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INCORPORATION OF LABEL INTO THE INTERCELLULAR SUBSTANCE OF STYLAR TRANSMITTING TISSUE FROM PETUNIA PISTILS LABELED WITH TRITIATED MYO-INOSITOL. AN ELECTRONMICROSCOPIC AUTORADIOGRAPHIC STUDY

M. KROH and C. H. J. VAN BAKEL

Botanisch Laboratorium, Universiteit Nijmegen

SUMMARY

Pistils of *Petunia hybrida* were labeled with tritiated myo-inositol. Electronmicroscopic autoradiographs of cross sections through the stylar transmitting tissue were made 72 hours after labeling. The silver grains were found predominantly above the intercellular substance and the cell wall region. These results provide additional evidence for the assumed carbohydrate nature of the intercellular substance.

1. INTRODUCTION

In plant species with solid styles pollen tubes grow through the intercellular substance of the stylar transmitting tissue towards the ovary. Electronmicroscopic (EM) studies suggest that the intercellular substance serves as nutritional material for growing pollen tubes (VAN DER PLUIJM & LINSKENS 1966). The intercellular substance is assumed to contain pectic compounds (VAN DER PLUIJM & LINSKENS 1966). In order to elucidate the role of the intercellular substance for pollen tube elongation it was necessary to get information concerning the nature of the intercellular substance. Myo-inositol is a precursor of uronosyl and pentosyl residues (LOEWUS 1969). Pectic substances as well as exudate polysaccharides are readily labeled by tritiated myo-inositol (LOEWUS 1969; LABARCA, KROH & LOEWUS 1970). The present paper describes the distribution pattern of silver grains on EM-autoradiographs of cross sections through the stylar transmitting tissue of pistils labeled with tritiated myo-inositol.

2. MATERIAL AND METHODS

Flower buds of *Petunia hybrida* from plants grown under greenhouse conditions were used. Petals, sepals, and stamens were removed and each pistil was labeled by placing it with the cut surface of the pedicel in a small vial containing 10^{-7}M myo-inositol-2-^{3}H (MI-2-^{3}H) (50 µl, 25 µC). The length of the styles of pistils to be labeled was between 16 and 17 mm. The small vial with labeled solution and pistil was placed in a glass vial filled with water. After the labeled solution had been taken up distilled water was added to keep the cut sur-

face submerged. At 72 hours after transfer of the pistils into the labeled solution stylar sections of 1 to 2 mm were cut out of the middle of the styles, which had then reached an average length of 23 mm. The sections were prefixed for 2 hours in 6.25% glutaraldehyde/0.1 M phosphate buffer (pH 7.2), postfixed in 1% osmiumtetroxide/water for 2 hours, and subsequently embedded in epon. Cross sections (± 600 Å) through the transmitting tissue were cut on an LKB ultratome using glass knives. For preparation of EM-autoradiographs the methods described by VRENSEN (1970a) were used. The ultrathin sections were mounted on slides coated with 0.7% collodion and stained with lead citrate for 3–5 min (REYNOLDS 1963). The specimens were then coated with a thin layer of carbon. The photographic emulsion (Ilford L 4) was applied by means of a semi-automatic coating apparatus (VRENSEN 1970b). After exposure for 4 weeks the submicroscopic autoradiograms were developed according to the gold latensification-elon-ascorbic acid procedure (WISSE & TATES 1968), fixed in 24% sodium thiosulfate, and rinsed in water. The autoradiographs were floated on a water surface, copper grids placed on the sections, and the grids picked up and air dried. The autoradiographs were examined in a Philips EM 300 (60 KV) and photographed at a magnification of 4600×.

According to the studies of BACHMANN, SALPETER & SALPETER (1968) and SALPETER, BACHMAN & SALPETER (1969) it seems justified to use a theoretical value of resolution in the localisation of radioactive material in biological specimens. For evaluation of the autoradiographs the radius of the circle was calculated within which 50% of the electrons emitted in the direction of the emulsion layer will give rise to a group of silver grains in the emulsion layer. The calculated radius is 1,500 Å. The analysis of the EM autoradiographs was carried out as follows: circles with a radius of 1,500 Å × photographic magnification were drawn around each group of silver grains on 45 randomly chosen micrographs. The cell material within these circles may be regarded as a probable carrier of radioactivity. The groups of silver grains were registered according to their position above one of the following four cell regions: A. intercellular substance, B. intercellular substance + adjacent cell wall and cytoplasm, C. cytoplasm, D. nucleus, chloroplast, or vacuole. To check whether the distribution of groups of silver grains above the cell regions merely reflects the distribution of the areas of these regions on the micrographs or whether the silver grains have indeed accumulated above some of the cell regions, an area measurement was carried out on the same micrographs that were used for the analysis of grain distribution (WILLIAMS 1969). Transparent plates with 100 randomly distributed circles (radius: 1,500 Å × photographic magnification) were placed above the micrographs and the number of circles above each of the four cell regions registered.

3. RESULTS AND DISCUSSION

Fig. 1 shows a micrograph of an autoradiogram that was developed after exposure for 7 weeks. The silver grains have accumulated above the intercel-

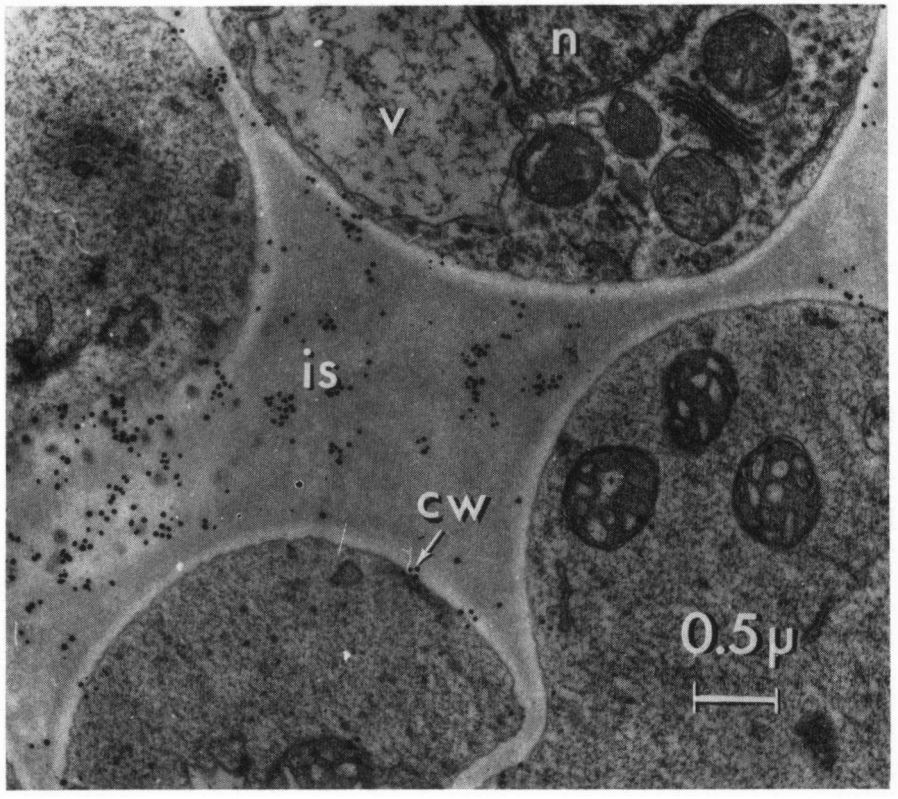

Fig. 1. EM-autoradiograph of a cross section through the transmitting tissue of a style labeled with MI-2-^{3}H. Exposure: 7 weeks. Note the accumulation of silver grains above intercellular substance (is) and adjacent cell walls (cw). n = nucleus, v = vacuole.

lular substance and the adjacent cell wall. However, for the evaluation of the autoradiographs a lower exposure time of four weeks was more favorable because single groups of silver grains can then be distinguished and counted. *Fig. 2* represents one of the 45 micrographs on which the distribution of the groups of silver grains above the cell regions of transmitting tissue was determined and the effective area analysis was carried out. *Table 1* summarizes the results of the evaluation of the autoradiographs.

A comparison of the total number of groups of silver grains above the four cell regions with that of the area distribution of these regions on the micrographs indicates that the groups of silver grains do not appear to be distributed randomly above the regions according to the four areas. Indeed, the number of groups of silver grains/area depends on the cell region (Friedman's test, $p < 10^{-5}$). In order to detect a possible dependency between concentrations of

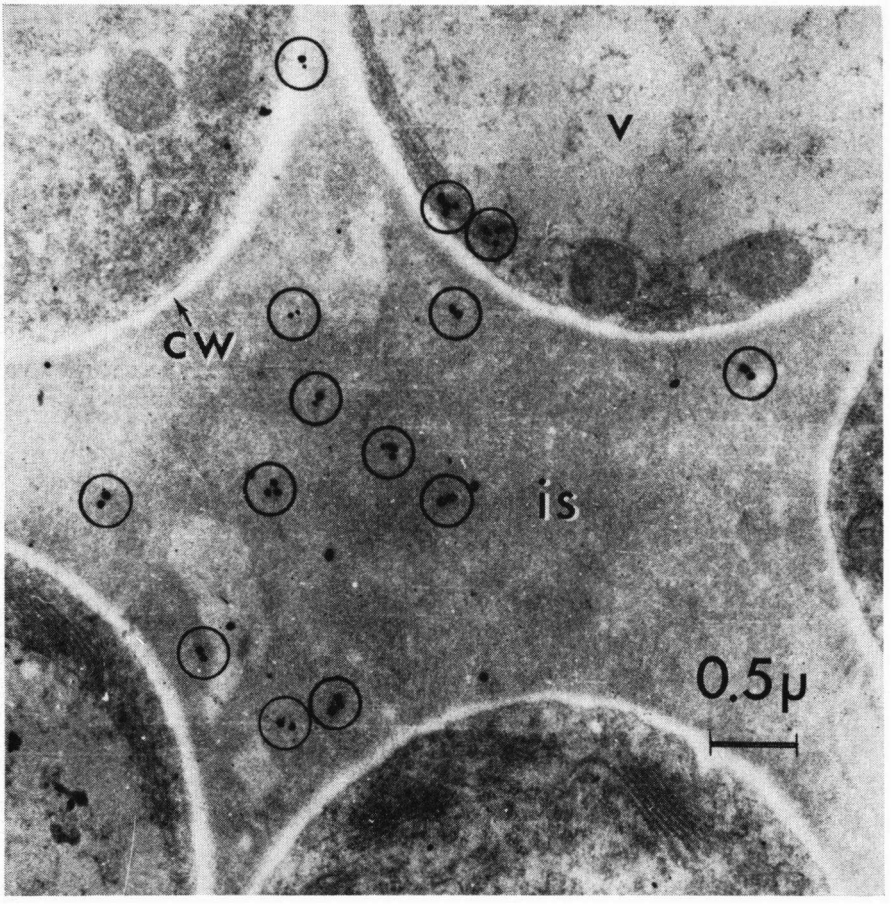

Fig. 2. EM-autoradiograph of a cross section through the transmitting tissue of a style labeled with MI-2-^{3}H. Exposure: 4 weeks. The material within the circles may be regarded as the probable carrier of radioactivity. is = intercellular substance, cw = cell wall, v = vacuole.

Table 1. Evaluation of 45 autoradiographs

Cell region	Number of silver grain groups	Number of circles	Silver grain groups/area
A. intercellular substance	76	467	0.16
B. intercellular substance + adjacent cell wall and cytoplasm	102	850	0.12
C. cytoplasm	46	1720	0.03
D. nucleus or plastid or vacuole	15	1463	0.01

silver grain groups and regions, all pairwise sign tests were carried out. In symbolic notation the results are: $A = B$, $A > C$, $A > D$; $B > C$, $B > D$; $C > D$. There were no significant differences in the concentration of label between cell region A and B ($p > 0.50$). The other relations indicate significance at 0.05 level. A considerably higher concentration of groups of silver grains is found above intercellular substance (A) and the region of intercelullar substance + adjacent cell wall and cytoplasm (B) than above cytoplasm (D) and the region of nucleus or plastid or vacuole (D).

One may conclude from these results that a considerable portion of the tritium from MI-2-^{3}H has been incorporated into pentosyl and/or uronosyl units of cell wall material and intercellular substance during the period between labeling of the pistils and fixation of the stylar sections. The bulk of the label in these cell regions does not originate from free MI-2-^{3}H (KROH 1973). The results confirm the assumption that the intercellular substance consists of carbohydrate material. It remains, however, an open question whether the intercellular substance is indeed pectin. Preliminary studies on its chemical composition indicate that it consists of a mixture of acidic carbohydrates of possibly low molecular weight with glucuronic acid predominating over galacturonic acid (KROH 1973).

A combination of EM-autoradiographic with biochemical work seems to be a convenient way of studying the synthesis of intercellular substance as well as the nutritional relations between pollen tube and style.

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