Cold Hardiness of Saltgrass Accessions

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ABSTRACT

Freezing tolerance is an environmental adaptation that significantly influences plant geographical distribution. Since differences in winter survival among saltgrass ecotypes have been observed in the field at Fort Collins, CO, the objective of this study was to determine the relative freezing tolerance, seasonal changes in the freezing tolerance, and winter survival of six accessions of saltgrass [*Distichlis spicata* var. *stricta* (L.) Greene]. Saltgrass accessions A65 and A29 were originally collected from Denver, CO, while C66 was from Humbolt Sink, NV, 32 from Wanship, UT, 55 from Hereford and 48 from Farmingdale, SD. These accessions were established in a field nursery at Fort Collins, CO. Rhizomes were sampled at monthly intervals from October 1999 through April 2000 and from October 2000 through April 2001 and subjected to laboratory freezing tests. Cold hardiness of the saltgrass accessions increased gradually during the fall with maximum hardiness occurring at midwinter. During midwinter, freezing tolerance was significantly different among accessions. Ranking of accessions for subfreezing temperature resulting in 50% mortality [LT_{50} (°C)] during January 2000 was A29 = 48 (−20.0) < 55 (−17.0) ≤ 32 (−15.5) ≤ A65 = C66 (−14.0). In January 2001, they were ranked with 48 = 55 (−26.0) < A65 = 32 (−23.0) < A29 (−20.0) = C66 (−18.5). In December and January sampling dates of the first season, accessions A29, 48, and 55 exhibited the highest relative regrowth when exposed to temperatures ≈ −20.0°C. In midwinter of the second season, accession 55 showed the highest regrowth after being subjected to temperatures ≈ −25.0°C. Accession C66 had the lowest regrowth potential after freezing treatments in both seasons. Winter survival in the field correlated negatively with LT_{50} value, with accessions 48, A29, and 55 demonstrating greater winter survival while C66 had the lowest percentage survival. The difference in freezing tolerance among accessions is in part associated with their origin-inherited adaptation. This information is useful for defining the potential adaptation range of saltgrass and in saltgrass breeding projects to select and develop freezing tolerant saltgrass.

The effects of freezing temperatures on living organisms have long interested biologists, both from practical and theoretical points of view (Levitt, 1980). Freezing tolerance is a significant environmental adaptation that controls geographical distribution of plants.

Inland saltgrass [*Distichlis spicata* var. *stricta* (L.) Greene], indigenous to America and Australia, is a dioecious, rhizomatous, perennial, salt tolerant, warm-season grass. Inland saltgrass is commonly found in areas of the western USA where salinity, alkalinity, and drought have eliminated many other types of vegetation (Hansen et al., 1976). It is a species with potential for revegetation of mine spoils and roadsides in the semiarid west (Pavlicek et al., 1977). In many of the saline-alkali basins of the western USA, saltgrass is an important forage species, providing the sole forage for cattle during the summer portion of the grazing season, although it is low in nutritive value (Cluff et al., 1983). A saltgrass breeding project has been initiated at Colorado State University, in cooperation with the University of Arizona, to develop saltgrass cultivars that can be used as turfgrass where soil and water salinity and alkalinity are high.

Saltgrass accessions have been collected from diverse climate zones, ranging from USDA climate zone 10 (Mexico) to 4 (South Dakota and Montana). A diversity of ecotypes of saltgrass have been found as some southern accessions suffered winter injury in field plots at Fort Collins, CO, while northern accessions were not injured. Information on cold hardiness is important for breeders as they develop new cultivars, as well as for the proper marketing and utilization of new cultivars. No scientific data related to saltgrass freezing tolerance are available. Data on subfreezing temperature resulting in 50% mortality (LT_{50}) and field winter survival would be valuable for accurately assessing the freezing tolerance of saltgrass accessions.

The objective of this study was to determine the relative freezing tolerance, seasonal changes in freezing tolerance, and winter survival of six accessions of saltgrass.

MATERIALS AND METHODS

Saltgrass accessions used in this experiment, A65, A29, C66, 32, 55, and 48, were collected from different regions of the western USA. The collection locations and climate zones at these individual locations were provided in Table 1, on the basis of the hardiness zone maps created by USDA (USDA, 1972, 1990). These accessions were established via rhizome plugs in 5-by-5 m field plots at the Horticulture Research Center, Fort Collins, CO, in July 1998. The soil in the field was a Nunn clay loam (fine, smectitic, mesic Aridic Argiustolls) with the initial soil N, P, and K content of 23.0, 14.0, and 497 mg kg^{-1}, respectively. The soil pH was 8.1. Entries were replicated two times in a randomized complete block design. Irrigation was provided once a year in early August by flooding the field with approximately 10 cm of water. Electrical conductivity of the irrigation water was about 3.0 dS/cm. No fertilizers were applied and the field was unmowed during 1999-2001 to serve as the breeding nursery. Daily maximum and minimum air temperature and soil temperature at 5 cm below soil surface were recorded via a weather station located 3.2 km north of the study area.

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Abbreviations: LT_{50}, the subfreezing temperature resulting in 50% mortality; GT_{50}, the subfreezing temperature that caused 50% rhizome regrowth reduction.

Laboratory Assessment of Freezing Tolerance

Accessions used in this experiment were sampled monthly (during the last week of each month) from October 1999 through April 2000 and from October 2000 through April 2001. On each sampling date, two pits were dug in each plot to 15 cm deep, soil and foreign matter were removed, and rhizomes were collected. After being washed with cold water to remove soil and plant debris further, rhizomes from each pit were divided into 7 to 8 fractions. Each fraction, containing at least 10 rhizomes (1–2 cm long, with 1–2 nodes), was individually wrapped in moist tissue paper (to prevent supercooling during freezing treatment), enclosed in aluminum foil to prevent moisture loss, and labeled for a targeted freezing temperature. Rhizomes samples were subjected to freezing treatments with a thermocontrolled freezer (Tenny Jr. Programmable Freezer, Tenny Inc., South Brunswick, NJ) as previously described (Qian et al., 2001). Initially, the samples were placed in the freezing chamber and maintained at 0°C for 16 h to allow temperature equilibration. The freezing chamber was then programmed to cool linearly at 2°C/h. One fraction of rhizomes was removed when each of the target temperatures was reached. Target temperatures (3°C intervals) were varied with sampling dates and expected acclimation to cover the range of expected 50% lethal temperatures. Thermocouples were inserted inside the wrapped samples to measure the temperatures individually. Following freezing treatments, samples were kept at 4°C over night for thawing. After thawing, individual rhizomes were planted in commercial potting soil (Scotts Metro-Mix 350, Scotts-Sierra Hort. Products, Marysville, OH) in a greenhouse. Irrigation was applied every 2 h by a mist system to provide approximately 3 mm d^{-1}. Saltgrass response to freezing temperature was evaluated on the basis of rhizome survival and total rhizome regrowth. Survival was recorded by observing regeneration of shoots from individual rhizomes 4 wk after planting. Rhizome aboveground regrowth was harvested 8 wk after planting, and oven-dried at 70°C for 3 d to determine total dry mass from all 10 rhizomes subjected to each temperature treatment.

Assessment of Winter Survival in the Field

In April and May 2000 and 2001, winter survival of saltgrass plots in the field were assessed by visually rating the percentage of the plot area that exhibited green-up.

Data Analysis

The PROC PROBIT procedure of the statistical analysis system (SAS Institute, 1989) was used to predict LT_{50}, which can be defined as the subfreezing temperature that resulted in 50% rhizome survival. The lower the LT_{50} the greater the freezing tolerance of the accession. Likewise, linear regression analysis was conducted to determine relationship between rhizome regrowth vs. temperature for each replication of each accession on each sampling date. Rhizome regrowth was evaluated as the subfreezing temperature that caused 50% rhizome regrowth reduction (GT_{50}). Since we had two replicated plots in the field and 2 sections (pits) were sampled in each plot, four LT_{50} and four GT_{50} values were generated from each accession on each testing date. The LT_{50} and GT_{50} of each replication were subjected to an analysis of variance (ANOVA) (SAS Institute, 1989) to test the differences among accessions over sampling dates. Significant interactions between accessions and sampling dates were found. Therefore, mean separations among accessions within sampling dates were performed with Fisher's LSD test at $P = 0.05$. Winter survival in the field was subjected to ANOVA, and means were separated by Fisher's LSD at $P = 0.05$. Correlation analysis between LT_{50} and winter survival in the field was conducted (SAS Institute, 1989).

Table 1. Original locations of the six accessions in the study.

Accessions	Original location†	USDA climate zone
A65	Denver, CO	5A
A29	Denver, CO	5A
C66	Humbolt Sink, NV	6B
32	Wanship, UT	5A
55	Hereford, SD	4B
48	Farmingdale, SD	4A

† Based on the hardiness zone maps created by USDA.

RESULTS

The seasonal minimal and maximal air temperatures and soil temperature were lower between November 2000 and February 2001 than during the same period in 1999-2000 (Fig. 1). Soil temperature from 8 November 2000 to 6 February 2001 fell below 0°C and the soil was constantly frozen during these 3 mo. In contrast, soil temperature from November 1999 to February 2000 fluctuated to a great extent and cycles of soil freezing and thaw were observed in the field.

Seasonal Lethal Low Temperature

October 1999 to April 2000

In October 1999, all accessions showed good survival from −5 to −8°C, while no survival was detected at –11°C (Fig. 2). The LT_{50} of all accessions decreased from October 1999 to January 2000 as air temperatures decreased. Accessions differ significantly in their LT_{50} during midwinter. The lowest individual LT_{50} for the season was –20°C, shown by accessions A29 and 48 in January 2000. Ranking of accessions for LT_{50} (°C) values during January of the first season was A29 = 48 (−20.0) < 55 (−17.0) ≤ 32 (−15.5) ≤ A65 = C66 (−14.0). From February to April 2000, all accessions showed an increase in LT_{50} with a significant difference among them during February and March but there was no difference between accessions in April.

October 2000 to April 2001

In October 2000, LT_{50} of all accessions ranged from –11.0 to –14.0°C with no significant difference among them (Fig. 3). In November, accessions 48 and 55 (−20.0°C) had lower LT_{50} than 32 and C66 (−14.0°C). In December, accession 55 had a lower LT_{50} than 32, 48, and A29. In January, accessions 55 and 48 achieved the lowest LT_{50} of –26.0°C. January LT_{50} value was 48 = 55 (−26.0°C) < A65 = 32 (−23.0°C) < A29 (−20.0°C) = C66 (−18.5°C). The LT_{50} values increased in February and were similar to October values without significant differences among accessions. In April, the LT_{50} values increased to between –5 to –10°C for all accessions except 55, which remained at –14.0°C.

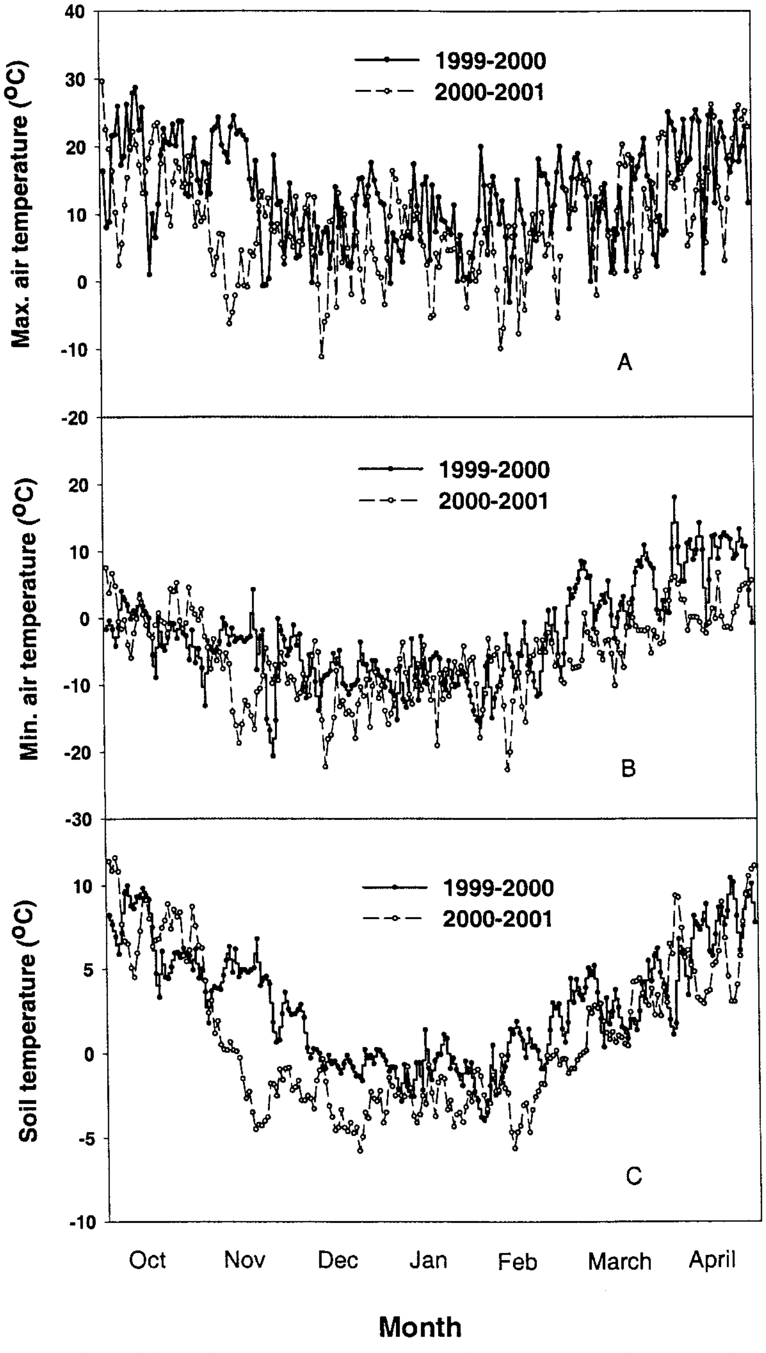

Fig. 1. The maximal air temperature (A), minimal air temperatures (B), and soil temperature (C) at Fort Collins, CO, from October to April in 1999-2000 and 2000-2001.

Total Rhizome Regrowth

October 1999 to April 2000

Regression analysis indicated that the relationship between total rhizome regrowth and freezing temperature was highly linear (R^2 = 0.67–0.97). In October 1999, GT_{50} was –10.4°C for A29 and 48, significantly lower than other accessions (Fig. 4). Regression predicted that C66 had the highest GT_{50} (–7.2°C). In November, the GT_{50} of A29 was –16.5°C, which was the lowest among all accessions. In December, 55 had a GT_{50} of –21.0°C while that of A29 experienced little change (–16.1°C). Ranking for accessions for GT_{50} in January 2000 was: A29 (−21.9°C) = 48 (−19.2°C) < 55 (−17.5°C) = 32 (−17.2°C) < A65 (−13.7°C) < C66 (−11.2°C) (Fig. 4). From February to April GT_{50} increased, with significant differences seen among accessions. In April, GT_{50} was about −6.3°C for A29, 32, 48, 55 and C66, which was higher than A65 (−8.1°C). Although the ranking of GT_{50} varied from month to month, in general A29, 48, and 55 exhibited lower GT_{50} than other accessions, indicating their better freezing tolerance.

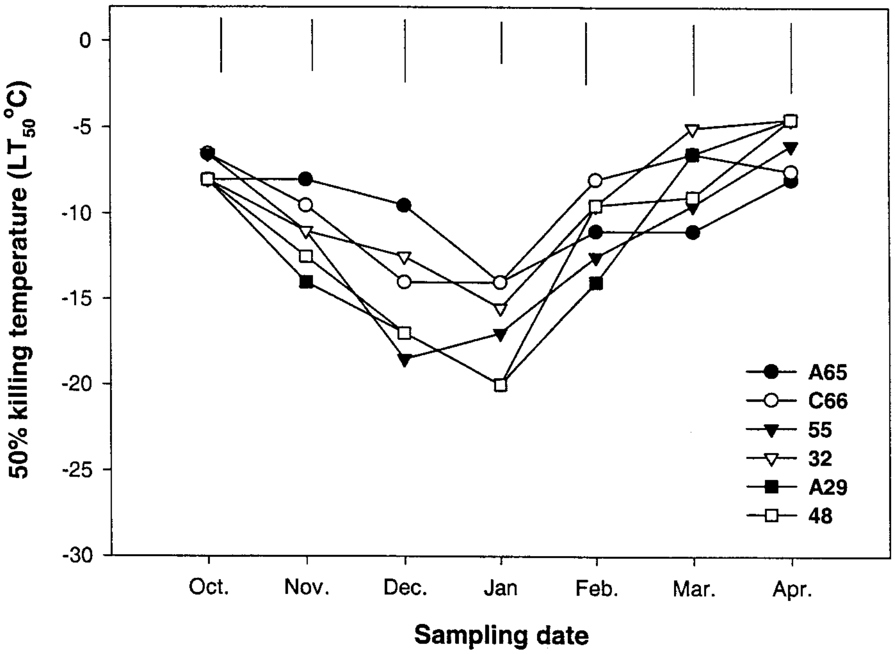

Fig. 2. Seasonal changes of LT_{50} (the subfreezing temperature resulting in 50% mortality) of six saltgrass accessions sampled monthly from unmowed field plots at Fort Collins, CO, during October 1999 and April 2000. Vertical bars at the top indicate LSD at $P = 0.05$ for accession comparison within each date.

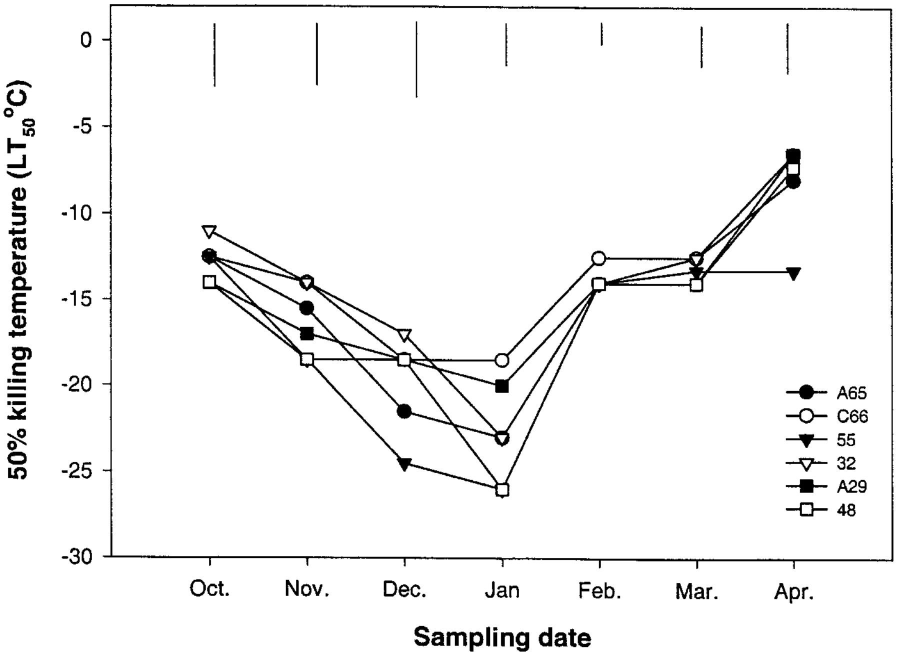

Fig. 3. Seasonal changes of LT_{50} (the subfreezing temperature resulting in 50% mortality) of six saltgrass accessions sampled monthly from unmowed field plots at Fort Collins, CO, during October 2000 and April 2001. Vertical bars at the top indicate LSD at $P = 0.05$ for accession comparison within each date.

October 2000 to April 2001

In the second season all accessions were more cold hardy and exhibited higher levels of regrowth than in the first season (Fig. 5). In October, the GT_{50} of 32 was −12.0°C, which was higher than other accessions (–15.6°C). In November, the GT_{50} was ≈ −19.0°C for 48 and 55, which were the lowest among all accessions. In midwinter 55, 48, and A65 exhibited lower GT_{50} than the other accessions. In April, the lowest GT_{50} was –10.9°C (Accession 55), which was lower than all other accessions (≈ –6.4°C).

Winter Survival in the Field

Winter survival in the field ranged from 85 to 94% in the first season and 73 to 86% in the second season (Table 2). In 2000, accessions 48 and A29 had greater

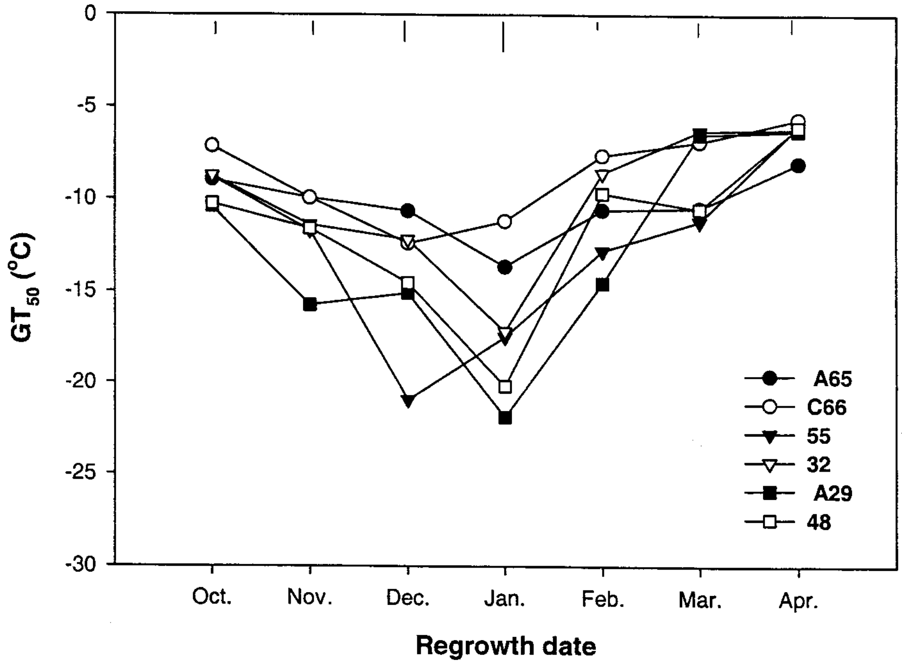

Fig. 4. Seasonal changes of GT_{50} (the subfreezing temperature that caused 50% rhizome regrowth reduction) of six saltgrass accessions sampled monthly from unmowed field plots at Fort Collins, CO, during October 1999 and April 2000. Vertical bars at the top indicate LSD at $P = 0.05$ for accession comparison within each date.

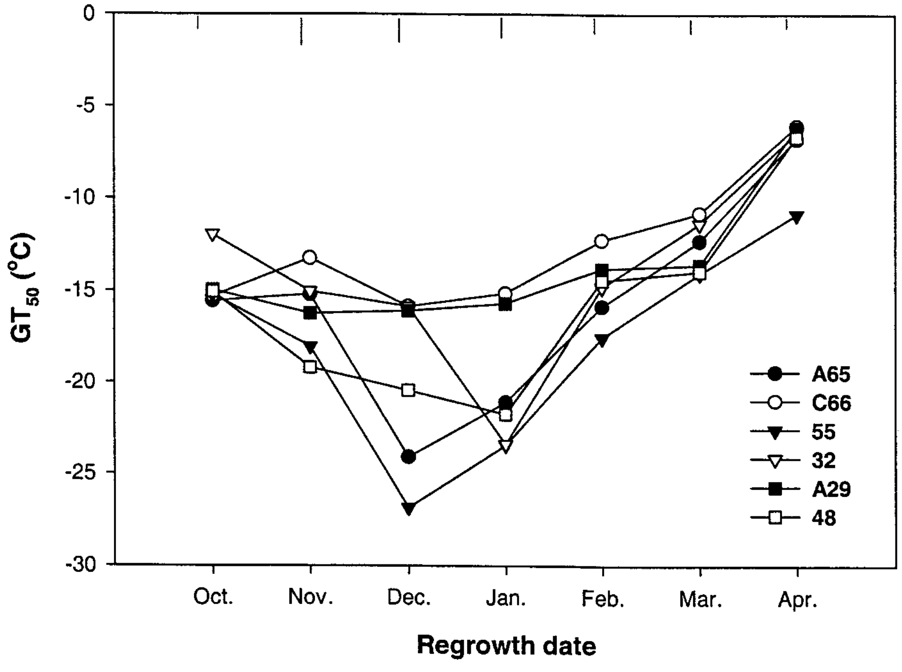

Fig. 5. Seasonal changes of GT_{50} (the subfreezing temperature that caused 50% rhizome regrowth reduction) of six saltgrass accessions sampled monthly from unmowed field plots at Fort Collins, CO during October 2000 and April 2001. Vertical bars at the top indicate LSD at $P = 0.05$ for accession comparison within each date.

survival than C66, A65, and 32 (Table 2). Accession 55 had higher survival than C66 but was not statistically different from 48 and A29 or 32 and A65. In 2001, accessions 48 and 55 had greater winter survival than C66, A65, and 32. Accession A29 had greater survival than A65 and C66 but was not different from 48 and 55 or 32. Winter survival in the field correlated negatively ($r = -0.81$) with LT_{50} value.

DISCUSSION

Considerable variations in LT_{50} and GT_{50} were found between the two seasons and among accessions and sampling dates. All accessions except A29 had greater freezing tolerance (lower LT_{50} and GT_{50}) in 2000-2001 when compared to 1999-2000. The difference in freezing tolerance between the two seasons likely be attributed to the climate conditions; the consistently frozen soil

Table 2. Winter survival of saltgrass accessions grown in the field in 2000 and 2001.

	Winter survival† (%)	
Accessions	2000	2001
48	94a‡	86a
A29	92a	84ab
55	90ab	85a
32	88bc	82bc
A65	88bc	80c
C66	85c	73d

† Winter survival was estimated visually in May as a green-up percentage for each sampled plot.

‡ Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ using Fisher's LSD test.

from November to February in 2000-2001 appeared to allow saltgrass to achieve and maintain greater freezing tolerance. The freezing and thawing cycles experienced during the winter of 1999-2000 likely reduced the freezing tolerance of saltgrass.

Significant differences in freezing tolerance were observed among accessions during mid-winter in both seasons. Accessions 48, 55, and A29 were usually the most cold hardy in the first season, with LT_{50} values as low as −20°C (Fig. 2). In the second season, A29 exhibited poorer freezing tolerance than 55 and 48, which exhibited an LT_{50} approaching −26°C (Fig. 3). Accession C66 had poor freezing tolerance with an LT_{50} ranging from –14 to –18°C during midwinter in both seasons. The difference in freezing tolerance among accessions is in part associated with their origin-inherited adaptation. In this study, the most cold hardy accessions were collected from South Dakota and Colorado, hardiness zones 4 and 5 based on the USDA hardiness zone map. The sensitivity of C66 over the two seasons can be explained by its origin in Humbolt Sink, NV, which is relatively warm (in hardiness zone 6). This accession may be genetically adapted to warmer climates, and thus not be as cold hardy. However, variations exist for accessions collected from the same climate zone. Both A65 and A29 were collected in Denver, Colorado, classified as USDA climate zone 5A. We cannot explain why A29 had lower LT_{50} and GT_{50} values than A65 during 1999-2000, but higher LT_{50} and GT_{50} during 2000-2001 than A65.

The range of LT_{50} values for saltgrass observed during 1999-2000 in this study is comparable to that reported for buffalograss during 1999-2000 at Fort Collins, CO (Qian et al., 2001). However, the range of winter survival in the field was higher for saltgrass than buffalograss. The deep rhizome growth of saltgrass is likely a low temperature avoidance mechanism, which contributes to greater field survival of saltgrass than buffalograss, a stoloniferous grass.

In summary, our results show that significant variability in freezing tolerance exists among saltgrass accessions. The difference in freezing tolerance among accessions is in part associated with their origin-inherited adaptation. This information will be useful for the development of cold hardy, turf-type saltgrass cultivars. Further study is recommended to determine the mechanisms of saltgrass freezing tolerance, which could include conversion of membrane stability (Cyril et al., 1998; Samala et al., 1998), carbohydrate content (Fry et al., 1991, 1993; Maier et al., 1994), and cold regulated protein synthesis (Gatschet et al., 1996).

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