Planta 133, 243–248 (1977)

Planta

Morphological Aspects of the Galactomannan Formation in the Endosperm of *Trigonella foenum-graecum* L. (Leguminosae)*

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Abstract. The mode of deposition (secretion) of galactomannan in the cells of the seed endosperm of *Trigonella foenum-graecum* has been studied by electron microscopy. In cells which are just beginning to secrete galactomannan there are stacks of rough endoplasmic reticulum (ER). The intracisternal space (containing the enchylema) of the rough ER then swells, becomes vacuolated and forms a voluminous network, with "pockets" of cytoplasm entrapped within poculiform rough ER. The enchylema contains material which reacts with periodate-thiocarbohydrazide-silver proteinate in a very similar manner to the galactomannan already deposited in the cell wall. It appears that the galactomannan is formed in the intracisternal space of the rough endoplasmic reticulum and then expelled outside the plasmalemma. This mode of deposition contrasts with that of other plant cell wall polysaccharides whose secretion is mediated by Golgi vesicles.

Key words: Endosperm – Galactomannan – Secretion – Ultrastructure – *Trigonella*.

Introduction

The *deposition* of galactomannan in the endosperm cells of seeds from *Trigonella foenum-graecum* (fenugreek) starts 3–4 weeks after anthesis and ends 4 to 6 weeks later, depending on culture conditions and plant variety (Reid and Meier 1970, 1973). It is obviously a very much slower process than the *mobilisation* of galactomannan during germination (Reid, 1971).

This paper describes an electron microscopic investigation of galactomannan deposition in the endosperm of fenugreek.

* This is part six in a series of papers dealing with galactomannan metabolism. Part five: Planta **133**, 219–222 (1977)

Abbreviation: ER = endoplasmic reticulum

Material and Methods

Seeds of *Trigonella foenum-graecum* L. in the course of maturation were harvested ca. 4, 6 and 8 weeks after anthesis. They had fresh weights between 12 and 32 mg corresponding to dry weights between 4 and 12 mg. After harvesting they were cut longitudinaly into 4 pieces; the embryos were removed and the endosperms with the seed coats still attached were fixed either with glutaraldehyde-osmium or with glutaraldehyde-acrolein-osmium.

For glutaraldehyde-osmium fixation tissue pieces were immersed overnight at 2° in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2, thoroughly washed with buffer and postfixed overnight at 2° in 2% osmium tetroxide in the same buffer. The fixed material was washed with buffer and dehydrated in an ethanol series.

For glutaraldehyde-acrolein-osmium fixation, tissue pieces were fixed for two hours at 2° in a mixture of 3% glutaraldehyde and 3% acrolein in 0.05 M collidine buffer, pH 7.1, thoroughly washed with buffer and postfixed for 2 h in 1.5% osmium tetroxide in the same buffer. The fixed material was washed with buffer and dehydrated in an ethanol series.

Fixed, dehydrated tissue pieces were treated successively with ethanol/propylene oxide (1:1), propylene oxide, propylene oxide/Araldite (1:1) and embedded in Araldite. Ultrathin sections were stained either with 2% aqueous uranyl acetate followed by Reynold's lead citrate, with 1% aqueous $KMnO_4$ or with periodate-thiocarbohydrazide-silver proteinate according to Thiéry (1967). Sections were examined with a Philips EM 300 electron microscope.

For light microscopy semi-thin sections (ca. 2 µm thick) of material embedded as for electron microscopy were stained for 2 min in Azur II-methylene blue [1% Azur II in water mixed (1:1) with a solution containing 1% methylene blue and 1% sodium borate].

Results

Light Microscopic Observations

Light microscopy of semi-thin sections of seeds at different stages of maturation revealed that galacto-

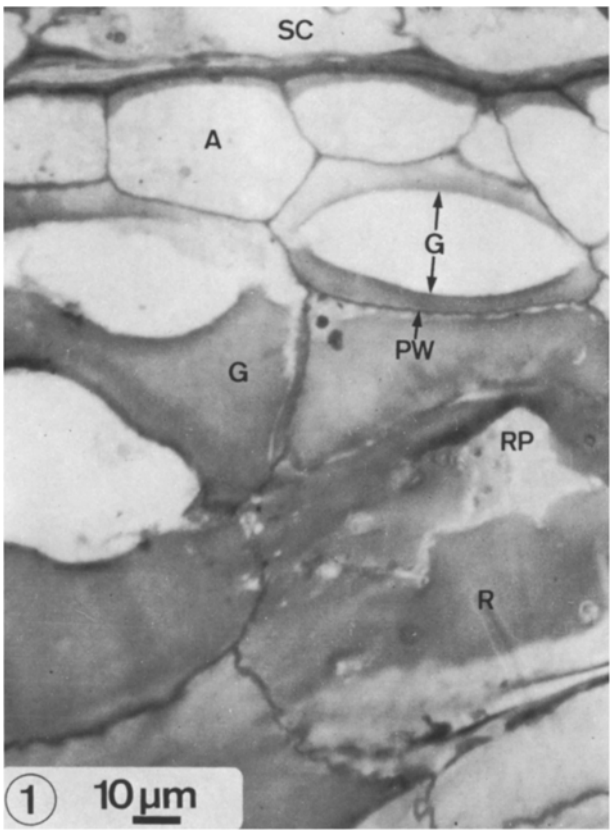


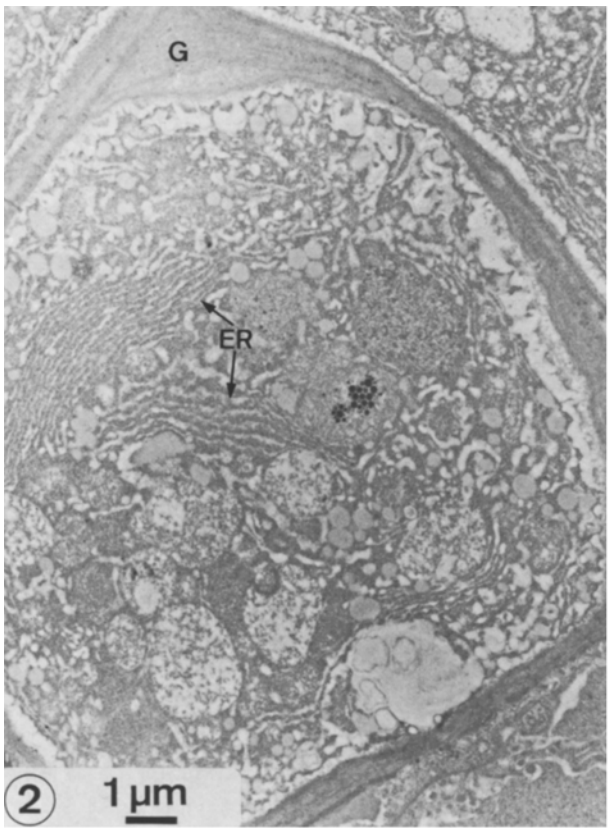
Fig. 1. Semi-thin section through the outer part of the endosperm of *Trigonella foenum-graecum*. In the cells adjacent to the aleurone layer the galactomannan deposition has just started. Seed coat (*SC*), aleurone cell (*A*), reserve cell (*R*), galactomannan (*G*), residual protoplasm (*RP*), primary wall (*PW*). Section stained with Azur II-methylene blue

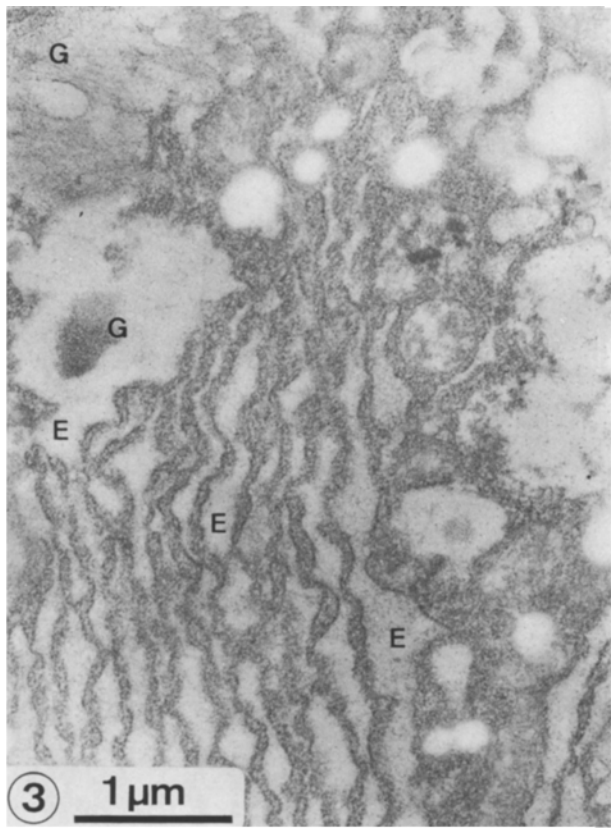
mannan deposition in the endosperm cells starts next to the embryo and proceeds outwards towards the seed coat. It is a cell by cell process. The deposition of the galactomannan onto the primary wall is very irregular but it often begins at the tangential walls (Fig. 1). All the cells of the endosperm, except those of the aleurone layer, become almost completely filled with the polysaccharide. Sometimes very small, irregularly distributed protoplasmic residues may persist. In the aleurone cells some galactomannan is deposited at the outer tangential wall, at the cell corners and occasionally at the side walls, but a large cell lumen filled with protoplasm always remains at the end of seed maturation.

Electron Microscopic Observations

Galactomannan Reserve Cells. In the earliest developmental stages investigated (3 to 4 weeks after anthesis) all the endosperm cells are highly vacuolated. Six weeks after anthesis, the endosperm cells near the embryo are already filled with galactomannan and the cells of the outer region of the endosperm are at different stages of galactomannan deposition. In cells which are just beginning to deposit galactomannan there are often stacks of rough endoplasmic reticulum whose membranes are more or less parallel (Fig. 2). Later the intracisternal space of the ER becomes highly swollen and vacuolated (Fig. 3), and the rough ER becomes "poculiform"—i.e. ER-membranes entrap cytoplasmic pockets. Ribosomes are attached to the *inner* side of the membranes bordering the pockets (Figs. 4 and 5).—Enchylema, which almost certainly contains galactomannan, appears to be discharged on the outside of the protoplast (Figs. 3, 5, 6). The rough ER-membranes do not appear to be continuous with the plasmalemma: the enchylema appears rather to be expelled from the cell with local disruption of the cell membrane. In the later stages of galactomannan deposition larger amounts of galactomannan remain within the protoplast inside the ER-vacuoles. It is probably precipitated within the cisternae during the dehydration of the samples after fixation and is then easily contrasted by the Thiéry reaction (Fig. 7).—Galactomannan is not laid down uniformly on the cell walls. In those cells where the deposition is almost complete, plasma-residues with enclosed galactomannan containing vesicles, are very irregularly distributed throughout the cell. The plasma-residues always appear however to maintain contact with the primary wall bordering on a neighbouring cell in which galactomannan deposition is not so far advanced (Fig. 8). This may reflect a mechanism of transport for cytoplasmic breakdown products. Finally almost the whole lumen of the cell is filled with reserve galactomannan. The cell junctions however are still easily discernible by the relatively dense primary walls, which have a wavy aspect, probably as a result of the dehydration of the galactomannan (Fig. 8).

Aleurone-cells. The aleurone cells form the outermost layer of the endosperm. Cytologically they are at first very similar to the other endosperm cells. They also form, in the enchylema of rough ER, a substance, which is probably galactomannan, and which is deposited on the outside of the plasmalemma. However this deposition is restricted. It occurs mainly on the outer walls of the aleurone cells where they are in connection with the seed coat and also to a small extent on the side walls. The inner cell walls adjacent to the galactomannan reserve cells usually remain relatively thin.





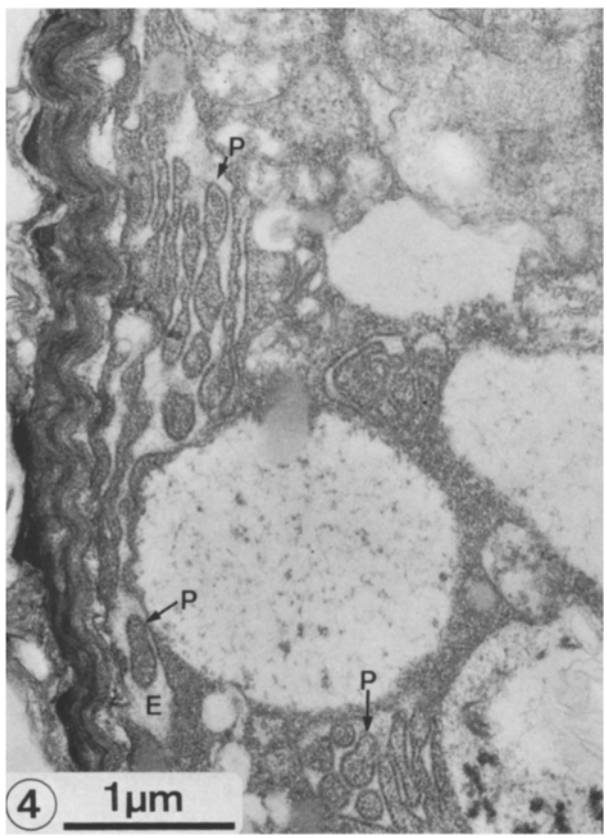
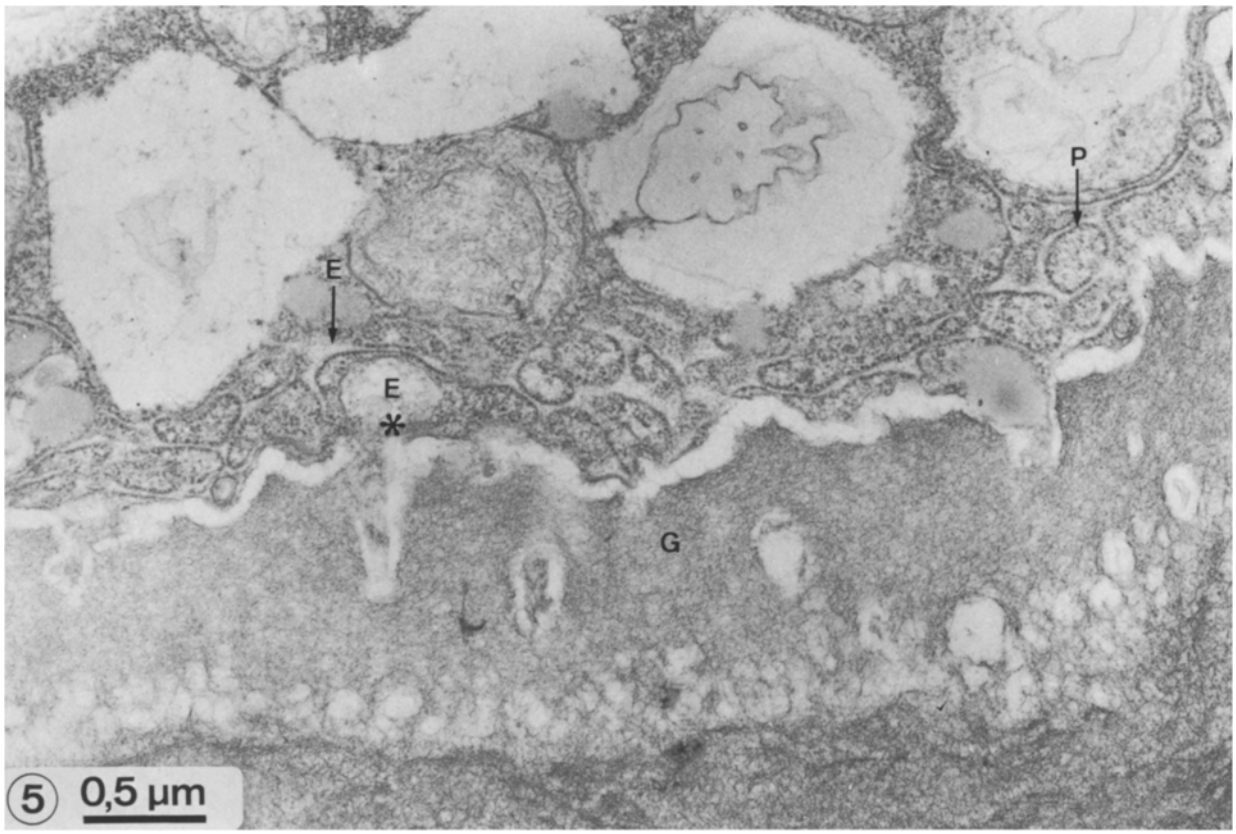
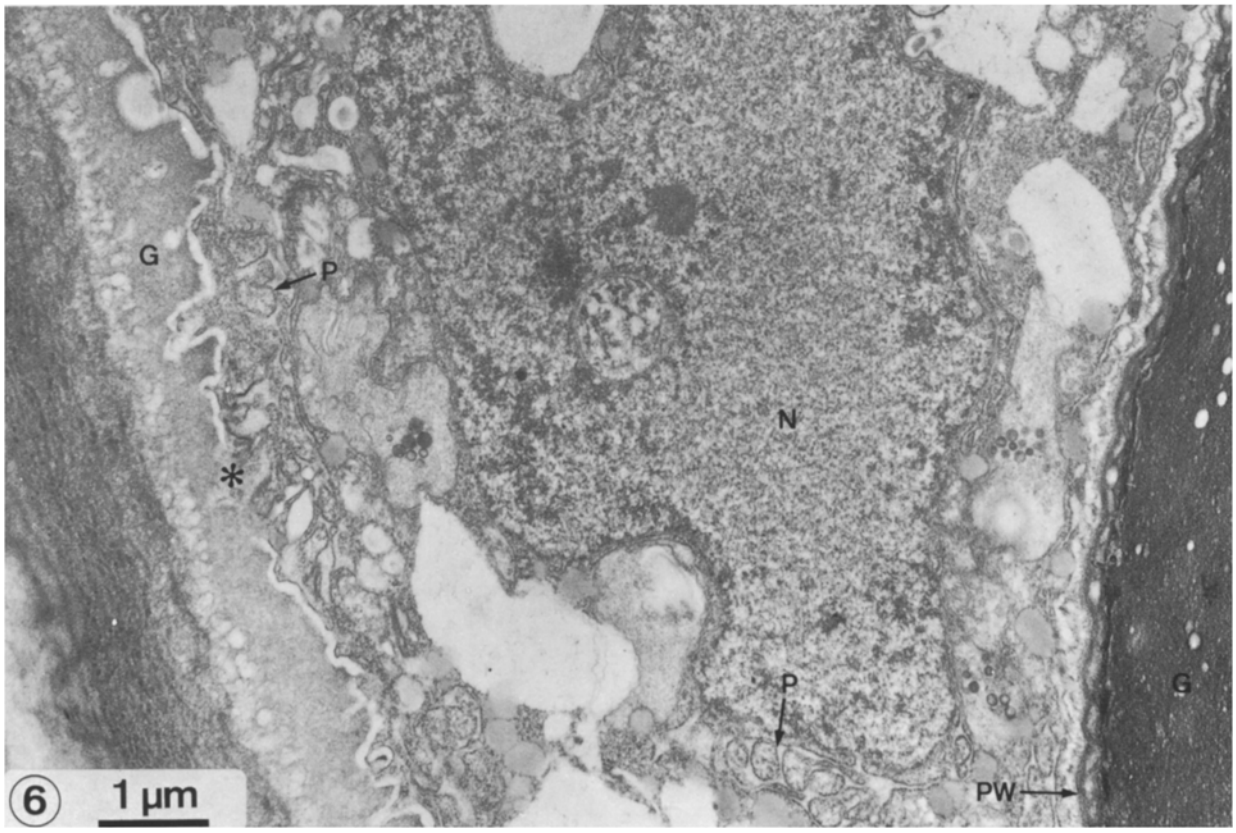


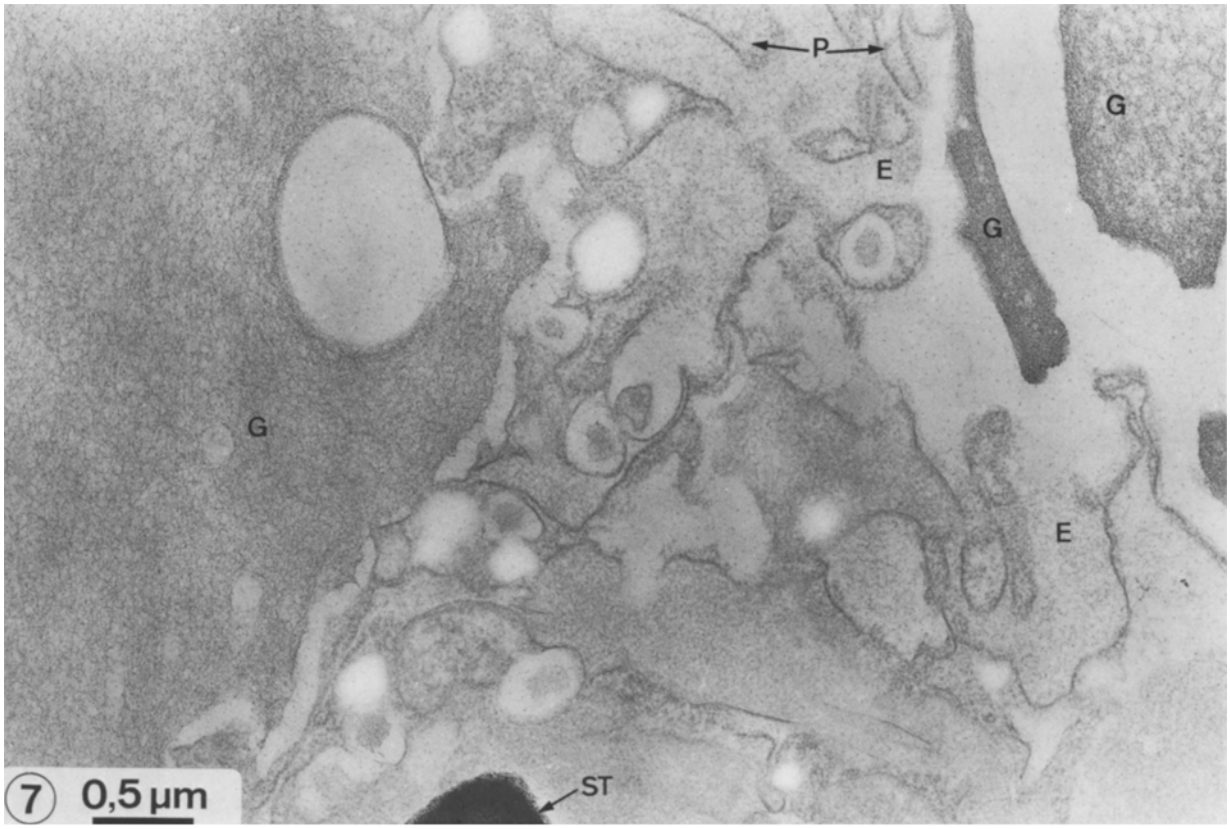
Fig. 2. Reserve cell at the beginning of galactomannan (*G*) secretion. Stacks of rough ER are visible. Section contrasted with potassium permanganate

Fig. 3. Reserve cell during galactomannan secretion. The enchylema (*E*) of the rough ER is highly swollen. On the upper part of the electron micrograph (*) a large ER-vesicle containing galactomannan (*G*) is in connection with the wall. Section contrasted by the Thiéry-reaction

Fig. 4. Reserve cell probably just before galactomannan deposition starts. "Poculiform" rough ER (*P*) lies near the cell border and the intracisternal space with the enchylema (*E*) forms a distended network. Section contrasted with uranyl acetate-lead citrate


P
E
E
*
G
5 0,5 µm


G
P
*
N
P
G
PW
6 1 µm



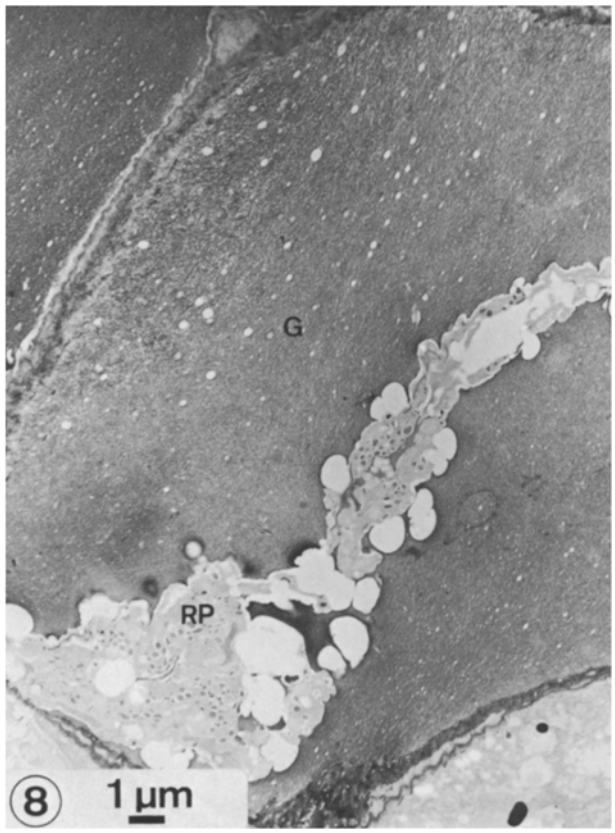


Fig. 5. Reserve cell after the secretion of galactomannan (*G*) has started. Asterisk indicates fusion of enchylema (*E*) with cell outer space. "Poculiform" rough ER (*P*). Section contrasted with uranyl acetate-lead citrate

Fig. 6. As Figure 5. On the left of the electron micrograph a thick layer of galactomannan (*G*) has already been deposited; on the right only a thin layer has been secreted on to the primary wall. Asterisk indicates connection between enchylema and outer space. Nucleus (*N*), "poculiform" rough ER (*P*). Section contrasted with uranyl acetate-lead citrate

Fig. 7. Reserve cell in a later stage of galactomannan (*G*) secretion. Galactomannan concentrations (probably precipitated and contracted during dehydration of the tissue) occur within the protoplast. Starch (*ST*), "poculiform" rough ER (*P*), enchylema (*E*). Section contrasted by the Thiéry-reaction

Fig. 8. Reserve cell almost filled with galactomannan (*G*) and with irregularly distributed residues of protoplasm (*RP*) enclosing small pockets of galactomannan. Section contrasted by the Thiéry-reaction

Discussion

In many cases it has been shown that polysaccharides are carried to the outside of cells within Golgi vesicles, whose membranes fuse with the plasmalemma. This mode of export has been well established by Rougier (1971) and others for slime-secreting root cap cells. Evidence has also been accumulating, to suggest that plant cell wall polysaccharides, especially the pectic and hemicellulosic components, are secreted by Golgi vesicles (e.g. Roland et Sandoz, 1969).

In other, probably fewer cases, however, secretion phenomena seem to be mediated by endoplasmic reticulum. Rachmilevitz and Fahn (1973) have shown that in nectar-secreting cells a swelling of rough ER occurs, followed by the formation of vesicles which probably secrete the sugar solution. According to Unzelman and Healey (1974) trichomes of *Pharbitis* secrete a protein-carbohydrate mucilage with the aid of rough ER. The latter makes frequent contact with the plasmalemma and discharges the enchylema outside the cell. Inside the cells, Golgi vesicles sometimes seem to fuse with the enchylema of rough ER. – Some of our electron micrographs look very similar to those of Unzelman and Healey. The similarity between the development of rough ER in trichomes of *Pharbitis* and in galactomannan-secreting endosperm cells of *Trigonella* is especially striking. Like Unzelman and Healey we have observed "poculiform" rough ER (Figs. 4 and 5) which seems to be responsible for the formation of the galactomannan that accumulates in the very distended and netlike enchylema space before it is released outside the plasmalemma. – In contrast to Unzelman and Healey, we have found no evidence for the participation of Golgi vesicles in the formation of the secreted material. Dictyosomes are very rarely observed in endosperm cells and do not seem to be very active.

It is tempting to speculate that the formation of the galactomannan in vesicles of rough ER may be an indication, that this polysaccharide, at least *in statu nascendi*, may be a glycoprotein.

We gratefully acknowledge the skilful technical assistance of Mrs. Monica Bennefeld and the financial support of the Swiss National Research Foundation.

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Received 29 August; accepted 7 October 1976