is advantageous in shallow floodwaters in which the shoot elongation allows plants to re-establish air contact (Luo et al., 2011). In contrast, when submerged, the shoots of quiescence type (T2) do not elongate to conserve energy, which could positively affect the survival rate and generation of new tissues after de-submergence (Panda et al., 2008; Colmer and Voesenek, 2009).

Relative to their respective controls, submergence significantly decreased WSC concentration by 57 %, 64 %, and 66 % for T1, T2, and ST, respectively. The relatively higher levels of WSC observed in T1 could be beneficial for submergence tolerance. In Kentucky bluegrass, shoot WSC concentration was reduced in some cultivars under soil waterlogging conditions along with a decline in grass quality (Wang and Jiang, 2007). The levels of non-structure carbohydrates after submergence are strongly associated with seedling survival in other species such as rice (*Oryza sativa* L.) (Das et al., 2005).

In summary, large phenotypic variations in HT, GR and WSC were found in diverse perennial ryegrass accessions in response to submergence. The escape and quiescence types of submergence responses were identified within perennial ryegrasses. The escape type generally had faster growth than the quiescence- and- susceptible types of plants under stress. The escape type had higher WSC concentration than the susceptible plants. The results can be used to further study physiological and molecular mechanisms contributing to submergence tolerance.

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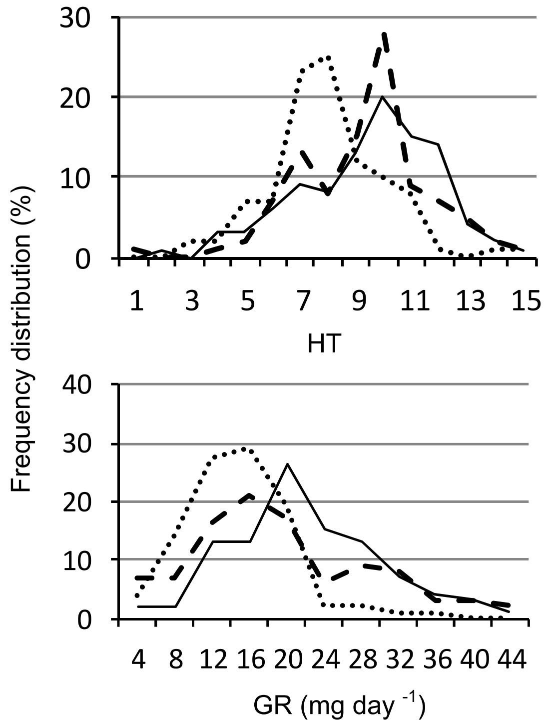

Fig. 1. Frequency distributions in maximum plant height (HT) and growth rate (GR) of 99 accessions of perennial ryegrass under the control (solid line), submergence (dots), and recovery (dash line) conditions.

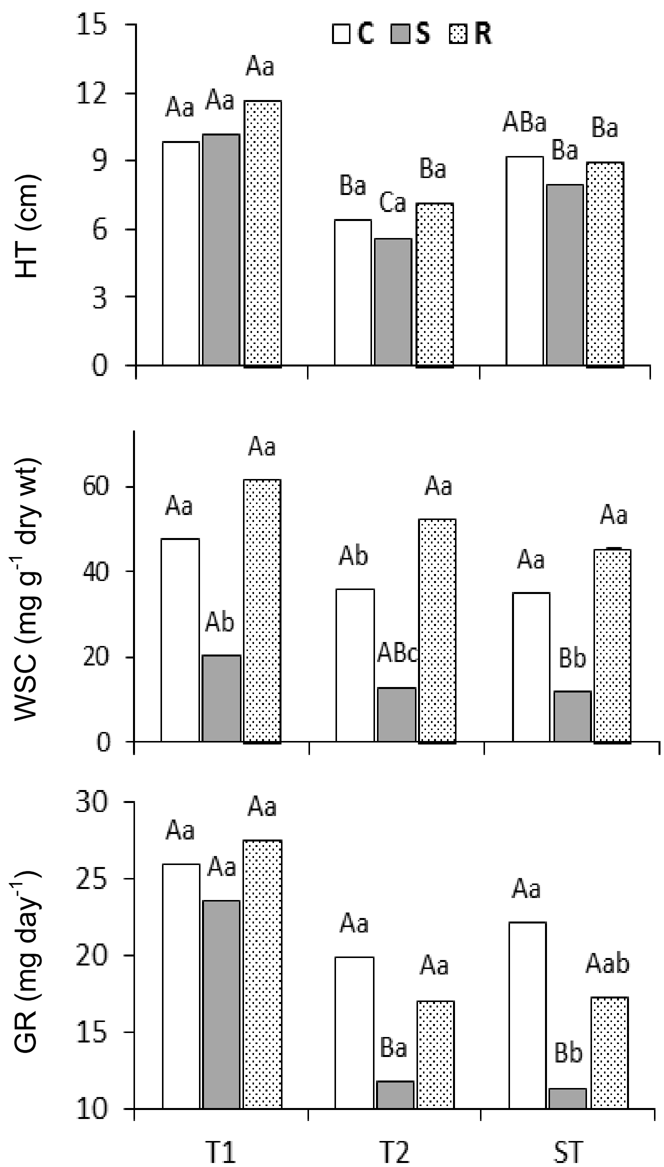

Fig. 2. Effects of submergence and recovery on plant height (HT), total water-soluble carbohydrate (WSC), and growth rate (GR) in perennial ryegrasses. C, S, and R represent the control, submergence and recovery, respectively. T1, T2, and ST represent escape tolerant, quiescence tolerant, and susceptible type, respectively. Data were averaged by four accessions for T1 and T2 and five accessions for ST, respectively. Means followed by same small letter are not significantly different at $P < 0.05$ within treatments for a given type of plant. Means followed by the same capital letter are not significantly different at $P < 0.05$ among type of plants for a given treatment.