韓草誌(J. Korean Grassl. Sci.) 21(4) : 259~264, 2001

Changes in Non-Structural Carbohydrate in Overwintering Creeping Bentgrass (*Agrostis palustris*)

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

크리핑 벤트그라스의 월동중 비구조적 탄수화물의 변화

김대현 · 정우진 · 이복례 · 김길용* · 김태환

Abstract

To investigate the physiological responses to naturally occurring winter freezing stress in creeping bentgrass, changes in carbohydrates were monitored during winter period. Turf quality and leaf growth was nearly parallel with temperature fluctuation. The concentration of glucose, fructose and sucrose in both shoot and root gradually increased from November to January, and then sharply decreased until April. Sucrose was the largest pool of soluble sugars. Fructan also slightly accumulated in both shoot and roots from November to February. Fructan hydrolysis in both organs was found to be much active between February to April. Shoot contained largely higher carbohydrate content in all compounds examined than roots did. Fructan was found to be a main carbohydrate storage form, showing the highest concentration (176.7 and 126.7 mg g^{-1} DW for shoot and root in February). The depolymerization of fructan from February coincided with the high declines in mono- and disaccharide. These results suggest that the accumulation of non-structural carbohydrate until January could be associated with freezing tolerance, and the active decrease from February with shoot regrowth.

(**Keywords** : Creeping bentgrass, Winter adaptation, Mono- and disaccharide, Fructan)

I. Intruduction

Creeping bentgrass (*Agrostis palustri*) is being widely used for golf course green in. Some cool-season turfgrasses including creeping bentgrass for golf course greens, Kentucky bluegrass (*Poa pratensis*) and turf-type perennial ryegrass (*Lolium perenne* L.) for polystand on fairways successfully provide a playing surface during winter period in Korea.

Low temperature below optimal growth temperature causes the decline in turf quality that has been associated with reductions in root growth, leaf water potential, cell membrane stability, photosynthetic rate, photochemical efficiency and carbohydrate accumulation (Olien and Lester 1985; Olien and Clark 1993; Dionne et al., 2001).

Major metabolic changes have been documented during the acquisition of cold tolerance including changes in carbohydrates, proteins, nucleic acids, amino acids, growth regulators, phospholipids and fatty acids (Levitt 1980). Among these, soluble sugars

전남대학교 농과대학 동물자원학과, 농업과학기술연구소 (Department of Animal Science & Institute of Agriculture Science and Technology, College of Agriculture, Chonnam National University, Gwangju 500-757, Korea)

* 전남대학교 농과대학 농화학과 (Department of Biological & Environmental Chemistry, College of Agriculture, APSRC, Chonnam National University, Puk-Gwangju P.O box 205, 500-600, Korea)

Corresponding Author: Prof. Tae-Hwan Kim, phone: +82-62-530-2126, fax: +82-62-530-2129, email: grassl@chonnam.ac.kr

are thought to be of important adaptive value for tolerance to subfreezing temperatures in plants through their interactions with membrane phospholipids (Anchordoguy et al., 1987) and the stabilization of proteins in freeze-desiccated cells (Carpenter and Crowe 1988). The depolymerization of polysaccharides at subfreezing temperature is thought to be an essential source of cryoprotective sugars (Olien and Clark 1993; Livingston 1996), although it has been claimed that there is no absolute requirement for sugars to accumulate in order for tissues to harden (Pollock et al. 1999).

Therefore, an investigation of the accumulation and utilization of non-structural carbohydrate during over-wintering is considered to be fundamental for understanding the winter adaptation of creeping bentgrass. The current work was conducted to establish the changes in pool size of different carbohydrate fractions in shoots and roots of overwintering creeping bentgrass.

Ⅱ. Materials and Methods

1. Sampling and collecting site

Sampling was made from green and fairway, established in 1998, of 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 −2.8 to 11.0℃ during the experimental period. It reached to 0℃ at mid-December, remained at below freezing temperature until end-February. Maximum daily temperature ranged 5.1 to 20.6℃ (Fig. 1).

Creeping bentgrass was sampled using a hole cutter (Ø108mm) at five randomly selected sites, from 20 November, 2000 to 23 April, 2001 with about one-month interval. The samples were obtained to a depth of approximately 20 cm. Thereafter, shoots were removed with a clipper, and roots were separated from the above-ground portion. Root samples were washed free of soil under a stream of cold water. Plant tissues were immediately frozen in liquid nitrogen, and lyophilized. Freeze-dried samples were finely ground and stored under vacuum for further analysis.

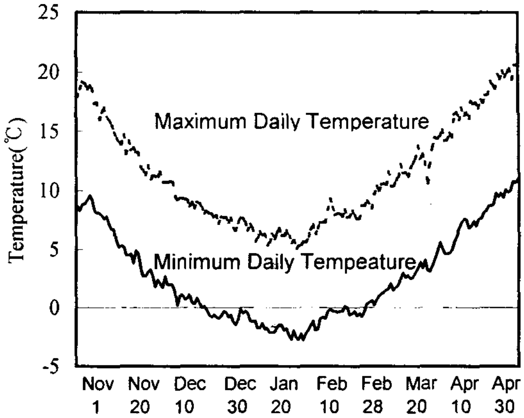

Fig. 1. Daily maximum temperature (---) and minimum temperature (—) during the winter of 2000~2001 at Muan city, Korea.

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 the color, density, and uniformity on a scale of 0 (worst, plants dead and brown) to 9 (best, plants healthy and green). Dry weight of lyophilized root sample was weighted 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 shaken for 10 min at room temperature, centrifuge 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 as standard using fructose (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 α-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 estimated as 0.9 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 as the sum of fructan glucose and fructose X 0.9.

Ⅲ. Results

1. Turf quality and root growth

Creeping bentgrass was maintained at least green color in mild-freeze during experimental period (Fig. 2A). *Turf quality decreased gradually by January* when temperature was the lowest, and then recovered with increasing temperature.

In November, shoot and root dry weight were 5.5g and 9.7 g DW per hold cutter, respectively (Fig. 2B). Shoot dry weight was less varied within 10g per hold cutter, under 2.5 cm height above ground level. However, root dry weight of creeping bentgrass decreased to the lowest level until January and then increased with a higher rate by the end of April, in parallel with air temperature. Between February and March, root dry weight increased about 26%. These results indicated that root growth of creeping bentgrass much sensitively responded to low temperature during winter compared to shoot.

2. Carbohydrates concentration

Changes of carbohydrates in creeping bentgrass are shown in Fig. 3 and Fig. 4. Mono- and disaccharides were accumulated from November (Fig. 3A, 3B and C), when the minimum daily temperature was about 5℃ (Fig 1). The highest concentration of these compounds was measured in January, when

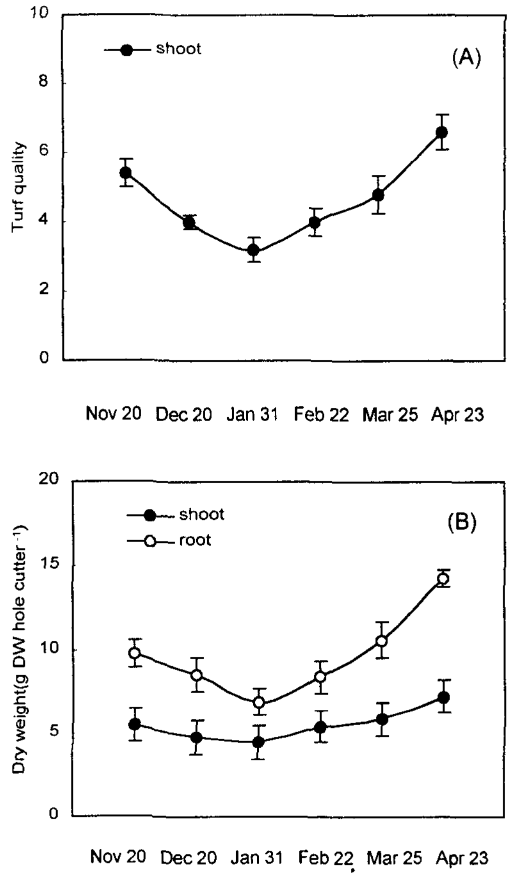

Fig. 2. Turf quality (A) and dry weight (B) in shoot (●) and roots (○) of overwintering creeping bentgrass. Each value is the mean±s.e. for n=5.

creeping bentgrass was exposed to their maximum cold stress. Glucose concentration of creeping bentgrass in January was 100.2 and 56.4 mg g^{-1} DW in shoot and root, respectively. From January to April, glucose concentration was declined about 77% and 79% in shoot and roots, respectively, showing the highest fall down between February and March. Fructose concentration in shoot gradually increased from November to January, while in root, it continued to increase until February. Thereafter these compounds decreased about 74% in both shoot and roots. The fructose concentration in shoot and roots at January was 100.1 mg g^{-1} DW and 58.9 mg g^{-1} DW, respectively, presenting the highest level.

Sucrose concentration increased until January, showing the highest concentration (137.5 and 70.42 mg g^{-1} DW for shoot and root, respectively) (Fig. 3C). From February to March, sucrose concentration largely decreased by 38% and 51% in shoot and root, respectively. Soluble carbohydrate such as glucose, fructose and sucrose was largely decreased from February to March. The net decrease for this period was much higher in shoot than that of roots.

Fructan concentration slightly increased from November to February, and then sharply decreased (Fig. 4). About 65% of the accumulated fructan was depolymerized in both shoot and roots 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 could be assumed that fructan was the main storage forms found in cold-hardened creeping bentgrass.

Ⅳ. Discussion

The concentrations of mono- and disaccharide during winter showed two distinct periods; the first accumulation phase from November to January and the second active reduction afterward (Fig. 3). The higher concentrations of glucose, fructose and sucrose in shoots during overwintering appeared to be associated with carbohydrate storage in stubble around root base. Firstly, they constitute carbohydrate reserves as a main storage in stubble (Smith and Dale 1974; Booyen et al., 1975; Kim et al., 2001). Secondly, their breakdown can provide sucrose and other sugars to increase osmotic potential and lower the freezing point of cells (Turner and Pollock 1998). Such decline has also been described for perennating organs of other plants (Li et al., 1996; Cunningham et al., 1998; Dionne et al., 2001). Especially, fructan in creeping bentgrass continued to accumulate by February (Fig. 4) with accompanying the increases in simple sugars and sucrose. Fructan accumulation 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 higher simple sugars and sucrose concentration compared to the roots at any given sampling date.

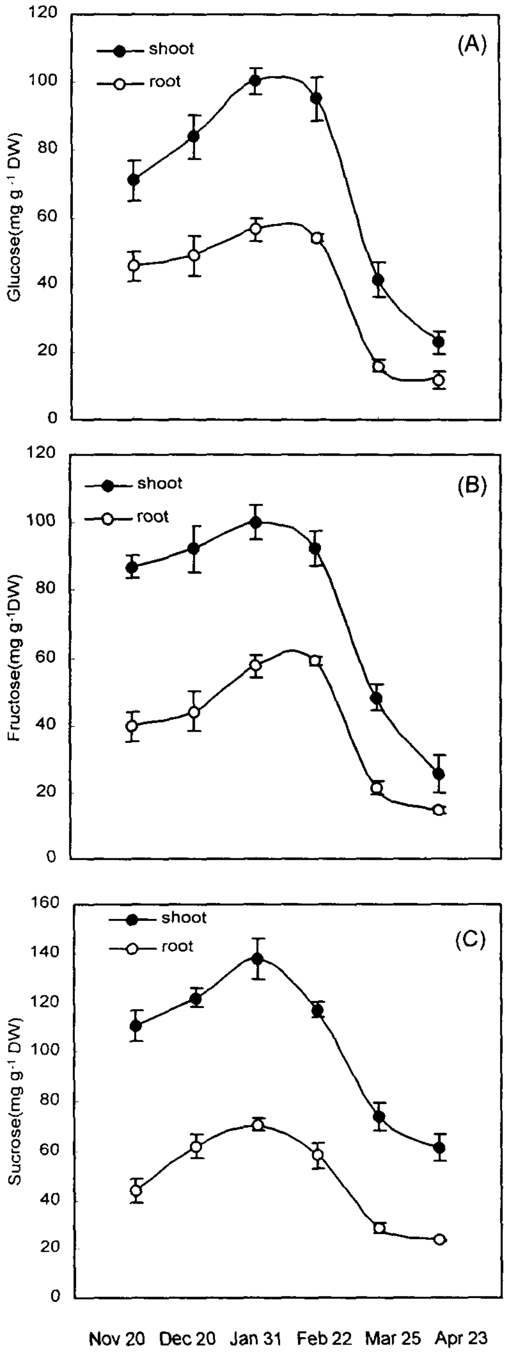

Fig. 3. Changes in mono- and disaccharide concentration in shoot (●) and roots (○) of overwintering creeping bentgrass. (A) Glucose, (B) Fructose, (C) Sucrose. Each value is the mean±s.e. for n=5.

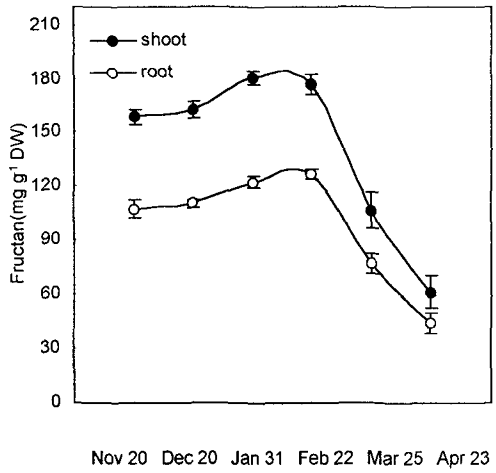

Fig. 4. Changes in fructan concentration in shoot (●) and roots (○) of overwintering creeping bentgrass. Each value is the mean±s.e. for n=5.

Fructan accumulation under cold stress has been documented in cool-season grasses (Chatterton et al., 1989), forage grasses (Pollock and Jones, 1979), and wheat and barley (Wagner et al., 1983). The data obtained have shown that fructan for creeping bentgrass mainly constitute carbohydrate reserves in shoot involving root base (Fig. 4). 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 current study, the depolymerization of fructan in creeping bentgrass coincided with the decline in mono- and disaccharide after February (Fig. 4). These indicate that the accumulation of simple sugars and sucrose by mild-freeze in creeping bentgrass results from mainly current photosynthesis and in part from fructan hydrolysis.

Sucrose started to accumulate from November when the mean temperature was between 5 and 10℃ (Fig. 3C). These temperatures are unlikely to have caused hardening (Dionne et al., 2001), and no tests for hardening were included in this study. The 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). Thus, sucrose levels in overwintering turfgrasses would be one of determinant factors for low temperature adaptation and freezing tolerance. A notably higher decrease in simple sugars and sucrose from February could be associated with an increase in the development of new foliage (Fig. 2A) and an increase in dry weight of roots (Fig. 2B).

In conclusion, carbohydrate accumulation by freeze temperature in winter is important compounds for declining winter damage. With increasing temperature, root regrowth dynamic was decided the reserves pool size to recover turf quality.

V. 적 요

한지형 잔디인 creeping bentgrass의 저온 스트레스에 대한 대사적 반응을 규명하기 위해 월동기간 동안 creeping bentgrass의 저장탄수화물의 변화를 분석하였다. 잔디의 녹색도와 잎의 성장은 온도의 변화와 거의 평행하게 변화하였다. 지상부와 지하부의 glucose, fructose, sucrose는 11월부터 1월까지 점차로 증가하였고, 1월 이후부터 4월까지 급격히 감소하는 경향을 보였다. Sucrose가 가용성 당류 가운데 가장 높은 구성 비율을 보였다. 지상부와 지하부에서 다당류인 fructan은 11월부터 2월까지 다소 증가하였으며, fructan의 가수분해는 2월과 4월 사이에 가장 활발하게 나타났다. 월동중 각 당류의 축적정도와 농도를 비교할 때, fructan이 비구조성 탄수화물의 주요 저장형태임을 보여주었다. Fructan은 가장 높은 농도를 보인 2월에 지상부와 지하부에서 각각 176.7 mg g^{-1} DW과 126.7 mg g^{-1} DW이었으며, 2월 이후 fructan의 감소 경향은 이 기간동안의 녹색도와 건물 축적율의 증가와 유기적 관련성을 보여주었다. 이상의 결과들은 1월까지 비구조적 탄수화물의 축적과 저온 내성, 2월 이후의 활발한 가수분해와 지상부의 재생과의 밀접한 생리적 관련성이 있음을 제시한다.

Acknowledgements

We thank Mr Gang, the head of green manager, and all staffs in Muan C.C for providing turfgrasses material and for helpful advices about turf management.

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. Booysen, P.V. and C.J. Nelson. 1975. Leaf area and carbohydrate reserves in regrowth of tall fescue Crop Science. 15:262-266.
3. Carpenter, J.F. and J.H. Crowe. 1988. The mechanism of cryoprotection of proteins by solutes. Cryobiology 25:244-255.
4. Castonguay, Y., P. Nadeau, P. Lechasseur and L. Chouinard. 1995. Differential accumulation of carbohydrates in alfalfa cultivars of contrasting winter hardiness. Crop Science 35:509-516.
5. Chatterton, N., P. Harrison, J. Bennett and K. Asay. 1989. Cabohydrate partitioning in 185 accessions of gramineae grown under warm and cool temperatures. Journal of Plant Physiology 134:169-179.
6. 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.
7. Davis, J.S. and Gander. 1967. A reevaluation of the Roe procedure for determination of fructose. Analytical Biochemistry 19: 72-79.
8. 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.
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. Kim. D.H., W.J. Jung, B.R. Lee, K.Y. Kim and T.H Kim. 2001. Effect of cutting height on C and N reserves and consequent regrowth in frequently defoliated turf-type perennial ryegrass (*Lolium perenne* L.). Journal of Korean Grassland Science 21(2):89-96.
11. Levitt, J. 1980. Response of plants to environmental stress. Vol. 1, 2nd ed. (Academic Press: New York)
12. Li, R., B.C. Joern. J.J. Volenec and S.M. Cunningham. 1996. Seasonal changes in non-structural carbohydrates, protein, and macronutrients in roots of alfalfa, red clover, sweet clover, and birdsfoot trefoil. Crop Science 36:617-623.
13. 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.
14. Olien, C.R. 1984. An adaptive response of rye to freezing. Crop Science 24:51-54.
15. Olien, C.R. and G.E. Lester. 1985. Freeze-induced changes in soluble carbohydrates of rye. Crop Science 25:288-290.
16. 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.
17. Pollock, C.J. and T. Jones. 1979. Seasonal patterns of fructan metabolism in forage grasses. New Phytology 38:9-15.
18. Pollock, C.J., A. Cairns, J. Harrison and J. Gallagher. 1999. Sources, sinks, and stress. In Crop Development for Cool and Wet Climate of Europe. (Eds M Sanchez-Daiz, JJ Irigoyen, J Aguirreolea and K Pithan) pp. 35-42. (European Communities: Belgium)
19. 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.
20. Smith, D. 1974. Growth and development of timothy tillers as influenced by level of carbohydrate reserves and leaf area. Annual Botony. 38:595-606.
21. 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.
22. 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.
23. Van Handel. E. 1968. Direct microdetermination of sucrose. Analytical Biochemistry 22:1341-1346.
24. Wanger, W., F. Keller and A. Wiemken. 1983. Fructan metabolism in cereals: induction in leavels and compartmentation in protoplasts and vacuoles. Z. Pflanzenphysiology 112:359-372.
25. Xu, Q. and B. Huang. 2001. Morphological and physiological characteristics associated with heat tolerance in creeping bentgrass. Crop Science 41: 127-133.