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Carbohydrate Concentration and Composition in Source and Sink Tissues of Two Tall Fescue Genotypes

Beom Heon Song

ABSTRACT

Carbohydrate metabolism and partitioning are dependent on relationships between sources and sinks which can be affected by rates of photosynthesis and respiration. Fructan, the major form of stored carbohydrate in tall fescue (*Festuca arundineacea* Schreb.), changes in concentration during growth and in response to the environment. Objectives of this study were i) to examine the content and the composition of carbohydrates in five tissues (mature leaf blade, immature leaf blade, leaf elongation zone, terminal meristem, and root tips) of two tall fescue genotypes, one with high yield per tiller (HYT) and one with low yield per tiller (LYT), and ii) to compare the reserved and utilized carbohydrates among above five different tissues, particularly between the leaf elongation zone and root tips. The established vegetative tillers of the HYT and LYT genotypes were grown in a controlled-environment growth chamber. Water-soluble carbohydrate (WSC) in the leaf elongation zone was about 22% of dry weight in the HYT and about 19% in the LYT genotype. The root tip also had high WSC, about 12% of dry weight in the HYT and 6% in the LYT genotype. Hexoses and sucrose were the major components of total WSC in all tissues except the leaf elongation zone. The growing tissues (sinks), i. e., the leaf elongation zone and root tip, had a high proportion of low degree of polymerization fructan, i.e., 3 to 8 hexose units.

Key words : leaf growth, root growth, fructan, carbohydrate, tall fescue.

Green plants utilize solar energy to synthesize organic compounds from carbon dioxide and water. Inorganic nutrients absorbed by roots are necessary for optimum functioning of physiological and biochemical reactions of the plant. For plant growth it is necessary to have efficient sources of energy, ability to utilize that energy, and to produce materials needed for growth (Rost et al, 1984; Marschner, 1989).

Tall fescue (*Festuca arundinacea* Schreb.) is a cool-season perennial grass that accumulates fructan as a major form of storage carbohydrate. Understanding the fructan metabolism in this species is important, because fructan accumulation is thought to be one mechanism by which plants adapt to environmental changes (Pollock, 1986). Fructan is important for winter survival, for regrowth after harvest, and during growth periods (Smith, 1972). The fructan concentration of meadow fescue varies with season (Pollock & Jones, 1979), and with temperature and light intensity (Labhart et al, 1983). Fructan synthesis, metabolism, and storage are probably located in the vacuole (Frehner et al, 1984; Pollock, 1986; Matile, 1987). Threshold concentrations of sucrose are required for fructan synthesis (Labhart et al, 1983, Schnyder & Nelson 1987), and accumulation occurs generally when sucrose supply exceeds demand (Pollock, 1986; Schnyder & Nelson, 1987). Interestingly, fructan synthesis was delayed if fructose was administered rather than sucrose (Pollock & Cairns, 1991).

Carbohydrate composition differs among tissues and among grass species. Tall fescue contained neokestose, 1-kestose, and higher oligosaccharides that comigrated on TLC plates with neokestose-based compounds and inulins (Spollen & Nelson, 1988). Fructan concentrations vary in tissues according to stage of growth and environmental conditions (Smith, 1972; Housley & Volenec, 1988). High irradiance or low temperature favors fructan accumulation in tissues (Schnyder & Nelson, 1989), while high N or low K^+ nutrition decreases fructan accumulation (Archbold, 1940).

In perennial species, fructan accumulation varies with season (Pollock & Jones, 1979; Volenec, 1986). Accumulation continues throughout the fall and early winter, then declines during winter with a transient accumulation of sucrose until spring regrowth has high enough photosynthetic capacity (Pollock & Jones 1979). Both fructan concentration and mean molecular weight increase with tissue age. In contrast, the increase in fructan concentration of a young leaf blade with age was not closely associated with increased mean molecular weight of the fructan. The variance of fructan concentration with tissue age was closely related to fructosyltransferase activity (Housley & Volenec, 1988).

Fructan occurs in abundant amounts in the growth zone of grass leaves, where cells are actively dividing and elongating. Carbohydrate composition in the leaf elongation zone varies with distance between basal and distal positions, and with temperature and light intensity. Sucrose concentration is high at the basal portion of the leaf elongation zone, whereas monosaccharide concentration is high at distal positions of the leaf elongation zone. Fructan concentration increases in the basal part,

* Dept. of Agronomy, College of Agriculture, Chungbuk National University, Chongju 361-763, Korea.
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then remains high through the remainder of the elongation zone (Schnyder & Nelson, 1987).

Reserve carbohydrates in the leaf elongation zone play important roles for crop growth as a short-term energy reserve. Considerable research has been published on energy metabolism of source and sink tissues of shoot growth. However, few studies have considered carbon metabolism in the root system, although many studies have been conducted regarding the balance of shoot and root growth in dry matter production. Objectives of this study were 1) to determine and examine the content and the composition of carbohydrates in source and sink tissues of two tall fescue genotypes, particularly the mature leaf blade, immature leaf blade, leaf elongation zone, terminal meristem, mature root, and root tip, 2) to compare the reserved and utilized carbohydrates among above five different tissues, particularly between the leaf elongation zone and root growth zone.

MATERIALS AND METHODS

Plant material and growth conditions

Two tall fescue genotypes, one selected for high yield per tiller (HYT) and one for low yield per tiller (LYT), were chosen because they exhibit a 50% difference in leaf elongation rate which is positively related with the economic yield (Horst et al., 1978; Jones & Nelson, 1979). Three vegetative tillers were transplanted into plastic pots which were 11 cm in diameter and 15 cm deep, and contained a mixture of Mexico silt loam soil, peat, and sand (2 : 1 : 1).

Plants were established for 16 weeks in the greenhouse at a mean daily temperature of 25℃ and a 14 h photoperiod extended with fluorescent lamps. Plants were transferred and grown in a controlled-environment growth chamber, where a 14 h photoperiod of 400 μmol $m^{-2}s^{-1}$ PAR was provided by cool-white fluorescent and incandescent lamps. Temperature was maintained at 22℃ /18℃ (day/night) and relative humidity was 70%. Plants were fertilized weekly with 50 ml of Hoagland's nutrient solution. About 40 ml of deionized water was applied daily to each pot. Plants remained vegetative throughout the experiment.

Tissue sampling

Samples for carbohydrate analysis were taken from five different tissues ; mature leaf blade (ML), immature leaf blade (IML), leaf elongation zone (EZ), terminal meristem (TM), and root tips (RT). The ML was represented by a 2 cm section from the middle of fully developed leaf blade on which the collar had already made the angle between the sheath and blade. The IML was represented by a 2 cm section of an elongating leaf blade (length about 15 cm above the whorl) taken midway between the tip and the collar of the previous leaf. The EZ of the elongating leaf blade (sample as IML) was carefully freed from surrounding leaf sheaths and the ligule, which was about 1.0 mm from the point of attachment of the unelongated sheath. A 2.5 cm segment for the HYT and 1.5 cm segment for the LYT genotype were sampled just above the ligule.

The TM consisted of a 0.5 cm to 1.0 cm section of the stem apex including lower nodes and internodes with the elongating leaf tissues being removed just below the ligule. The RT were carefully removed from the soil, and the terminal 1.0 cm from the root tip was sampled. During sampling the harvested tissues were maintained at low temperature within a box of ice. Samples were dried at 70℃ for 48 h. Dried samples were stored at -20℃ and analyzed for carbohydrates within 60 days.

Carbohydrate analysis

Dried tissue was ground with a mortar and pestle. The WSC was extracted with distilled water and filtered through Whatman No. 1 paper. Total WSC in an aliquot was determined by hydrolyzing with 1.0 M H_2SO_4 at 100℃ for 15 min, then measuring the reducing power using a copper reduction-iodate titration technique. Starch remaining in the residue following WSC extraction was hydrolyzed with mylase enzyme in a buffer solution of acetic acid and sodium acetate. Reducing power of the glucose liberated from starch was analyzed using a copper reduction-iodate titration technique (Smith, 1981).

Gel permeation chromatography (GPC) was performed using Sephadex G-50 in a 100 × 1.6 cm glass column using 0.2 M NaCl as the eluent (Labhart et al., 1983). Flow rate was 25 ml h-1. Samples containing about 1.0 mg of WSC were applied to the top of column, 3.2 ml fractions were collected, and the collected carbohydrate was measured by the anthrone procedure (Dimler et al., 1952). The WSC was also separated by silica gel thin layer chromatography (TLC) using Fisher Redi-Plates (Spollen & Nelson, 1988). About 80 μg of WSC was applied at the base of each lane on plate. Plates were developed three times with 1-butanol : glacial acetic acid : water (Thome & Kuhbauch, 1985) in the ratio of 50:25:25 which separates each component degree of polymerization, but can not separate the iso-structural fructan, and 55:30:15 (Spollen & Nelson, 1988) which can separate the iso-structural fructan. Plates were dried and WSC was visualized with urea-phosphoric acid which stains mainly ketoses (Wise et al., 1955).

This experiment was performed three times at the University of Missouri-Columbia, the United States, in 1988 and 1989. Four replications of each sample were measured. All data were analyzed with one-way analysis of variance, and among tissues if the F-test showed significance at $P<0.05$.

RESULTS AND DISCUSSIONS

Total non-structural carbohydrates

Table 1. Concentration of water-soluble carbohydrates and starch in five tissues of two genotypes of tall fescue, mature leaf blade (ML), immature leaf blade (IML), leaf elongation zone (EZ), terminal meristem (TM), and root tip (RT).

	Year	Genotype	ML	IML	EZ	TM	RT
			..... % of dry wt				
WSC	Exp. I	HYT	7.6	5.7	21.8	7.1	11.8
		LYT	14.1	4.9	19.0	3.7	5.6
		LSD (0.05)	2.0	NS†	NS	3.0	6.3*‡
	Exp. II	HYT	8.8	5.9	27.2	10.0	10.6
		LYT	8.4	5.4	21.2	4.4	16.8
		LSD (0.05)	NS	NS	NS	2.6	5.6
Starch	Exp. I	HYT	1.0	1.6	6.1	2.2	3.9
		LYT	4.2	3.5	5.8	3.0	3.1
		LSD (0.05)	1.3	1.2	NS	0.4	NS

†NS : Non-significant, ‡* : significant at $P<0.10$.

Water-soluble carbohydrate was the major carbohydrate component in all tissues (Table 1). The highest concentration occurred in the leaf elongation zone, ranging from 19 to 27% of dry weight. Starch was a small proportion of the carbohydrate component, about 6% of dry weight for both genotypes in the leaf elongation zone, which was the tissue with highest starch content.

Concentration of WSC in terminal meristem tissues was significantly different ($P<0.05$) between genotypes for both experiments. The WSC of root tips was different between genotypes at $P<0.10$ in experiment II and $P<0.05$ in experiment I. However, the WSC of the mature leaf blade was higher in the LYT than in the HYT genotype in experiment I ($P<0.05$), but not in experiment II. In both experiments, the highest WSC occurred in the leaf elongation zone, the second highest WSC occurred in the mature leaf blade and the root tips, and the third highest occurred in the terminal meristem and the immature leaf blade. Starch appeared in small amounts in all tissues, generally being less than 4% of dry weight, except for the leaf elongation zone.

The growing tissues, the leaf elongation zone and the root tips, had similar starch content in both genotypes. The mature tissue and non-growing tissues, namely the mature leaf blade, the immature leaf blade, and the terminal meristem, had higher starch in the LYT than in the HYT genotype, although in each case the concentration was low, about 1 to 2% of dry weight in the HYT and about 3 to 4% in the LYT genotypes. This is consistent with earlier data showing that starch is usually present in small quantities in the leaf elongation zones and herbage (Volenec & Nelson, 1984).

Fructan polymers

The elution profile from gel permeation chromatography (Fig. 1A) showed that hexoses and sucrose were the major components of total WSC in the mature leaf blade in both genotypes. Comparison of the two tall fescue genotypes suggests that the LYT genotype contained relatively more WSC as fructan than did the HYT genotype. Total WSC was about 8% of total dry weight in the HYT genotype and about 14% of total dry weight in the LYT genotype (Table 1). This profile is similar to that obtained from WSC of the leaf blade (Spollen & Nelson, 1988), although concentrations of both low and high DP fructan are lower than theirs. The WSC in the immature leaf blade was 5% and 6% of dry weight and consisted largely of hexoses and sucrose (Fig. 1B), with the small amount of low DP fructan being partially resolved in the one peak along with sucrose.

The terminal meristem had WSC contents between 4 and 7% of dry weight, and the WSC ranged from hexoses and sucrose to high DP fructan in both genotypes (Fig. 1C). The HYT genotype had slightly higher high DP fructan than did the LYT genotype, whereas the LYT genotype had slightly higher low DP fructan and sucrose than did the HYT genotype. This profile is similar to that obtained from Sephadex G-100 GPC of WSC from the stem base tissue of meadow fescue (Labhart et al., 1983).

The WSC of the leaf elongation zone was about 20% of dry weight for both genotypes, and contained a high proportion of low DP fructan relative to sucrose and hexoses (Fig. 2A). Little high DP fructan occurred in either genotype. The HYT genotype had slightly more hexose and sucrose than the LYT genotype, but they were similar for low DP fructan. The profiles are similar to those obtained earlier for the leaf elongation zone (Spollen & Nelson, 1988).

Root tips contained a much higher proportion of hexose and sucrose than low DP fructan (Fig. 2B). There was almost no high DP fructan in the root tips. The leaf elongation zone (Fig. 2A) had much higher concentration of low DP fructan than did the root tips (Fig. 2B). It

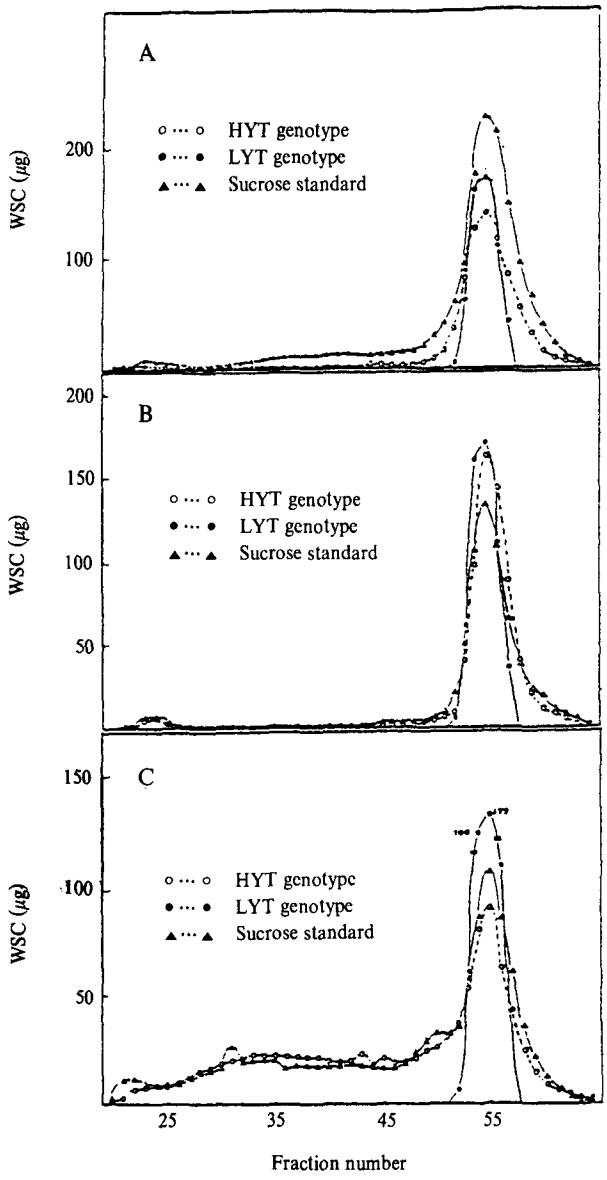

Fig. 1. Separation of water-soluble carbohydrate with gel permeation chromatography (Sephadex G-50) from the mature leaf (A), the immature leaf (B), and terminal meristem (C) of two tall fescue genotypes. The solid circle shows the elution profile of sucrose standard. High DP fructan generally eluted near fraction 40, low DP fructan between fraction 40 and 50, and sucrose plus hexoses greater than fraction 50.

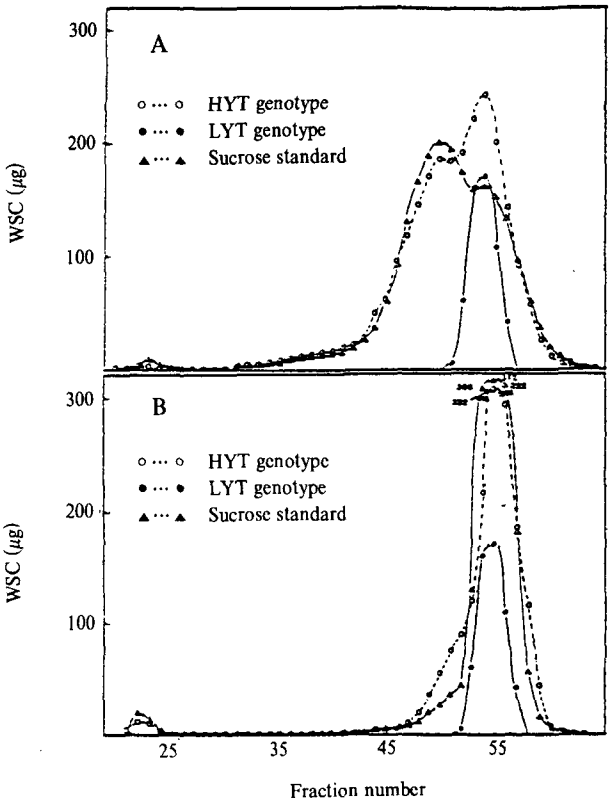

Fig. 2. Separation of water-soluble carbohydrate with gel permeation chromatography (Sephadex G-50) from the leaf elongation zone (A) and the root tips (B) of two tall fescue genotypes. The solid circle shows the elution profile of sucrose standard. High DP fructan generally eluted near fraction 40, low DP fructan between 40 and 50, and sucrose plus hexoses greater than fraction 50.

may speculate from these data that the leaf elongation zone has higher compartmentation ability for sequestering hexoses as fructan during the unloading of sucrose than do root tips. However, it is difficult to be definite at this stage because there are very few studies of carbohydrate metabolism and partitioning in root sinks compared to the leaf elongation zone, a leaf sink, which has been studied extensively in terms of carbon economy (Spollen & Nelson, 1988; Allard & Nelson, 1991). For example, in earlier study, the root tip was sampled to 1.0 cm long from the root cap, then recognized that was too long. It was sampled only 0.5 cm long in later experiment. Later experiments indicated the root growth zone was only about 0.3 cm long.

The leaf elongation zone ranges from 1.5 to 3.0 cm in length, depending on the leaf growth rate (Volenec & Nelson, 1981; MacAdam & Nelson, 1987). The leaf elongation zone of tall fescue contained the highest WSC of plant tissues which consisted largely of low DP fructan and sucrose plus hexoses. Tall fescue can accumulate WSC to 30% or more of dry weight in the leaf elongation zone, of which over 70% is fructan, predominantly of low DP (Schnyder & Nelson, 1987; Spollen & Nelson, 1988).

Fructan oligomers

TLC distinguishes monomers in the low DP fructan pools better than in the high DP pool (Fig. 3). All tissues of tall fescue had predominately hexoses and sucrose as WSC except for the leaf elongation zone, which contained a high proportion of low DP fructan, i.e., 3 to 10 hexose units. Root tips and the terminal meristem tissues also had some low DP fructan in both genotypes that chromatographed similar to fructan of the leaf elongation zone. Interestingly, the root tips contained a very high proportion of hexoses compared with other tissues, even the leaf elongation zone. A very little sucrose was detected in the root tips (Fig. 4) suggesting the peak eluting from GPC near fraction 55 was largely hexose (Fig. 2B).

The relative distribution of low and high DP fructan pools was consistent with the elution profiles from GPC (Fig. 1 and 2). All tissues contained a high content of trisaccharides, probably 1-kestose and neokestose (Spollen & Nelson, 1988), especially in the leaf elongation zone, the root tips, and terminal meristem. Double bands were clearly observed in all tissues for DP 4, 5, 6, 7, and 8 (Fig. 3). High DP fructan was most visible in the mature leaf blade of the LYT genotype and in the terminal meristem of both genotypes (Fig. 3). These data suggest that growing tissues, the leaf elongation zone and the root tips, have a higher proportion of low DP fructan, whereas the maturing and non-growing tissues contain a higher proportion of high DP fructan.

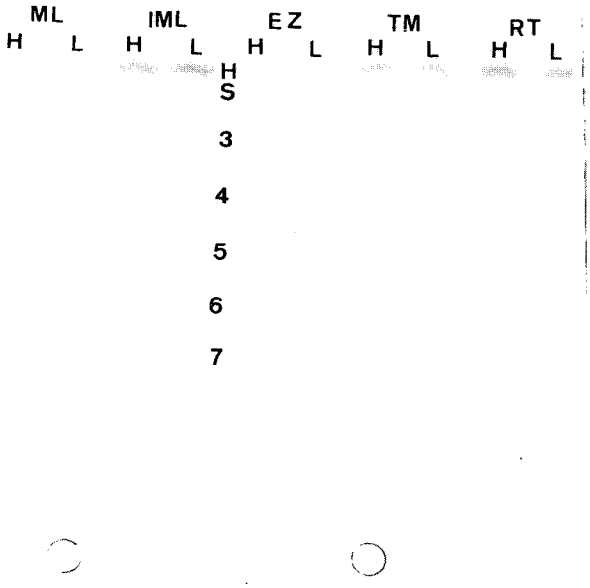

Fig. 3. Separation of water-soluble carbohydrate with thin layer chromatography from five different tissues of two tall fescue genotypes. 80μg of WSC were applied to each lane on the plate of silical gel. Tissues are the mature leaf blade (ML), immature leaf blade (IML), leaf elongation zone (EZ), terminal meristem (TM), and root tips (RT).

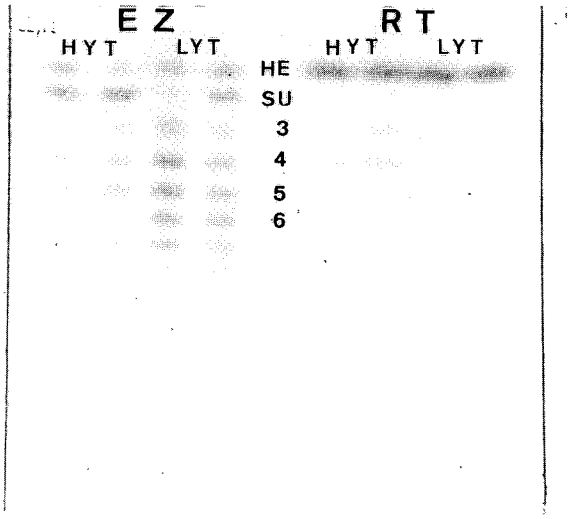

Fig. 4. Separation of water-soluble carbohydrate with thin layer chromatography from the leaf growth zone and root tips of two tall fescue genotypes. 80 μg of WSC were applied to each lane on the plate of silical gel.

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