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Carbohydrate Metabolism in Leaf Meristems of Tall Fescue[1]

I. RELATIONSHIP TO GENETICALLY ALTERED LEAF ELONGATION RATES

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ABSTRACT

The physiological bases for genetic differences in leaf growth rates were examined in two genotypes of tall fescue (*Festuca arundinacea* Schreb.) selected for a 50% difference in leaf elongation rate. Genotypes had similar dark respiration rates and concentrations of carbohydrate fractions in the leaf meristem and in each daily growth segment above the meristem. Dark respiration rates and concentrations of nonreducing sugars, fructans, and takadiastase-soluble carbohydrates were highest in leaf intercalary meristems and declined acropetally with tissue age. Concentrations of reducing sugars were 1.0% of dry weight in leaf meristems, 3.7% of dry weight in tissue adjacent to the meristem, then decreased progressively with distance from the meristem. Glucose, fructose, and *myo*-inositol comprised over 90% of the monosaccharides present in leaf meristems. Soluble protein concentration was 9.7 milligrams per gram fresh weight in leaf meristems, 5.5 milligrams per gram in tissues immediately above the meristem and, thereafter, increased linearly with distance from the meristem.

Leaf meristems of the genotype exhibiting rapid leaf elongation contained 30% more soluble protein than those of the genotype selected for slow leaf elongation. The 4-fold difference in size of the leaf meristem appeared to be more important in influencing leaf elongation than were other characteristics examined.

In tall fescue (*Festuca arundinacea* Schreb.), single-leaf net photosynthesis and herbage yield are not positively related (18); however, yield has been correlated positively with rapid rates of leaf elongation (6). Knowledge regarding partitioning to, and efficiency of utilization of carbohydrates within leaf intercalary meristems may be important in understanding leaf growth rates.

In monocotyledonous species, leaf intercalary meristems contain regions of cell division and elongation, and are located in bases of elongating leaves which are enclosed within a whorl of encircling leaf sheaths (3, 5). Lengths of leaf intercalary meristems are related positively to LER[3], whether such rates are modified environmentally (9, 10, 26) or through genetic selection (25). High R_Ds of meristematic tissues (7, 15, 20, 28) probably reflect the need for ATP in biosynthetic processes. High concentrations of nonstructural carbohydrates often occur in meristematic tissues providing substrates for anabolic and catabolic processes (7, 10, 12, 15). Low concentrations of nonstructural carbohydrate in the encircling leaf sheaths adjacent to the leaf intercalary meristem resulted in slow regrowth rates (3). Within the leaf intercalary meristem, the relationships between R_D, nonstructural carbohydrate concentration, and LER are not well understood.

In the present study, two genotypes of tall fescue differing in LER by 50% were studied to provide information concerning carbohydrate metabolism in relation to genetically altered LER. The genotype exhibiting slow LER generally has twice the number of tillers and approximately half the nonstructural carbohydrate concentration in its stubble of encircling sheaths when compared to the genotype exhibiting rapid LER. Thus, intraplant competition for a small pool of nonstructural carbohydrates may be related to the slow LER of the high-tillering genotype.

Our objectives were to (a) measure R_D of leaf intercalary meristems removed from elongating leaves of two genotypes of tall fescue with contrasting LER and (b) examine concentrations of reducing and nonreducing sugars, fructan, starch, and soluble protein in leaf intercalary meristems and relate these characteristics to R_D and LER. Segments of elongating leaves above the leaf intercalary meristem and center sections of recently collared leaf blades were included for comparison.

MATERIALS AND METHODS

Plant Culture. Two tall fescue genotypes, one selected for HYT and one for LYT were chosen because they exhibited a 50% difference in LER (7). Three vegetative tillers of each genotype were transplanted into 11- by 15-cm plastic pots containing a 2:1:1 mixture of silt loam topsoil, coarse sand, and peat moss, respectively. Plants were established in the greenhouse where temperatures were 25 ± 5°C with natural daylength. Plants received weekly 50 ml of a nutrient solution containing 189, 38, and 151 mg/l N, P, and K, respectively. Herbage was cut twice at 6-week intervals to a 5-cm stubble after which 36 uniform pots of each genotype were blocked into six replicates of six pots each and placed in a controlled-environment chamber. A 14-h photoperiod of 650 μmol m^{-2} s^{-1} PAR was supplied by cool-white fluorescent and incandescent lamps. RH was 50 to 70%. A temperature of 20°C was maintained at the leaf intercalary meristem by adjusting air temperature to 24/20°C (day/night) (25). Plants were fertilized weekly with Hoagland solution and on alternate weeks with 50 ml of nutrient solution containing 950, 950, and 1178 mg/l N, P, and K, respectively. Plants remained vegetative throughout the experiment.

Tissue Sampling. Length of three elongating leaves per pot was measured on 4 consecutive days, and slopes of the linear regressions of leaf length over time were used as an estimate of LER. Only elongating leaves whose exposed blades were less than one-half the length of the previous collared leaf blades were selected for measurement. This insured that increase in length over time would be linear, and that growth occurred as a result

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[3] Abbreviations: LER, leaf elongation rate; R_D, dark respiration rate; HYT, high yield per tiller; LYT, low yield per tiller; TNC, total nonstructural carbohydrates; SDW, structural dry weight.

of the leaf-blade meristem. Mean LER was calculated for each replication and used to estimate LER for other tillers at a similar stage of development in that replication.

Collared leaves and sheaths were removed from elongating leaves at the described stage of development (Fig. 1). The elongating blade was cut from the stems at the point of attachment of the most recently collared leaf. If the ligule of the elongating leaf had differentiated and moved into the intercalary meristem region, the leaf was discarded. This insured that measurements were made only on the leaf-blade meristem. The leaf intercalary meristem (M) was removed from the bottom of each elongating leaf, the length of this segment depending on the genotype (25, 26) (Fig. 1). Five consecutive segments above the intercalary meristem, each equivalent in length to the daily leaf elongation of that genotype in that replication, were cut from the remainder of the elongating leaf (Fig. 1). The result of sampling segment lengths equal to the LER is that segment one above the intercalary meristem was composed of cells that elongated to their final length during the 24 h previous to harvesting the leaf, while the fifth segment above the intercalary meristem was composed of cells that had elongated to their final length during the 5th d prior to harvest. Center segments of 2 cm length from the most recently collared leaf blade were included as a reference.

Measurement of R_D. Tissues were suspended immediately in 2 ml of 0.2 M phosphate buffer (pH 6.7) in the outer-well of 17-ml respirometer flasks. CO_2 was absorbed onto a filter paper wick saturated with 20% (w/v) KOH placed in the center-well of the flask. Consumption of O_2 at 20°C was measured manometrically on 5 to 15 segments per replicate using procedures described previously (7).

Carbohydrate and Protein Analyses. Harvested tissues were maintained at 4°C or less during sampling. Soluble proteins were extracted in a buffer (100 mM Tricine, 200 mM $MgCl_2 \cdot 6\ H_2O$, 10 mM $NaHCO_3$, and 1 mM DTT) by grinding segments and washed silica with a mortar and pestle. After filtering through Miracloth, soluble proteins were precipitated with 24% (w/v) TCA and analyzed using the Lowry technique as modified by Bensadoun and Weinstein (1). BSA was used as a standard.

Tissues for nonstructural carbohydrate analyses were microwaved for 1 min, dried at 70°C for 48 h, and ground to pass a 40-mesh screen. Mono- and disaccharides were extracted from tissue using 92% (v/v) ethanol. Following ethanol extraction, remaining water-soluble carbohydrates (fructans) were removed from the residue by shaking in deionized H_2O for 1 h. Starch remaining in the residue following water extraction was hydrolyzed with takadiastase (Clarase 900, Miles Laboratories). Reducing power of extracts was analyzed using the copper-iodometric technique of Smith (22).

Aldoses and *myo*-inositol were quantitatively identified as their aldono-nitrile acetate derivatives on 1.5% (w/w) diethylene glycol adipate in 100 to 200 mesh Chromosorb W using the GLC techniques of Mawhinney *et al.* (14). Ketoses were quantitatively identified as their *O*-trimethylsilyl oxime derivatives on 2% (w/w) SE-52 in 100 to 200 Chromosorb W. Methyl β-D-glucopyranoside was used as an internal standard. Qualitative identification of monosaccharides was confirmed using TLC techniques of Lato *et al.* (13) as modified by Streeter and Bosler (23).

Statistical Design. The experiment was analyzed as a randomized, complete, block design with six replications. Tissues from replicates 1 and 2, 3 and 4, and 5 and 6 were combined to provide sufficient sample for nonstructural carbohydrate analysis. Where the *F* test was significant ($P \leq 0.05$), an LSD was calculated.

RESULTS AND DISCUSSION

Dark Respiration. Though LER, length of leaf intercalary meristems, and tissue segments differed between genotypes (Fig. 1), R_Ds of respective tissues were similar; therefore, data were averaged over genotypes for presentation (Fig. 2). Comparisons were based on tissue age rather than position for more meaningful comparison. The R_D of leaf intercalary meristems (segment M) was 6-fold higher than that of collared leaf blades (Fig. 2). High R_D in lower segments of elongating leaves has been reported in corn (*Zea mays* L.) (20) and perennial ryegrass (*Lolium perenne* L.) (28), and in terminal meristem areas of tall fescue (7, 15). Cell division and expansion are limited to the leaf intercalary meristem; however, the elevated R_Ds of 1- and 2-d-old segments suggest that growth via dry weight accumulation and differentiation may be continuing in these tissues.

The R_D declined rapidly with tissue age, reaching constant rates in 3- to 5-d-old segments which were similar to that found in collared leaf blades (Fig. 2). The R_D of collared leaf blades is associated largely with maintenance processes (15, 19).

Monosaccharide Concentration. Averaged across segments, reducing sugar concentration of the LYT genotype was significantly greater than that of the HYT genotype, averaging 2.5 and 1.0% of dry weight, respectively (Table I). In both genotypes, reducing sugar concentrations were highest in the 1-d-old tissues, then declined rapidly as segment age increased resulting in similar concentrations in 4- to 5-d-old segments. Reducing sugar concentration of the leaf intercalary meristem of both genotypes was approximately 1.0% of dry weight, which was similar to concentrations found in collared leaf blades. Low concentrations of reducing sugars in basal segments of elongating grass leaves has been reported previously (4, 8, 16), and may reflect rapid utili-

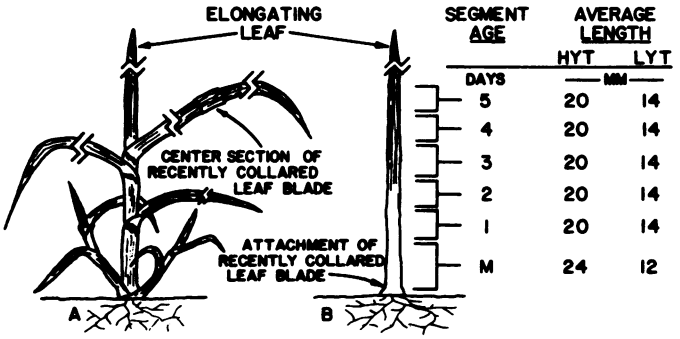

FIG. 1. Schematic of an intact tall fescue tiller (A) with partially emerged elongating leaf. The exposed elongating leaf (B) was cut from the plant at the point of attachment of the recently collared leaf. Elongating leaves were divided into the leaf intercalary meristem (M) and segments which had matured within 1 to 5 d. Daily leaf elongation was 20 and 14 mm d^{-1} for the HYT and LYT genotypes, respectively.

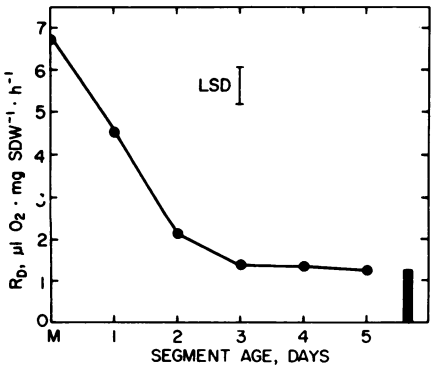

FIG. 2. Dark respiration rate (R_D) of leaf intercalary meristem (M) and segments of elongating leaves ranging from 1 to 5 d old. Vertical bar represents R_D of collared leaf blades. Data were averaged across genotypes selected for high (HYT) and low (LYT) yield per tiller.

Table I. *Concentration of Nonstructural Carbohydrates in Leaf Tissues of Tall Fescue*

Tissues examined included leaf intercalary meristems, 1- to 5-d-old segments of elongating leaves, and center sections of recently collared leaf blades. Genotypes were selected for high (HYT) and low (LYT) yield per tiller, and exhibited rapid and slow leaf elongation rates, respectively.

Tissue	Reducing Sugars		Nonreducing Sugars		Fructan		Starch		TNC	
	HYT	LYT	HYT	LYT	HYT	LYT	HYT	LYT	HYT	LYT
					% dry wt					
Meristem	0.96	1.12	7.27	7.54	23.6	23.1	5.39	3.24	37.2	35.0
Age (d)										
1	2.64	4.79	7.16	8.02	12.9	12.7	6.87	6.19	29.6	31.7
2	1.26	3.81	3.31	4.11	4.9	5.7	4.09	4.40	13.6	18.0
3	0.61	3.00	2.37	2.40	2.3	2.7	2.69	2.87	7.9	10.9
4	0.57	1.92	2.72	2.44	2.0	1.9	1.88	1.91	7.2	8.2
5	0.59	1.60	3.10	2.33	2.4	2.0	1.66	0.84	7.7	6.7
Collared blade	0.63	0.99	4.64	7.09	27.0	13.2	2.45	0.98	34.7	22.3
LSD[a]	0.51		0.93		1.8		0.99		2.1	

[a] Least signifciant difference between means at the 5% level of probability.

Table II. *Monosaccharides within Elongating Leaves of Tall Fescue*

Segments of elongating leaves above the meristem were 1 to 5 d old. Center segments of collared leaf blades were included for comparison. Genotypes were selected for high (HYT) or low (LYT) yield per tiller, and exhibited rapid and slow leaf elongation rates, respectively.

Tissue	Fructose		Glucose		*myo*-Inositol		Arabinose		Mannose		Galactose	
	HYT	LYT	HYT	LYT	HYT	LYT	HYT	LYT	HYT	LYT	HYT	LYT
					$mg\ g^{-1}$ dry wt							
Meristem	5.10	9.50	1.41	2.44	1.32	1.84	0.41	0.71	0.22	0.25	0.44	0.65
Age (d)												
1	25.74	52.99	3.47	11.05	0.79	1.63	1.48	2.65	0.38	2.01	1.17	2.19
2	9.11	31.67	1.05	4.95	0.56	0.70	0.45	1.29	0.44	1.19	0.51	0.58
3	5.06	24.66	0.86	6.52	0.37	0.65	0.27	0.65	0.12	1.92	0.45	2.08
4	4.39	16.47	1.35	5.51	0.45	0.55	0.32	0.43	0.16	1.27	0.51	1.49
5	4.02	18.16	1.90	5.26	0.59	0.62	0.44	0.45	0.12	0.81	0.18	1.62
Collared blade	5.32	11.06	2.11	3.62	0.23	0.19	0.22	0.33	0.04	0.11	0.26	0.78
LSD[a]	13.05		1.56		0.12		0.20		0.36		0.54	

[a] Least significant difference between means at 5% level of probability.

zation of these compounds in biosynthetic and metabolic pathways. Concentrations of reducing sugars in leaf intercalary meristems of genotypes were similar even though they differed nearly 50% in LER. This agrees with previous suggestions that concentration of these compounds may not greatly influence LER.

Glucose and fructose comprised over 90% of the reducing sugars present in segments (Table II). Small quantities of arabinose, mannose, and galactose were also present. As expected, concentrations of individual monosaccharides were low in leaf intercalary meristems, high in 1-d-old segments, then declined gradually with tissue age. The LYT genotype contained higher concentrations of each monosaccharide at a given age than did the HYT genotype. This agrees with the genotypic comparison of reducing sugar concentration (Table I).

Significant quantities of *myo*-inositol were found in all tissues (Table II). In contrast with the monosaccharides, concentrations of this cyclitol were highest in meristems and declined rapidly with tissue age. Further, concentrations of *myo*-inositol in the meristem and younger leaf segments of the LYT genotype were significantly higher than those of the HYT genotype. Functions of *myo*-inositol in leaf meristems are unknown, but involvement in synthesis of low mol wt esters of indol-3-yl acetic acid (2) and cell wall polysaccharides (21) has been suggested.

Sucrose and Fructans. Sucrose was the only disaccharide present in the nonreducing sugar fraction; however, small amounts of short-chain fructan may have been included in the extract. Nonreducing sugars were highest in leaf intercalary meristems and 1-d-old segments of the elongating leaf, then declined rapidly as segment age increased (Table I). Similar trends of di- and oligosaccharides have been reported in bases of elongating barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) leaves (8, 16). Within the elongating leaves, nonreducing sugar concentrations and R_D were correlated ($r = 0.88$, $P < 0.01$). However, high concentrations of nonreducing sugars do not cause high R_D as is evident by the low R_D-high nonreducing sugar relationship for collared leaf blades (Fig. 2; Table I).

Concentrations of fructan, the major water-soluble carbohydrate in tall fescue, were highest in leaf intercalary meristems,

then decreased rapidly with age reaching similar concentrations in 3- to 5-d-old segments (Table I). The high concentration of a 'storage' polysaccharide in actively growing tissue such as the leaf intercalary meristem was not expected even though Jones and Nelson (7) found high concentrations (about 50% of dry weight) of water-soluble carbohydrates in terminal meristem tissues of tall fescue. It was later determined that fructan comprised approximately 70% of the water-soluble carbohydrate of the terminal meristem (Moser, Volenec, and Nelson, unpublished).

Significant quantities of fructan have also been reported within bases of elongating leaves of other grasses (12, 16, 17, 27). The function of fructans in the leaf intercalary meristem is unknown; however, one could speculate that they serve as a pool of reduced carbon that is utilized when supply of carbohydrate from photosynthesis cannot meet demand. Alternatively, they may have a role in osmotic regulation within the meristem. Fructan concentrations were correlated with R_D ($r = 0.91$, $P \leq 0.01$) within the elongating leaves but, as with nonreducing sugars, no cause-effect relationships could be established.

Starch and Total Nonstructural Carbohydrates. Starch is usually present in tall fescue herbage in small quantities (about 2%). In both genotypes, 1-d-old segments contained the highest concentrations of takadiastase-soluble carbohydrate (Table I) and as with other fractions, concentrations declined with tissue age. Similar distributions of starch were reported within elongating leaves of barley (16) and tall fescue (27).

In contrast with other fractions, the leaf intercalary meristems of the HYT genotype contained more takadiastase-soluble carbohydrate than those of the LYT genotype (Table I). The physiological importance of this with respect to the genotypic differences in LER is unclear. However, accumulation of starch does precede cellular differentiation and organ formation in tobacco (*Nicotiana tabacum* L.) callus culture (24). Those authors suggested starch served as substrate for metabolism and biosynthesis and/or was involved in some system of osmotic adjustment. In the present experiments, takadiastase-soluble carbohydrate accounted for less than 15% of TNC (sum of all fractions) and, thus, may have had a minor influence in supplying substrates for growth and osmoregulation in leaf meristems of tall fescue.

Within the elongating leaf, TNC concentrations in both genotypes declined rapidly until segments were 4 d old (Table I). The high TNC concentration in intercalary meristems agrees with previous results with terminal meristems of tall fescue (7, 15). The 6% greater concentration of TNC in leaf intercalary meristems of the HYT genotype does not support the hypothesis that its 50% greater LER is due to it having a larger pool of nonstructural carbohydrates when compared to the LYT genotype. Within the elongating leaf, TNC concentrations were correlated with R_D ($r = 0.89$, $P \leq 0.01$). In other studies, positive relationships between TNC and R_D are often reported (15), but not always (7). These inconsistencies may reflect tissue-related differences in the relative contributions of the growth and maintenance components of dark respiration to total R_D, with the former being substrate-dependent (20, 24).

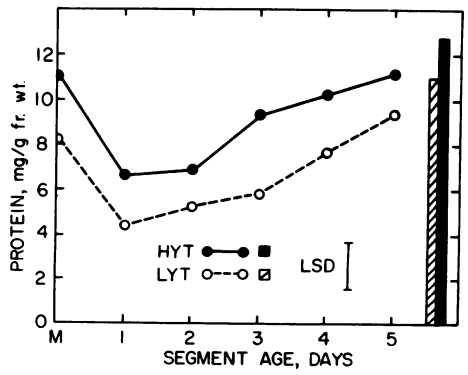

FIG. 3. Concentration of soluble proteins in leaf intercalary meristems (M) and segments of elongating leaves ranging from 1 to 5 d old. Vertical bars represent soluble protein concentrations of collared leaf blades. Genotypes were selected for high (HYT) and low (LYT) yield per tiller.

Soluble Protein. Averaged across segments, genotypes differed significantly in soluble protein concentration, averaging 9.8 and 7.4 mg/g fresh weight in the HYT and LYT genotypes, respectively (Fig. 3). Soluble protein concentrations were high in leaf meristems, low in 1-d-old segments, and thereafter increased linearly with tissue age. The high concentration in leaf meristems probably reflects the great number of unexpanded or partially expanded cells in this tissue, likely with a relatively low protein content per cell. High protein concentrations in leaf meristems have been previously reported (11, 27); however, no relationship between protein concentration and growth rate of leaves has been firmly established.

CONCLUSION

The data indicate that the 50% difference in LER of these genotypes was not related to nonstructural carbohydrate concentrations and R_D of leaf intercalary meristems. Soluble protein concentrations of leaf intercalary meristems for the two genotypes were significantly different but the impact of this on LER is difficult to assess, owing to the heterogeneous nature of proteins. It is unclear whether concentrations of nonstructural carbohydrates in leaf intercalary meristems of both genotypes are near a threshold concentration, above which an increase in TNC concentration results in no change in LER. Moser *et al.* (15) estimated the threshold concentration of TNC in terminal meristems for maximum LER to be approximately 20% of dry weight. Clearly, TNC concentrations of leaf intercalary meristems of both genotypes exceed this threshold. Thus, it is unlikely that LER of these genotypes was limited directly by the supply of carbohydrates from photosynthesis. The influence of nitrogen supply on LER, TNC, R_D, and soluble proteins is examined in a companion paper (27).

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