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# SUGAR COMPOSITION OF POLLEN GRAIN AND POLLEN TUBE CELL WALLS

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**Key Word Index**—*Camellia japonica*; *C. sasanqua*; *C. sinensis*; Theaceae; *Tulipa gesneriana*; *Lilium longiflorum*; Liliaceae; pollen grain wall; pollen tube wall; pollen growth.

**Abstract**—The sugar composition of pollen grain and pollen tube cell walls was studied for *Camellia japonica*, *C. sasanqua*, *C. sinensis*, *Tulipa gesneriana* and *Lilium longiflorum*. In all species, the main components of pollen grain walls were arabinose, galactose, glucose and uronic acid. On the other hand, the pollen tube walls consisted mostly of glucose. The pollen tube wall of *C. japonica* was fractionated into hemicellulose, α-cellulose and pectic substance fractions in yields of 61, 19 and 3%, respectively. The hemicellulose fraction was composed essentially of glucose. The sugar composition of the pollen tube wall was not influenced by the nature of exogenously supplied sugars. Rapid growth of the pollen tube seemed to correlate with the synthesis of hemicellulosic glucan.

## INTRODUCTION

Alterations in cell wall components relate closely to growth and differentiation in plants. Based on studies of the chemical composition, a model has been proposed for the structure of primary cell walls [1]. The pollen tube is perhaps the most rapidly growing cell in the plant world and one of the best materials for study of cell growth. The cell wall components of pollen tube have been studied only in *Lilium longiflorum* [2, 3], although those of pollen grain have been reported for some species [4–7]. Pollen growth *in vitro* is influenced by exogenously supplied sugars, the effect depending on the nature of the exogenous sugars [8, 9] and the plant species [10].

In this paper, the sugar composition of pollen grain and pollen tube cell walls was compared among five angiosperms, and the tube wall polysaccharides of *Camellia japonica* pollen were studied by fractionation and GC analysis.

## RESULTS AND DISCUSSION

The sugar composition of pollen grain walls has been reported for some gymnosperms [4–7] and angiosperms [4]. Table 1 shows the results of GC analysis of the sugars of pollen grain walls from five species of angiosperms. In all, the levels of arabinose, galactose, glucose and uronic acid (probably galacturonic acid) were higher than those of the other sugars, and a large amount of *myo*-inositol was detected in *C. japonica*, *C. sasanqua* and *L. longiflorum*. However, the ratio of these components differed among the species. Fucose was detected only in *T. gesneriana* pollen. In most of the specimens, unidentified weak or moderate peaks were found on GC: two between rhamnose and arabinose and one between arabinose and xylose peaks. Ribose has been found in the pollen grain walls of *L. lancifolum*, *Cucurbita maxima* and *Pinus thunbergii* [4], but we did not detect it in any of the pollens examined.

The pollen grain wall is composed of two layers, the intine and the exine. Since the exine is resistant to acid hydrolysis, the sugars detected probably originated from the intine. The intine is usually differentiated into microfibrillar (cellulosic) and pectin-rich zones and our analyses (Table 1) reflect the presence of these two components.

Table 2 shows the sugar composition of pollen tube walls from the same five species. In all, the main component was glucose and its level was more than 70% of the total sugars measured. The pentose and uronic acid levels of the pollen tube wall were lower than those of the grain wall. The sugar composition of the pollen tube wall has been reported only for *L. longiflorum* [2]. Our results were consistent with those data, except for the pentose levels. In *C. sinensis* and *T. gesneriana* pollen tube walls, pentoses and mannose were higher than in the others. This may result from inclusion of the grain wall in these specimens (see Experimental).

Electron microscopic studies on pollen germination [11, 12] suggest that the pollen tube wall is newly synthesized at the inner side of the intine, and that the new wall breaks through the grain wall and the tube growth is not a simple elongation of the intine. The fact that the pollen tube wall differed chemically from the pollen grain wall supports the above suggestion. It should be pointed out that Heslop-Harrison [13] takes a contrasting view and believes that the pollen tube wall is an elongation of one layer of the intine, at least in grasses and in *Crocus*.

The pollen tube growth depends on the nature of the exogenously supplied sugars [9, 10]. Therefore, the sugar composition of pollen tube wall may be influenced by exogenous sugars. The growth of *C. japonica* pollen tube is most rapid on a sucrose medium and is promoted significantly by the addition of bovine serum albumin (BSA) to various sugar media [10]. Maltose and cycloheximide (CHI) inhibit tube growth but not the germination of *C. japonica* pollen [9, 10]. The effects of

Table 1. Sugar composition of pollen grain walls

| Sugar | Amount, mg (% total) | | | | |
|---|---|---|---|---|---|
| | *Camellia japonica* | *Camellia sinensis* | *Camellia sasanqua* | *Tulipa gesneriana* | *Lilium longiflorum* |
| Rhamnose | 0.053 ( 3.4) | 0.055 ( 6.1) | 0.103 ( 7.5) | 0.195 ( 5.9) | 0.055 ( 5.7) |
| Fucose | nd | nd | nd | 0.060 ( 1.8) | nd |
| Arabinose | 0.188 (12.2) | 0.161 (17.8) | 0.303 (22.1) | 1.285 (38.9) | 0.118 (12.3) |
| Xylose | 0.022 ( 1.4) | 0.027 ( 3.0) | 0.038 ( 2.8) | 0.080 ( 2.4) | 0.118 (12.3) |
| Mannose | 0.075 ( 4.9) | 0.067 ( 7.4) | 0.038 ( 2.8) | 0.045 ( 1.4) | 0.013 ( 1.4) |
| Galactose | 0.193 (12.5) | 0.160 (17.7) | 0.215 (15.7) | 0.310 ( 9.4) | 0.113 (11.8) |
| Glucose | 0.300 (19.4) | 0.185 (20.5) | 0.200 (14.6) | 0.300 ( 9.1) | 0.200 (20.9) |
| Me-β-glucoside | 0.104 ( 6.7) | 0.021 ( 2.3) | 0.052 ( 3.8) | 0.045 ( 1.4) | 0.054 ( 5.6) |
| *myo*-Inositol | 0.376 (24.4) | 0.044 ( 4.9) | 0.233 (17.0) | 0.220 ( 6.7) | 0.222 (23.1) |
| Uronic acid | 0.232 (15.0) | 0.183 (20.3) | 0.190 (13.8) | 0.760 (23.0) | 0.066 ( 6.9) |
| Total | 1.543 (100.0) | 0.903 (100.0) | 1.372 (100.0) | 3.300 (100.0) | 0.959 (100.0) |
| Wall sample, mg (% hydrolysis) | 15.8 (81.6) | 15.2 (31.9) | 30.2 (29.2) | 23.5 (33.1) | 28.4 (80.3) |

nd: Not detected.

Table 2. Sugar composition of pollen tube walls

| Sugar | Amount, mg (% total) | | | | |
|---|---|---|---|---|---|
| | *Camellia japonica* (12.0 ± 0.5)* | *Camellia sinensis*† (7.5 ± 0.5) | *Camellia sasanqua* (12.8 ± 0.3) | *Tulipa gesneriana*† (4.5 ± 0.3) | *Lilium longiflorum* (6.0 ± 0.4) |
| Rhamnose | 0.060 ( 0.4) | 0.103 ( 2.3) | 0.033 ( 0.7) | 0.105 ( 2.4) | 0.020 ( 0.7) |
| Fucose | 0.020 ( 0.1) | 0.052 ( 1.2) | nd | 0.025 ( 0.5) | nd |
| Arabinose | 0.360 ( 2.4) | 0.270 ( 6.1) | 0.183 ( 3.7) | 0.713 ( 16.2) | 0.100 ( 3.6) |
| Xylose | 0.050 ( 0.3) | 0.052 ( 1.2) | 0.015 ( 0.3) | 0.100 ( 2.3) | 0.018 ( 0.7) |
| Mannose | 0.050 ( 0.3) | 0.062 ( 1.4) | 0.030 ( 0.6) | 0.010 ( 0.2) | 0.015 ( 0.5) |
| Galactose | 0.390 ( 2.6) | 0.640 ( 14.5) | 0.145 ( 2.9) | 0.230 ( 5.2) | 0.185 ( 6.7) |
| Glucose | 13.450 ( 90.8) | 3.000 ( 67.9) | 4.300 ( 87.5) | 3.000 ( 68.4) | 2.175 ( 79.3) |
| Methyl-β-glucoside | 0.091 ( 0.6) | 0.045 ( 1.0) | 0.041 ( 0.8) | 0.046 ( 1.0) | 0.055 ( 2.0) |
| *myo*-Inositol | 0.349 ( 2.4) | 0.192 ( 4.3) | 0.169 ( 3.4) | 0.160 ( 3.6) | 0.174 ( 6.3) |
| Total | 14.820 (100.0) | 4.416 (100.0) | 4.916 (100.0) | 4.389 (100.0) | 2.742 (100.0) |
| Wall sample, mg (% hydrolysis) | 20.9 ( 89.0) | 10.1 ( 80.2) | 24.6 ( 33.7) | 21.7 ( 53.9) | 18.5 ( 40.0) |

*Pollen was incubated on a sucrose (0.3 M)–agar (1.2%) medium at 25°. Numbers in parentheses indicate the average pollen tube length (mm) and standard deviation.

†Including pollen grain wall (see Experimental).

nd: Not detected.

these substances on the sugar composition of *C. japonica* pollen grown on a sucrose medium with or without BSA and on a glucose medium, where the pollen tube growth was excellent, are recorded in Tables 2 and 3. When the growth of *C. japonica* pollen tube was limited by the presence of CHI or maltose, or by the absence of exogenously supplied sugar, the level of glucose in the pollen tube wall decreased, while those of pentoses increased. The increased pentose levels seem to be due to the presence of the pollen grain wall in these specimens. However, since the glucose level in the tube wall preparations was higher than in the pollen grain wall (Table 1), the tube wall evidently became richer in glucose with growth of the tube.

Fractionation of the tube wall polysaccharides of *C. japonica* gave pectic substances, hemicellulose and α-cellulose in yields of *ca* 3, 61 and 19%, respectively (Table 4). The main sugars of the pectic substances were arabinose, galactose, glucose and uronic acid, and those of α-cellulose were glucose and uronic acid. Hemicellulose consisted mostly of glucose suggesting the presence of a homoglucan in the pollen tube wall. This hemicellulosic glucan appears to be callose, which has been demonstrated cytochemically to form an inner lining of

Table 3. Sugar composition of the tube walls of *Camellia japonica* pollen grown in various conditions

| Sugar | Amount, mg (% total) | | | | |
|---|---|---|---|---|---|
| | Medium | | | | |
| | 0.3 M sucrose + BSA (17.0 ± 0.6)* | 0.3 M glucose (11.4 ± 0.5) | 0.3 M glucose + CHI† (<0.1) | 0.01 M maltose† (1.5 ± 0.2) | None† (3.0 ± 0.2) |
| Rhamnose | 0.070 ( 0.8) | 0.035 ( 0.3) | 0.094 ( 4.7) | 0.075 ( 4.7) | 0.156 ( 6.3) |
| Fucose | nd | 0.015 ( 0.1) | nd | nd | nd |
| Arabinose | 0.365 ( 4.1) | 0.225 ( 2.2) | 0.278 ( 14.0) | 0.216 ( 13.5) | 0.438 ( 17.6) |
| Xylose | 0.030 ( 0.3) | 0.020 ( 0.2) | 0.015 ( 0.8) | 0.023 ( 1.4) | 0.044 ( 1.8) |
| Mannose | 0.020 ( 0.2) | 0.050 ( 0.5) | 0.044 ( 2.2) | 0.052 ( 3.2) | 0.058 ( 2.3) |
| Galactose | 0.345 ( 3.9) | 0.270 ( 2.7) | 0.190 ( 9.5) | 0.241 ( 15.0) | 0.466 ( 18.8) |
| Glucose | 7.100 ( 80.6) | 8.400 ( 83.0) | 1.130 ( 56.7) | 0.688 ( 42.9) | 0.835 ( 33.6) |
| Me-β-glucoside | 0.109 ( 1.2) | 0.064 ( 0.6) | 0.007 ( 0.4) | 0.041 ( 2.6) | 0.046 ( 1.9) |
| *myo*-Inositol | 0.444 ( 5.0) | 0.510 ( 5.0) | 0.168 ( 8.4) | 0.188 ( 11.7) | 0.265 ( 10.7) |
| Uronic acid | 0.322 ( 3.7) | 0.532 ( 5.3) | 0.066 ( 3.3) | 0.079 ( 4.9) | 0.177 ( 7.1) |
| Total | 8.805 (100.0) | 10.121 (100.0) | 1.992 (100.0) | 1.603 (100.0) | 2.485 (100.0) |
| Wall sample, mg (% hydrolysis) | 12.1 ( 91.7) | 21.1 ( 90.0) | 6.3 ( 43.8) | 4.9 ( 57.2) | 5.1 ( 85.4) |

*Average pollen tube length (mm) and standard deviation.
†Includes pollen grain wall (see Experimental).
BSA: Bovine serum albumin (5 mg/10 ml medium); CHI: cycloheximide ($5 \times 10^{-5}$ M); nd: not detected.

Table 4. Sugar composition of the pollen tube wall fractions of *Camellia japonica*

| Sugar | Amount, mg (% total) | | |
|---|---|---|---|
| | Fraction | | |
| | Pectic substance (3.0)* | Hemicellulose (61.0) | α-Cellulose (18.8) |
| Rhamnose | 0.505 ( 7.1) | 0.023 ( 0.1) | 0.133 ( 1.2) |
| Arabinose | 2.110 ( 29.8) | 0.125 ( 0.6) | 0.425 ( 3.9) |
| Xylose | 0.069 ( 1.0) | 0.025 ( 0.1) | 0.039 ( 0.4) |
| Mannose | 0.014 ( 0.2) | 0.037 ( 0.2) | 0.029 ( 0.3) |
| Galactose | 1.370 ( 19.3) | 0.120 ( 0.6) | 0.350 ( 3.3) |
| Glucose | 0.940 ( 13.3) | 19.200 ( 96.8) | 7.000 ( 65.0) |
| Me-β-glucoside | 0.025 ( 0.4) | 0.052 ( 0.3) | 0.011 ( 0.1) |
| *myo*-Inositol | 0.076 ( 1.1) | 0.192 ( 1.0) | 0.056 ( 0.5) |
| Uronic acid | 1.975 ( 27.9) | 0.060 ( 0.3) | 2.719 ( 25.3) |
| Total | 7.084 (100.0) | 19.834 (100.0) | 10.762 (100.0) |
| Wall fraction sample, mg (% hydrolysis) | 10.0 ( 87.0) | 20.0 (100.0) | 20.0 ( 79.5) |

*Yield(%) from the pollen tube wall.

the pollen tube wall [14, 15]. The pollen tube grows by deposition of wall materials at its apex. Rapid growth of the pollen tube appears to relate mainly to active synthesis of hemicellulosic glucan.

## EXPERIMENTAL

*Materials. Lilium longiflorum* pollen grains were collected from fresh open flowers and used immediately. Pollen grains of *Camellia japonica*, *C. sinensis*, *C. sasanqua* and *Tulipa gesneriana* were collected similarly but stored over Si gel at −15° before use [9].

*Preparation of pollen grain and pollen tube cell walls.* $Me_2CO$-treated pollen grains suspended in $H_2O$ were disrupted in a French press (1300–1500 kg/cm²) or a Potter teflon homogenizer. The homogenate was centrifuged (8000 rpm, 10 min) and the ppt. was collected. This procedure was repeated 3×. To remove starch, the ppt. was incubated with α-amylase (Type I-A, Sigma; 2.5 mg/ml) for 20 hr at 30° and washed with

$H_2O$ 5 ×. The ppt. was washed with EtOH and $Et_2O$ and dried *in vacuo* to use as a pollen grain wall prepn.

Pollen grains were sown in a straight line on sugar–agar media and incubated for 20–24 hr at 25°. Pollen tubes growing at 90° to the pollen grain-line were collected by cutting with a razor blade and the pollen tube walls prepared as above.

The germinating grains with a tube length below 0.1 mm, or those obtainable only in small quantities, were difficult to separate into tube and grain. Hence, the intact sample was washed with $Me_2CO$, dried *in vacuo*, and after sieving (No. 0.105 m/m) to remove ungerminated grains, employed to prepare 'the tube wall' (Tables 2 and 3).

*Fractionation of pollen wall polysaccharides.* According to the method of ref. [16], the pollen tube wall of *C. japonica* (1.720 g) was extracted consecutively with 0.25% ammonium oxalate–oxalate and 20% KOH to obtain fractions of pectic substances, hemicellulose and α-cellulose.

*Hydrolysis of wall materials and analysis of sugars.* Pollen wall materials were hydrolysed in 2 N TFA according to the method of Albersheim *et al.* [17]. The % hydrolysis was calculated from the dry wt of the sample used and the residue after hydrolysis. The hydrolysate was treated with $NaBH_4$ and then acetylated with $C_5H_5N$–$Ac_2O$ (1:1). The acetates were dissolved in $CH_2Cl_2$ and analysed by GC with $N_2$ at 40 ml/min on a glass column (100 × 0.3 cm) packed with 3% ECNSS-M on Gas Chrom Q at 180°. Uronic acid (as galacturonic acid) in the acid hydrolysate was determined by the carbazole method [18].

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