02 01

Relation of abscisic acid to dry matter acquisition and ethylene biosynthesis in ripening hybrid blueberry fruit (*Vaccinium darrowii* L. x *V. corymbosum* L.)

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Blueberries are an increasingly important horticultural crop in New Zealand and other countries. Fruit ripen over an extended period, with considerable differences in storage and other qualities. Tissue concentrations of abscisic acid (ABA), 1-aminocyclopropane-1-carboxylic acid (ACC), malonyl-ACC and also ACC oxidase activity and ethylene evolution were determined at 5 stages of colour development on 2 harvest dates. These were related to dry matter, sucrose and reducing sugar content. ACC concentrations, and the proportion conjugated varied between harvest dates, while ethylene evolution and ABA concentrations were similar. ABA concentrations were high relative to other fruit, exceeding 10 nmole/g fw prior to full ripeness. ABA was present in higher concentration in mesocarp than in seeds or pericarp/cuticle. It is proposed that ABA is implicated in dry matter acquisition, but is not a primary determinant of colour development or ethylene regulation.

02 02

Protein accumulation in pith explants of kale induced by hormone-like factor of vascular tissue

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The growth potential of young histologically organized tissues, namely meristems, is connected with the production of special growth stimulating metabolites. These metabolites may involve, in addition to well known compounds such as auxins and cytokinins, some other so far unidentified regulatory factors. A hormone-like factor (HF) differing from major classes of phytohormones was detected in stem explants of kale (Řeřábek et al. 1994, Biol.Plant. 36: 59-64). Explants in form of blocks were excised from the stem in such a way that they contained either pith parenchyma or pith parenchyma with adjacent vascular tissue including cambial meristem. When explants were cultured on a simple agar medium containing sucrose, HF was transported from vascular tissue to the pith, where it stimulated protein and starch accumulation. Electrophoretically separated soluble proteins contained a newly formed distinct protein of about 100 kDa , which was induced by HF and to a lesser extent also by a cytokinin added to the medium. Analogical analysis of glycoproteins by means of Con A-peroxidase test revealed three newly formed glycoproteins of 27, 35 and 50 kDa .

02 03

Content of endogenous cytokinins and cytokinin oxidase activity in *Petunia* leaf explants during shoot organogenesis

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Content of endogenous cytokinins (CKs) and cytokinin oxidase activity were compared in leaf explants of two *Petunia hybrida* lines (St40 and TLV1) during the early phase of commitment to shoot organogenesis. Free zeatin, isopentenyladenine (iP), their ribosides and dihydrozeatin were detected by HPLC-RIA method in both uncultured leaves and leaf explants cultivated for 6 or 10 days *in vitro* on shoot induction medium containing 4.4 μM BAP. *In vitro* cultivation resulted in the increased accummulation of CKs, namely iP (ca. 10 fold). Cytokinin oxidase activity was enhanced in parallel with higher cytokinin content in tissue cultured leaf explants (3-7 fold compared to uncultured leaves), which is in accordance with previous findings about the stimulation of the enzyme activity by exogenous cytokinins.

The results will be discussed in connection with different organogenetic responses of the two *Petunia* lines.

02 04

The effects of electric field on the growth of higher plants

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The experiments were carried out with 96-h-old intact *Zea mays L.* seedlings and 10 mm long coleoptile segments cut 4 mm below the tip. The electric fields were applied longitudinally along the mesocotyl region. It was found that: first, the electric fields (15V) caused inhibition of the elongation growth of intact seedlings. The amount of inhibition was dependent on both the polarity and the duration of the applied voltage. Second, the growth inhibition was greater when the tip of the shoot was positive relative to the roots. IAA and FC-induced growth of maize coleoptile segments excised from electrically stimulated seedlings showed that: first, the electric field caused inhibition of IAA and FC-induced growth. Second, IAA-induced growth of coleoptile segments was greater when the tip of the shoot was negative to the roots (not the case for FC and intact seedlings). It is suggested that the observed changes of growth of electrically stimulated maize seedlings are connected with mechanism of auxin transport.

02 05

Involvement of endogenous phytohormone levels in early pod shatter in oilseed rape (*Brassica napus*)

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Early pod shatter in oilseed rape (*Brassica napus*) is an important agricultural and economical problem. This pod shatter seems to be preceded by a rise in ethylene evolution. We investigated whether other phytohormones (*i.e.* auxins, cytokinins, abscisic acid and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) also are playing a part in this phenomenon. The ethylene peak showed to be well correlated with a rise in ACC level, especially in the dehiscence zone and for a lesser extent in the pod wall, indicating that both these parts may be responsible for the production of ethylene. Also a peak in auxin level seemed to be coinciding with the ethylene peak, again most notable in the dehiscence zone. This may raise the assumption that auxins themselves or via induction of ACC-synthase (followed by ethylene biosynthesis) are involved in early pod shatter. possible effects of cytokinins and ABA also will be discussed.

02 06

The content of endogenous zeatin and abscisic acid in etiolated cotyledons of lupine seedlings

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For studies of hormone participation in the plastogenesis and in chloroplast gene expression, the level of endogenous zeatin and ABA in lupine cotyledons were determined by competitive ELISA. In dry seeds, cytokinin was presented in quite low amounts and its content increased by a factor of 3 - 4 during the first three days after germination. Excision of cotyledons promoted a decrease in endogenous cytokinin level. A relatively high ABA content was found in cotyledons of dry seeds and on the early stage of seedlings development (up to 24 h). Significant decline of ABA level was observed thereafter in intact and isolated cotyledons. The observed changes in balance of endogenous zeatin and ABA correlated with acceleration of chloroplast differentiation and chloroplast genome expression.

02 07

Tissue sensitivity of "Madelon" roses for cytokinins

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In rose combination plants, bud break of the scion is influenced by the genotype of the rootstock, probably caused by differences in its cytokinin production. Branching of scions on one rootstock induces consequent differences in sensitivity of the scion tissue for cytokinins. The tissue sensitivity of "Madelon" and "Vivaldi" roses for BA, BAR, Z, ZR, iPA, iPAR, DHZ and DHZR was tested and reported here. Axillary rose buds from the second, third and fourth five-leaflet leaf counted from the apex were sterilized and placed on MS medium with glucose (45 g l^{-1}) and 0.32, 3.2 or 32 μM filter sterilized cytokinin. After 28 d fresh and dry mass and number of shoots were recorded. In general, at low concentration of cytokinin, the axillary bud developed into a primary shoot, increasing the concentration of cytokinin caused an increase in dry mass of the plant and formation of axillary shoots on the primary shoot (secondary shoots). Further increase in the concentration of cytokinin caused a decrease in the total dry mass; the share of the secondary shoots in the total plant dry mass increased up to approximately 50 %. All cytokinins appeared to be able to induce the maximal response, although each cytokinin required a characteristic amount to do so. The different cytokinins are either recognized by one, not specific receptor with different binding capacities for the cytokinins, or are converted to one form that is recognized by a specific receptor. Further research has to be done to elucidate this.

02 08

Interference between enzymes of auxin biosynthesis and growth retardants: Comparison between the mono- and dihydrazides of glutamic and aspartic acids

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It has been assumed that aminotransferase(s) is the primary enzyme(s) in the arylpyruvate pathways of auxins biosynthesis. The enzyme model systems from shoots of 7 to 9-d old etiolated pea plants were *in vitro* treated by different concentrations of mono- and dihydrazides of dicarboxylic acids. The results from enzyme kinetic analysis revealed the higher sensitivity of indolylacetic pathways towards mono- and dihydrazides of aspartic acid than phenylacetic acid pathway.

02 09

The influence of synergic retardant mixtures on the metabolism and producing capacity of the grape stock cv. BxR CO_4

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The action of chlorcholine chloride - 0.05 - 0.5 %, hydrel -.0.025 - 0.25 %, and mixtures of CCC + hydrel - 0.05 + 0.25 %; 0.05 + 0.05 %; 0.1 - 0.05 % on the metabolic process activity, growth and producing capacity of grape stock cv. BxR CO_4 was studied. Spraying grape with retardant products at the beginning of shoot intensive growth leads to the depression of growth processes in in the early and intermediate vegetative stage, acceleration of shoot ripening, increase in the standard vine size fit for grafting, standard cutting cutput by 33.5 - 44.2 % as compared with the control. The change of shoot anatomical structure under the action of retardants results in the increase in the regeneration activity of cuttings in the graft. The CCC + hydrel doses 0.025 + 0.05 %; 0.05 + 0.05 % are optimal.

02 10

Light intensity and the organ distribution of cytokinins in *Phaseolus vulgaris* cv. Provider

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The development of plants is much affected by the light intensity under which they are grown. Typically, a sub-optimal light intensity is associated with longer than usual stem growth and smaller leaf size. The possible relationship of such light effects to the cytokinins is being investigated in bean, *Phaseolus vulgaris* cv Provider. The cytokinin nucleotides, zeatin- 5′-monophosphate (ZMP) and isopentenyl adenosine-5′-monophosphate (iPMP), have been found to be the major cytokinins in all parts of the plant and at all light intensities so far examined. However, the levels of these cytokinins were found to differ between the organs and the levels were affected by light intensity.

02 11

Antisenescent activity of naturally occurring aromatic cytokinins

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Biological activity of naturally occurring N^6-benzyladenine analogues was tested to find the relationship between the position of hydroxyl group on the benzyl ring and relevant biological response. Free bases, 9-ribosides an 9-glukosides were tested. A modified senescence test was developed which increased the sensitivity and saved the consumption of cytokinin needed. The activities of all compounds tested were compared on the bases of E_{50} values, defined as the concentration at which 50 % of the maximum response was recorded, and on the bases of minimum concentration required for positive response. Unhydroxylated compounds ribosylated or glukosylated at 9-position of purine ring or relevant free base were used as standards. Hydroxylation at *meta*-position significantly increases the activity whereas hydroxylation at *ortho*-position leads to its decrease. There is no significant difference between response of free bases and 9-ribosides while 9-glukosides seem to be less active.

02 12

Developmental and stress control of alkaline hydrolysis-related ethylene releasing substances (AHRERS)

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Ethylene release during alkaline hydrolysis is a general property of plant tissues. In the present work we have specifically asked whether AHRERS (i) contributed to the physiological response of tissue to ethylene or (ii) are intermediates in the biological synthesis of ethylene. A series of experiments with cereal and conifer seedlings was intended showing both a developmental and stress control of AHRERS. A treatment with hydrogen peroxide increased ethylene production and AHRERS content both in detached needles of pine and spruce trees and in needles of intact pine seedlings. Yet the responses had different temporal characteristics. Induction of ethylene biosynthesis and protein synthesis de novo was essential for hydrogen peroxide-dependent rise of AHRERS. Gel filtration experiments with extracts from control and hydrogen peroxide-treated needles confirmed the existence of chromatographically distinct forms of AHRERS not identical with ACC and selectively induced by the stressor. A possible use of AHRERS as an indicator for the level of stress influence on plants will be discussed.

02 13

Properties of the ACC oxidase purified from pear fruit

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ACC oxidase (EFE) is the enzyme responsible for ethylene synthesis from its immediate precursor 1-aminocyclopropane-1-carboxylic acid (ACC). ACC oxidase was extracted, purified and characterized from pear fruits (*Pyrus communis* L cv Conference). The enzyme was purified 23-fold with a 21% recovery of the activity in a 2 step purification procedure. The specific activity of the pure enzyme was 190 µl ethylene mg $prot^{-1}$ h^{-1}. ACC oxidase from pear fruit showed an absolute requirement for ascorbate, Fe^{2+} and CO_2, and a Km of 225 µM for the substrate ACC. The enzyme has a temperature optimum at 30 °C. The ACC oxidase from pear fruit is a monomeric protein with a molecular weight of 36 kDa by SDS-PAGE and of 44 kDa by gel filtration. The enzyme has a pI of 4.8 by chromatofocusing. ACC oxidase activity increased 100% in the presence of 1 mg ml^{-1} of catalase. However, the 3-amino-1,2,4-triazole treated catalase, with less than 3% of the activity, was not able to stimulate the activity of the ACC oxidase. Thus, the catalytic activity of the catalase was required for the stimulatory effect of the ACC oxidase.

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02 14

The effect of jasmonic acid on the photosynthetic pigments of potato plants grown *in vitro*

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We analysed the effect of 1µM jasmonic acid (JA) in the media on the photosynthetic pigments of the leaves of potato cv. Sante and cv. Ulster grown in vitro. The pigments were identified from their light-absorbance properties, reaction with HCl, and high-performance liquid chromatography retention times. Application of JA resulted in increase of a fresh weight, stem elongation, leaves expansion and development of root system. These morphological changes were more pronounced in cv. Sante than in cv. Ulster. The leaves of JA treated potato cv. Sante contained reduced amounts of the total photosynthetic pigments calculated on a dry weight basis (40% compared with control). In contrast to cv. Sante, no significant reduction of the pigment's level was found in the leaves of the treated potato cv. Ulster. Antheraxanthin was the only photosynthetic pigment that was present in higher amount in the leaves of JA treated plants compared to the control plants.

02 15

Cadmium stress in growth reaction of plant cells

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Cadmium being one of the most toxic heavy metals upsets essentially a metabolism of plant cell. Substantial amount of announcements indicate its essential role in disturbances of structure of cell 's wall as well as of elongation growth process. In the connection with the above there has been studied a dependence between concentration of cadmium ions (10^{-7} - 10^{-2} M) and elongation growth of the coleoptile segments of *Zea mays* L. as well as acidification of incubation medium in the presence of IAA (10^{-5} M) or FC (10^{-6}). This problem is also important with regard to "acid-growth theory" proposed for clarification of elongation growth induced by IAA. Examinations were carried out with 10 mm long coleoptile segments of *Zea mays* L. cut from 3 mm below the tip. A first leaf was removed from segments. It was shown that cadmium in high concentrations inhibits elongation growth of coleoptiles both in presence of IAA or FC as well as without them. It was demonstrated also that cadmium does not change lag-phase of IAA-induced elongation growth. It was found that FC causes greater acidification of incubation medium of coleoptiles than auxin both in presence of cadmium ions or without them. Cadmium ions not only cause disturbances of the growth processes but also changes correlation between growth and acidification.

02 16

Role of cytokinin and abscisic acid in photosynthetic apparatus formation in isolated lupine cotyledons

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Cytokinins and abscisic acid (ABA) exert crucial effect on plastid development in detached lupine cotyledons. The effect of 6-benzylaminopurine (BAP, 22 μM) and ABA (67 μM) have been studied at the steady-state mRNA and protein levels using 15 gene-specific probes for plastid proteins and corresponding antisera. In BAP- and ABA-treated cotyledons the steady-state mRNA levels remain relatively unaffected for plastid-encoded polypeptides, but not for those nuclear-encoded genes that could be tested. On the other hand, synthesis and accumulation of proteins of nuclear and plastid origin vary significantly.
The results demonstrate that phytohormones influence the biogenesis of the thylakoid membrane mainly posttranscriptionally.It is concluded that there are very complex interactions between phytohormones and light in regulation of chloroplast proteins biosynthesis.

02 17

Induction of specific proteins in barley leaf segments by jasmonates and osmotic stressors

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Jasmonic acid induces a set of specific proteins (JIPs). These proteins appear also after treating the leaf segments with osmotic stressors like sorbitol, glucose, sucrose and PEG-6000. The treatment is accompanied by increased levels of endogenous jasmonates. But not all of the osmotic stressors are able to induce these proteins. NaCl, 6-deoxyglucose or 2-deoxyglucose are inactive in our system.
We conclude from the results that the chemical nature of the C-source is responsible for the jasmonate accumulation.

02 18

Hormone mutants in highly inbred lines of radish

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A genetic collection of radish *Raphanus sativus* consists of 36 highly inbred lines marked by genetic characters. Several lines can be treated as phytohormone mutants. *In vivo* gibberellin responsive dwarf line and giant line are thought of mutants in a deficiency and an excess of gibberellin. 5 lines showing abnormalities in seed dormancy or sensitivity to a water stress can be viewed as abscisic acid mutants. 2 lines possessing altered shoot geotropism are regarded as auxin mutants. 10 lines form spontaneous genetic tumors in storage roots. These tumorous lines exhibit altered *in vitro* reactions to auxin and cytokinin. Both auxin and cytokinin habituated tissues were obtained only for the tumorous inbred lines. Besides that, new cytokinin response mutants were revealed among the lines by in vitro screening reaction to cytokinin. Therefore, inbreeding has led to an emergence of hormone mutants existing in the population of radish plants.

02 19

Hormonal control of somatic embryogenesis in *Rauwolfia vomitoria* Afz.

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The role of ethylene and abscisic acid (ABA) in the growth of the callus and somatic embryogenesis in *Rauwolfia vomitoria* was investigated. The results of our studies confirmed the requirement of ABA and the action of endogenous ethylene for normal embryoid maturation.

02 20

The effect of Pb^{2+} ions on IAA and FC induced elongation growth and acidification of the incubation medium of maize coleoptile segments

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The experiments were carried out with 10 mm long maize coleoptile segments, excised from 4-day-old seedlings 3-4 mm below the tip. Segments were incubated for 4 hours in a basal medium of the folowing composition: 1.0 mM KCl , 0.1 mM NaCl , 0.1mM $CaCl_2$ in the presence or without growth regulators (IAA, FC) and $PbCl_2$. It was shown that IAA-induced elongation growth is more susceptible to lead ions than FC-induced growth and extension growth in the basal medium. It was also found that acidification of the incubation medium with or without IAA was greater in the presence of Pb^{2+} ions (not the case for FC). It is concluded that toxic effect of Pb^{2+} ions on elongation growth is not connected with inhibition of H^+-extrusion. It is probable that lead reduces extension growth by inhibition such processes induced by auxin as transcription, translation or exocytosis.

02 21

Effect of the treatment with kinetin on endogenous ABA content during Italia table grape ripening

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The table grape is one of the most important crop in the Region of Murcia (Spain) with great economical repercussions, but the ripening control is not clear yet. In this paper we study the effect of the kinetin treatment on ABA contents (t-ABA and ABA isomers) in Italia table grape (*Vitis vinifera* L.), a non climacteric fruit where the ethylene emission is not the trigger of the ripening but the changes of ABA levels.
In our experiments, we found that the both isomers levels are higher in control than in treated fruits at the veraison and the end of ripening, with a delay in the harvest date due to the treatment. These results confirm the cytokinin antisenescent role throught the modulation of ABA contents. Morever, an increase in the production with the treatment we observed.

02 22

Different molecular forms of cytokinin oxidase in tissue cultures of two tobacco cultivars

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Two distinct molecular forms of cytokinin oxidase were detected in tissue cultures of two tobacco cultivars (*Nicotiana tabacum* L., cv.Wisconsin 38 and cv.Virginia Bright Italia) differing in their requirements for exogenous cytokinins. Both enzyme forms exhibit variations in their glycosylation patterns, pH optima and *in vitro* responses to the presence of copper-imidazole complex in the reaction mixture.
With respect to the reported genotypic variation in molecular forms of cytokinin oxidase between *Phaseolus* species (Kamínek,M., Armstrong,D.J. [1990] *Plant Physiol.93*, 1530) it is suggested that the pH optimum and the glycosylation pattern of the enzyme are closely associated. Variation in characteristics between the two molecular forms of cytokinin oxidase, presented here for cultures of different tobacco cultivars, may be linked to tissue-specific differences in the compartmentation of the enzyme and will be discussed as one of possible regulatory mechanisms of cytokinin degradation in plants.

02 23

ACC oxidase from banana fruit, purification and biochemical characterization

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Ethylene is a plant hormone involved in the ripening of climacteric fruits. The last step of its biosynthesis is catalyzed by ACC (1-aminocyclopropane-1-carboxylic acid) oxidase (also known as Ethylene-Forming Enzyme, EFE).

We have purified ACC oxidase from banana pulp tissue (*Musa AAA* Group, Cavendish Subgroup) to homogeneity in 3 chromatographic steps: anion exchange (Mono Q), chromatofocusing (Mono P) and gel filtration (Superdex-75). The purified enzyme is active as a monomer with a molecular mass of 40 kDa estimated by gel filtration and 36 kDa by SDS/PAGE. A pI of 4.9 was estimated for the enzyme. The enzyme requires ascorbate and Fe^{2+} during the *in vitro* assay and its activity is stimulated by CO_2. The pH optimum was found to be 7.5 in Tricine buffer. A Km_{ACC} of 36 µM in the absence of CO_2 and 56 µM in its presence was determined. The activity is inhibited by Co^{2+}. The enzyme shares immunogenic epitopes with ACC oxidases from other fruits. This is the first report concerning the purification of ACC oxidase from a monocotyledonous fruit.

M.A.M-L. is a recipient of a fellowship from MIDEPLAN, CHILE.

02 24

Phytohormones of red, brown and green marine macroalgae

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Performed studies on the phytohormonal complex of red, brown and green marine algae-macrophytes enable to broaden the existing concept regarding the functioning of the cryptogamous plant regulatory systems specifically regarding the involvement of phytohormones in algae growth and development. The content and activity of auxins, gibberellins, cytokinins and ABA in 34 species of algae collected in the Black Sea, Indian and Atlantic Oceans were studied using specific bioassays and HPLC. Comparative studies on phytohormones of algae of *Gigartinales, Ulvales* and *Fucales* orders considerably different in their structure, morphology and type of reproduction made it possible to establish some differences in the qualitative composition of all investigated growth substances. Maximum concentrations of IAA were found in species of *Fucales* which were characterized by the presence of active growth zones. Brown algae showed a high cytokinin activity. Green and brown algae are characterized by a greater, as compared to red algae, activity of gibberellin-like substances. Increase in ABA content in brown and green algae during a transition from a vegetative growth to a reproductive development was shown. Seasonal changes in the phytohormonal content in red, brown and green algae which are correlated with the periodicity of their growth, development and reproduction were observed.

02 25

The effect of dehydration on chromatographic distribution of immunoreactive forms of cytokinins extracted from seedlings of *Zea mays* L.

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Maize seedlings grown in water culture were taken from water environment and were dried for 30 minutes. The aqueous residue of 80 % alcohol extract after freezing and melting was subjected to thin layer chromatography, and chromatographic distribution of immunoreactive material was evaluated with the help of antibodies to cytokinins. It is discovered that water deficit results in the changes of immunoreactive cytokinins spectra. The decrease of immunoreactive material content was observed in chromatogram regions corresponding to zeatin and zeatin-riboside standards in comparison to control plants. And synchronous immunoreactivity increase in other chromatogram zones under the influence of water deficit was observed. The influence of water deficit on cytokinins metabolism is discussed.

02 26

Levels of endogenous phytohormones in synchronous *Chlorella* culture

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The goal of our study was to examine levels of endogenous phytohormones in synchronous *Chlorella* sp. *K* culture. Intracellular IAA level over the cell cycle correlated positively with increase in dry biomass, augmentation of cell size, and DNA biosynthesis. Obtained results indicate that plant growth substances appear to control unicellular algae metabolism.

02 27

Auxin- and cytokinin-like compounds from some *Anabaena* strains

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Non classical auxins and cytokinins were investigated in some *Anabaena* (*Cyanobacteria*) strains with selective bioassay methods, such as in vitro tobacco leaf disc, soyabean callus, wheat leaf chlorophyll retention, cucumber cotyledon greening and expansion, oat coleoptile elongation methods. Various dilutions/concentrations of the supernatants and the centrifuged cyanobacterial suspensions were tested. On basis of the results it was established that all strains produce non-classical auxins at a concentration range of 10^{-6}-10^{-5} M, depending on the age and density of their cultures. The selective in vitro tobacco leaf disc method could not show the presence of cytokinin-like compounds, however the other bioassays -wheat leaf chlorophyll retention, cucumber cotyledon expansion tests- indicated the inferior role of the cytokinins.

02 28

Growth regulators of various algae associations of the Black Sea

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The report will cover the results of studying the phytohormonal status of marine algae-macrophytes of predominant species and subdominants of mono- and oligodominants associations in structurally different phytocenoses. Algae of annual local and perennial regional associations which are characterized by a high productivity were studied in different seasons of the year. Specific composition of associations and their productivity are liable to seasonal changes. Indole-3-acetic acid, zeatin, dihydrozeatin, riboside zeatin, izopentenyladenine, izopentenyladenosine and abscisic acid were identified and quantified using HPLC. Auxin-, gibberellin-, cytokinin and abscisic-like activity was found not only in algae thallus but also in surrounding sea water. This fact indicates that algae phytohormones might be exuded into the environment both in the process of algae life activities and as a result of washing out following the plant death. These seasonal changes found in the above associations suggest that phytohormonal regulation of the specific composition and phytocenosis productivity depend upon the phytohormonal balance of dominating species.

02 29

Immunolocalization of ABA by means of monoclonal antibodies in stressed leaves of *Lavandula stoechas* L.

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Plants of *Lavandula stoechas* L. were withdrawn of water for three days, showing ψ = -1.99 MPa and RWC = 62.98 %. Leaves of young shoots were fixed with EDC and embedded in Lowicryl K4M resin. Sections were incubated for two hours on different dilutions of monoclonal antibodies against abscisic acid (ABA), and then were floated on gold-labelled antibody (10 nm goat anti-mouse IgG Au) for one hour. Gold particles were localized in chloroplasts of parenchyma cells from the central mesophyll. This label was absent from the other cell compartments. ABA accumulation in chloroplasts due to water stress was visualized by immunocytochemistry techniques.

02 30

The effect of photoperiod and gibberellin on the apical shoot growth of *Salix pentadra* L.

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As in many other temperate trees the seasonal growth rhythm in willow is under photoperiodic control. The changes of daylength along with temperature serve as a signal, which is transformed into a growth response through altered metabolism of phytohormones, especially gibberellins (GA). Promoted growth results from accelerated divisions and elongation of cells. It has remained obscure, however, to what extent these mechanisms are involved in photoperiodic control of growth. In the present research the growth was studied in different areas of apical shoots in bay willow (*Salix pentandra* L.). The seedlings were given various photoperiodic or GA treatments. The morphological changes in the shoot apexes were observed with a scanning electron microscope. The cell lengths in apical and subapical internodes were measured with a light microscope combined with an image analyses system. The greatest difference between the treatments was found in the strength of the growth response. The elongation of internodes in the GA treated shoots was much faster than in the long day (LD) treated plants. In addition, the number of leaf primordia decreased as the apical growth was induced. Differences in shoot apex morphology between the different treatments could not be found. As a conclusion, the constant length of cells in different treatments indicate, that the GA and LD treatments promote equally both the elongation and the divisions of the cells in the subapical region of the willow shoots.

02 31

Molecular analysis of cytokinin action in *Physcomitrella patens*

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Within hours cytokinins induce cellular differentiation in the moss *Physcomitrella patens* as well as division of macrochloroplasts in a particular *Physcomitrella*-mutant. Concomitantly, cytokinins promote maturation of complex plastid transcripts and a transient occurence of plastid polypeptides. These cytokinin-induced molecular changes interfere with other internal and external stimuli, e.g. blue light and endogenous oscillators. Detailed analyses utilizing two-dimensional electrophoresis (IEF/SDS-PAGE) reveal a cascade of plastid proteins underlying chloroplast division in this particular mutant. Identification of these proteins by Western-analyses and microsequencing will be reported. Restriction enzyme- and Southern-analyses gave no hints for mutations in the plastid DNA of this chloroplast mutant but revealed its methylation around an open reading frame (ORF), possibly encoding a zinc-finger protein. Expression of the protein and analysis of its function will be reported. Four cDNAs representing novel genes have been isolated by molecular subtraction. Their expression is developmentally regulated and respond to cytokinin within one hour. Expression of these genes and behaviour of the mutant macrochloroplast was analysed in somatic hybrids and in transgenic plants overproducing cytokinin.
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02 32

The effect of phytohormones on the accumulation of nitrogen in barley

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The influence of IAA, GA_3 and kinetin, applied to leaves, with two levels of nitrogen nutrition, on biomass production and accumulastion of N depended on a genotype, of development phase and the levels of nitrogen nutrition. Out of three substances applied, kinetin increased the N content in the biomass of both varieties, cropped in the beginning of heading phase, more in presence of higher dose of N. On the other hand, GA_3 decreased the content of N also in plants of both varieties but independently on the levels of nitrogen nutrition. In contrast, at maturity the highest concentrations of N were confirmed in grain and straw of plants treated with GA_3. The effect of IAA on the N content in barley wasn't stated explicity.

02 33

Chemically controlled expression of hormone-related *Agrobacterium* T-DNA genes in transgenic tobacco plants

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To explore the consequences of an altered hormone metabolism in various tissues and at different developmental stages in otherwise normal plants, we have expressed the *rolB* and *rolC* ß-glucosidases, as well as the cytokinin-synthesizing *ipt* gene of *Agrobacterium* T-DNA under the transcriptional control of a modified 35S promoter, whose activity depends on tetracycline, in transgenic tobacco plants. Uninduced transgenic plants were phenotypically similar to wild type plants. After tetracycline treatment *rolB* transgenic plants ceased to grow and developed severe leaf necrosis. Following renewed repression of *rolB* expression normal growth continued, indicating a non-persistent nature of the *rolB* effects. This demonstrates that the gene expression system can be used for the transformation of genes that interfere with plant regeneration and normal growth. Gene specific transcripts were detectable two hours after induction by tetracycline. An increase of the hormonal content, as exemplified by the analysis of free cytokinins in *ipt* transgenic tissues, started five hours after gene induction and led to a twentyfold increase after 24 hours. In contrast, in *rolC* transgenic tissues no significant increase of free cytokinins was measured following gene induction.

02 34

Occurrence and physiological role of gibberellin-O-glucosides in seeds

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Gibberellin glucosyl conjugates, which are known to be formed especially during seed ripening, are considered to be both, waste products and depot pools of temporarily deactivated gibberellins. Reconversion of gibberellin conjugates into free gibberellins is assumed to occur during seed germination.
Using GC - MS analysis we have identified endogenous gibberellin-O-glucosides in a series of mature seeds, of e.g. maize, barley, and pea. So far, the identified glucosides of GA_1, GA_5 and GA_{20} represent potent candidates for reconversion.
Quantative changes of the pool of GA_{20}-13-O-glucoside have been measured during ripening of barley caryopses.

02 35

Plant hormones of the phototrophic non-sulfur bacterium *Rhodospirillum rubrum*

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Plant hormones were isolated from the biomass and culture medium of the purple non-sulfur bacterium *Rhodospirillum rubrum*. These compounds showed high physiological activities (300-350%) in a cytokinin bioassay. The cytokinins detected in the biomass were adenine derivatives according to their absorbance and flourescence spectra. One of them was identified as zeatinriboside by thin layer chromatography. One substance with UV absorbance was detected in the supernatant of *R. rubrum*. Its absorbance spectra was not typical of natural cytokinins and was red shifted about 15 nm. The effects of exogenous synthetic cytokinins on the growth of this bacteria was also investigated. In spite of its ability to produce cytokinin-like substances *R. rubrum* cultures were indifferent to the exogenous synthetic cytokinins, kinetin and benzylaminopurine. These cytokinins, with concentrations of 1-100mM, did not influence the growth rate or increase the cell biomass yield of *R. rubrum*.

02 36

Cytokinin effect on lectin and ABA content in wheat seedlings

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The effect of 5 nM benzylaminopurine (BAP) on wheat germ agglutinin (WGA) and ABA content determined by ELISA in germinating seeds of wheat (*T. aestivum L.*) was studied. WGA is involved in the development of unspecific resistance. ABA is known to induce unspecific plant resistance and at the same time was shown to regulate WGA synthesis. Cytokinins are characterized by protective action on plants under different stress conditions. However previously there was no information about the influence of cytokinins on lectin quantitative changes in wheat. BAP was shown to cause significant accumulation of WGA in 2-4- day seedlings. Certain accumulation of ABA content was followed in parallel with WGA content changes. Possibility is not excluded that BAP - promoted alteration of ABA content is involved in the mechanism of BAP action on lectin level.

02 37

The influence of cultivation conditions on the synthesis of cytokinins by the purple non-sulfur bacterium *Rhodospirillum rubrum*

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Cytokinins are important in regulating cell division both in vivo and in vitro. Four cytokinins with Rf values of 0.36, 0.44, 0.53, and 0.8 were isolated from the biomass and culture medium of the purple non-sulfur bacterium *R. rubrum*. A Cytokinin with Rf 0.53 was identified as zeatinriboside as shown by TLC and HPLC. The structure of the others was not identified. It was shown that the types and quantities of the cytokinins accumulated inside and outside the cells of *R. rubrum* were changed under various conditions of growth. Cytokinins with Rf 0.36 and 0.44 were present in the culture medium and biomass respectively. Furthermore, both of them were formed in detectable quantity under photoautotrophic conditions only. Zeatinriboside (Rf 0.53) was accumulated in the culture medium under all growth conditions. Cytokinin-like substances with Rf 0.8 are typical exogenous metabolites synthesized under photoautrophic conditions only. The quantity of cytokinins increased after a transition from photoheterotrophic to photoautotrophic growth conditions.

02 38

Growth responses to ABA of maize lines selected for ABA accumulation capacity

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Effect of ABA on root and shoot growth was investigated in maize lines selected for ABA content using two different experimental systems. In the first experiment ABA was fed to part of the roots of potted maize plants, while in the second young seedlings were grown in Petri dishes with ABA added in the substrate. Results show that ABA affected root and shoot growth rates in both experimental systems, while genotipic differences in the response were more expressed in early stages of development. Feeding experiment data will be additionally discussed in relation to stomatal reaction and endogenous leaf and xylem ABA content.

02 39

Immunoaffinity purification and analysis of cytokinins in transgenic plants by combined HPLC-ELISAs and HPLC-MS

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A precise and sensitive method for simultaneous analysis of a large number of isoprenoid and aromatic cytokinins in transgenic plants was developed. Immunoaffinity chromatography based on F22/32 monoclonal antibodies (ULVSKOV et al., Planta 188:70-77,1992) specific to the free bases, ribosides, ribotides and 3- or 9-glucosides of the isoprenoid and aromatic cytokinins was used to clean up enough the extracts from ipt, rol A, rol B and rol C transformed tobacco plants. Individual UV absorbing peaks of a single HPLC purification step were scanned by diode-array UV spectrum detection together with polyclonal antibody-based ELISAs. Quantitative cytokinin analysis were calibrated using twelve highly specific [2 - ^{3}H] labelled cytokinins as internal standards. The identity of cytokinins was subsequently examined by thermospray HPLC-MS using deuterium labelled standards. Usefulness of this methodology for the analysis of cytokinins and their metabolism in transgenic plants will be discussed.

02 40

Auxin autotrophy of *Nicotiana tabacum* tissue cultures originating from protoplasts

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The auxin metabolisms of habituated and auxin heterotrophic tissue cultures originating from protoplasts of three transgenic plant lines were compared. The transformed gene constructions contained GUS reporter gene with different promoters; CaMV (212), RUBISCO (312) and auxin-inducible mas 1' (412) promoter. The activity of β-glucuronidase (GUS) was measured. This reflects the auxin level in the tissue.
The auxin autotrophic tissue of line 412 exhibited high GUS activity on auxin-free medium, indicating the endogenous origin of the auxin. The results of the experiments in which autotrophic tissue was transferred on auxin-containing medium and the heterotrophic tissue was transferred on auxin-free medium provide new evidence suggesting that they differ in auxin sensitivity.
A derivativ of phenothiazine (802) strongly inhibited the growth of the auxin autotrophic culture as compared with the heterotrophic one. At the same time, the GUS activity indicated that the auxin level decreased. As concerns the growth reaction in response to this phenothiazine, the habituated culture is similar to some mammalian tumors.

02 41

The influence of antistress products on the phytohormone level in winter wheat seedlings depending on germinating temperature

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The data obtained on the qualitative composition of endogenous phytohormones using TLC indicate that the presowing seed treatment with antistress products (Payax, LHR-87) contributes to the increase in gibberellin, auxin and cytokinin contents in winter wheat seedlings at optimal germinating temperatures in comparison with the control, which is, evidently, associated with a stimulating effect of these products in the doses applied. When the temperature fell to +1 °C the products application resulted in the higher accumulation of abscisic acid as compared with the control and in the reduction of gibberellin and auxin content, while cytokinin content remained at approximately the same level.

02 42

Growth substances of phytopathogenic fungi genus *Septoria*

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An ability of fungi of the genus *Septoria* Sacc. to form growth substances *in vitro* in the process of metabolism was studied. The object of study were 24 isolates of fungi *Septoria tritici* Rob. and *S. nodorum* Berk. et Br. isolated from 8 different varieties of wheat. Cultivated filtrates of those fungi obtained on the 7-th and 14-th day of growing on the shaker with (290 rpm) at 22 - 24 °C were analysed. Indole acetic acid (IAA) and abscisic acid (ABA) were identified using HPLC after purification on TLC in all studied isolates. A maximum content of IAA and ABA amounted to 933 and 450 ng g^{-1} of mycelium raw mass, respectively. By applying bioassays there was also determined an activity of gibberellin-like substances (GLS) with the range of 10^{-6} - 10^{-7} M GA. In the process of ontogenesis the cultivated liquid was found to accumulate ABA and IAA continuously. However, GLS content reacted with maximum value on the 7-th day and later on remained constant. An intraspecific variability of phytohormone formation in *S. tritici* and *S. nodorum* was observed.

02 43

Influence of auxins and cytokinins on the regulation of nitrate reductase in chicory roots

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Almost all of nitrate reductase of chicory (*Cichorium intybus*) is localized in roots until they tuberise. In order to study whether auxins and cytokinins are involved in this tissue specificity, roots of 12-days old in vitro grown plantlets are subjected to 6-benzylaminopurine (BAP) or α naphtalene acetic acid (NAA) treatment in Heller's liquid medium.

In vivo nitrate reductase activity, after a drop induced by the changes of culture conditions, increased significantly after 24 hours with 10^{-6}M BAP and 48 hours with 10^{-6}M NAA. Northern analyses show that either BAP or NAA exert no transcriptional short-term effect. In situ hybridization shows that root apices are the major site of transcriptional activity of the nitrate reductase gene. On the other hand apices are the most susceptible regions of roots to the effect of auxins and cytokinins on nitrate reductase activity. These hormones induce new meristems, most of which differentiate into root apices.

Our current hypothesis is that hormonal effect on nitrate reductase is correlated to the induction of cell divisions.

02 44

The influence of gibberellin and cytokinin on growth and NO_3^- metabolism in salinity stressed *Lycopersicon esculentum* L. cv. F-144

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The influence of exogenous gibberellin and cytokinin supplied to the roots on the growth and NO_3^- metabolism of salinity stressed *Lycopersicon esculentum* L. cv. F-144 was investigated to determine whether changes in phytohormone levels could mediate responses of plants to salinity. Salinity increased the proportion of NO_3^- reduction in the roots and the ratio of organic N/NO_3^- in the xylem sap. The site of NO_3^- reduction was not altered by exogenous gibberellin, although gibberellin improved shoot growth and increased NO_3^- translocation in the xylem. Cytokinin was detrimental to shoot growth, but improved root growth. Xylem translocation of NO_3^- to the shoot was increased by cytokinin, perhaps as a consequence of increased root development. Salinity may affect plant growth by forcing a large extent energetically expensive NO_3^- reduction in the roots.

02 45

Auxin starvation of the auxin-dependent tobacco cell culture: dynamics of endogenous auxin, cytokinins and auxin reception

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The growth in terms of cell division was investigated in subcultures of the auxin-dependent and cytokinin-independent cell strain of tobacco *Nicotiana tabacum* L., cv. Virginia Bright Italia. Partial auxin starvation resulted in the decrease of frequency of cell division, however, the content of endogenous IAA was clearly increased during the exponential phase of the growth. A lack of auxin also led to substantial accumulation of endogenous cytokinins, preferentially in the form of free base N^6-(Δ^2-isopentenyl)adenine on the beginning of subcultivation period. In subcultures grown in medium with optimal concentration the maximum capacity of membrane-associated auxin-binding site was time-correlated with the maximum of mitotic activity. The auxin binding in the cells grown in partially auxin-deprived medium slightly increased and its maximum was shifted towards the onset of cell division. These results indicate the existence of mechanisms regulating both the endogenous levels of auxin and cytokinins and the auxin reception in relation to the maintenance of cell division.

02 46

Phloem transport of abscisic acid in *Ricinus communis* L. seedlings

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Analysis of the sieve-tube sap from castor bean seedlings shows that even slight water stress induces a drastic increase in abscisic acid synthesis in cotyledons and in basipetal translocation through the phloem. Since the young cotyledons do not yet have functional stomates, it has to be assumed that phloem transport of ABA is a signal for stem and roots. The phloem translocation rate of ABA depends linearly on the ABA concentrations in the medium up to 100 µM, in line with the thesis that uptake of external ABA may be by passive diffusion only influenced by the pH gradient across the plasmalemma of the sieve-tube. Besides its transport ABA does not have any short-time effect on the phloem loading of sucrose, Mg^{2+} or K^+ in castor bean seedlings.