15 01

Effect of synthetic growth regulators on the active ion transport in maize roots

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The low-toxic synthetic growth regulators, N-oxides of pyridine derivatives, activate growth of plants and affect the metabolic processes of plant cells. Uptake of minerals is one of the most important functions of roots. Ion uptake depends on the function of proton pump represented by plasma membrane H^+-ATPase. We have been investigated the influence of N-oxides of pyridine derivatives, differed in the position of the lateral methyl group, on the active transport processes in the seedling roots of *Zea mays* L. The treatment was provided by soaking seeds for 24 h. The proton leakage into the medium from roots of treatment seedlings was more intensive then in control ones. It was shown, that these compounds stimulated activity of the plasma membrane H^+-ATPase which provided energy for the secondary active ion transport. Simultaneously N-oxides of pyridine derivatives increased the nitrate uptake by the maize seedling roots. It has been found that the stimulation level depended on the lateral methyl group position in the structure of the pyridine ring.

15 02

Kinetic analysis of the sugar beet (*Beta vulgaris* L.) PM sucrose-H^+ symporter

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Sucrose uptake into plant cells is catalysed by a H^+-sucrose symporter. The kinetic behavior of this symporter was investigated in plasma membrane (PM) vesicles from sugar beet leaves (*Beta vulgaris* L.). At a saturating membrane potential ($\Delta\psi$), the apparent K_m for sucrose uptake increased 18-fold as the external pH (pH_o) increased from 5.5 to 7.5, while the apparent V_{max} for sucrose uptake remained constant. Conversely, when the H^+ concentration was varied at fixed sucrose concentrations ranging from 0.05 to 1.0 mM, the apparent K_m for H^+ decreased approximately 4-fold but the V_{max} increased > 6-fold. The effects of pH_i in the presence or absence of internal sucrose were exclusively restricted to changes in V_{max} with no statistically significant effect on the apparent K_m for sucrose. Thus, H^+_i behaved as a non-competitive inhibitor of sucrose uptake. The behavior of the sucrose symporter with respect to the $[H^+]_i$ can be interpreted as evidence for an ordered binding mechanism with the binding of sucrose to the carrier on the apoplastic side of the membrane and its release on the symplastic side precedes that of H^+ (i.e. first-on, first-off). The kinetic behavior of the carrier *in vitro* was used to predict its behavior *in vivo*. The K_m for $[H^+]_o$ was estimated to be pH 6.3, which would indicate that at physiological apoplastic pH, sucrose transport might be sensitive to changes in pH_o. On the other hand, the H^+_i concentration for half-maximal inhibition of sucrose uptake was determined to be in the range of pH 5.4, making regulation of sucrose transport through changes in $[H^+]_i$ unlikely.

15 03

Dependence of Ca^{2+} uptake on Na^+ gradient in *Dunaliella salina*

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^{45}Ca uptake was assayed in halotolerant alga *Dunaliella salina* cells (without cell wall). Simultaneously Na^+ efflux from cells was determined. Alga cells accumulated Ca^{2+} when Na^+ gradient (intracellular > extracellular) was formed across the membranes. Dissipation of the Na^+ gradient by the addition of Na^+ and monensin to the external medium decreased Ca^{2+} uptake and Na^+ efflux. The Ca^{2+} and Na^+ transport was increased at acidic pH but decreased at alkaline pH. The apparent K_m value for Ca^{2+} uptake was 33 μM. Probability of function Na^+/Ca^{2+} exchange in plant cells was supposed.

15 04

Analysis of the polysaccharide and glycoprotein synthetic pathways in monensin and brefeldin A-treated plant cells

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In plant cells, the Golgi complex is the site of the synthesis of N-linked glycans of glycoproteins and complex polysaccharides of the cell wall, hemicelluloses (e.g. xyloglucan) and pectins . Both types of molecules are transported in Golgi-derived vesicles to their final destination, the cell surface or vacuoles. We have used electron microscopical techniques in conjunction with highly characterized antibodies specific to N-linked glycoproteins and cell wall complex polysaccharides to examine the effects of monensin and brefeldin A (BFA) on the localization of these molecules within Golgi stacks and secretory vesicles in different plant cells. Electron microscope analyses show that, unlike monensin which induces the accumulation in the cytoplasm of large swollen, Golgi-derived vesicles, BFA leads to the formation of small dense vesicles originating from the *trans* side of the Golgi stacks. Both type of vesicles appear to accumulate large amount of N-linked glycoproteins and xyloglucan. In addition, our results demonstrate that some of the synthetic activities of the Golgi stacks (e.g. xyloglucan synthesis) are transferred to the drugs-induced vesicles. Supported by NIH-GM18639 to L.A.S and by la Région de Haute Normandie-France to A.D.

15 05

Researches on plasmodesmatal composition and function

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Symplastic transport is via gatable pore-like membranous structures called plasmodesmata (PD). The regulation of PD conductance may be by a reversible phosphorylation mechanism. Until recently little was know about PD composition. We have isolated an enriched PD fraction and identified ten putative Plasmodesmatal Associated Polypeptides (PAPs). Antibodies were generated against five putative PAPs with apparent molecular weights of 17, 26, 32, 41, and 64 kDa. The 26 kDa protein has previously been shown to be plasmodesmatal associated. The 41 kDa protein has now been immunolocalized to the PD. Immunolocalization studies for the 17, 32, and 64 kDa protein are in progress. Upon incubation of a wall fraction containing embedded PD or a PD fraction with ^{32}P-ATP, a number of putative PAPs were phosphorylated by an associated Ca^{2+}-dependent protein kinase. PAP17, PAP41, and PAP64 served as substrates for this kinase; PAP26 did not. A 51-53 kD calcium dependent protein kinase (CDPK) that is wall associated undergoes autophosphorylation in situ on nitrocellulose paper. This CDPK could not be extracted with 2% Triton X-100, 100 mM Na_2CO_3 pH 11, or 4 M LiCl, but was extracted with 8 M LiCl. This CDPK has been partial purified.

15 06

Molecular analysis of amino acid transport in higher plants

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Using yeast complementation systems, amino acid transporter genes were isolated from *Arabidopsis* and *Solanum tuberosum*. The different genes can be grouped into at least three different families based on sequence comparisons. The biochemical analysis in yeast indicates that the carriers are able to transport a broad spectrum of different amino acids, possibly by means of proton symport. The genes are differentially expressed as can be shown by Northern blot analysis, RNA *in situ* hybridization and promoter-GUS fusions. The function of the carriers was also studied by overexpression in transgenic tobacco plants.

15 07

Permeability of planar bilayers from chloroplast lipids

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Electrical measurements were carried out in order to investigate the contribution of chloroplast lipids to the membrane permeability. The permeability coefficients of H^+, K^+ and Cl^- were determined for MGDG, MGDG/DGDG/SQDG/PG and soybean lecithin bilayers formed either in the presence or in the absence of n-decane. The selectivity series is $P_H >>> P_K > P_{Cl}$. MGDG is responsible for the high conductance of chloroplast lipid bilayers. The diffusion of ions is voltage-dependent. The mechanism of conduction through planar bilayers was described using a trapezoïdal energy barrier but not by the Eyring model. Diffusion potentials measured in HCl gradients showed that HCl was not transported as neutral molecules.

15 08

Further evidence for sucrose/H^+-antiport across the tonoplast of red beet root vacuoles

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Beet roots accumulate sucrose at high concentrations in the vacuoles of their storage parenchyma cells. Sucrose transport across the tonoplast with Michaelis-Menten like kinetics was shown by Willenbrink and Doll (1979). A sucrose/H^+-antiport mechanism was postulated by Doll et al. (1982) from studies with isolated red beet root vacuoles. It was demonstrated by Briskin et al. (1985) with sugar beet light density membranes. Using monoclonal antibodies (mAb's), we have partially purified a sucrose carrier from red beet root tonoplasts (Getz et al. 1993). These antibodies inhibit stoichiometric H^+-export from isolated vacuoles and tonoplast vesicles that occurs upon addition of sucrose to a suspension medium with energized or non-energized membranes. About 100 sucrose carriers per μm^2 tonoplast surface can be immunogold labeled with carrier specific mAb's. We can estimate now a preliminary turnover rate of 10 sucrose molecules per carrier and second.

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15 09

Studies on phosphate uptake in the microalgae *Chlamydomonas reinhardtii*

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Initial rates of phosphate uptake were measured in phosphate-starved *Chlamydomonas. reinhardtii* using a rapid filtration technique. Two uptake mechanisms could be distinguished differing with respect to their affinities for phosphate and their response to inhibitors. The high-affinity system showed a K_M for phosphate in the range of 0.3 - 0.9 μM and was inhibited by pyridoxal-5-phosphate. The low-affinity system had a K_M in the range of 5-29 μM and was sensitive to valinomycin. Both systems were inhibited by the uncoupler FCCP.

15 10

Modification of algal cell wall properties by external carbohydrates and turgor-dependent sodium pump

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The presence of osmotically insignificant concentrations of D-glucose and 2-deoxy-D-glucose in cultivation medium, in which the alga *Hydrodictyon reticulatum* completes its growth in 9 - 10 d, dramatically reduces the water content of the cells. At the same time the two sugars have highly significant, albeit opposite effects on the intracellular sodium concentration. Elasticity properties of growing cell walls seem to be influenced by the sugars and the performance of the sodium pump affected by altered turgor pressure according to the general hypothesis by Bisson and Gutknecht.

15 11

Uptake of gibberellins in leaf protoplasts of *Alstroemeria pelegrina* L.

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Chlorophyll loss of *Alstroemeria pelegrina L.* is strongly delayed by various gibberellins (GA) in the water both in leaves attached to the cut flowering branches, in detached leaf tips and directly applied to the leaf surface.
We have investigated the characteristics of uptake of three radiolabelled gibberellins (GA_4, GA_9 and GA_{20}) in alstroemeria leaf protoplasts. The efficiency of uptake increases with increasing hydrophobicity of the molecule, is strongly stimulated at lower pH values of the external medium and is inhibited by the proton translocator FCCP. Furthermore, the uptake shows no saturable component at an external medium pH 5. To investigate the mechanism of GA-uptake in more detail we have isolated and characterized right-side-out plasmamembrane vesicles. Subsequently, we studied the interaction of gibberellins with monolayers composed of a lipid extract of plasmamembrane vesicles. The results are consistent with a model of passive partioning of undissociated molecules and subsequent alkaline trapping of anions within the cell.

15 12

Import and intraorganellar routing of PSI-3 and the Rieske Fe/S protein

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We have analyzed the mechanism of chloroplast import and assembly of subunit 3 of photosystem I (PSI-3; gene: *psa*F), a monotopic protein that is inserted from the lumenal side into the thylakoid membrane and is the docking protein for plastocyanin during photosynthetic electron transport. Using the authentic precursor protein as well as chimeric constructs with transit peptides and mature parts from different precursor polypeptides, it turned out that PSI-3 (i) is synthesized with a bipartite transit peptide with import and thylakoid-targeting properties, and (ii) is strictly depending on such a targeting signal for its correct intraorganellar routing. These requirements differ strikingly from those of the Rieske Fe/S protein of the plastid cytochrome complex which has a similar topology within the thylakoid membrane but is synthesized with a mere stroma-targeting transit peptide and integrates *via* uncleaved signals in the mature protein. Both proteins differ also in their physiological requirements (e.g., the proton gradient across the thylakoid membrane) for their thylakoid integration/translocation.

15 13

The cell wall study using the *Nitella* cell "ghosts"

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A simple technique for a preparation of "ghosts" of the *Nitella* cells is suggested. Persistence of turgor is provided by the introduced polyethyleneglycol (PEG) 20 000 with a concentration of 200 mg ml^{-1}. A device for registration of intact cells and "ghosts" thickness changes using the small shifts probe was constructed. Turgor in the "ghost" was shown to be close to one in intact cells that was checked flattening with mass. Experiments with PEG 600, 1 000, 2 000 and 3 000 have shown that the cell wall keeps inpenetrability for PEG 3 000 and more at least for a few hours. Comparing action of 5 % ethanol and saccharose solutions on the "ghost" (1 M) and the cell (100 mM) have shown similarity in ethanol action and difference in saccharose action. Submersion of the "ghost" into a saturated solution of LiCl induced fast dehydration which was reversable and repeatable. Stepwise increase of the LiCl concentration till saturation led to filling of the "ghost" by this solution and fast submersion into water led to burst of the "ghost". Experiments with compression of the "ghost" measured with one probe and thickness change registration with another one were carried out. Degradation of "ghost" was induced mainly (except ferments) by 5 - 10 mM EDTA and by temperature above 80 °C. We hope that the "PEG-ghost" can be widely used in a study of cell walls properties.

15 14

Two possible ways of plant cell injury under salt stress conditions

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The model experiments on giant *Nitella* cells have shown that cell injury under high salinity levels may proceed in different ways. Microelectrodes imposed in vacuole and cytoplasm were used for monitoring electrical parameters of both cell membranes. Turgor pressure was registered using small shifts probe flattened against the cell situated on the hard support. Mature *Nitella* cells when exposed to 100 mM NaCl solution are damaged in rather well-known way consisting in the plasmalemma leakiness and the following ionic homeostasis disturbance. The most interesting and new observation is that the young cells in model experiments burst or undergo osmotic lysis. The essential conditions of a cell burst are cell wall loosening and high turgor pressure. The former was achieved by young cell exposure to 100 mM NaCl for 10 - 60 min, the later - by returning the cell into artificial pond water. The cell burst took place turgor recovery and proceeded as a rule in a pulsewise regime namely by series of cytoplasm exhaustions each followed by plugging the hole and partial turgor recovery. The results obtained in model system makes us suggest whether young root cells might burst under salt stress conditions and wheather mechanisms of salt tolerance imply cell wall strengthening.

15 15

Varietal differences in Ca^{2+} accumulation of grapevine

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Ca^{2+} transport properties of grapevine varieties showing different lime tolerance were studied by growing plants under Ca^{2+} excess. Hydroponically rooted one node cuttings of Leányka, Georgikon 28 (Teleki 8BB x *V. vinifera*), Chasselas x Berlandieri 41 B and Rupestris du Lot were treated with 25 and 50 mM $Ca(NO_3)_2$ for ten days. Rupestris du Lot and Chasselas x Berlandieri 41 B damaged in part, wilting was observed. Leányka and Georgikon 28 grew vigorously and accumulated higher amount of Ca^{2+} in leaf blades. The majority of leaf calcium was deposited in the cell wall. Cell sap Ca^{2+}, including vacuolar Ca^{2+} increased considerable in the accumulator varieties but it did not change in the sensitive ones under Ca^{2+} excess. In the callus culture more intensive Ca^{2+} accumulation was found in the sensitive varieties. Lime tolerance of grapevine varieties can be achived by following different strategies: calcium exlusion under moderate stress (Chasselas x Berlandieri 41 B) or calcium accumulation and dilution by growth (Leányka, Georgikon 28).

15 16

Use of fluorescent probes to study the calcium ions transport across plasmalemma

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We have used fluorescence technique to monitor the Ca^{2+} transport across maize coleoptiles plasmalemma. The fluorescence of indo–1 and chlortetracycline in–creased when K^+ – diffusion potential on vesicles of plasmalemma had been generated. Similar effect in the presence of Ca – ionophore A–23187 proved Ca^{2+} transport into vesicles. The maximum rates of Ca^{2+} transport under the valinomycin treatment was found at potential, corresponding to the depolarized membrane potential of most plant cells. It was determined that passive Ca^{2+} influx is sensitive to verapamil. ATP – dependent Ca^{2+} uptake by the vesicles was observed during 2 min when Ca^{2+} (10μ M) had been added. This Ca^{2+} transport was inhibited by 70 % by DCCD, 80 % by DES (50μM both), 85 % by 100μM orthovanadate and 90 % by 10μM La^{3+}. Mg^{2+} – ADP gave one third of response that was in case of Mg^{2+} – ATP. This results indicate that the active Ca^{2+} transport is due to the plasma membrane Ca^{2+} – ATPase, while the passive Ca^{2+} transport has channel – like nature. The role of Ca^{2+} channels or Ca^{2+} – ATPase in the regulation of intracellular calcium level and possible function of Ca^{2+}ions as a second messenger in plants are discussed.

15 17

The necessity of PPi and H^+-PPiase in maintaining the vacuolar proton gradient in metabolic inhibitor-treated cells

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In *Acer pseudoplantanus* cells, the effect of metabolic inhibitors (KCN and 2-deoxy-D-glucose) on vacuolar proton gradient, monitored by acridine orange, was assayed. Potassium cyanide plus 2-deoxy-D-glucose slightly lowered this gradient, while strongly decreased cellular ATP level and halved inorganic pyrophosphate (PPi) content. Two inhibitors of phosphatases (imidodiphosphate and KF) restored the PPi level in metabolic inhibitor-treated cells, but decreased the vacuolar proton gradient by inhibiting H^+-PPiase. These results show, hence, that tonoplast H^+-PPiase is especially responsible for the maintenance of vacuolar ΔpH and that this enzyme is the major consumer of cytoplasmic PPi in metabolic inhibitor-treated cells.

15 18

Characterization of a 34 kD protein of the chloroplast outer envelope from *Pisum sativum*

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We cloned a 34 kD polypeptide from the outer envelope of pea (OEP 34). It behaves as an integral constitutent of the outer envelope. OEP 34 does not possess a cleaveable N-terminal transit sequence but it is target to the chloroplast by internal sequence information which seems to be located in the C-terminal part of the protein. Import of *in vitro* translated OEP 34 into the outer membrane of intact chloroplasts depended on protease-sensitive surface components. The orientation of the native OEP 34 was retained (N_{cyto} - C_{in}). That means that majority of the protein was accessible to externally added protease. Sequence comparison yielded only conserved motifs found in GTP-binding proteins. GTP-binding was demonstrated in vitro by photoaffinity labbeling. The cytosolic exposed topology, GTP-binding characteristics and the presence in a solubilized protein import competent complex results us to speculate that OEP 34 is involved in regulation of the posttranslational protein import into chloroplasts.

15 19

Ionic leakage of the plant cell plasmalemma - real ion pathway into the cell

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Present work was undertaken to reveal unselective plasmalemma permeability (leakage conductance), that is remained after all selective pathways (ionic channels, H -ATPase pump) are inhibited. On the cells *Nitella flexilis* we found appropriate experimental conditions: dark adaptation, plasmalemma hyperpolarization, low concentration of the permeating ions in solution, application of the potassium channel blockers. Under these conditions the lack of effects of the known channel blockers - ethacrynic acid (Cl -channel), La^{3+} (Ca -channels) are observed; and together with conductance increasing, caused by Cs^{+} , those are the proofs for validity of the found conditions. The loss of the leakage conductance selectivity among K^{+} , Na^{+}, Cs^{+} and Li^{+} ,was shown; the same was true for Ca^{2+} , Sr^{2+} and Mg^{2+} . The leakage conductance didn't display the potential dependence and the pH (5.5-8.2) had no effect on that. The temperature (+5-+30^{0} C) effect on the conductance is not large, and the value of the activation energy is about equal to that for KCl solutions. The obtained results suggest that under above conditions we in fact study the unselective leakage conductance, which is not of the remained channel permeability. This conductance appear to be due to dynamic defects of the membrane lipid matrix. The contribution of leakage conductance to the total one reached 20% under usual conditions.

15 20

The influence of various factors on the transport of oleanolic acid glycosides to isolated vacuoles

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Two series of oleanolic acid glycosides, i.e. derivatives of 3-O-monoglucoside (I) and derivatives of 3-O-monoglucuronide (F) have been found in leaves of *Calendula officinalis*. We have demonstrated effective transport of both monoglycosides (I and F) into isolated vacuoles, differing in $K_m$values, the dependence on pH, the presence of ATP and various inhibitors. Inhibition of glucoside I transport by nitrate, CCCP, DCCD, DIDS and PCMBS. and the saturation phase in the transport kinetics point to the presence of a carrier-mediated and ATP dependent mechanism. On the other hand, the saturation kinetics and sensitivity to protein-modifying agent PCMBS of the transport of glucuronide F would suggest a passive. carrier-mediated process. The present results concerning the dependence of the transport of I and F on the presence of various nucleotides and ions support the conception that the two oleanolic acid monoglycosides differ in the mechanism of their transport to isolated vacuoles.

15 21

The effect of the local anesthetic procaine on the electrical coupling between internodal cells of *Nitelopsis obtusa*

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In this study, we tried to elucidate possible mechanisms involved in the action of the local anesthetic procaine on the electrical coupling between *Nitellopsis obtusa* internodal cells. 15 mM procaine, applied extracellulary, has been found to induce a quite rapid transient decline in the node resistance - by about 30%; afterwards the resistance increases to about 120% of the initial value. Time course of the procaine induced response of the node resistance was compared to that of membrane resistance. The hypothesis has been put forward that in the first phase of procaine treatment, the dominating effect is a quite drastic decrease in the resistance of membrane areas of the node. This results in the decline of total node resistance. The subsequent increase in node resistance is thought to be due to the procaine-induced closing of plasmodesmata. Both processes (decrease in membrane resistance and closure of plasmodesmata) are supposed to be Ca^{2+}-mediated.

15 22

Ca^{2+}-pump and Ca^{2+}/H^+ antiport activities in plasma membrane vesicles of maize roots (*Zea mays* L.)

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ATP-dependent Ca^{2+}-uptake was investigated in plasma membrane vesicles of corn roots. We observed that in a Cl^--containing medium, about 30% of the total Ca^{2+} accumulation is CCCP-sensitive and corresponds to the fraction which is not accumulated in a $SO_4^=$-containing medium. Vesicles pre-loaded with H^+ take up Ca^{2+} more rapidly, suggesting that there is a mechanism of Ca^{2+} transport which depends on the magnitude of the proton gradient across the membrane. The fraction of Ca^{2+} uptake which is sensitive to the protonophore CCCP is increased from 30% to about 70% as the Ca^{2+} concentration in the medium increases from 50 µM to 250 µM, while the CCCP-insensitive fraction of Ca^{2+} accumulated is reduced from 70% to 30%, suggesting that different Ca^{2+} affinities exist in the two Ca^{2+} uptake processes. The used pH values of 6.6 or 7.2 are distinctly suitable for the Ca^{2+} uptake which is dependent or independent of the proton gradient, respectively. The sensitivity to Ca^{2+} and external pH indicates that the H^+ gradient-independent Ca^{2+} accumulation reflects activity of the Ca^{2+}-pump. The results indicate that the plasma membrane of corn roots contain two distinct mechanisms of Ca^{2+} transport: a high Ca^{2+} affinity, proton gradient-independent Ca^{2+} pump and a low Ca^{2+} affinity, proton gradient-dependent Ca^{2+}/H^+ antiport, which may be important to maintain low levels of cytoplasmic Ca^{2+}.

15 23

Isolation of one sucrose carrier clone and a family of 10 hexose carrier clones from *Ricinus communis* and their expression in various tissues

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With help of a cDNA probe for the sucrose carrier of *Arabidopsis* one clone from *Ricinus* was found, which codes for active sucrose uptake. In contrast, 10 different clones of the hexose carrier were isolated from *Ricinus*, using PCR. Transformation of full-length clones into bakers yeast or fission yeast allowed the determination of substrate specificity and the need for energization. The clones are differently expressed in various tissues of the castor bean plant, each clone showing its own, special distribution.

15 24

Energy-dependent cellular ion transport in wheat acclimated to drought and frost

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Evaluation of the extent of the energy-dependent ion transport, mainly Na^+/K^+ and Ca^{2+}, we have based on the assumption that estimation of the energy-consuming processes by selective inhibitors results in reduction of ATP production proportionally to the extent of ATP requirement. The inhibitor of Na^+/K^+-ATPase (0.5-1.0 mM ouabain), P-type ATPase (0.5-1.0 mM sodium vanadate) and inhibitor of Ca^{2+} uptake (0.5-1.0 mM lanthanum chloride) were applied to the incubation medium 1 to 3 hours prior to the manometric measurements of the dark respiratory rate. Lanthanum ions exerted the most spectacular inhibitory effect (up to 37%) in wheat leaves and crowns. The response to ouabain and vanadate was not as conspicuous (up to 13%).

Acclimation to frost and drought changed the pattern of energy expenditure. The sensitivity of crowns of the frost resistant wheat towards all the applied inhibitors was decreased whereas in the frost sensitive cultivars the inhibitory effects of vanadate and ouabain was lowered indicating the importance of the type of reaction of ion transport. Under water stress a reverse response of non-acclimated and acclimated leaves towards lanthanum ions was observed whereas the energy expenditure for ATPase action was only of quantitative character.

On the basis of the results obtained it seems that both quantitative and qualitative differences exist in the reaction to frost and drought whereas it seems that the common response to both stresses might involve Ca-mediated processes.