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Incorporation of Label into Pollen Tube Walls from Myoinositol-labeled *Lilium longiflorum* Pistils[1]

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M. KROH,[2] H. MIKI-HIROSIGE,[3] W. ROSEN, AND F. LOEWUS
Department of Biology, State University of New York at Buffalo, Buffalo, New York 14214

ABSTRACT

Compatible and incompatible pollen tubes growing on detached *Lilium longiflorum* pistils which had been prelabeled with myoinositol-U-^{14}C take up a portion of the label and utilize it for biosynthesis of tube wall substance. The label is transferred from pistil to pollen tubes apparently via the secretion products (exudate) of the pistil. The exudate thus appears to have a major nutritional role in pollen tube growth *in vivo*.

Pollen tubes produced *in vitro* seldom achieve the length needed for fertilization *in vivo*. One may assume that pollen tubes growing *in vivo* obtain additional substances necessary for their development from the pistil. A study of the nutritional role of the pistil in pollen tube development is described in this paper. We were interested especially in the utilization of pistil material for tube wall synthesis. Myoinositol, a precursor of uronosyl and pentosyl units of cell wall polysaccharides of higher plants, is utilized by detached *Lilium longiflorum* pistils for cell wall polysaccharide formation and for biosynthesis of exudate, the secretion product found on the stigma and in the style canal of pollinated pistils (2). As pollen tubes grow through the style canal, they are surrounded by this exudate, which may provide the tubes with carbohydrate material for wall synthesis. To explore this possibility, we have pollinated pistils that were previously labeled with myoinositol-U-^{14}C and examined the growing pollen tubes for uptake and incorporation of label into tube wall substance. The cultivars of *L. longiflorum* are self-incompatible. Advantage was taken of the fact to compare results obtained from compatible and incompatible pollinations.

MATERIAL AND METHODS

L. longiflorum pistils were labeled as described in the previous paper (2) and then pollinated (5 mg of pollen per pistil). Self-pollination experiments were carried out with Ace or Croft cultivars. Cross-pollinations were run with Ace ♀ × Croft ♂, Ace ♀ × Georgia ♂, or Croft ♀ × Georgia ♂. After self-pollination (72 hr) or cross-pollination (48 and 72 hr), styles were sectioned into 2-cm lengths (measured from the stigma), and pollen tubes were recovered by microdissection. Excised tubes were weighed immediately after dissection (±0.01 mg) and then washed either twice in 80% (v/v) ethanol (1.5–2.0 ml per wash) or five times in the same aqueous solution that had been used in our studies *in vitro* (1), designated as D-medium. Combined washes (identified in Table III as pregrind rinse) were assayed for ^{14}C. Washed tissues were ground in 80% ethanol, (0.5 ml) or D-medium (0.5 ml) in a conical glass homogenizer (Kontes K-88545B), transferred to a Gelman filter-centrifuge holder (No. 4305) fitted with a GA-4 membrane, filtered and washed in 80% ethanol, or in the case of tissue ground in D-medium, with water. Combined washes (identified in Table III as postgrind wash) were assayed for ^{14}C. Methods used for autoradiography and to determine radioactivity and chemical composition of samples were described in the previous paper (2).

RESULTS

Pollen tubes excised from labeled pistils 48 hr after cross-pollination contained about 0.5% of the ^{14}C recovered from the pistil (Table I). After 72 hr of pollination, this amount had increased to about 1%. In self-pollinated pistils where incompatible pollen tubes grow to less than one-half the length of compatible tubes in 72 hr, uptake amounted to about 1%, similar to that shown by the compatible tubes. These values were obtained from excised tubes washed in ethanol. Comparison of fresh weight of excised pollen tubes from five cross-pollinated and five self-pollinated pistils revealed a slightly higher average value for the former (8 ± 3 mg) as compared to that of the latter (6 ± 2 mg).

The radioactivity found in tube fragments that had been recovered from successive 2-cm sections of styles of labeled pistils was compared on a fresh weight basis in an effort to learn more regarding the distribution of ^{14}C in growing pollen tubes. As seen in Table II, the radioactivity, in counts per minute per milligram, fresh weight, of excised tube fragments was greater in tube fragments recovered from style sections distal to the region of the pollen grain, especially after 72 hr of pollination. Washing the ground tube fragments with 80% ethanol removed little radioactivity when fragments were excised from style segments rich in tube tips. Although incompatible tubes had grown only into the second style section in 72 hr (compatible tubes had reached section 5 in the same period), these tubes also contained a higher radioactivity per unit, fresh weight, in fragments excised from style section 2.

Since labeling of detached pistils with myoinositol-U-^{14}C resulted in formation of labeled exudate (2), we wished to determine if the increase in radioactivity per unit, fresh weight, of pollen tube was an expression of ^{14}C incorporation into tube

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[2] National Science Foundation Senior Foreign Scientist, 1967–1968. Permanent address: Department of Botany, University of Nijmegen, The Netherlands.

[3] Postdoctoral Fellow, State University of New York at Buffalo, 1967–1968. Permanent address: Biological Institute, Kanagawa Dental College, Yokosuka, Japan.

Table I. *Percentage of ^{14}C in Pollen Tubes Recovered from Labeled Pistils*

Pollination	Pollination Period	Total ^{14}C
	hr	%
Cross	48	0.2
Cross	48	0.4
Cross	48	0.9
Cross	72	0.9
Cross	72	1.3
Cross	72	0.7
Self	72	1.2
Self	72	0.8

Table II. *Radioactivity in Pollen Tube Material Excised from Style Sections of Labeled Pistils*

Experiment	Pollination	Pollination Period	Style Section				
			1	2	3	4	5
		hr	*cpm/mg, fresh wt*[1]				
1	Cross	48	240 (90)	850 (90)	570 (100)	...	...
2	Cross	48	170 (70)	130 (100)	...	...	...
3	Cross	72	860 (80)	1020 (90)	1090 (90)	2830 (90)	2330 (100)
4	Cross	72	460 (80)	300 (40)	620 (100)	680 (100)	1420 (100)
5	Self	72	650 (60)	3930 (80)	...	...	...
6	Self	72	610 (100)	3750 (100)	...	...	...

[1] Figures in parentheses correspond to the percentage of ^{14}C resistant to extraction with 80% ethanol.

Table III. *Effect of Extraction with 80% Ethanol or D-Medium on Pollen Tubes Excised from Cross-pollinated Pistils*
The pollination interval was 72 hr.

Extracting Medium	^{14}C in Excised Tubes[1]	Percentage of ^{14}C			Style Section				
		Pregrind Rinse	Postgrind Wash	Residue	1	2	3	4	5
	cpm				*cpm/mg, fresh wt*				
Ethanol	7,050	33	8	59	700	880	1100	2760	2200
Ethanol	6,360	30	15	55	425	240	590	...	...
D-water	10,000	57	4	39	730	550	705	740	...
D-water	8,950	62	4	34	360	115	380	...	...

[1] Total label recovered (pregrind rinse + postgrind wash + residue).

wall substance or merely an effect of adhering exudate. Pollen tubes from labeled pistils were examined 72 hr after cross-pollination. Tube fragments from successive style sections were washed and extracted with either 80% ethanol or D-medium and water. Results are summarized in Table III. D-Medium-rinsed tube fragments lost nearly twice as much ^{14}C in the pregrind washes as ethanol-rinsed fragments. Further extraction with ethanol after grinding (postgrind wash) removed appreciable ^{14}C from ethanol-extracted residues. The total amount of ^{14}C-labeled substance extracted from D-medium- and water-washed residues

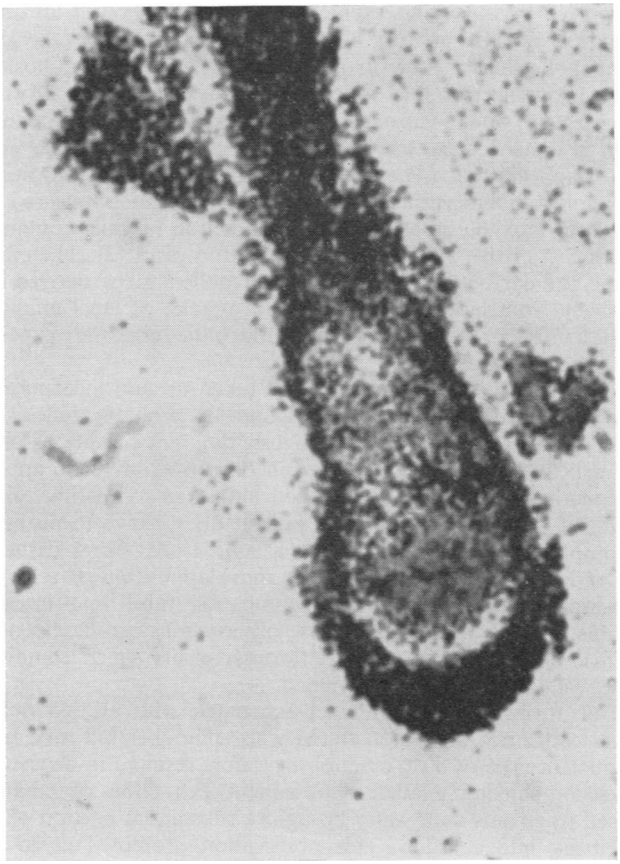

FIG. 1. Autoradiograph of a section through the tip region of a pollen tube grown in myoinositol-2-^{3}H-labeled detached pistil. × 920.

was about one-third greater than that of ethanol-washed residues. The D-medium-rinsed residues still retained 36% of their ^{14}C. Comparison of specific radioactivity in these water-washed residues obtained from tube fragments in successive style sections revealed little variation. Since a substantial portion of the radioactivity remained in tube residues even after extraction and grinding in D-medium followed by water washes, the chemical composition of this insoluble fraction was examined after enzymatic hydrolysis. Pectinase released 78% of the bound label. Of the pectinase-soluble material, 62% was neutral sugars and 38% acidic carbohydrate. Arabinose accounted for one-half of the neutral sugars. The other half consisted of unidentified compounds. The labeled acid material was identified as galacturonic acid.

Autoradiography of cross-pollinated pistils which had been labeled with myoinositol-2-^{3}H revealed that the walls of the pollen tubes were heavily labeled. Figure 1 shows a diagonal section through the tip region of such a pollen tube. Comparison of tip regions with regions closer to the pollen grain suggest that label is more concentrated in tube wall substance in the tip, but additional experiments are needed to verify this.

DISCUSSION

When *L. longiflorum* pollen is grown on pistils which had been prelabeled with myoinositol-U-^{14}C, a portion of the label could be recovered in an insoluble tube wall fraction. Since most of the label in this fraction could be solubilized by pectinase, and since the labeled products of the enzyme treatment are known products of pectin hydrolysis, it can be concluded that the label taken up by the pollen from the pistil is largely utilized for the biosynthesis of tube wall pectic substances. This conclusion is

supported by autoradiographic studies, which showed an accumulation of label in tube walls, especially near the tube tips, when pollen tubes grew in pistils prelabeled with myoinositol-2-^{3}H. From electron microscope studies on pollen tubes growing *in vivo* (5), and from observations that pollen tube growth *in vitro* is enhanced by secretory products (exudate) of the pistil (4), it appears that *Lilium* pollen tubes obtain substances necessary for wall biosynthesis from secretory products released by the pistil into the hollow style. The secretion product contained about 5% of the radioactivity applied to a pistil (2). Therefore, if the radioactivity present in excised pollen tubes derived exclusively from labeled exudate, actual uptake of label amounts to 10 to 20% of the radioactivity to which the tubes were exposed in the secretion product.

Variations in the amount of label taken up and incorporated into tube wall material (Tables I and II) probably reflect the use of flowers harvested under slightly different conditions or at different maturities. Nevertheless, incompatible tubes do appear to contain more label per unit tube length than compatible tubes. Electron microscopic studies of pollen tubes grown in pistils of incompatible species which have a solid transmitting tissue in the style have shown that there is more apposition of cell wall substance in the tip region of incompatible tubes than in compatible ones (3, 6). Whether these observations can be extended to incompatible tubes growing through pistils which contain a style canal remains to be examined.

The intrinsic nature of label associated with excised pollen tubes could not be demonstrated with ethanol-rinsed tube fragments since use of 80% ethanol invariably resulted in deposition of accompanying exudate. Subsequent extraction of ethanol-rinsed fragments with water brought a substantial portion of the ^{14}C back into solution. This observation prompted us to use D-medium as the rinsing solution and water as the final extracting solvent after grinding tube fragments. With this procedure it was possible to show that at least 34% of the ^{14}C was retained in water-washed tube residues as pectic substance.

Comparison of the specific radioactivity of ethanol-washed and water-washed tube residues from successive style sections of cross-pollinated pistils revealed the interesting observation that water was especially effective in removing ^{14}C from pollen tube residues of style sections rich in tube tips. Quite likely, newly formed tube wall substance in the vicinity of the tip as well as adhering exudate was removed by aqueous extraction whereas ethanol extraction resulted in the fixation of this highly labeled material. Structural evidence which supports the idea of an accumulation of bound pistil exudate in the region of the tube tip has been presented by Rosen and Gawlik (5). Further work on the carbohydrate nature of exudate from detached pistils of *L. longiflorum* and its utilization by growing pollen tubes will be described in subsequent papers (C. Labarca, M. Kroh, and F. Loewus; M. Kroh, C. Labarca, and F. Loewus, manuscripts in preparation). That the pistil supplies material for growing pollen tubes which is utilized for tube wall synthesis is clearly indicated in the present study.

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