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Research Article

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The role of short-term high temperature pretreatment on the UV-B tolerance of barley cultivars

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Abstract: The impact of pretreatment with high temperature (45 °C for 45 min) on the UV-B tolerance of 4 barley cultivars (*Hordeum vulgare* L. 'Bülbül-89', 'Kalaycı-97', 'Tarm-92', and 'Tokak-157/37') was examined. The response of the plants to treatment was evaluated by measuring the pigment content, chlorophyll a fluorescence, oxygen evolution, fraction of oxygen-evolving complex, proline content, UV-B-absorbing compounds (A_{535} and A_{300}), and stress markers (malondialdehyde, H_2O_2, and UV-B marker). Regardless of high temperature pretreatment, UV-B irradiation decreased the photosynthetic pigment content, photosystem II activity, oxygen evolution, and the fraction of oxygen-evolving complex in almost all of the barley cultivars. UV-B treatment significantly increased the proline content, UV-B-absorbing compounds, and stress markers. According to the findings, it can be deduced that short-term high temperature pretreatment might not provide a cross-tolerance to UV-B irradiation in the 4 barley cultivars studied; in fact, such exposure was found to aggravate the responses. In addition, although plants substantially accumulated the UV-B-absorbing compounds, the photosynthetic process might not be adequately protected from UV-B radiation.

2)

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Temperature dependence of resonance Raman spectra of carotenoids

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ABSTRACT

To understand the mechanism of the photoprotective and antioxidative functions of carotenoids, it is essential to have a profound knowledge of their excited electronic and vibronic states. In the present study we investigate the most powerful antioxidants: β-carotene and lutein by means of resonance Raman spectroscopy. The aim was to study in detail their Raman spectra in solution at room temperature and their changes as a function of temperature. To measure the spectra in their natural environment pyridine has been used as a solvent. It has been chosen because of its polarizability ($n = 1.5092$) which is close to that of membrane lipids and proteins. The temperature dependence of the most intensive ν_1 band in the range from 77 K to 295 K at 514.5 nm excitation has been obtained. It was found that in pyridine the C=C stretching frequency, its intensity, line shape, and line width are very sensitive to the temperature (the sensitivity being different for the two studied carotenoids). The observed linear temperature dependence of the C=C stretching frequency is explained by a mechanism involving changes of the vibronic coupling and the extent of π-electron delocalization. The different behavior of the temperature-induced broadening of the ν_1 band and its intensity for the two studied carotenoids can be associated with the different nature of their solid matrices: glassy for β-carotene and crystalline-like for lutein, owing to their different chemical structures.

3)

CHARACTERIZATION OF ENERGY TRANSFER PROCESSES AND FLASH OXYGEN YIELDS OF THYALAKOID MEMBRANES ISOLATED FROM RESURRECTION PLANT *HABERLEA RHODOPENSIS* SUBJECTED TO DIFFERENT EXTENT OF DESICCATION

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Abstract: The resurrection plants are unique with their extra desiccation tolerance. The physico-chemical properties of photosynthetic apparatus are of crucial importance for survival of plants upon water stress. In present work the effect of different extent of desiccation on energy transfer properties and oxygen evolving capacity of isolated thylakoid membranes from resurrection plant *Haberlea Rhodopensis* are investigated. The plants from different habitats in Bulgaria are compared in respect to energy distribution between. Energy distribution and spillover between both photosystems are studied by means of 77K chlorophyll fluorescence. The dependence of fluorescence ratio F735/F685 on the degree of desiccation of plants was also followed. Functionality of PSII and especially of oxygen-evolving apparatus under water deficit was estimated by flash oxygen yields and initial oxygen burst of thylakoid membranes isolated from desiccated to 50% and 8% RWC plants from the four habitats. Population of S_i states as well as the misses and the double hits were calculated according non-cooperative Kok's model and compared for plants from different habitats and desiccated to different extent. Desiccation-induced damages of Photosystem II and oxygen evolving complex are characterized by parameters of flash oxygen yields and initial oxygen burst. The results are discussed in terms of involvement of "fast" and "slow" centers from grana and stroma regions in oxygen evolution and alteration of their contribution as a result of desiccation.

Keywords: Desiccation, Energy transfer, Flash oxygen yields, *Haberlea Rhodopensis*, Resurrection plants,

4)

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BRIEF COMMUNICATION

UV-B induced stress responses in three rice cultivars

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Abstract

UV-B responses of three rice (*Oryza sativa* L.) cultivars (Sasanishiki, Norin 1 and Surjamkhi) with different photolyase activity were investigated. Carbon dioxide assimilation data support that Sasanishiki was less sensitive to UV-B than Norin 1 and Surjamkhi. UV-B radiation sharply decreased the content of Rubisco protein in Surjamkhi and has no effect in Sasanishiki. The photochemical activities of photosystem (PS) 1 and PS 2 was slightly affected by UV-B treatment. The content of H_2O_2 and the activities of antioxidant enzymes, catalase (CAT), peroxides (POX) and superoxide dismutase (SOD) were enhanced after UV-B treatment. The activities of CAT and POX isoenzymes in Sasanishiki were more enhanced by UV-B radiation than those in Norin 1 and Surjamkhi.

Additional key words: catalase, $^{14}CO_2$ fixation, hydrogen peroxide, peroxidase, Rubisco, superoxide dismutase.

5)

INVOLVEMENT OF REACTIVE OXYGEN RADICALS IN PHOTOINHIBITION OF PRIMARY PHOTOSYNTHETIC REACTIONS – EFFECT OF TEMPERATURE AND OXYGEN RADICAL SCAVENGERS

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ABSTRACT

Exposure of leaves or chloroplasts to high light intensity leads to inactivation of photosynthesis. Two processes are observed - inhibition of photochemical activity of both photosystems and photobleaching of pigments. Oxygen evolving complex, located at the oxidizing side of Photosystem II, is the most sensitive component of photosynthetic apparatus to environmental stress factors. In the present work the effect of high light treatment at room and low temperatures on kinetic parameters of flash oxygen yields and oxygen evolution were studied. Isolated thylakoid membranes were subjected to high light illumination for different periods of time at room (22^{0}C) and low (4^{0}C) temperature. Flash oxygen yields were determined using fast oxygen rate electrode. Photochemical activity of photosystem II was measured by Clark oxygen electrode using artificial electron acceptor. Data presented show that the damaging effect of high light treatment on oxygen evolution is lower at 4^{0}C than at 22^{0}C. When high light treatment was carried out in the presence of histidine and DMSO - scavengers of oxygen radicals, the inhibition process was retarded. Data are discussed in terms of different production rate and mobility of oxygen radicals at room and low temperature.

6)

Chapter 13
Physiological Responses of Higher Plants to UV-B Radiation

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13.1 Introduction

Seven percent of the electromagnetic radiation emitted from the sun is in the range of 200–400 nm. As it passes through the atmosphere, the total flux transmitted is greatly reduced, and the composition of the UV radiation is modified. Short-wave UV-C radiation (200–280 nm) is completely absorbed by atmospheric gases. UV-B radiation is often defined as 280–320 nm. However, the legal definition provided by the International Commission on Illumination sets the UV-B radiation range as 280–315 nm. UV-B radiation is maximally absorbed by stratospheric ozone and thus, only a very small proportion is transmitted to the Earth's surface, whereas UV-A radiation (315–400 nm) is hardly absorbed by ozone. In the past 50 years, the concentration of ozone has decreased by about 5%, mainly due to anthropogenic pollutants, such as chlorofluorocarbons, releasing Cl atoms that catalytically remove ozone molecules from the atmosphere. The surface concentration of ozone has risen from less than 10 ppb prior to the industrial revolution to a day-time mean concentration of approximately 40 ppb over much of the northern temperate zone. If current global emission trends continue, surface ozone might rise over 50% by this century. Ozone depletion is particularly severe over the Antarctic continent, where a dynamically isolated air mass cools down to extremely low temperatures during the austral winter, facilitating ozone photo destruction and formation of the so called spring-time "ozone hole". Depletion of stratospheric ozone has increased solar ultraviolet-B radiation at high- and mid-latitudes in both Southern and Northern hemispheres (Frederick et al. 1994). However, ozone destruction is more intense over the Southern hemisphere with measured solar UV-B fluxes up to 50% more than those at comparable latitudes in the Northern hemisphere (Seckmeyer et al. 1995). Consequently, enhanced solar UV-B may have a greater impact on plants in agricultural production and in natural ecosystems in the Southern than in the Northern hemisphere (Madronich et al. 1995). The global trend of increasing solar UV-B

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7)

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MISCELLANEOUS

Methyl Jasmonate Counteract UV-B Stress in Barley Seedlings

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Keywords
antioxidant enzymes; chlorophyll fluorescence; flavonoids; methyl jasmonate; oxygen evolution; ultraviolet B radiation

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Abstract

The role of exogenously applied phytohormone methyl jasmonate (MeJA) in counteracting the ultraviolet B (UV-B) stress in barley seedlings was investigated. Barley seedlings (*Hordeum vulgare* L., cv. Alfa) 4 days old were supplied with 5×10^{-5} M MeJA through the roots for 3 days and then exposed for 2 days for 5 h per day to UV-B (312 nm, biological effectiveness of UV-B radiation 28.8 kJ $m^{-2}day^{-1}$). The rate of $^{14}CO_2$ fixation, PSI and PSII activities and chlorophyll content decreased, but flavonoids, H_2O_2, malondialdehyde, proline and UV-B induced compounds increased after UV-B treatment. The rate of photosynthetic oxygen evolution was more strongly inhibited by UV-B-irradiation than PSI and PSII efficiency. MeJA itself increased the content of free proline, which acts as a stress protector due to its radical scavenging ability. Increased superoxide dismutase, catalase and peroxidase (POX) activities in the leaves and in the roots and the POX isoforms induction revealed the MeJA involvement in plant tolerance to oxidative stress caused by UV-B irradiation. It was shown that pre-treatment with MeJA counteracted UV-B stress. Therefore, it was suggested that MeJA could acts as a mediator in plant defense responses to UV-B irradiation by enhancing the activity of antioxidant system and free radical scavenging capability of plant cells.

8)

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UV-B RESPONSE OF GREENING BARLEY SEEDLINGS

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The relationship between the greening stage of barley seedlings and their response to UV-B irradiation was studied. Etiolated barley seedlings (*Hordeum vulgare* L., cv. Alfa) greened 12, 24 and 48 h were exposed to UV-B irradiation (312 nm) for 5 h. As a result of UV-B treatment the rate of CO_2 fixation and chlorophyll contents decreased but flavonoids, UV-B-induced compounds and carotenoids increased. The inhibition of photosynthesis in green plants was lower in comparison to greening ones. The 12 h greening plants were more sensitive to UV-B treatment than the plants greening 24 h and particularly 48 h, estimated by the quantum efficiency of PSII photochemistry and the oxygen production rate. The levels of flavonoids and UV-B induced compounds enhanced with increasing the greening time. Activity of antioxidant enzymes catalase, peroxidase and superoxide dismutase increased during the seedlings greening and as a result of UV-B irradiation, but the pattern of isoforms remained similar to those found in the controls. UV-B preferentially induced Cu,Zn-superoxide dismutase. Increase of UV-B induced synthesis of antioxidant enzymes is in line with their important role in the plant response to UV-B stress. Data presented show that the response of barley seedlings to UV-B irradiation is related to the development stage of photosynthetic apparatus.

Keywords: Antioxidant enzymes – chlorophyll fluorescence – flavonoids – oxygen evolution – UV-B radiation

9)

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Protective effect of histidine against pigment photobleaching in Photosystem I particles♣

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Photosystem I (PSI) is a supercomplex of a reaction centre and light-harvesting complexes. Photosystem I particles isolated from spinach leaves were studied by means of absorbance, 77 K fluorescence, and resonance Raman spectroscopy. During prolonged exposure to high-light intensities, various pigments in Photosystem I exhibited different susceptibilities to photodestruction. This work presents preliminary investigations on the effect of histidine on the photobleaching of pigments in isolated particles of photosystem I. Resonance Raman spectroscopy allowed us to obtain direct information about the effect of histidine on the photobleaching of the lutein molecules, upon excitation with a 514.5 nm laser line. Our results showed that histidine reduces the photobleaching of antenna pigments and especially luteins and the most long-wavelength absorbing chlorophylls located in the PSI antenna complex.

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Keywords: Pigments, Raman spectroscopy, Photobleaching, Photosystem I

10)

Research Article

Response of isolated thylakoid membranes with altered fluidity to short term heat stress

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ABSTRACT

The effect of alterations of lipid phase order of thylakoid membranes on the thermosensitivity of photosystem I (PS I) and photosystem II (PS II) was studied. Plant sterols stigmasterol and cholesterol were applied to decrease the fluidity in isolated membranes. After sterol treatment, a decrease of the temperature of 50 % inhibition of PSII activity was observed. Heat stress-induced stimulation of PSI-mediated electron transport rate was registered for control, but not for sterol-treated membranes. Effect of altered lipid order on oxygen evolving complex was evaluated by means of flash oxygen yields revealing changes in the stoichiometry of $PSII_\alpha$ and $PSII_\beta$ centers. The effect of sterol incorporation on the changes in the thermotropic behavior of the main pigment-protein complexes was studied by differential scanning calorimetry (DSC). DSC traces of control thylakoids in the temperature range 20-98 °C exhibited several irreversible endothermic transitions. Incorporation of cholesterol and stigmasterol results in superimposition of the transitions and only two main bands could be resolved. While high temperature band peaks at the same temperature after treatment with both sterols, the band that combines low temperature transitions shows different melting temperature (T_m) : 70 °C for stigmasterol- and 65 °C for cholesterol-treated membranes. The data presented here emphasise the crucial role of lipid order for the response of thylakoids to high temperatures, mediated not only by changes in the fluidity of bulk lipid phase as result of sterol incorporation but also by changes in the thermotropic properties of pigment-protein complexes. [Physiol. Mol. Biol. Plants 2009; 15(1) : 43-52] *E-mail : mayav@bio21.bas.bg*

Key words : Cholesterol, Fluidity, Heat stress, Oxygen flash yields, Thylakoid membrane, Stigmasterol

11)

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Silicon amelioration of manganese toxicity in Mn-sensitive and Mn-tolerant maize varieties

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ABSTRACT

Differences in tolerance to Mn excess and amelioration by Si were evaluated in two maize varieties. Dry weight, callose accumulation, chloroplast ultrastructure, and photosynthesis parameters were used as stress indicators. Variety Kneja 605 was much more Mn-sensitive than variety Kneja 434. In Kneja 605 excess Mn caused severe chloroplast damage and enhanced carotenoid production, symptoms similar to those triggered by photoinhibiton. In Mn-tolerant Kneja 434, in contrast, a Mn-induced decrease of the carotenoid concentrations, and only slight alterations in the chloroplasts were observed. These effects were similar to light Fe-deficiency symptoms. The threshold tissue concentration for Mn-induced callose accumulation was much lower in Kneja 605 than in Kneja 434. Therefore tolerance to excess Mn in Kneja 434 was not due to more efficient exclusion but to more efficient detoxification and compartmentation of Mn. The constitutively thicker epidermal layers in Kneja 434 and the observation that Si-induced amelioration of Mn toxicity in Kneja 605 substantially increased the thickness of the epidermal layers suggest that Mn storage in non-photosynthetic tissue could be a Mn tolerance mechanism in maize.

12)

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ORIGINAL PAPER

NaCl induced cross-acclimation to UV-B radiation in four Barley (*Hordeum vulgare* L.) cultivars

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Abstract The effect of pre-treatment with 200 mM NaCl on the response of four barley cultivars (*Hordeum vulgare* L. cv. Bülbül-89, Kalaycı-97, Tarm-92 and Tokak-157/37) to UV-B radiation was investigated. Salt stress as well as UV-B irradiation led to a decrease of the total chlorophyll (chl) content in all cultivars, except in Kalaycı-97. While carotenoids are almost not affected by NaCl treatment, UV-B irradiation caused an increase by 5–20% of carotenoid content of all cultivars. UV-B induced damages of photosynthetic apparatus were estimated by the rate of photosynthetic electron transport measured by chl fluorescence and the rate of oxygen evolution, the latter being more affected. Pre-treatment with NaCl alleviated harmful effect of UV-B irradiation on F_v/F_m and ETR, but not on oxygen evolution. UV-B-induced and UV-B-absorbing compounds with absorption at 300 and 438 nm increased as a result of UV-B treatment. The level of stress marker proline increased considerably as a result of NaCl treatment, while UV-B irradiation resulted in a pronounced increase of the level of H_2O_2. MDA enhanced in the seedlings subjected to salt and UV-B stress. Established cross-acclimation to UV-B as a result of salt treatment could be due to the increased free proline and the level of UV-B absorbing compounds in barley seedlings subjected to NaCl.

Keywords Barley · Chlorophyll fluorescence · NaCl · Oxygen evolution · UV-B inducing compounds

Abbreviations

Chl	Chlorophyll
ETR	Electron transport rate
F_o and F_m	Initial and maximum fluorescence yield

13)

Photochemistry and Photobiology, 2007, 83: 1–7

Selective Photobleaching of Chlorophylls and Carotenoids in Photosystem I Particles under High-Light Treatment

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ABSTRACT

Photosystem I particles (PSI-200) isolated from spinach leaves were studied by means of absorbance, 77K fluorescence and resonance Raman (RR) spectroscopy. The aim was to obtain better insight into the changes of the pigment spectral properties in those particles during prolonged exposure to high-light intensities and to reveal the involvement of these pigments in the photoprotection of the PSI. During prolonged exposure to high-light intensities of spinach PSI particles, a loss of a significant amount of photosynthetic pigments was observed. It was shown that various pigments exhibited different susceptibility to photodamage. In addition to bleaching of chlorophyll *a* (Chl *a*), bleaching of carotenoids was also clearly observed. RR technique allowed us to recognize the type and conformation of photobleached carotenoid molecules. Raman data revealed a nearly full photobleaching of the long-wavelength lutein molecules. The observed similar bleaching rate of the lutein molecules and the most-red shifted long-wavelength Chl *a*, located in the antenna membrane protein Lhca4, suggested that these molecules are located closely. Our results showed that the photobleached antenna pigments and especially luteins and the most long-wavelength absorbing chlorophylls are involved in photoprotection of PSI core complex.

LHCI are scarce and controversial to some extent. Relatively low amounts of neoxanthin and higher levels of lutein, violaxanthin and β-carotene are reported for LHCI and PSI core complex from barley, while low amounts of neoxanthin, some β-carotene and higher content of lutein and violaxanthin have been obtained in *Arabidopsis thaliana* (7–9). Data about carotenoid composition and configuration in spinach PSI particles have been reported recently (10). The knowledge of carotenoid composition and organization of light-harvesting and core complexes is important to understand in detail the mechanisms of carotenoid functions in the photosynthetic apparatus. The protection against high-light stress and reactive oxygen species, realized *via* the quenching of electronic excited states of Chl *a* molecules, is one of the main functions of carotenoids (11–13).

Light is required for photosynthesis, but excessive light may cause the inactivation of photosynthesis. Photosynthetic apparatus has evolved multiple photoprotective mechanisms as mentioned above to cope with the potentially damaging effect of light. However, despite these photoprotective defenses, photoinhibition occurs. Although it was believed that PSI is tolerant to strong light, it has recently been shown that PSI can also be photoinhibited (14,15). Besides the process of photoinhibition, photobleaching of photosynthetic pigments was also observed under high-light conditions. Photobleaching of pho-

14)

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UV-B response of green and etiolated barley seedlings

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Abstract

7-d-old etiolated and green barley seedlings (*Hordeum vulgare* L. cv. Alfa) were irradiated with UV-B for 30 min and then kept for 24 h in light or darkness. Chlorophyll (Chl) synthesis was inhibited by about 30 % as a result of UV-B irradiation, but there were no significant changes in photochemical activity measured by variable to maximum fluorescence ratio (F_v/F_m), quantum yield (Φ_{PS2}) and oxygen evolution rate. Electron transport of etiolated seedlings was similar to that of green ones, nevertheless, the Chl content was more then 2-fold lower. Ribulose-1,5-bisphosphate carboxylase/oxygenase large and small subunits were diminished as a result of UV-B irradiation in etiolated and green plants, especially in those kept in the darkness. Catalase activity decreased and total superoxide dismutase activity increased in green and etiolated plants following UV-B treatment. When benzidine was used as a substrate, an isoform located between guaiacol peroxidases 2 and 3 (guaiacol peroxidase X) appeared, which was specific for UV-B treatment. As a result of irradiation, the contents of UV-B absorbing and UV-B induced compounds increased in green seedlings but not in etiolated seedlings.

Additional key words: chlorophyll fluorescence, flavonoids, *Hordeum vulgare*, oxygen evolution, ribulose-1,5-bisphosphate carboxylase/oxygenase.

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Quality control of Photosystem II: Cleavage and aggregation of heat-damaged D1 protein in spinach thylakoids

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Abstract

Moderate heat stress (40 °C, 30 min) on spinach thylakoids induced cleavage of the D1 protein, producing an N-terminal 23-kDa fragment, a C-terminal 9-kDa fragment, and aggregation of the D1 protein. A homologue of *Arabidopsis* FtsH2 protease, which is responsible for degradation of the damaged D1 protein, was abundant in the stroma thylakoids. Two processes occurred in the thylakoids in response to heat stress: dephosphorylation of the D1 protein in the stroma thylakoids, and aggregation of the phosphorylated D1 protein in the grana. Heat stress also induced the release of the extrinsic PsbO, P and Q proteins from Photosystem II, which affected D1 degradation and aggregation significantly. The cleavage and aggregation of the D1 protein appear to be two alternative processes influenced by protein phosphorylation/dephosphorylation, distribution of FtsH, and intactness of the thylakoids.

Keywords: Photosystem II; D1 protein; FtsH proteases; Phosphatases; Protein aggregation; Spinach

16)

Light induced changes in Raman scattering of carotenoid molecules in Photosystem I particles

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ABSTRACT

The photosynthetic antenna systems are able to regulate the light energy harvesting under different light conditions by dynamic changes in their protein structure protecting the reaction center complexes. The changes modulate the electronic structure of the main antenna pigments (chlorophylls and carotenoids) and distort the characteristic planar structure of carotenoids, allowing their forbidden out of plane vibrations. Electronic absorption and low-temperature resonance Raman spectroscopy were used to study the changes in composition and spectral properties of the major carotenoids in spinach Photosystem I particles due to high light treatment. The duration of the applied intensity of the white light (1800 $\mu E\ m^{-2}\ s^{-1}$) was 30, 60 and 120 minutes. We used Raman scattering in an attempt to recognize the type and conformation of photobleached carotenoid molecules. The resonance Raman spectra were measured at 488 and 514.5 nm, coinciding with the absorption maximum positions of the carotenoids neoxanthin and lutein, correspondingly. The results revealed nearly a full photobleaching of the long wavelength lutein molecules, whereas the bleaching of neoxantin molecules is negligible. The involvement of these changes in the photoprotection and photoinactivation of the Photosystem I particles was discussed.

Keywords: carotenoids, Photosystem I particles, photobleaching, absorption, resonance Raman spectroscopy

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Effect of Membrane Fluidity on Photosynthetic Oxygen Production Reactions

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The effect of changes of membrane fluidity on the oxygen evolving capability of isolated thylakoids was investigated. Alteration of the lipid phase fluidity was achieved by incorporation of the plant sterol stigmasterol. Incorporation of stigmasterol in the lipid bilayer of thylakoid membranes results in rigidization of the hydrophobic phase of thylakoid membranes and decreases the degree of packing of the lipid head groups. These changes of lipid order are accompanied by a reduction of oxygen evolution, measured with 1,4-benzoquinone as an electron acceptor, and by a more pronounced inhibition of PSI-mediated electron transport. By analysis of the parameters of oxygen flash yields and oxygen burst under continuous illumination it was shown that after treatment with stigmasterol: 1.) the number of active oxygen-evolving centres decreased; 2.) the remaining active oxygen-evolving centres were not affected in respect to the oscillation pattern; 3.) the contribution of the slow oxygen-evolving centres in oxygen burst yield was increased. The effect of stigmasterol was compared with the well-studied effect of cholesterol. Results were discussed in terms of determining the role of lipid order for the organization and functioning of the photosynthetic machinery.

Key words: Thylakoid Membrane Fluidity, Oxygen Evolution, Stigmasterol

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Effect of pretreatment of barley seedlings with different salts on the level of UV-B induced and UV-B absorbing compounds

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Abstract

The relationship between the level of UV-B-induced and/or UV-B-absorbing compounds and stress tolerance of barley seedlings (*Hordeum vulgare* L. cv. Alfa) was investigated. A physiological response to UV-B irradiation was evaluated by measuring the oxygen evolution rate and chlorophyll fluorescence. UV-B irradiation led to an increase of the amount of UV-B absorbing compounds, including flavonoids, measured in acidified methanol extract at 300 nm and of UV-B induced compounds, with maximum absorbance at 438 nm, extracted in 0.1% trichloracetic acid. The content of free proline, malondialdehide and H_2O_2 increased as a result of 4 days treatment with 150 mM NaCl, KCl or $NaNO_3$. Salt pretreatment resulted in considerable decrease of the level of UV-induced and UV-B absorbing compounds measured 24 h after UV-B irradiation. In the meantime chlorophyll fluorescence parameters and oxygen evolution in salt pretreated seedlings were less affected by UV-B in comparison to the control. Damaging effect of UV-B measured by the MDA and H_2O_2 generation and electron transport activity corresponded to the increased levels of UV-B induced and UV-B absorbing compounds. We do not necessarily exclude UV-inducing compounds from an important role in overall UV-B protection but the data presented here showed that the accumulation of these compounds could be a consequence of stress-induced damage to the cells and probably they may serve as stress markers.

Keywords: Chlorophyll fluorescence; Flavonoids; *Hordeum vulgare*; Oxygen evolution; Salt stress; UV-B induced compounds; UV-B absorbing compounds

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EFFECT OF CHLORAMPHENICOL AND CYCLOHEXIMIDE ON THE LEVEL OF UV-B INDUCED COMPOUNDS IN BARLEY SEEDLINGS

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Key Words: Chlorophyll fluorescence; Flavonoids; *Hordeum vulgare*; Oxygen evolution; UV-B; UV-B absorbing compounds

***Abbreviations used*:** CHL, chloramphenicol; CHI cyclohexymide; F_0, chlorophyll fluorescence in dark-adapted state; F_m, maximal fluorescence yield in dark-adapted state; F_m', maximal fluorescence yield in light-adapted state; F_v, variable chlorophyll fluorescence; PPFD, photosynthetic photon flux density; PSII, photosystem II; TCA, trichloracetic acid.

ABSTRACT

Barley seedlings (*Hordeum vulgare* L., cv. Alfa) were treated with 25-μg ml^{-1} cyclohexymide (CHI) or with 100-μg ml^{-1} chloramphenicol (CHL) for 3 h and 24 h and then were irradiated with UV-B at the rate of 49KJ.m^{-2}.d^{-1} for 30 min. Both antibiotics had no effect on the level of UV-B induced compounds (A_{438}) and flavonoids in non-irradiated seedlings. In UV-B irradiated seedlings antibiotics had some non-specific stimulating effect on the content of A_{438} and did not alter the level of UV-absorbing compounds. CHI and CHL acts as a stress factors and induced proline accumulation both in control and irradiated plants. The photochemical efficiency of PSII was not influenced by CHI and CHL treatment but the oxygen evolution rate was decreased. UV-B irradiation reduced both PSII activity and oxygen evolution in non-treated barley seedlings and no synergic effect in antibiotics-treated seedlings was detected.

The data showed that UV-B induced compounds are not proteins and their accumulation is not related with protein synthesis. It is possible for these compounds to accumulate as a result of UV-B cell damage.

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High light-induced changes of 77 K fluorescence emission of pea thylakoid membranes with altered membrane fluidity

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Abstract

The effect of lipid phase order of isolated thylakoid membranes on fluorescent characteristics of both photosystems during illumination with high light intensity at 22 °C and 4 °C was investigated. For artificial modification of membrane fluidity two membrane perturbing agents were applied—cholesterol and benzyl alcohol. 77 K fluorescence emission and excitation spectra of control, cholesterol- and benzyl alcohol-treated thylakoid membranes were analysed in order to determine the high light-induced changes of emission bands attributed to different chlorophyll–protein complexes—F735, emitted by photosystem I—light-harvesting complex I; and F685 and F695, emitted by photosystem II—light-harvesting complex II. Analysis of emission bands showed that high light treatment leads to a decrease of the area of band at 695 nm and a concomitant increase of intensity of the band at 735 nm. The involvement of different pigment pools (chlorophyll *a* and chlorophyll *b*) in the energy supply of both photosystems before and after photoinhibitory treatment was estimated on the basis of excitation fluorescence spectra. The dependence of the ratios F735/F685 and the band areas at 685 and 695 nm on the illumination time was studied at both temperatures. Data presented indicate that cholesterol incorporation stabilized the intersystem structure in respect to light-induced changes of fluorescence emission of PSI and PSII. It was shown that the effect of fluid properties of thylakoid membranes on the 77 K fluorescence characteristics of main pigment protein complexes of pea thyalkoid membranes depends on the temperature during high light treatment.

Keywords: Benzyl alcohol; Chlorophyll fluorescence; Cholesterol; Excitation energy; Photoinactivation; Thylakoid membrane fluidity

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Resonance Raman spectroscopy of carotenoids in Photosystem I particles

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Abstract

Low-temperature resonance Raman (RR) spectroscopy was used for the first time to study the spectral properties, binding sites and composition of major carotenoids in spinach Photosystem I (PSI) particles. Excitation was provided by an argon ion laser at 457.9, 476.5, 488, 496.5, 502 and 514.5 nm. Raman spectra contained the four known groups of bands characteristic for carotenoids (called from ν_1 to ν_4). Upon 514.5, 496.5 and 476.5 nm excitations, the ν_1–ν_3 frequencies coincided with those established for lutein. Spectrum upon 502-nm excitation could be assigned to originate from violaxanthin, at 488 nm to 9-*cis* neoxanthin, and at 457.9 nm to β-carotene and 9-*cis* neoxanthin. The overall configuration and composition of these bound carotenoid molecules in Photosystem I particles were compared with the composition of pigment extracts from the same PSI particles dissolved in pyridine, as well as to configuration in the main chlorophyll *a*/*b* light-harvesting protein complex of photosystem II. The absorption transitions for lutein, violaxanthin and 9-*cis* neoxanthin in spinach photosystem I particles are characterized, and the binding sites of lutein and neoxanthin are discussed. Resonance Raman data suggest that β-carotene molecules are also present in all-*trans* and, probably, in 9-*cis* configurations.

Keywords: Resonance Raman spectroscopy; Carotenoids; Photosystem I particles

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UV-B-induced compounds as affected by proline and NaCl in *Hordeum vulgare* L. cv. Alfa

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Abstract

From the leaves of barley seedlings (*Hordeum vulgare* L. cv. Alfa) UV-B induced compounds, with maximum absorbance at 438 nm (A_{438}) were extracted. The relationship between the level of UV-B induced compounds and UV-B tolerance of barley seedlings was investigated. The level of these compounds depended on the time of UV-B irradiation. They increased 4 h after UV-B treatment, reached maximum after 24 h and then declined. Contrary, the syntheses of UV-absorbing compounds extracted in acidified methanol continued for a long period after UV exposure and after 120 h the values of A_{300} are higher. The content of UV-induced compounds enhanced in the plants treated with proline before UV-B irradiation and decreased as a result of NaCl pretreatment in a concentration depending manner. A physiological response to UV-B irradiation was evaluated by measuring the oxygen evolution rate, chlorophyll fluorescence and chlorophyll/carotenoids ratio. No correlation was found between the level of A_{438} and UV-B tolerance of barley seedlings. It is possible these compounds to play a subtle role in plant UV-B protection than simple UV-B screening or to serve as stress markers.

Keywords: Chlorophyll fluorescence; Flavonoids; *Hordeum vulgare*; Oxygen evolution; UV-B; UV-B absorbing compounds

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EFFECT OF MEMBRANE LIPID ORDER ON THE DEGREE OF FREEZING DAMAGE OF THYLAKOID MEMBRANES

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Abstract

The extent of freezing damage of the photosynthetic apparatus of isolated thylakoid membranes, control and after modification of their membrane fatty acid acyl chain order by cholesterol and benzyl alcohol, was studied. The photochemical activity of photosystems I and II and the energy transfer between the main pigment protein complexes had been determined. Cholesterol-treated membranes are less susceptible to freezing damage, expressed by minor changes of the photochemical activity and retaining the 77K fluorescent characteristics. Benzyl alcohol incorporation enhanced the degree of freezing damage. The photochemical activity of both photosystems was severely decreased (by 80%) and considerable changes in the fluorescent properties were observed, mainly in the pigment pool associated with Photosystem 1. The effects of different freezing media (artificial stroma medium, trehalose, glycine betaine and NaCl) were compared in respect to the maintaining of the activity of photosynthetic apparatus.

Keywords: thylakoid membranes, freezing damage, cholesterol, benzyl alcohol, lipid acyl chain order.

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Photobleaching of photosynthetic pigments in spinach thylakoid membranes. Effect of temperature, oxygen and DCMU

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Abstract

The time dependence of photobleaching of photosynthetic pigments under high light illumination of isolated spinach thylakoid membranes at 22 and 4 °C was investigated. At 22 °C, the bleaching at 678, 472 and 436 nm was prominent but lowering the temperature up to 4 °C during illumination prevented the pigments from bleaching almost completely. The accelerating effect on pigment photobleaching by the presence of 3-(3,4 dichlorophenyl)-1,1-dimethyl-urea)—(DCMU), a well-known inhibitor of the electron transport and known to prevent photosystem I (PSI) and photosystem II (PSII) against photoinhibitory damage, was also suppressed at low temperature. At 22 °C in the presence and absence of DCMU, the decrease of the absorption at 678 and 472 nm was accompanied by a shift to the shorter wavelengths. To check the involvement of reactive oxygen species in the process, pigment photobleaching was followed in anaerobiosis. The effects of the three different environmental factors—light, temperature and DCMU—on the dynamics of photobleaching are discussed in terms of different susceptibility of the main pigment–protein complexes to photoinhibition.

Keywords: Environmental factors; Photobleaching; Pigments; Spectroscopy; Thylakoids

25)

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Different kinetics of photoinactivation of photosystem I-mediated electron transport and P700 in isolated thylakoid membranes

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Abstract

Photoinactivation kinetics of photosystem I (PSI)-mediated electron transport rate was compared to that of P700 content at room (22 °C) and low (4 °C) temperatures in isolated spinach thylakoid membranes. The high light treatment was carried out under aerobic and anaerobic conditions. At 22 °C the decrease of electron transport rate showed first order exponential kinetics. The amount of P700 decreased linearly, being less affected in the first hours of illumination. During photoinhibition at 4 °C in the presence of oxygen, the kinetics of inactivation of PSI photochemical activity and the content of P700 were different. It was found that 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) had different protective effect on the electron transport rate and on P700 content at both temperatures. Treatment with high light intensity under N_2 atmosphere had no effect on the electron transport rate or P700 content. The possible degradation of PSI reaction centre proteins was determined using immunoblot methods. In the presence of linear electron transport at 22 °C correlation between formation of toxic hydroxyl radicals and inhibition of oxygen uptake was observed.

Keywords: Electron transport rate; Oxygen radicals; P700; Photoinhibition; Photosystem I; Thylakoid membranes