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NEUTRAL SUGAR COMPOSITION OF POLLEN TUBE WALLS OF LILIUM LONGIFLORUM

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SUMMARY

The tube walls of lily grown *in vivo* (in the stylar canal) contain more neutral sugars than the walls of pollen grains and the cell walls of roots. Pollen tubes grown *in vitro* are intermediate. Besides neutral sugars, β-1,3- and β-1,4-glucans, uronic acids and proteins are found. *In vivo* grown tube walls contain much more arabinose, galactose and glucose, and much less xylose than pollen grain walls and root walls. The *in vitro* grown pollen tube walls are slightly lower in arabinose, galactose and glucose, and higher in xylose content than *in vivo* grown pollen tubes. The ratio glucose/arabinose is much higher in the walls of *in vitro* grown pollen tubes, than in roots, pollen grains or *in vivo* grown pollen tubes.

No difference was found in the sugar composition between compatible and incompatible lily pollen tube walls.

Some differences were found between the wall composing neutral sugars of pollen tube walls of the cultivars (Mount Everest and Arai 5), indicating genotype controlled tube wall composition.

1. INTRODUCTION

The analysis of pollen tube walls up till now was done with material of *in vitro* germinated pollen grains (NAKAMURA & SUZUKI 1981, VAN DER WOUDE et al. 1971, NAKAMURA et al. 1980). From morphological studies (ROSEN et al. 1964; ROSEN 1971; KROH 1967) there is some evidence that there are differences in the wall fine structure between *in vitro* and *in vivo*-grown pollen tubes. From X-ray diffraction studies (HERTH et al. 1974) there is evidence for the presence of both β-1,3- and β-1,4-glucans in pollen tube walls of lily. In continuation of our investigation on the chemical composition of *in vivo* grown pollen tubes in lily (LI et al. 1983; LI & LINSKENS 1983) we looked for the sugar composition of pollen tube walls in comparison with ungerminated grains, *in vitro* grown pollen tubes, and with root walls.

2. MATERIAL AND METHODS

Plant material: incubation of self- and cross-pollinated lily pollen tubes and isolation of the tube walls are the same as described earlier (LI & LINSKENS 1983). Collection of lily roots, incubation of *in vitro* lily pollen tubes and isolation of the wall materials are the same as reported earlier (LI et al. 1983). All the wall preparations were negative to iodine test (I-KI solution) and can be supposed to have no contamination of starch.

Determination of neutral sugar composition of the walls: 10 mg of the dried walls were hydrolyzed in 1 ml of 2 N trifluoroacetic acid, with 0.5 mg of *myo*-inositol as an internal standard, in a sealed tube at 121 °C for 1 h. After hydrolysis the soluble portion was evaporated to dryness at 50 °C under a stream of N_2. The sugar mixture obtained from hydrolysis was reduced to the corresponding alditols with sodium borohydride in 0.5 ml 1 N ammonia at room temperature for 1 h. The excess of borohydride was decomposed by dropwise addition of glacial acetic acid until effervescence had ceased (ALBERSHEIM et al. 1967). The alditols were then acetylized in pyrridine-acetic anhydride (1 : 1) in a sealed vial at 100 °C for 2 h. The acetylation reagent was then evaporated and the alditol acetate mixture was dissolved in ethyl acetate. Gas liquid chromatography was carried out according to Klok (KLOK et al. 1981) with some modification. A Varian 2700 gas chromatograph was used, equipped with a glass capillary column (25 m × 0.25 mm I.D.) coated with OV-275 (Chrompack, Middelburg, The Netherlands). The temperature was programmed as following: 190 °C for 2 min, from 190 to 220 °C at the rate of 2 °C per min and then remained at 220 °C for 6 min. The temperature of the injector and the flame ionization detector was 250 °C. The carrier gas was N_2 at a flow-rate c. 1.5 ml per min. The split ratio was 1:20 and the attenuator was set at 1.10^{-11} amps.mv^{-1}. All the data were processed with a Shimadzu CR-1 integrator. Identification of the acetates was based on the retention times of the individual alditol acetates, purchased from Applied Science Laboratories Inc. U.S.A.

3.. RESULTS

3.1. Comparison of the composition of pollen tubes, pollen grains and root cell walls

After acid hydrolysis with trifluoroacetic acid different amounts of neutral sugars were liberated from the cell walls of roots, from ungerminated pollen grains and from *in vitro* and *in vivo* germinated pollen tubes of *Lilium longiflorum* (*table 1*). The highest percentage was found in pollen tube walls with a maximum being more than one third of the dry weight for pollen tube walls grown *in vivo*. In all samples 8 different neutral sugars could be detected: arabinose (ara), fucose (fuc), galactose (gal), glucose (glu), mannose (man), rhamnose (rha), ribose (rib) and xylose (xyl).

Table 1. Weight percentages of neutral sugars in cell walls.

Cell walls	Percent content of neutral sugars in the walls (%)
roots	17
pollen grains	9
in vitro pollen tubes	19–24
in vivo pollen tubes	34–39

Table 2. Neutral sugar composition of lily roots, pollen grains and *in vitro* pollen tube walls, with standard deviation.

Sugar	Roots of 'Arai 5'		Ungerminated pollen grains of 'Arai 5'		'Mount Everest' pollen tube walls		'Arai 5' pollen tube walls	
	($\mu g.mg^{-1}$)	(%)	($\mu g.mg^{-1}$)	(%)	($\mu g.mg^{-1}$)	(%)	($\mu g.mg^{-1}$)	(%)
(1) Rha	2.51 ± 0.17	1.50 ± 0.10	4.11 ± 0.43	4.55 ± 0.49	$4,84 \pm 0.09$	2.01 ± 0.04	5.85 ± 0.20	3.04 ± 0.10
(2) Fuc	1.71 ± 0.03	1.02 ± 0.02	1.89 ± 0.04	2.09 ± 0.05	2.40 ± 0.04	1.00 ± 0.02	2.76 ± 0.12	1.43 ± 0.06
(3) Rib	3.81 ± 0.03	2.27 ± 0.02	3.41 ± 0.17	3.77 ± 0.19	5.70 ± 0.56	2.37 ± 0.23	5.11 ± 0.32	2.66 ± 0.17
(4) Ara	51.50 ± 0.45	30.71 ± 0.27	27.06 ± 0.29	29.93 ± 0.31	57.38 ± 0.56	23.84 ± 0.23	60.78 ± 1.31	31.60 ± 0.68
(5) Xyl	76.33 ± 0.21	45.52 ± 0.13	30.94 ± 0.30	34.22 ± 0.34	20.54 ± 0.13	8.53 ± 0.05	25.08 ± 0.46	13.04 ± 0.24
(6) Man	3.83 ± 0.03	2.28 ± 0.02	2.00 ± 0.08	2.21 ± 0.09	1.52 ± 0.02	0.63 ± 0.01	1.83 ± 0.04	0.95 ± 0.02
(7) Gal	13.15 ± 0.03	7.84 ± 0.02	10.66 ± 0.13	11.79 ± 0.15	20.26 ± 0.04	8.42 ± 0.02	19.64 ± 0.12	10.21 ± 0.06
(8) Glu	14.84 ± 0.03	8.85 ± 0.02	10.34 ± 0.21	11.44 ± 0.23	128.08 ± 0.13	53.21 ± 0.05	71.31 ± 0.28	37.07 ± 0.15
Total	167.68	99.99	90.41	100.00	240.72	100.01	192.36	100.00
(4):(5):(8)		1.00:1.48:0.29		1.00:1.14:0.38		1.00:0.36:2.23		1.00:0.41:1.17

Ara: arabinose, fuc: fucose, gal: galactose, glu: glucose, man: mannose, rha: rhamnose, rib: ribose, xyl: xylose.
All the data are the means of 3 determinations. See text for experimental condition.
The content of each sugar is presented as µg per mg of walls, dry weight.

Table 3. Neutral sugar composition of *in vivo* lily pollen tube walls after self- and cross-pollination, with standard deviation.

Sugar	A × M		A × A		M × M		M × A	
	($\mu g.mg^{-1}$)	(%)	($\mu g.mg^{-1}$)	(%)	($\mu g.mg^{-1}$)	(%)	($\mu g.mg^{-1}$)	(%)
(1) rha	9.48 ± 0.42	2.77 ± 0.12	9.76 ± 0.39	2.65 ± 0.11	5.61 ± 0.50	1.53 ± 0.14	15.14 ± 0.56	3.90 ± 0.14
(2) fuc	5.78 ± 0.06	1.69 ± 0.02	6.39 ± 0.08	1.74 ± 0.02	4.60 ± 0.23	1.26 ± 0.06	5.61 ± 0.11	1.45 ± 0.03
(3) rib	14.80 ± 0.60	4.32 ± 0.18	9.11 ± 0.89	2.47 ± 0.24	31.67 ± 1.12	8.66 ± 0.31	10.89 ± 0.28	2.81 ± 0.07
(4) ara	157.98 ± 2.04	46.14 ± 0.60	185.06 ± 4.94	50.27 ± 1.34	168.30 ± 4.42	46.01 ± 1.21	203.34 ± 1.83	52.43 ± 0.47
(5) xyl	18.93 ± 0.06	5.53 ± 0.02	19.05 ± 0.61	5.17 ± 0.17	17.04 ± 0.92	4.66 ± 0.25	18.11 ± 0.72	4.67 ± 0.19
(6) man	8.06 ± 0.15	2.35 ± 0.04	6.87 ± 0.36	1.87 ± 0.10	4.93 ± 0.08	1.35 ± 0.02	9.03 ± 0.33	2.33 ± 0.09
(7) gal	31.88 ± 0.15	9.31 ± 0.04	36.81 ± 1.28	10.00 ± 0.35	26.63 ± 0.27	7.28 ± 0.07	34.79 ± 1.92	8.97 ± 0.50
(8) glu	95.50 ± 1.17	27.89 ± 0.34	95.11 ± 2.94	25.83 ± 0.80	107.02 ± 1.54	29.26 ± 0.42	90.95 ± 2.67	23.45 ± 0.69
Total	342.41	100.00	368.16	100.00	365.80	100.01	387.86	100.01
(4):(5):(8)		1.00:0.12:0.60		1.00:0.10:0.51		1.00:0.10:0.64		1.00:0.09:0.45

A: Arai 5; M: Mount Everest (*Lilium longiflorum*); all the abbreviations are the same as in *table 2.*
All the data are the means of 3 determinations. See test for experimental condition.
The content of each sugar is presented as μg per mg of dry weight walls.

Table 4. Comparison of sugar content of cell walls from germinated and ungerminated pollen and of root cells.

Cell walls	Content of sugars in total neutral sugars		
(1)	ara + gal + glu (2)	xyl (3)	(2) + (3) (4)
in vivo pollen tubes	83–86%	5– 6%	88–91%
in vitro pollen tubes	79–85%	9–13%	92–94%
pollen grains	53%	34%	87%
roots	47%	46%	93%

Table 5. Differences in dominant sugar composition between *in vivo* and *in vitro* tubes.

in vivo Sugar	'Mount Everest' tubes	'Arai 5' tubes
ara.	46%	50–52%
gal.	7– 9%	9–10%
glu.	28–29%	23–26%
rib.	4– 9%	2– 3%
in vitro Sugar	'Mount Everest' tubes	'Arai 5' tubes
ara.	24%	32%
xyl.	9%	13%
gal.	8%	10%
glu.	53%	37%

3.2. Sugar composition of the walls

Comparison of the neutral sugar composition of walls from *in vitro* germinated (*table 2*) with *in vivo* (*table 3*) shows in general (*table 4*) a higher content in *in vivo* grown tubes. Ara, gal and glu were found to be the predominant neutral sugars in pollen tube walls, while in the walls of pollen grains or roots, xyl is the most abundant component. The remaining four sugars (rha, fuc, rib and man) only amount to 6–13% of the total sugars in all the samples although in the sample of self tube (M × M) about 9% of the neutral sugars is rib.

No significant difference in sugar composition has been found between pollen tube walls resulting from self and cross pollination (*table 3*).

Both *in vivo* and *in vitro* tube walls have different sugar composition, depending upon the cultivar used for pollination, either 'M.E.' or 'Arai 5' (*table 5*). 'Arai' pollen tube walls have a higher ara content both in *in vivo* and *in vitro* tubes. Glu is higher in 'Mount Everest' tubes for both *in vivo* and *in vitro* germinated, whereas gal in general is higher in 'Arai' tubes both *in vivo* and *in vitro* germinated.

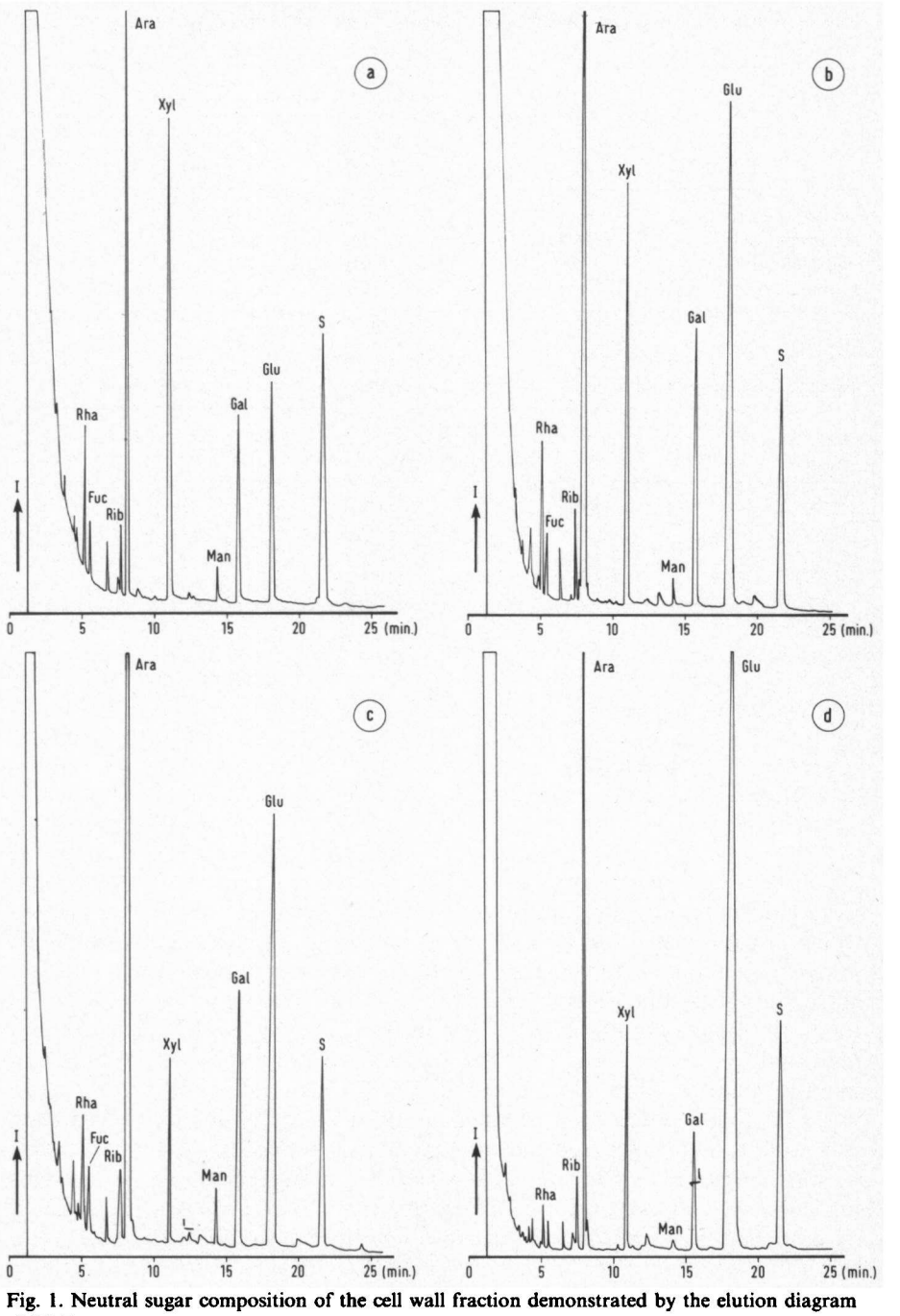

Fig. 1. Neutral sugar composition of the cell wall fraction demonstrated by the elution diagram of GLC.

(a) pollen grains of 'Arai 5', ungeminated
(b) *in vitro* germinated pollen (grains and tubes) of 'Arai 5'
(c) *in vivo* pollen tubes (only tubes) of self-pollinated 'Arai 5'
(d) *in vitro* germinated pollen (grains and tubes) of 'Mount Everest'
S = internal standard

The neutral sugar composition of the cell wall fraction is quite clear when the elution diagrams of the gas-liquid chromatography are compared (*fig. 1, a–d*).

4. DISCUSSION

The finding that after acid hydrolysis of cell wall material from pollen and pollen tubes 8 neutral sugars are liberated is in good agreement with similar results of the cell wall analysis of suspension cultures of monocot cells (BURKE et al. 1974). That ara, xyl and glu are the main components of the neutral sugars from walls was found in oats, rye flour and wheat bran (SELVENDRAN & PONT 1980) and in labelled lily tubes (LABARCA & LOEWUS 1972; NAKAMURA & SUZUKI 1981).

In general the amount of neutral sugars of *in vivo* pollen tubes is higher than in the wall fractions of ungerminated pollen grains and roots. Besides the neutral sugars there are β-1,3- and β-1,4-glucans, uronic acid and proteins.

Taking the high protein content in the walls of *in vivo* pollen tubes into account (LI et al. 1983, LI & LINSKENS 1983) it might be true that in the walls there is a smaller amount of cellulose than in roots, pollen grains and *in vitro* pollen tubes. Differences in the amount of total wall material in beans liberated as neutral sugars by acid hydrolysis was also found (NEVINS et al. 1968): at the age of 4 days cell walls of bean hypocotyls yield almost 70% of their total weight as neutral sugars while at the age of 28 days only 18–19% is liberated. There may be a difference in the neutral sugar content along the pollen tube, with e.g. in the tip region a lower cellulose content, than near the germination pore of the pollen grain. Whether a link can be made with the substrate, either stigmatic exudate or stylar exudate is an open question.

The walls of pollen tubes contain much more ara, gal and glu, and much less xyl than roots and pollen grains. The walls of *in vitro* pollen tubes are slightly lower in ara, gal and glu content and higher in xyl than *in vivo* pollen tubes. The ratio of glu/ara is much higher in the walls of *in vitro* grown tubes than in roots, pollen grains and in *in vivo* pollen tubes. This is not in agreement with the finding that before rapid cell elongation the cell walls of corn and bean seedlings are higher in ara, gal and glu, but lower in xyl. Along with the increase in age xyl becomes higher, and ara, gal and glu content becomes lower (NEVINS et al. 1968). Also in the cell walls of the meiospores of *Allomyces arbuscula* a change in the carbohydrate composition with age was observed (KROH et al. 1977) along with the increase of incubation time.

The much higher glu content in the walls of *in vitro* pollen tubes of lily found by VAN DER WOUDE et al. (1971) disagrees with our results. They found 80–86% of the total neutral sugars are glu. NAKAMURA et al. (1980) supposes that this is because of the high sugar content in the medium used. The probable reason for this differences between their results and ours is that they used another medium (sucrose-B or sucrose-agar) and different clones of lily pollen.

There is also evidence from morphological studies for different chemical com-

position of the tube walls between *in vivo* and *in vitro* germinated pollen (ROSEN 1971; KROH 1967). Rosen mentioned also from his electron microscopical studies that there is no clear difference between *in vitro* pollen tube wall-tips and self-incompatible pollen tube tips. Also we could not find significant differences in sugar composition between compatible and incompatible lily pollen tube walls.

Finally there was no substantial difference between the sugar composition among the 4 different *in vivo* pollen tubes, even not between compatible and incompatible pollen tube walls. This is in agreement with LABARCA & LOEWUS (1973) finding that the incorporation of ^{14}C-glucose into cytoplasm and tube walls of compatible and incompatible tubes is not different.

There are some differences in the neutral sugar composition between those generated from 'Mount Everest' and 'Arai 5' cultivars. This suggests that the genotype of the plants producing the pollen controls the wall composition of the pollen tubes. Wall composition seems to be a species specific property, varied by the substrate of germination, either stigmatic fluid, stylar exudate or the synthetic substrate of *in vitro* germination.

Surprisingly enough no significant differences could be found between self- and cross-pollen tubes. This is in agreement with the fact that the pattern of degradation of phytic acid depends on whether pollen and style from a compatible or incompatible combination (JACKSON et al 1983). This suggests that the tube wall composition, so far as it concerns the glucan skeleton, is not linked with the recognition reaction of incompatibility (LINSKENS 1983a, b) but rather part of the rejection reaction in the later phase of the timecourse of the incompatibility reaction.

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