

10 01

Light stress proteins in chloroplasts; their expression and possible functions**I. ADAMSKA and K. KLOPPSTECH***Institut für Botanik, Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany*

The early light-inducible proteins (ELIPs) are nuclear-encoded proteins localized in the chloroplasts and related to *cab* (light harvesting chlorophyll *a/b*-binding) gene family. Light stress controls ELIP expression at different levels, at the level of transcription and the accumulation of the protein in the thylakoid membranes. The induction of ELIP transcription in mature pea plants is mediated via blue light receptor(s) activated by blue and ultraviolet-A light. Additionally, ELIPs are transcribed and translated under the control of the circadian oscillator triggered by light absorbed by phytochrome. Accumulation of ELIPs in the thylakoid membranes correlates with the photoinactivation of PS II, degradation of D1 protein and changes in the level of pigments. We thus propose that ELIPs are related to light stress syndrome and may have protective roles by interaction with pigments involved in energy dissipation and free radical scavenging.

10 02

The flexible organization of the thylakoid membrane**B. ANDERSSON***Department of Biochemistry, Arrheniuslaboratories for Natural Sciences, Stockholm University, S-106 91 Stockholm, Sweden*

The plant thylakoid membrane which is the reaction vessel for the conversion of light into chemically stored energy, shows a unique complexity. Apart from the typical transverse asymmetry of biomembranes it has an lateral heterogeneity in its organization. Photosystem I and the ATP-synthase are excluded from the appressed regions of the grana and confined to the stroma exposed regions. Photosystem II, on the other hand, is mainly located in the appressed regions. More recently the regions of the grana margins have been suggested to possess their own characteristics distinct from those of the two main membrane domains. This lateral organization is not static however. There is a pronounced lateral rearrangement of components between the appressed and stroma exposed regions. Such lateral migration is required for biosynthesis and assembly of photosystem II including the repair of photoinhibition. Moreover, lateral rearrangements of the LHCII antenna occur as a mechanism for short and long term acclimation of the light-harvesting process. The lateral migration of the cytochrome *b/f* complex are thought to be involved in the so-called state transitions. The mechanisms and enzymology of these events providing for a flexible thylakoid membrane will be discussed.

10 07

Fast reorganizations in thylakoid systems of chloroplasts of mature leaves under action of heat shocks**L.S. BUBOLO and V.E. SHARKOVA***Komarov Botanical Institute, Russian Academy of Sciences, Prof. Popov st. 2, 197376 St. Petersburg, Russia*

Exposure of young wheat plants to 40 ° and 42.5 °C for 10 min resulted in an increase of a relative content of large grana, while the number of small grana fell. The width of grana was diminished mainly due to disturbances of interactions between thylakoids along grana edges. Hence the ratio of the length of appressed to non-appressed thylakoid membranes increased moderately. These redistributions of the thylakoids occurred on retention of the overall length of photosynthetic membranes and of the total content of chlorophylls. Both heat shocks inhibited the rate of PS2-mediated electron flow which partially recovered over the next 24 h period after the high-temperature treatment. By this time the chloroplast structure returned slightly to the original state, but the overall length of thylakoids decreased.

10 08

Chlororespiration in the chlorophyll-c-containing alga *Pleurochloris meiringensis* detected by flash-induced absorbance changes**C.BÜCHEL* and G. GARAB*****Institute for General Botany, University of Mainz, 55099 Mainz, FRG***Institute of Plant Biology, Biological Research Center, 6701 Szeged, Hungary***

The existence of an oxygen dependent pH-gradient across the thylakoid membrane in the dark led to the hypothesis of a chlororespiration in *Pleurochloris meiringensis*. Measuring flash-induced absorbance changes at different wavelength and mathematical fitting of the transient spectra absorbance changes around 515 nm were attributed to an electrochromic shift in these chl-*b* and lutein-less organisms. Adding 2 mM KCN increased the slow phase of the electrochromic absorbance change. The enhanced activity of the cytochrome (cyt) *f* due to KCN was further demonstrated by the absorbance changes at 553 nm, where a stronger oxidation of the cyt as well as an accelerated reduction became visible favouring the idea of an oxidase competing with the cyt *f* for electrons. The KCN-concentration needed to induce this effect was 0.25 mM for half saturation, whereas mitochondrial respiration was completely blocked adding 0.1 mM. Therefore we conclude that there is a KCN-sensitive oxidase located in the thylakoids of *P. meiringensis*.

10 09

Characterization of the functional states of the energy transformation systems in chloroplasts of different plant genotypes under the change of environmental conditions**N.V. BUDAGOVSKAYA***Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya 35, Moscow 127276, Russia*

Energy transformation processes in chloroplasts of different plant genotypes (different pea and maize varieties) have been investigated under the change of plant growing and experimental conditions. It has been shown that chloroplasts with a genotypically high concentrations of ironporphyrins and SH-compounds (pea) had high activities of light induced proton uptake and photophosphorylation coupled with electron transport as compared to chloroplasts with a low content of these compounds (maize). Conditions causing a decrease in ironporphyrins (iron deficiency) and SH-groups (low pH) lead to uncoupling of the electron transport with energy conservation processes. The disturbances are expressed stronger in maize chloroplasts. Normalization of the conditions results in the recoupling of the processes. Thus, the level of energy transformation processes in chloroplasts depends on the genotypically and phenotypically determined content of ironporphyrins and SH-compounds. Participation of these components in mechanisms of regulation of energy transformation in chloroplasts is considered and illustrated schematically.

10 10

The photosynthetic apparatus biogenesis in greening etiolated seedlings of barley (*Hordeum vulgare* L.)**M.T. CHAIKA, G.E. SAVCHENKO, L.M. ABRAMCHIK, E.V. SERDUCHENKO
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Coleoptiles and leaves of etiolated greening barley seedlings of different ages were used to investigate biogenesis of the photosynthetic pigment apparatus. The Protochlorophyllide (Pde) content in etiolated leaves was 10-20 times higher than in coleoptiles. High relative content of the Photosystem 1 reaction center apoprotein and active oxidoreductase (PCR) detected by ELISA in etiolated barley seedling coleoptiles was shown. The levels of both proteins in total homogenates and plastid fraction of coleoptiles was substantially higher than ones in such fractions of etiolated leaves. The PCR destroy after irradiation (3.5 h, 3500 lx) in coleoptiles was more than in leaves of young etiolated seedlings. In spite of the high content of intraplastid Pde the negative light influence on the PCR was especially significant in old badly greening leaves. So, chlorophyll biogenesis is limited not only by size and substrate supply of intraplastid PCR pool. The important regularity link of the photosynthetic apparatus biogenesis is the incorporation of PCR in the polyenzyme system of chlorophyll biosynthesis which protects it from destruction.

S137

10 15

On the possibility of existence of the terrestrial C₃ plants with CO₂ concentrating mechanism

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The possibility of existence of the terrestrial C₃ plants with CO₂ concentrating mechanism (CCM) was investigated. The efficiency of CCM action in dependence on the CO₂ permeability through chloroplast envelope was analyzed. A possible CCM with the participation of membrane-bound and soluble carbonic anhydrases was computer simulated, with the CO₂ concentration increasing from the border to the center of chloroplast. The conclusion was made that the search of CCM by screening of terrestrial C₃ plants with low CO₂ compensation concentration cannot be successful. Specific decrease in the rate of photosynthesis at supraoptimal CO₂ concentration was observed in several wheat species. It can be explained by the possible existence of some CCM in wheat.

10 16

Protein phosphorylation affects the sensitivity of Photosystem II to high irradiance

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Four types of differently phosphorylated thylakoids isolated from field grown spinach (*Spinacia oleracea* L.) were tested for the sensitivity of photosystem II (PSII) to photoinactivation. Phosphorylation of light-harvesting II complexes protected PSII electron transfer from photoinhibitory damage, while the phosphorylation of the PSII core polypeptides slightly accelerated the decline of electron transfer during high irradiance treatment. Dephosphorylation of the CP43 apoprotein and PsbH protein by an alkaline phosphatase resulted in an extreme sensitivity of the thylakoids to strong illumination. The PSII photoinactivation of thylakoids with the impaired oxygen-evolving complex was found to be independent of phosphorylation.

The thylakoids of the thermophilic cyanobacterium *Synechococcus elongatus* were used in order to compare the plants with an organism where LHCII complexes are missing and the PSII core proteins are not phosphorylated.

10 17

Biochemical evidence that a main part of heterogeneity observed *in vivo* by fluorescence induction arises from PSII core phosphorylation heterogeneity of thylakoid grana regions

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In the present work we study the regulation of distribution of the phosphorylated PSII core populations present in grana regions of the thylakoids from several plant species. The heterogeneous nature of photosystem II core phosphorylation has previously been reported (Giardi et al. *Plant Physiol* 100, 1948-1954, 1992; *Planta* 190, 107-113, 1993). It is shown, in accordance with the observation that photosystem II is heterogeneous *in vivo* also in the dark, that at least two phosphorylated PSII core populations are always detected. The pattern of phosphorylated PSII core populations appears to be ubiquitous in higher plants. Nevertheless a mutant of wheat that shows monophasic room-temperature chlorophyll fluorescence induction curves when leaves are treated with DCMU, also lacks the usual pattern of phosphorylated PSII core populations probably due to the absence of phosphorylation on D-2 and CP43 proteins. The experimental results, correlated with previous experiments *in vivo* strongly support the idea that the phases observed in fluorescence induction arises from grana phosphorylation heterogeneity.

10 18

Photosynthesis and carbon partitioning in mature leaves of *Apium graveolens* L.

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Photosynthetic rates (A) in mature leaves *Apium graveolens* L. are relatively high for C3 species and high A may be related to the capacity to synthesize mannitol in addition to sucrose. Short-term, ¹⁴C pulse-chase experiments, whereby different A were obtained by simultaneously imposing a light gradient across opposite leaflets of mature leaves, consistently showed a 1-10% increase in the mannitol-sucrose labeling ratio in the lit leaflet. Label recovered in the starch fraction was higher in the lit than in the shaded leaf tissue (19% vs 10% of total label), but no differences in the lipid or soluble sugars fractions were apparent. A comparison of pool sizes showed that the mannitol-sucrose ratio generally increased in shaded leaflets and starch content was consistently lower in the shaded portion of the leaf. These results may indicate that higher A in *A. graveolens* is reflected in photosynthetic carbon being preferentially partitioned towards mannitol, in agreement with a hypothesis that polyol biosynthesis effectively recycles reductant and triose-P in the cytosol, which may be critical to achieving or maintaining high A in polyol-synthesizing species.

S141

10 19

Photorespiration of tobacco leaf is enhanced by glycine feeding

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The rate of CO₂ evolution after switching the light off (postillumination burst, PIB) was measured in tobacco leaves fed by 0.1M glycine. Markedly enhanced PIB was observed in normal air (300 ppm CO₂) and especially under low CO₂ (about 100 ppm CO₂). Glycine fed leaves exhibit strong PIB also under non-photorespiratory conditions in the air with 1% oxygen. The rate of gross photosynthetic CO₂ uptake and the rate of O₂ evolution were unchanged as compared to control plants fed with 0.1M salt). The rate of dark respiration was markedly stimulated by glycine feeding as indicated either by measurement of CO₂ evolution or by O₂ consumption. Chlorophyll *a* fluorescence measurement showed the increased non-photochemical quenching of fluorescence. We conclude that 1) the capacity of glycine decarboxylation is far from being saturated even under photorespiratory conditions, 2) enhanced glycine metabolism affects the activity of photosystem 2. We suggest that glycine feeding followed by PIB measurement could be a useful approach to test the photorespiratory glycine decarboxylating capacity of the plants.

10 20

Annual changes in the photosynthetic capacity of spruce needles due to light and temperature stress

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Norway spruce needles (*Picea abies* L. Karst) were submitted to the following environmental stresses throughout the year: i) frost stress (winter); ii) chilling, photochilling (spring); iii) high light, high temperature, drought (summer). Depending on the stress pattern, needles displayed significant depressions in the photochemical capacity (Fv/Fm, dark adapted), indicating photoinhibition. In particular, photochilling events induced a reduction of Fv/Fm to 0.4. Much smaller depressions of Fv/Fm (0.72) were observed in high light or high temperature. On the upper (adaxial) side of needles, photoinhibition was more pronounced (+140%) than on the lower (abaxial) side. During recovery studies, frost-stressed and photochilled needles were limited in their ability to recover within 24 hours. P₇₀₀ absorption measurements showed that PSI reaction centers are less influenced by light and temperature stress. Spruce needles in winter and early spring showed a strong reduction in the amount of functional PSII reaction centers present. Apart from the slow recovery, the stress induced no major changes in the quenching pattern of these centers.

10 21

Purification and characterization of an intracellular Carbonic Anhydrase from the green microalga *Coccomyxa*.

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Initial studies showed that *Coccomyxa* contain an intracellular CA activity about 100 times higher than that measured in high CO₂ grown cells of *Chlamydomonas reinhardtii*. Purification of a protein extract containing the CA activity was carried out using ammonium sulphate precipitation followed by anion exchange chromatography. Proteins were then separated by native (non-dissociating) polyacrylamide gel electrophoresis, with each individual protein band excised and assayed for CA activity. Measurements revealed CA activity being associated with two discrete protein bands with similar molecular masses of 80 kDa. Dissociation by denaturing PAGE showed that both proteins contained a single polypeptide of 26 kDa. The purification protocol will be presented as well as the isoelectric point of the protein and its sensitivity to inhibitors. Two internal polypeptide fragments were N-terminal sequenced and the alignment of these sequences will also be presented.

10 22

Chloroplast biogenesis and chlorophyll formation in dark grown seedlings of *Pinus mugo*

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Some gymnosperms can synthesize chlorophyll in the dark, and this character enables them to develop some kind of chloroplasts. This ability is very variable among different species and limited to the very young seedlings. To this group of plants belongs *Pinus mugo*. 12 days old seedlings of *Pinus mugo* grown in the dark are intensely green and contain chlorophyll a and b. The amounts of chlorophylls is elevated after 24 hrs cultivation of the seedlings at the continual illumination. Dark grown seedlings possess chloroplasts with well developed membrane system, small prolamellar bodies and starch grains.

10 23

Do mitochondria control directly chloroplast functions?

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Isolated chloroplasts from leaves of pea and other species of plants and mitochondria from leaves and roots were objects of our experiments. It has been shown that *in vitro* mitochondria [$0.1 - 1.0 \text{ mg}(\text{protein}) \text{ ml}^{-1}(\text{reagent medium})$] suppressed electron transport in chloroplasts and photophosphorylation. It has been found that the mitochondrial effectors are water-soluble proteins. They influence chloroplasts strongly. Inhibition of electron transport in chloroplasts preincubated with mitochondria sets was due to formation of some short-living photoproducts in the dark ($<20 \text{ s}$). The decrease in the electron transport rate occurs through the partial thermal dissipation of absorbed quanta. There is a protein in cell cytoplasm of leaves which removes inhibition. Experiments have shown that chloroplasts can regulate *in vitro* the mitochondria functions, too. These facts allow to suppose that chloroplasts and mitochondria can control directly the functions of each other *in vivo*.

10 24

Fluorescence induction investigations on chilling injury to young maize (*Zea mays* L.) plants under different light conditions

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The effect of low temperature on the photosynthesis of young maize (*Zea mays* L.) plants was investigated by measuring the chlorophyll fluorescence induction. If the cold treatment was carried out in the light, there was a decrease in the level of F_v/F_m and in the quantum yield of photosynthetic electron transport. In the case of cold treated maize plants, not only the variable (F_v) but also the initial fluorescence (F_0) was quenched during the measurement. The results show that the degree of damage depends on the ambient temperature and on the illuminating light intensity. This suggests that photoinhibition has an important role in the cold damage to young maize.

Although there was no damage in complete darkness even at low temperature, the repair processes need a higher temperature.

10 25

Optical properties of cotyledons of sycamore *Acer pseudoplatanus* L.**B.R. JOVANIĆ, M.T. BOGDANOVIĆ* and M.N. TOMAŠEVIĆ***Institute of Physics, Pregrevica 118, 11080 Zemun, Yugoslavia**Institute for the Application of Nuclear Energy, Banatska 31 b, 11080 Zemun, Yugoslavia**

Fluorescence emission and excitation spectra were measured at 296 K during germination of sycamore (*Acer pseudoplatanus* L.) in the dark and in the light. Reflection spectra were recorded for the same material as well. The preliminary results indicate that energy migration in pigment assembly of sycamore cotyledons is related to the different pattern of chlorophyll biosynthesis in the light and in the dark.

10 26

The effects of cold hardening on the photosynthetic carbon metabolism in leaves of winter rye (*Secale cereale* L.)**O. KEERBERG[†], P. GARDESTRÖM^{††}, H. IVANOVA[†], H. KEERBERG[†] and T. PÄRNIK[†]***Institute of Experimental Biology, EE3051 Harku, Estonia[†]**Department of Plant Physiology, University of Umeå, S-901 87 Umeå, Sweden^{††}*

Leaves of cold-hardened (CH, grown at 5°C) and nonhardened (NH, grown at 25°C) rye were exposed to $^{14}\text{CO}_2$ under saturating light at 25°C and the kinetics of ^{14}C incorporation into the products of steady-state photosynthesis was determined. From kinetic data the rates of carbon fluxes and the pool sizes of metabolites were calculated. The total rate of CO_2 fixation was approximately equal in NH and CH rye, despite the stronger stomatal limitation in CH rye where the lower intracellular level of CO_2 (c_i) was compensated by the larger pool of ribulose biphosphate. Due to reduced c_i the rate of carbon flux through the glycolate cycle in CH rye was about twice of that in NH rye while the specificity factor of Rubisco remained unchanged. The ratio of glycine/serine pools was significantly lower in CH rye suggesting an increased activity of glycine decarboxylase system in cold-hardened plants. Acclimation to low temperatures also resulted in an increased ratio of sucrose/starch synthesis.

S145

10 27

Effect of S-methylmethionine on cold tolerance of inbred maize lines**J. KISSIMON*, D. LÁSZTITY ** and E. PÁLDI****Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, P.O.B. 19, H-2562, Hungary***Dept. Plant Physiol., Eötvös Loránd Univ., Budapest, P.O.B. 330, H-1445, Hungary***

The late spring freeze can cause serious frost damages or/and different problems in flowering of maize (*Zea mays* L.). Two inbred lines, a cold tolerant (F 7) and a cold sensitive (CM 174) were treated with S-methylmethionine (SMM) in 0,01% (w/v) concentration of nutritive solution medium at different stages of development in a fitotron chamber. The cold treatment (5°C 24 hours) was carried out at stage of 3-4 leaves old plants. Reactions to cold were controlled by checking of fluorescence induction activity after 30 minute dark adaptation. The effect of decreasing temperature can be observed at all treatments but the Fv values were higher at the cold tolerant line (F 7) than those of the cold sensitive one (CM 174). The SMM treatment were more successful in the tolerant line at treatment of all time and at the freezing.

10 28

Phosphorylation of membrane proteins in chloroplasts from pea plants grown at various irradiances and temperatures**S.M. KOCHUBEY***Institute of Plant Physiology and Genetics, Ukraine Academy of Sciences, Vasilkovskaya 31/17, 252022 Kiev, Ukraine*

Changes of the rearrangement of pigment-protein complexes in chloroplast membranes were studied for pea plants grown at irradiance 9 or 30 W m⁻² and temperature 15 or 28 °C. It has been found that mainly LHCII proteins became phosphorylated and migrated to photosystem 1 (PSI) for variant with 9 W m⁻² irradiance and 15°C temperature. For variant with 30 W m⁻² and 28 °C phosphorylation and migration of both LHCII and PS2 core were observed. Association of phosphoproteins with PS1 was investigated by the fluorescence methods such as measuring of fluorescence emission and excitation spectra. Two fractions of PS1 particles with different ability of PS1 to associate with phospho-LHCII were associated with PS1 in those PS1 particles. The possible physiological role of membrane pigment-protein rearrangement induced by phosphorylation is discussed.

10 29

The experimental evidences of the epoxylutein cycle in isolated chloroplasts

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The reactions of epoxidation and de-epoxidation of xanthophylls were studied in isolated pea (*Pisum sativum* L.) chloroplasts. Lutein-5,6-epoxide was accumulated in darkness in the presence of ferricyanide, but on the light there occurred de-epoxidation. The latter was inhibited by diuron. The reaction of the lutein epoxidation took place on the light also in the presence of diuron. So the experiments with diuron showed firstly the connection between the reaction of epoxylutein de-epoxidation and Hill reaction i.d. with PSII and secondly cyclic character of lutein-5,6-epoxide lutein conversions. The absence of the visible changes of the xanthophylls in the conditions of the Hill reaction is probably the result of equilibration of two reactions: epoxidation of lutein and the de-epoxidation of lutein-5,6-epoxide. The epoxylutein cycle has two peculiarities: 1. It may operate in the presence of the uncoupler, that it is impossible for the violaxanthin de-epoxidation. 2. The rate of the lutein de-epoxidation is much higher, than the rate of the ascorbate dependent violaxanthin de-epoxidation. These two facts let us to suggest the different functions of these two cycles.

10 30

Delay in chlorophyll biosynthesis of a maize line sensitive to low temperature (12 °C)

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In the fluorescence spectra of etiolated samples, protochlorophyllid and protochlorophyll bands can be observed. The shape of the spectra is almost identical, the only deviation being observed for the cold-treated cold-sensitive line, where the protochlorophyll(ide) band emitted at short wavelengths is considerably greater than in the other three samples. The flash applied in the experiments was always followed by the process of photoreduction. The fluorescence emission bands of the protochlorophyllide forms almost disappeared after the flash, being replaced by bands characteristic of chlorophyllide-*a* forms. In optimum cases, the 30 min period following the flash is sufficient for the main emission band to shift from 696 nm to 680 nm, i.e. for the Shibata shift to take place, which is generally accompanied by the phytolisation of the chlorophyllide. It can be clearly seen from the figures that this process occurs for the control plants and for the cold-treated tolerant line. In the case of the cold-sensitive line, however, the Shibata shift is delayed after cold treatment, with a retardation or only partial occurrence of the phytolisation process.

S147

10 31

Effect of phosphorus deficient on the metabolism of glycolate in *Chlorella vulgaris***B. KOZŁOWSKA and S. MALESZEWSKI***Warsaw University, Branch in Białystok, Institute of Biology, Świerkowa 20B, PL-15-950 Białystok, Poland*

Cells of the green alga *Chlorella vulgaris* Beijer. were cultivated under atmospheric levels of O₂ and CO₂ in complete or phosphorus-deficient inorganic medium. The growth of the cells, their content of phosphorus, photosynthetic and respiratory oxygen exchange, and glycolate concentration in the medium were assayed. Suspensions of cells from both types of cultures were prepared in fresh medium, glycolate-free or containing exogenous glycolate, and the rate of glycolate excretion under light and of glycolate uptake in the dark were analysed. Phosphorus deficiency resulted in lowering of phosphate content in cells and increased glycolate level in the growth medium. It enhanced also the excretion and metabolism of glycolate by the cells. The results are discussed in relation to the role of the photorespiratory carbon oxidation cycle in photosynthetic recycling of phosphorus and in regulation of inorganic orthophosphate concentration within the chloroplasts.

10 32

The susceptibility of photosynthesis of hardened and non-hardened pine seedlings to Cd toxicity - a preliminary fluorescence characteristics**Z. KRUPA*, G. ÖQUIST**, A. SIEDLECKA****Department of Plant Physiology, M. Curie-Skłodowska University, 20-033 Lublin, Poland*
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Hardened (**H**) and non-hardened (**NH**) pine seedlings (*Pinus sylvestris* L.) cultivated as described in [1], were transferred to the nutrient solutions containing increasing concentrations of Cd - 0, 50 and 100 µM. Additionally, part of hardened seedlings was subjected to an ambient growth temperature of +23°C (**H**→**23**°) as well as part of non-hardened ones to low temperature of +4°C (**NH**→**4**°). On the 4th day the metal concentration in the growth medium was doubled. Fast fluorescence induction kinetics was measured with PAM Chlorophyll Fluorometer. Our results show that low growth temperature sensitized pine seedlings to Cd toxicity. F_v/F_m ratio, reflecting PSII photochemical efficiency, was distinctly lower in **NH**→**4**° plants and diminished in **H** seedlings. The F_v/F_m values, a measure of PSII photochemical efficiency under steady-state light conditions, was also highly depressed both in **NH**→**4**° and **H** plants. This was responsible for the considerable decrease in quantum yield for PSII electron transport, especially in **NH**→**4**° seedlings. Moreover, the analysis of Cd content in roots and upper parts of seedlings (stems+needles) showed that **NH**→**4**° plants accumulated about 50% more Cd in roots than other individuals. The level of Cd in the overground parts of **H** and **NH**→**4**° was up to 2 times higher than in other experimental variants (**H**→**23**° and **NH**, respectively).
1. Öquist G., Huner N.P.A., 1991. *Funct. Ecol.* 5: 91-100.

10 33

Phosphinothricin inhibition of ribulose 1,5-bisphosphate carboxylase and its consequence on photosynthesis

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The effect of phosphinothricin (PPT) an inhibitor of glutamine synthetase on ribulose 1,5-bisphosphate carboxylase activity and on other aspects of photosynthesis has been studied in whole lucerne (*Medicago sativa*) plants. Under atmospheric conditions photosynthesis is quickly inhibited. Initial and total RuBPCase activities have been measured in PPT sprayed plants. Both types of activities are inhibited, especially after 48 h treatment. However, this inhibition is about 30-40% whereas photosynthesis is inhibited by 90%. The data indicate that neither ammonia accumulation nor RuBPCase inhibition are the only cause of photosynthesis inhibition by phosphinothricin. The decrease of PGA observed after 48 h PPT treatment would be involved in the decrease of CO₂ assimilation.

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10 34

Hormonal regulation of photosynthetic function development

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The photochemical activity of chloroplasts isolated from detached cotyledons of yellow lupine (*Lupinus luteus* L.), exposed to light for different periods of time in the presence of 67 μ M ABA or 22 μ M BAP, was studied. Comparisons of their delayed fluorescence inductive curves and the ESR spectra revealed a marked changes in the abundance of the both PS II and PS I reaction centers. Electron transport through the whole chain and PS II was assayed; it was found that ABA and BAP had opposite effects on the activity of photosystem reaction centers. BAP also accelerated the appearance of coupling between electron transport and photophosphorylation. The observed changes in functional activity are discussed in relation to the data on hormonal-induced changes in content of different chloroplast polypeptides and their transcripts.

10 35

Response of runner bean primary leaves to excess copper, depending upon their growth stage

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Runner bean (*Phaseolus coccineus* L., cv. Piękny Jaś) seedlings growing hydroponically in Knop solution were supplied with excess Cu (20 mg l⁻¹) in five different stages of primary leaf growth and analyzed 10 days after the treatment. Cu applied at the early stage of growth decreased the leaf area and its fresh weight and doubly increased the plastid pigments level. At the end of intensive leaf growth a slight decrease of the leaf area but a significant increase of fresh weight correlated with specific leaf area increase and pigment level decrease were observed. With the development of primary leaves increased sensitivity of the photosynthetic apparatus to Cu was shown. This was manifested by a partial decrease in the photochemical O₂ evolution; the highest inhibition was observed in leaves treated with Cu at the end of their intensive growth. Analyses of fast Chl a fluorescence kinetics did not reveal considerable changes in F_v/F_m (reflecting PSII efficiency) on the beginning, but a strong decrease was shown at the end of leaf growth. In plants treated with Cu after the transition from intensive to slow growth stages of primary leaves a partial decrease in half rise time from F_0 to F_m ($t_{1/2}$), quantum yield for photochemistry in PSII (ϕ) and so-called "vitality index" (R_{id}) were also observed.

10 36

Characteristics of fluorescence emission by leaves of water stressed *Setaria sphacelata*

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Water stress was slowly imposed on potted *Setaria sphacelata* var. *splendida* (Stapf) Clayton plants by withholding watering. During the stress period fluorescence emission was measured under actinic light intensity of 850 $\mu\text{Em}^{-2}\text{s}^{-1}$, using a PAM fluorometer. From the data obtained several fluorescence parameters were computed and correlated with relative water content (RWC) of leaves. The ratio between variable and maximum fluorescence (F_v/F_m) was approximately constant until RWC=50%, and then decreased linearly with RWC. Photochemical quenching (qP) decreased slightly, but linearly, with decreasing RWC. Non-photochemical quenching (qN) and high-energy state quenching (qE) slightly increased when RWC changed from 100 to 70%, and then decreased sharply with RWC. State transition quenching (qT) did not correlate with RWC, and photoinhibition quenching (qI) showed a small decrease between 95 and 90% RWC, and a slight increase at RWC below 70%.

10 37

The change in the dark stage of photosynthesis in isolated spinach chloroplasts affected by exogenous quercetin

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The influence of an exogenous flavonoid, quercetin, on the dark stage of photosynthesis was studied. Radiobiochemical investigations on photosynthesis and the kinetics of primary carbon metabolism in spinach chloroplasts revealed a 70% inhibition of $^{14}\text{CO}_2$ assimilation by quercetin. A relative quantity of labeled hexophosphates in the presence of quercetin increased 95% by the sixth minute of exposure and remained above the control level. The action of the flavonoid increased a relative quantity of ^{14}C in PGA and glycolate, particularly in the first three minutes of light exposure. In our early investigations, it was shown that quercetin has an effect on the light stage of photosynthesis by changing the ATP/NADPH relationship and decreasing the activity of the glyceraldehyde-3-phosphate dehydrogenase complex, therefore playing an important role in the further processes of photosynthesis. Thus, quercetin is a good instrument for studying photosynthesis as it is able to displace the dynamic equilibrium of the carbon reduction cycle.

10 38

The influence of ABA on the light and dark reactions of photosynthesis

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The effect of the phytohormone ABA on the light and dark states of photosynthesis was investigated. ABA in concentrations from 10^{-7} to 10^{-4} did not have a significant effect on the photoreduction of NADP^+ and the synthesis of ATP in isolated spinach chloroplasts. Radiobiochemic investigations of photosynthesis and primary carbon metabolism in isolated chloroplasts showed that the photoassimilation rate of $^{14}\text{CO}_2$ moderately decreased up to the tenth minute of exposure. The results of measuring the primary products of photosynthesis showed that the relative hexophosphate content decreased from 84% to 65% during a ten minute period. The accumulation of ^{14}C in alanine increased especially in the first 5 minutes of exposure. The content of labeled PGA in the first minute was lower than that in the control, on the contrary, relative content of radioactive glycolate in the first minute was three times as many as that in the control variant. This shows that ABA only slightly influences the light phase of photosynthesis. It changes the relationship between reactions of carboxylation and oxygenation of RBP, and decreases the flow of carbon through the PGA-DHAP reaction.

S151

10 39

Effects of supplementary ultraviolet-B radiation on light use efficiency of photosynthesis in *Pisum sativum* leaves

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Pea plants (*Pisum sativum* L., cv. Meteor) were grown in a glashouse under supplementary lighting (minimum PPFD of $450 \mu\text{mol m}^{-2} \text{s}^{-1}$). Half of the plants were exposed to supplementary UV_B radiation (biologically effective UV_B irradiance of 0.74 W m^{-2}) during 15 h protoperiod after 14 d from sowing. On exposure to UV_B radiation Φ_{PSII} , Φ_{CO_2} and A_{sat} decreased after 4 d, however the decrease in Φ_{CO_2} (ca 55 %) was considerably greater than the decrease in Φ_{PSII} (ca 40 %). These changes were accompanied by only a very small decrease in Fv/Fm. The light-saturated rate (A_{sat}) was also found to decrease by ca 60 % of the controls after 4 d exposure to the UV_B treatment. The primary factor determining UV_B-induced decreases in CO₂ assimilation in pea leaves does not appear to be photoinhibition of PSII photochemistry.

10 40

Study of C₂-metabolism in dark and light in absence of photorespiration

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Measuring the change of formaldehyde, glyoxylate, free glycine and serine in intact leaves in dark and at low light intensity, with and without HCO_3^- one might reach the conclusion that C₂ photosynthesis exists in determined conditions. In plants raised at low light intensity (10-20 W/m²) the formaldehyde, free glycine and serine amounts increased significantly in dark, when 5 mM NaHCO₃ was taken into leaves by vacuum infiltration, and glyoxylate content increased when hydroxylamine (HA) was present simultaneously. Aminoacetonitrile (AAN) and isonicotinic acid hydrazide (INH) are inhibitors of serine hydroxymethyltransferase and glycine decarboxylase. Amino-oxyacetate (AOA) and probably HA are inhibitors of transamination. According to our own experiments HA increases the amount of glyoxylate both with and without HCO_3^- . AAN, INH and AOA effect significantly on the amounts of glycine and serine though the circumstances influence the direction and extent of change.

10 41

Organization of kinetin-treated maize mesophyll thylakoids

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Cytokinins have an important regulatory role in the development of plants including chloroplast biogenesis. They effect on the synthesis and/or stability of some nuclear encoded m-RNA increasing the level of the corresponding polypeptides (Parthier 1989, BPP 185, 289). We have investigated the effect of kinetin on the development and organization of maize mesophyll thylakoid membranes greened under different light condition. Kinetin treatment promoted the synthesis and/or assembly of some components of thylakoid membranes (chlorophyll, LHCI and II) allowing a more developed structure (i.e. more complete light-harvesting system) and higher efficiency of photosynthetic function (CO_2 fixation). Kinetin facilitated the normal formation of thylakoids under extreme low-light illumination compensating partly the absence of higher light intensity. In conclusion, structure and function of hormon-treated thylakoids seemed to be in a more advanced developmental stage reflecting the synergic cooperation of the light and hormonal factor during the thylakoid biogenesis. (Granted by OTKA T-5503).

10 42

Seasonal changes in Photosystem II activity and D1-protein content in *Pinus sylvestris*

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Current year needles were regularly sampled from a pine tree in an open natural stand for one year. Changes in Photosystem II efficiency were measured by chlorophyll fluorescence. Needles were stored at $-80\text{ }^{\circ}\text{C}$ for subsequent immunoblot analysis of the D1-protein and the chlorophyll-protein complexes of both photosystems. It was confirmed that winter stress inhibited the photochemical efficiency of Photosystem II. Degradation of the D1-protein also occurred. The interaction of high light and low temperatures resulted in a strong reduction of the variable fluorescence (Fv/Fm of 0.1). In March, branches were brought indoors and recovery at $50\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ and $20\text{ }^{\circ}\text{C}$ was followed. The recovery of the photochemical efficiency was completed within three days. The content of the immunologically detectable D1-protein per mg chlorophyll changed in parallel with Fv/Fm , indicating that recovery from photoinhibitory damage required protein synthesis.

S153

10 43

Functioning of photosystems I and II under Cu-stress
Analysis by in vivo chlorophyll fluorescence and photoacoustic measurements

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Fluorometric and photoacoustic techniques were used to measure in vivo various functional aspects of the photosynthetic apparatus in intact leaves of a Cu-tolerant *S. compacta* ecotype after 15 days exposure to Cu. Low Cu-treatment (8 μ M) resulted in an increase in photochemistry activity of PS II (Fv/Fp) and stimulation of the vitality index (Rfd) of *S. compacta* leaves. Moreover, the increased chlorophyll content under 8 μ M of Cu was accompanied by an enhanced oxygen evolution (Aox) and increased capacity for cyclic electron flow around PS I, as indicated by the high value of the photochemical energy storage (PES). These results confirmed the necessity of Cu presence for more vigorous photosynthetic function by the Cu-tolerant ecotype. However, high Cu-level (160 μ M) induced strong inhibition of the Fv/Fp and Rfd ratios as well as retardation in chlorophyll biosynthesis rate. A differential sensitivity of the two photosystems in Cu was found. PS II seemed to be more sensitive to Cu inducing decline in yield of O₂ evolution, while PS I was intact or less inhibited as it was shown by the small reduction of PES values.

10 44

Manganese interaction on copper toxicity in pea chloroplasts

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Copper is an essential microelement for higher plants. On plant leaves the main pool of Cu is located in the chloroplast. The well known blue copper-protein, plastocyanin, contains 50% of chloroplastidial copper. Localization and biochemical function of the remaining Cu is a controverse matter. On previous studies we have looked for effects of Cu toxicity on photochemical reactions in pea chloroplasts. The results suggested that Cu action may be associated with photosystem II (PSII).

In order to better understand the site and the mechanism of Cu action on photochemical reactions, we have studied manganese interaction in Cu treated chloroplasts. Determinations of oxygen evolution and chlorophyll fluorescence measurements were made simultaneously. Inhibition of oxygen evolving capacity and changes in the fluorescence parameters (namely photochemical quenching) increased with copper concentration (5 μ M to 20 μ M CuSO₄ were used). Inhibition of Mehler reaction is consistant with other results. Manganese interaction was tested by adding Mn during incubation time and experimental running process. Prevented and reversed effects on copper toxicity were observed. The results suggest an interference of Cu at the level of the PSII reaction center, probably related with the oxygen evolving system. It is known that this complex contains polypeptides involving manganese. Considering the similar ionic radius of the divalent form of Cu and Mn a possible competition of Cu for Mn sites may occur.

10 45

Photosynthesis and saccharide accumulation in frost-hardened leaves of *Hedera helix*

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The aim of this study was to prove whether an impairment of photosynthetic activity following frost hardening is associated with an accumulation of soluble saccharides usually occurring during the development of frost tolerance. The rates of photosynthetic O_2 evolution and the contents of glucose, fructose and saccharose in ivy leaves were determined after the plants were hardened for three weeks at 5/0°C day/night temperatures. Furthermore, the effect of P_i feeding via the petioles on photosynthesis and the recovery at 20/15°C were recorded. Photosynthesis was reduced by about 30 % after hardening accompanied by an increase in the content of soluble saccharides by almost 100 % (mainly saccharose). Photosynthesis and saccharides normalized after three days at 20/15°C. Since feeding frost-hardened leaves with P_i did hardly restore their photosynthetic activity, we conclude that the photosynthetic impairment was not the result of a feed back inhibition via assimilate accumulation.

10 46

Responses of photosynthesis and ribulose-1,5-bisphosphate carboxylase/oxygenase to limitations in water availability in sunflower (*Helianthus annuus* L.)

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The effects of water stress on photosynthesis of two sunflower hybrids which are differently tolerant to water stress have been examined.

Gas exchange parameters and fluorescence quenching parameters which have been determined at ambient CO_2 and light, indicate that water stress induced decrease of photosynthesis is not caused by altered photochemical efficiency and quantum yield of electron transport, but rather by decreased stomatal conductance.

Photosynthesis data obtained at saturating CO_2 and light indicate the contribution of nonstomatal limiting factor. The nature of this mesophyll regulation of photosynthesis in water stress conditions is discussed in terms of changed ribulose-1,5-bisphosphate carboxylase/oxygenase content and activity.

10 47

Effect of lead ions on the photoelectric potential of *Zea mays* L.**K. PAZURKIEWICZ-KOCOT***Department of Plant Physiology, Faculty of Biology, Silesian University,
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The relationship between the photoelectric potential of *Zea mays* L. plants and concentrations of lead ions in the external medium was investigated. The experiments were carried out with the use of conventional electrophysiological technique. The effect of Pb^{2+} on the acidification of external medium containing fragments of leaves was also studied. Detailed studies were carried out on the chlorophyll a and b participation in the process of excitation of plants by light. The results suggest that in plants treated with the lead ions the photoelectric reaction was significantly reduced. The effect of Pb^{2+} ions on the photoelectric potential at the low concentrations (10^{-6} - 10^{-4} mol) was mainly quantitative. High, sublethal concentrations of this metal (10^{-3} - 10^{-2} mol) induced strong inhibition of the photoelectric reactions. The pH variation of the incubation medium showed that lead ions caused inhibition of light induced alkalisation and that H^{+} electrogenic proton pump probably participated in generation of the photoelectric response in the green plant cells. The correlation between the electric potential value and decrease in chlorophyll content induced by lead ions was as well studied.

10 48

Photoinhibition in photoautotrophic culture cells of *Marchantia polymorpha* is characterized by the accumulation of xanthophyll-cycle pigments and by changes in the thylakoid-membrane protein pattern**S. PETER, M. ROOS and C. SCHÄFER***University of Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany*

The physiological and biochemical effects of high light treatment were studied in photoautotrophic cells of *M. polymorpha*. Reductions of both maximum photochemical efficiency and - to a lesser extent - light-saturated photosynthetic capacity could be observed during high light treatment. These changes were partly (maximum photochemical efficiency) or completely (light saturated capacity) reversible in low light. The xanthophyll cycle pigments were located mainly in the minor chlorophyll binding proteins of photosystem II and in photosystem I. The xanthophyll cycle pigment pattern was affected in two ways by high light treatment: On a short-term basis violaxanthin was deepoxidized, and zeaxanthin and antheraxanthin accumulated. Over longer periods the total amount of xanthophyll cycle pigments (and of lutein) increased by *de novo* synthesis. The increases in carotenoid contents were accompanied by changes of the pigment compositions of several pigment-protein complexes.

The effects of high light stress were more pronounced in the stroma thylakoid membranes than in the grana thylakoid membranes, and they were accompanied by changes in the protein composition and pigment contents will be discussed in relation to the reduction in photosynthetic performance.

10 49

Photosynthetic characteristics of different sex types of *Plantago lanceolata* grown under optimal, low light and N-stress conditions

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Plantago lanceolata is a gynodioecious species; most natural populations consist of both hermaphrodites (H) and male steriles (MS). MS plants can only reproduce via their ovuli, while H plants can pass on their genes to the next generation via ovuli as well as pollen. Clearly MS plants have a reproductive disadvantage and in order to maintain themselves they should compensate for this disadvantage. Since the inheritance of MS in *P. lanceolata* is nuclear-cytoplasmic, both nuclear and chloroplast/mitochondrial genes are involved in the development of the MS trait. In order to check whether there are physiological differences in chloroplast/mitochondrial performance between MS and H phenotypes photosynthesis was measured. MS and H plants were grown in a climate chamber on hydroponics under high light, low light and N-stress conditions. Light and CO₂ response curves were made on the youngest fully grown leaves.

10 50

Chlorophyll fluorescence and CO₂ exchange in bean plants grown under conditions simulating *in vitro* culture

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French bean plants were grown in nutrient solution in closed vessels where environmental conditions simulated cultivation *in vitro*. CO₂ concentration in the vessels decreased during plant ontogeny in dependence on the degree of autotrophy. Photosynthetic parameters were measured during the whole life span of primary leaves. The content of chlorophyll (Chl) *a*+ *b* was lower, and the content of carotenoids and the Chl *a/b* ratio were higher in these model plants in comparison with control plants. In the model plants, the number of grana in chloroplasts was initially low and increased with plant age; while the number of grana decreased with the age of control plants. The ratio of variable to maximal fluorescence was not significantly affected, photochemical and nonphotochemical fluorescence quenching was lower in model plants and differently affected by elevated CO₂ concentration during measurement. Net photosynthetic rate (P_N) measured under optimal conditions was 50 to 70 % higher in model plants than in control plants. This increase in P_N was connected with considerably higher conductances of adaxial and abaxial epidermes found in model plants in comparison to control plants. The transpiration rate was higher and the rates of dark and light respiration, and CO₂ compensation concentration were lower in model plants than in control plants.

S157

10 51

Growth and photosynthetic properties in natural grassland and managed pasture in southern Thailand

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Natural grasslands in southern Thailand are dominated by *Eulalia trispicata*. During recent years most of these have been converted to managed pastures, frequently using *Brachiaria mutica* which originates from Australia. In one years growth, natural grassland accumulated 10 540 kg dry matter / ha (shoots 6991 kg, roots 3549 kg), whereas the managed pasture was found to produce 10 050 kg (shoots 9 0043 kg; roots 1 007 kg). The two dominant species are quite different in their growth pattern and energy-storing capacity. *Eulalia* grew throughout during both rainy seasons, accumulating more biomass below ground. *Brachiaria* grew much faster initially, making use of the first rainy season for the production of, predominantly, above-ground biomass. Photosynthetic rates of flag leaves demonstrated clearly the better performance of *Brachiaria* (dry 12-20; wet 20-40 $\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in comparison to *Eulalia* (dry 3-5; wet 5-10 $\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$). If growth was limited by environmental factors (e.g. mild drought stress), stored reserve substances were 10% to 20% higher in their energy content. Chlorophyll fluorescence measurements indicated a higher light-stress tolerance and faster recovery from photoinhibition in *Brachiaria* compared to *Eulalia*. It was concluded that the high productivity of *Brachiaria* was achieved by deep rooting in managed soil, supported by adequate fertilisation.

10 52

Low CO₂ inducible protein synthesis in *Dunaliella tertiolecta*

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The green marine alga *Dunaliella tertiolecta* can grow at very low levels of CO₂ due to induction of a CO₂ concentrating mechanism (CCM). To identify proteins induced under low CO₂ conditions cells were labelled with $^{35}\text{SO}_4^{-2}$ and seven polypeptides with molecular masses of 45, 47, 49, 55, 60, 68 and 100 kDa were detected. The induction of these polypeptides was observed when cells grown in high CO₂ (5% CO₂ in air) were switched to low CO₂, but only in the light. Western blot analysis of plasma membrane fraction against plasma membrane ATPase antibodies showed an immunoreactive 100 kDa band that occurs only in low CO₂ cells. There are two different forms plasma membrane ATPase, and that one of them can be regulated by the CO₂ concentration in the culture medium.

10 53

The function of zeaxanthin in q_E amplification and its control by the proton gradient

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We found a complex interrelation between zeaxanthin, the proton gradient across the thylakoid membrane, and the extent of q_E amplification by zeaxanthin. The proton gradient is a central part of the mechanism of q_E and by this way it is also a prerequisite for the occurrence of q_E amplification in zeaxanthin containing thylakoids. However, our results show that through an independent mechanism the proton gradient is also important at the stage of zeaxanthin synthesis to get zeaxanthin functional in q_E amplification. Large amounts of zeaxanthin synthesized in a dark deepoxidation at pH 5.5 without a Δ pH were much less efficient in q_E amplification than smaller amounts of zeaxanthin formed in a dark treatment at pH 7.6 in thylakoids having a Δ pH from the hydrolysis of ATP. From our results we can conclude on distinct roles of the proton gradient in the activation of the zeaxanthin dependent q_E amplification at the stage of zeaxanthin synthesis on the one hand and in supporting the q_E amplification at the stage of zeaxanthin function on the other.

10 54

Characterization of the cytochrome *b/f* complex from spinach chloroplasts

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The cytochrome *b/f* complex was isolated from spinach grana and stroma lamellae vesicles. Electrophoretic analyses indicate that the complexes are in dimeric form and composed of six polypeptides with molecular masses of 34/33, 23, 20, 17, 12 and 4 kDa. The 12 kDa protein was identified as plastocyanin by immunoblotting. Plastocyanin was present in the cytochrome *b/f* complex even after a second repeated sucrose density gradient centrifugation. There is significantly more plastocyanin and 4 kDa protein in the cytochrome *b/f* complex isolated from stroma lamellae than from grana. In additions there is a 15 kDa protein in the complex isolated from the stroma lamellae fraction. Immunoblot analysis after crosslinking indicated that the 4 kDa protein and the plastocyanin are associated in the cytochrome *b/f* complex. We suggest that a difference in composition of the cytochrome *b/f* complex between the two membranes might be important in the regulation of the cyclic and non cyclic electron flow.

10 55

D1 protein turnover in higher plants is not strictly light-dependent

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An almost linear relationship between light intensity and turnover of the D1 protein, one of the PSII reaction centre proteins, was reported some years ago in *Spirodela* cultures. In terrestrial higher plants, this relationship appears to be more complex. Firstly, the rate of turnover is saturated at a light intensity close to growth light intensity, as indicated by uptake of radiolabelled methionine into the protein. Incorporation of label thereafter appears to remain the same, as in our studies with intact *Schefflera arboricola* and *Arabidopsis thaliana* leaves. Secondly, other factors affect the relationship between D1 protein turnover and light. These factors include growth light intensity and, apparently, growth CO₂ concentration, as demonstrated in our experiments with *Schefflera arboricola* leaves.

These observations suggest that leaves develop a certain basic rate of D1 protein turnover, which is related to growth conditions. We suggest that this basic rate of turnover plays a role in regulating photosynthesis in the environment in which the plant grows. Changes in D1 protein turnover, or the uncoupling of its component parts, do not appear to be responsible for photoinhibitory losses of quantum yield in higher plants. Instead, photoinhibition seems to be related to heterogeneity of function within the PSII population. Possible regulatory mechanisms behind this heterogeneity are discussed.

10 56

The effect of doubled ambient CO₂ concentration on photosynthesis, daily carbon balance and growth of wheat plants.

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Winter wheat plants were grown under controlled atmospheric and light conditions for five weeks to assess the response of photosynthesis, respiration and root/shoot carbon partitioning to doubled ambient CO₂ concentration. Doubled CO₂ (700 μmol mol⁻¹) stimulated photosynthetic CO₂ uptake and dark respiration rate when calculated on the leaf area basis. However, total daily carbon gain per one plant and total dry matter of shoot was lower for high-CO₂-grown plants due to reduced leaf area. After about 3 weeks of exposure to high CO₂, photosynthesis was depressed probably by limiting regeneration of ribulose biphosphate. Starch accumulation in chloroplasts and daily osmotic cycling was checked to assess the cause of CO₂ acclimation. Both stomatal resistance and water use efficiency were markedly higher in high-CO₂-grown plants. Stomatal frequency did not differ significantly between control and high-CO₂-grown plants. Higher evaporative demand in low-CO₂-grown plants promoted root elongation. Differences in root exudation and development of root microflora are reported elsewhere.

10 57

Organization of photosystem I antenna

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Photosystem I (PSI) particles of different composition were isolated from maize mesophyll and bundle sheath (in purer form) thylakoids by mild Deriphat PAGE: (i) complete PSI particles containing PSI core (69,68,18,16,15,11-13 kDa), light-harvesting complex I (LHCI: 25.5,24,22,17 kDa) and a complex built of 27 kDa polypeptides; (ii) PSI particles composed of core and LHCI or (iii) 27 kDa complex, respectively; and (iv) PSI core complex. Changes in the antenna composition were accompanied by the shift of the fluorescence emission maximum of the complex from 730-3 nm (i, ii), to 720-5 nm (iii) and to 715-20 nm (iv), as well as by a gradual decrease in the amount of longer wavelength excitation (682, 688, 712 nm bands). Stronger solubilisation resulted in a relative increase in the amount of smaller complexes. Second-dimensional mild PAGE of complete PSI particles separated PSI reaction centre (69,68 kDa), as well as 27 kDa species and LHCI-730 (22 kDa) in oligomeric state, and LHCI-680 (24,25.5 kDa) in monomeric state. The 27 kDa complex found in PSI bands did not contained any 25 kDa polypeptide, showed fluorescence emission at 680 nm and had higher chlorophyll a/b ratio than its LHCI counterpart. As both LHCI and 27 kDa species containing PSI particles were isolated it was concluded that both types of antenna were connected directly to the PSI core. (This research was supported by OTKA Grant T-5503.)

10 58

A nonregulatory isozyme of chloroplast glyceraldehyde-3-phosphate dehydrogenase

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Plastid glyceraldehyde-3-phosphate dehydrogenase (NAD(P)-GAPDH) is a 600 kDa oligomer constituted by 36 kDa (A) and 39 kDa (B) subunits, i.e. (A₂B₂)₄. Effectors such NADP(H), ATP, reduced thioredoxin, and glycerate-1,3-bisphosphate contribute to enzyme dissociation to 150 kDa protomers (A₂B₂) endowed with high NADPH-dependent activity. Similar events occur in illuminated chloroplasts (1). A minor A₄ isoform has also been reported for several species. The biochemical function and regulatory properties of A₄ isozymes are unknown. We have purified the A₄ isozyme from spinach chloroplasts, by a procedure including anion exchange chromatography (Q-Sepharose). The native A₄ isoform exhibits M_r 150 000, it fails to associate to high molecular mass oligomers and is invariably more active with NADP(H) than NAD(H), contrary to the (A₂B₂)₄ isoform. The present and other results suggest that only A-subunits of NAD(P)-GAPDH have a catalytic function whereas B-subunits are probably regulatory.

1. Scagliarini S., Trost P., Pupillo P. and Valenti V. (1993) *Planta* 190: 313-319

10 59

Redox modification of stromal enzymes: Variations of a regulatory principle during oxygenic photosynthesis

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Various chloroplast enzymes are subject to covalent modification by reduction/oxidation mediated by the ferredoxin/thioredoxin system. The reversible reaction is linked to photosynthetic electron flow and to the simultaneous presence of O_2 . The thermodynamic equilibrium between the oxidized and the reduced forms exhibiting differential properties varies between the enzymes and depending on the presence of specific effectors, thus allowing for the individual fine-tuning of the light regulated stromal enzymes. By this way, a strong feed-forward activation of fructose-1,6-bisphosphatase upon increase of fructose-1,6-bisphosphate and an effective feed-back inhibition of the malate-valve enzyme NADP-malate dehydrogenase by decreasing the reduction state of the NADP(H) pool is achieved. Furthermore the activity of NADP-glyceraldehyde-3-phosphate dehydrogenase is adjusted to the level of 1,3-bisphosphoglycerate that initiates the formation of the high-affinity heterotetrameric form by disaggregation. *In vivo*, this is the case only in the light or when the enzyme has been previously reduced, but not in the dark, since activation of the oxidized enzyme can be achieved only with unphysiologically high concentrations of the effector.

10 60

A fast change in photosynthate accumulation, formation of plastids and plant morphogenesis caused by Ethephon

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40-d-old cucumber (*Cucumis sativus* L.) plants were sprinkled with Ethephon (2-chloroethyl-phosphonic acid) in a concentration of 4×10^{-3} M causing a sharp two stage process: from inhibition of growth and from apical dominance to promotion of several side shoots - new attracting centres. During growth inhibition (2 - 7 d) the chloroplast overfilling with starch and plastoglobules in the palisade parenchyma cells of the leaves of the basic shoot was typical. Later (7 - 10 d), in the same cells of the same leaves in large chloroplasts the starch granules disintegrated, in others - macrograna formed: chloroplast division and proplastids was frequent. That is a reflection of renewal and increase of heterogeneity of the fund of chloroplasts in the same cell at the same time, an evidence of several ways of formation, accumulation, export of photosynthates of each chloroplast and different its zones according to specific demands of different acceptors.

10 61

Thermotolerance of photosynthesis and chloroplast heat shock granules in wheat plants after heating

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A partial inhibition of photosynthesis and a simultaneous acquisition of thermotolerance of this function occurred following a high-temperature treatment (40 °C for 10 min) of young wheat plants. Rare heat shock granules (HSGs) appeared in approximately 2 % of chloroplasts immediately after this heating. The level of thermotolerance remained higher in heated plants compared with control ones 1 d later, whereas the HSGs disappeared. Exposure of the plants at 42.5 °C resulted in a nearly complete inhibition of photosynthesis and a formation of numerous HSGs in the majority of chloroplasts, as well as in mitochondria, microbodies and cytoplasm. Photosynthesis being strongly inhibited, its high-temperature tolerance could not be measured at once, but only after the partial recovery of the function within 2 days. Thermotolerance of photosynthesis in both heated plants and in control ones was equal. The chloroplasts still contained HSGs. Thus, in wheat there is no correlation 1) between the duration of acquired thermotolerance of photosynthesis and the persistence of chloroplast HSGs; 2) between the induction of acquired thermotolerance and HSGs formation as the result of heat shock.

10 62

Kinetic parameter change of light-induced P700 absorption signal at *Pisum sativum* chloroplast protein light phosphorylation

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Oxidation and reduction rate changes of light-induced P700 absorption signal of chloroplast light particles have been investigated by chloroplast protein light phosphorylation of pea plants grown at different temperatures. It was shown that oxidation rate of phosphorylated sample increased in comparison with control in plants grown at temperature of 14 °C, while in phosphorylated sample of plants grown at temperature of 28 °C oxidation and reduction rate increases were observed. Chlorophyll/P700 ratios were found to increase in both samples. These results explained phosphorylated light-harvesting complex transfer and functional association with photosystem I (PS I) in plants grown at 14 °C, and reaction center photosystem II phosphorylated proteins transfer and functional association with PS I in plants grown at 28 °C.

10 63

The dependence of *Phaseolus vulgaris* L. photosynthesis upon variable Fe and Cd concentrations in the growth environment

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8-days old etiolated bean seedlings (*Phaseolus vulgaris* L. cv. Słowianka) were grown for subsequent 6 days on modified Hoagland full strength nutrient solution. After this period they were transferred into fresh nutrient media containing different Fe (0, 0.5, 1, 2 and 4 normal doses; 1 dose=2.5 mM) and Cd (0, 10, 20, 50, 100 μ M) concentrations. All analyses were performed on the primary leaves after 1, 3, 5 and 7 days of treatment. Under normal Fe supply (1 dose) its uptake and accumulation in plants was diminished by the increasing Cd, probably due to Cd/Fe interaction causing Fe deficiency. This was accompanied by the decrease in primary leaves area. This effect was enhanced under insufficient external Fe supply (0 and 0.5 dose). While doubling the normal Fe dose the detoxicating effect of this metal on Cd-treated plants was observed - the leaf area as well as Fe accumulation increased and Cd content decreased. Although further increasing of Fe supply (4 doses) enhanced this metal accumulation in plants and diminished that of Cd, the decreasing leaf area suggested that excess Fe became toxic for organisms. Primary PSII photochemistry as evaluated by F_v/F_m ratio was only slightly diminished by high Cd concentrations in plants grown under Fe deficit. However, we observed very strong effect of variable Fe and Cd concentrations on the so-called "vitality index" (R_{fd}) which reflects the CO_2 fixation efficiency in the Calvin cycle. Under Fe deficit the R_{fd} values dropped to 50% of control and increasing Cd concentrations caused the consecutive strong decrease in this parameter. High Fe supply showed very distinct detoxification effect on Cd-treated plants increasing R_{fd} values. This points to the Calvin cycle as one of the primary targets of Cd toxicity towards the photosynthetic apparatus.

10 64

Does the growth stage of primary leaves play a role in response of the photosynthetic activity of runner bean plants to Cd treatment?

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The effect of 25 μ M Cd on primary leaves of runner bean plants (*Phaseolus coccineus* L., cv. Piękny Jaś), applying the metal to the nutrient solution at different growth stages, was studied after 10 days of its action. Early Cd application to the growth medium of plants was found to be related to the reduction of the leaf area, chlorophyll increase calculated on leaf area basis, 30% PSII activity decrease ($H_2O \rightarrow FeCN_6$), partially reversed in the presence of BSA, and to unaffected F_v/F_m ratio. Cd added in the stage of slow leaf growth only slightly reduced the leaf area but significantly decreased the chlorophyll level, PSII activity and F_v/F_m ratio. These changes were accompanied by a decrease in half rise time of a fluorescence signal ($t_{1/2}$) and vitality index (R_{fd}). No significant changes in PSI activity (DCPIP \rightarrow MV) were found in plants of all experimental variants. The obtained results suggest a gradual inhibitory action of Cd, depending on the primary leaf growth stage, beginning with the donor side of PSII through its reaction centre to the acceptor side of this photosystem.

10 65

Disconnection between chlorophyll *a/b* protein complexes and core-complexes of both photosystems in barley leaves exposed to high irradiance is blocked by dithiothreitol (DTT)

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Excitation spectra of chlorophyll *a* fluorescence at 77 K, non-photochemical fluorescence quenching (qN) at room temperature and pigment composition were measured in 7 d old barley leaves. Immediately after illumination at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 90 s 10 % decrease of chl *b* absorption band around 477 nm of excitation spectra was observed both at 685 nm and 695 nm emission wavelengths, compared with dark adapted leaves. This inhibition of transfer of excitation energy from LHCP to PS II core-complex was not caused by "state 1 - 2" transition but rather by general disconnection between part of LHCP and cores. When 2 mM DTT was infiltrated into the leaves before illumination, the same effect on excitation spectrum was observed at 685 nm emission. This findings are used in interpretation of qN.

10 66

The physiology and ecophysiology of the green algal lichens *Lobaria pulmonaria* and *Platismatia glauca*

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The metabolic activity of the two green algal lichens *Lobaria pulmonaria* (L.) Hoffm. and *Platismatia glauca* (L.) W. Club & C. Club was measured as CO₂ gas exchange under controlled laboratory conditions and in relation to the abiotic factors light, water and temperature. The ability of dry lichen thalli to absorb water from humid air was also investigated. The growth of these lichens under natural conditions was followed in a parallel field study where the microclimate was also monitored. Growth of transplanted lichens were followed at two climate stations, one situated at a forest edge adjacent to a 15 year old clearcut and the other in the interior of a spruce forest. Data is presented for a 2 month period (Aug 25 - Oct 28 1993). These data indicates that the lichens were most productive at the forest edge. From the laboratory measurements it could be concluded that the availability of light and the water content of the lichen thallus were equally important for the metabolic activity of both lichens. There was no great difference in the RH between the forest edge and the forest interior. It is therefore suggested that the observed differences in growth between the two sites were mainly related to the increased availability of light at the forest edge. Finally, there was no data to support the hypothesis that *L. pulmonaria* should be a more sensitive species than *P. glauca*.

10 67

Comparison of photosynthetic induction and acclimation between plants from different habitats

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The photosynthetic response to light variation was investigated, both in the short term through induction and in the long term through acclimation. This was done for three different groups of plants: shade plants, fast-growing sunplants and slow-growing sun plants. All plants were grown under a low irradiance of $200 \mu\text{mol m}^{-2}\text{s}^{-1}$ at 20°C . The photosynthetic induction following transition from 30 to $800 \mu\text{mol m}^{-2}\text{s}^{-1}$ was analysed as net CO_2 uptake, stomatal conductance and Rubisco activation. The same parameters were analysed during photosynthetic acclimation following transition from 200 to $800 \mu\text{mol m}^{-2}\text{s}^{-1}$. General differences were found between the different groups that reflected the degree to which light was expected to be a limiting factor for growth in the particular environment: species from shaded and productive environments showed faster photosynthetic responses than those from unproductive environments where nutrients and water are more likely to be more limiting than light. The relative limitation exerted by stomata and Rubisco activation during photosynthetic induction will also be discussed.

10 68

Expression of P700 apoprotein in heterologous system

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The light inducible PsaB protein of P700 chlorophyll-protein complex of photosystem I from maize has been expressed in *E. coli*. PCR method was used to amplify psIA2 gene from chloroplast genome and adding new restriction sites to both end of the gene. The 2 260 bp long fragment was cloned into pET-3d expression vector. The vector carrying *E. coli* (strain BL21) produced a new protein molecule of right molecular mass detected by polyacrylamide gel electrophoresis.

10 69

Photosynthetic activity of tobacco plants under O₂ and CO₂ deficiency**F. VÁCHA and M. DURCHAN***Institute of Plant Molecular Biology, Branišovská 31, 370 12 České Budějovice, Czech Republic*

The tobacco plants were transferred into growing chambers and grown hydroponically under defined conditions. The CO₂ in the atmosphere was lowered minimum and O₂ concentration was adjusted to 20 % and 10 %, respectively. The activity of photosystem 2 and xanthophyll cycle was measured by means of PAM fluorometer, leaf disc electrode and HPLC pigment analysis. The photosynthetic apparatus is more susceptible to damage at standard oxygen concentrations in the air (20 %) than at reduced oxygen concentrations during CO₂ deficiency. The results are discussed in terms of photorespiration and high-energy fluorescence quenching.

10 70

Photosynthetic characteristics of transgenic *Pssu-ipt* plants**K. Van LOVEN*, H. SYNKOVÁ** and R. VALCKE****Limburgs Universitair Centrum, Universitaire Campus, B-3590 Diepenbeek, Belgium***Institute of Experimental Botany, Na Karlovce 1a, CZ-160 00 Praha 6, Czech Republic***

Photosynthetic characteristics of transgenic tobacco plants (grafts and rooted plants in soil) with the *Pssu-ipt* chimeric gene construction are studied. In the transgenic leaf tissue, the course of chlorophyll fluorescence hardly shows any oscillation upon dark-light transition. Quenching analysis reveals that non-photochemical quenching in particular is very low. The *in vitro* capacity of the whole electron transport chain is reduced and is less tightly coupled to the H⁺-gradient in the transgenic plants. In the transgenic grafts photophosphorylation (ATP-production) is also shown to be reduced. Partial reactions of the electron transport around PSI and PSII, measured with different electron acceptors and donors, are severely affected in transgenic plants. When DBMIB is added, in the grafts only, the electron transfer capacity of the whole chain is increased compared to the capacity in the absence of DBMIB. It is known that DBMIB can act either as inhibitor or as electron acceptor at the cytochrome *b₆/f* complex, depending on pH and concentration. This could indicate that there is a structural difference in the cytochrome *b₆/f* complex between the wild type plants and the transgenic grafts. The reoxidation of P700 also indicates that in young leaves of grafts during actinic illumination a smaller pool of reductive power is build up.

S167

10 71

Rubisco activity and localization of Rubisco in birch leaves during early ontogeny

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Changes in the specific activity of Rubisco were followed in the young leaves of cloned birch (*Betula pendula* Roth) saplings, grown at 3 different nitrogen concentrations, until the leaves were fully expanded. Irrespective of the nutrient availability, the specific activity of Rubisco increased as the leaves matured. This increase was positively correlated with chlorophyll content of the leaves which suggested a role of the photosynthetic light reactions for the specific activity of Rubisco. As the result of this observation a second, similar experiment was conducted to test if the binding of Rubisco to the thylakoid membranes increases during leaf maturation. Samples were taken to localize Rubisco in thin sections using the immunogold labelling technique. Additionally, two different buffers and repeated grinding of the samples were used to study how Rubisco is solubilized during the extraction procedure. The immunogold labelling studies showed an increasing attachment of Rubisco into the thylakoid membranes with ageing. However, we were unable to verify this observation with the used extraction techniques.

10 72

Xanthophyll cycle pattern and *in vivo* photoinhibition in atrazine-resistant biotypes of *Conyza canadensis* and *Chenopodium album*

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Xanthophyll cycle and *in vivo* photoinhibition were investigated in the wild (S) and atrazine-resistant (Atr-R) biotypes of *Conyza canadensis* and *Chenopodium album* as well as in the paraquat-atrazine co-resistant (PQAtr-R) biotype of *Conyza canadensis*. A remarkably reduced level of zeaxanthin determined by HPLC was observed under photoinhibitory conditions in all atrazine-resistant (Atr-R and PQAtr-R) biotypes as compared to the S biotype and PQ-R biotype of *Conyza* and S biotype of *Chenopodium*. An enhanced susceptibility to *in vivo* photoinhibition was detected in Atr-R and PQAtr-R biotypes by variable chlorophyll fluorescence (F_v) decrease and constant fluorescence (F_o) increase. It is suggested that the increased susceptibility to photoinhibition in these typical D1 protein mutant biotypes with altered PSII electron transport maybe a consequence of the reduced rate of violaxanthin deepoxidation.

10 73

CO₂-induced changes in absorbance of intact leaf and in chlorophyll fluorescence. Effect of acidification**S. VELJOVIĆ-JOVANOVIĆ*, Ž. VUČINIĆ*, U. HEBER**, W. BILGER*****Center for Multidisciplinary Studies, University of Belgrade, Slobodan Penezić-Krcun 35, 11000 Belgrade, Yugoslavia***Julius-von-Sachs-Institut für Biowissenschaften der Universität Würzburg, Mittlerer Dallenbergweg 64, D-8700 Würzburg, Germany***

Exposure of intact spinach leaf (*Spinacea oleracea* L. cv. Yates) to high partial pressure of CO₂ in the darkness induced an acidification of leaf interior, a broad spectral absorbance change in the blue-green region and a quenching of chlorophyll (chl) fluorescence. The absorbance changes induced by CO₂ were complex comprising two phases. The fast phase lasted for few seconds and the slow phase continued to increase in the presence of CO₂. The fast absorbance changes at 540 and 505 nm were suppressed by nigericin. The slow phase of CO₂-induced absorbance change at 505 nm was inhibited up to 40 % by dithiothreitol (DTT). Neither a nigericin-dependent nor a DTT-dependent absorbance increase were required for a CO₂-dependent decrease in quantum yield of photosystem 2. A non-photochemical quenching of Chl fluorescence occurring in the presence of 20 % CO₂ was suppressed up to 40 % by DTT and stimulated by nigericin. A dramatic drop in the ATP/ADP ratio in leaves exposed to 30 % CO₂ was obtained. We propose that the acidification induced these changes in the thylakoid reactions.

10 74

Chlorophyll fluorescence induction measurements to evaluate chilling tolerance of tomato hybrids grown at two different temperatures**J.H. VENEMA and P.R. VAN HASSELT***Department of Plant Biology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands*

Tomato hybrids, obtained after protoplast fusion between the domestic tomato (*L. esculentum* cv. Large Red Cherry) and the more chilling tolerant wild species (*L. hirsutum* LA 1777) were analysed for their chilling sensitivity after growth under optimal (24/18°C) and suboptimal (18/12°C) temperatures. The hybrids possess the nucleus of *L. esculentum* and the chloroplasts of *L. hirsutum*, which we expect to be less chilling sensitive compared to the domestic tomato. Apart from this unique plant material other more cold tolerant species (*L. peruvianum* LA 2157, *L. penellii* LA 716 and *L. pimpinelli-folium*) were also studied. To determine the chilling tolerance and the role of the growth temperature the initial fluorescence rise upon illumination was accessed during incubation at 0.5°C. We also measured the effects of decreasing temperature (30° to 0°C) on chlorophyll fluorescence induction (F_p). The data will be discussed with emphasis primarily on the chilling sensitivity of the hybrids in comparison to the other genotypes and secondly the role of the growth temperature will be reviewed.

10 75

Characterization of a functional covalent complex between ferredoxin and glutamate synthase from *Monoraphidium braunii*

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The iron-sulfur flavoprotein, ferredoxin-dependent glutamate synthase (GOGAT, EC 1.4.7.1) from *M. braunii*, is a single polypeptide chain, of 157 kDa, which catalyzes the synthesis of glutamate from 2-oxoglutarate and glutamine, using the dithionite-reduced ferredoxin (Fd, 11.8 kDa), as electron donor. The incubation of purified Fd and GOGAT, in the presence of the carboxyl groups activator, *N*-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), at 4 °C during 3 h, leads to the formation of a stable covalent Fd:GOGAT complex, of 168 kDa, with an estimated stoichiometry of 1:1. Complex formation was favored at low ionic strength and acid pH, and it was functional for the synthesis of glutamate (0.45 U/mg) in absence of exogenous Fd, using sodium dithionite as reductant. When the Fd carboxyl groups were blocked with glycine ethyl ester and EDC, the protein lost the capacity to bind GOGAT and thus was unable to give electrons for glutamate synthesis. Treatment of purified GOGAT with arginine- or lysine-modifying reagents results in a significant loss of the enzymatic activity and also its capacity to form the covalent complex with Fd. These experiments support the role of Fd as the physiological electron donor for GOGAT because specific and direct protein-protein interaction. (Supported by DGICYT, PB90-0880* and CICYT, BIO90-1124**. Spain).

10 76

Viability of *Chlamydomonas reinhardtii* cells immobilized in agar and used for nitrate uptake

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C. reinhardtii is a photosynthetic alga used for contaminants elimination, like inorganic nitrogen, phosphate and/or sulphate. Cell immobilization is a commonly used technique because of its easy preparation and control in continuous reactors, and the high stability and metabolic efficiency of the cells for chemicals production or consumption. Cells viability was determined by the photosynthetic and respiratory activities, and also following the cell growth in the matrix by electron microscopy. Cells immobilized in agar (2x2 mm pieces) showed photosynthetic values between 80-100 $\mu\text{mol O}_2\cdot\text{mg}^{-1}\text{Chl}\cdot\text{h}^{-1}$, and respiratory activity between 20-30 $\mu\text{mol O}_2\cdot\text{mg}^{-1}\text{Chl}\cdot\text{h}^{-1}$, corresponding the highest value to the lowest agar concentration. When the cell systems were stored at 4°C, both activities increased reaching a maximum after one week, and then decreasing with the time course. Electron micrographs showed that outer cells grow forming colonies, releasing the oldest cells to the medium and thus maintaining constant the immobilized biomass. The cells showed a maximum nitrate uptake rate of 20 $\mu\text{mol}\cdot\text{mg}^{-1}\text{Chl}\cdot\text{h}^{-1}$ at agar concentration of 1% (w/v), decreasing until 12 at 3% (w/v) of agar. In all cases, the nitrate uptake rate shown by immobilized cells was higher than that corresponding to free suspended ones (10 $\mu\text{mol}\cdot\text{mg}^{-1}\text{Chl}\cdot\text{h}^{-1}$). Supported by DGICYT. PB90-0880. Spain.

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10 77

Photosynthetic performance of Scots pine (*Pinus sylvestris* L.) during the course of the year as related to frost hardiness**G. VOGG, R. HEIM, J. HANSEN, C. SCHÄFER and E. BECK***University of Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany*

3-year old Scots pine grown under natural temperature conditions were subjected to three different illumination regimes. Thus, the efficiency of both parameters, photoperiod and temperature, on the induction of frost hardiness could be studied. Several photosynthetic and membrane parameters were assayed as potential physiological markers for frost tolerance.

Frost hardening, induced by 'short-day' conditions (9 h daylength) as well as by low temperatures was accompanied by a reduction in chlorophyll content and a concomitant increase in xanthophylls. Prolonged daylength (16 h), despite of low ambient temperatures, resulted in a small change of these pigments.

Pine trees kept under a 'long-day' photoperiod showed a significantly higher photosynthetic rate compared to 'short-day' trees. This phenomenon is predominantly attributable to the reduction in chlorophylls. However, the photosynthetic performance under frost was better in 'short-day' plants. This result together with chlorophyll fluorescence data indicate structural changes of the photosynthetic apparatus which accompany frost hardening.

In addition to seasonal changes of the photosynthetic apparatus, its capacity is impaired by frost events. A positive correlation was observed between the maximum photosynthetic rates and the minimum temperature to which the plants were exposed one day prior to examination.

10 78

Carotenoid and chlorophyll bleaching in thylakoids isolated from greening cucumber (*Cucumis sativus* L.) cotyledons and exposed to strong red light**A. WALOSZEK, E. SIKORSKA and S. WIĘCKOWSKI***Laboratory of Physiology and Biochemistry of Plants, Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31-120 Kraków, Poland*

Photobleaching of carotenoids and chlorophylls in thylakoid membranes isolated from 6- or 24-h greening cotyledons were studied. Strong red light treatment (600 - 750 nm, $3.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ for 15 min) caused bleaching of carotenoids and chlorophylls by ca. 40 and 20 %, respectively. Two scavengers of the active forms of oxygen, ascorbate and α -tocopherol, appeared to protect considerably the pigment bleaching. The results obtained suggest that apart from carotenoids some other compounds are involved in the protection of photosynthetic pigment molecules against photodamage.

10 79

Influence of exogenously applied 6-benzylaminopurine on structure of chloroplasts and arrangement of their membranesN. WILHELMOVÁ¹, J. KUTÍK² and Z. ŠESTÁK¹

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The influence of a cytokinin, 6-benzylaminopurine (BAP), on chloroplast structure was studied by biochemical and electron microscopic methods. The average degree of thylakoid stacking was determined by digitonin fractionation and differential centrifugation in chloroplasts of tobacco plantlets after treatment with different concentrations of BAP in agar medium during cultivation *in vitro*. An elevated level of BAP in the medium induced an increase in grana stacking. This was in accordance to the lowering of chlorophyll *a/b* ratio in such chloroplasts. The relative amount of proteins and carotenoids in both stromal and (to a lesser extent) granal chloroplast thylakoid fractions increased with BAP concentration in the medium. The electron microscopic studies revealed the same volume density of thylakoid membranes within chloroplasts of the BAP-treated plantlets and control ones. Chloroplasts in leaf mesophyll cells were smaller in the BAP-treated plantlets. Profound accumulation of starch inclusions in the chloroplasts and a more flattened chloroplast shape in BAP-affected plantlets, were the main differences as compared to the chloroplasts of control plantlets. The volume density of plastoglobules in chloroplasts did not decrease under the influence of BAP.

10 80

Nitrogen nutrition and photosynthetic productivity

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The effect of soybean nitrogen nutrition on the plant growth, some leaf characteristics, carbon dioxide exchange parameters and dry matter production was investigated. Nitrogen stress (deficiency and, to some degree, surplus) caused restriction in the biomass production and also in chlorophyll and soluble protein synthesis. The rate of photosynthesis and ribulose-1,5-bisphosphate carboxylase (RuBPC) activity increased with increasing of nitrogen concentration in the nutrient solution up to 180 ppm and then did not change significantly or even decreased. Light and dark CO_2 evolution and CO_2 compensation concentration increased with nitrogen level even more than photosynthesis and RuBPC activity. The results furnish with some explanation of the positive effect of nitrogen dressing on plant productivity as well as of the decrease of nitrogen treatment effectivity with increase in nitrogen status of plants.

Changes in PSII and PSI activity in response to changes in ambient light intensity

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By means of a modulated fluorimeter (MFMS, Hansatech) photochemical capacity of photosystem II (PSII) (Fv/Fm), photochemical quenching (q_p), non-photochemical quenching (q_{NP}), quantum yield of electron transport (Φ_e) and P_{700} -absorbance changes were determined. After an exposure time of 6 min to light intensities (actinic light) between 150 and 550 $\mu\text{mol m}^{-2}\text{s}^{-1}$, low light grown (150-200 $\mu\text{mol m}^{-2}\text{s}^{-1}$) grown, attached leaves of *Schefflera aboricola* were measured. With rising light intensity, an almost linear decrease in q_p from 0.86 to 0.57, was accompanied by a continuous increase in q_{NP} from 0.29 to 0.60. Simultaneously, Φ_e decreased from 0.72 to 0.44. These changes were thought to be due to the formation of down-regulated, heat-deactivating and non-functional PSII centres. After a treatment with 150 $\mu\text{mol m}^{-2}\text{s}^{-1}$ of actinic light, a long-lasting pulse of weak, red light (24 W. RG-9, Schott, 90 s) was able to convert 80% of modified centres back to normal, whereas with 550 $\mu\text{mol m}^{-2}\text{s}^{-1}$ only 68% had become functional again. It can be concluded that with rising light intensity more PSII centres were converted to non-functional ones, which required more time for replacement. The redox state of P_{700} is mainly influenced by the electron transport from PSII. Nevertheless, the oxidation status of P_{700} at 150 and 550 $\mu\text{mol m}^{-2}\text{s}^{-1}$ reached 25 and 75%, respectively. In part, the high-light-induced oxidation status was due to a 40% reduction in Φ_e the remaining 28% could be attributed to a decrease in P_{700} activity.