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Developmental changes of sugar contents in the gall on the leaf of elm (*Zelkowa serrata* Makino) formed by *Paracolopha morrisoni* Baker (Homopetra)

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To understand cell wall polysaccharide synthesis and the role of gall in interaction with aphids, the changes of sugar contents in the galls during their growth and development were determined from May 2 to June 8, 1996. The sugar content in the symplastic (MeOH and hot water) fractions decreased as the developmental stages progressed. In the cell wall fraction, the amount of pectic substances (2-3 mg per gram fresh weight) did not change. The hemicellulosic substance increased by 40% from May 14 to May 31. Among the neutral sugar components of hemicellulosic polysaccharides, xylose and arabinose contents increased during development of the gall, suggesting that xylans with arabinose residues were massively synthesized. On the other hand, glucose content decreased during development of the gall. The cellulose substance consistently increased 5 folds from May 2 to 31. The relationship between the aphid and the changes in sugar contents of cell walls during the development of aphid and the gall formation was discussed.

Keywords: *Zelkova serrata*, Gall, Sugar content, Cell wall polysaccharide, *Paracolopha morrisoni*

Galls are induced by a great variety of organisms including insects, mites, nematodes, fungi, bacteria, and viruses. The association of gall with gall-inducing organisms was probably recognized in the earliest cecidological studies. However, it was not until recently that the gall development and growth were correlated with the feeding activities and nutritional requirements of gall-inducing organism. It has also been recognized that the development of a gall is contingent on the continued presence and feeding activity of the gall inducer (Mani, 1992).

The sugars of plant cells are classified into two groups, i.e., symplastic and apoplastic sugars. Not only symplastic sugars but also apoplastic sugars are metabolized extensively during growth and development in plant cells (Labavitch, 1982; Taiz, 1984; Masuda, 1990; Sakurai, 1991; Hoson, 1993). The turnovers may be tissue- or organ-specific. Among apoplastic sugars, cellulose is the major constituent and plays an important role in the regulation of cell shape and rigidity. Massive synthesis of cellulose takes places during plant growth and development, although the mechanism of cellulose synthesis and its regulation remain unclear and in dispute (Bacic *et al.*, 1987; Delmer, 1988; Delmer *et al.*, 1993; Okuda *et al.*, 1993; Albersheim *et al.*, 1997).

To understand not only the mechanism of cell wall polysaccharide synthesis but also the role in interaction between gall and aphid, the changes in symplastic and apoplastic sugar contents of the gall during its development were determined.

MATERIALS AND METHODS

Plant materials

The elm tree (*Zelkova serrata* Makin), which is growing on the field at Chonbuk National University and observed to have frequent occurrence of gall induced by pemphigid aphids (*Paracolopha morri-*

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soni Baker) on the young leaves, was selected. The galls and leaves were sampled on May 2, 7, 14, 31, and June 8, 1996. The aphids were removed from the galls. The galls were immediately weighed one g, and fixed in 10 ml methanol at 65°C for 15 min.

Fractionation of symplastic and apoplastic sugars

The gall (1 g fr wt) sample was fractionated by the modified method of Sakurai *et al.* (1987). The sample stored in MeOH was centrifuged for 10 min at 1,000 g. The extract in supernatant was designated as symplastic, MeOH fraction. This fraction contains mono- and oligosaccharides such as glucose, fructose, and sucrose (Wakabayashi *et al.*, 1991). The residue was rehydrated with deionized water for 10 min and homogenized in deionized water with a mortar and pestle. The homogenate was boiled for 10 min, then washed twice with deionized water by a centrifugation for 10 min at 1,000 g. The supernatant was designated as symplastic, hot water (HW) fraction. This fraction contains polysaccharides of non-cell wall components such as starch and arabinogalactan (Fincher and Stone, 1983).

The portion (200 mg) of the residue was treated with 2 ml (10 unit) of porcine pancreatic α-amylase solution (Type I-A; Sigma, St. Louis, Mo, USA) in 50 mM sodium acetate buffer (pH 6.5) for 2 h at 37°C to remove symplastic, contaminated starch. Crude cell-wall materials were washed three times with deionized water. Pectic substances were extracted three times for 15 min with 50 mM EDTA in 50 mM sodium phosphate buffer (pH 6.8) at 95°C. Next, hemicellulosic substances were extracted for 18 h at 25°C with 17.5% NaOH containing 0.02% $NaBH_4$. In a previous study (Yeo *et al.*, 1995), the residue was found to contain appreciable amounts of neutral sugars other than glucose. Therefore, the residue was further hydrolyzed with 2 ml of 2 M TFA (trifluoroacetic acid) for 1 h at 121°C in a screw-capped tube. The TFA-soluble fraction was collected by centrifugation for 10 min at 1,000 g. The residue was washed twice with 2 ml of deionized water. The supernatant was combined with the TFA fraction. The TFA-insoluble fraction was washed three times with a mixture of ethanol and ether (1:1, v/v) and dried for one day at 25°C and for two days at 40°C. The dried material was designated as cellulose fraction.

Measurement of sugar contents

Total sugar contents of each fraction was determined by a phenol-sulfuric acid assay (Dubois *et al.*, 1956). Before determination, the cellulose fraction was hydrolyzed with 7.5 M H_2SO_4 for 2 h in ice bath and 1 M H_2SO_4 for 1 h at 100°C. Data from one experiment with triplicated samples are given.

Analysis of neutral sugar components of TFA fraction

The neutral sugar components of TFA fraction was analyzed with a gas liquid chromatography. A portion of TFA hydrolysate was placed in a screw-capped tube and dried with a stream of filtered air at 50 °C. One ml of 2 M TFA containing 300 μg of *myo*-inositol as a standard was added to the tube. The tube was autoclaved for 1 h at 121°C. Hydrolyzed monosaccharides were reduced by $NaBH^4$ and acetylated with acetic anhydride in the presence of 1-methylimidazole as a catalyst (Blakeney *et al.*, 1983). Acetylated monosaccharides were dissolved in 200 μL of acetone and introduced into a gas liquid chromatography system (M600D, Young-Lin Instrument Co., LTD.) equipped with a capillary column (SP-2380, Supelco, Park, Bellefonte, PA. 16823 USA). Data from one experiment with three determinations are given.

RESULTS AND DISCUSSION

Aphid inhabitants in galls

The changes of pemphigid aphid (*Paracolopha morrisoni* Baker) inhabitants in the gall is shown in Table 1. Only 1st instar larvae of fundatrix were found on May 2. This stage seems to be a gall forming period. Five days later, on May 7, fundatrix adults began to appear. On May 14, most of inhabitants were fundatrix adults with a few fundatrix 3rd instar and emigrant larvae. It seems a growth period of the second generation (emigrant) lasts until May 31 and we assumed that aphids depended mostly on nutrients from the galls. On June 8, emigrant alates (final stage of emigrant) appeared in the brown galls.

Developmental changes of symplastic sugar contents

More than one galls were formed on the vein of young leaves in the beginning of May. The leaf and gall were greening in developmental stages. Fig. 1 shows the changes in total sugar contents of symplastic, MeOH and hot water (HW) fractions of the gall during its development. The sugar content in MeOH fraction decreased from May 2 to 14, whereas, that of hot water fraction decreased more sharply after May 7. However, the sugar contents (18.0±0.6 mg per gram fresh weight) of MeOH fractions of the

Table 1. Number of aphid (*Paracolopha morrisoni* Baker) inhabitants during gall development of elm (*Zelkowa serrata* Makino)

Morph	Stadium	Date of observation				
		May 2	May 7	May 14	May 31	June 8
1st generation (Fundatrix)	L1	4 (100)	8 (30.8)			
	L2		9 (34.6)			
	L3		7 (26.9)	1 (5.9)		
	Adult		2 (7.7)	11 (64.7)	25 (83.3)	8 (22.2)
2nd generation (Emigant)	Larvae			5 (29.4)	25 (83.3)	23 (63.9)
	Alate					23 (63.9)
No. of gall sampled		4	26	17	30	36

L1, first instar larva; L2, second instar larva; L3, third instar larva.
Numbers in parentheses represent the percentages of stadium appeared in the sampled galls.
Mixed life stages of aphid were found in some of the galls.

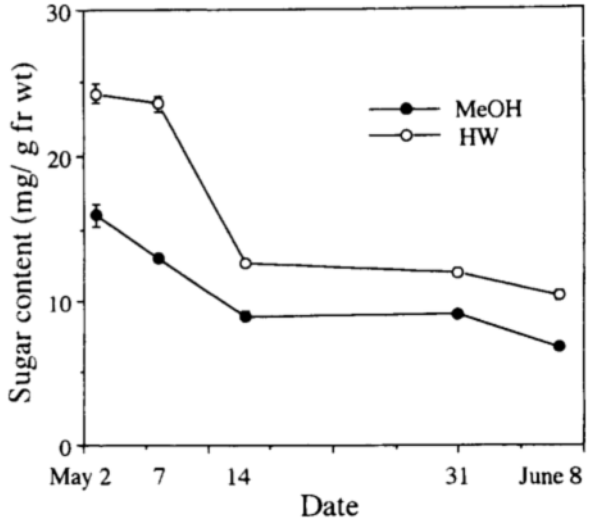

Fig. 1. Changes in sugar contents of symplastic (MeOH and HW) fractions of the galls at different developmental stages. Bars represent SE (n=3).

leaf was similar to that (18.6±0.4 mg) of HW fractions on May 2 and the content (25.6±1.8 mg) increased and that (14.1±0.8 mg) of HW fractions decreased on June 8. These results indicate that the symplastic sugars of the gall were used either for the precursors of cell wall polysaccharides or foods of the aphid.

Changes in apoplastic sugar contents during gall development

Table 2 shows changes in total sugar contents of apoplastic, pectin, hemicellulose, TFA and cellulose fractions of the gall and leaves during their development. Pectin consisted of less than 10% of cell wall polysaccharides in gall and leaf, and the amount remained constant during the gall development. Contents of hemicellulose and TFA fractions in the galls commenced to increase from May 14. Since TFA fraction consisted of heterogeneous polysaccharides (see below), it should belong to hemicellulosic fraction. Sum of the hemicellulose and TFA fraction increased by 50% from May 14 to 31. Leaf also contained higher level of hemicellulose and TFA fraction on June 8 than on May 2. Cellulose content consistently increased 5 folds from the beginning of May till the end of May.

Fig. 2 shows changes in the percentage of total sugar contents of apoplastic, cell-wall fractions of the galls during its development. The percentage of pectic substance, having 2-3 mg per gram fresh weight, did not change. The percentage of TFA-soluble substances gradually decreased. Hemicellulosic substances increased till May 7, and then decreased. Relative amount of cellulose consistently increased till the end of experiment and reached 40% of the total sugar content. It is generally accepted that the plant cell walls that develop during the proliferation and elongation of cells are primary cell walls, while those that are synthesized after the cessation of cell elongation are secondary cell walls and, moreover, that the secondary cell walls are often rich in cellulose (Heigler, 1985). Transient increase in hemicelluloses found in the beginning of experiment apparently corresponds to an active turnover of hemicelluloses in primary wall during rapid development of galls (Table 2 and Fig. 2).

The changes in the neutral sugar components of TFA fraction

To determine whether or not there was an increase or a decrease in a specific neutral sugar components of hemicellulosic fraction, the monomeric sugars of the TFA fractions belonging to hemicellulose sub-

Table 2. Changes in sugar contents of cell wall fractions during development of galls formed by pemphigid aphids (*Paracolopha morrisoni* Baker) on leaf of elm (*Zelkowa serrata* Makino)

Tissue	Harvest Day	Cell Wall Fraction				
		Pectin	Hemicellulose	TFA	Cellulose	Total
			mg (g fr wt)$^{-1}$			
Gall	May 2	2.9±0.1	22.7±0.1	17.0±0.2	8.1±0.2	50.7±0.3
	May 7	3.0±0.1	28.3±0.8	13.5±0.2	11.8±0.2	56.6±0.9
	May 14	2.1±0.1	22.7±0.4	17.3±0.5	23.6±0.4	65.7±0.8
	May 31	2.4±0.1	36.7±0.9	23.5±0.6	41.4±0.4	104.0±1.2
	June 8	2.3±0.1	35.4±0.3	20.5±0.4	37.9±0.5	96.1±0.7
Leaf	May 2	3.8±0.1	18.6±0.2	14.0±0.2	13.0±0.3	49.4±0.4
	June 8	2.9±0.1	28.8±0.7	21.5±0.3	31.5±0.1	84.7±0.8

Sugar contents of each fraction were determined by a phenol-sulfuric acid assay.
The means and S.E. of triplicates with three measurements are shown in each case.

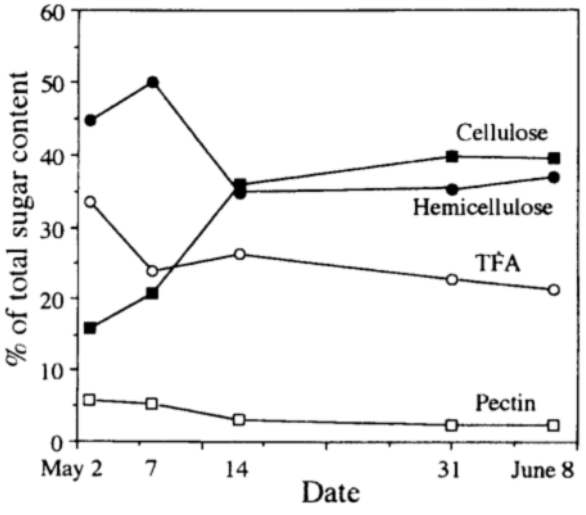

Fig. 2. Changes in total sugar contents of apoplastic (cell wall) fractions of the galls at different developmental stages.

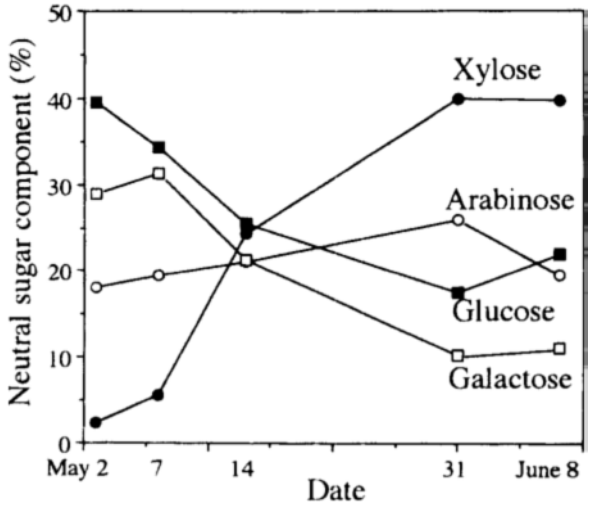

Fig. 3. Changes in neutral sugar components (%) of hemicellulosic TFA fractions during gall devel opment.

stances were analyzed (Fig. 3). The components which comprised over 10% of the total neutral sugars in the early developmental stage (May 2) were glucose, galactose, arabinose, and xylose in a descending order, but in the late developmental stage (May 31), xylose was the major component of neutral sugars, suggesting that xylan was accumulated in the cell wall as the gall developed. The data also imply that xylan is difficult to be extracted even concentrated alkaline solution used for a common extraction method for hemecellulose (Pazur, 1986). A low level of xylose is indicative of young cell walls, while a high level of xylose and a low level of glucose in non-cellulosic polysaccharides seems to be indicatives of old cell walls (Sakurai, 1991).

In conclusion, the symplastic and apoplastic sugar contents of the gall changed drastically during its growth and development. In the early developmental stage, symplastic sugar content of HW fraction of the gall was higher than that of the leaf, while the apoplastic sugar content was less than that of the leaf (Table 2). In the late developmental stage, the symplastic sugar content of the gall was much less than that of the leaf, while the apoplastic sugar content, especially hemicelluloses and cellulose was higher than that of the leaf. Among apoplastic sugars, the pectic substance did not change and seems to be not associated with gall development. Transient increase in hemicelluloses content implies that an active development of gall tissue is associated with a massive synthesis and breakdown of hemicelluloses in the cell walls as in the case of growth process of young

seedlings of dicot and monocot (Sakurai, 1991). Cellulose substance increased in the developmental stage, probably being accompanied by the rigidity of the secondary cell walls.

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