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ABSTRACT OF PRESENTED INVITED LECTURE
(not submitted as paper)

ETHYLENE-INDUCED SHOOT ELONGATION IN *RUMEX*: A MECHANISM TO AVOID FLOODING STRESS

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Rumex palustris has the capacity to respond to complete submergence with hyponastic (upward) growth and stimulated elongation of petioles. These adaptive responses allow survival of this plant in habitats with sustained high water levels by re-establishing contact with the aerial environment. Accumulated ethylene in submerged petioles interacts with ethylene receptor proteins and operates as a reliable sensor for the under-water environment. Further downstream in the transduction pathway, a fast and substantial decrease of the endogenous ABA concentration and a certain threshold level of endogenous auxin and GA are required for hyponastic growth and petiole elongation. Interactions of these plant hormones result in a significant increase of the *in vitro* cell wall extensibility in submerged petioles. Furthermore, the pattern of transcript accumulation, measured with quantitative PCR, of a *R. palustris* α-expansin gene correlated with the pattern of petiole elongation upon submergence.

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ABSTRACTS OF ORAL PRESENTATIONS
(not submitted as papers)

GENETIC ENGINEERING FOR IMPROVED ABIOTIC STRESS TOLERANCE

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Drought, low and high temperatures are the major environmental factors that affect plant growth and crop productivity. In the frame of an extensive biotech program, we transformed tobacco (*Nicotiana tabacum*) cultivars with genes, coding for accumulation of osmoprotectants. Cultivars belonging to all three commercially grown types were used: oriental (Nevrokop 1146), burley (Burley 21) and flue cured (Coker 254). Overproduction of proline was achieved by transformation with two genes – from *Arabidopsis thaliana* (AtP5Cs) or *Vigna acconitifolia* (VacP5Cs). The gene for synthesis of fructans (SacB) has been isolated from *Bacillus subtilis*. The gene for glycine betaine accumulation (codA) has been isolated from *Arthrobacter globiformis*. All genes were introduced separately into tobacco genome and effectively expressed.

Several stable transgenic lines, along with wild type tobacco were tested for freezing tolerance at early stage of development. At a later stage, they were subjected to high temperatures and drought under controlled conditions. The transformants performed much better than wild type plants under stress conditions. The biometrical, physiological and molecular data under normal and stress conditions will be discussed.

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WHY COTTON LEAVES MAY TURN RED: CAUSES AND MECHANISMS OF REDDENING

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Reddening of cotton leaves occurring in some locations of Aegean region of Turkey brings about reduced growth, yield and fiber quality as well as economical losses. Abiotic constraints are supposed to provoke reddening; K deficiency in soil resulting in K shortage and Na accumulation in plants are shown to be one of the reddening-causing factors. The importance of water, temperature and light conditions is also admitted. Inhibition of CO_2 assimilation, transpiration, stomatal conductance, water use efficiency and photosystem II photochemical activity in reddening leaves was established, this being related to the reduced yield. Significant biochemical changes underly reddening: strong accumulation of total phenols and proline, drop of chlorophylls, increase of peroxidase activity, and decrease of lipid peroxides. HPLC reveals the non-involvement of coumaroyl derivatives (C_6-C_3) and flavonoids (C_6-C_3-C_6) phenol types in reddening; on the contrary, anthocyanin (C_6-C_3-C_6^+) types, namely cyanidin glycosides, were shown to be dramatically increased. Protective role of anthocyanins, proline and peroxidase is proposed, due to their active oxygen scavenging properties; the conserved membrane integrity in reddening leaves (as judged from the lowered lipid peroxidation) lends support to this assumption. Other protective functions of the above compounds, such as photoprotection, osmoregulation, structure stabilisation, are also suggested.

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EFFICIENCY OF UTILIZATION OF SOIL NITROGEN, WATER AND SYMBIOTICALLY FIXED ATMOSPHERIC N_2 IN THE COMBINED GROWING OF BLACK LOCUST (*ROBINIA PSEUDOCACIA* L.) AND POPLAR (*POPULUS EUROAMERICANA*/DODE/GUINIER/ DEPENDING ON DIFFERENT SOIL NITROGEN LEVELS

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The efficiency of the methods for increasing nitrogen fixation in thelegume plant black locust (*Robinia pseudoacacia* L.) – a decidious tree species of great forestry importance for the maritime climate countries – grown on soil with a high content of mineral nitrogen (N_{soil}) by introducing a system of combined growing (intercropping) with other non-nitrogen fixing species with high nitrogen and growth rates like poplar (*Populus euroamericana* /Dode/ Guinier/ has been studied. Relationships between assimilation of inorganic N (soil and fertilizer) and fixation of atmospheric N_2 in the nodules

of black locust compared with N assimilation rate of intercropped poplar saplings were assessed by time course measurments of activities of nitrate reductase, nitrogenase, CO_2 assimilation and transpiration rates, dry matter and total N accumulation during the vegetative growth of two species. Using the ^{15}N-dilution method and introducing into the soil labeled $^{15}KNO_3$ salt during the intensive growth period of plants, the uptake rates of soil, fertilizer and atmospheric N by plants were determined. Analysis of data show that the system of mixing with poplar plants can change the efficiency of nitrogen and water use during vegetative growth periods. These alterations were connected with the changes of relative growth rates and accumulation of dry matter of the two species. It was found that the higher N use efficiency of nodulated black locust in the beginning of growth period upon mixing is combined with a decreased water use efficiency which favoured nitrate assimilation but delayed nodule formation, while intercropped poplar extracts soil nitrogen more intensively during the second part of the vegetation period at the expense of decreased water use efficiency.

CONSTITUTIVE AND INDUCIBLE DESICCATION TOLERANCE IN SEEDS

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During seed development, desiccation tolerance is usually acquired long before the onset of water loss associated with maturation drying. This suggests that desiccation tolerance in seeds is constitutive and a part of the developmental programme. The initiation of the desiccation tolerance programme is thus associated with internal signalling rather than with signalling water loss. Seeds are often well protected from water loss during moderate drought stress. This protection is generally caused by anatomical structures around and inside seeds. However, long lasting, severe drought might cause premature water loss. In such cases desiccation tolerance can be prematurely induced. We show using an EPR spin probe technique that there is no critical limit to the developmental age at which wheat grains can be induced by slow water loss to acquire desiccation tolerance. Even non-differentiated wheat embryos can acquire desiccation tolerance at the cellular level, although such poorly developed embryos fail to germinate. This means that the programme of desiccation tolerance, which normally succeeds the programme of morphogenesis, can be induced by environmental stress independent of seed developmental age. Moreover, seed development is accelerated under drought conditions. We obtained viable dwarf grains after slow drying on ears that were cut from the plant as early as 5 days after anthesis. This ability for seed accelerated development allows viable seeds to be produced even under the conditions of early interruption of water supply.

LUMINESCENT CONTROL OF BIOTIC AND ABIOTIC STRESS EFFECTS IN PLANTS

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Application of biotechnology in agriculture for the creation of plants tolerant to unfavourable environments requires appropriate methods to analyse their physiological state and vitality under stress conditions. Suitable methods could be variable and delayed chlorophyll a fluorescence because of their possibility to work with intact objects *in vivo* and in situ, high sensitivity, and exceptionally information-rich signal. Luminescent methods are based on the intrinsic feature of the photosynthetic machinery to emit a small amount of the absorbed energy as prompt and delayed fluorescence quanta. By applying different experimental approaches one can register various parameters of both types of luminescence that describe structural and functional properties of the photosynthetic apparatus as well as the kinetics of individual reactions in the light energy transformation chain.

Photosynthesis is a target process in the plant cell for most types of stress influence. This makes luminescent methods suitable for studying primary stress response and long-term stress-induced effect. The applicable fields include biotic stress studies (bacterial and virus infinfections in plants) as well as abiotic stress (low and high temperature, drought, salinity, herbicides and other biologically active substances, high light).

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DIFFERENTIAL MECHANISMS OF CELLULAR PROTECTION DURING WATER LOSS IN ANHYDROBIOTIC PLANTS

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Anhydrobiosis (“life without water”) is the remarkable ability of certain organs/organisms to survive almost total dehydration. It requires a coordinated series of events during dehydration that are mainly associated with preventing oxidative damage and maintaining the native structure of macromolecules and membranes. Adaptations in

cell wall shape and in plasma membrane surface area occur by folding or rippling, and in the case of membranes, also by vesiculation. During dehydration, cytoplasmic amphiphiles redistribute over the lipidic and aqueous phases. This may lead to membrane disturbance, but in the case of partitioning of amphiphilic antioxidants, also to membrane protection. We found that the acquisition of desiccation tolerance coincided with the insertion of endogenous amphiphiles into membranes upon drying. Desiccation tolerance correlates with considerable reduction in metabolism. The production of reactive oxygen species during dehydration is thus reduced. Loss of water leads to molecular crowding, which may be harmful if molecules abound that preferentially interact with macromolecular surfaces – causing proteins to unfold. To counteract this effect, compatible solutes may be produced that force macromolecules to be preferentially hydrated – which keeps proteins in the native conformation. The mechanisms listed above are particularly important above 0.3 g H_2O/g dry matter, i.e. in the presence of bulk water, whereas hydrogen-bonding interactions become an issue when the water shell is gradually lost. Sugars play an important role in macromolecular stabilization through hydrogen-bonding interactions. On reaching approx. 0.1 g H_2O/g dry matter, the cytoplasm turns into an amorphous glass that is characterized by an extremely high viscosity. Sugars and biopolymers are constituents of such a cytoplasmic glass. In principle, a glass will be formed in the cytoplasm of any cell on drying, but glasses in anhydrobiotes are special in that they are tightly packed and highly stable.

THERMAL IMAGING FOR THE STUDY OF PLANT STRESS AND STOMATAL CLOSURE

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Leaf or canopy temperature has been used for some years as a measure of plant stress. This presentation addresses the problems of estimating stomatal conductance using new techniques based on thermal imaging, and discusses their application in the field. Stomatal conductance is only one of the factors affecting the leaf or canopy energy balance, and hence the canopy temperature, through its control of transpiration rate. It follows that canopy temperature can be used to estimate stomatal conductance as long as other components of the energy balance are known or can be estimated. One

approach to estimation of the other components of the energy balance is to use temperatures of wet and dry reference surfaces (Jones 1999). In this paper we outline improvements to the approach that are possible using thermal imaging rather than infrared thermometry and their application to the study of stomatal conductance in a vineyard (*Vitis vinifera*) in Portugal.

Reference: H. G. Jones (1999) *Agric. Forest Meteorol.* **95**: 139–149.

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OVER-EXPRESSION OF AN *ATH-ALDH3* GENE IN TRANSGENIC *ARABIDOPSIS* PLANTS CONFERS TOLERANCE TO DROUGHT AND HIGH SALINITY

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Drought and salt stress are major and closely related environmental challenges influencing plant growth and productivity worldwide. Improved tolerance to environmental stresses in genetically engineered plants is a promising way to reclaim farmlands lost due to environmental challenges. In order to identify and elucidate the regulatory mechanism of genes that are critical for drought and salt stress tolerance in plants, database searches for corresponding genes to a dehydration-inducible aldehyde dehydrogenase gene (*Cp-ALDH*) from the resurrection plant *Craterostigma plantageneum* have revealed a putative *Arabidopsis*-ALDH (*Ath-ALDH3*) gene, which shows 70% similarity to the *Cp-ALDH* sequence (Kirch et al., 2001). ALDHs catalyze the oxidation of toxic aldehydes into their corresponding non-toxic carboxylic acids. Transgenic *Arabidopsis* over-expressing *Ath-ALDH3* was shown to be much more resistant to high concentrations of salts (NaCl, KCl) and water deprivation than the isogenic wild type as result of biomass production, chlorophyll content and lipid peroxidation under various growth conditions. Transcript analysis revealed that *Ath-ALDH3* is constitutively produced in transgenic plants unlike in the wild type. These results demonstrate that *Ath-ALDH3* is a crucial gene for abiotic stress-tolerance in plants and its over-expression in transgenic plants can improve plant tolerance to various stresses. Thereafter, our interest is to improve abiotic stress tolerance in a crop plant such as rice with a sense contruct of *Ath-ALDH3*.

Reference: Kirch H-H, Nair A, Bartels D. (2001) Novel ABA- and dehydration-inducible aldehyde dehydrogenase genes isolated from the resurrection plant *Craterostigma plantagineum* and *Arabidopsis thaliana*. The plant Journal, 28**,** 555-567.

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STEM RADIAL GROWTH AS A TOOL TO ANALYZE THE PLANT RESPONSES TO ENVIRONMENTAL CONDITIONS

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It is reported from the 19th century that the elastic living tissues of trees could change their size in relationship to environmental conditions.

To evaluate the stem diameter change the radial rings with elastic component was the first device applied. In the second half of the 20th century the introduction of electronic dendrometers with chart recorder permitted to increase the frequency and accuracy, also if the dimension and the apparatus complexity limited the applications.

Now a day, the modern technology in microelectronic, computer hardware software and storage memory, gives the possibility to realize small device with light weight that can be installed for months or years on the same plant, and to collect thousands of data with 0.1 mm precision.

Data and results of application to evaluate stem and root size variation in relation to vegetative growth stage and to the environment are presented. Experiments carried out under different stresses (drought, flooding, frozen) give indication of different answer of different tissues and of influence of the plant part directly stressed to the other plant organs.

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STRESS DETECTION IN LEAVES OF *Chinese cabbage* INFECTED WITH TURNIP YELLOW MOSAIC VIRUS BY FLUORESCENCE SPECTRA, INDUCTION AND IMAGING

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Virus infection induces changes in host plant metabolic processes, including the most basic one, photosynthesis. Chinese cabbage (*Brassica pekinensis* cv. Pach Choi) plants were infected with turnip yellow mosaic virus (TYMV). In the emission spectra of isolated chloroplasts from the green tissue area of infected leaves maxima and their amplitudes were similar to the control. Controversially, in the spectra of chloroplasts isolated from the yellow tissue area, the ratio of the two main bands altered. The short-wavelength band (680 nm region) had a lower amplitude compared to the control,

which shows changes in certain PSII complexes. This is correlated with the results on chlorophyll-protein complexes obtained earlier by PAGE. When excited with UV-A radiation, plant leaves show a fluorescence emission in the visible range with maxima around 440 nm (F440), 520 nm (F520), 690 nm (F690) and 740 nm (F740). Certain stress factors alter not only the red and far red fluorescence emission of Chl *a*, but cause significant changes also in the blue and green region. The blue-green fluorescence is thought to be primarily due to the accumulation of stress related substances. The strength of the stress effects exerted by TYMV infection on the activity of the photosynthetic apparatus (red and far-red fluorescence) and on the blue-green fluorescence were investigated by fluorescence imaging. The higher values of the blue/red, blue/far red and red/far red ratios observed in infected tissues were accompanied with a decrease in F_v/F_m, the maximal quantum efficiency of PSII. It can be concluded that the higher fluorescence ratios may correlate with the lower PSII activity, which is also reflected in reduced CO_2 fixation. The work was supported by OTKA (Hungarian National Scientific Research Fund No. T032497), which is gratefully acknowledged.

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Mo-CONTAINING ENZYMES RESPONSES TO LOW TEMPERATURE STRESS OF WINTER WHEAT GROWN ON ACID SOIL

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The effect of cold acclimation and freezing stress on the activity of nitrate reductase (NR, EC 1.2.2.1), aldehyde oxidase (AO, EC 1.2.3.1), and xanthine dehydrogenase (XDH, EC 1.1.1.204) was investigated in winter wheat (*Triticum aestivum* L.) grown on acid soil (pH 4.2, KCl) and supplied additionally with molybdenum (Mo). All the enzymes contain Mo bound to a unique pterin named Mo-cofactor (MoCo), which is presented either as a dioxo form (in NR) or a sulfide form (in AO and XDH). NR catalyses the first rate-limiting reaction of nitrate reduction in plants; AO is considered to catalyse the last step of biosynthesis of abscisic acid and indolylacetic acid; XDH is suggested to produce superoxide radicals in plants. We found that Mo supply resulted in increased levels of NR, AO and XDH activities. During cold acclimation, the activity of NR increased due to both *de novo* synthesis of NR protein and activation by dephosphorylation of the synthesised NR. Studies with inhibitors of protein synthesis (CHI) and dephosphorylation (okadaic acid) have showed that the cold-induced NR activation preceded the *de novo* NR synthesis. In contrast to NR, no change in the activities of AO (tested with indole-3-aldehyde as a substrate) and XDH was

observed during cold acclimation. After freezing stress, the level of NR activity remained higher in Mo-treated compared to Mo-nontreated plants. A sharp increase in AO activity was established in Mo-treated plants only after the 4th day after freezing. These results will be discussed with respect to a) a positive correlation between the increased NR activity and protein accumulation in plants during CA and b) the involvement of AO in the biosynthesis of plant hormones abscisic acid and indolylacetic acid.

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ABSTRACTS OF PRESENTED POSTERS
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ISOLATION AND PURIFICATION OF NUCLEAR DNA FROM EXCISED COTYLEDONS OF *CUCURBITA PEPO* L. (ZUCCHINI) AND INVESTIGATION OF METHYLATION PATTERN OF rRNA GENES

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High molecular wight nuclear DNA was isolated from excised cotyledons of *Cucurbita pepo* L. treated for different periods of time (6, 12, 24 h) in darknes with 6-benzyladenine (BA), abscisic acid (ABA) or the methyl ester of jasmonic acid (MeJA). DNA was purified by retaining from celite column in order to eliminate the polysaccharide contaminants. Thoroughly purified nuclear DNA was completely digested with a number of restriction enzymes (Eco RI, Hind III, Bam HI) and the changes of the methylation pattern of the intergenic spacer (IGS) of rRNA genes was studied after subsequent digestion with the restriction enzymes Msp I and Hpa II and by the method of "indirect end labelling". As rDNA probe was used cloned ^{32}P-labelled Eco RI 2.1 kb fragment spanning the most part of 18S rRNA gene of flax rDNA (pBG35). Results showed heavy methylation of the rRNA genes. As judged from the almost total lack of digestion with Hpa II, in the repeating rDNA units there are no methylation free regions or either just a few are observed. Methylation pattern of the IGS is not changed upon treatment of the cotyledons *in vivo* with BA, ABA and MeJA. This suggests that previously observed changes in the rRNA gene activity are not accompanied by any significant DNA methylation changes. DNAse I hypersensitivity in the promoter region of rRNA genes will be also reported.

A REGULATORY ROLE OF SALICYLIC ACID IN PARAQUAT INDUCED-OXIDATIVE DAMAGE IN BARLEY PLANTS

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Twelve-day-old barley seedlings were supplied with 500 μM Salicylic acid (SA) or 10 μM Paraquat via the transpiration stream and kept in dark for 24 h. They were then exposed to 100 $\mu mol.m^{-2}.s^{-1}$ PAR and samples were taken 24 h after dark and 6h after light exposure. The relationship between the role of SA, paraquat toxicity and the

degree of oxidative damage was examined by measuring the activities of several antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR).Treatment with 10 µM paraquat significantly increased the level of H_2O_2 followed by a reduction in the activities of APX, GR and a slight increase in the activity of SOD. Pre-treatment with 500 µM SA for 24 hours in the dark improved the capacity of the antioxidative defence system, which in its turn increased paraquat tolerance, when barley plants were exposed to paraquat for 6 hours in light. The possible involvement of SA in the plant response to paraquat toxicity will be discussed.

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CHANGES IN ELECTRON TRANSFER PATHWAYS IN THYLAKOID MEMBRANES FROM PEA MUTANTS WITH MODIFIED CHLOROPHYLL CONTENT

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The properties of the 77 K chlorophyll fluorescence in thylakoid membranes isolated from pea mutants are investigated. The mutants have modified pigment content, altered structural organization, different functional and surface electric properties. A method for decomposition of the total fluorescence spectrum of thylakoid membranes into the bands belonging to the individual pigment-protein complexes is proposed. Using this method the changes in the energy transfer between the two photosystems and within the framework of each photosystem, due to the structural rearrangement of their pigment-protein complexes as a result of the mutation are revealed in thylakoid membranes. It is shown that the excitation energy transfer to core and RC complexes of PSII is in proportion to the total amount of pigments in mutants, whereas this one to PSI is inversely in proportion. In the investigated mutants a clear correlation between the part of aggregated LHCII and the pigment content (chlorophyll *a*, chlorophyll *b*, and lutein) exists. It suggests that the closer packing in the complexes with higher extent of aggregation regulates the energy transfer to the core and the RC complexes decreasing it. Based on the reduced energy transfer to PSII, accompanying with the increased energy transfer to PSI, we suppose that the aggregation of LHC II switches the energy flow to LHCI in proportion with its extent. The results reveal an additive regulatory photo-protective mechanism, which redistributes the excitation energy between two photosystems operating at low light intensities but at reduced pigment content.

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ISOENZYME PROFILES OF PEROXIDASE, CATALASE AND SUPEROXIDE DISMUTASE AS AFFECTED BY DEHYDRATION STRESS AND ABA

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Germinating seeds are exposed to many unfavorable environmental conditions which lead to the increased accumulation of damaging concentrations of ROS. Plants cope with stress by activation of the cell antioxidant system. In the present investigation data are presented about the effect of dehydration stress on germinating wheat seeds.

Germination of seeds in the presence of ABA was used as a marker for dehydration stress. A strong inhibition of germination rate and growth of seedlings by all stress factors applied was established The high temperature of germination activated moderate and fast moving anodic peroxidase isoenzymes in the endosperm as well as basic peroxidases in the roots of seeds and activated all SOD isoenzymes. ABA application activated moderate moving basic peroxidases and catalase in the roots. A strong inhibitory effect of H_2O_2 on catalase activity was observed for both endosperm and roots.

ABA and H_2O_2 inhibited SOD activity in the roots. All stress factors applied decreased the band intensity of total soluble proteins in the endosperm. Thermostabile proteins were established only in tne endosperm of seeds. Data presented show that the changes in the electrophoretic profiles depend on the seed organ and the stress factor applied.

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ON THE ESTIMATION OF THE QUANTUM EFFICIENCY OF PHOTOSYNTHESIS. I. THEORETICAL GROUND AND EXPERIMENTAL APPROACHES

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All preceding investigations on the quantum efficiency of photosynthesis, starting with the early experiments of Warburg and Negelein (1922a, b) and Emerson and Lewis (1939, 1941) are performed under low irradiances, even under light compensation point, assuming that the photosynthetic “light curves” are linear and the quantum efficiency of photosynthesis is maximum under these conditions.

However, as was shown in our investigations (Zeinalov, 1977a, 197b; Zeinalov and Maslenkova, 1980, 1996) "light curves" of photosynthesis are non-linear or "S"-shaped. In this work a special polarographic oxygen electrode system is described allowing irradiation with background light, and some principal problems existing in the experiments dealing with the maximum value of photosynthetic quantum efficiency are considered and some experimentally obtained results will be presented.

MATHEMATICAL MODELLING OF STRESS EFFECTS IN PLANTS MONITORED BY LUMINESCENT METHODS

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Variable and delayed chlorophyll *a* fluorescence are suitable methods for analysis of a plant's physiological state and vitality under stress conditions. Luminescent methods provide exceptionally information-rich signals, which require a thorough mathematical analysis in order to understand the structural and functional aspects of stress influence. The use of mathematical models of photosynthetic processes, monitored by luminescence methods, allows the simulation and analysis of most types of stress influence (low and high temperature, herbicides and other biologically active substances, high light).

Kinetic models describing the redox reactions in the donor and acceptor side of Photosystem II, responsible for the luminescence properties, are designed. A computer program is developed to numerically solve the differential equations, and to fit the model curves to the experimental ones. In this way, the exact place of action of different stress factors can be assessed. Combined with the information obtained with the JIP-test, the additional use of the delayed chlorophyll *a* fluorescence allows an extended analysis of stress effects in plants.

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THE EFFECT OF COPPER ON GROWTH, SOLUBLE PROTEINS AND PEROXIDASE ACTIVITY OF MAIZE PLANTS: THE ROLE OF SUCCINATE IN TOLERANCE TO COPPER

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Maize plants (*Zea mays* L., cv. Kneja 2L611*)* were treated with various copper concentrations (0.78; 1.5; 4.7; 31; 78; 156 μM Cu) in the presence and absence of succinate (200 μM Na-succinate) to evaluate its potential role in the tolerance to Cu. Plant growth expressed as RGR – relative growth rate, IT – index of tolerance, R/Sh – root/leaf ratio, DW/FW – dry weight/fresh weight, the electrophoretic patterns of soluble proteins, polypeptides and isoenzyme spectrum of peroxidase in leaves and roots of maize plants were investigated.

Treatment with copper led to a reduction in the plant growth, to an inhibition in the activity of anodic and cathodic peroxidase isoenzymes in the leaves and roots. The quantitative changes in protein spectrum and the negative correlation between the intensity of polypeptide bands and Cu concentrations were observed.

Treatment with succinate had an amelioration effect on the Cu-induced inhibition of plant growth. Succinate-treated plants showed an increase of RGR and an improvement of IT compared with the Cu-treated plants. Addition of succinate restored in a significant degree polypeptide spectrum of Cu-treated plants.

These results provide indirect evidence for a possible role of succinate in Cu-resistance of plants.

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IS THERE AN INFLUENCE OF PHLOEM INTERRUPTION AND SUCCINATE ADDITION ON THE DIURNAL VARIATIONS IN THE ACTIVITY OF PHOSPHOENOLPYRUVATE CARBOXYLASE AND NADP-MALIC ENZYME DURING THE EARLY STAGE OF INTERACTION BETWEEN *GLYCINE MAX* AND *BRADYRHIZOBIUM JAPONICUM?*

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Two important enzymes in organic acid metabolism, phosphoenolpyruvate carboxylase and NADP-malic enzyme, show marked diurnal rhythms in their activities during the establishment of the soybean – *Bradyrhizobium japonicum* symbiosis. The pH of the nutrient solution changes in parallel with NADP-malic enzyme activity, being maximal during the night periods, whereas activity of phosphoenolpyruvate carboxylase was highest during the day periods. Nitrogen fixation is dependent on a regular supply of photosynthetic products from the host plant leaves to the roots. We established that the diurnal variations in the activity of phosphoenolpyruvate carboxylase and NADP-malic enzyme were saved when carbon supply to the roots was interrupted in the early stage of interaction between *Glycine max.* and *Bradyrhizobium japonicum.* It was also established that succinate application in the nutrient solution during ino-

culation altered significantly the pattern of enzyme activities. Our experiments indicated that the diurnal variations in the activity of phosphoenolpyruvate carboxylase and NADP-malic enzyme during the first 72 h postinoculation and resulting alkalization of the rhizosphere are essential for establishment and functioning of the symbiosis.

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RESURRECTIONS GO FOR REGENERATION

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The small group of resurrection plants is known for their extreme tolerance to dehydration. Their vegetative tissue can tolerate loss of as much as 98% of its water content, yet return to active metabolism and growth within hours after rehydration. *Craterostigma plantagineum* (Scrophulariaceae) is recognized as a model in studies of stress signal perception and transduction. The Bulgarian endemic halophyte *Haberlea rhodopensis* could give additional opportunities for in depth investigations. We developed systems for *in vitro* regeneration and propagation of both rare plants. Possible relations between stress response and regeneration ability will be discussed.

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RESPONSE OF SOME ESSENTIAL OIL AND MEDICINAL PLANTS TO SEED AND FOLIAR APPLICATION OF PLANT GROWTH RETARDANTS

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Four plant growth retardants: PBZ – α-[(4-chlorophenyl)methyl]-α′-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol; Prohexadione-Ca – (calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate); MEIA – β-monomethylester of itaconic acid and TDZ – N-phenyl-N′-(1,2,3-thiadiazol-5-yl)-N′-phenylurea – were used for seed and foliar treatments on *Primula officinalis* (L.), *Matricaria chamomilla* (L.) and *Datura metel* (L.).

The results obtained showed that all substances have an inhibiting effect on the growth of plant seedings, depending on the concentration used. Optimum rates per kg of seed were about 5.0 mg for PBZ, 15.0 mg for Prohexadione-Ca, 250.0 mg for MEIA and 25.0 mg for TDZ. Positive effects of plant growth retardants on the germination of seeds with low germinating capacity and protective effect against low temperature were obtained.

The foliar application of all substances resulted in shortening of plant height, but markedly increasing number of leaves and branches, fresh and dry weights of leaves, stems and flowers. The highest pod number, pod weight and economic yield were attained by spraying with the optimal level of plant growth retardants: PBZ – 12 mg/l; Prohexadione-Ca – 25 mg/l; MEIA – 180 mg/l and TDZ – 20 mg/l.

INFLUENCE OF PROHEXADIONE-Ca ON GROWTH, MATURITY AND SOME ENDOGENOUS GROWTH REGULATORS ON *HIBISCUS SABDARIFFA* (L.)

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Hibiscus subdariffa L. (Roselle) is a subtropical plant, which is known under the name of Karkade in Egypt. The epicalyx and calyx of its fruits are used to prepare soft drinks and extraction of a red pigments used in cosmetics, pharmacy and food industry.

Greenhouse and field experiments were carried out during two successive seasons to investigate the effect of Prohexadione-Ca on growth and chemical composition of *Hibiscus subdariffa* L. The results could be summarized as follows:

- Prohexadione-Ca inhibited plant height and stimulated the number of branches, flowers and pods as well as yield components. The maximum values of these characters were obtained as a result of 200 mg a.i./l.
- The substance used increased fixed oil content, anthocyanine content and flavone yield.
- Application of Prohexadione-Ca decreased endogenous GA and IAA in the plant.

NON-PHOTOCHEMICAL QUENCHING AND ACCLIMATION TO HIGH TEMPERATURE IN BARLEY MUTANTS

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The influence of high temperature on the efficiency of photosystem II was investigated in plants with different pigment content and light harvesting components. The role of zeaxanthin-mediated non-photochemical quenching (NPQ) in high temperature acclimation was also studied. Wild type barley plants and two chlorina mutants, (chlo.

f2 and chlo. 126), were exposed to high temperature treatments (38°C) for 7 days. The quantum efficiency of PSII, measured as F_v/F_m, was not significantly different in different barley genotypes. Exposure to 38°C did not cause considerable changes in F_v/F_m in the different barley lines, but caused significant differences in the actual efficiency of PSII electron transport (Φ_{PSII}). Φ_{PSII} was higher in the two mutants than in the wild type before high T treatment. Consistently, the two mutants were able to maintain a high electron transport activity at high temperature conditions, whereas Φ_{PSII} decreased significantly in the wild type after 7 days at 38°C. Decreased PSII photochemical activity in the wild type after prolonged exposure to 38°C correlated with an increased level of NPQ and zeaxanthin content (Z). NPQ, measured in control chlo. f2 leaves, was lower than that of wild type and it increased slightly after 5–7 days at 38°C. However the amount of Z (plus antheraxanthin) expressed on the basis of chlorophyll a content was much higher in the mutants both before and during heat stress. Apparently, these mutants appear perfectly efficient under conditions of high temperature and high light.

REGENERATION PROCEDURES FOR BULGARIAN RASPBERRY CULTIVARS AND HIBRIDS

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Raspberry production is strongly limited by environmental factors among which drought is the most damaging. In order to improve abiotic stress tolerance of Bulgarian cultivars a large-scale breeding programme is in progress in our Institute. In this respect, studies on regeneration and osmotic stress tolerance *in vitro* are performed. Efficient regeneration system is an important prerequisite for successful transformation procedure. In this respect, we tested several media, explants and genotypes of various origins to develop such system for Bulgarian raspberry genotypes.

Leaf segments and petioles from *in vitro* cloned plants of four Bulgarian cultivars (Shopska Alena, Lyulin, Samodyva and Bulgarski Rubin) and two elite hybrids were tested for regeneration ability. Seven variants of nutrient media were used in the study. The best genotype x media combinations and explants will be discussed.

TEMPERATURE EFFECTS ON CHARACTERISTICS OF DARK RELAXATION OF DELAYED FLUORESCENCE IN *ZEA MAYS* LEAVES

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Effect of temperatures in range from 5 to 60°C on dark relaxation kinetics of millisecond delayed chlorophyll *a* fluorescence was studied. The dark relaxation of delayed fluorescence (DF) between 0.35 and 5 ms is poly-exponential and can be approximated by 3 components with life-times around $\tau_1 \sim 0.6$, $\tau_2 \sim 3.5$ ms and $\tau_3 \sim 20$ ms. Both the amplitudes and the life-times of the DF components drastically changes during the induction. The contribution of different DF components depends on registration temperature also. At low temperature (5ºC) the main part in the fast phase of DF induction curve has the millisecond component, at high (38ºC) – the sub-millisecond and at room temperature (22ºC) the deal of the both components is approximately equal. The photoinduced PQ pool reduction is accelerated with temperature increase – at 38ºC it closes about three times faster than at 5°C. The termogramms of DF dark relaxation kinetics are recorded at stationary light conditions. The amplitudes of all three components show very different temperature dependencies – L_1 is maximal at 40ºC, L_2 – at 15ºC, and L_3 – at about 55ºC. The life-time of sub-millisecond DF component decreases with temperature growth contrary to millisecond component that slows down with temperature up to 55ºC. It is concluded that temperature strongly modifies the rate constants of electron transfer reactions in PSII acceptor side whose determine DF relaxation kinetics.

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TREATMENT WITH METHYL JASMONATE ALLEVIATES THE EFFECTS OF PARAQUAT ON PHOTOSYNTHESIS IN BARLEY PLANTS

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The present study investigated the possible mediatory role of methyl jasmonate (JaMe) in paraquat (Pq) toxicity on photosynthesis. Twelve-day-old barley seedlings were supplied with 23 μM JaMe or 10 μM Pq via the transpiration stream and kept in the dark for 24 h. Then they were exposed to 100 $\mu mol.m^{-2}.s^{-1}$ PAR and samples were taken 1,2,3, and 6 h after the light exposure. Leaf gas exchange parameters, the activity of ribulose-1,5 bisphosphate carboxylase (RuBPC, EC 4.1.1.39) and of the photorespiratory enzymes phosphoglycolate phosphatase (PGP, EC 3.1.3.18), glycollate oxidase (GO, EC 1.1.3.1), and catalase (EC 1.11.1.6) were determined. Treatment of seedlings with JaMe alone resulted in decreased levels of chlorophyll and protein, CO_2 assimilation and transpiration rates. Pq treatment led to a decrease in chlorophyll and protein contents and to a very strong inhibition of photosynthesis, the effects were manifested

by 1 h of illumination. Pq-treatment did not affect the activity of RuBPC but highly increased the activity of the photorespiratory enzymes. Pretreatment of seedlings with JaMe fully blocked the inhibitory effect of Pq on photosynthesis and provide protection against subsequent Pq-induced oxidative damage. This observation was confirmed by gas exchange parameters, chlorophyll and protein content and by changes in some important parameters associated with oxidative stress, namely lipid peroxidation and electrolyte leakage. The data suggest that JaMe might protect cells against oxidative damage and prevent photosynthesis upon paraquat treatment.

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EFFECT OF TMV ON THE GROWTH, LEVEL OF OXIDATIVE STRESS MARKERS AND ACTIVITY OF SOME DEFENCE ENZYMES IN SENSITIVE AND RESISTANT TOBACCO CULTIVARS

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Two tobacco (*Nicotiana tabacum* L.) cultivars, Nevrokop 1146 (resistant, hypersensitive located), and Samsun (sensitive) were inoculated with TMV prepared from infected tobacco leaves. At the end of the vegetation the biometric prameters, virus concentration (by ACP-ELISA), oxidative stress markers (MDA, hydrohen peroxide), free proline, activity of some defence enzymes (catalase, peroxidase, and superoxide dismutase) were determined. In inoculated plants of both cultivars typical virus symptoms were observed - fresh weight, height of overground parts and number of leaves decreased by half (compared to the relative controls). In tobacco plants, cv. Nevrokop, there were no significant changes in the amount of stress markers and activity of defence enzymes. However, in the sensitive cultivar the infection provoked an accumulation of hydrogen peroxide, strong inhibition of catalase and superoxide dismutase activity and an increase in guaiacol peroxidase activity. The results presented showed that TMV caused an oxidative stress in all leaves of sensitive tobacco plants and this effect was enhanced with time. In the hypersensitive-located cultivar, despite the inhibited plant growth and even after the death of innoculated leaves there were no oxidative events.

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LIPID COMPOSITION OF CALYSTEGIA SOLDANELLA – A HALOPHYTIC PLANT FROM BULGARIAN BLACK SEA COAST

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Although Bulgaria is situated in a moderate climatic zone, there are some areas along the Black Sea where the soil may contain up to 700–900 mg salts in 100 g soil. A plant which can grow under these extreme conditions (salinity and temperature up to 36ºC) is Calystegia soldanella. In these work we present the fatty acid profiles in the main lipid classes of C.soldanella. As in all higher plants, the main lipid classes are glycolipids (57.1% of total lipids). The main glycolipid is sulphoquinovosyl diacylglicerols (SQDG) – 31.7%, followed with almost equal content of monogalactosyl diacylglycerols (MGDG) – 11.1% and digalactosyl diacylglycerols (DGDG) – 14.3%. It is to note that the content of triacylglycerols (TAG) is very high (31.7% of total lipids), while the content of phospholipids (PL) is relatively low (11.1%). TAG are not constituents of lipid membranes and their present in so high quantities in C. soldanella is still unexplicable. The dominated fatty acid (FA) in all lipid classes is linolenic (18:3), followed by palmitic (16:0) and linoleic (18:2) acid.The membrane fluidity of the higher plants depends on the content of the unsaturated 18:2 and 18:3 acids. A membrane with lower content of these unsaturated FA would resist better the harmful salinity. In the case of *C. soldanella* the content of 18:2 and 18:3 acids is very high (more than 50% of total FA). This could be indication that the halophyte plants can react to different ways to harmful environment.

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PRODUCTION OF REACTIVE OXIGEN SPECIES IN YOUNG PEA PLANTS TREATED WITH SUBHERBICIDE CONCENTRATION OF ATRAZINE, HIGH TEMPERATURE AND COMBINATION OF THEM

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Pea plants (*Pisum sativum* L.,) cv. Manuela, were grown as a water culture on Hoagland-Arnon medium (25±1°C, 12/12 day/night photoperiod, 70 μmol.m^{-2}.s^{-1} photon

flux density). At the stage of fully developed 2nd leaf the plants were treated with 10^{-6} M atrazine supplied by the roots (added to the nutritional medium). Five days later, part of the seedlings were subjected for 2 days to a high temperature stress regime. The accumulation of superoxide radicals ($^{\bullet}O_2^-$) were measured indirectly by the reduction of tetrazolium chloride (NBT spectrophotometric test). The generation of hydroxy radicals ($^{\bullet}OH$) was estimated by the formation of 3- and 4-hydroxybenzoic acid. In the experiments the intact leaves from 2nd internode were used. It was found that the separate application of herbicide and high temperature caused and increase in the level of $^{\bullet}O_2^-$ (1.7 and 4 times respectively) but the effect was amplified in the plants subjected to combined stress. Atrazine did not influence significantly the concentration of $^{\bullet}OH$. However, the high temperature applied alone and in combination with atrazine considerably elevated the $^{\bullet}OH$ levels, and the effect was more pronounced by the action of both stress factors applied together.

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POLYSACCHARIDES OF *RHODELLA RETICULATA* AND STRESS

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The cells of *Rhodella reticulata* (Rhodophyta) are encapsulated within sulfated polysaccharide, the external part of which dissolves in the medium. The polysaccharides have a wide range of industrial applications and occupy an important position in the strategies of *Rhodella* to avoid different stresses: protection against salt stress, desiccation , metal binding capacities and others. The significance of the heteropolysaccharide is linked to its key role in protecting the integrity of the cell and the fact that it must be disrupted, penetrated or destroyed before an attacker has access to the protoplast and cause death of the organism. Although the production of heavy mucilaginous layer of polysaccharide around the cells of *Rhodella* a bacterial contaminant causing lysis of the algal cells was isolated. SEM investigations demonstrate the abilities of *Cytophaga* bacteria to adhere and produce microcolonies within the polysaccharide layer of extensive and intensive cultivated algal cells. The bacterial pathogen overcomes the polysaccharide barrier (host defense mechanism) by specific production of adhesion fimbriae and lytic enzymes. The persistence of *Rhodella reticulata* to the bacterial metabolites depended on the factors controlling the amount of polysaccharide production during the different development stages – temperature, light intensity, presence of different mineral salts in the cultivation medium.

VOLATILE SUBSTANCES FROM *SCENEDESMUS* GROWN IN MEDIA WITH DIFFERENT SALINITY

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Volatile substances obtained from the green unicellular alga *Scenedesmus incrassatulus* were investigated. The habitat of this species is fresh water and it is an important subject of large-scale cultivation. The algae were grown in fresh water and in a medium containing 17 g/l NaCl. Volatile substances were isolated by steam distillation. They were analysed by gas chromatography / mass spectrometry. The main components in both samples were saturated and unsaturated hydrocarbons, fatty acids and phytol. Significant differences were observed in products of secondary metabolism. These compounds were almost absent in the sample grown on the NaCl – containing medium. In the freshwater sample, significant amounts of diterpenes, benzyl benzoate and other secondary metabolites were found. It seems likely that salt stress downregulates secondary metabolism in this species.

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THE INFLUENCE OF EXCESS OF Cd AND Cu ON THE PHOTOSYNTHESIS, TRANSPIRATION AND BIOLOGICAL PRODUCTIVITY OF PEA-PLANTS (*PISUM SATIVUM*)

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The influence of toxic concentrations of Cu (10 mg/l), Cd (5 mg/l) separately and together (3 mg/l) on the intensity of assimilation of CO_2, transpiration and biological productivity of young pea-plants (*Pisum Sativum*), sort "Manuela" have been studied. Under conditions of metal toxicity, the protective (revitalizing) effect of humic acid (natural product) gained through the extraction of brown coal, possessing physiologically-active impact and their addition to the nutritious solution of Helrigel at a concentration of 250 mg/l has also been considered.

A powerful inhibition of the formed leaf - area, has been detected as regards young pea-plants It was reduced with Cd by 49%, by Cu – 35%, and by Cd+Cu by 42%. When humic acids are applied along with heavy metals, the leaf-area increases by up

to 10%. This stronger inhibition in the intensity of CO_2 assimilation, under metal stress leads to a decrease of the photosynthesis/ transpiration ratio. Humic acids increase the photosynthetic assimilation of CO_2. Humic acids enhance the photosynthetic assimilation of CO_2 by the pea leaves, increase the intensity of transpiration and decrease the stomatal- resistance.

CHANGES IN THE QUANTITY OF AMINIC ACIDS AND SOLUBLE PROTEINS AS REGARDS PEA PLANTS IN AN ANSWER TO Cd TOXICITY IN THE PRESENCE OF SOME NATURAL PHYSIOLOGICALLY - ACTIVE SUBSTANCES

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Three types of natural products, concerning their effect on young pea-plants' physiology (*Pisum Sativum*), sort "Citrina" and grown in conditions of Cd toxicity (4 mg/l). To the nutrient solution of Helrigel one by one have been added: humic acids (100 mg/l), suspension of green algae – *Chlorella* and bluegreen *Plectonema* in 1g/l dry substance.

In the presence of toxic concentrations of Cd both three examined products exert positive physiologically active impacts. A recovery of the plants' habitus and of the values of the soluble proteins and amino acid content to a considerable degree are witnessed. Humic acids influence most, followed by the green *Plectonema*, and weakest is the *Chlorella* suspension. The quantative changes in the three interrelated amino acids: glutamic: glutamine; serine and asparginine correlate to the variations of the soluble protein's quantity.

EFFECT OF INCREASING UV-B RADIATIONS ON GROWTH PARAMETERS AND STRESS MARKERS IN PEA

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Pea (*Pisum sativum,* L., cv. Secado) seedlings, grown in Hoagland's nutrient medium at 12/12 h photoperiod, 25/20°C day/night temperature, PFD 70 $\mu mol.m^{-2}.s^{-1}$, were daily irradiated with UV-B (obtained from UV-b emitting lamp STYLO STY 115, Italy, and fluence rate 0.29 mW/cm^2 normalized to 300 nm) for 0 s, 20 s, 60 s, 120 s or 180 s for 20 running days. The growth parameters: fresh weight (FW), dry weight

(DW) and electrolyte leakage were determined for an over ground plant; but stress markers: H_2O_2, malondialdehyde (MDA) and proline were determined in the youngest leaves after cessation of the stress programme.

No significant differences between control plants and these irradiated with 20 s UV-b, in growth parameters, electrolyte leakage, MDA and proline levels were detected. The only observed change in plants irradiated for 20 s was decreased H_2O_2 levels down to 77% as compared to the control.

With the increase of UV-B irradiance duration, FW, and DW decreased, but H_2O_2, MDA and proline levels unexpectedly decreased linearly down to 10%, 28% and 42% respectively as compared to the control, in plants irradiated for 120 s. Changes in membrane structure and permeability in all UV-B irradiated for 180 s plants were indicated by both the extreme increase in electrolyte leakage, as compared to the control, and the severe desiccation of upper leaves.

We suggest that declined products of lipid peroxidation (estimated as MDA levels) and H_2O_2 levels are due to an increase in glutathione-S-transferase activities, which deserve future investigations.

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EFFECT OF NITROGEN NUTRITION TYPE ON THE RESPONSE OF SOYBEAN PLANTS TO WATER STRESS. CHANGES IN ELECTROPHORETIC SPECTRA OF SOLUBLE PROTEIN, SUPEROXIDE DISMUTASE AND PEROXIDASE

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Effect of the nitrogen source, nitrate or atmospheric nitrogen, on the response of soybean (*Glycine max.* L., cv. Hudson) to gradual (20–50%) water stress has been investigated. The changes in electrophoretic spectra of soluble proteins and polypeptides as well as isoenzyme spectra of peroxidase and superoxide dismutase were studied at different stages of plant development (5th-8th leaf). It was established that the changes in the electrophoretic protein spectra began at 30% of water deficiency in the leaves of inoculated plants. In the leaves of uninoculated plants these changes were more pronounced at 50% of water deficiency. The same was the effect of water deficiency on the polypeptide spectrum. The influence of water stress on the spectrum of superoxide dismutase, isolated from uninoculated plants was manifested as a lack of the most active fast moving isoenzyme. In the case of inoculated plants there were no significant differences between the variants investigated. The number of anodic peroxidase isoenzymes in inoculated plans was higher compared to the uninoculated plants and the activity of some isoenzymes decreased with the progress of

water stress. There was no effect of water deficiency on the anodic peroxidase spectrum in the leaves of uninoculated plants. A high active basic peroxidase isoenzyme was revealed which did not show differences between inoculated and uninoculated plans.

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EFFECT OF THERMODASE ON NITROGEN FIXATION, PHOTOSYNTHESIS AND LEGHEMOGLOBIN CONTENT

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Thermodase is an enzyme isolated from hen egg white. It is a HEWL (hen egg lyzozyme) modification, which is obtained by physicochemical treatment accompanied by a change of its conformation (proved by the fluorescence characteristics of tryptophan fluorescence). The modified enzyme acquires new properties and the lysis of the cell walls of Gram-positive and -negative bacteria is interrupted and the eukaryote cells are reversibly immobilised without damage.

HEWL and some of its modifications are used in medical practice as antimicrobial agents, which destroy the cell walls.

There is no literature data on the effect of thermodase on the physiological processes occurring in plants. We have studied the effect of thermodase on the symbiotic system *Br. Rhr. Japonicum273 – Glycine max* var. *Corada*. For this purpose, soybean plants grown in pots on an area of 75 cm^2 were treated by 0.1% thermodase solution during the initial blooming phase. The comparison of the results obtained with the control reveals an enhanced nitrogenase activity – 29.2%, an increased $^{14}CO_2$ fixation rate – 28% and increase of the total Lb content – by 36%. Some changes in the electrophoresis profile of the total Lb have been observed.

HOT NEWS! TOBACCO TOLERANCE TO DROUGHT AND HIGH TEMPERATURES

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Drought and extreme temperatures are the most serious handicaps for agriculture. Accumulation of osmolytes appears to be a prerequisite for tolerance to hot and dry weather. To improve the abiotic stress tolerance of tobacco we triggered overaccumu-

lation of proline and induced fructan or glycine-betaine accumulation. Two months old plants (8–9 leave stage) of T3 transgenic stables along with wild type plants were subjected to high temperatures (38–40°C) and drought for 6 days under controlled conditions. We followed the dynamics of leaf relative water content (RWC) and electrolyte leakage. The transformants preformed much better than wild type plants.

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ACTIVITY OF PHOTOSYNTHETIC HERBICIDES IN INTACT PEA LEAVES MEASURED BY PROMPT AND DELAYED CHLOROPHYLL FLUORESCENCE

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The goal of the present work was to explore the possibility of using chlorophyll *a* fluorescence and delayed fluorescence as a test to analyse the action of photosynthetic herbicides in intact pea leaves. Detached leaves of 14-days pea plants were treated with different concentrations of the herbicides diuron or atrazine, both binding to the Q_B-site on the D1 protein of PS2. Two application approaches were compared – passive diffusion through the leaf tissue (by incubating leaves in herbicide solution) and vacuum infiltration (using a plastic syringe). Various parameters of the chlorophyll a fluorescence induction curves were calculated and their concentration dependencies and half-inhibition concentrations were compared. Comparing the two herbicides, diuron had a greater inhibiting potency than atrazine in each case, but the difference between them depended on the type of treatment – atrazine was 10-fold weaker than diuron when applied by diffusion and 3-fold weaker when applied by infiltration. Both herbicides induced a greater effect when applied by infiltration (half-inhibition concentrations were 4 μM for diuron and 35 μM for atrazine) than by diffusion (2 μM and 5 μM for diuron and atrazine respectively).

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DETERMINATION OF SOME ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN BEAN PLANTS EXPOSED TO DIFFERENT LIGHT INTENSITY AND CO_2 CONCENTRATIONS

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The present study aims to follow the changes in the enzymatic activity of the antioxidant system of bean plants (*Phaseolus vulgaris* L.) under different light intensities at ambient and elevated CO_2 concentration. 19-day-old plants were exposed for 4 days (8 h daylight period) under low (100 µmol.m^{-2}.s^{-1}) and high (1100 µmol.m^{-2}.s^{-1}) photosynthetic photon flux density (PPFD) and ambient (350 ppm) or elevated (1300 ppm) CO_2 concentration. Extracts were prepared from each experimental group and the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and peroxidase (POD) as well as the concentrations of endoperoxides and malondialdehyde (MDA) were determined. The presence of two Cu,Zn- and one Mn-SODs and eight forms of POD was shown by disc electrophoresis. SOD activity was almost unchanged in all samples. High PPFD significantly changed APX and POD activity and the peroxides content – the increase was about two-fold, while MDA concentration increased slightly. Elevated CO_2 diminished the light-induced increase of APX activity and endoperoxides content, however overall APX and POD activity was higher than under ambient CO_2.

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CHANGES IN THE ACTIVITIES OF ANTIOXIDANT ENZYMES IN MARROW SEEDS GERMINATED IN THE PRESENCE OF ABA AND METHYL JASMONATE

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Abscisic acid (ABA), jasmonic acid and methyl jasmonate (MeJa) influence plant growth and development as well as plant responses to different stress factors. The effect of ABA and MeJa on the germination, seedling growth and isoenzyme spectra of peroxidase, catalase and superoxide dismutase was investigated. The activity of anodic peroxidases increased with the progress of seed germination.

The more pronounced effect of ABA and MeJa was revealed at 72 h of germination. Both substances activate moderate moving isoenzymes but in addition ABA decreased the activity of fast moving isoenzymes.

A slight stimulatory effect of ABA was expressed on the activity of basic peroxidases in the roots. High SOD and catalase activities were manifested in the embryonic axes of dry seeds. A promotive effect of MeJa on fast moving SOD isoezymes in the roots was observed. ABA slightly inhibited catalase activity in the roots. It was established an organ specificity of the activity of enzymes investigated both in the course of germination and in respect to plant growth substances used.

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CHANGES IN THE ACTIVITY OF SOME STRESS DEFENCE ENZYMES IN PEA AND WHEAT PLANTS TREATED WITH GLYPHOSATE, 2,4-D, AND ATRAZINE

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A study was undertaken to compare the effect of the widely used herbicides glyphosate, atrazine and 2,4-D on the physiological response of plant species characterised by different sensitivity. Pea (*Pisum sativum* L.) and wheat (*Triticum aestivum* L.) plants were grown as water cultures and treated at the 1st (wheat) or 3rd (pea) leaf stage by leaf spraying as follows: 10 mM glyphosate, 1 mM 2,4-D, 15 mM atrazine. Concentrations applied were calculated on the basis of the field rate of each and were preperad as water solutions containing 0.5% TWEEN-80. Guiacol peroxidase (POD, EC 1.11.1), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione reductase (GR, EC.1.6.4.2.) and glutathione-S-transferase (GST, EC 2.5.1.18) activities were measured on the 2nd, 5th, and 9th day after the treatment (1st leaf of wheat; and 2nd and 3rd leaf of pea plants). With minor exceptions all herbicides applied caused an increase in the activity of SOD and POA in both plant species . Herbicides did not change significantly the CAT activity in wheat plants but in pea a tendency of inhibition was observed, and the effect was more pronounced after 2,4-D treatment. The herbicides acted in different ways on the GST activity in both species used – they enhanced it in wheat but in pea plants an inhibition was detected.

COMPARATIVE EFFECT OF THE ACTION OF GLYPHOSATE, 2,4-D, AND ATRAZINE ON STRESS MARKERS IN PEA (*PISUM SATIVUM* L.) AND WEAT (*TRITICUM AESTIVUM* L.*)* PLANTS

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The effect of the widely used herbicides glyphosate, atrazine and 2,4-D on two plant species with different sensitivity to them were studied. Plants were grown as water cultures and treated at the 1st (wheat) or 3rd (pea) leaf stage by leaf spraying as follows: 10 mM glyphosate, 1 mM 2,4-D, 15 mM atrazine. The concentrations applied were calculated on the basis of the field rate of each and were prepared as water solutions containing 0.5% Tween. Levels of lipid peroxidation (as MDA equivalents), free proline content and endogenous concentration of hydrogen peroxide were measured

in first and second (wheat) and second and third leaf (pea) nodes on the 2nd, 5th, 9th day after the treatment. The herbicides did not change significantly the free proline content in pea, but in the wheat plants, which are more tolerant to the herbicides applied, an increase in this parameter was found, implying its eventual protective function. In both plant species, glyphosate provoked an accumulation of all stress markers measured. Application of atrazine and 2,4-D did not influence MDA and H_2O_2 concentration in young or in older wheat leaves. Similar results were found in relation to the atrazine treated pea plants. However, 2,4-D caused an increase of H_2O_2 in pea plants at the end of the experimental period, which corresponded to plant death.

CORRELATION BETWEEN RESPONSE OF CALLUS GROWTH TO ABA AND PLANT RESISTANCE TO CORRESPONDING STRESS IN ROMANIAN WINTER WHEAT GENOTYPES

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Efficiency of *in vitro* selection for stress resistance depends on the proportion of the plant stress resistance mechanisms that are present and can be selected for at cell level. This proportion can be evaluated by correlating callus growth response to the addition of stress factors in the media, with the *in vivo* plant response to corresponding stresses in the field or laboratory tests.

Calli of several winter wheat cultivars, representative for the germoplasm present in Romanian winter wheat breeding programs, were cultured on MS media, for 4–5 weeks, and then transferred to the same media, to which abscisic acid (ABA – 6 mg/l, 8 mg/l and 10 mg/l) as a stress factor were added. Callus growth was estimated by weighing calli after 4 weeks of culture media with stress factor, and compared with the growth on media without stress factor.

A variation of callus weight among genotypes, as expressed in percentage from the average across genotypes, in the presence of ABA, was registered. This suggests that the applied stress reveal genotypic differences, which are not present, when calli are cultured on media without stressing factor.

Best in *vitro-in vivo* correlation was found between callus growth on media with 8 and 10 mg/l ABA, and frost resistance. Also a significant correlation between callus growth on media with 10 mg/l and a drought susceptibility index was registered. Our results suggest that the above mentioned stress factor can be used for *in vitro* selection programs in winter wheat.

RECOVERY EFFECT OF α-NAPHTHYLENEACETIC ACID AND/OR IRON(III)-ETHYLENEDIAMINETETRAACETIC ACID FOLIAR SPRAYS ON IRON DEFICIENT MAIZE PLANTS

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The possibility of α-naphthyleneacetic acid to ameliorate iron supply in iron deficient plants or to improve the effectiveness of the foliar application of iron was studied.

Young maize plants (*Zea mays L.*, hybrid Knezha – 2L 611), grown hydroponically, were supplied with 1/10 the optimal amount of iron (0.75 mg/l).The iron deficiency brought about reduced growth, low chlorophyll and carotenoids content, decreased activities of catalase and peroxidase in the leaves. Chlorotic plants were treated with solutions, containing 50 mg/l iron under the form of FeEDTA (Fe), 25 mg/l α-naphthyleneacetic acid (NA) or combined solution of both substances (Fe + NA). All three treatments increased the chlorophyll content and the dry biomass of the chlorotic plants, but to a lesser extent as compared to the plants supplied with optimum amount of iron. The recovery effect of the combination Fe + NA was not greater than the recovery effect of Fe alone. The plants, treated with Fe + NA, were higher and heavier than those, treated with NA alone. The activities of both enzymes tended to increase in the treated plants as compared to chlorotic untreated plants.

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POPULAR SCREENING FOR OSMOTIC TOLERANCE *IN VITRO*

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Populars are a model tree species for plant biotechnology and ecophysiology, because of their fast growth, tolerance to various unfavorable conditions and industrial use. They became the subject of intensive studies. We developed a system for *in vitro* testing the osmotic stress tolerance in *Populus* sp: *Populus tremula x tremuloides*, *Populus tremula x alba*, *Populus alba*, *Populus euphratica*. Plants were treated with various concentrations of PEG 6000. Several periods of treatment were tested. Different physiological parameters were screened under stress conditions. Survival, growth, L-proline levels, relative water content (RWC), electrolyte leakage were measured. The results and specific performance of each genotype will be discussed.

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THERMOLUMINESCENCE STUDY OF PHOTOSYSTEM II ACTIVITY IN *HABERLEA RHODOPENSIS* AND SPINACH LEAVES DURING DESICCATION

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The Balkan endemic *Haberlea rhodopensis Friv.* belongs to the group of "resurrection" plants that are able to survive after severe desiccation. In order to elucidate the possible reasons for desiccation tolerance of *Haberlea* we made a set of comparative experiments with desiccation-sensitive spinach leaves on PSII reactions. Thermoluminescence glow curve parameters have been used to assess the functional features of PSII in the investigated species. An unusually high temperature of thermoluminescence emission from *Haberlea* leaves after excitation by 1 flash at 5ºC was observed. The position of so-called "B"-band (S_2Qb^-) was at 42–46ºC while this temperature was at 32–35ºC in spinach leaves. Consistent with the up-shift in TL emission the lite-time of S_2 was also increased showing a stabilization of charge storage in PSII complex in "resurrection" plant that may represent some PSII peculiarities, related to *Haberlea* desiccation tolerance. This suggestion was supported by the different way that dehydration affected thermoluminescence properties in *Haberlea* and spinach leaves.

THE EFFECT OF HYDRIC STRESS ON SOME CHARACTERISTICS OF SUNFLOWER PLANTS

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The evolution of leaf area, root depth and volume, chlorophyll content and peroxidase activity in leaves of some Romanian sunflower hybrids, under optimal and drought conditions were established. Five Romanian sunflower hybrids were grown in the greenhouse. Two watering regimes for each genotypes were used*: control variant* – [in which plants were maintained at 70% from TSWC (total soil water capacity)] and *stress treatment* where (sunflower seedlings were irrigated no more than 40% from TSWC) were used.

The results showed that hydric stress induced the decrease of leaf area, root length and volume, and chlorophyll content, but a significant increase of peroxidase activity under hydric stress was registered. Some differences between the sunflower hybrids were registered.

Also, a significant correlation between drought resistance and modification in fatty acid composition from seeds of sunflower plants was found.

In the normal sunflower hybrids, like the hybrids from this study, in addition to the reduction in oleic acid concentration was registered a simultaneous increase in the content of linoleic acid in drought conditions.

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THE EFFECT OF HYDRIC STRESS ON SUNFLOWER PLANTS GROWN IN ACID SOIL

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In this paper the results concerning the effects of acid stress on the Select sunflower hybrid under normal watering and water stress conditions are presented.

The experiment was conducted in green-house; sunflower plants were grown in PVC tubs filled with: (i) cambic cernozem soil (pH = 6.2), (ii) albic luvisol (pH=5.27) and (iii) albic luvisol fertilized with phosphorus (100 kg/ha), potassium (100 kg/ha) and nitrogen (150 kg/ha) (pH = 4.83).

Two watering regimes were used: five replications were watered daily and maintained at 70% from TSWC (total soil water capacity) (control variant) and five replications were watered once aevery couple of days no more than 40% from total TSWC (hydric stress variant).

Significant decrease of leaf area, volume of root and dry matter accumulation was registered in sunflower plants under drought conditions. These modifications were more obvious on albic luvisol as compared with cambic cernozem soil.

The effect of fertilizers applied in albic luvisol on sunflower growth was significant. As results, the grown of sunflower plants on this soil in normal watering conditions was higher than in cambic cernozem soil and albic luvisol. For all that, sunflower plants grown under drought conditions showed a significant decrease in leaf area, volume of root and dry matter accumulation were registered as compared with cambic cernozem.

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THE REACTION OF SOME ROMANIAN GENOTYPES TO *IN VITRO* STRESS INDUCED BY *PHOMOPSIS HELIANTHI* FILTRATE

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The immature embryos from 14 sunflower inbred lines were aseptically prelevated and inoculated on MS media supplemented with *Phomopsis helianthi* filtrate.

7 plants from the 4019/1c inbred line and 24 plants from the 4011/1c inbred line, with an average of 14.5 plants for all the studied genotypes.

The lowest germination percentage of immature embryos was registered at 4006/1c genotype (26.9%), followed by 4005/1c (40%) – in control variant. For the variant with *Phomopsis* filtrate, the genotype with the lowest results was 4005/1c inbred line, followed by 40024/2c inbred line, underlining a high sensitivity to the pathogen used in experiment. The genotypes 4001/1c, 4002/1c, 4018/2c, 4022/1c and 4023/2c imbread line manifested an increased degree of tolerance to the culture filtrate, the germination ability being superior versus control variant. In the literature, has been noticed explanations regarding the possible stimulatory effect of culture filtrate on seed germination as well as plants in the first stages of vegetation, fact signaled during the present experiment.

THE BEHAVIOUR OF SOME VARIETIES LOCATED IN VITICOL CENTRE MURFATLAR-ROMANIA ON THE CLIMATIC STRESS CONDITIONS

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At Murfatlar, last few years where characterised by stressing climatic conditions for the vine growing, consequently it was necessary to take supplementary observations in the plantations with specific Murfatlar centre varieties: Pinot gris, Chardonnay, Riesling italian, Columna, Cabernet Sauvignon and Mamaia.

The late frost from 2000 year, 4 may, (-2.2ºC in the air), unfortunately like a repetition of the precedent frost from the year 1999 (may 7–9, –4ºC), leaded at the compromising of more than a half of the planed harvest, existing plantation located on the down valley or on the plane with 80–100% frozen shoots.

The high temperatures from the next months forced secondary buds, the shoots growing being very intense. On the other hand, their fertility was diminished with approximate 20% in comparison with the average of the normal years. On the summer, because of the high temperatures and low precipitation appeared a strong water stress in plantations. Therefore, vines with nutritional deficiency and vines with viral or mycosis diseases slowly are drying, first the grapes, then the leafs and shoots. The most affected varieties are Riesling italian and Columna where 3–5% from vines are dead.

Concerning grapes quality, it is observed a good accumulation of sugar in grapes-juice, a low acidity and an adequate colouring for to obtain high quality red wines.

Some of the mains physiological processes were monitories using Lci portable system.

EFFECT OF CU AND MN TOXICITY ON GROWTH PARAMETERS, PHOTOSYNTHETIC PIGMENTS, LEAF PROTEIN PATTERN AND RUBISCO CONTENT OF BARLEY SEEDLINGS

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The growth parameters, photosynthetic pigments, leaf protein pattern and Rubisco content of 10-d old barley seedlings (*Hordeum vulgare* L. cv. Obzor) exposed for 5 days to toxic concentrations of Cu (15, 150 and 1500 μM) and Mn (183, 1830 and 18300 μM) in the nutrient solution were investigated. Controls contained 1.5 μM Cu and 18.3 μM Mn in the growth medium. Along with the appearance of toxicity symptoms, the treatment significantly depressed the growth and the fresh biomass of the barley plants. Reduction in the fresh weight was accompanied by desiccation of the aboveground plant organs. All growth parameters were influenced more by toxic concentration of Cu than by excess Mn. The content of chlorophyll a and b considerably decreased without change in chlorophyll a/b ratio. Cu excess increased the carotenoid content whereas Mn excess decreased it. Reduction of protein content and diminution in Rubisco quantity after treatment with increasing concentrations of Cu or Mn was observed, which was more evident on DW basis. Cu toxicity effects on the protein and Rubisco content were stronger. Using 12.5% SDS-PAGE diminution in the intensity of the polypeptide bands corresponding to LS and SS of Rubisco was observed. Partial degradation of the LS could be seen in the protein pattern after treatment with the highest Cu concentration. Appearance of two polypeptide bands with MW 13 and 16 kDa and enhancement of the protein band with MW of 25 kDa was detected in the variants with highest Cu and Mn excess. These observations suggest that common as well as specific mechanisms could take part in the biochemical basis of Cu and Mn toxicity.

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BICYCLIC COMPOUND-INDUCED ALLEVIATION OF SENESCENCE DAMAGE IN RELATION TO ANTIOXIDANT ACTIVITY

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2-(2´-Oxo-1´-imidazolidinyl)-pyridine was synthesized. Its effect on the time-dependent changes in the levels of pigments (chlorophyll and carotenoids), free proline, hydrogen peroxide, malondialdehyde and in catalase and peroxidases (ascorbate and guaiacol) activities was determined in senescing barley leaf segments. It was found that the cyclization of ureido-group in the imidazolidinone ring modified the mode of protective action compared to urea derivatives. The anti-senescence effect of the bicyclic compound is mediated by greatly increased levels of carotenoids (natural antioxidants). The protected senescing tissues have developed an active oxidative defense system which may prevent the senescence syndrome.

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SUBCELLULAR EVIDENCE FOR ADAPTATION TO SALT AND LIGHT IN THE ALGA *DUNALIELLA SALINA*

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The alga *Dunaliella salina* (Volvocales, Chlorophyta) was cultivated in different conditions of salinity and light intensity. Resistance to long-term stress conditions was enhanced by the addition of exogenous sulfur-containing peptidnucleotide complex isolated from *Scenedesmus incrassatulus* R-83. The changes in fine structure of the cellular components were compared and evaluated using transmission electron microscopy. The importance of chloroplasts to provide cellular integrity *via* structural changes determining functional changes in their synthetic activity during stress conditions was demonstrated.

Ca FREE AND BOUND Ca IN PLANTS IN RELATION TO NICKEL AND CALCIUM CONTENT IN THE SUBSTRATE.

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In a three-year pot experiment the influence of different nickel and calcium concentrations in the substrate on Ca fractions in plants were determined. The examination object were made by spinach, bean, zucchini and sugar maize. The experiment was differentiated in regard to nickel and calcium content, the following were introduced into 1kg substrate: 0 (control); 10; 40 or 60 mg Ni (in form of nickel sulfate) and 250

(basic dose) or 400 mg Ca (fortified dose) (in form of calcium carbonate). In dry plant material free and bound Ca content by manganometric method as well as nickel concentration by atomic spectrophotomentric method were determined. Increasing nickel content in the substrate caused statistically significant nickel increase in all examined plant species. Intensive calcium fertilization (400 mg Ca per 1 kg of the substrate) the most of all reduced nickel content in shoots at 40 and in roots at 60 mg Ni per 1 kg of the substrate. It was found that increasing nickel dose in the substrate (10–60 mg per 1 kg) caused more intensive changes free Ca in shoots and bound Ca in roots of examined plant species. After additional calcium application there was recorded distinct increase of both calcium fractions in spinach plants and maize roots as well as their decrease in zucchini roots. Moreover statistically significant bound Ca increse in roots of zucchini, bean roots and maize shoots, bound Ca decrease in bean shoots as well as free Ca decrease in maize shoots was observed.

EFFECT OF UV-B-IRRADIATION ON GROWTH SOME STRESS MARKERS AND ENZYMES OF MAIZE SEEDLINGS

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There has been considerable concern over the reduction of stratospheric ozone leading to an increase of ultraviolet-B (UV-B) radiation causing plant damage. The aim of the study was to estimate the physiological and biochemical response of maize seedlings to UV-B irradiation. The experiments were carried out with maize seedlings grown hydroponically. 14 day old seedlings were subjected to UV-treatment.

It was found that UV-irradiation decreased fresh and dry weights of plants and damaged them, especially the first and second leaves that were subjected to UV. Despite strong injury the plants survived that stress. However, the application of UV-treatment caused an increase in the free proline content during stress and recovery period. Anthocyanin content was decreased but during recovery period it increased sharply. The only exception (at 48 h) was the lower malondialdehyde content in UV-treated seedlings compared to untreated ones. Forty min UV-irradiation increased hydrogen peroxide level but during the first days of recovery it was lower and at the end of the experimental period it was again higher than that of the control. During UV-stress the plants possessed higher catalase activity while peroxidase activity (GPOX) was opposite. During recovery period these enzymes changed the pattern of their activity. Superoxide dismutase (SOD) activity was significantly higher at the end of experimental period. Chlorophyll peroxidase was absolutely depressed during stress while

throughout period of recovery its activity was restored even in some cases exceeded that of control plants.

The conclusion is that proline and anthocyanins are UV-induced factors possessing a protective role against UV-B. The enhancement of SOD and GPOX activities also take part in alleviating UV damage.

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LOW TEMPERATURE ENHANCED PHOTOINHIBITION OR PHOTOSYNTHETIC DOWN-REGULATION IN BEAN PLANTS: ACCLIMATION AND RECOVERY

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The mechanisms of photosynthetic adaptation to different growth temperature and irradiance combinations, and especially the consequences on exposure to brief high-light treatments (2000 $\mu mol.m^{-2}.s^{-1}$ PPFD for 5 min) simulating natural sunflecks was studied in bean plants (*Phaseolus vulgaris* L.). High light at low temperature (10°C) led to significant down-regulation of photosynthetic electron transport capacity (as measured by the efficiency of PSII) with the protective acclimation to low light allowing the simulated sunflecks to be used more effectively for electron transport in low-light grown plants. The greater energy dissipation by thermal processes (revealed by lower F_v'/F_m' ratio) at low temperature was related to differences in the xanthophyll de-epoxidation state and to the fact that photosynthetic carbon fixation was more limiting at low temperatures and could not respond sufficiently to changes in irradiance. A key objective was to investigate the role of photorespiration in acclimation to irradiance and temperature by comparing the effect of normal (21%) and low (1.5%) O_2 concentrations. Low O_2 enhanced the PSII down-regulation in acclimated plants, expressed by decreasing F_v/F_m ratio, F_{PSII} and ETR, but minimised the further inhibition by the mild "sunfleck" treatment used. Our results support the hypothesis that photorespiration provides a 'safety-valve' for excess energy. There was also evidence for greater oxidative stress at low temperature/high light conditions with an increase in H_2O_2 and slight decreases in activities of catalase and peroxidase.

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WHY DO PLANTS MAKE ISOPRENE?

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Isoprene is emitted by a variety of plant species, but its role remains still unclear. Several experimental evidences indicate that isoprene may have a protective role under stressful conditions

The role of endogenous isoprene in *Phragmites australis* leaves exposed to ozone and high temperature was studied. We inhibited the synthesis of endogenous isoprene by feeding fosmidomycin and observed that *Phragmites australis* leaves became more sensitive to ozone and high temperature than those leaves forming isoprene. Photosynthesis, stomatal conductance, and fluorescence parameters were significantly affected by ozone in leaves on which isoprene was not formed. The protective effect of isoprene was more evident when the leaves were exposed for a long time (8 h) to relatively low (100 nl.l^{-1}) ozone levels than when the exposure was short and acute (3 h at 300 nl.l^{-1}). At temperatures increasing from 30 to 48°C, photosynthesis decreased and this reduction occurred both in isoprene-emitting leaves and in leaves in which isoprene was inhibited. However, isoprene inhibition was incomplete especially at elevated temperatures (38–44°C). The residual emission may have been sufficient to induce a certain thermal protection. The results obtained show that the inhibition of isoprene biosynthesis leads to an increased H_2O_2 content, a higher catalase and peroxidase activities, and an accumulation of MDA, indicating that a state of oxidative stress is induced and is likely related to membrane damage. In addition, isoprene effectively reduces the formation of H_2O_2 and MDA consequent to the ozone and thermal stresses. When the oxidation potential becomes high, such as under acute or prolonged ozone exposures, isoprene quenches ozone-dependent reactive oxygen species reducing the damage at the membrane level and probably the consequent damage at biochemical and physiological levels. Irrespective of the mechanism, our results suggest that endogenous isoprene has an important antioxidant role in plants.

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CRYOPROTECTION OF THYLAKOID MEMBRANES WITH ALTERED MEMBRANE FLUIDITY

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The stability of photosynthetic apparatus in the course of freezing of control thylakoid membrane and after alteration of their fluidity has been studied. The photochemical activity of photosysthems 1 and 2 and the energy transfer between pigment protein complexes have been determined. After incorporation of cholesterol, thylakoid membranes retained 70–90% of the photochemical activity of PS1 and this extent was dependent on the medium used for freezing. Membranes with increased fluidity, due to manipulation with benzyl alcohol, showed dramatic decrease in the activity of PS1 independently on the freezing medium. The activity of PS2 was less affected by freezing in comparison with that of PS1. Cholesterol-treated membranes are less susceptible to freezing damage whilst benzyl alcohol manipulation enhanced the damaging effects of freezing. The freezing effects on the distribution of excitation light energy between both photosystems and energy interaction of the main pigment-protein complexes were analyzed by 77K fluorescence spectroscopy. Cholesterol - treated membranes retained their fluorescent characteristics after freezing. In benzyl alcohol treated thylakoids considerable changes of fluorescent properties were observed, mainly in the pigment pool associated with PS1. The cryoprotective properties of different freezing mediums (artificial stromal medium, trehalose, glycine betaine and sodium chloride) were compared in respect to the maintaining of the activity of photosynthetic apparatus. Trehalose protects to a higher extent the activity of photosystem 1 in cholesterol treated membranes while glycine betaine is the better protector for control membranes. These mediums do not exhibit protective effect on the activities of both photosystems in benzyl alcohol treated membranes.

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ALDEHYDE OXIDASE AND XANTHINE DEHYDROGENASE ACTIVITIES IN WHEAT PLANTS – EFFECT OF COLD STRESS

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Aldehyde oxidase (AO, EC 1.2.3.1) and xanthine dehydrogenase (XDH, EC 1.1.1.204) are Mo-cofactor containing enzymes and here were studied in connection with the increased frost tolerance of plants grown on acid soil after Mo application. AO and XDH proteins in leaves, roots and seeds were investigated. Plants were grown on soil with pH 5 and part of them were treated with Mo. One protein band for AO in leaves, 3 for seeds and minim 3 bands for roots, capable of oxidizing indole-3-aldehyde and heptaldehyde were proved. Only one protein band for XDH was detected in leaves,

roots and seeds. No difference in substrate specificity and mobility in native PAGE in two cultivars differing in their frost resistance (Sadovo 1 – sustainable and San Pastore – sensitive) was found. Mo-treatment caused strong increase in the activities of these two enzymes. The differences in the activities of leaf AO were greater in the light than in the dark. No effect of transition of the temperature 20°C→2°C (7-days cold acclimation, CA) and freezing (–7°C, 24 h) of plants was established. Very clear increase in AO activity during recovery of Mo-treated plants was observed 5 days after the stress action compared with non-stressed ones. ABA treatment of San Pastore during CA had clear cryoprotective effect.The results are discussed in connection with the key regulatory role of ABA, last step of which biosynthesis is catalysed by AO containing sulfurylated form of MoCo.

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ANTIOXIDATIVE ENZIMES IN BARLEY SEEDLINGS SUBJECTED TO ROOT HYPOXIA

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Roots of barley plants (*Hordeum vulgare L., cv. Alfa*) were flooded with tap water 25 mm above level of the soil surface. Other plants remained well watered (60% soil moisture) as controls. Samples were taken 72, 96 and 120 hours after the start of treatment.

Changes in components related to the antioxidative system of barley seedlings subjected to root oxygen deficiency have been studied. These include: superoxide dismutase (SOD), catalase (CAT) and peroxidase activities (POD). We also investigated the two major enzymes involved in ascorbate-glutathione cycle operating in chloroplasts – APX (ascorbate peroxidase) and GR (glutathione reductase). A progresive decrease in the activity of SOD was found (up to 75% of the controls) depending on the duration of flooding. Observed decrease in total SOD activity was mainly due to decreasing of chloroplasts Fe-SOD. On the course of treatment the activity of POD remarkably increased and total activity at 120 h after start of flooding was 2 fold higher than the control. The same trend was found for the catalase activity too.

GR activity was influenced differently over the course of the treatment. It was slightly increased at 72nd hour and declined at 96th and 120th. Both total and thylakoid bound APX activity increased during hypoxia.

It is suggested that root oxygen deficiency caused photoxidative damage to barley leaves via an increased generation of active oxygen species. Possible reasons for the response of antioxidant system to hypoxia will be discussed.