Journal of Agricultural Science (2002), **139**, 37–45. © 2002 *Cambridge University Press*
DOI: 10.1017/S0021859602002319 *Printed in the United Kingdom*

Sugar metabolism in expanding husk leaves of flint corn (*Zea mays* L.) genotypes differing in husk leaf size

K. G. OFOSU-BUDU[4], H. EL-SHEMY[2] AND K. FUJITA[1]*

[1] *Faculty of Applied Biological Science, Hiroshima University, 1-4-4, Kagamiyama, Higashihiroshima, 739-8528 Japan*
[2] *Faculty of Integrated Arts and Sciences, Hiroshima University, 1-7-1, Kagamiyama, Higashihiroshima, 739-8521 Japan*
[3] *Hokkaido Tokachi Agricultural Experiment Station, Memuro-cho, Hokkaido, 082-0071 Japan*
[4] *Agricultural Research Station-Kade, Faculty of Agriculture University of Ghana, Legon, Accra, Ghana*

(*Revised MS received 20 May 2002*)

SUMMARY

Relationships between leaf expansion and MeOH-soluble (cytosol) and cell-wall fractions, and their sugar composition prior to silking in flint corn lines were studied. A greater husk leaf area of one genotype, X-15 is mainly due to prolonged and higher rate of expansion. Prior to rapid expansion of husk leaf area, neutral sugars in the cytosol fraction accounted for most of the non-starch carbohydrates (56–62 %), while hemicellulose and cellulose fractions accounted for less than 20 %. In mature leaf parts, however, sugars in the cytosol fraction decreased but those in hemicellulose and cellulose fractions increased by 30 % and 42 %, respectively. The predominant sugar in the cytosol fraction was glucose (Glc), while in the hemicellulose fraction xylose (Xyl) and arabinose (Ara) dominated. During rapid expansion of husk leaves, ^{13}C was incorporated at a higher rate into hemicellulose than cellulose, and this process was more active in X-15 than in other genotypes. During an identical period, ^{13}C atom % excess in Xyl increased markedly in the hemicellulose fraction, however it remained low in the cytosol one. The current results suggest that synthesis of Xyl and xylan plays an important role in renewal of hemicellulose, which may be required for expansion.

INTRODUCTION

Husk leaves in some corn genotypes contribute to grain yield more efficiently than culm leaves due to a higher percentage of photosynthate translocation (Sawada *et al.* 1995), however, their total contribution of photosynthate to grain production is smaller than culm leaves because of their small leaf area. The husk leaf area is correlated with leaf dry weight (Fujita *et al.* 1995) and expands in parallel with increases in the amount of cell-wall components such as the hemicellulose and cellulose fractions (Sato *et al.* 1997). However, how they relate to each other has not yet been elucidated. Therefore, it is intended to test a hypothesis that relative changes in carbohydrate metabolism play a role in synthesis of cell-wall components, leading to leaf area expansion among corn genotypes.

Prior to emergence of husk leaves in some corn genotypes, we observed a slowed expansion and a paling of the leaves (Sato *et al.* 1997). Immediately after the husk leaf tip emerged from the ear leaf sheath, however, the leaf area increased rapidly until silking and photosynthetic activity increased. Furthermore, husk leaves sustained photosynthate levels more by their photosynthesis than by influx from culm leaves (Sato *et al.* 1997). Thus, the photosynthate supply may not restrict the expansion of husk leaves, and the extent to which the photosynthate is utilized in the developing husk leaves may give rise to differences in leaf area.

The cells of plants are surrounded by a hydrated wall composed of complex carbohydrates, glycoproteins and phenolics (Darvill *et al.* 1980; Fry 1988).

* To whom all correspondence should be addressed. Email: fujiko@hiroshima-u.ac.jp

Although alterations in the physical properties of the primary wall are clearly responsible for plant cell enlargement (Cleland 1977), the chemical bases for such alterations are far from understood. Several structural and architectural changes accompany elongation of grass cell walls. During isodiametric expansion of meristematic cells, the major non-cellulosic polysaccharides are glucuronoarabinoxylan (GAX) and the pectic polysaccharides (Carpita 1984). In dividing and elongating cells, highly branched GAXs are abundant, whereas after elongation and differentiation more unbranched GAX accumulates (Carpita 1984; Gibeaut & Carpita 1991). The appearance of β-D-glucan during cell expansion, the association of hydrolysis of β-D-glucan *in muro*, and acceleration of its hydrolysis by growth regulators further point to the direct physical involvement of the polymer in cell growth (Carpita 1996). We have deduced from data on a flint corn genotype that the production of cell wall components such as hemicellulose and cellulose is related to leaf expansion (Sato *et al*. 1997).

Leaf growth involves the production and expansion of leaf cells, with the latter suggested to be of overriding importance (Ferris & Taylor 1994; Gardner *et al*. 1995). Expansive growth is co-regulated by the rate at which cell walls loosen and extend, and the rate at which water and solutes are accumulated by the growing cell (Lockhart 1965). The cell wall consists of two continuous interpenetrating systems, one of which is the cellulose fibrils and the other the continuous system of microcapillary spaces. These spaces may be filled with hemicellulose and other organic substances (Bailey 1957). The properties of the cell wall are determined by those of cellulose and other substances which may add some characteristics or alter slightly those of the cellulose.

It is known that cell wall relaxation and turgor pressure are prerequisites for cell elongation (Cosgrove 1987). For instance, expansin plays a vital role in cell wall expansion through loosening cell wall materials (Cosgrove 1999). When the amount of hemicellulose in *Gramineae* is reduced by degradation with 1,3;1,4-β-glucanase, the cells elongate by turgor pressure (Sakurai 1991). Sequential loosening, expansion and subsequent tightening of the cell walls along the growing zone play a vital role in cell elongation (Carpita 1996). However, the relation between cell expansion and synthesis of cell wall polysaccharides in the husk leaves is not well understood. Detailed analyses of husk leaf composition during the rapid phase of development may give a clue, but these have not been made.

This report documents the changes in the composition of the cell components, particularly the hemicellulose fraction, for the different growth phases (expanding and matured) and correlates these changes with the rapid expansion phase of the husk leaf.

MATERIALS AND METHODS

Seeds of the pure lines of flint corn X-15, CM-80 and CE-78 were grown in the experimental field of Hiroshima University, Higashihiroshima City, Japan from May to June 1997. The soil consisted mainly of granite regosols, and received basal dressings as follows; 150 kg N/ha, 98 kg P/ha and 139 kg K/ha. The soil pH was adjusted to about 6·5 with dolomitic lime prior to fertilization. Nitrogen was top dressed at a rate of 100 kg/ha about 1 month after planting.

Whole plots consisted of 60 rows spaced at 100 cm apart with 40 cm between hills. Three seeds were planted per hill on 18 May 1997 and the plants were thinned to one plant per hill 21 days after planting. Main plots consisted of pure lines while the subplots consisted of sampling periods at 11, 5 and 4 days before silking (DBS). Three replications were adopted.

Three plants from each replication were harvested at 11, 5 and 4 DBS when the husk leaf tip started to emerge from the leaf sheath of the first ear (located at the uppermost position on the main culm), a period referred to as presilking hereafter, and at silking. For determination of dry weights, the harvested plants were separated into leaves on the main culm, husk leaves, culm and other parts including ears. These were dried at 70 °C in a forced-air oven for more than 3 days and the dry weights taken.

Photosynthetic rate and leaf area measurements

Photosynthetic rate was measured by a portable infrared gas analyser (Model LI-6400, LICOR, Lincoln, NE) under natural light conditions as described in Sawada *et al*. (1995). The photosynthetically active radiation (PAR) was above 1700 μmol/m²/s during the measurements. Photosynthetic rates for about eight leaves on the first ear (uppermost ear) and ear leaves were measured at 11, 5 and 4 DBS, and at silking.

The leaf area of 8–11 husk leaves was individually determined by an auto leaf area meter (Model AAM-5, Hayashi Denko Co. Ltd., Osaka, Japan). Specific leaf weight was calculated as mg leaf dry weight/mm² leaf area. All the measurements were made with three different plants of each genotype.

Water potential, osmotic potential and turgor measurements

The leaf water potential (ψ_w) and osmotic potential (ψ_s) were measured by thermocouple psychrometry using a sample chamber (Model C-52, Wescor, Logan, UT, USA) and microvolt meter (HR-33T, Wescor) attached to a chart recorder to read the microvolt output. Calibration was performed at 25 °C with 10 μl volumes of NaCl solutions of a known molarity.

Leaf discs, 0·5 cm in diameter, were cut from the husk leaf and immediately placed in sample chambers for equilibration. After reading of the ψ_w, the sample chamber was transferred to a −20 °C freezer, and kept overnight. The sample chamber was returned to the experimental room and ψ_s was measured after 3 h of equilibration at 25 °C. Turgor potential (ψ_p) was calculated as the difference between ψ_w and ψ_s.

Fractionation and measurement of sugars in each fraction

Samples of husk leaves (1 g) were taken from the 1st ear immediately after harvesting, separated into the emerged (matured) part (the husk leaf tip only) and the unemerged (expanding) part (0–3 cm from the base of the leaf blade), and a portion of each was immersed in a vial containing 10 ml of 99·5% methanol and kept below 4 °C until analysis. The overall length of the husk leaf at the presilking stage was about 6–10 cm in all the genotypes.

Cell walls which were prepared from the husk leaves on each ear and ear leaf were fractionated according to the method described in Sakurai *et al.* (1987). Analyses of cell wall components were conducted on emerged and unemerged husk leaf parts at presilking and on the whole husk leaves and ear leaves at silking. Fresh leaves were fixed in MeOH and boiled for 10 min. The MeOH extract was designated as the MeOH-soluble fraction and contained neutral sugars including glucose, fructose and sucrose in the cytosol. The husk leaves were rehydrated and homogenized in liquid nitrogen. The homogenate was centrifuged at 1000 ***g*** for 10 min. The resultant supernatant was designated as the water-soluble (WS) fraction. This fraction also consisted of neutral sugars in the cytosol, but was collected after denaturation of the proteins with TCA (trichloroacetic acid) (50 g/l, weight/volume). The wall residue was washed with deionized water, acetone and a methanol:chloroform mixture (1:1, volume/volume) and air dried. In this procedure, the dried cell wall material, starch, is gelatinized in boiling water and then removed by enzymatic digestion with α-amylase (Sigma). Proteins were removed by treating the residue with 200 μg/ml pronase (Actinase Kaken Kagaku Co., Tokyo, Japan) in Na-phosphate buffer (pH 6·5, 50 mM) containing 5% (v/v) ethanol for 18 h. The pectic fraction was extracted from the wall residue (cell wall) by treating with 50 mM EDTA at 95 °C for 15 min. The hemicellulose fraction was extracted with a solution of 175 g/l NaOH containing 0·2 g/l $NaBH_4$ for 18 h. The alkali-insoluble fraction was designated cellulose. The hemicellulose fraction was neutralized with glacial acetic acid. The pectic and neutralized hemicellulose fractions were dialysed against deionized water for 20 h. The total sugar content of each fraction was determined by the phenol-sulphuric acid method (Dubois *et al.* 1956). The neutral sugar composition of the MeOH soluble and hemicellulose fractions was determined by GLC, according to the method of Albersheim *et al.* (1967).

$^{13}CO_2$ feeding

A $^{13}CO_2$ feeding experiment was conducted to determine changes in the labelled assimilates in the various plant parts and in the cell wall composition for a short period (6 to 72 h after the introduction of $^{13}CO_2$). The experiment was carried out as described in Sawada *et al.* (1995). An ear leaf was exposed to $^{13}CO_2$ feeding 5 to 11 DBS. A leaf was enclosed in a transparent plastic bag in which a beaker containing 10 g of $Ba^{13}CO_3$ (99·5 atom % ^{13}C) was placed. Four millilitres of 30% $HClO_4$ was injected and $^{13}CO_2$ was evolved. The leaf was then allowed to assimilate the $^{13}CO_2$ under natural light conditions for 2 h. During $^{13}CO_2$ assimilation, the PAR was greater than 1700 μmol/m²/s. Five plants were harvested at 6, 24 and 72 h after the end of $^{13}CO_2$ assimilation. Harvested plants were immediately separated into the $^{13}CO_2$ fed ear leaf, husk leaves and other parts. Husk leaves were put into a vial containing 10 ml of 99·5% (v/v) methanol. Other plant parts were separately dried at 70 °C in a forced-air oven for 3 days. The plant parts were weighed, and ground to fine powder with a vibrating sample mill (Model T1-100 Heiko Co. Ltd., Iwaki, Fukushima, Japan).

^{13}C analysis

The ^{13}C abundance in the ground plant materials, cellulose and hemicellulose fractions was determined with a mass spectrometer (Delta plus, Finnigan, San Jose, CA, USA). The ^{13}C atom % excess in the plant sample was calculated as the difference in ^{13}C atom % between the sample and standard pure chemical, glycine. The amount of labelled C in the plant sample was calculated as described by Sawada *et al.* (1995). The ^{13}C abundance in respective sugars in MeOH and hemicellulose fractions was determined with GC/C/MS (MAT 252, Finnigan, San Jose, CA, USA).

Statistical analysis

All the experiments were conducted with 3 or 4 replications. The effect of treatments was assessed according to the expected mean squares given by McIntosh (1983). For mean separation, the treatment sum of squares was partitioned by the method of orthogonal contrasts. The coefficients of variation for all response variables were less than 10%. The standard error was calculated according to the methods described by Snedecor (1959).

RESULTS

Leaf expansion and photosynthetic rate

Husk leaf area increased rapidly after the tip portion emerged and until silking. There was a difference in the husk leaf area for the first ear in the order of X-15 > CE-78 > CM-80 (Fig. 1). The duration of increase in husk leaf area on the first ear was 4, 5 and 11 days in CM-80, CE-78 and X-15, respectively (data not shown). The rate of increase in husk leaf area was similar for X-15 and CE-78, which was greater than in CM-80 (Fig. 1). The specific leaf weight of husk leaves was almost identical in the unemerged expanding part of the husk leaf at presilking, however it was greater in CM-80 than in X-15 in the distal husk leaf part and at silking (data not shown). Whole plant dry weight did not differ significantly among genotypes but husk leaf weight was greatest in X-15, followed by CE-78 (Fig. 2). Such genotypic differences were consistent during 2 consecutive years (data not shown). Photosynthetic rate was slightly higher in the culm leaves than in the husk leaves (Fig. 2).

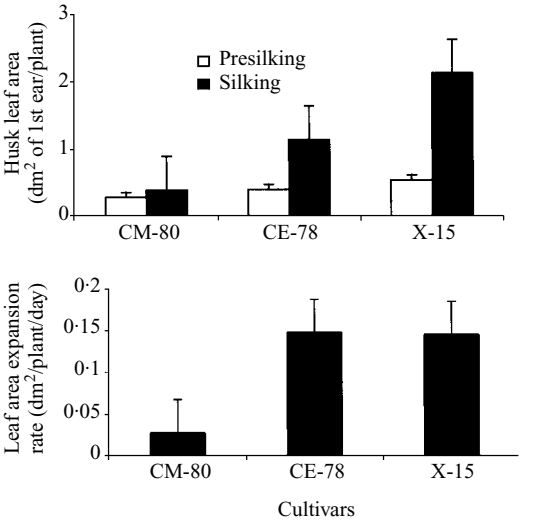

Fig. 1. Leaf area at silking and its expansion rate in flint corn genotypes differing in husk leaf size.

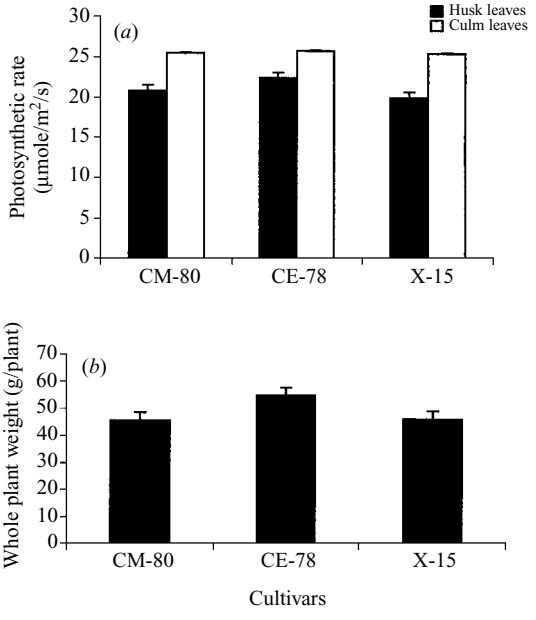

Fig. 2. Apparent photosynthetic rate of husk leaves (*a*) and whole plant dry weight (*b*) at silking in flint corn genotypes differing in husk leaf size.

Table 1. *Water potential (ψ_w), osmotic potential (ψ_s) and turgor (ψ_p) of expanding part of husk leaves at presilking from flint corn genotypes differing in husk leaf size*

Cultivar	ψ_w	ψ_s	ψ_p
		(Mpa)	
CM-80	−0·38	−0·96	0·58
CE-78	−0·97	−1·06	0·09
X-15	−0·98	−1·27	0·28
LSD (0·05)	0·31	0·19	0·18

Water potential, osmotic potential and turgor measurements

Water potential (ψ_w) was higher in CM-80 than other genotypes, while osmotic potential (ψ_s) was higher in X-15 than the genotypes (Table 1). Turgor (ψ_p) was in the order of: CM-80 > X-15 > CE-78.

Cytosol and cell wall composition

Analyses were made to associate the rapid expansion of husk leaves with cell and cell wall properties. The sugar content of husk leaves was highest in the MeOH-soluble (21–28 % relative to total sugars) and lowest in the pectin (0·7–1·5 %) fraction in the unemerged expanding part (Table 2). While the sugar content of MeOH decreased in the emerged and matured parts, WS and pectin fractions remained stable despite differences in leaf age, that of the hemicellulose and cellulose fractions recorded significant increments in the emerged parts.

The sugar content of various fractions during the rapid expansion stages varied among genotypes as indicated by the differences in the values for the matured part and that of the expanding part in Table 2. Although there was no significant difference in increase in sugar concentration in the cellulose fraction, the increase in the hemicellulose fraction was greater in X-15 than in other genotypes.

Table 2. *Sugar content of Me-OH, water soluble and cell wall fraction (mg/g fresh weight) of expanding part and matured part of the same husk leaves at presilking from flint corn genotypes differing in husk leaf size*

Cultivars	Cytosol		Cell wall		
	Me-OH	Water soluble	Pectin	Hemicellulose	Cellulose
Expanding part					
CM-80	28·3 ± 1·4	2·4 ± 0·9	0·7 ± 0·2	10·8 ± 1·4	10·4 ± 0·2
CE-78	21 ± 1·6	3·3 ± 0·6	1·5 ± 0·2	6·2 ± 0·3	4·1 ± 1·5
X-15	25·1 ± 1·0	2·9 ± 0.6	1·3 ± 0·1	6·4 ± 1·3	3·8 ± 1·5
Matured part					
CM-80	21·8 ± 0·8	6·2 ± 1·4	1·3 ± 0·3	19·3 ± 2·8	35 ± 1·2
CE-78	16·3 ± 1·5	4·4 ± 0·9	1·2 ± 0·1	14·7 ± 1·1	24·1 ± 1·4
X-15	18·5 ± 0·4	2·7 ± 0·3	1·5 ± 0·2	25·8 ± 2·1	29·4 ± 1·4

Although there was no increase in hemicellulose except in CM-80, cellulose fractions continued to increase after the leaves emerged (Table 2). The sugar content of the pectin fraction was the lowest among the various components during leaf expansion.

Constitutional sugar compositions of MeOH soluble, pectin and hemicellulose fractions

In the MeOH soluble fraction, the Glc content was generally high, but it was lower in the emerged and matured husk parts of the leaf (Table 3). The major components of the neutral sugar of pectin were Glc, Ara and rhamnose (Rha) (data not shown). The predominant sugar components of the hemicellulose fraction were Xyl, Ara and Glc (Table 3). The Xyl content was higher in the emerged and matured distal portion than in the unemerged expanding portion, however the Ara and Glc content showed no definite trend. A greater husk leaf area in X-15 tended to be associated with an increase in Xyl content during leaf area expansion.

^{13}C partitioning among cell components and constitutional sugars

The ^{13}C atom % excess in husk leaves tended to be higher than that in the other plant parts for 72 h after $^{13}CO_2$ feeding (data not shown), and was higher in X-15 than in other genotypes (Fig. 3). The excess in the hemicellulose fraction increased rapidly 6–24 h after the $^{13}CO_2$ feeding in X-15 but remained stable in other genotypes (Fig. 3). Although the ^{13}C atom % excess in the cellulose fraction of the husk leaves increased up to 72 h, it decreased in CM-80 (Fig. 3).

At presilking, the ^{13}C atom % excess was greater in Ara in the MeOH fraction 24–48 h after $^{13}CO_2$ was fed, however it remained lower in Xyl (Fig. 4). On the other hand, in the hemicellulose fraction, the ^{13}C atom % excess in Xyl and Glc was consistently higher than in Ara, and increased rapidly, attaining a peak at 48 h. At silking, the ^{13}C atom % excess in all the sugars tended to decrease with time after $^{13}CO_2$ feeding, with that in Xyl the lowest (data not shown). A similar tendency was found in the culm leaves.

The ^{13}C atom % excess in various constitutional sugars in the MeOH-soluble and hemicellulose fractions in the expanding husk leaf varied among flint corn genotypes (Fig. 4). In the MeOH-soluble fraction, it was higher in Ara than in other sugars, and remained lower in Xyl except for a temporary increase at 72 h after $^{13}CO_2$ feeding. In general, X-15 showed higher values than other genotypes except for Xyl up to 48 h. In the hemicellulose fraction, the ^{13}C atom % excess tended to be higher in Xyl, and Glc in X-15 than other genotypes, but lower in CM-80 in all the sugars.

DISCUSSION

These results suggest that husk leaf area differed among corn pure lines (Fig. 1), however, no significant difference in the culm leaf area and photosynthetic rate was found (Fig. 2) suggesting that the difference in the husk leaf area may not be due to the supply of photosynthate to developing husk leaves. This can be supported by our earlier report that defoliation of all the culm leaves just before the husk leaves emerged had no detrimental effect on the area expansion of husk leaves (Fujita *et al*. 1995).

The rapid increase in sugar content in the cellulose fraction continued even after the rate of leaf expansion slowed down (Fig. 1), suggesting that cellulose synthesis contributes not only to leaf expansion but also secondary growth of leaf.

The Xyl, Glc and Ara were predominant in the hemicellulose fraction (Table 3); these and other results suggest that the synthesis of hemicellulose which occurs in parallel to leaf expansion is closely linked to synthesis of Xyl, Glc and Ara, particularly Xyl synthesis.

The ^{13}C tracer experiments revealed that during the expansion of husk leaves, while the ^{13}C atom % excess in Xyl increased at 72 h in the MeOH-soluble fraction,

Table 3. *Sugar composition of Me-OH-soluble and hemicellulose fractions* (μg/*g fresh weight*) *of expanding part and matured part of the expanding same husk leaves at presilking from flint corn genotypes differing in husk leaf size*

	Me-OH						Hemicellulose					
Cultivars	Fuc	Ara	Xyl	Man	Gal	Glc	Fuc	Ara	Xyl	Man	Gal	Glc
Expanding part												
CM-80	38·1±2	108·6±3·1	43·9±1·9	75·2±2·5	136·5±5·6	12651·6±100	0·0±0	1757·3±96·4	7669·6±112	46·1±2·7	473·7±5·9	1147·3±88·9
CE-78	0·0±0	142·6±2·9	39·2±2	84·4±4·1	202·7±7·2	8668·9±99	24·9±2	1091·2±83·7	2577·8±213	66·0±3·1	242·1±12	739·3±35
X-15	0·0±0	170·2±1·7	45·9±4	30·7±3·1	220·6±6·3	15066·2±154	15·7±1·7	1571·2±91·6	3085·3±300	118·0±9·5	630·2±24	1116·6±355
Matured part												
CM-80	57·9±8	181·0±13	88·7±4·5	85·9±6·8	366·2±9·9	12781·3±524	0·0±0	2374·6±430	140161·1±3233	159·6±8·9	316·4±12·3	758·4±23·5
CE-78	0·0±0	132·9±11	42·9±3·8	63·6±5·5	514·8±105	8105·6±354	11·2±2	741·5±22	8667·7±121	46·7±5·7	225·5±9·8	942·6±13·7
X-15	0·0±0	55·3±5·6	22·7±0	41·4±0	461·1±0	14075·6±0	15·9±10.2	2897·2±241	17130·4±1211	105·1±10	471·3±8·7	1595·3±24·0

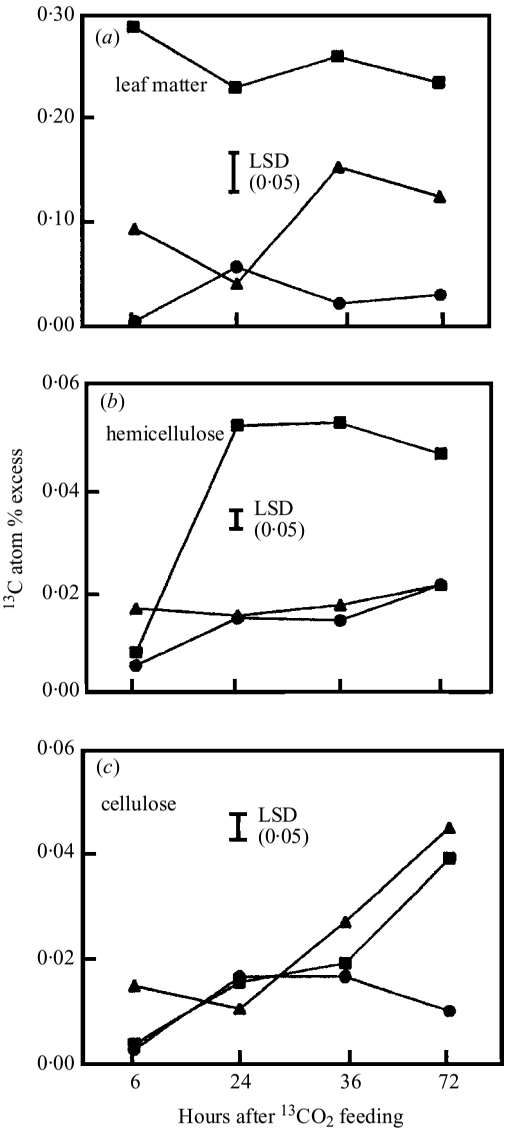

Fig. 3. ^{13}C atom % excess in (*a*) whole leaf matter, (*b*) hemicellulose and (*c*) cellulose fractions of expanding husk leaves from flint corn genotypes differing in husk leaf size. ●, CM-80; ▲, CE-78; ■, X-15.

it increased much earlier (8 h) and was maintained at a higher level in the hemicellulose fraction (Fig. 4). This result suggests that although renewal of Xyl in the cellular pool is slower, incorporation of Xyl synthesized newly into the hemicellulose in the Golgi apparatus is active. From these results, together with the phenomena showing an active synthesis of Xyl in the developed and emerged husk leaves, it can be concluded that synthesis of Xyl occurs specifically in the Golgi apparatus during leaf expansion. The

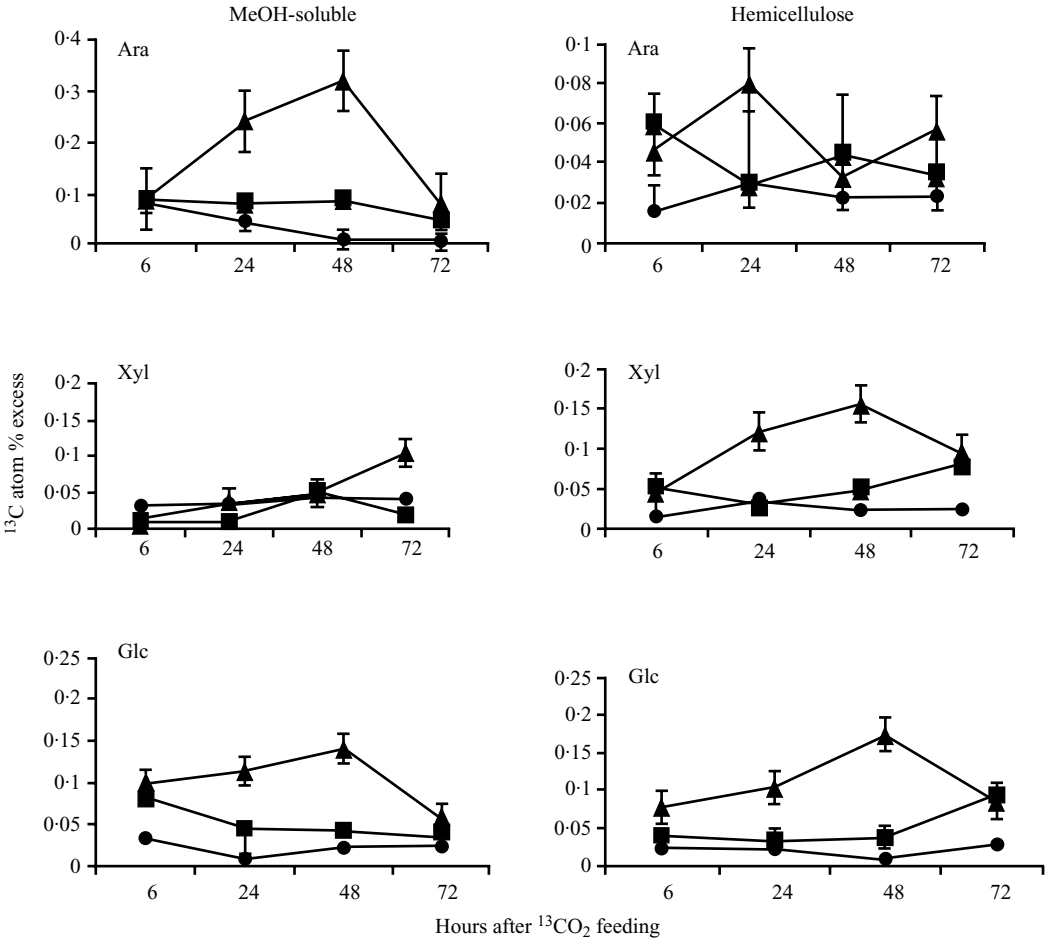

Fig. 4. ^{13}C atom % excess in sugar species of MeOH-soluble and hemicellulose fractions of expanding husk leaves from flint corn genotypes differing in husk leaf size. Ara, Arabinose; Xyl, Xylose; Glc, Glucose; ●, CM-80; ▲, X-15; ■, CE-78.

current results confirm that synthesis of UDP-xylose from UDP-glucose is conducted in Golgi membrane (Hayashi *et al.* 1988).

Although the concentration and ^{13}C atom % excess of Xyl was low in the cytosol of the expanding husk leaves, it remained high in the expanded leaves (Fig. 4). This is consistent with the finding that little Xyl is synthesized in the cellular pool in the developing leaves (Hayashi *et al.* 1988). The increase in concentration of Xyl in the cytosol fraction in the expanded leaf seems to be due to an acceleration of the release of Xyl from Golgi owing to a decrease in the requirement for synthesis of hemicellulose.

The ^{13}C labelling pattern varied depending upon the constitutional sugar species: the ^{13}C atom % excess in Gal was high after 24 h in the MeOH-soluble as well as in the hemicellulose fraction, while in Man it increased after 24 h in the former but only at 72 h in the latter fraction (data not shown). Furthermore, that in Ara was high after 6 h in the MeOH-soluble fraction but low in the hemicellulose fraction (Fig. 4). These results suggest that some sugars such as Gal, Man and Ara are produced in the cytosol, and subsequently transferred to the hemicellulose. The results of the current study support an assumption that UDP-Xyl is synthesized from UDP-Glc by UDP-glucose dehydrogenase and UDP-glucuronate decarboxylase in the Golgi membrane as reported by Hayashi *et al.* (1988) and Tenhaken & Thulke (1996).

It has been reported that cell loosening through digestion of hemicellulose by 1,3;1,4 glucanase is a prerequisite for cell elongation (Sakurai 1991). Breakdown or metabolic turnover of cell wall constituents during growth has been reported (Machet & Nance 1962). Wada *et al.* (1968), suggesting that enzymatic degradation of some hemicellulose polysaccharides, which are the major components of the cell wall matrix, possibly of β-1,3-glucan, could lead to

weakening of bonds in the cell matrix, or to relaxation of stress exerted by turgor, thus leading to elongation and growth. Sato *et al.* (1997) suggest from a ^{13}C tracer experiment that a progressive decrease in the percentage of ^{13}C in the hemicellulose fraction after feeding with the $^{13}CO_2$, is related to wall turnover, an important process in cell expansion. A similar phenomenon was observed in the current study showing a depression of ^{13}C atom % excess at 48 h after attaining the highest value in the hemicellulose fraction (Fig. 3).

It has also been reported that cell expansion, which is the basis for root extension, could be controlled by turgor pressure, wall rheology and the hydraulic conductivity of the water pathway to the growing cell wall (Lockhart 1965). Although cell turgor pressure also plays an important role by providing the driving force for cell growth (Taylor & Davies 1986; Boyer 1993; Nonami & Boyer 1993), it is generally considered that some cell wall characteristics limit the growth rate in plants (Cleland 1967; Pritchard 1987; Passioura & Fry 1992; Cosgrove 1993). The cell wall appears to control or modulate that extension (Ferris & Taylor 1994). A similar conclusion can be drawn from our results indicating no significant difference in turgor pressure during an active expansion of husk leaves among flint corn genotypes (Table 1).

In summary, our data suggest that synthesis of Xyl changes in parallel to hemicellulose synthesis, and may contribute in part to the formation of cell wall, leading to leaf expansion. From current data, it infers that X-15 is capable of producing larger husk leaves than other flint corn genotypes mainly due to a greater capability to synthesize Xyl and Ara, leading to the synthesis of hemicellulose, especially arabinoxylan.

REFERENCES

Albersheim, P., Nevins, D. J., English, P. D. & Karr, A. (1967). A method for the analysis of sugars in plant cell-wall polysaccharides by gas liquid chromatography. *Carbohydrate Research* **5**, 340–345.

Bailey, I. W. (1957). Aggregation of microfibrils and their orientations in the secondary wall of coniferous tracheids. *American Journal of Botany* **44**, 415–418.

Boyer, J. S. (1993). Temperature and growth-induced water potential. *Plant, Cell and Environment* **16**, 1099–1106.

Carpita, N. C. (1984). Fractionation of hemicelluloses from maize cell walls with increasing concentration of alkali. *Phytochemistry* **23**, 1089–1093.

Carpita, N. C. (1996). Structure and biogenesis of the cell walls of grasses. *Annual Review Plant Physiology Plant Molecular Biology* **47**, 445–476.

Cleland, R. E. (1967). Extensibility of isolated cell walls: measurement and changes during cell elongation. *Planta* **74**, 182–191.

Cleland, R. E. (1977). The control of cell enlargement. Integration of activity in the higher plant. Symposium. *Society of Experimental Biology* **31**, 101–115.

Cosgrove, D. J. (1987). Wall relaxation and the driving forces for cell expansive growth. *Plant Physiology* **84**, 561–564.

Cosgrove, D. J. (1993). How do plant cell walls extend? *Plant Physiology* **102**, 1–6.

Cosgrove, D. J. (1999). Enzymes and other agents that enhance cell wall extensibility. *Annual Review Plant Physiology Plant Molecular Biology* **50**, 391–417.

Darvill, A. G., McNeil, M., Albersheim, P. & Delmer, D. P. (1980). The primary cell walls of flowering plants. In *The Biochemistry of Plants* (Ed. N. E. Tolbert), pp. 91–162. New York: Academic Press.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–356.

Ferris, R. & Taylor, G. (1994). Elevated CO_2, water relations and biophysics of leaf extension in four chalk grassland herbs. *New Phytologist* **127**, 297–307.

Fry, S. C. (1998). Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. *Biochemical Journal* **332**, 507–515.

Fujita, K., Sato, H. & Sawada, O. (1995). Husk leaves contribution to dry matter and grain production as well as N distribution in flint corn (*Zea mays* L.) genotypes differing in husk leaf area. *Soil Science and Plant Nutrition* **41**, 587–596.

Gardner, S. D. L., Bosac, C. & Taylor, G. (1995). Leaf growth of hybrid poplar following exposure to elevated CO_2. *New Phytologist* **131**, 81–90.

Gibeaut, D. M. & Carpita, N. C. (1991). Tracing cell wall biogenesis in intact cells and plants: selective turnover and alteration of soluble and cell wall polysaccharides in grasses. *Plant Physiology* **97**, 551–561.

Hayashi, T., Koyama, T. & Matsuda, K. (1988). Formation of UDP-xylose and xyloglucan in soybean Golgi membranes. *Plant Physiology* **87**, 341–345.

Lockhart, J. A. (1965). An analysis of irreversible plant cell elongation. *Journal of Theoretical Biology* **8**, 264–275.

Machet, W. H. & Nance, J. F. (1962). Cell wall breakdown and growth in pea seedling stems. *American Journal of Botany* **49**, 311–319.

McIntosh, M. S. (1983). Analysis of combined experiments. *Agronomy Journal* **75**, 153–155.

Nonami, H. & Boyer, J. S. (1993). Direct demonstration of a growth-induced water potential gradient. *Plant Physiology* **102**, 13–19.

Passioura, J. B. & Fry, S. C. (1992). Turgor and cell expansion: beyond The Lockhart equation. *Australian Journal Plant Physiology* **19**, 565–576.

Pritchard, J. (1987). *The control of wheat root growth*. Ph.D. Thesis, University of Wales.

Sakurai, N. (1991). Cell wall functions in growth and development – A physical and chemical point of view. *The Botanical Magazine, Tokyo* **104**, 235–251.

Sato, H., Sakurai, N., Sendo, S., Saneoka, H., Nobuyasu, H. & Fujita, K. (1997). Factors affecting leaf area development in husk leaf of flint corn. *Crop Science* **37**, 1826–1831.

Sawada, O., Ito, J. & Fujita, K. (1995). Characteristics of photosynthesis and translocation of ^{13}C-labelled photosynthate in husk leaves of sweet corn. *Crop Science* **35**, 480–485.

Snedecor, G. W. (1959). *Statistical methods, 5th Edn*. Ames, Iowa: Iowa State College Press.

Taylor, G. & Davies, W. J. (1986). Yield turgor of growing leaves of *Butula* and *Acer*. *New Phytologist* **104**, 13–19.

Tenhaken, R. & Thulke, O. (1996). Cloning of an enzyme that synthesizes a key nucleotide-sugar precursor of hemicellulose biosynthesis from soybean: UDP-glucose dehydrogenase. *Plant Physiology* **112**, 1127–1134.

Wada, S., Tanimoto, E. & Masuda, Y. (1968). Cell elongation and metabolic turnover of the cell wall as affected by auxine and cell wall degrading enzymes. *Plant and Cell Physiology* **9**, 369–376.