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Communication

Fructan Content and Fructosyltransferase Activity during Wheat Seed Growth[1]

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ABSTRACT

The objective of this research was to determine the changes in fructan content and the activity of fructosyltransferases during the growth of wheat seeds (*Triticum aestivum* L. Thell, cv Caldwell). The total fructan content of the seeds decreased significantly during seed growth. The trisaccharide and tetrasaccharide content increased from 6 to 28 days post anthesis (DPA) and then declined, but these changes are not statistically significant. The content or concentration of longer chain polymers did decline significantly (64.55–6.52 milligrams per gram dry weight). Free fructose also decreased significantly during seed growth indicating that the fructose liberated from the decrease in fructan content was utilized by the seed. Sucrose increased significantly from 6 to 12 DPA, then declined significantly from 12 to 28 DPA. Sucrose:sucrose fructosyltransferase activity was greatest from 6 to 12 DPA (averaging 0.16 micromole of fructose transferred per seed per hour), then declined rapidly (0.04 micromole of fructose transferred per seed per hour). The estimated activity of fructan:fructan fructosyltransferase followed a similar pattern. The increase in sucrose concentration and high enzyme activity suggests that fructans were synthesized during the lag phase of seed growth.

Wheat species (*Triticum* sp.), like other grasses of Poales, accumulate polymers of fructose called fructans. They have been isolated in stems (1, 6), leaves (23), and seeds (2, 4). Of particular interest here is the kernel or seed accumulation of fructans. The quantity of fructan in the seed has been reported to decline during growth from about 1.0 to 0.25 mg per seed (2, 4). It is not clear whether the initial fructan content of the seed results from accumulation before anthesis or from synthesis during seed growth.

Fructans are believed to be synthesized by SST[2] and FFT. SST transfers a fructose moiety from one sucrose molecule to another producing a trisaccharide and glucose. FFT catalyzes the synthesis of higher order polymers. The research published on SST indicates the only substrate is sucrose since UDP-fructose does not act as a substrate (17, 18). Second, localization of the enzyme suggests it is exclusively vacuolar (5, 22). The pH optimum ranges from 5.0 to 5.6 (19). The correlative changes in SST activity and fructan synthesis, and the fact that it catalyzes the synthesis of the first fructan (the trisaccharide) suggests SST controls carbon partitioning into the fructan pool (18, 19).

The enzyme synthesizing fructan polymers beyond the trisaccharide is FFT. Little is known about this enzyme. Much of what is known has been deduced from work on Jerusalem artichoke (*Helianthus tuberosus*) or dandelion roots (*Taraxacum officinale*) (19). The pH optimum of FFT is higher (6.1–8.5) than that of SST. Both dandelion and Jerusalem artichoke produce inulin-type fructans. *Lolium* (11) and wheat blades (22) are the only Gramineae tissue for which FFT activity estimates have been made. In wheat blades the tetrasaccharide was synthesized in cell-free extracts, although higher-order polymers were extracted from the tissue (22).

The objectives of our study were to examine the changes in fructan content and the enzymes of fructan synthesis during maturation of wheat seeds. We have been studying carbon accumulation and metabolism in wheat seeds and noticed the presence of fructans in developing kernels. We were interested in estimating both the changes in fructan content and the potential for synthesis of fructan during seed growth.

MATERIALS AND METHODS

Plant System. A soft red winter wheat cultivar (*Triticum aestivum* L. em Thell., cv Caldwell), was grown in the field at the Purdue University Agronomy Farm (West Lafayette, IN) in a randomized complete-block design. Each plot contained four 2.5 m rows which were spaced 30 cm apart. The seeding rate was 1 seed per cm. The time of anthesis was considered to be when anthers first became visible in the spike. Individual spikes were tagged at anthesis and harvested at 2 to 4 d intervals from anthesis until maturity. Spikes were frozen in liquid N_2, placed in plastic bags and transferred on dry ice to a −80°C freezer until used. Grains from the central spikelets were weighed, lyophilized, and reweighed to obtain fresh weight, dry weight and water content measurements. Data presented are the means of 8 replications.

Separation of Fructans. Grains were extracted with 80% ethanol (v/v) and water to obtain sugars. The sugar extractions were flash evaporated and reconstituted with distilled H_2O. Sugars were separated by passing the solution through a 110 × 1.5 cm column of Bio-Gel P2 (200–400 mesh). Aliquots were collected by elution with 4 g/L NH_4HCO_3 and fractions were assayed colorimetrically for free and combined fructose by reaction with anthrone (13). The optical density obtained by reaction of fructose standards with anthrone was utilized to determine the fructose equivalents of the separated sugar fractions. The free

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[2] Abbreviations: SST, sucrose:sucrose fructosyl-transferase EC 2.4.1.9; DPA, days post anthesis; FFT, fructan:fructan fructosyl-transferase EC 2.4.1.10; SS, sucrose synthase EC 2.4.1.13; LCF, long chain fructans, sucrose plus 3 or more fructose moieties; gdw, grams dry weight; BSLD, Bayes least significant difference.

fructose, sucrose, trisaccharide and tetrasaccharide peaks were identified by retention time and glucose to fructose ratios after acid hydrolysis. Glucose and fructose were estimated using the liberation of NADPH in a coupled enzyme assay (14). Statistical differences in fructan content were estimated when F-test analyses were significant ($P < 0.10$ or 0.05). Bayes least significant difference (BLSD) was calculated for significance using either a k ratio of 50 or 100 (21). Data were based on 4 replications.

Measurement of Transferase Activity. Grains at various times after anthesis were extracted and SST assayed after the procedure of Pollock (16), and Shiomi and Izawa (20). Briefly the grains are extracted in a 100 mM sodium phosphate buffer containing 20 mM DTT, 20 mM EDTA, 20 mM β-mercaptoethanol, and 100 mg g^{-1} PVP. We have found that Bio-Gel P6 desalting gels do not separate the fructans from the proteins in crude extracts so that $(NH_4)_2SO_4$ fractionation was necessary. Over 90% of the activity is recovered with a 60% $(NH_4)_2SO_4$ fractionation, but the fructans do not precipitate. After addition of the $(NH_4)_2SO_4$, the extract is stirred for 30 min, centrifuged at 20,000g for 15 min and the pellet resuspended in 10 mM sodium phosphate buffer (pH 5) containing 1.8 mM ethane diol, 2.5 mM EDTA. The solution was desalted by passage through Bio-Gel P6 DG (1 × 5 cm column). The assay buffer included 20 mM sodium acetate (pH 5), 600 mM sucrose, 0.5 μM DTT, 0.2 mg BSA, and enzyme solution in a final volume of 250 μl. The reaction was begun with the addition of sucrose, incubated at 30°C for 1 h, and terminated by boiling for 3 min. The excess of glucose over fructose (measured in coupled enzyme assay [14]) was used to estimate the activity of SST (15). Data presented are means of 3 replications.

The activity of FFT cannot be estimated on the same crude protein extract as that used for SST since the extraction buffers differ in pH. The FFT extraction is at pH 6, whereas that of SST is pH 5. Essentially other than the difference in pH (FFT at 6) the assay is similar to that above with the exception that the incubation period is 24 h, and the activity was calculated as the mg of fructose containing polymers of greater mol wt than the trisaccharide as determined after separation on Bio-Gel P2. This procedure is similar to that reported for *Lolium* (11). The estimated SST activity of these long term incubations was in agreement with the activity estimates obtained from measurement of excess glucose, so we feel that other metabolic reactions which influence the ratio of glucose to fructose did not bias our results. Data presented represent 3 replications.

RESULTS AND DISCUSSION

The fresh weight, dry weight, and water content of the maturing kernels (Fig. 1) from the central spikelets were typical of many soft red winter wheats (10). Generally, the increase in fresh and dry weight may be divided into a lag phase lasting from the beginning of anthesis to about 10 to 12 DPA, followed by a linear phase of growth (12–26 DPA), then a maturation phase. The increase in seed fresh weight up to about 16 DPA results from the accumulation of both water and dry matter. Beyond 16 DPA the water content remained fairly constant, so that increases in weight are a result of the accumulation of dry matter. While not illustrated by these data, other work suggests that carbon arriving in the seed during the lag phase is utilized in metabolic events associated with increase in cell number (TL Housley, EM Dale, CST Daughtry, unpublished data). During the linear phase of seed growth, starch is the major sink for arriving carbon. Near maturation, water content and starch synthesis begin to decline (10).

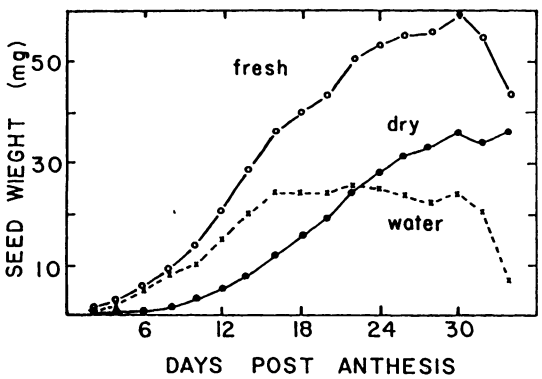

FIG. 1. Fresh and dry weight, and water content of field-grown Caldwell wheat from the central portion of the spikelet during seed growth.

The amount of extractable fructose containing compounds in the seed declines during seed growth. In the lag phase prior to starch synthesis, these compounds comprise 8.9% of the dry weight of the seed, while at 28 DPA at the end of the linear phase of growth they comprise only 2.1% (Table I). At all time periods in seed growth, fructans comprise 50 to 95% of the fructose containing compounds extracted from the seed. Sucrose, the major carbon source arriving in the seed, is utilized to support metabolic events. Sucrose is also necessary for fructan synthesis (8). In the leaves of *Lolium temulentum* increasing sucrose concentration results in fructan synthesis (11). Indeed in Caldwell seeds there is a significant increase in sucrose content from 6 to 12 DPA (Table I) with slight, but nonsignificant increases in the trisaccharide and tetrasaccharide, which are products of the first (SST) and second (FFT) enzymes of fructan synthesis, respectively. LCF (fructan containing more than 3 fructose moieties), free fructose, and total fructan (tri, tetra, and LCF) decline significantly during seed growth. Our data agree with that of Escalata and Moss (5) as well as that of Cerning and Guilbot (2) who also found a decline in fructan concentration during seed growth, although our values are initially higher and ultimately lower than theirs. This steady decline indicates that there is net hydrolysis of fructans during seed growth. The lack of increase in fructose concentration during seed growth suggests that, if hydrolysis is occurring, the released fructose is being utilized by the seed. Since the LCF content steadily declines, it may be that at least some of the trisaccharide and tetrasaccharide in the seed arises from LCF hydrolysis.

Correspondent with the changes in seed growth pattern and sugars are anatomical changes. Initially the pericarp comprises a large proportion of seed tissues, but this proportion declines as the proportion represented by endosperm tissues increases. In wheat and barley (*Hordeum vulgare* L. cv Gerbel) leaf blades (23), as well as Jerusalem artichoke tubers (23) the fructans are localized in the vacuole. Ho and Gifford (7) reported that fructans are present in the *nucellar* fluid of immature wheat seeds. They report that the concentration of these fructans is about 200 mM. Their data suggest that some of the seed fructans are between the pericarp and the endosperm. Beyond this report we do not know how the fructans are compartmentalized within the seed, but experiments are in progress to delineate this distribution.

The enzyme SST forms the trisaccharide, utilizing sucrose as a substrate. The estimated activity of SST on a fresh weight or per seed basis is highest during the lag phase of growth (Fig. 2). The range of measured activity (20.2–0.7 μmol fructose/gdw·h) is similar to the values reported for *Lolium* (7.7–44.1 μmol fructose/g fresh weight·h [16]), barley (0.09–0.89 nkat/ml [23]), and tall fescue (*Festuca arundinacea*) (1.91–6.31 μmol fructose/gdw·h [12]) leaf tissues. The activity that we report is for the whole seed; as yet we do not know if the pattern of activity results from changes in the metabolism of different tissues in the seed or is relative to a change in the proportion of seed tissues. At 12 DPA, the activity of SST in the whole seed is distributed

Table I. *Sugar Content of Wheat Seeds during Maturation*

Time Post Anthesis	Fructose		Sucrose		Trisaccharide		Tetrasaccharide		Long Chain Fructans		Total Fructan
d	*mg/gdw*	*mg/seed*	*mg/gdw*	*mg/seed*	*mg/gdw*	*mg/seed*	*mg/gdw*	*mg/seed*	*mg/gdw*	*mg/seed*	*mg/gdw*
6	6.45	0.32	6.47	0.32	5.20	0.26	6.33	0.32	64.55	3.23	76.10
8	5.91	0.30	8.50	0.43	4.49	0.22	6.87	0.34	64.21	3.21	75.57
12	4.29	0.21	20.15	1.01	7.34	0.37	7.55	0.38	41.02	2.05	55.91
16	3.08	0.17	16.50	0.83	7.22	0.36	3.96	0.20	20.67	1.03	31.84
20	1.15	0.06	9.53	0.50	4.58	0.26	1.79	0.10	7.00	0.39	13.36
24	1.00	0.05	8.05	0.40	3.38	0.17	4.11	0.21	6.53	0.33	14.01
28	0.45	0.02	10.09	0.50	5.59	0.28	1.70	0.09	3.56	0.18	10.86
BLSD	1.32[a]		8.40[b]		NS[c]		NS		6.52[a]		6.64[a]

[a] Bayes least significant difference using a k ratio = 100. [b] Bayes least significant difference using a k ratio = 50. [c] Not significant.

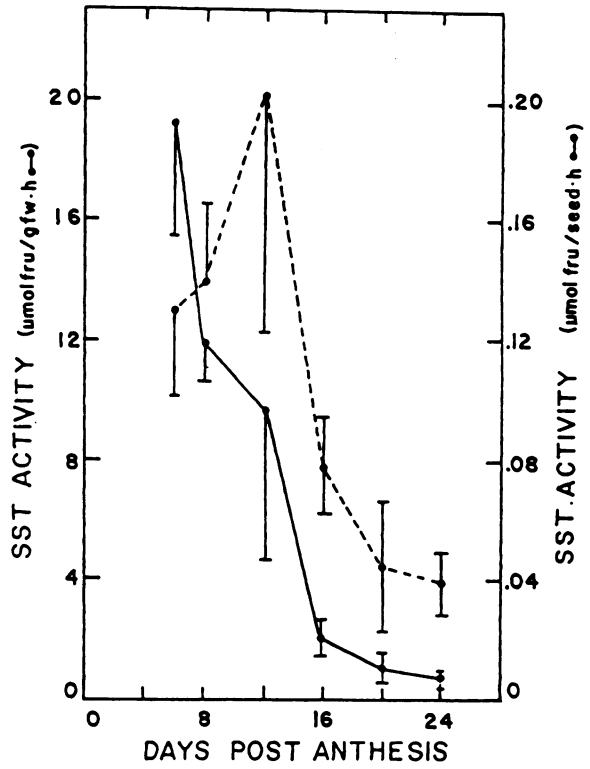

FIG. 2. The activity of SST in Caldwell wheat seeds during growth from 6 to 24 DPA. Activity is expressed as μmol of fructose (fru) transferred from sucrose per g fresh weight or per seed per h. The bars represent one standard deviation about the mean.

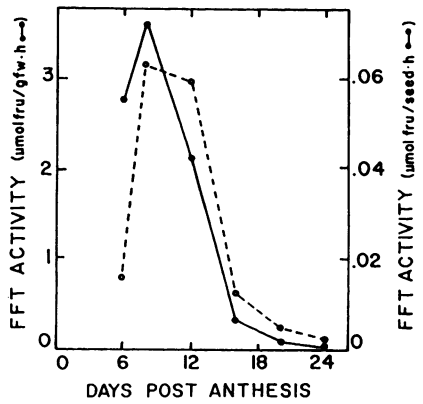

FIG. 3. The relative changes in activity of FFT in Caldwell wheat seeds during growth from 6 to 24 DPA. The activity is expressed as μmoles of fructose (fru) transferred from sucrose per g fresh weight or per seed per h.

about equally between pericarp and endosperm tissues (9). The decline in activity is likely to be related to the maturation of the pericarp and the turnover of SST. SST activity in barley leaf tissue increases during a 24 h light period and then decreases in the subsequent dark period (23). The increase in activity in barley was inhibited by cyclohexamide, suggesting protein synthesis is a prerequisite to an increase in activity. Additionally, SST activity increased in darkened barley leaf blades if sucrose was exogenously supplied. The increase in sucrose content of wheat seeds could be responsible for the increase in SST activity from 6 to 12 DPA. The decrease in sucrose in the seed after the dark period may also have contributed to the loss of SST activity beyond 12 DPA. During the linear phase of seed growth there is an increase in SS activity (3) which could lead to competition for the common substrate, sucrose, by SST and SS, which would contribute to the drop in sucrose concentration and the subsequent decline in SST activity of the seed.

The apparent activity of FFT follows a similar pattern to that of SST (Fig. 3). While we feel the relative pattern of the FFT activity is representative of that during seed growth, the *in vivo* values of the activity are likely to be higher than those reported for the following reasons. The activities reported are estimates utilizing sucrose as the substrate rather than the trisaccharide so the assay depends on SST to produce the substrate for FFT. Sucrose can compete with the trisaccharide as an acceptor for fructose moieties so that production of oligomers is inhibited (19). In our FFT assays we found only the tetrasaccharide as a product, perhaps a result of sucrose inhibition. In extracts from the seed, however, higher order polymers are present (Table I). Unfortunately the substrate for the synthesis of oligomers and polymers is not commercially available. Even if we could purchase the trisaccharide it is not known whether kestose (β-2–6 linked fructose), or isokestose (β-2–1 linked fructose) is the preferred substrate for the wheat seed FFT. In those plants where the product of SST has been structurally identified, isokestose has been found (18). Structural analysis of wheat seed fructans suggests a mixed-linked polymer (NC Carpita, TL Housley, JE Hendrix, LO Sillerud, unpublished results), so that perhaps both trisaccharides are present and necessary in particular proportions for oligomer synthesis. Additional research is underway to resolve some of the problems associated with the assay of FFT activity.

The measurement of fructosyltransferase activity *per se* does not prove that fructans are synthesized during seed growth. However, the increase in sucrose concentration and the general pattern of SST and FFT activity suggests that at 12 DPA some

synthesis of oligomers occurs. Experiments are in progress to examine the *in vivo* synthesis of fructans and to estimate the fructosyltransferase activity of the pericarp and endosperm tissues of the seed.

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