45 Progress in Botany

Morphology Physiology Genetics
Taxonomy Geobotany

Fortschritte aer Botanik

Morphologie Physiologie
Genetik Systematik Geobotanik

45 Progress in Botany

Morphology · Physiology · Genetics
Taxonomy · Geobotany

Fortschritte der Botanik

Morphologie · Physiologie · Genetik
Systematik · Geobotanik

Editors/Herausgeber

Karl Esser, Bochum
Klaus Kubitzki, Hamburg
Michael Runge, Göttingen
Eberhard Schnepf, Heidelberg
Hubert Ziegler, München

Springer-Verlag
Berlin Heidelberg New York Tokyo 1983

Vorwort

Der Senior unserer Herausgebergemeinschaft HEINZ ELLENBERG hat sich leider aus der Redaktion zurückgezogen. Nach dem Tode von ERNST GÄUMANN hatte er 1963 (ab Band 26) zusammen mit ERWIN BÜNNING die Herausgeberschaft der "Fortschritte der Botanik" übernommen. Seiner Aufgeschlossenheit und auch seiner Initiative ist die dann später erfolgte Erweiterung des Herausgebergremiums und die damit verbundene inhaltliche Umgestaltung zu verdanken.

In seiner nunmehr fast zwanzigjährigen Betreuung des Abschnittes "Geobotanik" hat er dazu beigetragen, daß dieses Teilgebiet zu einer wesentlichen Säule unserer Reihe geworden ist.

Herausgeber und Verlag möchten ihm für seine Redaktionsarbeit danken und hoffen, daß Herr ELLENBERG ihnen auch weiterhin in alter Freundschaft verbunden bleibt.

An seiner Stelle hat von diesem Band ab Herr MICHAEL RUNGE die Schriftleitung des Kapitels Geobotanik übernommen.

Die Herausgeber

With 23 Figures

ISBN 3-540-12997-9 Springer-Verlag Berlin Heidelberg New York Tokyo
ISBN 0-387-12997-9 Springer-Verlag New York Heidelberg Berlin Tokyo

Printed in Germany

Offsetprinting and bookbinding: Brühlsche Universitätsdruckerei, Giessen
2131/3130-543210.

List of Editors

Section A: Professor Dr. E. SCHNEPF, Zellenlehre, Universität Heidelberg, Im Neuenheimer Feld 230, D 6900 Heidelberg

Section B: Professor Dr. H. ZIEGLER, Institut für Botanik und Mikrobiologie, Technische Universität München, Arcisstr. 21, D 8000 München 2

Section C: Professor Dr. Dr. h.c. K. ESSER, Lehrstuhl für Allgemeine Botanik, Ruhr-Universität, Postfach 10 2148, D 4630 Bochum 1

Section D: Professor Dr. K. KUBITZKI, Institut für Allgemeine Botanik und Botanischer Garten, Universität Hamburg, Ohnhorststr. 18, D 2000 Hamburg 52

Section E: Professor Dr. M. RUNGE, Lehrstuhl für Geobotanik, Systematisch-Geobotanisches Institut der Universität, Untere Karspüle 2, D 3400 Göttingen

Contents

A. Morphology

I. Cytology

a) General and Molecular Cytology

By ANTHONY W. ROBARDS

This contribution presents some recent information on the general topic of cytoplasmic streaming, with some specific comments about the role of calmodulin, which may well be involved in regulatory mechanisms of cytoplasmic streaming, in plants. It also concerns itself with the structure and growth of root hairs, which have often been used to study intracellular movements.

1. Root Hairs

a) Development and Structure of Root Hairs

The asymmetric division leading to the formation of a trichoblast was noted by GUNNING et al. (1978) to be predicted by the presence of a pre-prophase band of microtubules in *Hydrocharis* roots. This division passed acropetally along every cell in the dermatogen layer, pre-prophase bands being noted up to eight cells in advance of the last completed division. KAWATA and CHUNG (1979), studying the crown roots of rice plants, found that the length of trichoblasts could not be distinguished from that of other epidermal cells. However, trichoblasts had more E.R., mitochondria, and ribosomes than other epidermal cells. ROTHBERG and CUNNINGHAM (1978) observed membrane transformation in the roots hairs of *Raphanus*, which they found to elongate at a rate of about 0.1 mm h^{-1} (cf. COOPER and BROWN, below). Numerous vesicles were found adjacent to the plasmalemma and complex interfoldings of the wall were noted, which expanded as the cells grew. Freeze-etching, to reveal intramembrane particles, indicated the possibility of membrane flow, small Golgi vesicles having 1250 to 1400 7-8 nm diameter P-face particles μm^{-2}.

SORAN and LAZAR-KEUL (1978) investigated the relationship between growth and length of barley root hairs and the rate of streaming within them. In young root hairs there was a good correlation between growth and increase in streaming rate but after the end of the grand period of growth this relationship is lost. The continuing increase in streaming rate must then be sought in biochemical changes rather than in the physical strain of the cytoplasm during growth.

The surface area of root hairs is obviously a critical parameter in relation to uptake capacity and yet it is often a difficult value to obtain. SMITH et al. (1979) made measurements on root hairs of *Zostera* and *Halodule* from estuarine sediments. The root hair basal cells of *Zostera* had an average exposed surface area of 7.98×10^{-3} mm^2. The average *Zostera* root and root hair surface areas were 48.2 and 138.8 mm^2 per root respectively. *Halodule* roots and root hairs were shorter than those of *Zostera* with areas of 34.8 and 19.2 mm^2 per root respectively.

Progress in Botany, Vol. 45

2 Morphology

The subject of root hair wall synthesis has been a matter of particular interest, as these plant cells elongate rapidly and are relatively accessible to study. SEAGULL (1978a,b,c) and SEAGULL and HEATH (1980a) have made a detailed study of the events surrounding root hair wall synthesis and deposition in *Raphanus*. Cortical microtubular arrays were analysed from reconstructions of serial thin sections by SEAGULL and HEATH (1980a) to test further and extend the hypotheses concerning the relationship between microtubular orientation and the direction of microfibril deposition (SEAGULL and HEATH, 1979a). Approximately 25 µm behind the tip of the root hair, microfibrils change from a random to an orientated arrangement. It was confirmed that microtubules increase in number, from the tip, until they reach a plateau at about 25 µm. They also change in length from having approximately 60% less than 1.0 µm long in the tip to approximately 40% less than 1.0 µm long at 60 µm behind the tip. They maintain a pattern of angular deviation from the longitudinal axis of the cell which is similar to the angular deviation of cell-wall microfibrils, and they maintain a constant (approximately 70% of tubules) close (within 50 nm) proximity with the plasma membrane. There is a low (approximately 20%) degree of intermicrotubule proximity (within 50 nm of each other) but some variable long range (> 50 nm) association. Fixation with glutaraldehyde in a "complete microtubule polymerization medium", or pretreatment with cytochalasin B, caused an approximate twofold increase in the proportion of long microtubules in the tip region and microtutules within 50 nm of one another.

Bridges were seen joining microtubules to the plasmalemma. Microfilaments ranging from single fibres to large bundles, paralleled the microtubules and treatment with 1% dimethylsulphoxide, while not affecting microtubules, increased the observed number of microfilaments. It was concluded that there were too few long microtubules to coordinate cell-wall microfibril orientation and, further, microtubule orientation did not change within the first 60 µm from the tip although microfibrillar orientation shifts from a random arrangement to an orientated one. The suggestion was made that actin bridges between long and short microtubules might produce a meshwork which would allow a few long microtubules to direct the synthesis of many microfibrils, thus resulting in an orientated microfibrillar deposition. A population of single, actin-like microfilaments was found specifically associated with the cortical microtubules of root hairs (SEAGULL and HEATH, 1979a). Extensive filament bundles were not specifically associated with the tubules although close proximity was observed. The addition of tannic acid to the fixative (SEAGULL and HEATH, 1979a,b,c) preserved more microtubule-associated microfilaments but did not reveal any extra non-microtubule-associated microfilaments. The tannic acid increased the apparent microfilament diameter in proportion to its concentration in the fixative.

SASSEN et al. (1981) studied wall texturing in hairs of *Equisetum*. They identified two general types of wall texture in root hairs: that in terrestrial plants where there is a fibrillar texture, and that in aquatics where the arrangement is helicoidal. *Equisetum* was found to be an exception as the primary wall had randomly arranged microfibrils and the secondary wall was helicoidal. The microtubules ran more or less longitudinally and no clear correlation between microfibrillar and microtubular orientation was found. BROWN (1981) and COOPER and BROWN (1981) experimentally altered microfibril deposition in *Raphanus*, using the wall brightener *Calcofluor*. They found that, when growing root hairs were treated with *Calcofluor*, the tips became rounded although cyclosis was unimpeded; when the *Calcofluor* concentration in the medium dropped below a certain threshold level, normal tip growth resumed. COPPER and BROWN (1981) showed that the growth rate of radish root

hairs was about 0.05 to 0.1 mm h^{-1} and that this rate was reduced by inhibitors of cellulose synthesis such as coumarin and 2-6-dichlorobenzonitrile, which eventually caused the hair tips to burst. Cyclosis continued up to the time of bursting. Prolonged treatment with *Calcofluor* resulted in the deposition of acid- and alkali-resistant masses, possibly callose, at the tips of the hairs. It was suggested from these, and other, experiments that *Calcofluor* prevents the lateral aggregation of cellulose chains, thus impeding crystallisation.

If root hairs are functional in nutrient uptake and transport, then their connections to adjacent cells are of some importance. VAKHMISTROV and KURKOVA (1979), KURKOVA (1981) and VAKHMISTROV et al. (1981) have addressed themselves to this problem. In the aquatic plant *Trianea* it was found (VAKHMISTROV and KURKOVA, 1979) that the highest frequency of plasmodesmata was on the inner tangential wall of the hair cell where there were more than 17 times as many plasmodesmata as in the equivalent position in hairless epidermal cells (see also HARRIS 1979, for *Equisetum* trichoblasts). Similarly (VAKHMISTROV et al. 1981), the frequency of plasmodesmata in root hairs was twice as great as that in other epidermal cells of *Raphanus*. A comparison between the data from *Trianea* and *Raphanus* led VAKHMISTROV et al. to conclude that, whereas in the aquatic species the root hairs provided a preferential channel for K^+ uptake, root hairs conferred no special advantages in the absorption of ions by the terrestrial plant.

Using 3,3'-diamonobenzidine, ZAAR (1979) studied the distribution of peroxidase activity in root hairs of *Lepidium* and found it to be high in the dictyosomes and associated vesicles, in ribosomes on E.R. cisternae and in the cell wall. It was proposed that the peroxidase in the root hairs is synthesised in the E.R. and dictyosome cisternae, packaged into vesicles and transported to the tip region of the hairs. Peroxidase activity seemed to be stimulated by stress incubation in distilled water and was 20 times higher in the root hairs than in cells of the root body.

b) Microbial and Other Associations with Root Hairs

Numerous recent papers have concerned themselves with structural changes related to the penetration of root cells by fungi or during the formation of mycorrhizal associations with roots. AIST and ISRAEL (1977) reported the formation of papillae on root hair cell walls during host cell penetration by the fungus *Olpidium brassicae*. Most papillae were initiated after the penetration tubes had appeared from the zoospore cysts and were attached to the tubes, but some were produced before tube development and were attached to the host walls. Although "tube" and "wall" papillae were initiated at about the same absolute time after inoculation, cysts which induced wall papillae were significantly later than other cysts in producing tubes. Failure of some tubes to penetrate was clearly unrelated to papillae formation. The penetration efficiency of cysts that induced wall papillae was merely half that of cysts that induced only tube papillae. AIST (1977) also found that mechanical wounding of root hairs of *Brassica oleracea* prior to incubation with zoospores of a compatible fungus produced localized wall appositions resembling those commonly induced by fungal attack. These induced appositions were effective in preventing fungal penetration at wound sites.

TURGEON and BAUER (1982) followed the early events during infection of *Glycine* and *Rhizobium*. They found that bacteria became attached to

epidermal cells and root hairs within minutes of inoculation and marked root hair curling occurred within 12 h. Light microscopy revealed infection threads within 24 h in short, tightly curled root hairs which had not emerged at the time of inoculation. Infection threads, apparently originated in pockets, formed by contact of the cell wall of the curled root hair with itself. By 48 h, the infection threads had progressed to the base of the root hair but had not penetrated into the cortex. Increase in cortical cell cytoplasm and mitotic activity occurred in advance of thread penetration. A nodule meristem developed in the outer cortex next to the infected root hair by 4 days and was accompanied by cell division across the cortex.

The pattern of calcium localization in clover root hair cells associated with the infection process was studied by SETHI and REPORTER (1981) using the Ca^{2+}-binding antibiotic, chlorotetracycline. Some hairs from the immature zone showed increased Ca fluorescence distributed through most of the wall and these cells had "notched" side walls typical of cells infected with bacteria. Maturing roots hairs, with growing infection threads, similarly showed increased fluorescence especially around the site of origin of the infection thread and at the tip of the root hair.

HIGASHI and ABE (1980) used scanning electron microsopy to study *Rhizobium trifolii* infection sites on root hairs of *Trifolium repens*. Three morphological types of root hair retaining infection threads were recognized. The bacteria were strongly attached between the surfaces of two cell walls in one of the following ways: between surfaces of a root hair curled back on itself; between a protruberance from a root hair and its cell surface; or between two root hair tips clinging together. The structural basis for infection of root hairs of *Trifolium* by *Rhizobium* was investigated by CALLAHAM and TORREY (1981). Most infected root hairs had in common an enclosed region at the site of thread origin formed by specialized root hair growth or contacts with, in every case studied, a degradation of the root hair wall at the site of thread origin within the enclosure. The thread wall is a new layer formed by the apposition of material by the host cytoplasm near the penetrated wall and surrounding the break as encapsulation of the invading rhizobia. Rhizobial enzymes probably provide for degradative penetration of the root hair cell wall. Localized concentrated activity of hydrolytic enzymes as well as protection from cell lysis is probably favoured by physical constraints provided by the deformed root hair enclosures.

BHUVANESWARI et al. (1981) determined that the roots of four different leguminous plants were only transiently susceptible to nodulation by *Rhizobium*. Initially susceptible regions of host roots became progressively less susceptible if inoculation was delayed by a few hours. Apparently, a fast-acting regulatory mechanism prevents overnodulation. Nodulation in white clover may occur in two distinct phases: in addition to the transient susceptibility of pre-emergent and developing root hair cells, there appeared also to be an induced susceptibility of mature clover root cells brought about by a substance exuded from the bacterial cells.

NEWCOMB et al. (1978) made a detailed study of the structure and host-actinomycete interactions in developing root nodules of *Comptonia*. They found that the fungus enters the host via a root hair infection and then the hyphae perforate the root cortical cells by a local degradation of host cell walls and penetration of the host cytoplasm. WERKER and KISLEV (1978) demonstrated small drops of mucilaginous material near the tips of root hairs of several *Sorghum* species. Electron microscopy demonstrated that the secretion was made up from two dis-