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Cobalt effects on root nodulation, nutrients content and yield of faba bean (*Vicia faba* L.)

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Introduction: It has been known that cobalt is an essential element for humans, animals and microorganisms. The essentiality of Co in plants has not been recognized, although it is needed by rhizobia in root nodules of leguminous plants. The mechanisms by which Co affects plants are not yet clearly known, although it is known, that Co inhibits many physiological processes, such as photosynthesis, seed germination, plant growth and yield (Walsh 1995, Liu *et al*. 2000). The present investigation was to study the influence of rates from Co on nodulation, nutrients content and yield of faba bean (*Vicia faba* L.) cv. Giza 461.

Materials and Methods: In a preliminary experiment we studied different rates of Co addition to faba bean plants. We found that over 20 PPM cobalt inhibited growth of faba bean. Based on our preliminary experiment we used 5 rates of Co. The rates of Co addition were 0, 5, 10, 15 and 20 PPM at 14-days old at nursery. The seeds of faba bean cv. Giza 461 were sown in nursery. One week later faba bean plants were transplanted to the field in two successive seasons 2004/05 and 2005/06. The plants of faba bean were grown in sandy loam soil at Nubarai Experimental farm, National Research Centre, Egypt. At flowering, one sample was collected for morphological, physiological and biochemical investigation. The total N concentration in plants was estimated by macro Kjeldahl method.

Results: Co addition increased plant height, stem diameter, branch number, leaf number/plant and leaf area/plant and the increase was in parallel with increasing Co rates. Co increased significantly ($p < 0.05$) root nodule number and nodule dry weight/plant. Root, shoot and total biomass and their TN (%), and TN (mg/plant) of sample at flowering stage were significantly increased with increasing Co rates. Co addition resulted in increasing nutrients content (P & K(%), and Co, Fe, Mn, Zn and Cu (PPM)) of roots and shoots of plants at flowering, Co addition increased significantly ($p < 0.05$) total biomass, seed yield and seed content of nutrient (P,K, Fe, Mn, Zn, & Cu) at harvest. In addition, content of seeds from TSS %, total crude protein% at harvest was significantly ($p < 0.05$) increased due to application of cobalt.

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Influence of nickel stress on ammonium assimilating enzymes in wheat seedlings

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Introduction: Nickel, although considered to be an essential micronutrient, at excess concentrations becomes toxic for most plant species. Mechanisms on Ni phytotoxicity are not fully understood; however they seem to result mostly from the alternations of the primary plant metabolism induced by this heavy metal. The purpose of the present work was to study the influence of Ni on nitrogen metabolism in wheat shoots, particularly on the activities of enzymes participating in ammonium assimilation.

Methods: Wheat plants (*Triticum aestivum* L.) cv. Zyta were grown hydroponically in the nutrient solution containing 0 (control), 50 and 100 μM Ni. After 1, 4 and 7 days the shoots were harvested and growth parameters, Ni content, activities of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) were estimated. The activity of GS was determined by a biosynthetic assay based on glutamylhydroxamate synthesis. The activity of GDH was measured with NADH (NADH-GDH aminating activity) and NAD (NAD-GDH deaminating activity).

Results: Treatment of wheat seedlings with Ni caused a significant decrease in length and fresh weight of shoots. The activity of GS did not change in response to Ni application. However, both aminating and deaminating GDH

activities were significantly influenced by Ni stress. The activity of NADH-GDH showed an increase at the end of experiment and 7 days after Ni treatment it was 68 % and 76 % higher than in the control, at 50 and 100 μM Ni, respectively. On the contrary, NAD-GDH activity after 1 and 4 days of exposure to the higher concentration of Ni was reduced by 24 % and 37 %, respectively. On the 7th day the activity of this enzyme was enhanced by 68 % and 76 % over the control level, at 50 and 100 μM Ni, respectively.

Conclusions: Exposure of wheat seedlings to Ni considerably alters the processes mediated by GDH. It can be suggested that increase in the aminating GDH activity may support the biosynthesis rate of glutamate required for production of glutathione which has been shown to play an important role in tolerance of plants to Ni. In addition, it may participate in detoxification of ammonium, which has been found to accumulate in plant tissues under stress conditions.

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The morphological, biophysical and biochemical changes in the salinity-stressed tomato plant roots

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Introduction: Salinity causes disturbances in the water balance, photosynthesis and plant growth (Romero-Aranda *et al*. 2001). Plant salt tolerance might be the result of the increase in water use efficiency achieved by the development of morphological, anatomical and physiological modifications (Delphine *et al*. 1998, Romero-Aranda *et al*. 2001) including the L-proline accumulation (de Lacerda *et al*. 2005).

Methods: 5-week-old tomato (*Lycopersicon esculentum* cv. *Perkoz*) roots were harvested 1, 3 hours and 1-14 days after the application of 50 or 150 mM NaCl. The fresh (FW), dry weight (DW) and water content (WC) were estimated according to the methods described by Munne-Bosch and Alegre (2002). The L-proline concentration was assayed according to the method of Bates *et al*. (1973).

Results:. Only the severe salt stress lowered FW and DW during the whole experiment. 150 mM NaCl-treatment reduced the intensity of lateral root growth and caused root browning since 2nd day of the experiment. During the following days those roots degenerated. At the beginning and end (7-14 days) of the experiment both solutions of NaCl diminished WC, however on 2nd and 5th day only the higher NaCl-treatment slightly decreased it. The salinity induced gradual increase in L-proline content, especially in the severe-stressed roots.

Conclusions: The obtained data suggest that even mild salinity causes changes in the water balance, but only severe stress impairs root growth. Higher L-proline content during salt stress, not directly accompanying lower root hydration, suggests others roles of this compound in tomato roots than osmoprotection.

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ABA level in *Arabidopsis thaliana* L. plants as affected by heavy metals

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Introduction: Heavy metals (HMs), if in higher concentrations, are a source of abiotic stress for plants and can create a serious threat to human health. Abscisic acid (ABA) plays an important role in plant responses to various environmental stresses, especially to those leading to tissue dehydration. In this work we aimed to check whether level of ABA in *Arabidopsis thaliana* L. plants changes also due to exposure to HMs.

Materials and methods: Plant of *Arabidopsis thaliana* L., Columbia 4 were grown in continuously aerated hydroponics culture with Hoagland's nutrient solutions, renewed weekly. Five-week-old plants were exposed during 14 days to HMs added at nutrient renewal. The plants were treated with Pb^{2+} (0, 120, 240, 360, 480 μM), Cu^{2+} (0, 50, 100 μM) and Cd^{2+} (0, 25, 50 μM). At harvest plants were divided into rosette and roots, sub-samples for ABA determination were collected and kept in -80 ˚C till analysis and accumulated biomass was recorded. After freeze-drying, plant material was ground in ball mill and cold distilled water was added. The samples were heated for three minutes in boiling water and then shaken overnight at 4 ˚C. On the following day the extracts were centrifuged for 20 minutes in cool centrifuge at 18 000 *g*. ABA determination was done in the supernatant by an indirect ELISA using the antibodies MAC 252.

Results: Arabidopsis plants were negatively affected by all three used in this study HMs except of Pb^{+2} in lower concentration (120 μM), at which stimulatory effect on plant vigour – phenomenon known as hormesis was observed. At higher concentrations effect of lead was also harmful though to less degree that treated with cadmium and copper. Plants treated with lead at toxic concentrations and with Cd^{2+} and Cu^{2+} were delayed in development, had smaller rosettes and accumulated less biomass. Cadmium and copper treated plants exhibited cholorosis, necrotic spots and lesions followed by leaves decay.
ABA concentration in roots, shortly after exposure to HMs increased in plants treated with Cu^{2+} and Cd^{2+} at 25 μM, while in plants treated with Pb^{+2} it was lower than in control. After 14d of plants exposure to HMs ABA concentration in roots, was lower except Cd^{2+} at 25 μM. ABA concentration in leaves of plants treated, with all HMs, within 3h of exposure decreased but in longer exposure (14d) it was higher than in control plants.

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Early-season defoliation of *Sorbus aucuparia* and *Acer platanoides* induce resistance to spider mite (*Tetranychus* sp.)

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Introduction: Simulated defoliation is often used in studies of induced defense reactions of plants to grazing. However, very little is known about the role of various external factors that may determine the nature of inducible defenses. Here we tested whether light levels under which plants grow could affect the natural invasion of *Tetranychus* sp. a polyphagous, parenchyma cell feeding spider mite with a broad range of host plants. Depending on their degree of shade tolerance, plants may express different morphological, physiological and defense traits under low light conditions. Therefore we used two host species that differ widely in shade tolerance for this study: shade-adapted *Acer platanoides* and sun-adapted *Sorbus aucuparia*. The purpose of this study was to explore the pattern of spider mite invasion and to test whether light conditions and the host species differences in nutritive constituents, secondary metabolites and leaf structure play a role in invasion success.

Methods: One-year-old Norway maple (*Acer platanoides* L.) and European mountain ash (*Sorbus aucuparia* L.) seedlings were grown in pots with mixed forest soil and peat. At the beginning of growing season (April 2006) seedlings were randomly divided into two groups. The first group was placed into two shade houses covered with polypropylene shade cloth that produced a treatment with 5 % light transmittance. The second group of seedlings was placed in two blocks with full sunlight. The defoliation was made by manual removal of 0 (control), 25, 50, 75 and 100 % of leaves.

Results: Spider mite (*Tetranychus* sp.) invasion took place in mid-July and was observed only on seedlings growing in the shade. Mites were reared on leaves of almost all seedlings, but intensity of feeding was related to the level of earlier defoliation. Control and lightly defoliated seedlings were most affected by spider mites. Mite feeding behaviour was directly linked to changes in concentration of leaf nutrients and secondary metabolites. Carbon content was correlated with the percent of damaged seedlings in both species, R^2= 0.80; $p<0.05$ for maple and R^2= 0.86; $p<0.05$ for ash. The concentration of phenolic compounds varied with the level of defoliation; the highest concentrations were observed in leaves from seedlings with 50 % and 75 % prior defoliation.

Conclusion: Spider mites prefer the leaves of plants grown in a shade environment with low content of phenolic compounds and high content of carbon. Both plant species were able to survive early season leaf damage inflicted by spider mite (*Tetranychus* sp.). Inducible secondary metabolites (soluble phenolics) were among factors contributing to low spider mite success in plants recovering from spring mechanical defoliation.

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Effects of the ATH-rich extract on lead absorption and localization in *Allium cepa* L. roots

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Introduction: Our earlier studies revealed the role of the ATH-rich extract from red cabbage leaves in the protection of the meristematic cells of onion roots against mitodepressive and turbogenic effects of Cd, Cr and Pb (Glińska *et al*. 2007). It would be interesting to examine to what extent that effect was caused by changes in the metal absorption.

Materials and methods: The two-day old adventitious roots of *Allium cepa* L. (cv. Polanowska) were treated with the aqueous solution of $Pb(N0_3)_2$ at the concentration of 100 μM with or without preliminary incubation in the extract from *Brassica oleracea* L. *var. capitata rubra* leaves (250 μM, 3 h) or with the mixture of these solutions for 2 hours. The roots kept in distilled water were the control. Lead localisation in whole roots and in meristematic cells was determined as described earlier (Glińska and Gabara 2002).

Results: The ATH-rich extract from red cabbage leaves added to the lead solution suppressed metal accumulation in onion roots as was shown by the rhodizonian method. Moreover, lead absorption was slightly lowered after root preincubation in the ATH-rich extract as compare to the not pre-treated ones.
Ultrastructural studies revealed that root preincubation in the ATH-rich extract diminished the number of meristematic cells with lead deposits in vacuoles, cytoplasm, mitochondria, plastids, Golgi apparatus and nucleus. Moreover, the number of lead deposits in those cell compartments was lower. On the other hand, although the lead deposits were less numerous in cell walls, the number of cells with the lead deposits in cell wall has not decreased. Contrary, in ER the lead deposits were visible only in the cells of not pre-treated roots.

Conclusions: The ATH-rich extract limited lead absorption by roots probably due to metal chelatation in the incubation medium. However, also in roots preincubated in that extract the number of lead deposits decreased mainly in a cell symplast.

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Changes in the properties of membranes and lipoxygenase activity in the roots of *Pisum sativum* L. in response to the action of hydroxycinnamic acids

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Introduction: Hydroxycinnamic acids are allelopathic compounds evoking biochemico-physiological changes. Their action mechanism has not been fully explained so far. The work objective was to investigate whether in pea roots there occur any changes in the properties of membranes and lipoxygenase induction under the influence of ferulic and p-coumaric acids.

Methods: The objects of studies were 7-day old 'Bohun' pea seedlings subject to allelochemical stress by treating their roots with hydroxycinnamic acids (ferulic and p-coumaric ones). The control consisted of roots not treated with the acids. Experiment was carried out in dynamic design. In the period of 4 hours after stress introduction, in the roots of seedlings, the degree of cell membranes injury was examined basing on the leakage of electrolytes (in acid concentration range from 0.1 to 1.0 mM) and the peroxidation of lipids (at 1.0 mM acid concentration). Furthermore, during the first 90 minutes after stress introduction, in roots, the activity of lipoxygenase was determined in cytosolic and membrane-bound fraction (at 1.0 mM acid concentration).

Results: In the roots of seedlings subject to stress, an increase of cell membrane permeability was found proportionally to the concentration of the applied hydroxycinnamic acids. This effect increased with the lapse of time. Under the influence of ferulic and p-coumaric acids, it was also found that in pea roots, there increased the degree of lipids peroxidation and the lipoxygenase activity of cytosolic and membrane-bound fractions.

Conclusions: Obtained results indicate that under the effect of hydroxycinnamic acids in pea roots, there comes to lipids peroxidation causing in effect changes in membranes permeability which partially can also be the result of increased activity of lipoxygenase which has been found in this work.

Chlorophyll fluorescence response of corn seedlings to lead stress

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Introduction: All environmental factors, such as excessive irradiance, water or heavy metal stress either directly or indirectly affect the photosynthetic function of leaves, the pigment composition and the ultra-structure of the photosynthetic apparatus. Early stress detection in plants, before visual damage symptoms are noticeable, is required in order to reactivate the plant's vitality by suitable countermeasures. Today the non-destructive fluorescence methods are the most promising techniques for early stress detection in plants (Lichtenthaler *et al*. 2005).

Methods: Corn (*Zea mays* L. cv. Theresa H) seeds were germinated for 7 days at Petri dishes containing different concentrations of Pb^{2+} (50, 300, 500, 1000 and 1500 mg Pb/l solution). Lead was provided as lead nitrate $Pb(NO_3)_2$. Petri dishes were placed in the growth chamber and kept under constant conditions (20 °C, 60-70 % humidity, PPFD 800 μmol m^{-2} s^{-1} during 12/12 h. day/night period). Chlorophyll fluorescence measurements were realised by GFPCam kinetic fluorescence camera (PSI, Czech republic) according to Trtílek (1997). We determined minimal fluorescence (F_0), maximal fluorescence (Fm) and maximum quantum yield efficiency (Fv/Fm) by fluorescence method. Results were analysed by ANOVA statistical method (SigmaStat programme).

Results: Statistical analyze confirmed significant influence of lead on minimal fluorescence (F_0) of corn seedlings between F_0 of control plants and all lead treatments. Treated plants had higher F_0 values than control plants. Although without insignificant differences between Fm of control and treated plants (with exception of 1500 mg Pb treatment with significant difference) lead application induced Fm increasing. The maximum quantum yield efficiency (Fv/Fm) decreased with increasing lead concentration, significant differences between Fv/Fm of control and treated plants were confirmed by statistical analyse. Average values of Fv/Fm lead treated plants were lower from 15.6 % to 20.4 % with increasing lead concentration comparing with FV/Fm of control plants.

Conclusions: We dealt with lead influence on selected chlorophyll fluorescence parameters of corn seedlings by fluorescence spectrophotometry method. Lead had significant influence on minimal, maximal fluorescence and

also on maximum quantum yield efficiency. We observed also impact of lead on decrease of photochemical effectiveness of photosystem PSII.

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Possibile factors of increased resistance of triticale (x*Triticosecale* Wittm.) to pink snow mould (*Microdochium nivale*) infection

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According to the regular reports from Europe, *Microdochium nivale* is the most widespread snow mould fungus. It is the most virulent at 0-2 °C, due to its cold-active enzymes. Among other, it attacks winter triticale, new cereal with growing economic importance therefore, quality and quantity of its yield is regularly under the threat of *M. nivale* infection. The resistance of the best triticale cultivars is far from being satisfactory. No molecular analyses of triticale response to *M. nivale* have been done so far and understanding of the infection process in all cereals is poor. Thus, there is a need to perform studies providing better insight into host-pathogen interactions in order to elaborate efficient breeding strategies towards resistance against pink snow mold. Seven winter triticale cultivars were subjected to an artificial infection with *M. nivale* mycelium in „cold chamber method". After initial screening, two resistant and two susceptible cultivars were selected for further studies. Cold-hardening, efficiency of photosystems I and II, and activity of antioxidative enzymes was studied in plants as possible factors influencing plant resistance to fungus attack.

Toxicity of mixture herbicides MCPA and chloridazone in Lemna test

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Introduction: MCPA (auxin-like growth inhibitor) and chloridazon (photosystem II inhibitor) are extensively used in European agriculture. These substances may enter freshwater ecosystems by leaching or runoff and may cause potential risk for aquatic biota. However, the data on the toxicity MCPA and chloridazon on water plants are scare and no investigations of their mixtures. Since aquatic organisms are usually exposed to many substances at the same time, the assessment of their possible interactions (antagonism, synergism) are necessary. *Lemna minor* (L.) is regularly used for ecotoxicological tests. It's free floating organism, typical of fresh surface waters around the globe. The purpose of this study was to determine the toxicity of MCPA and chloridazon alone, and in combination, on the growth of *Lemna minor* St..

Methods: The experiments were performed according to International Standard (ISO 200079:2005(E)). Toxicity assessment was based on inhibition of growth, fresh weight and dry weight after 7 days. In the first step EC_{10} (inhibition growth by 10 %) and EC_{50} (inhibition growth by 50 %) values for MCPA and chloridazon were evaluated separately. Then the influence of binary mixtures (EC_{10} and EC_{50}) on *L. minor* was characterized. Statistical calculation were based on the variation analysis (ANOVA) and Abott's formula (Teisseire *et al*. 1999).

Results: Values of EC_{10} and EC_{50} were 0.8 and 5.4 mg/dm^3 for MCPA and 0.7 and 10.4 mg/dm^3 for chloridazon, respectively. When chloridazon was applied at EC_{50} value, antagonistic interaction between MCPA and chloridazon was observed, irrespective of MCPA concentration. However, antagonism, synergism and as well as addition revealed all other binary mixtures of tested substances.

Conclusions: The comparison of EC_{50} values indicates that chloridazon was less toxic to duckweed than MCPA. Both herbicides showed interaction. Chloridazon reduced inhibitory effect of MCPA on growth of Lemna minor.

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Effect of combined drought and heat stress on heat shock proteins in wheat varieties

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Introduction: The response of crop plants exposed on drought or heat shock is related to decrease in the synthesis of normal proteins, accompanied by increased translation of heat shock proteins (HSPs). Though drought and heat stress have been studied individually, little is know about their combined effect on plants.

Methods: The wheat (*Triticum aestivum* L.) varieties (Katya- tolerant, Sadovo or Mladka-susceptible) were potted in soil. Eight-day-old plants were exposed to withdrawing water for seven days. Heat shock was realized in growth chamber at 40 °C for 6h. A combination of drought and heat shock was performed by subjecting drought-stressed plants to heat shock treatment. Expression of HSPs in the first leaf of wheat varieties was analyzed by SDS electrophoresis and immunoblotting. Polyclonal antibodies against HSP20, HSP60, HSP110 and mononclonal antibodies against HSP70 were used to distinguish the mentioned HSPs.

Results: The leaf relative water content (RWC), which indicated the level of plant dehydration decreased significantly (34 %) under drought stressed conditions The electrolyte leakage of ions (EL), representing the level of the cell membrane stability increased markedly (68 %), especially under combination of drought and heat. Maximum EL was observed in drought susceptible varieties Sadovo and Mladka. Drought and heat shock combination in the wheat plants resulted in the induction of specific HSPs.

Conclusions: Our results demonstrate that the response of the wheat plants to a combination of drought and heat stress is different from the response of plants to each of these stresses applied separately. Induction of synergetic effect on HSP expression in case of combination between drought and heat was discussed in the case of two contrasting wheat varieties.

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The effect of low or high soil water content on the root system architecture of triticale and maize seedling

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Introduction: Studies on the qualitative and quantitative changes of the plant root system features of plants exposed to different moisture conditions are infrequent. Cereal plants develop two types of the root system: the scattered and the concentrated, and two types of 1st order lateral roots. The aim of this experiment was to study the effect of low (drought) and high (waterlogging) soil water content on the number, length and dry matter of the particular components of the root system.

Methods: Plants were grown in root-boxes and the pin board set was used. Treatments: C - (control) - soil water content was 65 % FWC by 28 days, D - (drought) - soil moisture was maintained at 35 % FWC from 7 to 28 day after sowing and W - (waterlogging) soil flooding was established by paddy condition of soil from 7 to 28 day after sowing

Results: Drought caused higher decrease in number and length of each component in developed root system and entire root dry mass in comparison to waterlogging. Comparing to the control, in case of waterlogging, the increase in number and length lateral and seminal-adventitious roots was observed. The drought condition as well as waterlogging slightly influenced the ratio of dry weight of above-ground part to root dry matter (S/R), especially for drought resistant genotypes no significant differences comparing to control were observed. Harmful effects of drought and waterlogging on the root dry matter, S/R ratio and number and length of seminal, seminal adventitious, lateral and nodal roots was higher for genotypes included to group of drought sensitive comparing to the resistant to drought. Results confirmed that drought sensitive genotypes of triticale and maize were also more sensitive to the soil flooding. The observed modification of the internal root morphology caused by water deficit in plant tissues may partly influence on water transport within roots. The drought resistant maize and triticale genotypes developed more extensive root system and stress-induced changes in root morphology were less pronounced than in drought-sensitive genotypes. Results confirmed that drought-susceptible genotypes were also more sensitive to temporary soil flooding. Tolerance to unfavorable soil conditions was, presumably, bestowed by a more efficient water use and a lower shoot-to-root ratio in the resistant genotypes. The different responses of triticale and maize genotypes to soil drying or waterlogging may be due to a more economical water balance and better relations between the shoot and root dimensions in the drought-resistant genotypes. The results suggest that the morphological traits of the plant root system may be useful selection criteria in the breeding. (The impact of drought or waterlogging on morphological features of developed root system is shown in table and photographs).

Conclusions: Among all plant organs root is the organ that is directly influenced by different water conditions in soil. Nodal and laterals were most sensitive to stresses than the seminal and seminal adventitious roots. The drought sensitive genotypes were more sensitive to the soil flooding**.** Precise comprehension of various aspects of plant growth under stress conditions should encourage the improvement of the technology for crop production management under adverse field conditions and contribute to the progress of breeding program aiming to improve the stress tolerance of cereals crop

Effect of different soil compaction on growth and root architecture in maize and triticale seedlings

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Introduction: Plant roots are strongly modified by the soil-environmental physical factors. A root extending through soil may force aside overcoming the mechanical impedance, or may be diverted to move around the particles, if the impedance is too great and if the space allows it (Yamauchi 1993, Iijima *et al.* 1994, Masle 2002). Soil mechanical impedance is caused by various factors such as the use of heavy machinery combined with more intensive tilling and also by natural processes; untimely cultivation with poor timing of field operation has led to reduced soil aggregate stability.

Methods: Plants were grown in root boxes, which enabled non-destructive isolation of all compartments of the root system and the pin board set was used. The effect of different soil compaction level (1.30, 1.47 and 1.58 g/cm^3) on a triticale and maize seedlings shoot and root dry matter, leaf number and area, number and length of particular components of the root system, leaf water potential (ψ), maximum quantum yield of PS II (Fv/Fm) and

gas exchange (Pn, E, g_S, C_i) parameters was examined.

Results: After 5 weeks of growth in conditions of higher soil compaction in comparison to the control treatment, decrease in leaf number, leaf area, dry matter of shoot and roots, and increase in shoot to root (S/R) dry matter ratio were observed. For both examined species high level of soil compaction strongly affected the length of seminal and seminal adventitious roots and also number and length of lateral roots developed on the seminal root. Similarly as in case of growth characters in triticale, comparing to maize, slightly weaker impact of soil impedance on number and length of particular components of developed root system was observed. The changes in number and length of particular components of the root system under high soil impedance were accompanied by changes in leaf water potential (ψ), maximum quantum yield of PS II (Fv/Fm) and as well as by gas exchange parameters (Pn, E, g_S, C_i). Similarly as in case of shoot and root growth characters for these parameters, more adverse was impact of the high soil compaction for maize than triticale. In high soil compaction the values of ψ, Fv/Fm, and Pn, E and g_S were lower comparing to the control. Distinct differences in response to soil compaction stress between triticale and maize were observed in internal concentration of CO_2 (C_i) *i.e.* increase for triticale and decrease for maize under high soil impedance in comparison to the control.

Conclusions: Better understanding of plant growth under high soil compaction stress should encourage the improvement of the technology for crop production management under adverse field conditions and contribute to the progress of breeding program aiming to improve the stress tolerance of crop plants.

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Application of sewage sludge to improve of soil quality by make use of model plant energy

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Introduction: Stockyards of sewage are one of the civilization problems, which result from purifying processes of industrial and sanitary sewage. Now sewage can be used as an ecological fertilizer for energy plant cultivation and as biofuel in heating production. It can be also used to improve wastelands. This research shows the influence of sewage on changes of ground quality on the basis of growth and development of plant.

Methods: Different concentration of stabilized sewage added to two types of soil and two kind of plants: *Salix vinimalis* and *Sida hermaphrodita* were used. Seeds of *Sida hermaphrodita* and seedlings of *Salix vinimalis* were used in each variants. *Sida hermaphrodita* and *Salix vinimalis* were cultivated for 14 and 5 weeks, respectively. Than *Salix vinimalis* was subjected drought stress and the influence of sewage on wetness of ground was tested. Enzymatic activities of RN-ase and dehydrogenises as well as chlorophyll (a+ b) content were measured in the tissues of *Sida hermaphrodita* leaves. The control was ground without sewage.

Results: Changes in the intensity of growth, which were induced by different concentrations of sewage were observed. Both stimulation and inhibition were notes. In the case of *Salix vinimalis* wetness was directly proportional to the sewage concentration.

Conclusions: The results show that appropriately selected concentrations of sewage beneficially influence growth of plants by increasing the content of soil nutrients. The use of sewage may contribute to increasing plant yield on poor soils. Sewage may improve ground wetness, so it can be used on dry soils (for example: lower content of ground water). This sewage may cause the reduction of degraded and waste areas. Recycling and elimination of slag heaps may increase the scenic and touristic advantages.

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Application of *Cyanobacteria* in ornamental plants cultivation on the basis of China aster (*Callistephus chinensis*)

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Introduction: Ornamental plants are mainly annual and biennial plants. Invention of a simple and not expensive method of fertilizing their ground seems to be beneficial. Supplying them with nitrogen as the building element, conditioning plant growth and development is of particular importance. Of notion might be the application of various *Cyanobacteria* species with the ability to bind molecular nitrogen.

Methods: The selected seeds of China aster (*Callistephus chinensis*), common annual plant, were used in the studies. The seeds were sowed on filter paper rings placed on Petri dishes and in the metal moulds filled with standard garden soil. All seeds were treated with suspensions containing one of the following algae: *Anabaena* sp (Treboń), *Anabaena variabilis, Oscillatoria, Microcystis aeruginosa* (Treboń). Additionally, the mixture of all specified *Cyanobacteria* species was applied as the MIX variant. The seeds cultivated without the algal suspensions, supplied only with water were the control. Observations were carried out within the period of four days, every 24 hours. The seed germination dynamics was measured every day and fresh plant matter was taken in order to evaluate putative enzymatic activity alterations as well as the chlorophyll (a+b) content. Three key enzymes were used as markers: ribonuclease, alkaline phosphatase, and acid phosphatase. In the experiment with soil the lengths of seedlings were measured during 10 following days.

Results: Our experiment shows positive effect of *Cyanobacteria* cell suspension on China aster (*Callistephus chinensis*) seed germination and seedling growth, comparing to the control. In the tissue of seedlings treated with *Cyanobacteria*, increased ribonuclease, alkaline phospahatse, and acid phosphatase enzymatic activities as well as alterations in photosynthetic pigment (chlorophyll a+b) content were shown.

Conclusions: Application of *Anabaena* sp (Treboń), *Anabaena variabilis, Oscillatoria, Microcystis aeruginosa* (Treboń) in the form of aqueous cell suspension to soil significantly increase of seed germination dynamics and seedling growth in higher plants. This contributed to production, intensification and to reduction of the costs. It also provided possibility of using the unwelcome, seasonal cyanobacterial blooming as an ecological fertilizer.

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Adaptive changes of the content of phytohormones *Persicaria amphibia* (L.) Delarbre in different conditions of growth

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Introduction: The reaction of plants on the changes of the environmental factors mediated by phytohormones (Yurekli *et al*. 2004). Nowadays the hormonal control of growth and development of plants that are able to grow under natural adverse conditions for some time remains almost unexplored (Vedenicheva *et al*. 2004). That is why

the aim of our researches was the study of the quantitative changes of phytohormones in *Persicaria amphibia* (L.) Delarbre – plant species that are characterized by high degree of adaptation to the water-level fluctuation.

Methods: Separation and analysis of phytohormones (ABA, GA and ethylene) were made according to the methods (Methodical recommendations to the definition of the phytohormones, 1988).

Results: The researches show that in the beginning of the vegetation in the young organs of water forms of P. amphibia free ABA prevailed, comparing to the ground forms, while at the circumstances of the moderate water deficiency in all investigated organs – conjugated. In inflorescence water deficiency results in rising ABA in internodes and flowers, and declining activity in excretion of the ethylene in investigated organs. The GA activity in the beginning of the vegetation was lesser in organs of the ground forms than in water forms, while in the inflorescence vice-versa.

Conclusions: The above mentioned results of the researches allow to assume the participation of ABA, ethylene and GA in adaptive reactions of P. amphibia.

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Changes in total phenolics, ferulic and cinnamic acids content and PAL activity in seedlings of winter oilseed rape inoculated with *Phoma lingam* picnospores

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Introduction: Stem canker is a damaging disease of oilseed rape worldwide and it is also one of the most important diseases of this crop in Poland. The disease is caused by two pathogens: *Leptosphaeria maculans* (Desm.) Ces. Et de Not (vegetative form *Phoma lingam*) and *L. biglobosa* nov. Shoemaker and Brun, which are regarded as aggressive and nonaggressive. The aim of the present work was to study whether some phenylpropanoid compounds are involved in plant responses to pathogenesis

Materials and methods: The experiment was performed on seedlings of winter oilseed rape cultivar Lisek. Plants were grown at 16 °C for 10 days in 12h-photoperiod. Next plants were artificially inoculated with picnospores *Phoma lingam*. The phenolic content (Singleton and Rossi 1965), phenylalanine ammonia-lyase activity (PAL) (Peltonen and Karjalainen 1995), the level ferulic acid and cinnamic acid in seedlings was analyzed 24, 48 and 72 hours after inoculation.

Results: No statistically significant differences were found in the total pool of phenolic compounds during subsequent hours of pathogenesis. The level of phenolics was lower than those observed in control plants. Inoculation with picnospores *Phoma lingam* induced increase in the activity of PAL, the key enzyme in the synthesis of phenylopropanoids, however statistically significant differences between infected and control plants were noted 48 and 72 hours after inoculation. The measurement exhibited higher content of cinnamic than ferulic acid in shoots. The general increase in ferulic acid content during pathogenesis was observed, however differences were statistically significant only 48h after inoculation. Compared to the control cinnamic acid content increased initially (24h) and then decreased (48 and 72h) to the level measured in control plants.

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The 24-epibrassinolide in soybean cultivation

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Introduction: Soybean is an important global crop grown for oil and protein, however is not commonly cultivated in Poland, because badly tolerates cold and drought stress. BR27 is one of brassinosteroids – regulators which can protect plants against stressors (Krishna 2003). The influence of 24-epibrassinolide (BR27) on quality and quantity of soybean yield in field condition was studied.

Material and methods: Soybean was cultured in the field (season 2006, considered as a drought). BR27 was applied by soaking of seeds, spraying and drenching plants. Yield of seeds and chemical meal composition (fatty acids, sterols, soluble proteins, macroelements, phytoestrogens, brassinosteroids) were estimated. Additionally laboratory tests of activity of BR27 in soybean plants growing in conditions of osmotic stress (caused by PEG) have been made.

Results: Increase in yielding of soybean was found in plants sprayed with BR27 and in plants of which the seeds were soaked in BR27 solution before sowing (27 % and 43 % respectively). The chemical composition of seeds, content of BR27 after application as well as some physiological bases of BR27 activity especially in drought condition will be discussed.

Discussion: Brassinosteroids as "antistress protectants" may be used in agriculture to minimize stress effect especially in susceptible varieties as soybean. In conditions of drought prevents of yield decrease.

Conclusions: The 24-epibrassinolide modifies the yield and seed composition of soybean.

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Quantity and quality of grain yield of wheat cultivated in season 2006 – impact of 24-epibrassinolide

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Introduction: Brassinosteroids are steroidal regulators which stimulate plant yielding and protect plants against stressors. The aim of work is study the modification of quantity and quality of grain of wheat after application of 24-epibrassinolide (BR27) in field condition.

Material and methods: Spring wheat was cultured in the field in vegetation season 2006. BR27 was applied by soaking of seeds, spraying and drenching plants. Analyses of brassinosteroids in plants after application of BR27 were done using HPLC/MS (Swaczynová *et al*. 2007). Grain yield per plant and meal quality (starch, fatty acids, soluble proteins and makroelements) were estimated.

Results: BR27 stimulated yielding in cultivar with lower potential of crop production mainly by increasing percent of tillers with ears per plant and number of grains per plant. The chemical composition of grains, residues of BR27 after application as well as some physiological bases of BR27 activity especially in drought condition will be discussed.

Disscusion: Vegetation season 2006 in Poland has been considered as a natural drought. The present studies may help to answer the question about antistress protection of 24-epibrassinolide in natural drought condition.

Conclusions: The 24-epibrassinolide stimulates the yield of wheat and modifies component of grains.

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The effect of low temperature on the plasma membrane oxidoreductase activity in cucumber seedlings

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Introduction: Low temperature is one of the most important environmental factors limiting the growth of the plants. To respond to cold stress, plants must perceive low temperature signals and transduce them into biochemical responses. The primary site of low temperature injury is plasma membrane and cell membranes are directly involved in cold acclimation.. Our earlier investigations suggested the crucial role of plasma membrane H+-ATPase for plant survival of the cold stress. The activity of this proton pump directly depends on the activity of oxidoreductase, ananother important plasma membrane associated enzyme . This enzyme generate the transplasma membrane electron transport and activates the plasma membrane H^+-ATPase. In the present study we have examined the effect of low temperature on the plasma membrane oxidoreductase activity in cucumber seedlings.

Material and Methods: Cucumber seedlings were grown for two days at control conditions (25 ˚C during the day and 22 ˚C during the night). After that part of plant plants were transferred to the 10 ˚C for 3 or 6 days. Additional set of plants were after 3 days of growth at 10 ˚C were placed in control conditions for another 3 days (acclimated plants). Roots of plants were used to isolation of highly purified, inside out oriented plasma membrane vesicles by phase partitioning in an aqueous polymer two-phase system according to Kłobus, 1995. The activity of plasma membrane oxidoreductase was assayed according to Kłobus, 1995. Reduction of ferricyanide by NADH in plasma membrane vesicles was measured spectrophotometrically as a change of absorbance at 420 (A_{420}). Determination of H_2O_2 was made by fluorescence quenching of the pyranine as described by de Donato and Chiavazza 1994.

Results: Oxidoreductase activity determined in plasma membranes isolated from cucumber roots growing 3 or 6 days at 10 ˚C was the same as in the control, whereas activity of enzyme found in membranes obtained from acclimated plants was higher about 40% than in control. The accumulation of H_2O_2 closely followed the plasma membrane oxidoreductase activity and the highest quenching of pyranine was observed in acclimated plants .

Conclusions: Plants have the ability to acclimate to a variety of environmental stresses. Our results showed that modification of plasma membrane oxidoreductase activity was only in acclimated plants. These changes may bre related to increase tolerance of plants to cold stress. However the meaning of oxidoreductase in acclimationplants to low temperature is still under investigation.

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Physiological reactions of tomato plants to different watering strategies

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Introduction: The pot experiment on fresh tomato plants (cv. Cedrico) with different watering strategies was performed in a glasshouse to determine the reaction of tomato plants to various deficit irrigation types.

Methods: After replanting to special split root pots, plants grew in a mixture of sand and soil substrate in a glasshouse at 26/20 °C day / night. From flowering seven PRD (partial root-zone drying, 50 %) and DI (deficit irrigation, 50 %) cycles as well as a treatment with full irrigation (FI, 100 %) were performed. In FI the whole root system was irrigated daily close to pot capacity based on daily volumetric soil water content (VWC). For PRD and DI only 50 % of the FI water was applied. The reduced water amount was applied to the whole root system in DI and to one half of the root in PRD, and irrigation was shifted when VWC of the dry side had decreased to ca. 5 % (every six days). During the treatment, soil water content (TDR system), chlorophyll fluorescence, stomatal conductance, leaflet elongation and diameter of chosen fruits were measured. After 3 and 7 PRD cycles, leaf and stem biomass, number and fresh weight (FW) of fruits, number of mature fruits and dry weight (DW) of roots were measured.

Results and conclusions: Three and seven PRD cycles reduced the DW of the upper part of plants in comparison to FI but the reduction was more pronounced in DI than in PRD. Similarly, FW and number of fruits were reduced. The reduced number of mature fruits after 7 PRD cycles in comparison to FI and DI showed a clear 'green stay' effect of PRD plants. The effect is probably connected with the higher nitrogen uptake and content in plants. DW of roots was significantly higher in PRD than in FI plants after seven drying cycles. DI reduced root DW in comparison to FI. Irrigation water use efficiency in the production of DW of the upper part was highest in PRD. It was significantly higher not only compared to FI but also to DI plants. The reason for this was probably the partial closed stomata in PRD plants during drying cycles. The stomatal closure was more pronounced in PRD than in DI. The partial closed stomata did not, however, limit the photosynthetic efficiency in PRD plants as measured by chlorophyll fluorescence parameter effective quantum yield of PSII. In DI plants a decrease in photochemical fluorescence quenching was observed suggesting a biochemical (non-stomatal) limitations for photosynthetic efficiency.

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Modification of vacuolar pyrophosphatase (V-PPase) activity in cucumber roots stressed with heavy metals

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Introduction: The vacuolar sequestration of heavy metals is one of the detoxification mechanisms developed by plants. Accumulation of metals could be mediated by secondary active transporters energized by vacuolar proton pumps: V-ATPase and V-PPase. It was shown that activities and/or gene expression of both enzymes have been induced in response to environmental stresses. Chilling, anoxia and phosphate starvation markedly increased the protein amount and specific activity of V-PPase. Thus, it has been postulated that vacuolar pyrophosphatase is an important element of the plant survival strategies under stress conditions especially when cellular ATP levels are

reduced. In this study we demonstrate the effect of heavy metals (Cd and Cu) on the function of PPase at the tonoplast isolated from cucumber roots.

Materials and methods: Six-day-old cucumber seedlings were treated with 100 μM $CdCl_2$ or $CuCl_2$ for 2, 12 and 24 hours. After two hours of metal exposure part of plants was transferred into fresh nitrate-containing medium for subsequent 16 hours. Tonoplast-enriched fraction was prepared from cucumber roots as described by Kabała and Kłobus (2001). Hydrolytic activity of V-PPase was assayed according to modified method of Maeshima and Yoshida (1989). Total RNA was isolated from roots and expression of pyrophosphatase gene (*CsVP*) was measured with semi-quantitative RT-PCR.

Results: V-PPase activity was significantly stimulated in tonoplast vesicles isolated from roots treated with copper. This activation was time-dependent. Slight increase in the enzyme activity has been observed after 2 and 12 h exposition of plants to cadmium. But treatment of seedlings with Cd for 24 h decreased it. When 100 μM Cd or Cu was included into the incubation medium the inhibition of PP_i hydrolysis has been observed. To explain if heavy metals could modulate the expression of gene encoding proton pump the level of specific transcript for vacuolar PPase (CsVP) was determined. The constitutively expressed gene encoding elongation factor EF1 was used as an inner standard. It was shown that mRNA level for tonoplast enzyme in roots treated with 100 μM Cu was similar as in control roots.

Conclusions: Copper ions, contrary to cadmium, markedly stimulated V-PPase activity after treatment of plants with metal. This activation was not a result of direct Cu effect on the enzyme protein nor changes in the expression of gene encoding V-PPase.

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Chemical leaf composition affects insect grazing on shrubs growing under different light conditions

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Introduction: Based on long-term observations under natural conditions, we found significant differentiation in susceptibility to insect attack among six understory shrub species. The aim of our study was to determine the relationship between leaf chemistry and vulnerability to grazing insects.

Methods: At monthly intervals we assessed the average percentage of herbivorized leaves in understory shrubs growing in full sun and shade conditions. The total content of soluble phenols (TPh), condensed tannins, lignin, nitrogen and nonstructural carbohydrates (TNC) was measured for leaves unaffected by insect herbivory.

Results: We found that leaves of black elder (*Sambucus nigra* L.) and bloodtwig dogwood (*Cornus sanguinea* L.) were least affected by insects, and that leaves of glossy buckthorn (*Frangula alnus* Mill.) were only slightly damaged. Hazel (*Corylus avellana* L.) and black cherry (*Prunus serotina* Ehrb.) exhibited intermediate vulnerability to leaf grazing insects and European bird cherry (*Prunus padus* L.) was the most affected among those species studied. There were no significant correlations between leaf perforation by insects and concentration of nitrogen and metabolites analyzed in intact leaves of the species studied. Our result has showed that TPh and condensed tannins played a significant role in the defense of *P. padus*, *P. serotina* and *C. avellana* leaves. Leaves of *C. sanguinea* exhibited relatively high concentrations of TPh, whereas leaves of *S. nigra,* which showed a similar level of vulnerability to insects, were low in TPh and tannins. In all species, leaves of plants growing in high light conditions showed higher concentrations of defense metabolites. In all species of shrubs except of *C. avellana*, leaves growing in full sunlight were less susceptible to insect grazing than those from shade conditions. Within each species (except *C. avellana*) significantly lower leaf herbivory of plants from well-lit conditions may be explained by low concentrations of nitrogen and higher of defense metabolites (TPh and tannins). Similar patterns among light conditions, leaf herbivory and concentrations of N and defense metabolites were also observed within the crowns of individual shrubs in the study. None of the metabolites studied or nitrogen concentrations explained the higher vulnerability to insect grazing of leaves grown in high light as compared to shade leaves of *C. avellana.*

Conclusions: We found that interspecific differences in leaf herbivory among the six species of shrubs studied were not systematically related to concentrations of defense metabolites (TPh and tannins). Contrary to expectation, concentrations of these metabolites were higher in leaves of species vulnerable to insect grazing and lower in those species resistant to grazing. For five out of six species leaves developed in high light conditions were less susceptible to insect grazing.

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Carbohydrates content in *Clematis* shoots cultured *in vitro* depending on temperature and sucrose/nitrogen level in the medium

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Introduction: In plants, it is know that sugar and nitrogen are critical nutrients. Numerous studies have found a negative relationship between nitrate fertilization and starch level. It was stated that starch synthesis is regulated by nitrate (Scheible *at al*., 1997). Our previous results showed that sucrose and nitrogen compounds concentration in the medium strongly influenced the growth and development of *Clematis pitcheri in vitro.* The effect was also temperature dependent. The aim of the present work was to determine the carbohydrates content in plantlets from such experiments.

Methods: Shoot tips with 2-3 nodes derived from *in vitro* grown plants were used as explants. They were placed on MS media with m-Topolin 0.2 $mg \cdot l^{-1}$, sucrose 10 or 30 $g \cdot l^{-1}$, and two levels of KNO_3 and NH_4NO_3 (normal strength – x 1; half strength – x 1/2). In each treatment
6 jars x 7 explants were used. After 8 weeks of growth at the temperature of 15 ˚C, 20 ˚C or 25 ˚C plantlets were collected, lyophilized, weighed and grounded. Contents of glucose, fructose, sucrose and starch were determined with the use of enzymatic methods.

Results and Conclusions: Starch, glucose and fructose were present in shoots. Regardless of the treatment no sucrose was found in plantlets. In shoots growing at 15 ˚C on medium with 10 $g \cdot l^{-1}$ of sucrose the starch content was low, while glucose and fructose level was higher. With the increase of temperature (20 ˚C, 25 ˚C) shoots accumulated more starch. The highest level of starch occurred when shoots were cultured at 20 ˚C on medium with high sucrose (30 $g \cdot l^{-1}$) and low nitrogen (1/2 strength). Generally, the higher concentration of sucrose in medium the higher content of carbohydrates in plantlets. Lower nitrogen compounds level in the medium (˝ strength) led to increase of glucose and starch content in shoots, and had a residual effect on the level of fructose.

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Effect of temperature and sucrose/nitrogen level in the medium on chlorophyll and anthocyanin contents in *Clematis* cultured *in vitro*

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Introduction: In plants propagation with tissue culture the efficiency of micropropagation but also the quality of microcuttings are important. The aim of the present work was to determine the chlorophyll and anthocyanin levels in *Clematis pitcheri*. It is a part of the study concerning the effect of temperature and sucrose/nitrogen level in the

medium on *C. pitcheri* growth and development *in vitro*.

Methods: The apical shoot explants (2-3 nodes) were placed on MS media with m-Topolin 0.2 $mg \cdot l^{-1}$, sucrose 10 or 30 $g \cdot l^{-1}$, and two levels of KNO_3 and NH_4NO_3 (normal strength – x 1; half strength – x 1/2). In each treatment 6 jars x 7 explants were used. After 8 weeks of growth at the temperature of 15 ˚C, 20 ˚C or 25 ˚C plantlets from 1 jar were taken for chlorophyll analysis in fresh material. The chlorophyll content in the acetone extracts was determined using the Bruinsma (1963) method. Plantlets from 5 jars were collected and lyophilized. Then, samples were macerated and anthocyanins were extracted at 2 ˚C in 1 % HCl in methanol for 24 h. The content of anthocyanin was determined spectrophotometrically at 530 nm.

Results: Plantlets grown at 20 ˚C contained the highest and at 15 ˚C the lowest content of chlorophyll. The sucrose concentration in medium had no effect or only a little effect. Lower nitrogen compounds level in the medium (1/2 strength) stimulated accumulation of chlorophyll in shoots as compared to a normal strength, with the highest differences at 15 ˚C. On the other hand, shoots grown at 15 ˚C produced more anthocyanins. However, in the case of medium with low sucrose (10 $g \cdot l^{-1}$) the effect of temperature on anthocyanins content was limited. In the case of medium with 30 $g \cdot l^{-1}$ of sucrose a decrease of anthocyanins accumulation occurred as the temperature increased.

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Stomatal characteristics, leaf gas exchange and growth of strawberry plants as affected by various growing conditions

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Introduction: Numerous studies have shown that stomatal size and density can be modified by environmental factors such as light, humidity, CO_2 and drought. However, there are contradictory results in the literature regarding the effect of environmental factors on stomatal development (Bańon *et al*. 2004). The current study was undertaken to investigate the effects of irrigation regime, light and humidity levels on stomatal characteristics, leaf gas exchange and growth of strawberry cultivar 'Salut' grown under greenhouse conditions.

Methods: Four groups of strawberry plants were grown in separate compartments of the greenhouse with modified growing conditions: (i) limited intensity of solar radiation (approx. 50 % of light compared to control), (ii) high relative air humidity (approx. 90 %), (iii) deficiency of water in growing medium (water doses were reduced by approx. 50 % compared to control), (iv) typical greenhouse conditions, optimal irrigation (control). After harvest, all the plants were transferred to uniform (control) conditions to characterize stomata, collect morphological parameters and to measure leaf gas exchange.

Results: Differentiation of growing conditions affected significantly morphological response of strawberry plants. Biomass and leaf area were the lowest in the plants grown under conditions of water deficiency. No differences in the length of root system were observed between control and water stressed plants. The water shortage resulted in a decrease of density and reduction of dimensions of stomata on plant leaves. On the other hand, higher number of bigger stomata was found on leaves of plants grown under high humidity. It is possible that the variations in stomatal density and size found in the plants grown under different conditions may account for different rates of gas exchange observed in these plants.

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Response of two winter wheat cultivars to nitrogen deficiency

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Introduction: The effect of nitrogen deficiency on the parameters of gas exchange at leaves and yielding of two winter wheat cultivars were investigated.

Materials and methods: The experiment was conducted in the years 2003-2004, in greenhouse, in Mitscherlich pots, containing 7 kg of soil. Examined two winter wheat cultivars (Begra and Juma) showed different responses to nitrogen addition. The following doses of N element were applied per pot: 1200 and 2400 mg N (as NH_4NO_3). The other mineral nutrient components were given in amounts appropriate to assure the normal growth of winter wheat plants. Ten plants were grown in each pot. At successive stages of plant development (in 3 stages), the net photosynthesis rate, transpiration rate and stomatal conductance were measured on flag leaves of winter wheat using gas exchange system Li- 6400 (Li-COR). Plants were harvested at fully ripeness.

Results: N deficiency in the soil decreased the gas exchange and yielding at two investigated winter wheat cultivars. Independently of N doses Juma variety was characterized by higher photosynthetic activity at leaves and higher biomass and seed yield than Begra variety.

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Extreme conditions for proceeding photosynthesis in stem of herbaceous plants

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Introduction: Leaves proceeding active and efficiently gas exchange with atmosphere by a stomata apparatus. In order of stomata absence the stem surface of lignified plants is very little pervious (Pilarski 1994). In consequence the high level of intercellular CO_2 and low level of O_2 can occur. In this extremely conditions efficient photosynthetic apparatus is observed (Pfanz *et al*. 2002). Cortex of herbaceous plants absorb more irradiation than bark of lignified plants. So, if diffusive resistance of epidermis herbaceous plants are this same as resistance of cork lignified ones probability of stress increase.

Material and Methods: The diffusive resistance of leaves and stems for water vapor was measured on plants growing in field conditions, by means of a Delta porometer MK-3. The efficiency of photosynthesis apparatus was measured by Hansathech fluorometer Handy Pea.

Results: On the experiments occurred the absence of the stomata apparatus in stems of both species. In effect of lack stomata gas exchange of steam was poor. Some lenticels witch occur on the surface of Japanese knotweed did not change this gaseous impermeabilities. The different situation occurred in topinambur which stems are equipped in lot of trichomes. In this case, exchange of steam was little higher. Even though tests done by fluorometer parameters described good state of photosynthetic apparatus. Especially maximal efficiency of PS II (Fv/Fm) was close to standard level (0.815 – 0.837).

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Lipids composition of plasma membranes from root of corn seedlings under salt stress and synthetic compounds treatment

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Introduction: The salt stress is complex and involves changes in ion and osmotic homeostasis and also oxidative stress. It is now generally accepted that the first deteriorating change during stress injuring is an alteration in the lipids composition of plasma membrane. The pervious investigation showed that synthetic compounds (Methyure and Ivin) treatment decreased lipid peroxidation intensity in corn seedlings exposed at NaCl presence. The aim of our study is investigation effect of Methyure and Ivin treatment on lipids composition of plasma membrane from root of corn seedlings and lipoxygenase activity under salt stress.

Methods: Seeds of corn were treated 10^{-7} M Methyure and Ivin. 7-old-days seedlings were exposed at 0,1M NaCl presence. The phospholipid fraction from plasma membrane was quantified for individual species using TLC with glass plates. Fatty acids of total membrane lipid extract were analyzed as fatty acid methyl esters on GC. Lipoxygenase activity was assayed spectrophotometrically at 234 nm with linolic acid as substrate.

Results: The salt exposition decreased the level of major phospholipids phosphatidylcholine and phosphatidylethanolamine and increased the level of phosphatidic acid and lysophosphatidylcholine. The Methyure treatment with salt exposition increased the level of phosphatidylcholine and decreased the level of phosphatidylethanolamine, phosphatidic acid and lysophosphatidylcholine. The Ivin treatment had contrast effect. NaCl induced a decrease in the plasma membrane unsaturated fatty acids (16:1, 18:1, 22:1) and increased saturated fatty acids (16:0, 18:0) resulting in a lower unsaturated/ saturated ratio. The synthetic compounds treatment caused a increased in the plasma membrane unsaturated fatty acids that resulting in alteration unsaturated/ saturated ratio. The NaCl exposition decreased the level of lipoxygenase activity relative control plants, but synthetic compounds treatment increased lipoxygenase activity in corn seedlings exposed at NaCl presence.

Conclusions: Results indicate that NaCl exposition and synthetic compounds treatment change lipids composition of plasma membrane and lipoxygenase activity. The synthetic compounds treatment likely increase salt tolerance of plants.

ADH expression in aerial-aquatic plants in response to different water environment

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Introduction: Aerial-aquatic on its ecology, *Sium latifolium* L. (*Apiaceae*) and *Alisma plantago-aquatica* L. (*Alismataceae*) are highly plastic species. They can grow both under flooding and moderate water deficit, forming morphologically different ecotypes. Their phenotypic structural traits are aerenchyma in aerial-aquatic plants and its absence in terrestrial ones. Adaptation in ontogenesis (operative adaptation) of these plants, first of all, to the contrast water supplying is realized by differential gene expression. Activity of alcohol dehydrogenase (ADH) plays a critical role in the metabolism of plants under hypoxia increasing energetic status of the cells. This study was aimed to characterize localization of ADH activity and its isozyme spectrum in roots of the aerial-aquatic and terrestrial plants.

Methods: Roots of two natural *S. latifolium* and *A. plantago-aquatica* ecotypes were used to compare the expression and localization of ADH activity. Cytochemical ADH staining in roots as well as native 6% PAG following by staining for ADH activity in gels were carried out.

Results: Cytochemical localization revealed that the ADH activity was associated with the root tip in aerial-aquatic plants. The ADH spectrum consisted of 2 isozymes in the *A. plantago-aquatica* roots and 4 isozymes in the *S. latifolium* ones. At the same time ADH activity was not observed in the roots of terrestrial plants. However, the weak ADH activity appeared in the roots of terrestrial plants after temporary flooding.

Conclusions: The established changes in ADH gene expression in different water environment correlate with the root anatomical structure and demonstrate its significant role in plant phenotypic plasticity.

Arabidopsis halleri (L.) from metalliferous and non-metalliferous soils in southern Poland – heavy metal accumulation

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Introduction: Some plant species can grow on soils with high level of heavy metals. They have developed tolerance strategies against metals. *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz is a hyperaccumulator of Zn, and it is able to accumulate huge amount of metal in its above –ground parts without any sign of toxicity (Bert *et al*. 2000, Bert *et al*. 2002). *A. halleri* belonging to Brassicaceae is perennial, stoloniferous species frequent in Europe. As pseudometallophyte, it can occur on contaminated and normal soils. In Poland, species occurs in mountains and in the Silesia - Kraków Upland. The aim of study was to compare ability to accumulate heavy metals (Zn, Cd, Pb) by several *Arabidopsis halleri* populations from metalliferous (M) and non-metalliferous (NM) soils in southern Poland.

Methods: In July 2005, 9 populations of *A. halleri* on the Silesia – Kraków Upland (5 on contaminated soils, 4 on clean soils) were sampled. In five samples of each population concentrations of Zn, Pb, Cd in leaves and roots, and pH, macronutrients and Zn, Pb, Cd in soils were determined.

Results: Zinc content in leaves of all studied *A. halleri* populations and Cd in M populations exceeded threshold of hyperaccumulation for these metals (1 % of Zn, 0.01 % of Cd). Level of Pb in leaves were lower than roots. All populations accumulated similar amounts of Zn (Kruskal-Wallis H test, $p>0.05$) although they grow on soils with different Zn content (Kruskal –Wallis H test, $p<0.05$). Content of Zn in leaves did not correlate with total and extractable metal content in soils (Spearmen test, $p>0.05$). Concentrations of Cd in plants depended on Cd concentration in soils (Spearman test, $p<0.05$).

Conclusions: Presented results are similar to results from other studies (Bert *et al*. 2000, Bert *et al*. 2002) and confirm that hyperaccumulation of Zn is a constitutive trait in *A. halleri.* Very interestingly the accumulation of Cd appeared to be a characteristic of M populations which suggest that Cd accumulation could be non constitutive in *A. halleri* and specific from Cd containing sites.

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Growth reaction of maize coleoptile segments modified by lead and fusicoccin

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Introduction: Lead can influence various physiological processes in plants including root and shoot elongation, cell division and seedling development. Plant growth and development are tightly regulated by plant growth regulators (auxin - IAA) and other growth substances e.g. fusicoccin – FC. In agreement with the "acid growth theory" the fungal phytotoxin fusicoccin induces plant cell elongation *via* activation of plasma membrane H^+-ATPase. The goal of the present study was to investigate interrelations between the action of FC and lead on elongation growth and proton extrusion in maize coleoptile segments.

Methods: The studies were performed on the 10-mm long maize coleoptile segments obtained from 96-h-old etiolated seedlings grown in dark, at 27±1 ˚C**.** Intact coleoptile segments with the first leaves removed were excised 3 mm below the tip and incubated in solution (control) of the following composition: 1.0 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$. Fusicoccin (FC) at a final concentration 1 μM was introduced into the incubation medium at 120 min. $PbCl_2$ (0.1 mM) was added to the incubation medium before (0, 60 min), simultaneously (120 min) or after application (240 min) of FC. Growth experiments were carried out in an apparatus, which allowed simultaneous measurements of elongation growth and pH of the incubation medium

Results: It was found that FC-induced growth of maize coleoptile segments was enhanced ca. 2.8-fold as compared to endogenous growth (growth without FC). Pb caused different effect depending on the time of lead addition. Lead applied to the incubation medium at 0 time reduced FC-induced growth by 30 %, whereas Pb added to the incubation medium at 60, 120, 240 min not significantly affected FC-induced growth. Acidification of the incubation medium observed in the presence of FC was not changed by lead.

The accumulation of raffinose in vegetative tissues of legume seedlings in response to cold and desiccation stress

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Introduction: The participation of raffinose in dehydration tolerance of plant tissues exposed to osmotic stresses, caused by desiccation, drought or cold was well studied in developing seeds, especially in the case of legumes, naturally accumulating a large amounts of raffinose family oligosaccharides. However, there is only scare information on the participation of raffinose in response of vegetative tissues to dehydration stress conditions.

Methods: In the preliminary studies 7-days old seedlings of pea, vetch, soybean, chick pea and lentil were fast desiccated (at 25 ˚C and 25 % RH) for screening of their ability to formation of raffinose in response to stress. Then seedlings of pea and vetch (accumulating the highest amounts of raffinose) were exposed for cyclic dehydration/rehydration or cold/warm treatment. Soluble sugars were estimated by high resolution gas chromatography (HRGC).

Results: The common response of seedlings of all investigated species to desiccation stress was the accumulation of sucrose and raffinose. Galactinol and raffinose appeared at 4-8 hour of dehydration and coincided with detectable activity of galactinol synthase and raffinose synthase (both activities were not detectable in control seedlings). Accumulation of raffinose rapidly increased from 12 to 48 h of desiccation, mainly in epicotyl tissues. However, the rehydration of seedlings for 1-3 days caused completely disappearance of raffinose. The next two repetition of seedlings dehydration/rehydration cycle temporary induced accumulation of raffinose, but to lower level than in the first dehydration). Similar results were obtained during seedlings exposition to cyclic treatment by 4 ˚C and 20 ˚C.

Conclusions: Results indicate that beside sucrose, raffinose participates in legume seedlings response to dehydration or cold stress. The similarity in the induction of raffinose accumulation by both stresses and disappearance of raffinose according to removal of stress conditions also suggest the possible existence of common factor(-s?) triggering biosynthesis of raffinose in vegetative tissues.

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Effect of indole-3-acetic acid (IBA) on redox activity and proton extrusion in maize coleoptile segments

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Introduction: Auxins are defined as organic substances that promote cell elongation growth when applied in low concentrations to the adequate plant tissue. Natural auxins IAA, IBA and 4-CL-IAA are found in plants as the free acids and in conjugated form. Indole -3-butyric acid has been identified as a natural product in many plant species from maize (*Zea mays*) and pea (*Pisum sativum*) to *Arabidopsis*. IBA was definitively shown to occur naturally in plants in 1989 (Ludwig-Müller 2000).

Methods: Indole-3-butyric acid was tested at different concentrations (10^{-6} - 10^{-4}M) and times for their capacity to change the redox activity and medium pH of maize coleoptile segments. The experiments were carried out with 10 mm long coleoptile segments cut from four-day-old etiolated maize seedlings. Before experiments the coleoptiles were abraded in an aqueous suspension of 1200 mesh SiC power. Redox activity and pH changes in incubation medium were measured simultaneously according to the method described by Carrasco-Luna *et al*. (1995)

Results: Results indicate that both the redox activity and pH changes in coleoptile segments dependent on concentrations of IBA and the time after its addition to the incubation medium.The maximal values of redox activity and pH changes of the incubation medium (expressed as Δ pH) were observed at 10^{-5}M of IBA. Both the proton extrusion and the redox activity were strongly enhanced in abraded coleoptile segments.

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Effects of elevated temperature and CO_2 on *Lepidium sativum* L. under ozone pollution

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Introduction: Global increase of CO_2 level and warming can have both positive and indirect negative effects on plants (Qaderi *et al*. 2006). Moreover, plants grow in multifactorial environment where tropospheric (ground level) ozone is one of phytotoxic air pollutants. The aim of the present work was to investigate the influence of higher temperature and increased CO_2 level (700 ppm), as components of global warming, on plants cultivated under monitored conditions of elevated air pollution with ozone.

Materials and methods: Garden cress (*Lepidium sativum* L.) seedlings germinated 7 days were cultivated for 7 days in phytotron chambers during a 12 h photoperiod at 21/14 ˚C and 25/16 ˚C day/night temperature with CO_2 ambient (350 ppm) or elevated (700 ppm) levels without or with 40 and 80 ppb ozone. Biometrical and morphological analyses were performed.

Results: Increase of temperature from 21/14 ˚C to 25/16 ˚C and concentration of CO_2 from 350 to 700 ppm inhibited elongation of cress seedlings. However, higher temperature promoted genesis of leaves by 6.5 %, caused foliage injures and decreased dry biomass by 22.8 % at ambient CO_2 level. Elevation of CO_2 level did not significantly change these parameters. The applied O_3 increased the number of leaves in present-day temperature with 350 ppm CO_2, nevertheless 49.2 % and 65.3 % of leaves were injured under 40 and 80 ppb O_3, respectively. The effect of increased temperature and elevated CO_2 significantly diminished the extent of O_3-induced injuries (by 60.1 % and 78.3 % under 40 and 80 ppb O_3, respectively) and increased parameters of leaves.

Conclusions: Increase of temperature to 25/16 ˚C and elevation of CO_2 concentration to 700 ppm suppressed the growth of cress seedlings and diminished the extent of negative effect of O_3 on leaf morphology and biomass.

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Isothermal Calorimetry as a tool for estimating resistance maize genotypes to cold temperatures

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Introduction: The aim of the presents study was to establish whether isothermal calorimetry can be used as a method of assessment of the early impact of thermal shock of maize. To this purpose, two genotypes of maize (cold-resistant and cold-sensitive) were used to examine the low temperature response.

Methods: Cold-sensitivity of maize was checked as follows: seeds germinated in the 26 ˚C were then put into the calorimeter for 24 hours of three temperatures: 10 ˚C, 17 ˚C and 25 ˚C. During this time heat production by seeds was measured continuously.

Results: At 10 ˚C, a sudden decrease in germination was observed and accompanied with low heat production. At 17 ˚C the germination process didn't influence. Finally at 25 ˚C the rate of germination and heat production was the biggest as compare to lower temperatures. Significant differences in heat production were found for the both genotypes only at 17 ˚C. It's possible, that 10 ˚C was too low and 25 ˚C was too high for the differentiation of plant response to temperature. Thus 17 ˚C can be used as a testing temperature.

Conclusions: Isothermal calorimetry is useful to investigate to low temperature shocked of maize genotypes, but testing temperature must be earlier established

Water relations, gas exchange, nodulation, nitrogen fixation and growth of faba bean (*Vicia faba* L.) in relation to the drying of surface roots

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Introduction: Drought stress near the soil surface is common in the field, whereas water availability deeper in the soil profile may be adequate for plant uptake; this is particularly true in Mediterranean-type environment. Soil drying in the upper profile may have a profound impact on plant growth and seed yield (Mwabanwebge *et al*. 1998). Studies in the field and glasshouse suggested that stomata close and photosynthesis, transpiration and leaf growth all decrease when only a portion of the root system is in drying soil (Turner and Henson 1989). In the present study the segmented soil column system separated into distinct layers was utilized to test the hypothesis that stomatal conductance and leaf gas exchange were/were not reduced in proportion to the fraction of roots in dry soil.

Materials and methods: The segmented soil column system in which the root system divided into two layers, each 24 cm in diameter and 33 cm high used. The experiment was conducted, in a glasshouse at the University of Western Australia, from June to August 2006. Faba bean (*Vicia faba* L. var. *minor*) breeding line SP98002 was subjected to 2 watering regimes. The watering regimes were: (i) well-watered, and (ii) water withheld at flower-

ing, from 61 to 81 days after sowing (DAS). The treatments imposed as, (i) all two layers adequately watered (control), and (ii) the upper layer un-watered with the remaining layer kept adequately watered (droughted). The treatments have been applied at flowering (i.e. where 50 % of the plants had one open flower), exactly at 61 days after sowing. Measurements were done at 1, 4, 7, 11, 14, 17 & 21 days after drying (DAD).

Results: Withholding water resulted in significant increase in LWP in all days of measurements compared to control except of measurement at 1 DAD. Osmotic potential (OP) did not differ significantly between treatments. Drying surface roots significantly resulted in reduction in net photosynthetic rate and stomatal conductance in all measurements except of measurements at 7 DAD. Plant height, branch number/plant, leaf number/plant, green area and shoot biomass at 61 and 81 DAS, respectively were not significantly affected by drying surface roots. Drying surface roots resulted in significant reduction in nodule number/plant and nodule biomass (mg/plant) on top layer and total pot. N_2 fixation (%) was significantly reduced in droughted plants and the reduction was representing 20.1% compared to well-watered plants.

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Evaluation of the influence of soil drought on yellow lupine using some physiological markers

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Introduction: Lupine is considered as an alternative crop to soybean, which possess allergenic properties. Many soybean cultivars are transgenic and their introduction into cultivation is controversial and socially not accepted. In recent years in Europe and over the world, drought is one of the most serious problem for breeders and agriculture. Many physiological, biochemical and genetical studies are continued toward the increase in the resistance to this kind of streess.

Methods: Physiological tests for yellow lupine (*Lupinus luteus* L.) grown under 30 % soil drought were performed. Chlorophyll *a* (Chl) fluorescence, superoxide radical generation and free polyamines (putrescine – Put, spermidine – Spd and spermine – Spm) contents were measured in the youngest expanded leaves, after 15, 30 and 45 days of drought treatment, which was initiated at the anthesis. Chl measurements were performed by FMS2 fluorimeter (Hansatech Instruments, King's Lynn, UK). Intensity of O_2 generation *in situ* was determined by the reduction of nitroblue tetrazolium (NBT) to monoformazane according to Doke and Ohashi method (1988). Polyamines were quantified by HPLC (Agilent Technologies 1100) equipped with mass spectroscope with API-ESI.

Results and conclusion: The highest decrease in photochemical quenching (qP) and electron transport rate (ETR) of PSII was observed. Water defficit probably caused the damage of photosynthetic apparatus leading to photoinhibition (Baker and Rosenqvist 2004). Similarly, superoxide radical generation was enhanced when the stress was prolonged. Drought treatment induced also an increase of Put and decrease of Spd and Spm level in leaves. These changes are coincident with measurable signs of stress, such as wilting and the decrease in the values of various chlorophyll fluorescence parameters. Physiological test used in our experiment seem to be useful in evaluation of the impact of drought and for the comparison with future studies using molecular markers.

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