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Photosynthesis in Tall Fescue[1]

IV. CARBON ASSIMILATION PATTERN IN TWO GENOTYPES OF TALL FESCUE DIFFERING IN NET PHOTOSYNTHESIS RATES

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ABSTRACT

We previously reported that the net photosynthetic rate of a decaploid genotype (I-16-2) of tall fescue (*Festuca arundinacea* Schreb.) was 32 to 41 *versus* 22 milligrams CO_2 per square decimeter per hour in a hexaploid genotype (V6-802) (Randall, Nelson, Asay Plant Physiol 59: 38–41). The high rate was later correlated with increases in total ribulose 1,5-bisphosphate carboxylase protein (17%) and activity (27%) (Joseph, Randall, Nelson Plant Physiol 68: 894–898). This report characterizes photosynthesis with respect to light saturation and early products of photosynthesis in an attempt to identify regulatory metabolic site(s) in these two genotypes. Analysis of the early products of photosynthesis indicated that both genotypes fixed CO_2 via the Calvin-Benson cycle with phosphoglyceric acid as the initial primary product. Both genotypes had similar ^{14}C-labeled intermediates. Sucrose was the primary sink of $^{14}CO_2$ assimilation. After 10 min of $^{14}CO_2$ assimilation with attached leaves, sucrose accounted for 89% (decaploid) and 81% (hexaploid) of the total ^{14}C incorporated. In 10 min, this amounted to 1.3 (decaploid) and 0.8 (hexaploid) μmol [^{14}C]sucrose formed g fresh weight^{-1} and reflected the observed differences in photosynthetic rates. There was limited labeling of starch (1%) and fructan (1%). Results of total nonstructural carbohydrates and P_i analysis also demonstrated sucrose was the predominant carbohydrate in fescue leaves. Quantitative differences in sucrose and P_i between the two genotypes may reflect changes in partitioning and this possibility is discussed.

Considerable effort is being directed toward identifying factors which contribute to increased photosynthetic performance by polyploidization in certain species of higher plants. Increased CER[2] per unit leaf area or leaf weight has been reported in nonisogenic polyploid tall fescue (4, 17, 22), and higher CER per leaf but no change per unit leaf area in isogenic polyploid of alfalfa (24). The increased CER per unit leaf area with polyploid tall fescue has been correlated with (a) increased PSI electron transport activity (18) and (b) higher total RuBPCase activity and total carboxylase protein (17, 22). The concentration of RuBPCase in plastids also increases with ascending nuclear ploidy in a nonisogenic series of wheat (R.M. Leech, personal communication). Since RuBPCase is the primary site for carboxylation in C_3 plants, the increased amount of RuBPCase, whose synthesis is under nuclear control (2, 11, 14), could influence photosynthetic capacity and photosynthetic performance in leaves. Other work on CER of polyploids has been done with plants that are normally diploid (3, 10, 16). These investigations measured significantly lower CER in colchicine-doubled tetraploid plants. The lower CER may have been due to poor adaptation to the tetraploid level in those species or because of inbreeding associated with doubling in ploidy (24). However, altered ploidy level is known to affect the morphological and anatomical features as well as the content and types of protein in higher plants (3–6, 10, 12, 13, 16–18, 24, 25).

The consequences of polyploidization on photosynthetic carbon assimilation pattern is not well characterized. Champigny *et al.* (7) reported that the distribution pattern of newly assimilated $^{14}CO_2$ was notably similar among diploid, tetraploid, and hexaploid species of *Triticum* and their wild relative, *Aegilops*, with sucrose being the principal sink for photosynthetic carbon. However, no rates of CO_2 fixation and its relation to polyploidy were reported.

This study was initiated to identify whether there are any obvious differences in assimilation products and patterns of photosynthetic $^{14}CO_2$ fixation which might explain the difference in CER found between a typical cultivated hexaploid tall fescue genotype, V6-802, and a decaploid genotype, I-16-2, which consistently exhibits an unusually high photosynthetic rate (4, 17, 22). The higher photosynthetic capacity of I-16-2 would be desirable for increasing potential for commercial production of *Festuca* forage species.

MATERIALS AND METHODS

Plants. Clones of two genotypes of tall fescue (*Festuca arundinacea* Schreb.), one a hexaploid (2N = 6× = 42 chromosomes) and the other a decaploid (2N = 10× = 70 chromosomes), were vegetatively propagated into soil in 10-L plastic pots and were maintained in greenhouse. Mature leaf blades that had newly formed collars were used for determination of TNC and P_i. For $^{14}CO_2$ fixation by intact attached leaves, single tillers of each genotype were transplanted into 1-L plastic pots and maintained in the greenhouse for 10 to 12 weeks before the experiment. The CER experiments were conducted on plants which had been transferred to the growth chamber at least 2 months in advance. Photoperiod was 12 h at 25/20°C (day/night) with a RH of 70%. A light intensity of about 500 μE m^{-2} s^{-1} was provided by cool-white fluorescent and incandescent lamps. Newly collared leaves were used in all experiments to minimize differences in the age of leaf between genotypes.

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[2] Abbreviations: CER, CO_2 exchange rate; RuBPCase, ribulose-1,5-bisphosphate carboxylase; PGA, glyceric acid 3-phosphate; TNC, total nonstructural carbohydrates.

CER Measurement *versus* Light Intensity. Rate of CO_2 exchange was measured as described previously (31), starting at a light intensity of 1400 $\mu E\ m^{-2}\ s^{-1}$. Leaves reached new CER equilibrium in about 10 min, after which light intensity was reduced by increments of 200 $\mu E\ m^{-2}\ s^{-1}$. Leaf temperature ranged from 28 to 24°C between the highest and the lowest intensity used. Radiation was provided by 16 150-w floodlights, filtered through 5 cm of running water and regulated by a rheostat. A Lambda Instruments LI-170 quantum sensor was used to measure light intensity at the leaf surface. Three individual determinations with two plants per determination of each genotype were conducted and the mean reported.

Photosynthesis by Attached Leaves in the Presence of $^{14}CO_2$. Uptake of $^{14}CO_2$ by the mature leaf was used to compare fixation patterns and products. Attached leaves were enclosed in a Plexiglas assimilation chamber that was sealed with foam rubber. The assimilation chamber proper had an internal volume of 0.95 L, together with the mixing chamber, pump, and the connecting tubing; the total internal volume of the closed system was 1.3 L. It took approximately 8 s for the injected $^{14}CO_2$ to circulate through the system once. The leaves were equilibrated at 28°C for 45 min in air with a light intensity of 800 to 1,000 $\mu E\ m^{-2}\ s^{-1}$. The pump was turned on after five ml of $^{14}CO_2$ (9.0 μmol containing 100 μCi) were injected through a serum stopper into a mixing chamber equipped with a stir bar. Fixation periods were 5 s, 30 s, 1 min, 5 min, and 10 min. One to two leaves from each genotype were enclosed at any one time. At the end of fixation, the blade was cut from the plant and dropped into boiling 80% ethanol for 3 min.

Extraction of Samples and Identification of Photosynthetic Intermediates. Samples were homogenized in 80% ethanol with a Brinkmann Polytron for 1 min. The homogenate was filtered through Whatman No. 1 paper with suction. The residue was successively washed with 2 × 5-ml aliquots of boiling 80% ethanol, then 2 × 5 ml of hot water, the filtration being repeated after each wash. Filtrates and rinses were combined and the volume measured. The fraction, termed the alcohol-soluble fraction, was evaporated to dryness at 30°C in a Brinkmann Buchi Rotavapor-R. The dried material was washed with 2 × 5-ml aliquots of chloroform to remove chloroform-soluble materials, which constituted the lipid fraction.

Material insoluble in chloroform was dissolved in 10 ml distilled H_2O and termed H_2O-soluble fraction. The H_2O-soluble fraction was separated by two-dimensional electrophoresis and chromatography on cellulose thin-layer plates MN 300, 250 nm (Analtech, Inc.) as described by Schurmann (23). After autoradiography for 4 to 14 d, the products were identified from chromatograms published by Schurmann (23) and by cochromatography with authentic compounds. Radioactivity of all fractions and spots was determined by liquid scintillation counting in 5 ml Brays solution with a Beckman LS7000 Scintillation System. Counting efficiency was 90%. Percent recovery of radioactivity from chromatograms ranged from 75 to 95%. Results were expressed as a percent of the total ^{14}C incorporated unless otherwise stated.

The residue retained on the filter paper was dried at 70°C overnight. The whole filter paper was cut into small pieces and treated thereafter as the sample using a clean filter paper as a control. The residue was hydrolyzed with 15 mg of α-amylase (Sigma, type III-A) in 5 ml distilled H_2O during incubation at 50°C overnight. After centrifugation, the residue was washed twice and the decanted extracts were combined. This fraction was designated the starch fraction and was verified as a single [^{14}C] glucose spot which comprised 96% of the applied radioactivity on TLC using a solvent system of ethyl acetate:glacial acetic:formic acid:water (18:3:1:4, v/v) (15).

TNC Determination. Six pots each of the hexaploid (V6-802) and decaploid (I-16-2) genotype were transferred from the greenhouse to a dark-growth chamber. After remaining in darkness (destarched) at 25°C for 48 h, the plants were transferred back to the greenhouse for an additional 72 h. Due to diurnal variation in carbohydrate contents, leaf samples were harvested between 1330 and 1400 h each day for maximum TNC in tall fescue leaves (19). Leaf samples were killed by immersion in liquid N_2 and then stored at −80°C for later analysis.

Reducing sugars, total sugars, fructan, and starch were extracted and determined from triplicate samples of 200 to 300 mg of freeze-dried, ground leaf tissue according to the procedure of Lechtenberg *et al.* (19), with some modification from Smith (27, 28). Alternatively, the neutral sugars were analyzed by GLC as their acyclic *O*-trimethylsilyloxime per-*O*-trimethylsilylated derivatives according to the procedure of Mawhinney *et al.* (20).

P_i and Chl Determination. Lyophilized and ground leaf samples were extracted with 10% TCA at 4°C and centrifuged. The supernatant was used for P_i determination after neutralization with 5 N KOH. Two colorimetric methods were used for P_i determination (9, 29) with similar results. Chl was determined according to Arnon (1).

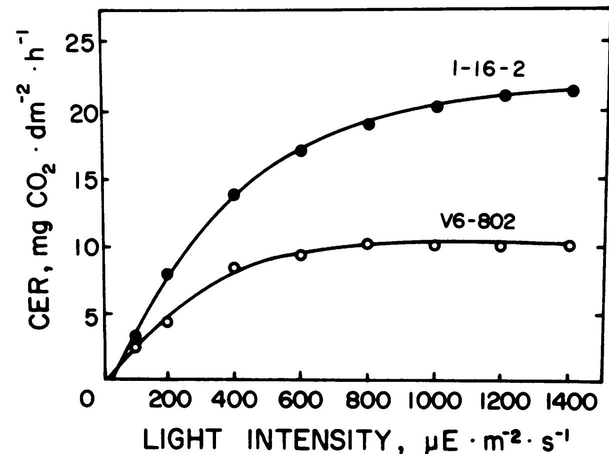

FIG. 1. CER in air as a function of incident light intensity in the 6× and 10× genotypes of tall fescue. Plants were transferred and grown in growth chamber with a photoperiod of 12 h at 25/20°C (day/night) for two months. The light intensity was about 500 $\mu E\ m^{-2}\ s^{-1}$.

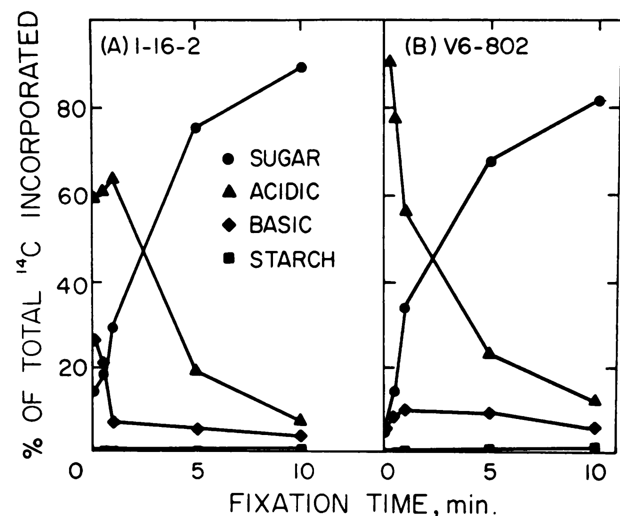

FIG. 2. Percent distribution of ^{14}C into various fractions in $^{14}CO_2$ fixation by attached leaves of two genotypes of tall fescue as a function of time. CO_2 concentration was 457 μl/l. Light intensity was 800 to 1,000 $\mu E\ m^{-2}\ s^{-1}$ and percentage of each fraction was summation of products (Table I) from that fraction as follows: acidic (organic acids), basic (amino acids), and sugar (sucrose and fructan).

RESULTS

CER *versus* Light Intensity. The effect of light intensity on rates of CO_2 exchange in air by 6× and 10× genotypes of tall fescue is shown in Figure 1. In the studies shown using growth chamber grown tissue, maximum rates of photosynthesis in air were 10.5 mg CO_2 dm^{-2} h^{-1} for the 6×, 21.5 mg CO_2 dm^{-2} h^{-1} for the 10×, and light saturation occurred at an intensity of about 800 $\mu E\ m^{-2}\ s^{-1}$ for 6× and 1,200 $\mu E\ m^{-2}\ s^{-1}$ for 10×.

$^{14}CO_2$ Fixation by Attached Leaves. Photosynthetic $^{14}CO_2$ fixation in the 10× genotype was linear up to 10 min, and in 6× genotype at least up to 5 min (data not shown). The amount of CO_2 fixed (mg) per h on various bases by 10× *versus* 6× were as following: leaf area, dm^2 (28.4 *versus* 13.7), mg Chl (17.8 *versus* 10.9), and g fresh weight (10.0 *versus* 5.6), respectively. These photosynthetic rates for the 10× were 1.6- to 2.1-fold greater than that of 6× and were in agreement with previous reports (17, 22).

Fractionation of ^{14}C Assimilates. The percentage distribution of ^{14}C among various fractions is shown in Figure 2. The overall distribution pattern was very similar for the two genotypes; however, minor differences were observed. The acidic and basic fractions were the primary recipient of label with 59% acidic and 26% basic for the 10× genotype, and 90% acidic and <5% basic for the 6× genotype after 5 s of $^{14}CO_2$ fixation (Fig. 2). However, the general precursor-product relationship was not different despite the initially higher percentage of total label that was incorporated into the acidic fraction. In the 10× genotype, after a slight increase within the 1st min, the acidic fraction showed the characteristic decline from 65 to 7% after 10 min fixation (Fig. 2). In the 6× genotype, the decline of the acidic fraction started from 90% at 5 s to 12% after 10 min (Fig. 2). Concomitantly, the ^{14}C in the neutral fraction of the 10× genotype rose from 14 to 89% and that of the 6× genotype from 5 to 81% in the same period. The percent of label in the sugar fraction was consistently higher for the 10× genotype, *i.e.* from an initial difference of 10% at 5 s to 8% at 10 min. Label in the basic fraction decreased from 26 to 4% in the 10× genotype, whereas the basic fraction in the 6× genotype increased slightly from 4 to 10% in the first min, leveled off, and then decreased. Less than 1% of the total radioactivity appeared in starch after 10 min of $^{14}CO_2$ fixation in both genotypes.

The distribution of ^{14}C products in the H_2O-soluble fraction is shown in Table I. The overall pattern and products were very similar for both genotypes. PGA was the initially labeled product which rapidly turned over and was followed by sugar phosphates. This was consistent with an earlier report which included tall fescue (8). Minor quantitative differences occurred, *i.e.* the initial labeling (1st 30 s) of amino acids accounted for 20% of the total ^{14}C incorporated in 10× and 6% in 6×. Less than 1% of label was found in malate after 30 and 60 s of $^{14}CO_2$ fixation.

Sucrose was the major product of photosynthesis. The percentage of [^{14}C]sucrose increased from 15 to 89% in 10× and from 5 to 81% in 6× over 10 min of $^{14}CO_2$ fixation. Because of differences in photosynthetic rates, this increase of label in sucrose represented an actual increase of newly synthesized [^{14}C]sucrose, from 0.01 to 1.29 and 0.001 to 0.8 μmol/g fresh weight for 10× and 6×, respectively (Table II). The calculated rates (mean of three) of sucrose synthesis were 8.03 and 4.67 $\mu mol\ g^{-1}$ fresh weight h^{-1} for 10× and 6×, respectively.

Amount of TNC and P_i in Tall Fescue Leaves. The content of TNC in the leaf over 2 dark and 3 normal d was determined (Fig. 3). No notable difference in content of reducing sugars was observed between genotypes (Fig. 3A). However, nonreducing sugar (predominantly sucrose) showed a consistent difference between genotypes. The content of sucrose in both genotypes decreased in the dark as would be expected. However, from day 0 to day 2, the 6× genotype maintained a 2- to 4-fold higher sucrose content (% dry weight) than did the 10× genotype (Fig. 3B). Upon returning to the light, the rate of sucrose increase was similar for both genotypes, but the relative difference between genotypes was reduced. Despite its lower photosynthetic rate, the 6× genotype maintained a higher percentage of sucrose on a dry weight basis than did the 10× genotype throughout the 5-d experimental period.

As for polysaccharides in tall fescue leaves, about 2% each of fructan and starch on a dry weight basis was detected (Fig. 3, C and D). The content of these two fractions remained fairly constant over the 5-d period. The P_i content of these samples were also determined (Fig. 4). The 10× genotype had about 2-fold higher P_i than the 6× genotype.

In a separate experiment, the H_2O-soluble carbohydrates were analyzed by GLC as *O*-trimethylsilyloxime trimethylsilylated derivatives (20), and the results (Table III) were in agreement with the above observation (Fig. 3) that the 6× had 23% higher sucrose content than 10× on a g fresh weight basis.

DISCUSSION

The $^{14}CO_2$ labeling studies demonstrate that both 10× and 6× genotypes of tall fescue fix CO_2 into PGA by the Calvin-Benson

Table I. *^{14}C Distribution in the Products of Water-Soluble Fraction in $^{14}CO_2$ Fixation by Attached Leaf*

Time	Genotype	^{14}C Incorporated													
		Sucrose	Fructan	HMP[a]	HDP	PGA	PEP	Glyceric	Malic	Glycolic	Ala	Ser	Gly	Other Amino Acids	Unknown
		% total													
5 s	I-16-2	14.5	0.07	30.0	0.6	22.5	0.1	5.0	0.3	0.08	17.4	2.7	1.4	4.6	0.5
	V6-802	4.7	0.09	42.4	3.2	39.6	0.8	3.7	0.4	0.1	0.3	1.2	1.5	1.4	0.6
30 s	I-16-2	17.9	0.2	24.7	0.7	26.4	0.2	6.7	0.7	0.1	13.2	0.8	6.5	0.3	1.4
	V6-802	14.2	0.2	36.5	3.0	31.7	0.8	3.8	0.5	0.1	0.4	3.7	3.6	0.5	1.1
1 min	I-16-2	29.4	0.2	25.3	0.7	29.1	0.8	6.3	0.3	0.1	0.8	0.8	5.1	0.2	1.1
	V6-802	33.4	0.5	22.3	4.1	21.9	0.6	4.6	0.8	0.2	0.3	3.2	6.1	0.2	1.8
5 min	I-16-2	74.9	0.6	5.2	0.6	7.8	0.2	2.5	1.7	0.04	0.5	1.8	2.2	1.0	1.0
	V6-802	67.0	1.2	9.0	0.8	7.5		2.8	1.6	0.3	0.07	1.2	6.2	1.5	1.0
10 min	I-16-2	88.9	0.3	1.5	0.2	2.6	0.09	1.3	1.2	0.04	0.3	1.0	0.7	1.7	0.3
	V6-802	80.7	1.0	4.5	1.4	3.1	0.1	0.9	1.7	0.06	0.2	1.7	2.4	1.4	0.5

[a] HMP, hexose monophosphates; HDP, hexose diphosphates; PGA, phosphoglyceric acid; and PEP, phosphoenolpyruvic acid.

Table II. *[^{14}C]Sucrose Formation from $^{14}CO_2$ Fixation in Tall Fescue Leaves*

Estimation of sucrose formed by, assuming all 12 carbon atoms are uniformly labeled, calculating the total count recovered from the sucrose spot on TLC using the specific activity of $^{14}CO_2$ and the measured leaf area, g fresh weight, and mg Chl. Specific activity was 1.2×10^7 cpm/μmol and 12 μmol CO_2/μmol sucrose.

Time	Genotypes	[^{14}C]Sucrose Synthesized		
		μmol/dm²	*μmol/g fresh wt*	*μmol/mg Chl*
5 s	I-16-2	0.030	0.011	0.014
	V6-802	0.003	0.001	0.002
30 s	I-16-2	0.088	0.031	0.038
	V6-802	0.048	0.019	0.031
1 min	I-16-2	0.314	0.110	0.134
	V6-802	0.077	0.031	0.118
5 min	I-16-2	2.294	0.810	0.985
	V6-802	1.477	0.599	0.945
10 min	I-16-2	3.680	1.293	1.577
	V6-802	2.032	0.823	1.298

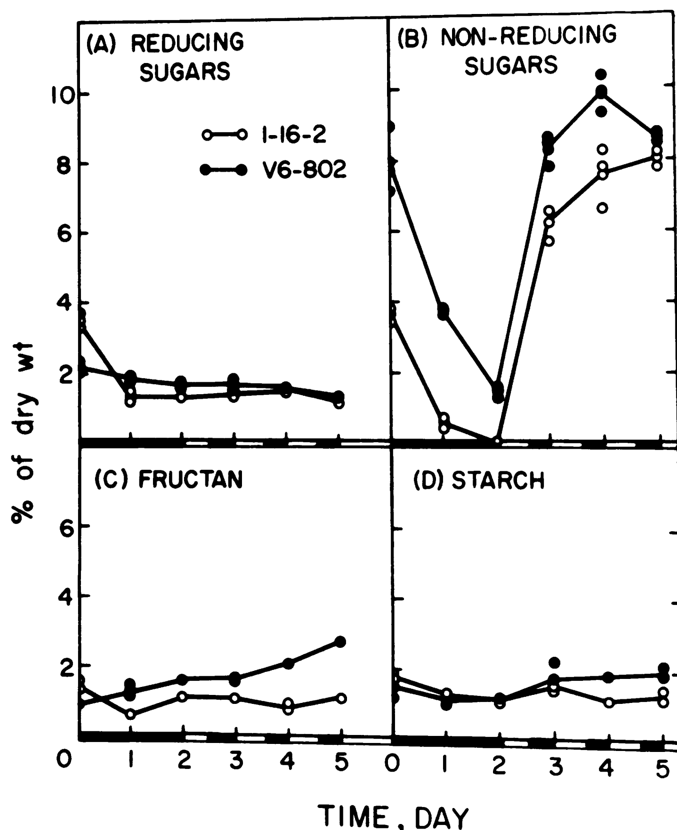

FIG. 3. TNC from leaves of two genotypes of over a 5-d period. Days 1 and 2 were in darkness and days 3 to 5 in greenhouse. Leaves were harvested between 1330 and 1400 h each day and analyzed for: A, reducing sugars; B, nonreducing sugars; C, fructan; D, starch.

cycle (Table I) and this observation is consistent with others (8, 21) despite differences in light response curves and photosynthetic rates (Fig. 1). The lower photosynthetic rate is fairly typical of growth chamber-grown plants; however, the ratio of CER between the two genotypes from growth chamber-grown tissue is the same as those from field-grown tissue. Newly collared leaves were used in all experiments in order to minimize leaf age between genotypes. The 6× genotype has a faster leaf elongation rate than the 10× genotype; *i.e.* approximately 47% under controlled environment (C. J. Nelson, unpublished observation). Rate of CER is maximum at collar formation and is relatively constant in the next 10 to 14 d (32) with a decrease of 15 to 20% per week for the 6× genotypes.

The kinetics of $^{14}CO_2$ incorporation into cellular intermediates is very similar in both genotypes and there are no unusual patterns of CO_2 fixation. Minor differences are observed, *e.g.* alanine is more highly labeled initially in 10× than 6× which then turns over rapidly and eventually reaches the same level. There is less than 2% [^{14}C]malate detected following 10 min of $^{14}CO_2$ fixation in both genotypes. These results suggest that the direct utilization of recently fixed CO_2 for amino and organic acid synthesis is not a major path of carbon metabolism under the experimental conditions. The large percentage of ^{14}C which appeared in sucrose, and which increased with time, indicates that this disaccharide is a major photosynthetic product of tall fescue. This increase in percent sucrose label represents a net synthesis of [^{14}C]sucrose (Table II). This is a minimal estimation of [^{14}C]sucrose formation assuming uniform labeling of each carbon atom in sucrose. The calculation in Table II is justified because the actual amount of labeled sucrose formed may be higher since this disaccharide molecule could potentially have between 1 and 12 labeled carbon atoms if there is exchange between labeled and nonlabeled pools of metabolites. The amounts and rates of sucrose formed in the two genotypes reflect the observed differences in photosynthetic rate (Fig. 1; Table II). Starch and fructan are only slightly labeled in the same period.

Results of $^{14}CO_2$ fixation indicate that sucrose plays a major role in photosynthetic carbon metabolism of tall fescue leaves. The sucrose content present in leaves is the result of synthesis, translocation, and utilization. We would expect higher sucrose content in leaves of 10× genotypes relative to 6× genotype at maximal carbohydrate accumulation in the light because of the higher rates of photosynthesis (Fig. 1) and of sucrose synthesis (Table II and III) in 10× genotype, if other processes are equal between the two genotypes. Therefore, TNC and P_i were determined in leaf tissue of these two genotypes (Fig. 3) during maximal accumulation at 1400 h (19). The low content (2% or less on dry weight basis) of reducing sugars, fructan, and starch are consistent with our labeling experiments and in agreement with others (26–28). Sucrose (nonreducing sugar) is the major component of TNC in both genotypes. However, in contrast to our expectation, the 6× genotype has higher sucrose content (% dry weight) than the 10× genotype (Fig. 3B). This is further supported by the GLC analysis of the H_2O-soluble carbohydrates of leaf tissue on a g fresh weight basis (Table III). The leaf tissues used for the latter GLC analysis of H_2O-soluble carbohydrates were from similar, newly collared leaves as those used for $^{14}CO_2$ fixation experiments.

In separate $^{14}CO_2$ experiments using leaf segments, we have been able to show that tall fescue leaf tissue accumulated up to 10% of the total ^{14}C incorporated into starch after 5 min exposure to a high level of $^{14}CO_2$ (1.1%) in both genotypes (data not shown). This observation indicates that tall fescue leaves have the enzymic capacity for starch synthesis in addition to fructan synthesis under high CO_2 concentration or in the presence of high sucrose concentration. However, less than 1% of the total label is incorporated into starch with attached leaves (Fig. 2) after 10 min of $^{14}CO_2$ fixation.

The disparity between the amount of sucrose synthesized, *e.g.* a ratio of 1.6 (Table II), and the total sucrose content, *e.g.* a ratio of 0.8, per g fresh weight (Table III) in leaves of 10× and 6× may reflect differences in pool size and/or utilization of sucrose for partitioning, translocation, respiration, and growth. It has been reported that both 10× and two other 6× genotypes have similar percentage of photorespiration, *i.e.* about 30% of the total photo-

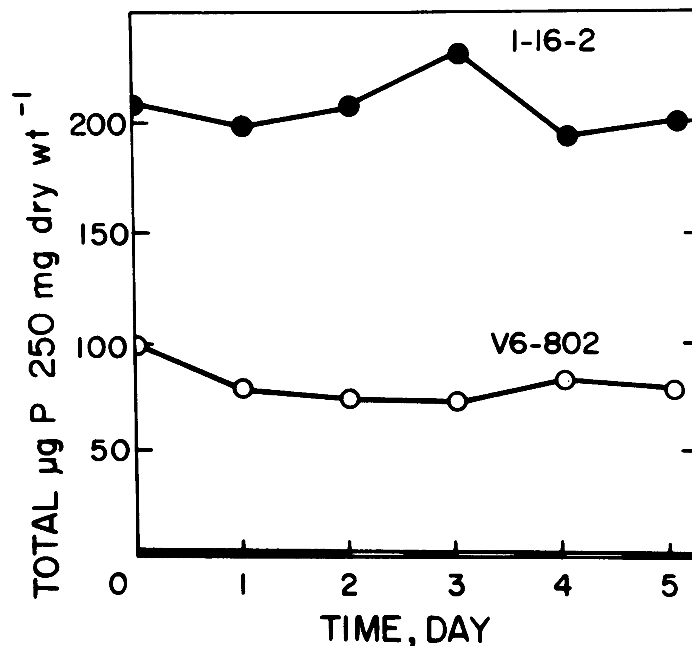

FIG. 4. Total inorganic phosphorus from tall fescue leaves of two genotypes. Dark/light treatment was the same as in Figure 3. Lyophilized and ground leaf samples were assayed for P_i by two procedures as described in "Materials and Methods."

Table III. *GLC Analysis of H_2O-Soluble Carbohydrates of Tall Fescue Leaf*

Compound	I-16-2 (10×)	V6-802 (6×)
	μmol·g⁻¹ fresh wt	
Sucrose	14.37 (85.0)[a]	17.69 (80.6)
Fructose	0.92 (5.5)	2.72 (12.4)
Glucose	0.76 (4.5)	0.84 (3.8)
Inositol	0.53 (3.1)	0.19 (0.9)
Sugar alcohol[b]	0.29 (1.7)	0.51 (2.3)

[a] Numbers in parentheses, percentage of total carbohydrates per g fresh weight within each genotype.

[b] Mol wt of mannitol (182.17) was used to estimate the μmol of sugar alcohol formed.

synthesis (22). But 6× has a slightly higher dark respiration relative to 10× genotype (C. J. Nelson, unpublished observation). Clearly, further efforts are needed to clarify these relationships. Nevertheless, these differences in respiration only partially account for the dissimilarity between the two genotypes. The different P_i content (Fig. 4) between the two genotypes may be coincidental but it further enhances the possibility of the difference in partitioning and/or translocation, since Walker and coworkers (30) have suggested that P_i may be critical in determining the photosynthate partitioning. We are investigating this aspect, and initial results show that the 10× genotype translocates a higher percentage of photosynthate than does the 6× genotype (J. H. H. Wong, unpublished observation).

At present, the similarity in assimilation pattern and products does not allow us to explain the difference in photosynthetic rate between these particular 6× and 10× genotypes of tall fescue. In theory, the difference in sucrose content should result from, rather than cause, the photosynthetic rate differential. Other factor(s) may be capable of affecting photosynthesis directly, *e.g.* stomatal conductance since increased nuclear ploidy levels in plants are frequently correlated with changes in morphology and anatomy (3, 4, 10, 13, 16, 24). However, there appears to be differences in utilization of sucrose between the two genotypes.

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