韓草誌 (J. Korean Grassl. Sci.) 25(1) : 57~64, 2005

Comparison on the Changes in Non-structural Carbohydrate Concentration with Regard to Cold Tolerance of Zoysiagrass and Creeping Bentgrass

Dae-Hyun Kim*, Woo-Jin Jung**, Bok-Rye Lee*, Eun-Ju Kim*, Kil-Yong Kim***
and Tae-Hwan Kim

월동 기간 중 Zoysiagrass와 Creeping Bentgrass의 비구조 탄수화물 농도의 변화 비교

김대현* · 정우진** · 이복례* · 김은주* · 김길용*** · 김태환

적 요

월동기간 동안 zoysiagrass[*Zoysia matrella*(L.) Merr]와 creeping bentgrass(*Agrostis palustris* Hunds)의 저온 스트레스에 대한 탄수화물 대사 반응을 구명하기 위해 11월부터 이듬해 4월까지의 식물체내의 탄수화물 대사산물의 변화를 비교 분석하였다. Creeping bentgrass의 녹색도와 뿌리 성장은 온도변화와 거의 평행하게 변화하였으나 zoysiagrass는 뿌리 성장에 거의 변화가 없이 3월 말까지 담황색을 나타냈다. 11월부터 이듬해 1월까지 zoysiagrass에서 glucose와 sucrose 함량은 크게 높았고, 1월에서 4월까지 starch를 제외한 모든 탄수화물 화합물들의 감소의 정도가 현저하게 낮았다. Reeping bentgrass에서 fructan의 depolymerization이 2월 이후 단당류와 이당류의 감소와 일치했으나 zoysiagrass에서는 starch 가수분해가 11월부터 일찍이 시작되었다. 이상의 결과들은 creeping bentgrass와 비교 했을때 zoysisagrass 뿌리의 높은 비구조적 탄수화물의 농도가 저온 내성과 밀접한 관계가 있음을 잘 나타내어 주고 있다.

(**Key words** : Creeping bentgrass, Zoysiagrass, Overwintering)

Ⅰ. INTRODUCTION

Zoysiagrasses (*Zoysia japonica*, *Zoysia matrella* and *Zoysia tenuifolia*) are widely used for tee and fairway in golf course and for home lawn in Korea. Although the grass is very cold hardy, it turns to straw-colored turf in the first frost and does not green up until April in early spring. Some cool-season turfgrasses including creeping bentgrass (*Agrostis palustris*) for golf course green, kentucky bluegrass (*Poa pratensis*) and turf-type perennial ryegrass (*Lolium perenne*) for polystand on fairways provide successfully a playing surface during winter period in Korea.

In exposure to cold stress, plant cells properly undergo dramatic morphological changes such as fragmentation of vacuoles, formation of small vesicles of endoplasmic reticulum and thickening

* Department of Animal Science, Institute of Agricultural Science and Technology, College of Agriculture & Life Science, Chonnam National University, Gwangju 500-757, Korea.
** Glucosamine Saccharide Materials Laboratory(NRL), Institute of Agricultural Science and Technology, Chonnam National University, Gwangju 500-757, Korea.
*** Department of Biological and Environmental Chemistry, College of Agriculture & Life Science, APSRC, Chonnam National University, Gwangju 500-757, Korea.
Corresponding author : Tae Hwan Kim, Tel : +82-62-530-2126, Fax : +82-62-530-2129; E-mail : grassl@chonnam.ac.kr

of cell walls (Griffith et al., 1985; Fujikawa and Takebe, 1996) along with the enhancement of freezing tolerance. These morphological changes in plant cells in concert with the actions of accumulated compatible solutes such as soluble sugars and amino acids (Wanner and Junttila, 1999) are thought to contribute to the development of maximum freezing tolerance of plant cells.

Levitt (1980) reported that carbohydrate levels and composition influenced the sensitivity of plant tissues to low temperature. The importance of soluble carbohydrates, especially sucrose, in freezing tolerance induced by cold acclimation in grass species has been shown (Dionne et al., 2001). Sugars are also involved in the acclimation response to subfreezing temperatures in plants through their interactions with membrane phospholipids (Anchordoguy et al., 1987). Dionne et al. (2001) reported that the higher starch content is a prerequisite for freezing tolerance in green-type annual bluegrass. Fructan is the major nonstructural carbohydrate in many plant species and has an important function in the temporary storage of assimilates (Pollock and Cairns, 1991). Fructan content and its metabolism have been shown to be closely related to freezing tolerance in cereals (Tognetti et al., 1990).

Although susceptibility to freezing temperatures has been pointed out as a major factor for winter damage of turfgrasses in golf course, little information exists on physiological response to cold temperature with regard to freezing tolerance and winter adaptation. Eventually, when no green lamina remains during overwintering as like zoysiagrass, carbon assimilation in leaves ceases and the reserves in surviving rhizomes and roots become the main source of energy. The experiment was designed to investigate the difference of carbohydrate metabolism in response to naturally occurring winter freezing stress between zoysiagrass and creeping bentgrass used often for golf course in Korea.

Ⅱ. MATERIALS AND METHODS

1. Sampling and collecting site

Sampling was made from green and fairway established in 1998 at Muan Country Club (35° 05' 32" N, 126° 17' 12" E) in Korea. The turfgrasses were maintained by general winter management. Minimum daily temperature in green field ranged from －2.8 to 11.0 ℃ during the experimental period. It reached to 0 ℃ at mid-December remained below freezing temperature until the end of February. Maximum daily temperature ranged from 5.1 to 20.6 ℃ (Fig. 1).

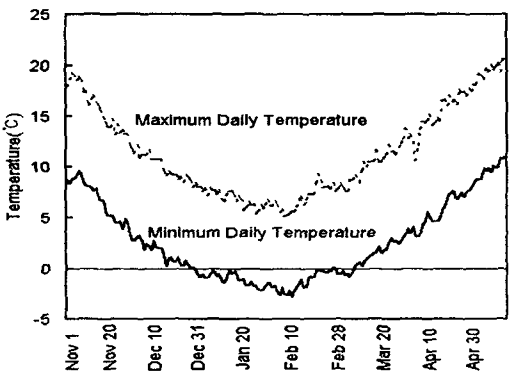

Fig. 1. Daily maximum temperature (---) and minimum temperature (—) during the winter of 2000～2001 at Muan city, Korea.

Creeping bentgrass and zoysiagrass were sampled using a hole cutter (108 mm) at five randomly selected sites from November 20 in 2000 to April 23 in 2001 with about one month interval. Root samples were obtained to a depth of approximately 20 cm. Roots were severed from crown, packed with ice andtransported to the laboratory. Roots were free from soil by washing with cold running water, immediately frozen in liquid nitrogen and lyophilized. Freeze-dried samples were finely ground and stored under vacuum for further analysis.

2. Turf quality and root growth

Turf quality was visually rated as described by Xu and Huang (2001). It was estimated on the basis of color, density and uniformity on a scale of 0 (the worst: plants turned brown and dead) to 9 (the best: plants turned healthy and green). Dry weight of lyophilized root sample was weighed to estimate root growth.

3. Carbohydrate analysis

About 30 mg of finely ground sample was extracted with 1 mL of 92 % (v / v) ethanol. Tubes were shakenfor 10 min at room temperature and centrifuged at 14,000 rpm for 10 min at 4 ℃. The ethanol extraction was repeated three times, and the combined supernatant was diluted to a final volume of 10 mL with 92 % (v / v) ethanol.

The glucose concentration in the ethanol extracts was determined with anthrone reagent (Van Handel, 1968) using glucose as a standard. Fructose concentration in ethanol extracts was determined using fructose asstandard (Davis and Gander, 1967). Sucrose concentration in the ethanol extracts was determined with anthrone reagent (Van Handel, 1968) using sucrose as a standard.

The residue was dried at 80 ℃ to remove ethanol. Deionized water was added and heated to gelatinize the starch. The pH of the solution was adjusted to 5.1 by adding 0.2 N Na-acetate buffer. Starch was digested by adding amyloglucosidase (Sigma product A3514) and -amylase (Sigma product A0273) in the acetate buffer to each sample. Tubes were incubated at 50 ℃ for 24 h with occasional shaking and centrifuged. Glucose in the supernatant was determined using glucose oxidase (Glucose Trinder, Sigma product 315 - 100). Starch concentrations were calculated multiplysing a factor of 0.9 to glucose concentration. Fructan present in the starch extracts was hydrolyzed with 0.1 N H_2SO_4 and fructose released quantified using resorcinol (Davis and Gander, 1967). Glucose liberated from the fructan was determined as described above. Fructan concentration was calculated by multiplying a factor of 0.9 to glucose and fructose concentrations.

Ⅲ. RESULTS

1. Turf quality and root growth

Creeping bentgrass maintained much better turf quality than zoysiagrass did during experimental period (Fig. 2A). Turf quality of creeping bentgrass decreased gradually by January when temperature was the lowest and then recovered with increasing temperature. Discoloration of zoysiagrass began with the advent of 10 to 13 ℃ in the minimum temperature (at the end of October in the this experimental site). This resulted in a straw-colored turf during most of the winter season. It was started to green up from the end of March.

In November, root dry weight of creeping bentgrass and zoysia grass were 9.7 g and 3.7 g DW per hold cutter, respectively (Fig. 2B). Root dry weight of creeping bentgrass slowly decreased to the lowest level until January and then increased with a higher rate by the end of April in parallel with air temperature. However, root dry weight of zoysiagrass was less changed within 5 g per hold cutter. These results indicated that

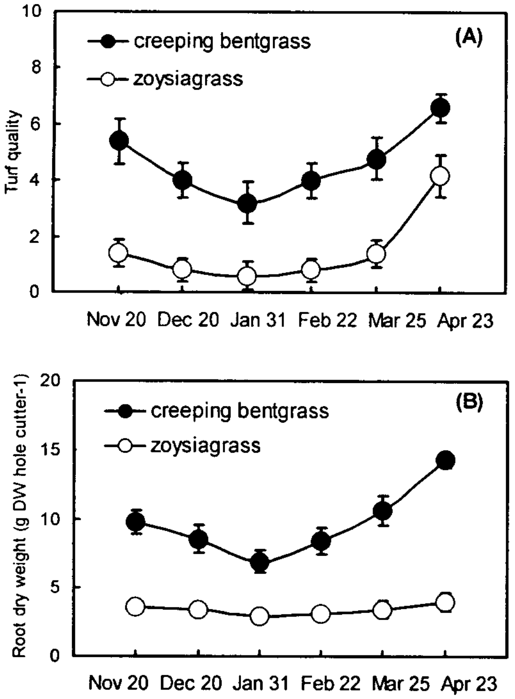

Fig. 2. Turf quality (A) and root dry weight (B) of overwintering creeping bentgrass and zoysiagrass. Each value is the mean ± s.e. for n = 5.

root growth of creeping bentgrass responded much sensitively to low temperature during winter compared to that of zoysiagrass.

2. Carbohydrates concentration

Changes in soluble carbohydrates in roots of creeping bentgrass and zoysiagrass are shown in Fig. 3. In both species, mono- and disaccharides started to accumulate from November, when the minimum temperature was about 5 ℃. The highest concentrations of these compounds were detected in January when both species were exposed to their maximum cold stress. Glucose accumulation from November to January was much higher in zoysiagrass than that of creeping bentgrass and glucose concentration of creeping bentgrass and zoysiagrass in January was 56.4 and

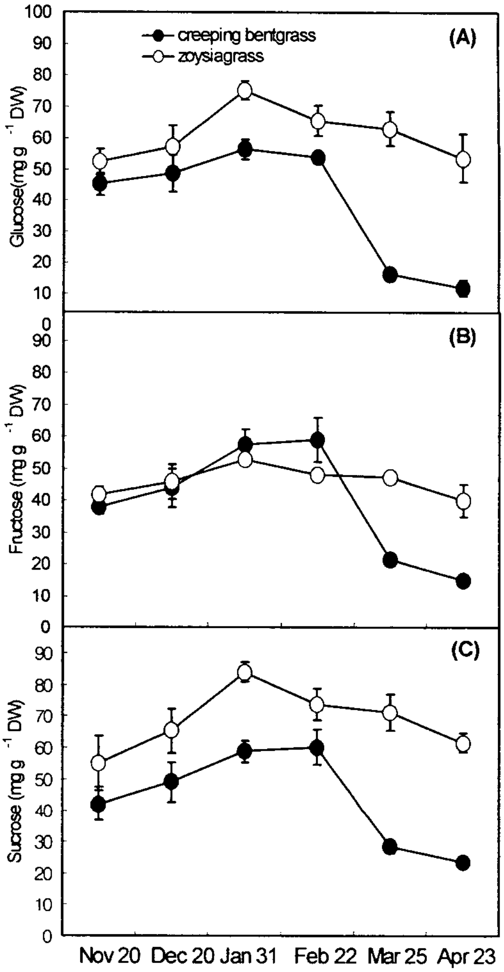

Fig. 3. Changes in glucose (A), fructose (B) and sucrose (C) in roots of overwintering creeping bentgrass and zoysiagrass. Each value is the mean ± s.e. for n = 5.

75.0 mg g^{-1} DW, respectively (Fig. 3A). From January to April, a remarkable decline in glucose concentration (about 79 % decline) occurred in creeping bentgrass, but a small declinedid (only 28 %) in zoysiagrass. Fructose concentration was not significantly different between creeping bentgrass and zoysiagrass from November to January (Fig. 3B). From February to March, fructose in creeping bentgrass decreased rapidly whereas that of zoysiagrass was less varied in the same period.

Sucrose concentration increased with a similar rate for both species until January showing the highest concentration in January (70.4 and 90.6 mg g^{-1} DW for creeping bentgrass and zoysiagrass, respectively) (Fig. 3C). From January to April, sucrose largely decreased in both species. The decline concentration for this period was about 3 times higher in creeping bentgrass.

Starch concentration of zoysiagrass in November was about 4 times higher than that of creeping bentgrass (Fig. 4A). Starch concentration in zoysiagrass continuously decreased from November (173.2 mg g^{-1} DW) to April (67.1 mg g^{-1} DW), whereas that in creeping bentgrass tended to increase until January and then decreased slowly. Fructan concentration in creeping bentgrass slightly increased from November to February representing at least 2 times higher concentration compared to zoysiagrass (Fig. 4B). About 65 % of the accumulated fructan in creeping bentgrass was depolymerized from February, when turf quality and root growth began to recover. Considering the pool size at the end of autumn and the extent of degradation during winter, it has been shown that starch for zoysiagrass and fructan for creeping bentgrass were the main storage forms found in cold-hardened roots.

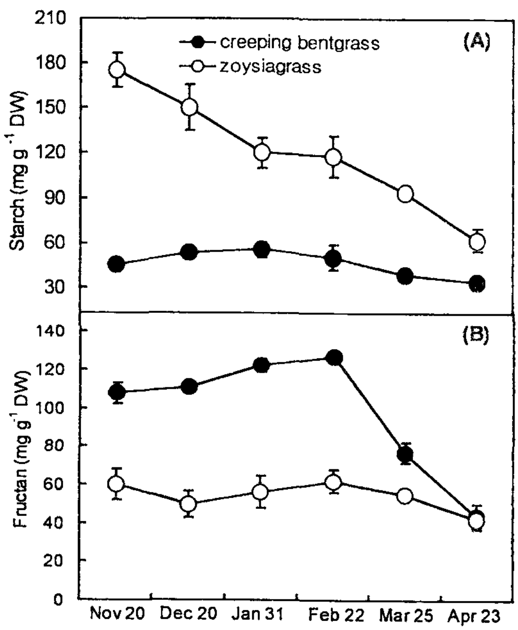

Fig. 4. Changes in starch (A) and fructose (B) in roots of overwintering creeping bentgrass and zoysiagrass. Each value is the mean± s.e. for n = 5.

The changes in total non-structural carbohydrate through experimental period indicated that creeping bentgrass much responded to the temperature fluctuation showing higher accumulation until January and greater degradation afterwards when compared to that of zoysiagrass (Fig. 5).

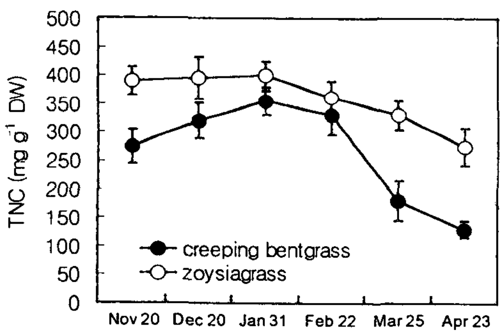

Fig. 5. Changes in total non-structure carbohydrate (TNC) in roots of overwintering creeping bentgrass and zoysiagrass. Each value is the mean ± s.e. for n = 5.

Ⅳ. DISCUSSION

The concentrations of mono- and disaccharide in both species during winter showed two distinct periods; the first accumulation phase from November to January and the second was that of active reduction after January (Fig. 3). The higher concentrations of glucose and sucrose in zoysiagrass during the first phase (Fig. 3A and 3 C) appeared to be associated with the higher decline in starch at the same period (Fig. 4A). This indicates, in zoysiagrass which is being dormant and dose not have active photosynthetic leavesduring this period, that starch reserves have at least two functions. Firstly, they constitute carbohydrate reserves as a main storage form to supply the energy for basal metabolism ifthe rate of photosynthesis is low (Sagiska, 1995). Secondly, their breakdown can provide sucrose and other sugars to increase osmotic potential and lower the freezingpoint of cells (Turner and Pollock, 1998). Such a decline has also been described for perennating organs of otherplants (Li et al., 1996; Cunningham et al., 1998; Dionne et al., 2001). However, fructan in creeping bentgrass continued to accumulate until February (Fig. 4B) with accompanyingthe increases in simple sugars and sucrose. Fructan accumulation in creeping bentgrass is considered to be a sink for carbon from current photosynthesis which is in excess of growth requirement. Indeed, the stubble of creeping bentgrass contained simple sugars and sucrose concentration of at least the same level as the roots at any given sampling date (data not shown).

The data obtained have shown that fructan for creeping bentgrass and starch for zoysiagrass mainly constitute carbohydrate reserves (Fig. 4A and 4B). Depolymerization of reserves at subfreezing temperature is thought to be an essential source of cryoprotective sugars in grasses (Olien and Clark, 1993; Dionne et al., 2001) and forage legumes (Turner and Pollock, 1998; Cunningham et al., 1998). In the current study, the depolymerization of fructan in creeping bentgrass coincided with the decline in mono and disaccharide after February whereas the starch hydrolysis in zoysiagrass started much earlier from November (Fig. 4). These indicate that the accumulation of simple sugars and sucrose by mid-winter in creeping bentgrass results from mainly current photosynthesis and in part from fructan hydrolysis, but nearly all of them does from starch hydrolysis in zoysiagrass. It is probable that the differences in the extent of accumulation and hydrolysis of non-structural carbohydrates between creeping bentgrass and zoysiagrass are associated with the different responses to temperature, irradiance and photoperiod during winter.

One possible explanation for this is species-related difference in carbohydrate accumulation associated with fall dormancy. It is generally believed that the continued shoot growth of non-dormant species or cultivars may prevent accumulation of high levels of root total non-structural carbohydrates (TNC) necessary for adequate winter survival. Alternatively, the premature resumption of shoot growth during mild winter weather may consume TNC reserves necessary for plants to survive the remainder of winter (Cunningham et al., 1998). Root TNC levels of zoysiagrass, dormant and a warm-season grass exceeded those of creeping bentgrass, non-dormant and cool-season grass, throughout experimental periods (Fig. 5) indicating that root TNC reserve level is negatively associated with fall dormancy related difference in cold tolerance. Castonguay et al. (1995) reported that crowns of fall-dormant alfalfa cultivars accumulated higher concentrations of sucrose, raffinose and stachyose than non-dormant alfalfa cultivars. Other studies with alfalfa (Sheaffer et al., 1992; Cunningham et al., 1998) have shown that cultivars differences in fall dormancy and winter survival were associated with carbohydrate concentrations in roots.

Sucrose started to accumulate in both species from November when the mean temperature was between 5 and 10 ℃ (Fig. 3C). Thesetemperatures are unlikely to have caused hardening (Dionne et al., 2001), and hardening test was not included in this test. For both species, peak sucrose concentration was shown in January, the coldest period. This result agrees with previous observations in grass species (Tronsmo et al., 1993; Dionne et al., 2001) and winter cereals (Olien and Clark, 1993; Livingston, 1996). It has been suggested that sucrose plays an important cryoprotective role by stabilizing cell membranes and proteins (Hoekstra et al., 1989), preventing adhesion of ice to critical cellular tissue during freezing (Olien, 1984).

In conclusion, the availability of cryoprotective sugars in the roots of overwintering zoysiagrass is largely enough in conferring winter hardiness as a consequence of sufficient synthesis of starch reserves by late autumn and their active hydrolysis of starch reserves. There are some evidences that cold tolerance mechanism is very active in roots of zoysiagrass during winter in comparison to those of creeping bentgrassas manifested by higher concentration of sugars.

V. ABSTRACT

To compare the carbon metabolic response to low temperature stress in zoysiagrass [*Zoysia matrella* (L.) Merr] and creeping bentgrass (*Agrostis palustris* Huds) with respect to cold tolerance, carbon metaolites were determined from November 20 in 2000 to April 23 in 2001. Turf quality and root growth of creeping bentgrass were nearly parallel with temperature fluctuation, while zoysiagrass showed a straw-colored turf until the end of March with little change in root growth. Glucose and sucrose accumulation from November to January was much higher in zoysiagrass, while the reduction from January to April in all carbohydrate compounds except starch was remarkably less for this species. The depolymerization of fructan in creeping bentgrass coincided with the decline in mono and disaccharide after February, whereas starch hydrolysis in zoysiagrass started much earlier from November. These results suggest that the higher amount of carbohydrate reserves (e. g. total non-structural carbohydrate) in zoysiagrassroots could be associated with higher cold tolerance when compared with that of creeping bentgrass.

VI. REFERENCES

1. Anchordoguy, T.J., A.S. Rudolph, J.F. Carpenter and J.H. Crowe. 1987. Modes of interaction of cryoprotectants with membrane phospholipids during freezing. Cryobiology 24: 324-331.
2. Bouchart, V., J.H. Macduff, A. Ourry, M.M. Svenning, A.P. Gay, J.C. Simon and J. Boucaud. 1998. Seanal pattern of accumulation and effects of low temperatures on storage compounds in Trifolium repens. Physiologia Plantarum 104:65-74.
3. Castonguay, Y., P. Nadeau, P. Lechasseur and L. Chouinard. 1995. Differential accumulation of carbohydrates in alfalfa cultivars of contrasting wintrhardiness. Crop Science 35:509-516.
4. Cunningham, S.M., J.J. Volenec and L.R. Teuber. 1998. Plant survival and root and bud composition of alfalfa population selected for contrasting fall dormancy. Crop Science 38:962-969.
5. Davis, J.S., Gander. 1967. A reevaluation of the Roe procedure for determination of fructose. Analytical Biochemistry 19: 72-79.
6. Dionne, J., Y. Castonguay, P. Nadeau and Y. Desjardins. 2001. Freezing tolerance and carbohydrate changes during cold acclimation of green-type annual bluegrass (*Poa annua* L.) ecotypes. Crop Science 41:443-451.
7. Fujikawa, S. and K. Takabe. 1996. Formation of multiplex lamellae by equilibrium slow freezing of cortical parenchyma cells of mulberry and its possible relationship to freezing tolerance. Protoplasma 190:189203.
8. Griffith, M, N.P.A. Huner, K.E. Espelie and P.E.

Kolattukudy. 1985. Lipid polymers accumulate in the epidermis and mestome sheath cell walls during low temperature development of winter rye leaves. Protoplasma 1985:125:5364.

9. Hoekstra, F.A, L.M. Crowe and J.H. Crowe. 1989. Differential desiccation sensitivity of corn and pennisetum pollen linked to their sucrose contents. Plant, Cell and Environment 12:83-91.

10. Levitt, J. 1980. Response of plants to environmental stress. Vol. 1, 2nd ed. (Academic Press: New York).

11. Li, R., B.C. Joern, J.J. Volenec and S.M. Cunningham. 1996. Seasonal changes in nonstructural carbohydrates, protein, and macronutrients in roots of alfalfa, red clover, sweetclover, and birdsfoot trefoil. Crop Science 36:617-623.

12. Livingston, D.P. III. 1996. The second phases of cold hardening : Freezing tolerance and fructan isomer changes in winter cereal crowns. Crop Science 36:1568-1573.

13. Olien, C.R. 1984. An adaptive response of rye to freezing. Crop Science 24:51-54.

14. Olien, C.R and J.L. Clark. 1993. Changes in soluble carbohydrate composition of barley, wheat, and rye during winter. Agronomy Journal 85:21-29.

15. Pollock, C.J. and A.J. Cairns. 1991. Fructan metabolism in grasses and cereals. Annual Review of Plant Pysiology and Plant Molecular Biology 42: 77-101.

16. Sheaffer, C.C, D.K. Barnes, D.D. Warnes, W.E. Leuschen, H.J. Ford and D.R. Swanson. 1992. Seeding-year cutting affects winter survival and its association with fall growth score in alfalfa. Crop Science 32:225-231.

17. Togentti, J.A., G.L. Salerno, M.D. Crespi and H.G. Pontis. 1990. Sucreose and fructan metabolism of different wheat cultivars at chilling temperatures. Physiologia Plantarum 78:554-559.

18. Tronsmo, A.M, G. Kobro, S. Morgenlie and R. Flengsrud. 1993. Carbohydrate content and glycodidases activities following cold hardening in two grass species. Physiologia Plantarum 88:689-695.

19. Turner, L.B and C.J. Pollock. 1998. Changes in stolon carbohydrates during the winter in four varieties of white clover (*Trifolium repens* L.) with contrasting hardiness. Annals of Botany 81:97-107.

20. Van Handel, E. 1968. Direct microdetermination of sucrose. Analytical Biochemistry 22:1341-1346.

21. Wanner, L.A and O. Junttila. 1999. Cold-induced freezing tolerance in Arabidopsis. Plant Physiology 120:391399.

22. Xu, Q. and B. Huang. 2001. Morphological and physiological characteristics associated with heat tolerance in creeping bentgrass. Crop Science 41: 127-133.