SECTION 4 - ROOT PHYSIOLOGY AND PLANT STRUCTURE

Differentiation of tracheids in explant cultures of some tree species

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Explants from single trees of *Abies concolor* (Gord. et Glend) Lindl. × *Abies grandis* (mature embryo), *Quercus robur* L. (embryonic axis, young stem segments), *Quercus rubra* L. (young stem segments) and *Castanea sativa* L. (embryonic axis of mature embryo) were cultured on a callus-inducing medium with NAA or IBA (in *Castanea*) and BAP. Differentiation of tracheids in both callus and explant tissues was preceeded by formation of cambium-like cells forming wound procambium zones of different shape. These zones produced tracheidal cells either on their convex side (cambium near to callus surface) or concave side (virtually wound cambium zones), where tracheid nests were produced. These were composed of irregulary arranged short or long and wound tracheids surrounded by cells of the wound cambium. Tracheid nests originated in callus produced on hypocotyl-radicula region of *Abies* explants were composed of nodal tracheids irregular in shape surrounded by the layer of the meristematic cells isodiametric in shape, capable of differentiation into tracheid cells. These tracheids as well as others differentiated in *Abies* explants had thickened secondary side walls with large circular bordered pits and sometimes, in addition, wound helical thickenings. On the other hand, in tracheids differentiated in callus of *Quercus* sp. and *Castanea* small circular or oval non-bordered pits or step-like thickening of secondary wall prevailed.

Preliminary results in determining the individual root profiles of maize growing in seminatural conditions

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Recently, a great attention is paid to the architecture of the plant root system. Root maps regularly do not describe the real branching character of roots. Our aim was to get the branching profile by manual measuring of the lengths of the parent roots, the lateral roots (LR), and by measuring the distances between the successive LR. As the model plant we used maize (*Zea mays* L. cv. CE 380) in which the differences in the branching pattern of the different morphological types of root were measured. After washing up the root by a stream of water on the 61st d from sowing, the individual roots were excised and the length characteristics were measured. It was found, that on nodal roots the longest LR grew out in the middle part of the branched region. Since the basal part in this type of root was most frequently branched, the LR were shorter than in the middle part and the same was true in the apical part of the branched region. Seminal roots - primary seminal root and adventive seminal roots were characterized by a lower frequency of branching compared with the nodal roots. The adventive seminal roots showed the tendency to produce the longest LR in the basal part of roots and apically the length and the number of LR decreased.

Analysis of cell cycle kinetics in *Vicia faba* root tips synchronized with hydroxyurea

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Populations of synchronized plant cells represent an attractive system for various studies ranging from karyology and cytogenetics to the study of the mechanisms of cell cycle regulation. The aim of this study was to develop an efficient technique for cell cycle synchronization in meristem root tip cells of *Vicia faba*. The cells were synchronized after treatment with hydroxyurea (HU). Cell cycle kinetics was followed by the analysis of mitotic activity and by flow cytometric analysis of nuclear DNA content. It was found that 18 h treatment with 2.5 mM HU resulted in accumulation of a large part of cell population at the G_1/S interface. 2 h after the removal of HU, large fraction of meristem cells was found to be in S phase, and after 4 h the cells were in late S phase and in G_2 phase. At this time, only 10.2 % of cells were found to be in G_1. Simultaneously, the frequency of cells in various stages of mitosis was gradually increasing and reached its maximum 8 h after the release from the block when 44.5 % of cells were found in mitosis. The technique which allows to achieve a high degree of synchrony is currently used in experiments directed towards the isolation of intact chromosomes and in the study of proteins specific for individual phases of the cell cycle.

Changes of hydraulic conductivity of root system of *Zea mays* L.

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Uptake activity of root system as a major organ of water absorption substantionally changes during the plant development. Main factors influencing water uptake are transpiration of shoot, absorption capacity of the root system, which is determined by surface area and permeability of individual roots. Participation of individual root types in water uptake has not been satisfactorily documented, so entire conception about uptake characteristics of the total root system in relation to growth and demand for shoot has not yet been developed. *Zea mays* L., cv. CE 380 plants were grown in Knop nutrient solution and in the vessels with soil during the first stages of ontogenesis up to 30 d, and 70 d from sowing, respectively. Hydraulic conductivity (Lp) of individual root types (primary seminal root, seminal adventive roots, nodal adventive roots) and the total root system were studied. Lp was measured by applying negative pressure (20 kPa) on the basal part of roots. The Lp changed with the age of the root and the developmental stage of the plant within 0.5×10^{-8} to 9.8×10^{-8} cm s^{-1} kPa^{-1}. The individual root types during the first 10 d of development showed a rapid increase and then decrease of Lp, which correlated with the first formation of lateral root primordia and their emergence. Lp values of total root system showed a fluently increasing trend. Confidently correlation coefficients between Lp on one side and root surface, specific root surface, volume flow, and shoot:roots surface ratio (S/R) on the other side were established. S/R surface ratio seems to be a dominant parameter influencing the rate of water influx into the plant roots. Similar results were obtained on plants from soil conditions.

Development phases of short roots and mycorrhizas in seedlings and cuttings of *Picea abies* (L.) Karst.

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During rhizogenesis of Norway spruce cuttings and seedlings, five consequent phases were distinguished: 1) The proper rhizogenesis - a formation of the first roots, 2) Roots with fully developed root hairs, 3) Club-shaped short roots originating from older short roots after the loss of root hairs. These roots are similar to roots created during the growing of plant under the influence of growth substances. Fine roots appear to be more or less hyaline, 4) Fine roots turn darker. The first mycorrhizal structures (mostly Hartig net) are formed in fine roots, sometimes the cells of rhizodermis are edged by dark fungal hyphae, 5) Fully developed mycorrhizal root tips differentiate in shape and colour according to fungal symbiont. At the stage of intensive root growth, the tip of all short roots can be classified as phase 1. The phase of short root development was estimated according to the status of prevailing older part of the root. When the tip of the short root is outgrowing from an infected primary root cortex (through Hartig net), this tip possesses mycorrhizal structures from the beginning and needs to be classified as phase 4. After the first growing period all five phases were observed in spruce seedlings but only phases 1, 2, 3 were distinguished. In cuttings at this time roots of all present phases turned mature and darker.

Respiratory patterns in the maize roots

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The root respiration is the main source of energy, reducing equivalents (NADH) and other intermediates recurred for biosynthetic reactions and transport processes in the root cells. The rate of root respiration and especially its efficiency therefore, might be regulated not only by the carbohydrate supply as it has been established by many researchers but also by the energy requirements and the requirements for intermediates. In the present study, we measured the rates of total and cytochrome-mediated respiration as well as the participation of the non-phosphorylating, alternative pathway in the different root tissues and correlated them with the carbohydrate level and some tissue activities (*in vivo* and *in vitro* NO_3^- reduction and synthesis of glutamine) in *Zea mays* L. The growth region of root is characterized by the relatively high rate of respiration where the cytochrome pathway is mainly operating. There is the highest ATP production and the participation of the alternative pathway is very low. With gradual maturation of root cells the total rate of respiration increases and the participation of the alternative pathway is the highest in the stele. In spite of the highest proportion of the alternative pathway in the stele, cytochrome-mediated respiration is still more active in this tissues complex compared with cortex. High activity of cytochrome pathway is also confirmed by the relative high level of ATP measured in the stele. Activity of cytochrome oxidase is another evidence of it. The rate of respiration in the different root tissues does not correlate with the supply of soluble carbohydrates. *In vivo* and *in vitro* nitrate reductase activities together with the activity of glutamine synthetase indicate that the work performed by root tissues is also independent on the level of soluble carbohydrates. Our results suggest that the contribution of the alternative pathway to root respiration is more related to the work demand of root cells than to the actual carbohydrate level in the root.

Effect of metabolic inhibitors on anion uptake and radial transport in maize roots

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Metabolical and non-metabolical aspects of anion uptake and radial transport were studied by using inhibitors CCCP (carbonyl cyanide m-chlorophenyl hydrazone), 2,4-DNP (2,4-dinitrophenol) and CH (cycloheximide). ^{35}S-sulphate and ^{32}P-phosphate uptake and transport were examined in primary seminal roots of maize seedlings (*Zea mays* L., cv. CE 330) treated with various inhibitors from 30 min up to 24 h. The treatment with specific inhibitors of oxidative phosphorylation, respiration and proteosynthesis changed the pattern of uptake and radial transport to various degree. Both sulphate and phosphate uptake were principally affected by 2,4-DNP, resulting in the rapid decline of uptake already after 30 min of the treatment. The decline appeared to be permanent up to 6 h. CCCP and CH were apparently different in their effect on the uptake: compared with sulphate a more significant inhibition was found for phosphate absorption, suggesting the higher sensitivity of plasma membrane transport system of phosphate to metabolic inhibitors. Radial transport of anions changed in a different way in dependence on the specific role of applied inhibitor in cell metabolism. The results obtained with phosphate suggest that in short time intervals all inhibitors could reduce the centripetal transport of phosphate practically to the same rate, but later the negative effect of various inhibitors differed. For sulphate the highest inhibition of centripetal transport was found after CH treatment. 2,4-DNP principally affected the uptake of sulphate: It is worth notice that once uptaken, sulphate, though markedly reduced, was not inhibited in the following radial transport too much, suggesting the more important role of this inhibitor for the activity of sulphate uptake mechanisms than the radial transport to the stele.

An ultrastructural study of plant protoplasts

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Changes of ultrastructure of cell organells caused by isolation conditions were studied. Protoplasts were isolated from leaves (*Lilium*, cv. Black Beauty), pericarp (*Vitis vinifera*), cell suspensions (*Vicia faba, Beta vulgaris, Triticum timopheevi*) and callus cultures (*Vitis vinifera*) by incubation of cells in various combination of cellulases and pectinases. For electronmicroscopical studies, protoplasts were fixed by glutaraldehyde and osmium tetroxide solutions with osmoticum. After dehydration protoplasts were embedded in a low viscous medium. After fixation, washing and during dehydration, protoplasts were collected by centrifugation. Another procedure was to embed protoplasts into agar before or after fixation. Subsequently the agar blocks with protoplasts were processed as pieces of tissue. In general, this technique resulted many disrupted protoplasts and therefore unsatisfactory results. The structure and number of organellae in protoplasts generally corresponded to those in the source tissues. But some changes in the fine structure were observed. The most noticeable of them was that membrane systems were negatively stained. At the same time

plastids and mitochondria showed an increase in matrix electrondensity and somewhat constricted shapes. The cytoplasm was also more electrondense with the results that cytoplasm and organellae become difficult to distinguish. In the case of leaf protoplasts, fragmentation of chloroplasts could be seen. In cytoplasm, aggregations of electrondense material were present and they were located mainly in larger electrontransparent spaces. Heterochromatin aggregates were larger and more electrondense in protoplasts than in the cells of source tissues. Shape abnormalities of plastids, mitochondria and nuclei were a characteristic feature of protoplasts isolated from *in vitro* cultivated tissue. However, such abnormalities were typical for original cells as well. All changes in ultrastructure mentioned above were not observed in every kind of protoplast but their occurrence was dependent on the source tissue.

Functional implications of root system architecture

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The functional implications of root system architecture are reflected in the cost of root systems on their construction, maintenance and activities, including the soil exploration and exploitation. Most of these questions were studied in our experiments with *Zea mays* L. cultivars growing in water culture and in soil, which differ in their root architecture. These were cv. Microsperma var. melanese, inbred line VIR 17, hybrids CE 270, CE 330, CE 380, CE 420, TOMv335, ORNELA. The number of seminal adventive roots (RAS) as an intergenotype characteristic variability was studied in hybrids. It was found that from emergence during vegetative phase, the root growth rate and the penetration into depth of soil was higher in the plants with a lower number of RAS. But, the root growth of the plants with higher number of RAS continued in generative phase and reached significantly higher absolute maximum in the milk-wax phase. The absorption surface maximum has primary seminal root and this characteristics decreased successively in the nodal roots of superposed nodes. Maximum of construction cost proportional to biomass, which is in turn a function of tissue volume was found in nodal roots of the fifth whorl.

Submicroscopic structure of the shoot apex cells of wheat (*Triticum* sp.)

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The ultrastructural analysis of the shoot apex cells was undertaken during the first three phases of morphogenesis on five wheat varieties. The analysed cells of the shoot apex of the main stem differed in individual cell organelles contents in the individual phases. In the first morphogenesis phase the cells were tunica and corpus conspicuous with high content of dense bodies of 0.3 - 0.7 μm diameter. We also registered the occurrence of high amount of vacuoles with irregular shape. In the second morphogenesis phase we observed reduced number of dense formations and even vacuole number. In the third morphogenesis phase we observed considerable activity of the endoplasmic reticulum and dictyosomes. We did not identify any variety differences.

Seminal adventitious roots of maize (*Zea mays* L.)

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The whole root system of *Zea mays* consists of different roots, only one of them being the primary seminal root (RPS). The other roots, growing adventitiously, are of different origin. One group of them originates very early in embryo (the adventitious seminal roots - RAS). The aim of present work is to characterise RAS. On transversal sections of maize embryo (after staining on starch - PAS or J+KJ reactions) two dorsal primordia of RAS situated on the site of provascular trace deviated to apical part of scutellum, and one ventral primordium on the opposite side of embryo axis can be distinguished. One or two intermediary RAS can emerge between the dorsal and ventral primordia of RAS. Embryos of sugar maize have only primordia of dorsal RAS or the RAS primordia are missing. RAS are permanent and integral part of the root system during the whole life span of maize. The proportion of cortex in the area of cross-section through the basal part of young and older roots is approximately the same. The cortex of RAS is less porous ("internal aeration") than the cortex of RPS and with age this difference increases. As shown by our experimental data (we measured the relative conductivity and conducting capacity) RAS are effective in water absorption and translocation and keep their potential conductance for the longitudinal water transport occurs primarily in the outer early differentiated metaxylem vessels and in protoxylem. The vessels of the late metaxylem participate in the conduction of water as the last. In the root system of young maize plant, water flow occurs mostly in conducting vessels of RPS and RAS.

Morphology of the female organs of some species of the *Pinaceae*

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The development of the morphogenic processes of the female organs and the pollination mechanism of some species of the *Pinaceae* has been observed by scanning electron microscope. The female buds of various species have a different form and localization in the region of the crown. The seed cones are formed one year before the pollination. The sequence of all the morphogenic processes is the same: the initiation of the seed cone promordia, and of the protuberances-like origin of the bractea and ovuliferous scales realise till the winter dormancy in the year before the pollination. In the second year the primordia of ovules are formed. The last stadium is the formation of the stigmatic apparatus. There are three types of this apparatus: 1) bilobate or *Pinus* - *Picea* type, 2) funnel-like or *Abies* type, 3) papilous or *Larix* type. In the process of the capturing and the transport of the pollen grains on the nucellar to the following takes a part: the pollination drop secreted by nucellus through micropylar canal, the microdrops secreted from the cells of the stigmatic apparatus, the small blisters on these cells or the electrostatic forces.

Heterogeneity of protein bodies in different tissues of seeds

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Protein bodies (PBs) are reserve cell structures present mainly in tissues of mature seeds. These bodies are spherical or oval and range in size from 0.1 to 22 μm and are enveloped by a single membrane. Inside of the proteinaceous matrix different inclusions may be present. The PBs are classified into several types depending upon the presence or absence of inclusions and characteristics of these inclusions. The most frequent of the inclusions are globoid crystals (GCs). In EM these spherical structures appear as electron dense spherical particles. They are composed mainly of salts of K, Mg, Ca and occasionally other elements of phytic acid. GCs are suitable objects for energy-dispersive X-ray microanalysis (EDX). The focus of our study was PBs in some tissues of cereal seeds: barley (*Hordeum distichum*), maize (*Zea mays* and *Z. diploperennis*) and sorghum (*Sorghum bicolor*). Structural analyses showed great heterogeneity of PBs among the species, but also among different tissues of the same species and even, on occasion, within a single cell (Mikus and Lux 1990). The variability of structure was found within the scutellum, coleorhiza and the primary cortex of the radicle. PBs in radicular epidermis were structurally uniform. EDX analyses of GCs in barley and maize germ tissues revealed that the majority of them contained P, K and Mg. Some tissues also contained traces of Fe, Ca and Zn (Mikus *et al.* 1992). We have also analyzed PBs of two species from the family *Cactaceae* (Lux 1990). In different tissues and organs of embryos of *Echinocactus platyacanthus*, EDX analysis showed the presence of P, Mg and K but little or no Ca, Fe or Zn. We also found interesting differences in spatial distribution of some elements in relation to the position of the tissue within the embryo. These results are reported here for the first time.

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Mechanism of nitrate uptake by the maize roots (*Zea mays* L.)

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Nitrate influx into the cells of roots is coupled to symport of proton and usually is accompanied by alkalinization of the external solution. The rate of the nitrate uptake appears to be determined by the activity of the transport system, which is regulated by the concentration of nitrate in the uptake medium and by the activity of H^+-ATPase localized in plasmalemma. The aim of our work was to investigate the uptake of nitrate by the root system of 13 to 15 d old maize plants (cv. TO 360), which were grown on $CaSO_4$ (0.2 mmol l^{-1}) without nitrate. During the measuring of NO_3^- influx the uptake medium contained $CaSO_4$ (0.2 mmol l^{-1}) and $Ca(NO_3)_2$ (1 mmol l^{-1}). Nitrate uptake was estimated from depletion of NO_3^- ions from the uptake medium. The changes of pH and concentration of K^+ ions in this medium were also registered. Mechanism of nitrate uptake was studied in the presence of some inhibitors: DES, VO_3^--inhibitors of proton pump: DIDS-inhibitor of anion carriers and CCCP-protonophore. The uptake of nitrate was inhibited by CCCP, DES and

VO_3^-, but it was not affected by DIDS in used concentration (0.5 mmol l^{-1}). In the same time after application of inhibitors a rapid efflux of K^+ occurred. This was later expressed by the change of pH values in the uptake medium. Our results confirm mechanism of influx into the maize root cells in symport with a proton.

Plastid stroma structure

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At growth phase 2 and 3 weeks from the start of flowering, structure of plastid stroma in terminal leaf and in aleurone cells of grain of various cultivars of wheat were observed. A plastid stroma is considerably heterogenic also within the framework of one plastid, after fixation by glutaraldehyde and osmium acid. A granular structure dominates. Also plastid ribosomes were included to this category. Granules of various size and density occurred isolately, in groups or in oriented linear formation. This order entered into relations with thylakoid system. In plastid stroma were noted also vesicles, mostly in groups.

Ontogeny of mesophyll arm palisade cells in the first wheat leaf

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The arm palisade cells are the main photosynthetic cells in the mesophyll of the majority of the cereals. The ontogeny of these cells during the growth of the first leaf of *Triticum aestivum* L., cv. Grana was determined. The leaves were fixed in FAA, macerated in 5 % H_2CrO_4. The leaf cells were separated by shaking in distilled water and stained by mixture of acid fuchsin, fast green aniline blue, glycerin, hydrochloric acid, formaldehyde and water. The following parametres were studied: leaf length, cell length, number and depth of the protuberances of the cell wall. At the beginning of the leaf development the meristematic cell (without protuberances) were present above all. Cells with three, four, five, resp., shallow and middle protuberances gradually replaced the meristematic ones. During further leaf ontogeny the frequency of these cells decreased and the main part of the mature leaf was constituted of the cells with six, seven, eight, resp., shallow, middle and deep protuberances. No cells with more than twenty protuberances were noticed. The obvious longitudinal gradient of the cell differentiation was demonstrated by analysis of the basal, middle and apical leaf parts. In the apex highly differentiated cells were found already in 4 mm long leaves. Their differentiation finished in 17 mm long leaves. The cell shape was more complicated. Differentiation of the leaf base was the latest, the final shape comparing with other leaf parts was the simplest. The middle part of the leaf represents the transient zone between the basal and apical parts.

Proton-translocating ATPase activity in reconstituted vesicles of plasma membrane from maize roots

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The plasma membrane was isolated from corn roots by sucrose gradient centrifugation. Plasma membrane H^+-translocating ATPase was solubilized from the plasma membranes with procedure using 0.8 % *n*-octylglucoside and proteins were precipitated by adding saturated ammonium sulfate to reach 45 % saturation. The specific activity of vanadate-sensitive ATP hydrolysis increased about 3 fold from 333 nmol Pi min^{-1} mg^{-1}(protein) in fraction from sucrose gradient to a final 1066 nmol Pi min^{-1} mg^{-1}(protein) in reconstituted vesicles. Partially purified ATPase was reconstituted into soybean phospholipid (lecithin) liposomes by the *n*-octylglucoside dilution method. The ability to drive protons into reconstituted proteoliposomes was examined. Intraliposomal acidification was monitored by the decreasing absorbance at 495 nm of pH probe acridine orange in presence of Mg^{2+} and K^+. The specific activity for proton transport by reconstituted vesicle was 0.014 *A* min^{-1} mg^{-1} protein.

Internal secretory structures in the leaves of *Karwinskia humboldtiana* Zucc.

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Karwinskia humboldtiana is a shrub from the family *Rhamnaceae* which in Mexico is widely distributed within the type of vegetation called "matorral xerofito". This plant is significant for the presence of toxins which cause paralysis and occasionally death in ruminants and humans, mainly children. The illnesses and deaths are caused by eating the fruit, in which are localized several of these toxins. One of these toxins has been shown to have selective toxicity *in vitro* against certain kinds of tumors (Pineyro Lopez 1990). The evergreen leaves of *Karwinskia* are relatively large and thin in comparison with other plants from these arid zones. As was shown by Lux and Earl (1989), these plants do not have the typical characteristics of xeromorphic leaves apart from a thick cuticle and epicuticular wax. Chlorenchyma is organized into palisade and spongy parenchyma. Crystals and druses are present in idioblasts (Ruiz Ordonez *et al.* 1991). Two different kinds of secretory structures were observed in the leaves: mucilage cells and secretory canals or cavities. Mucilage cells frequently are found in members of the family *Rhamnaceae* (Metcalfe and Chalk 1983). Secretory canals and cavities of *K. humboldtiana* are unusual in that they normally have a papilous epithelium. This kind of secretory cavity is present in some members of the *Fabaceae* and they are found in leaf veins of *Reynosia* spp. from the family *Rhamnaceae* (Metcalfe and Chalk 1983). In *K. humboldtiana* these structures are present beneath leaf veins, in the mesophyll and also in petioles. Ontogeny of these structures and the chemical composition of the secreted substances will be the aim of a future study.

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