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# Meetings of the Royal Botanical Society of The Netherlands

## MEETING OF THE SECTION FOR PLANT MORPHOLOGY, ANATOMY AND CYTOLOGY ON 19 JUNE 1992

### Localization of Extensins and/or Extensin-like Proteins in the Pistils of *Nicotiana tabacum* Flowers

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Extensins are a class of hydroxyproline-rich glycoproteins, and are one of the best characterized classes of structural cell wall proteins present in a wide variety of plants and algae. Recently our group isolated and characterized pistil-specific genes encoding extensin-like proteins in *Nicotiana tabacum* (Goldman *et al.* 1992, *Plant Cell*, 1041–1051). Some of these genes, identified as class III genes, were highly expressed in stigmas and styles and weakly expressed in ovaries. The class III genes were developmentally regulated and not induced in the vegetative tissues under stress conditions like wounding, pathogenic infection or ethylene treatment. The extensin-like proteins encoded by these genes differed from previously described extensins by a lower copy number of the Ser-$Pro_4$ motif and a lower content of tyrosine residues. These residues in particular are thought to play a role in cell wall architecture. *In situ* hybridization experiments showed that the class III mRNA is specifically localized in the cells of the transmitting tissue.

Indirect immunofluorescence was used for the localization of extensins and/or extensin-like proteins. Using a polyclonal antibody raised against soybean seedcoat extensin (kindly provided by Dr J. Varner), we could show the presence of extensins and/or extensin-like proteins in the intercellular matrix and cell walls of the transmitting tissue. Currently, we are undertaking experiments to overexpress the pMG15 protein in *E. coli* to generate specific antibodies.

### Origin and Function of Micropylar Exudate of *Gasteria verrucosa* (Mill.) H. Duval.

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Ovules of *Gasteria verrucosa* may contain a micropylar exudate even before the stigma is receptive. Exudate droplets appeared at first on ovules in the basal part of the ovary. Large quantities of micopylar exudate, which had a smooth cauliflower appearance, were observed on nearly all ovules at the late phase of stigma receptivity.

The micropylar exudate is viscous and contained among others carbohydrates and proteins. The composition of the exudate showed a strong resemblance to that of the filiform apparatus and it was observed that the exudate was connected with the filiform apparatus. Therefore, the micropylar exudate most probably originated from the filiform apparatus. However, it cannot be excluded that the nucellus cells also formed exudate.

During the receptive period the embryo sac became pear-shaped. The amount of starch in the synergids decreased. The micropylar exudate might well be squeezed by the increasing osmotic pressure of the embryo sac generated on its surrounding tissue. The micropylar exudate reacts with the passage of the pollen tube by the formation of an extra layer between the exudate and the wall of the pollen tube. The exudate may nourish the pollen tube but it may also be a signal of recognition for the pollen tube.

In general, the ovules at the very base of the ovary were not fertilized during the period in which the stigma was receptive because only few pollen tubes reached this part of the ovary. When pollen were placed on a cut style after the removal of the stigma at a stage in which the stigma was not yet receptive, the pollen tubes mainly grew to the basal part of the ovary. As a consequence the most basal ovules were fertilized in particular. Pollen tubes show a preference to penetrate micropyles with relative fresh exudate. The relationship between seed setting and the presence of fresh exudate points to preferential penetration. It is also reasonable to assume that the exudate has a function in the attraction of the pollen tube.

### Interpretation of Cell Wall Textures

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The main function of the cellulose skeleton is to provide mechanical strength for the cell and for the plant as a whole. Cell wall textures vary, depending on the type and function of the cell.

We can distinguish two fundamental orientations of cellulose microfibrils (CMFs), viz. 'parallel' and 'non parallel'. The term 'random' should only be used if the cell wall is examined with polarized light. The parallel orientations form either lamellae or layers. Lamellae are sheets of parallel CMFs with a thickness of one microfibril, while layers consist of a three-dimensional bundle of parallel CMFs.

Primary walls can be divided into 'meristematic walls' and 'growth walls'. The former, occurring for instance in meristematic cells and in the tips of tip-growing cells, show a non-parallel texture. Growth walls have a surrounding meristematic wall, but in addition they also show helicoidal, cross-layered and multinet types of texture. All these textures can absorb the stress of turgor pressure, except for the meristematic wall, which is very thin and is not subject to turgor pressure.

Secondary walls of root hairs of aquatic and terrestrial plants show helicoidal and parallel textures, respectively. This difference in texture may be explained as follows. Terrestrial plants can be swayed by the wind, and this movement is transferred to the roots and root hairs. A longitudinal orientation of CMFs in the secondary wall layer provides the greatest strength in that direction. In aquatic plants, the turgor pressure can increase suddenly as result of rainfall, and the helicoidal texture provides the greatest resistance against this pressure.

The secondary walls of fibres have a layered system with S- and Z-orientations in successive layers. As a result of this construction these cells, and consequently the entire plant, are not only proof against longitudinal stress by bending, but also against torsion.

## Immunodetection of Spectrin-like Proteins in Plant Cells

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In animal cells, spectrin is a membrane-bound cytoskeletal protein linked to actin filaments that contributes to the mechanical stability of the plasma membrane. The occurrence of spectrin-like proteins in plant cells was studied with immunoblotting, and these proteins were localized in paraformaldehyde fixed tissues. Proteins from a variety of plant tissues were separated in native and denatured form by, respectively, iso-electric focussing and SDS-PAGE. Electrophoretograms were silver stained or immunoblotted and compared with blots loaded with commercially available purified chicken erythrocyte spectrin, run in parallel lanes. Western blots were incubated with polyclonal antibodies, raised against either human or chicken erythrocyte spectrin. Detection of peroxidase-conjugated secondary antibodies with DAB and $H_2O_2$, visualized the spectrin(-like) proteins. Native plant spectrin was found at pI of 4·8 in extracts of proembryonic masses of *Daucus carota* L. grown in suspension and in embryogenic calli and root tips of *Zea mays* L. Blots from SDS-gels showed immunoreactive breakdown products of the purified spectrin at 150, 100 and 85 kDa. Similar staining patterns were observed on immunoblots of many plant extracts. This indicates the presence of spectrin-like proteins in plant cells. The breakdown of spectrin may be diminished by the use of anti-oxidants and protease blockers. Both antibodies gave similar data. Tissues were embedded in PEG or cryo-preserved, sectioned and indirectly labelled. The secondary antibody was conjugated with FITC or Bodipy 503/512. Suspension cells of carrot and protoplasts of potato were immunolabelled as whole cells. The different techniques resulted in similar cellular localizations.

Fluorescence was found not only at the plasma membrane, but also at the periphery of plastids and in some nuclei. Potato protoplasts, single cells of carrot cell suspension and calyptra cells of maize root tips were labelled strongly at the plasma membranes. We hypothesize that there is a relation between the presence of spectrin-like proteins and cell wall absence or low cell wall rigidity.