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Short Communication

Labeling of Fructans in Winter Wheat Stems

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ABSTRACT

Fructans synthesized from newly formed assimilates accumulate in wheat stems as nonstructural carbohydrates. Experiments performed tested the hypothesis that the fructose moiety from translocated sucrose is used preferentially in biosynthesis of these fructans. Results indicated: (a) a large percentage of labeled sucrose was translocated and unloaded in an unaltered state; and (b) sucrose contributed its fructose moiety to fructan synthesis in stems.

Many temperate (cool season) forage grasses and cereal crops accumulate phlein fructans (β-[2-6]-linked polyfructosylsucrose) as the predominant nonstructural carbohydrate during vegetative growth (10, 13). These compounds are a significant component of the nonstructural carbohydrate fraction in extended stem internodes of winter wheat (14). They accumulate when photosynthetic production exceeds utilization, reaching a maximum near the time of anthesis (6). Fructan synthesis may increase as a result of environmental stresses such as drought, low light intensities, or suboptimal temperatures (4, 5, 8).

In wheat, little is known of either the biosynthesis of these polymers or the mechanism of remobilization. Data of Robinson (11) indicate that the fructose moiety of translocated sucrose was used selectively by wheat stems. She hypothesized that her observation resulted from synthesis of fructans. Edelman and Jefford (2) presented a biosynthetic scheme for fructans of *Helianthus tuberosus* involving two enzymes. SST[1] catalyzes the transfer of a fructose moiety from one sucrose molecule to another via a dismutation reaction forming a trisaccharide (DP = 3). A second enzyme, FFT, transfers a terminal β(2-1)-linked fructofuranosyl residue from the trisaccharide to a similar position on a growing fructan chain (DP $\geq$ 3). Wagner *et al.* (15) have isolated an SST from vacuoles of wheat protoplasts. Pollock *et al.* (9) have shown that sucrose is the major precursor of fructan in *Lolium temulentum*. Fructans from wheat have a β-(2-6)-linked backbone with β-(2-1) branching (7). This structure is analogous to a soluble glucan synthesized by the bacterium *Streptococcus mutans* (12). *S. mutans* also used sucrose as a substrate. The present study tested the hypothesis that the fructose moiety of translocated sucrose is used preferentially in the biosynthesis of fructans in winter wheat stems. To our knowledge, the work reported here is the first attempt to confirm *in vivo* the proposed mechanism of synthesis of phleins larger than trisaccharides in wheat.

MATERIALS AND METHODS

Plant Material. Grains of *Triticum aestivum* L. var 'Duke' were treated with Terra-Coat, a commercial fungicide, and germinated in 9-cm filter paper-lined Petri dishes. Seedlings were subjected to an 8-week cold treatment (4°C) to induce vernalization. Vernalized seedlings were transferred to soil then placed in a growth chamber with a 16 h daylength, 24°C daytime, and 16°C night temperature. The light source was a bank of 12 Westinghouse fluorescent lamps (F48T12/CW/HO) plus eight GE 100 W incandescent bulbs. Irradiance ranged from 170 to 200 μmol m^{-2} s^{-1} (PPFD) at the flag leaves.

Experimental Methods. Flag leaves of six intact plants were labeled at anthesis for 3 h by reverse flap method (1) using (glucose-^{14}C[U])-sucrose and (fructose-1-^{3}H[N])-sucrose[2] in combined aliquots. Specific activities of stock solutions were 11.4 Ci/mmol ^{3}H and 0.2 Ci/mmol ^{14}C. Radiolabeled sugar aliquots were dissolved in 50 mM Mes buffer (pH of 5.2) to yield 4 μCi/μl ^{3}H and 1 μCi/μl ^{14}C based on supplier's specification. However, measured ratios based on dpm are reported for each experiment. Fifty μl of this solution were supplied to each plant. Three h after initiation of labeling, stem sections were harvested, placed in boiling 95% (v/v) ethanol then extracted in 80% (v/v) ethanol for 48 h in a microsoxhlet apparatus to remove ethanol-soluble sugars. The sections were subsequently extracted with water (20°C) for 48 h to dissolve fructans. One-half ml aliquots of extracted samples were placed in dioxane-based scintillation cocktail and analyzed with a dual channel Beckman LS 7500 microprocessor-controlled liquid scintillation spectrometer. Aliquots of water extracts were hydrolyzed with 2 M TFA and chromatographed using 1-dimensional paper chromatography in 1-butanol:ethanol:water (9:1:10, v/v/v, organic phase). Radioactive peaks representing glucose and fructose were determined from positions of standards. Chromatograms were cut into 1-cm strips, eluted with water (20°C) and analyzed using liquid scintillation spectroscopy.

RESULTS AND DISCUSSION

Fructans were the only detectable labeled compounds extracted with water following an 80% ethanol extraction (11). This procedure undoubtedly eliminated smaller fructans from water extracts. We wanted to be certain that no sugars were included in the 'fructan' sample. Ratios of ^{3}H:^{14}C in unhydrolyzed water extracts were similar for each stem section within one plant and conformed closely to the ^{3}H:^{14}C ratio of supplied sucrose (data not shown).

[1] Abbreviations: SST, sucrose-sucrose 1-fructosyltransferase; FFT, β-(2-1)-fructan:β-(2-1)fructan 1-fructosyl transferase; PPFD, photosynthetic photon flux density; DP, degree of polymerization.

[2] Labeled sucrose purchased from New England Nuclear. Supplier reports that 100% of the ^{3}H was attached directly to carbon one. That statement based on MS analysis of precursor hexose from different lot than used.

Table I. *Radioactivity of ^3H and ^{14}C and Ratios of ^3H:^{14}C of Hexose Moieties of Hydrolyzed Water Extracts of Plant I*

Sample[a]	Glucose		Fructose		^3H:^{14}C	
	^3H	^{14}C	^3H	^{14}C	Glucose	Fructose
	dpm				*ratio*	
1-1	46	175	267	28	0.26	9.5
1-2	85	321	1097	16	0.26	68
1-3	95	384	1080	180	0.25	6.0
2-1	96	245	2477	67	0.37	37
2-2	164	244	987	52	0.68	19
2-3	1	105	333	54	0.01	6.1
3-1	621	1024	1212	121	0.61	11
3-2	63	174	733	61	0.37	12
3-3	825	1069	3606	570	0.77	6.4
4-1	518	658	2200	76	0.75	29
4-2	14	141	1105	43	0.10	28
4-3	694	1122	3940	173	0.59	23

[a] Stem section designation: first number indicates internode, starting directly below the head, second number indicates section within internode, each counting from apex. Ratio of ^3H:^{14}C was 6.0 in the supplied sucrose.

Table II. *Radioactivity of ^3H and ^{14}C and Ratios of ^3H:^{14}C of Hexose Moieties of Hydrolyzed Water Extracts from Five Plants for the Most Radioactive Internodes*

Experiment/ Sample	^3H:^{14}C Supplied	Glucose		Fructose		^3H:^{14}C	
		^3H	^{14}C	^3H	^{14}C	Glucose	Fructose
	ratio	*dpm*				*ratio*	
Plant 2[a]							
1-3	5.04	97	255	320	64	0.38	5.0
2-1		63	113	537	26	0.56	21
Plant 3							
1-3	2.67	83	114	43	17	0.72	2.6
2-1		16	80	846	19	0.21	45
Plant 4							
1-3	3.85	3620	7388	1233	325	0.49	3.8
2-1		3332	3830	5469	290	0.87	19
Plant 5							
1-3	3.62	406	688	1366	367	0.59	3.6
2-1		172	905	2047	56	0.19	37
Plant 6							
1-3	4.05	53	125	1443	346	0.42	4.0
2-1		34	92	1888	70	0.37	27

[a] Stem sections 1-3 and 2-1 (above and below the node of insertion of the flag leaf, respectively) are listed for each plant.

To determine if there was selective labeling in the hexose moieties of water soluble fructans, the fructans were hydrolyzed, and the resultant hexoses separated. Table I lists the dpm value of ^3H and ^{14}C and the ratios of ^3H:^{14}C from the hydrolyzed water extracts from all stem sections of a representative plant (plant 1). Table II lists similar data from five other plants for internode sections directly above and directly below the insertion of the labeled leaf.

By separating the hexoses derived from the water-soluble fructans it was possible to observe the labeling pattern in glucose and fructose, *i.e.* the ratio of ^3H:^{14}C in each. These data indicate that the water-soluble fractions from these winter wheat stems at anthesis contained more ^3H in fructose than in glucose. In each experiment, the ratio of ^3H:^{14}C in the fructose fraction was greater than the ratio of ^3H:^{14}C in the supplied sucrose except in the specific sections (Table I: 1-3, 2-3, and 3-3; Table II: all 1-3 sections).

The lower third of the top three internodes was less mature than the remainder of the stem. Immaturity may account for different labeling patterns (Table I, stem section 1-3, 2-3, and 3-3). In less mature sections, metabolism is directed toward reactions contributing to active growth *i.e.* degradation of translocated sucrose. These reactions would lead to greater randomization, producing ^3H:^{14}C ratios in all compounds closer to that in supplied sucrose. However, the ratios in the more mature sections of each plant indicate that there was selective use of the fructose moiety from supplied sucrose (Tables I and II). This pattern was followed in all mature sections of all plants (additional data plants 2-6 not shown). In all sections, mature and immature, the glucose derived from fructans (Tables I and II) had ^3H:^{14}C ratios below the ratios of the supplied sucrose.

These data indicate that, in mature sections, sucrose was transferred unaltered from the sieve tubes to the site of fructan synthesis. That was followed by preferential use of the fructose moiety of sucrose in the synthesis of the fructose portion of fructans. In addition, in all sections, the single glucose moiety of

each fructan was incorporated into fructans by direct use of translocated sucrose. However, in the youngest sections, much randomization of label occurred before fructose was incorporated into fructans.

Labeling patterns, especially for the mature tissue, indicate that enzymes responsible for fructan synthesis in wheat function similarly to those proposed for *H. tuberosus* (2), or as proposed for glucan synthesis by *S. mutans* (12). Further evidence that the fructose moiety was transferred directly to a growing fructan polymer in wheat awaits purification and identification of appropriate enzymes.

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LITERATURE CITED

1. BIDDULPH O 1941 Diurnal migration of injected radio-phosphorus from bean leaves. Am J Bot 28: 348–352
2. EDELMAN J, GT JEFFORD 1968 The mechanism of fructosan metabolism in higher plants as exemplified by *Helianthus tuberosus* L. New Phytol 67: 517–531
3. GIAQUINTA RT, W LIN, NL SADLER, VR FRANCESCHI 1983 Pathway of phloem unloading of sucrose in corn roots. Plant Physiol 72: 362–367
4. KOOMANOFF NE 1981 Relationships among stem carbohydrate reserves, grain yield and yield components in winter wheat. MS thesis, Colorado State University, Fort Collins
5. LABHART CH, J NOSBERGER, CJ NELSON 1983 Photosynthesis and degree of polymerization of fructan during reproductive growth of meadow fescue at two temperatures and two photon flux densities. J Exp Bot 34: 1037–1046
6. MCCRAIG TN, JM CLARKE 1982 Seasonal changes in nonstructural carbohydrate level of wheat and oats grown in semiarid environment. Corp Sci 22: 963–970
7. MEIER H, JSG REID 1982 Reserve polysaccharides other than starch in higher plants. *In* FA Loewus, W Tanner, eds, Encyclopedia of Plant Physiology, 13A, Plant Carbohydrates I, Intracellular Carbohydrates. Springer-Verlag, Heidelberg, pp 434–471
8. POLLOCK CJ, T JONES 1979 Seasonal patterns of fructan metabolism in forage grasses. New Phytol 83: 9–15
9. POLLOCK CJ, EJ LLOYD, JL STODDARD, H THOMAS 1983 Growth, photosynthesis and assimilate partitioning in *Lolium temulentum* exposed to chilling temperatures. Physiol Plant 59: 257–262
10. PORTER HK 1962 Synthesis of polysaccharides in higher plants. Annu Rev Plant Physiol 13: 303–328
11. ROBINSON NL 1980 Translocation of ^{14}C-sucrose in wheat. MS thesis. Colorado State University, Fort Collins
12. ROBYT JF, PJ MARTIN 1983 Mechanism of synthesis of D-glucans by D-glucosyltransferases from *Streptococcus mutans* 6715. Carbohydr Res 113: 301–315
13. SMITH D 1973 The nonstructural carbohydrates. *In* GW Beutler, RW Bailey, eds, Chemistry and Biochemistry of Herbage, Vol I. Academic Press, New York, pp 105–155
14. STOY V 1965 Photosynthesis, respiration and carbohydrate accumulation in spring wheat in relation to yield. VI. Accumulation and transformation of soluble carbohydrates during late ontogenesis. Physiol Plant Suppl 4, 92–125
15. WAGNER W, F KELLER, A WIEMKEN 1983 Fructan metabolism in cereals: induction in leaves and compartmentation in protoplasts and vacuoles. Z Planzenphysiol 112: 359–372