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Perennial ryegrass is a cool-season turfgrass species that can exhibit significant freezing injury in northern climatic regions (note freezing injury of perennial ryegrass plots above at University of Massachussets research plots). The objective of this study was to quantify physiological and biochemical changes occurring in overwintering perennial ryegrass crowns during the cold acclimation period. To date, superior cold adaptation in perennial ryegrass was associated with higher accumulation of water soluble carbohydrates and greater capacity to alter membrane lipid composition.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 400 projects at a cost of $31 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

Editor

Jeff Nus, Ph.D.
1032 Rogers Place
Lawrence, KS 66049
jnus@usga.org
(785) 832-2300
(785) 832-9265 (fax)

Research Director

Michael P. Kenna, Ph.D.
P.O. Box 2227
Stillwater, OK 74076
mkenna@usga.org
(405) 743-3900
(405) 743-3910 (fax)

USGA Turfgrass and Environmental Research Committee

Physiological Factors Associated with Perennial Ryegrass Freezing Tolerance

Michelle DaCosta, Lindsey Hoffman, J. Scott Ebdon, and Eric Watkins

SUMMARY

Perennial ryegrass (*Lolium perenne*) is a cool-season turfgrass species that can exhibit significant freezing injury in northern climatic regions. Field and growth chamber evaluations of perennial ryegrass germplasm collected from the United States, Asia, and Europe led to identification of accessions with superior freezing tolerance compared to many commercially available cultivars. The objective of this study was to quantify physiological and biochemical changes occurring in overwintering perennial ryegrass crowns during the cold acclimation period. Four perennial ryegrass accessions with contrasting freezing tolerances were selected, including two freezing-tolerant accessions (Tol-1 and Tol-2) and two freezing-susceptible accessions (Sus-1 and Sus-2). Results include:

- Tol-1 exhibited the most rapid accumulation of water soluble carbohydrates in crowns during acclimation, followed by Tol-2.
- Tol-2 exhibited higher capacity to alter membrane composition (individual lipid classes and unsaturated fatty acid levels) that help to maintain cell membrane integrity at low temperatures.
- Based on the analysis of two tolerant accessions, superior cold adaptation in perennial ryegrass was associated with higher accumulation of water soluble carbohydrates and greater capacity to alter membrane lipid composition.

Perennial ryegrass (*Lolium perenne*) is a cool-season turfgrass species that is widely used on home lawns, sports fields, and golf courses due to its rapid establishment and superior traffic tolerance. Compared to other cool-season turfgrasses, however, perennial ryegrass exhibits higher levels of freezing injury in northern climatic regions. Although there has been limited progress in the development of perennial ryegrass cultivars with improved winter hardiness (12), recent investigations from the University of Minnesota turfgrass breeding program have identified perennial ryegrass germplasm with superior freezing tolerance compared to many commercially available cultivars, suggesting that significant progress could be made toward the development of perennial ryegrass cultivars better adapted to northern climatic regions (4, 5).

Perennial ryegrass can be affected by any number of winter stresses, which represents a major challenge for breeders. Winter injury can result from direct low temperature kill, ice cover (suffocation), crown hydration, desiccation, and/or low temperature diseases (snow molds). Each winter presents different mechanisms of injury, which further complicates field screening for tolerance to these stresses. For example, perennial ryegrass may experience damage from ice cover in one year, while the next year may bring damage from direct low temperature kill.

In spite of the different potential causes for winter injury, however, research conducted using perennial ryegrass has demonstrated that the overall level of plant freezing tolerance is an important

Plants were harvested weekly (0, 7, 14, and 21 days) throughout the cold acclimation period to obtain crowns for quantification of water soluble carbohydrates, proline, and changes in membrane composition.

M. DaCOSTA, Ph.D., Assistant Professor; L. HOFFMAN, Graduate Student; and J. S. EBDON, Ph.D., Associate Professor; Dept. of Plant, Soil, and Insect Sciences, Univ. of Massachusetts, Amherst, MA; and E. WATKINS, Ph.D., Associate Professor, Dept. of Horticultural Science, Univ. of Minnesota, St. Paul, MN.

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Winter injury can result from direct low temperature kill, ice cover (suffocation), crown hydration, desiccation, and/or low temperature diseases (snow molds). *(Photo credit: Alden Maddocks)*

component of how effectively grasses overwinter (6, 16). More recently, Hulke et al. (4) also reported an improved method for screening perennial ryegrass freezing tolerance that was well correlated with field winter survival evaluations. Therefore, based on the close relationship between perennial ryegrass freezing tolerance and overwintering capacity, it is important to identify important plant-associated factors that might lead to intraspecific differences in perennial ryegrass freezing tolerance.

Plant overwintering capacity is associated with a period of cold hardening that is necessary for turfgrasses to develop freezing tolerance. The cold hardening (acclimation) period is associated with decreasing temperatures and daylength during fall months, which trigger many physiological and structural changes inside of plant cells (7, 10). For example, plants accumulate numerous protective compounds, such as carbohydrates, amino acids, and proteins, which help to lower the cell freezing point and stabilize cell structures under conditions of freezing-induced dehydration. In addition, modifications in phospholipid classes and increases in unsaturated fatty acid content are necessary to enhance membrane fluidity and facilitate membrane function at low temperatures (8, 13, 14).

For cool-season grasses, the initial cold hardening period generally occurs at temperatures of approximately 32 to 46° F, followed by second stage of hardening as temperatures drop below freezing (approximately 22 to 32° F). Environmental conditions and management practices (light levels, mowing height, nutrient availability, etc.) during the cold acclimation period can be important factors in determining freezing tolerance levels going into winter months. In addition, turfgrass species and cultivars vary in their level of freezing tolerance, which has been associated with the capacity of plants to alter their cellular structure and accumulate protective compounds during the cold acclimation period (2, 9, 11).

The evaluation of perennial ryegrass germplasm collected from the United States, Asia, and Europe confirmed significant variability in freezing tolerance and winter hardiness among perennial ryegrass accessions in both field and controlled environment experiments (4, 5). However, the underlying factors responsible for these differences were unknown. Therefore, our objectives were to evaluate perennial ryegrass accessions with contrasting freezing tolerance during the cold acclimation process, focusing on important physiological and biochemical changes occurring in the overwintering crowns. Information gained from this research may then be used to develop more efficient screening procedures that could lead to improved winter hardiness in perennial ryegrass.

Materials and Methods

The perennial ryegrass accessions were selected based on differences in winter survival ratings from a two-year field trial in Minnesota and estimates of LT_{50} (lethal temperature resulting in 50% mortality of plants) from a controlled freezing chamber study (4, 5). The plants consisted of two freezing-tolerant accessions, including Tol-1 (PI 598433, LT_{50} approximately 7 °F) and Tol-2 (PI 610806, LT_{50} approximately 8 °F), and two freezing-susceptible accessions, Sus-1 (PI 223178, LT_{50} approximately 13 °F) and Sus-2 (PI 229476, LT_{50} approximately 14 °F).

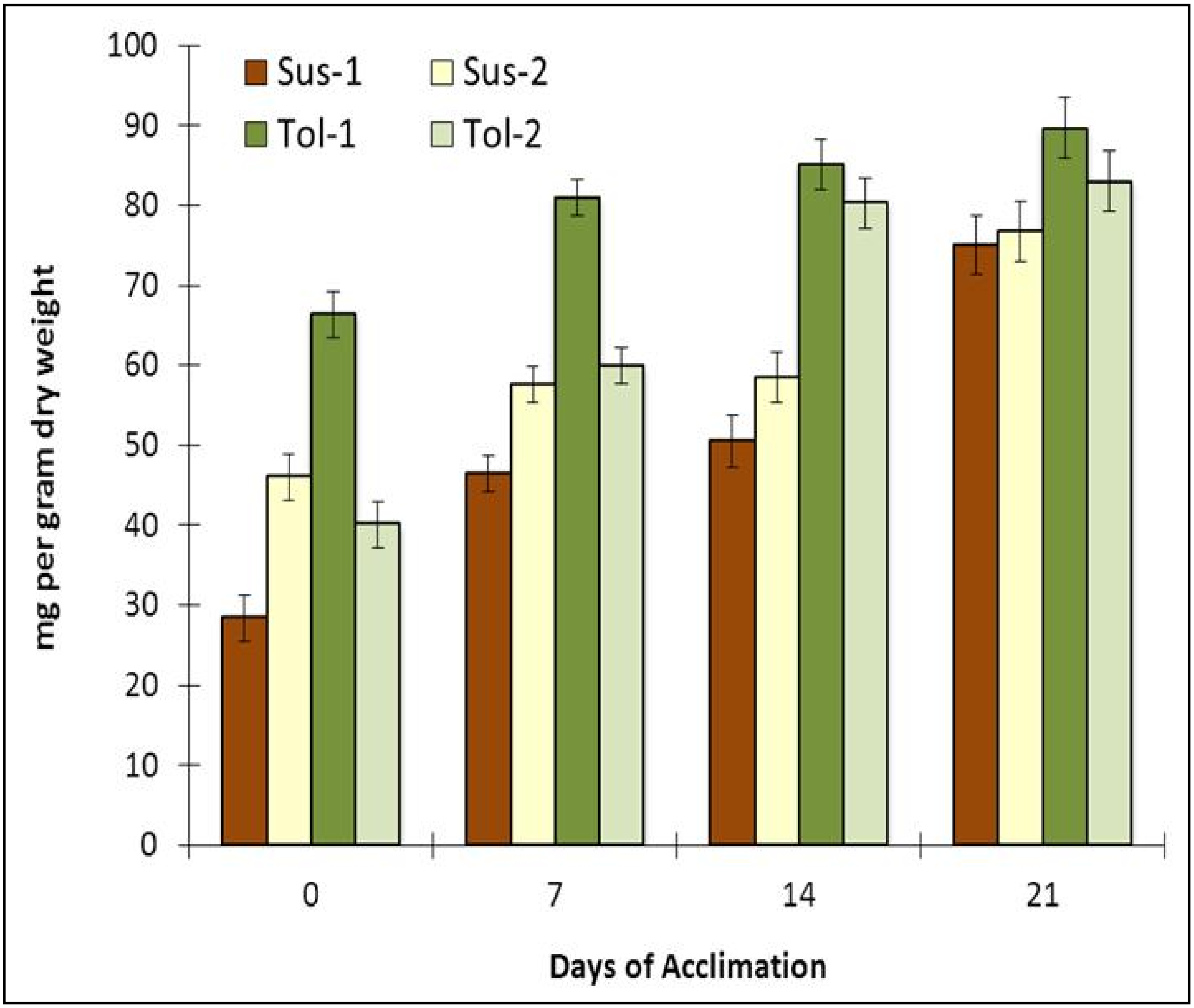

Figure 1. Accumulation of water soluble carbohydrates in crowns of four perennial ryegrass accessions in response to cold acclimation at 36° F for 21 days.

Accession	PC/ (PE+PA)	18:3/18:2
Sus-1	1.0 c	0.64 b
Sus-2	1.2 b	0.67 b
Tol-1	1.2 b	0.65 b
Tol-2	1.4 a	0.87 a

Table 1. Changes in the phospholipid composition and fatty acid content in crowns of freeze-susceptible (Sus-1 and Sus-2) and freeze-tolerant (Tol-1 and Tol-2) perennial ryegrass accessions at 21 days of cold acclimation. The ratio of phosphatidylcholine (PC) phospholipids to total phosphatidylethanolamines (PE) and phosphatidic acids (PA) provides information on the degree of membrane stabilizing lipids, and the ratio of linolenic (18:3) to linoleic (18:2) acids (18:3/18:2) provides information of the ratio of unsaturated to saturated fatty acids. Accession means followed by the same letter within a column are not significantly different based on Fisher's protected LSD (P=0.05).

Single seeds of each perennial ryegrass accession were planted into pots filled with a commercial potting medium. After growing in the greenhouse for approximately three months, perennial ryegrass plants were moved to a controlled environment chamber and exposed to constant 36° F temperature and 10-hour light period for 21 days to initiate cold acclimation.

Plants were harvested weekly (0, 7, 14, and 21 days) throughout the cold acclimation period to obtain crowns for examination of water soluble carbohydrates, proline, and changes in membrane composition. Water soluble carbohydrates (sucrose, glucose, fructose, and raffinose family oligosaccharides) and proline were analyzed using high-pressure liquid chromatography. Changes in the composition of major polar lipid classes were determined using electrospray ionization tandem mass spectrometry (ESI-MS/MS). In particular, we focused on changes in the levels of phosphatidylcholines (PC), phosphatidylethanola-

The accumulation of protective compounds such as water soluble carbohydrates along with changes in lipid composition during cold acclimation represent critical mechanisms that help to lower cellular freezing points and improve cellular stability of perennial ryegrass at low temperatures. These physiological features allowed freeze-tolerant accessions (right) to tolerate low temperatures that killed susceptible accessions (left).

mines (PE), and phosphatidic acids (PA), since these lipid classes made up the largest fraction (over 60%) of total polar lipids.

Based on the arrangement of the lipid head-group and fatty acid domain, PC is generally considered to be a bilayer-forming lipid, whereas PE and PA have a greater tendency to form non-bilayer structures. Consequently better cold tolerance has been associated with higher proportion of membrane-stabilizing PC compared to levels of PE and PA, which may help to maintain membrane integrity at low temperatures (15). In addition, modifications in fatty acid composition were determined using gas chromatography. Higher freezing tolerance in grasses has been associated with an increase in unsaturated fatty acid content of membrane lipids during cold acclimation (1, 11).

Summary

We found that differences in the capacity to modify certain physiological traits in the crowns during cold acclimation may contribute to differences in freezing tolerance among accessions of perennial ryegrasses. Freezing-tolerant accession Tol-1 exhibited the most rapid accumulation of water soluble carbohydrates in crowns during acclimation, followed by Tol-2. These differences were mostly accounted for by higher sucrose content during acclimation, although raffinose family oligosaccharides were also higher for the freezing-tolerant accessions by 14 days of acclimation. Future work should also evaluate the contribution of storage carbohydrates, such as fructans, to differences in freezing tolerance among these accessions since fructans have been shown to be important for freezing tolerance of temperate grasses, including perennial ryegrass (3).

Although proline levels generally increased during cold acclimation, we did not detect significant differences among the four perennial ryegrass accessions following 21 days of acclimation. As cold acclimation progressed, there were significant decreases in saturated fatty acids, including palmitic acid (16:0) and stearic acid (18:0), and significant increases in unsaturated fatty acids, including linoleic acid (18:2) and linolenic acid (18:3). Among the different lipid classes, we found significant increases in membrane stabilizing PC, and decreases in PA. Overall, Tol-2 exhibited higher capacity to alter membrane composition (individual lipid classes and unsaturated fatty acid levels) that could help to maintain cell membrane integrity at low temperatures. Specifically, Tol-2 exhibited a higher proportion of membrane-stabilizing lipids and a higher proportion of unsaturated lipids by 21 days of cold acclimation.

Based on the analysis of two tolerant accessions, the accumulation of protective compounds such as water soluble carbohydrates along with changes in lipid composition during cold acclimation may represent critical mechanisms to help to lower cellular freezing point and improve cellular stability of perennial ryegrass at low temperatures. Therefore, selecting for a combination of these traits may lead to superior cold adaptation in perennial ryegrass.

Additional investigations are necessary to determine additional factors that contribute to contrasting winter survival among these perennial ryegrass accessions, including differences in deacclimation sensitivity, crown hydration potential, and tolerance to ice cover (anoxia). This information will be crucial for the identification of important physiological and/or biochemical traits that could serve as selection criteria for perennial ryegrass breeding programs.

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