# STRESS IN ANTHER AND MICROSPORE CULTURES OF RAPE

## Zuzana BEŇOVÁ\*

In anther and microspore cultures it is necessary to change gametophytic development of microspores to sporofytic to create embryos and then plants. In this case, temperature stress is important. Temperature of 32°C is suitable for the first time of an incubation. This information has been proved in our experiment. According to CORDEWENER et al. (1998) heat treatment at 32°C is sufficient to achieve a quantitative and irreversible induction of the microspores into embryogenic pathway. According to CUSTERS et al. (1994) culture at 32,5°C leads to sporophytic development and culture at 17,5°C leads to gametophytic development. Early stages of both developmental pathways are observed in the culture at 25°C. Our experiment in this work included two variants of incubation temperatures. Anthers were cultured according to KELLER and ARMSTRONG (1978). Induction of embryoids, their growth and regeneration of plants, were conducted according to the work of NALECZYNSKA (1991). We obtained 352 embryoids from 1360 anthers at 32°C during the first 48 hours and then at 25°C of incubation of all genotypes. We obtained 51 embryoids from 1040 anthers at 25°C at whole time of incubation and from the same genotypes. Based on our results, we can see that the number of the obtained embryoids depends on the temperature of an cultivation. Temperature of 32°C is better than 25°C and this result offers a chance to use this temperature to create embryoids. And so in the first moment, the stress temperature becomes factor of optimatization to create embryoids in the next moment.

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# PHOSPHOLIPIDE SIGNALLING IN PLANT DEFENCE RESPONSES

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Phospholipide signalling is now recognised as one of the main signalling systems which probably play role during pathogen attack. Besides biotic activation, several chemical compounds, like salicylic acid (SA) and its functional analogue, benzothiadiazole (BTH), have been shown to mimic pathogen attack triggering the same biochemical consecutive events as do plant pathogens. Nevertheless detailed understanding of early events in signalling cascade leading to systemic response is still missing.

We have studied phosphatidylinositol specific phospholipase (PI-PLC) and phospholipase  $A_2$  (PLA<sub>2</sub>) activities and induction of extracellular proteins on model material of tobacco cell culture VBI-0 strain after BTH and SA treatment. An increase of PLC activity and no changes of PLA2 activity were observed after two hours of treatment. The pattern of extracellular proteins determined by native page, showed quantitative changes corresponding with the concentration of BTH added to medium within 24 h. In next experiments we plan to follow phospholipase D activity during the same treatment and estimation of enzyme activities of induced proteins.

#### Acknowledgements

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# EXTRACELLULAR ALGAL PRODUCTS COULD MODIFY TOXIC EFFECTS OF HERBICIDE 2,4-D

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Freshwater planktonic algae release into the environment many organic substances, mainly carbohydrates, amino acids, peptides, phenols and vitamins. This process is a result of normal physiological activity of algal cells. The amount and quality of extracellular products depend on species, the level of mineral compounds in the environment and photosynthesis intensity. In our previous work we noted that the media after batch cultures of unicellular green algae contained active substances and stimulated the growth rate of the other algae (BURKIEWICZ, SYNAK, 1996). The exuded substances have a number of different functions. It was shown that the amount of organic compounds produced by algae increased when ecosystems were polluted with pesticides or heavy metals ( ARŠÁLEK, ROJIČKOVÁ, 1996). Since planktonic algae are the basic link of food chains in water environment, their ability to accumulate and/or metabolize different kinds of contaminants plays an important role in proper function of water ecosystems.

The aim of our investigations was to determine the combined effect of extracellular algal products and commonly used herbicide, 2,4-D. We found that the diluted filtrates obtained from the stationary phase of *Scenedesmus subspicatus* culture showed a significant growth promoting effect on the other unicellular green alga, *Scenedesmus armatus*. Moreover, the degree of stimulation depended on the concentration of released compounds. On the other hand, 2,4-D caused a strong reduction in growth activity of *S. armatus* as compared to the control culture. We also observed that the interaction between the extracellular algal substances and 2,4-D resulted in partial or total suppression of the inhibiting influence of 2,4-D on *S. armatus* growth. It could be presumed that the planktonic algae, due to adaptation processes, developed mechanisms protecting them against the accumulation of xenobiotics in their cells.

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# EFFECTS OF GRADED CONCENTRATION OF AL<sup>3+</sup> IONS ON THE PRODUCTION OF ASSIMILATION PIGMENTS AND ETHYLENE IN EXPLANTAT CULTURE OF MAGNOLIA X SOULANGIANA SOUL.-BOD.

## Angelika FILOVÁ\*, Zuzana JUREKOVÁ\*

Every reaction of a cell, structural or functional, is conditioned by its efforts to resist against the stress effects according to the Le Chatelier principle: "If a system in equilibrium is subjected to a stress, the system tends to react in such a way as to undo the effect of the stress" (CARMAK, HORST, 1998). When all the abilities of protection and energy on their assurance are exhausted, the structural components and their functions in the cells are damaged. Ions stress is understand as activiting of ions, whose direct effect is a change in a normal ions balance in the live cell. Alike in case of other stresses, the ion stress be included a stress from excess and a stress from deficiency of ions (RENGEL, 1992).

There were studied the influence of graded concentration of  $Al^{3+}$  ions on the production of assimilation pigments and ethylene in explantant culture of *Magnolia x soulangiana Soul.-Bod*. Primary explantants of *Magnolia x soulangiana Soul.-Bod*. were to take for stability sterile culture. Organ culture were cultivated on the agar medium WPM, which was composed of Lloyd-Mc Cown. Into the medium was added AlCl<sub>3</sub>, which concentrations were gradually increased (over a range of 5, 10 and 20 mg Al<sup>3+</sup>.dm<sup>-3</sup>). The control group was cultivated on the basic mineral substrate and the pH of cultivation medium was always adjusted to 5,5.

The results from our experiments have confirmed different reactions of *Magnolia x soulangiana Soul.-Bod.* evokely for gradually concentrations. The presence of low  $Al^{3+}$  concentrations in the cultivation medium have positive effects on the production of chlorophylls <u>a</u>, <u>b</u> and <u>a+b</u>. In cultures whose growth was modified by high concentrations of  $Al^{3+}$  ions was increased the production of ethylene.

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## THE RESPONSE OF ZEA MAYS ROOT SYSTEM TO LOW TEMPERATURE

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Responses of shoot growth depend to a large extent on root growth and activity. A higher degree of chilling tolerance of maize seems to be related to the ability of a genotype to maintain a close ratio between the leaf and the root ratio area, at the expense of a somewhat higher allocation of biomass to the roots. The larger primary root diameter due to larger area of cortical and stelar tissues including xylem conducting area are intrinsic characteristics of chilling-tolerant genotype (CIAMPOROVA and DEKANKOVA, 1998) and correlate with higher hydraulic conductivity of this genotype. The maintenance of high hydraulic conductivity during stress is the first defence against a leaf water deficit, while stomatal closure can be regarded as the final defence mechanism against desiccation when there is already a water deficit. Another fundamental difference between sensitive and tolerant plants seems to be in the ability of resistant plants to reduce the generation of superoxide and/or peroxide hydrogen in the root mitochondria during exposure to chilling stress. In the roots of tolerant genotype the capacity of cyanideresistant alternative pathway of respiration increased about twice during chilling stress that otherwise inhibited the main phosphorylating pathway (LUXOVÁ and GAŠPARÍKOVÁ, 1999). Thus, respiratory electrons can flow through the alternative pathway, thereby decreasing the potential for the generation of superoxide and/or hydrogen peroxide in stressed root tissues and keeping the production of these toxic reactive oxygen species in balance with the levels of antioxidants and active oxygen scavenging enzyme systems. The potential of roots to reduce the destructive effects of free radicals is also discussed. It is suggested that activities of superoxide dismutase, catalase and especially glutathione reductase in maize roots may also contribute to their enhanced chilling tolerance.

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# STRESSES OF WAREHOUSED AGRICULTURAL CROPS

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The endurance of many agricultural crops is prolonged by their storage in chilling room at temperature from 12°C to  $-2^{\circ}$ C. Doubtless this temperature has retardatory effect on the set of enzymatic reaction of respiration and ripening processes. The conditions of optimal conservation are strictly individual by the kind and variety of the crop. Mostly it cannot be said, "the lower temperature is the better it is". The cooling stress can occurred on the crops there, if the temperature of warehousing is lower than an optimal temperature, but higher than the zero degree. The most complexe knowledge is available from conservation of apples and pears, which are collectively cultivated and warehoused especially in Europe, the USA, Canada, but also in Asia and Australia as well. The first (inside) responses of the cooling stress: The liquid phase is changed to the solid gel in the cells of twines, enzymes that are bound onto the cells' membrane are activated, there is a change in the structure of the cells' walls. After the first responses follow second responses (inside and outside), by the effect of cooling stress: Increases the production of ethylene and the intensity of respiration, increases permeability of cells membranes, arises change in the twines structures. Short effects of the cooling stress causes reversible changes which alter into irreversible by another exposition of improper lower temperature with the following consequence: Maillardion of skin and pulp (notably in the central zone), surface freckles, wilting of crops, post-ripening blockade (notably pears), accumulation of phenolic substances, skin and pulp fading below its skin and hydrated pulp. The warehoused crops are producing more ethylene in the cells and therefore its determination can be criterion for fault metabolism of substance. Of course, there exist processes and custodies which conciliate or even prevent cooling stress. It is possible to avoid unpleasant consequences of cooling stress by consistent application.

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# THE REACTION OF ISOLATED EMBRYONIC AXIS OF PEA (PISUM SATIVUM L.) ON TREATMENT BY ULTRASOUND

## Katarína HRUBÍKOVÁ<sup>1</sup>, Milan BEŽO<sup>2</sup>

Cytological changes of tissue of embryonic axis after treatment by ultrasound were caused by mechanical and heat effects of ultrasound waves. These changes were similar for individual segments as well as for individual genotypes. Distinct deformation of protoplasts were observed in tendency of longer time effect of ultrasound (20 and 40 minutes). The induction of micropores in cell wall resulted in decrease of cell turgor and consequently contraction of protoplast. Protoplasts were gradually separated from cell wall, decreased its volume, changed strong its shape and position in cell. From the original isodiametric shape of cells, typical for parenchymatic tissue, after treatment by ultrasound cells acquired irregular square shape. In the cause of change of shape the intercellular space increased. In case of undivided embryonic axis or specific segments which content meristematic cells like apex and hypocotyl of embryonic axis the induction of multishoots were observed. The ultrasound with self-cleavage effect dislocates intercellular interactions and so it enables to stimulate separation of meristematic cells, what resulted in multiple shoots formation.

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# CHANGES IN CHLOROPHYLL FLUORESCENCE OF TRITICOSECALE WITTMACK PLANT SUBJECTED TO DROUGHT STRESS

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The aim of the study was to determine the effect of water deficit (14 days) and rehydratation (14 days) on the intensity of chlorophyll fluorescence. Using luminescence spectrometer Perkin-Elmer LS 50B fluorescence emission spectra were collected from the flag leaves. Under water stress significant increase in the intensity of red and far-red fluorescence was found. During rehydratation plants return to the level of fluorescence in control plants. Although the red chlorophyll fluorescence yield was lower during rehydratation. The higher intensity of red and far-red fluorescence are connected with injures of tylakoid components, especially PSII and LHC. We suggest that low level of red fluorescence during rehydratation is connected with regeneration of the photosynthetic apparatus and better its function after removal of the stressor (LICHTENTHALER, 1996, 1996a, SCHWEIGER, 1996, STARCK, 1997).

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# INFLUENCE OF PHOMA LINGAM (TODE EX FR.) DESM. TO SECONDARY METABOLITES ON METABOLIC ACTIVITY OF OILSEED WINTER RAPE CALLUS

## Katarzyna HURA<sup>1</sup>, Iwona ŻUR<sup>2</sup>, Marcin RAPACZ<sup>1</sup>

The aim of the present work was to investigate the influence of metabolites produced by Tox<sup>0</sup> isolate - Ph3 on callus of winter oilseed-rape cv. Górczański.

Callus was cultured on Murashige and Skoog (1962) medium with 1 mg·dm<sup>-3</sup> BAP and 0,5 mg·dm<sup>-3</sup> 2,4-D (MSH), at 23°C and 8h/16h (day/night) photoperiod. After 4 weeks calluses were transferred to the MSH medium supplemented (1:4 volume ratio) or not with elicitor, obtained from liquid cultures of the fungus. The toxic effect of metabolites was tested after 14 days of tissue culture by measuring changes in fresh and dry weight of callus, cell respiration rate, heat emission rate and cell viability.

Secondary metabolites of *Phoma lingam* considerably decreased the viability of cells. The rate of dry mass accumulation was significantly different. The influence on cell respiration rate and heat emission were not observed.

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# EFFECT OF LOW TEMPERATURE ON CALLUS TISSUE OF BRASSICA NAPUS L. RESPONSE TO SECONDARY METABOLITES OF PHOMA LINGAM (TODE EX FR.) DESM.

## Katarzyna HURA<sup>1</sup>, Iwona ŻUR<sup>2</sup>

Resistance in plants against fungal pathogen may be induced by various even nonbiotic stress factors. We study if low temperature stress could increase the resistance of *Brassica napus* (L.) callus to secondary metabolites of *Phoma lingam*.

Calluses of cv. Górczański were exposed to low temperature stress (2°C for two weeks), then treated with secondary metabolites of isolate PL47 of *Phoma lingam*. The level of resistance was estimated by cell respiration and heat emission rates measured 0, 3, and 6 hours after elicitor treatment.

Compared with the control calluses, elicited tissues significantly differed in respiration rate and heat emission 3 hours after elicitation. Unhardened calluses increased the respiration rate and heat production. Whereas, low temperature stress caused decreased heat emission and slightly (not significantly) reduced respiration rate.

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# UTILIZATION OF PHYTOEXTRACTION KNOW-HOW IN TECHNOLOGIES OF ENVIRONMENTAL REMEDIATION

### Zuzana JUREKOVÁ\*

There are enough knowledges about many plant species, which are tolerant and able to vegetate on locations, that has been contaminated with heavy metals, metal isotopes, environmental polutants with high concentration of CO, NO<sub>2</sub>, benzene or formaldehyde. Defense mechanisms of these plants are according to GAWRONSKI (verbal information) in their abilities:

- to change pH in root environment,
- to retain metal ionts on root surface,
- to synthesize metalotionein
- to synthesize fytochelatin

With knowledge of above mentioned facts, there are developing technologies of phytoremediation, which are based on ability of superior plants to absorb elemets contaminating soils, waters and air. Success of these technologies consists in selection of suitable genotype or in creation of transgenic plants (RASKIN et. al, 1994), that are not only tolerant to polluted environment, but also able to absorb for example metal ions and metabolise them by their binding with a specific protein (RAUSER, 1990).

Understanding of physiologicaly-biochemical and molecular mechanisms of these plants is a current scientific problem in present. From physiological point of wiew, it will be necessary to obtain information about growth activities of particular organs, abilities of biomass production per unit area, to identify organs of translocation etc. It will be important to obtain information about changes in concentration of substances after remediation, respectively about waste that remains in environment. Attention will be paid to further handling of organic material from plants used on phytoremediation.

We believe, that more effective research work in this field will bring some positive results for science and consecutively will enable to use this modern technology as an alternative for improvement of environment.

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# STRESS IN TREES GROWING AT DIFFERENT ALTITUDES

Ewa KĘPA\*

Trees growing in the mountains at high altitudes are usually exposed to extreme and often contrasting conditions with prevailing cool climate, high irradiance and a number of other limiting factors.

Exposure of plants to high irradiance, especially during the period of low temperature, is known to initiate oxidative stress and causes photoinhibition or photodestruction in plants. Plants have developed several defence systems that prevent or retard the demaging processes thus enabling the trees to survive. Photoinhibition of PSII is one of the photoprotective and acclimatory strategies of plants under excessive light. This strategy is realized by plants occupying higher stands in the mountains.

Oxidative stress and damage of PSII reaction centers lead to a decline in the photosyntetic quantum yield. The ratio of Fv/Fm is a useful parameter in evaluating the PSII photoinhibitory damage in higher plants.

Needles of *Picea abies* were collected in spring, when the trees were partially covered with snow. Needles collected from the plants at the highest stands were characterized by partial photoinhibition. The decrease of fluorescence parameters was reversible, at least partly, after the branches had been kept for some days in the laboratory conditions. The reactivation of photosynthesis, measured at higher temperatures, was quicker in branches from below the snow cover in comparison with those exposed to irradiation above the snow cover.

Two main SOD isoforms were determined in needles of *Picea abies* and classified as CuZnSODs. The activity of both SOD isoforms, evaluated using semiquantitive method of PAGE, increased with increasing altitude. The plants from higher stands cure the strong oxidative stress and have developed higher detoxification capacity as a response to the prevailing, uncomfortable environmental conditions.

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# EFFECTS OF FOTOMODIFIED FLUORANTHENE ON LACTUCA SATIVA (L.), ALLIUM CEPA (L.) AND SOLANUM LYCOPERSICUM (L.) DURING GERMINATION AND EARLY SEEDLING DEVELOPMENT

## Eva KMENTOVÁ<sup>1</sup> and Marie KUMMEROVÁ<sup>2</sup>

Polycyclic aromatic hydrocarbons (PAHs) are group of ubiquitous environmental organic polutants (FERNANDEZ et al., 1992; POTHULURI et al., 1992). These persistent organic compounds penetrate into the food chain and cooperate as potential carcinogens, mutagens and teratogens (WANG et FREEMARK, 1995). In this connection it is necessary to study the effect of selected PAHs on higher plants which are the dominant components of terrestrial ecosystems. The fate of PAHs in environment is influenced by many of abiotic and biotic factors (e.g. UV radiation) (MACKAY et al., 1992). Photomodified products of PAHs are more water-soluble than parent compounds and therefore they can migrate to the environmental compartments which are not exposed to the sunlight (e.g. to the soil) and exert toxic effects on the germination and growth higher plants (REN et al., 1996). The influence of intact and photomodified fluoranthene (2 000, 5 000 and 10 000 g.l<sup>-1</sup>) on germination (energy of germination and germination rate) and growth of seedlings (elongation, fresh and dry weight of root and hypocotyl). The obtained results demonstrated that the changes of length and dry weight were more significant responses of the phytotoxicity of the intact and photomodified fluoranthene than the germination. The phytotoxicity of the intact and photomodified fluoranthene than the intact fluoranthene. The *Solanum lycopersicum* (L.) was the most sensitive species.

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# BIOINDIKATION OF THE PHYSIOLOGICAL STATE OF BEECH (FAGUS SYLVATICA L.) IN DIFFERENT STRESS CONDITIONS

### Jaroslav KMEŤ\*

At present, it seem very urgent to find the ways for discovering the stress load on forest tree species, determining latent damages and diseases, because they could warn us about the possibility of development of more progressive phases of diseases. The objective of a practiceoriented ecophysiological research of damages on forest tree species and forest stand must be providing a clear answer to the question, what are the mechanisms by which stressors and stressor patterns of a particular site disturb the physiology of trees and forest ecosystems. To describe the symptoms and causal factors of latent damage, and to predict the consequences of damage for tree growth and survival, we started a research on tolerance and dynamics of damage on young beech stands on two localities under a different pollution load (localities Kovacova and Ziar nad Hronom).

There were chosen 5 individual beech trees in age of about 12 years on both localities. We carried out the sampling for quantitative analysis of *a* and *b* chlorophyll using the disc method. The total chlorophyll and chlorophyll *a* and *b* of the extracts were determined spectrophotometrically accordind to LICHTENTHALER (1987). To determine the parameters of chlorophyll *a* fluorescence fast kinetics ( $F_0$ ,  $F_m$ ,  $F_v$ ,  $F_v/F_m$ , Area,  $T_m$  – nomenclature by KOOTEN & SNEL 1990), a portable fluorometer was used (PEA Hansatech Ltd.).

We consider the phenomenon of generally higher concentrations of chlorophylls *a* and *b* at the locality in the Žiarska valley, which is under a strong pollution load, to be important. The dynamics of the course of selected parameters of fast kinetics of chlorophyll fluorescence  $(F_v/F_m)$  shows a better physiological state of assimilatory organs and consequently the observed beech trees on the EES Kováčová locality.

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# EFFECT OF SELENIUM ON THE ACCUMULATION OF CALCIUM IN ZEA MAYS L. PLANTS

### Krystyna Pazurkiewicz-Kocot<sup>1</sup>, Witold Galas<sup>2</sup>

Selenium (Se) is a trace element that has several essential functions in living organisms. Although for the animals its role is well defined - in the case of plants still needs further investigations (BROWN and SHRIFT 1982, ARVY, 1993). In this paper the effect of the selenium (SeO<sub>2</sub>) on the accumulation of calcium in the leaves and roots of Zea mays L. was investigated. The experiments were carried out with 8-9 days old maize plants (Zea mays L. var K33xF2) grown on the Hoagland's medium at temperature about 25°C. Seeds of maize were cultivated in the darkness on the moist filter paper and individual seedlings were transferred into an aerated solution containing the macro- and microelements. The concentrations of selenium in external medium were: 10<sup>-5</sup> mol (SeO<sub>2</sub>)·dm<sup>-3</sup>, pH of the medium was 6,5. Accumulation of selenium and calcium were studied as function of exposition time of the plants of Zea mays L. to the SeO<sub>2</sub> solution. The strongest effect of selenium accumulation was observed for 5 days and longer treated plants. On the other hand, selenium ions in concentration  $10^{-5}$  mol (SeO<sub>2</sub>)·dm<sup>-3</sup> change the uptake and accumulation of calcium ions in plant leaves in comparison to control. The accumulation of calcium and selenium in the leaves and in the roots of maize was measured by emission spectroscopy using sequential spectrometer with excitation by argon inductively coupled plasma technique (ICP-AES). Analytical lines were: Se 196,026 nm; Ca 317,933 nm. Standard solutions Ca and Se (supplied by Merck) of 1 mg·ml<sup>-1</sup> were used as a reference. Each sample of ca. 0,5 g dry matter was treated by conc. HNO<sub>3</sub> and digested. At the and of mineralization 30 % H<sub>2</sub>O<sub>2</sub> was added until mineralization was completed. Selenium ions probably interfere with calcium ions, thus causing deficiencies or adverse ion distribution within the plant.

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# DIFFERENT ACTIVITY AND RESISTANCE OF BRADYRHIZOBIUM JAPONICUM STRAIN IN SYMBIOSIS UNDER WATER STRESS

### Anna KUBOVÁ\*

One of the most important sites of biological N2 fixation are nodules that form on root systems as a result of symbiosis host plant and bacteria. Both organism and their activity are extremely sensitive to drought stress (SERAJ et al., 1999; KIRDA et al., 1989). The goal of our trial was to obtain the characteristics of the water deficit and water stress influence on host plant and on microsymbiont. The results are obtained from several seasonal vegetative trials. The symbiosis efficiency was observed on soybean (Glycine Max. Merrill (L) cv. Maple Arrow) inoculated by Bradyrhizobium japonicum strains (D 216, D 536, D 538, D 574, D 575 and D 597) and by the commercial agent Rizobin from the Rhizobium collection at Research Institute of the Plant Production in Prague-Ruzyně. We supposed, that it is possible to find a rhizobial strains more resistant to the conditions of temporary drought and these could be manifested by the total production increasing, growth and protein accumulation. Rhizobial activity was evaluated according to modulation and nitrogenase activity determined by the acetylenethylen reduction method (HARDY et al., 1968.). The value of water saturation deficit (WSD) was used for characterisation of water deficit in the plant tissues under water stress conditions. It was found, that under the influence of soil water deficit (during hot summer day - 38°C) the water deficit in leaves of soybean plants was increased from 8 % up to 30-35 %. Under reduced watering (35-40 % MWC - maximum water capacity) the water deficit even increased by 5-7 % in comparison with the optimum watered trial. The 40 % WSD is accompanied with hard wither and damage of leaves parts what corresponds to symptoms of sub-letal water deficit. After rehydration the plants with reduced watering were able to liquidate the water deficit faster then optimally watered plants. Nitrogenase activity indicating the ability of nodules to fix N2 was significantly higher under the conditions of reduced watering. The highest activity was shown by D 216, D 575 and D 597 strains. Repeated stress influenced very negatively not only Rhizobium activity but, it even caused nodules dying. In the frames of treatment C the highest activities were at D216 strain and Rhizobín.

Reactions of the plants to the water stress are different in individual growth stages. 7-days water stress during the stage of flowering reduced biomass production by 21 %, seed production by 6 %. Soil water deficit (21 days of 1/2 dose of optimal watering) since the start of seeds filling up to ripening led to biomass reduction by 50,8 % and seed production by 45,22.

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# THE PROTECTIVE EFFECT OF ELEVATED ATMOSPHERIC CO<sub>2</sub> CONCENTRATION OF MAIZE HYBRIDS SUBJECTED TO CHILLING

## Renata BACZEK-KWINTA\*, Janusz KOŚCIELNIAK\*

The aim was to investigate the effects of an enhanced  $CO_2$  (700 µL L<sup>-1</sup>) on  $O_2^-$  production and antioxidants activities in leaves, leaf membrane injuries and photosynthesis after 7 and 11 days of continuous chilling (7 °C) of maize hybrids differing in chilling sensitivity. Control plants were chilled under ambient  $CO_2$  concentration (350 µL L<sup>-1</sup>). Following 11 days of stress, membrane disruption (measured as electrolyte leakage), the drop in photosynthesis, and chill-dependent inhibition of catalase activity were diminished by elevated  $CO_2$  in both genotypes, compared to control.  $CO_2$ -treated seedlings of chilling-sensitive genotype K103 x K85 showed reduced  $O_2^-$  generation (visualised by NBT staining) in leaves, comparing to plants of ambient concentration. In chilling-resistant KOC 9431, a drop in photochemical efficiency of PS II (Fv/Fm) was inhibited by  $CO_2$ . Superoxydismutase activity and soluble protein level was influenced by genotype, and constitutionally higher in chilling-resistant than in chilling-sensitive plants.

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# THE EFFECT OF FROST HARDENING OF MOTHER PLANTS ON THE FROST RESISTANCE OF WINTER OILSEED-RAPE CALLI

## Elzbieta MARKOWSKA-KOZAK\*

It is known, that the physiological state of a donor plant influences callus tissue. The aim of the present work was to answer the question whether the hardening of mother plants affected frost resistance of callus tissue. Callus of winter oilseed rape *cv* Górczański was induced on petioles from 8 week-old seedlings and grown on the MS agar medium supplemented with 1 mg dm<sup>-3</sup> BAP and 0,5 mg dm<sup>-3</sup> 2,4-D in 16h/8h (day/night) photoperiod at 20° C for 4 weeks. Next calli were cut off and cultured during 8 months (passages every 2 weeks). Mother plants were grown under different thermal conditions and differed in their frost resistance: 12/20 °C, 12/12 °C (day/night) – prehardened plants (higher frost resistance), and 20/12 °C, 20/20 °C- non-prehardened plants (lower frost resistance). After 8 weeks some of the plants were cold acclimated for 4 weeks at 2°C – reached the high level of frost resistance in comparison with not acclimated control plants. Frost resistance of callus tissue was estimated using several methods: 1) decrease in triphenyltetrazolium chloride reduction rate during freezing, 2) increase in callus fresh and 3) dry weight during 14 days after freezing. The results indicated that callus tissue preserved cold acclimation of mother plants.

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# THE EFFECT OF PREHARDENING OF EXPLANT SOURCE ON FROST RESISTANCE OF WINTER OILSEED-RAPE CALLI

## Elzbieta MARKOWSKA-KOZAK\*

The aim of the present work was to answer the question whether the prehardening of mother plants affected frost resistance of callus tissue. Callus of winter oilseed rape cv Górczański was induced on petioles from 8 week-old seedlings and grown on the MS agar medium supplemented with 1 mg dm<sup>-3</sup> BAP and 0.5 mg dm-3 2,4-D in 16h/8h (day/night) photoperiod at 20 °C for 4 weeks. Next calli were cut off and cultured during 8 months (passages every 2 weeks). Mother plants were grown under different thermal conditions and differed in their frost resistance: 12/20 °C, 12/12 °C (day/night) – prehardened plants (higher frost resistance), and 20/12 °C, 20/20 °C- non-prehardened plants (lower frost resistance). Frost resistance of callus tissue was estimated using several methods: 1) decrease in triphenyltetrazolium chloride reduction rate during freezing 2) increase in callus fresh and dry wight during 14 days after freezing, 3) decrease in metabolic heat production during freezing. The results indicated that callus tissue derived from prehardened plants was more resistant to frost and repaired faster their damages after freezing in comparison with callus derived from not prehardened mother plants.

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## ALUMINIUM TOXICITY IN PLANTS: STRUCTURAL AND FUNCTIONAL ASPECTS

#### Igor Mistrík\*

Aluminium toxicity is a serious problem in acid soils. Several recent studies have shown that Al resistance involves mechanisms reducing the Al concentration in both apoplasm and symplasm (excluders). Especially, exclusion of Al from the root apex due to the release of Al-chelating ligands (e.g. malate) or root induced increases in rhizosphere pH show a good correlation with Al-resistance. Mechanisms of Al tolerance which enable plants to tolerate high Al concentration inside the cell (accumulators) are more fragmentary but significant progress have been made in these area over the past 10 years. The progress in understanding of both Al-resistance and Al-tolerance mechanism will be the starting point for the generation of agriculturally important crop species able to grow in acidic soils all over the world. The initial and most dramatic visual symptom of Al toxicity is inhibition of root growth which can occur within 1-2 h after exposure to Al (DELHAIZE & RYAN, 1995). Other visible symptoms of Al toxicity include swelling of the root tip, and sloughing off of the root epidermis (BUDÍKOVÁ, 1999). However, several physiological and biochemical processes precede morphological changes. Among the earliest symptoms of Al toxicity in different plant systems, reduction of net  $Ca^{2+}$  uptake a decrease in Mg<sup>+</sup> uptake (RENGEL, 1990), reduction in K<sup>+</sup> efflux, blockage of plasmalemma Ca<sup>2+</sup>-channels, as well as changes in root cell membrane potential (PAVLOVKIN & MISTRÍK, 1999). Each types of Al resistance which decrease the concentration of toxic form of Al, can reduce the frequency of interaction of Al with apoplastic side of plasma membrane. Synthesis of callose and lignification of cell walls also belong to the well documented structural adaptation affecting Al concentration in root apoplast (HORST et al., 1997). Among the long-term responses that take at least several hours to develop and influence the resistance of plant cells to Al are changes in synthesis and accumulation of low-molecular binding polypeptides (phytochelatins) that might play a role in metal tolerance. Based on the results of our studies on maize and barley cultivars it is evident that changes in polypeptide patterns of extracellular and intracellular proteins of roots and leaves usually appear after 24 h exposure of plants to Al (HUTTOVÁ et al., 1998). Specific induction of apoplasmic polypeptides in resistant barley cultivar support the view about the importance of apoplasm in Al resistance (TAMÁS et al., 1999).

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## CAM PLANTS AS A MODEL IN STRESS STUDIES

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There are three main types of photosynthesis:  $C_3$ ,  $C_4$  and CAM (Crassulacean Acid Metabolism). CAM metabolism was for the first time described for Crassulacean family but this type of metabolism is taxonomically widespread among vascular plants. Now, there are known more than 16000 species representing 33 familes, ranging from the ferns through gymnosperm to eight families of monocotyledones and 21 families of dicotyledones (WINTER and SMITH, 1996). Some plants are able to switch from  $C_3$  photosynthesis to CAM. The shift from  $C_3$  photosynthesis to CAM is accompanied by changes in physiology, biochemistry, and gene expression (LUTTGE, 1993). Using these plants for experiments we are able to compare two different modes of metabolism in one species. Here, we present some chosen problems which we are able to solve using CAM plants as a model system. CAM plants offer a very useful model in studies on the effects of different stresses. For example, *Kalanchoe daigremontiana* plant which is very resistant to sulphur dioxide gives very clear and typical increase in SOD activity as a response to the pollutant presence in the air. The huge changes in activity are easy to follow in both acidified and deacidified leaves. The higher increase in SOD activity (due to SO<sub>2</sub> fumigation) in the light in comparison to the dark condition points to the increase of sensitivity in light parallel to decrease in CO<sub>2</sub> concentration. Using acidified and deacidified leaves we can also estimate the penetration of some stressors into the cell and cell compartments (MISZALSKI, 1995).

CAM-intermediate plants depending on the metabolism type, show different sensitivity to stressors, like low light, excess light, high and low temperatures and air pollutants. In our earlier paper we were able to show that in *Mesembranthemum crystallinum* the C<sub>3</sub>-CAM transition is accompanied by increase in activity of antioxidative SOD enzymes at three cell compartments (cytoplasm, mitochondria and plastids). This makes it easy to localize the stress in the cell. This enzyme has conservative sequence and it is similar to the same protein in the other plants. However, it seems to be more resistant to high temperature and other denaturating factors. This makes it easy to test them.

By exposing CAM plants to stress factors at different times of the day it is possible to evaluate the effect of many physiological factors on stress reaction. For example, if we want to estimate the effectiveness of some factors as dependent on photorespiration, we can simply compare the effect of the chosen factors in different photosynthetic phases of CAM (NIEWIADOMSKA et al., 1999).

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# GROWTH DEPRESSIONS IN WHEAT CAUSED BY COMMON BUNT FUNGUS (TILLETIA CARIES (DC.) TUL.)

## Peter PAULECH\*

Tilletia caries (DC.) Tul. is next to Tilletia controversa Kühn the economically important pathogen of winter wheat from the genus Tilletia. One of the visual symptoms of bunt infection is reduction in the wheat plant height (PAULECH, 1998a). In this contribution, I studies on some other parameters not yet analysed in a tests host plant parasite combination fare dealt with. Seed of three wheat differentials cultivars normally used for detection powdery mildew virulence against wheat resistance genes (ŠVEC, MIKLOVIČOVÁ, 1998): Axminster/8xCc (Pm1), Asosan/8xCc (Pm3a) and Khapli/8xCc (Pm4a) were inoculated by dusting spores of the fungus T. caries, race T-22 (PAULECH, 1998b). Infected seed was sown in the field of our Institute during 1999/2000 season. At the maturity, percentage of smutty spikes and smutty plants were detected. After harvest percentage in reduction of whole plant height height, the culm depression, the spike depression and the depression of upper internode, were determined. Percentage of smutty spikes was 76,94 % (Khapli), 74,44 % (Asosan) and 69,56 % (Axminster). Percentage of smutty plants was 78.91 % (Asosan), 76,17 % (Khapli) and 68,75 % (Axminster). Depression in the whole plant height moved (depending on cultivar) from 17,88 to 23,27 %, the culm depression from 17,74 to 20,45 %, the spike depression 11,46 to 13,30 % and the depression of upper internode moved from 17,10 to 19,40 %. There was high significance in all parameters pertaining to the differences between healthy and infected plants. In test host - parasite combination no great differences in of depression percentage between parameters in comparison to the host - parasite combination Tilletia controversa - was noticed. The greatest differences occurred in the upper internode depressions ranging from 43,80 to 72,20 %. This parameter can be used as the first visual symptom for distinguishing this two fungal pathogens.

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## EFFECT OF ALUMINIUM ON MEMBRANES OF MAIZE ROOT CELLS

## Ján PAVLOVKIN<sup>1</sup>, Igor MISTRÍK<sup>2</sup>

Aluminium (Al) is a major component of soils which becomes soluble and phytotoxic when soil pH decreases below 5. For plants that are sensitive to Al, detrimental effect of Al occur primarily in reduction of root growth. Among the earliest symptoms of Al toxicity belong reduction of net Ca<sup>2+</sup> uptake, decrease in Mg<sup>+</sup> uptake (RENGEL, 1990), reduction of  $K^+$  efflux, blockage of plasmalemma Ca<sup>2+</sup>-channels, and changes in membrane potential (KINRAIDE et al., 1992). The aim of presented electrophysiological experiments was the characterisation of the Al-induced membrane potential (Em) changes in intact maize root cells. The Em differences were measured on 3-day old maize roots in epidermal and cortical cells along the whole root axis using standard microelectrode technique (PAVLOVKIN, MISTRIK, 1999). The Em values of root cells ranged between -115 and -146 mV. Em of older cells towards to root base was considerably more negative than that of the young root tip cells. In short term experiments Em has been rapidly and significantly depolarized by Al. This depolarization was concentration dependent and reached the maximum at 150  $\mu$ M Al. The extent of membrane potential depolarization by 100  $\mu$ M Al decreased continuously from the apex to the base of the root. Simultaneously, the number of cells without any response to Al considerably increased in the region from 10 to 35 mm behind the root tip. No Em changes have been observed in cells more distant than 40 mm from the apex. The sensitivity of the cells to Al decreased in radial direction and when the tip of microelectrode reached the interior of the central cylinder Al induced no Em changes. Both the P-ATPase activator fusicoccin (FC) and glucose diminished the depolarizing effect of Al on Em. Exposure of roots to Al treatment retarded potassium efflux from the root tip segments and had no effect on potassium efflux from segments of the root base. The presented results suggest that, in short-term experiments Al does not directly affect the activity of the electrogenic plasma membrane H<sup>+</sup>-ATPase.

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# CHANGES IN ELECTRIC POTENTIAL OF SPRING BARLEY AND MEADOW FESCUE CALLUS TISSUES TREATED WITH FUNGAL ELICITOR

## Agnieszka PŁAŻEK<sup>1</sup>, Maria FILEK<sup>2</sup>

Fungal toxins cause a disturbance in many metabolic processes of host plants. In previous works greater membrane permeability (PŁAŻEK and FILEK, 1998), a decrease in soluble carbohydrate content (PŁAŻEK, 1998), changes in heat emission and phenolic content (PŁAŻEK et al., 2000) in callus tissue under fungal phytotoxins were demonstrated. The electrophysiological measurements can be used as an indicator of plant response to a stress condition. The aim of the present work was the estimation of changes in electric potential of *Hordeum vulgare* (L) and *Festuca pratensis* (Huds.) cells under influence of *Bipolaris sorokiniana* metabolites. The measurements of cell electric potential were performed for 9 days of pathogenesis.

The obtained results showed that barley responded to the elicitor with a disturbance of electric potential of cells generally within all studied time. The most significant increase in potential from 20  $\mu$ V to 45  $\mu$ V was observed within 30 min since the start of elicitation. Then the values of potential decreased up to -25  $\mu$ V (10 hrs after elicitation). In the case of fescue the significant decrease in electric potential of elicited cells (from 30  $\mu$ V to 0) were noted only within the first hour of pathogenesis. Described effects proved earlier observation that barley tissue is more sensitive to *B. sorokiniana* metabolites than fescue one.

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# WATER STRESS AND REHYDRATION: EFFECT OF CYTOKININS

## Jana POSPÍŠILOVÁ\*, Jana RULCOVÁ\*

It is almost impossible to find a single process in plant life that is not affected by both stress and phytohormones directly or indirectly, and phytohormones are considered as main signals in root-to-shoot communication and *vice versa* during stress. Water stress reduces growth and induces stomatal closure which decreases the water efflux and the photosynthetic CO<sub>2</sub> influx. The severe water stress affects carboxylation, electron transport chain activities, many other metabolic pathways, mineral uptake, membrane structure, chloroplast ultrastructure, *etc.*, and accelerates leaf senescence. Cytokinins (CK) have the opposite effects, *e.g.*, they delay leaf senescence or stimulate stomatal opening. In addition, endogenous CK content and activity in xylem sap and/or leaves decreases during water stress. These facts led us to propose a new hypothesis that recovery of plants after water stress might be accelerated by application of synthetic CK. The aim of this research was to determine whether the application of benzylaminopurine (BAP) can speed up recovery of photosynthetic rate of bean plants after water stress by promoting of stomatal opening and/or by retarding senescence.

BAP in concentrations 1 and 10  $\mu$ M was applied to the substrate (sand + nutrient solution) or sprayed on primary leaves of 14-d-old *Phaseolus vulgaris* L. plants sufficiently supplied with water or water-stressed for 4 d (RWC decreased to 70 %). The latter ones were fully rehydrated during following three days. Parameters of photosynthesis and water relations were measured in primary leaves of 7-, 10-, 14-, and 17-d-old plants.

Application of 1  $\mu$ M BAP slightly delayed leaf senescence. In 17-d-old control plants, net photosynthetic rate (P<sub>N</sub>), chlorophyll (Chl) content, and when sprayed on leaves also some of Chl *a* fluorescence kinetic parameters of BAP-treated leaves were slightly higher than those of untreated leaves.

Application of 1  $\mu$ M BAP either to the substrate or sprayed on leaves slightly improved recovery of plants during rehydration after water stress in terms of increased g<sub>ad</sub>, g<sub>ab</sub>, and P<sub>N</sub>, *i.e.*, parameters which were markedly affected by mild water stress. However, contents of Chl *a*, Chl *b*, and carotenoids and Chl *a* fluorescence kinetic parameters were not markedly affected by mild water stress and after rehydration were not stimulated by 1  $\mu$ M BAP.

Higher BAP concentration (10  $\mu$ M) had mostly negative effects on the measured parameters.

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# THE INFLUENCE OF ABIOTIC STRESS FACTORS ON SELECTED PHYSIOLOGICAL CHARACTERISTICS OF BEECH

## **Tibor PRIWITZER**

The aim of the presented paper is a basic analysis of possible impact of abiotic stress factors on selected physiological characteristics (especially photosynthesis and transpiration) of beech foliage. This contribution is based on the results gained within the framework of ecological and ecophysiological research being realized in the research and demostration object (RDO) Pol'ana - Hukavský grúň. More detailed description, as well as the whole spectrum of problems being solved are presented in ČABOUN et al. (1997). For measurement of physiological characteristics we used the gasometric method with portable photosynthesis system Li - 6200 (Li-cor, Nebraska, USA). The measurement were performed in the field conditions directly within the crown of mature beech, from June to September, during favourable weather on physiologically mature leaves. The results of our analysis can be expressed as follows:

- The main abiotic stress factors, which could influence photosynthetic and transpiration rate of beech foliage on our research site were: high air temperature, low air humidity, high irradiance, enhanced ozone impact and drought,
- the threshold value of PhAR, which can be regarded as potentionally responsible for decrease of photosynthetic rate in beech leaves was low, approximately 1000 mol m<sup>-2</sup> s<sup>-1</sup>,
- the photosynthetic rate declined with rising leaf temperature over 32°C and with decrease air humidity under 50 %,
- the values of maximal transpiration rate were lower during the dryer growing season (precipitation 30 % below normal) compared to wet growing season (precipitation 24 % above normal),
- as far as the influence of ozone on photosynthesis is concerned, considerable reduction is at ozone concentration over 50 ppb.

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# **ELECTRICAL CHANGES INDUCED BY T-2 TOXIN**

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Various pathogenic fungi produce biologically substances (elicitors, toxins) which in the course of pathogen-plant interactions induce disease processes. T-2 toxin is chemically defined as trichothecene which has an epoxy grouping at 12,13 level so he is often referred as 12,13-trichothecene. Biologic activity of T-2 toxin is optimum at 8°C and has effect on the stimulation of lipid peroxidation, the change of structure of erythrocytes, the effect on respiration processes, depolarizations of active component of membrane potential and oxidative metabolism of maize root mitochondria.

Corn seeds (*Zea mays* L.) were germinated in darkness at 25  $^{0}$ C in wetted filter paper by the standard solution (2 mmol/l KCl, 0.5 mmol/l CaCl<sub>2</sub>). T-2 toxin was dissolved in UV-grade EtOH. Aliquots of this stock solution were added to standard solution (2 mmol/l KCl, 0.5 mmol/l CaCl<sub>2</sub>). The possible effect of T-2 toxin on corn root was investigated by help the plexiglass measuring chamber. A root current monitor (current to voltage converter) could be subsequently connected to each of the subchambers to record the current passing through the respective part of the root. Since the respective subcompartment was virtually grounded by the current monitor the simultaneously recorded root potential could be referenced to the particular root section being in the respective subchamber the current monitor was connected to. The electrical conductivity versus the root lenght is not linear, mostly in the range of 0,9 to 4  $\mu$ S. In the presence of the toxin increased values of root conductance could be observed. Since in the radial part of the root current path the ion strength of the apoplastic space determines the root current these results indicate a leakage of cytoplasmic electrolytes through the plasma membranes of the cortex cells into the apoplasm, and/or reduced uptake capabilities of that cells. In any case the toxin causes increased conductance figures in the apoplastic space by modifizing the permeability behavior of the cortex cell membranes. T-2 toxin probably entrances to interior root and is transported. Different degree of onthogenesis of xylem elements can be important for transport T-2 toxin by plant.

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# CHANGES IN PHOTOSYNTHETIC APPARATUS OF OILSEED RAPE SUBJECTED TO FROST DEHARDENING IN VARIOUS THERMOPERIODIC REGIMES

## Marcin RAPACZ\*

Cold deacclimation is a relatively little known process. One of the questions is a mechanism of temperature-rise sensing. Spring (Górczański) and winter (Star) cultivars of oilseed rape were cold-acclimated at +5°C and deacclimated during 4 weeks at combinations of +12 and +20°C operating in the light or/and dark with 12-h photoperiod. Deacclimation was faster and more effective when +20°C was applied during the light period. It may suggest the participation of photosynthetic apparatus in perception of temperature during dehardening. Deacclimation was accompanied with changes in some properties of PSII measured at low temperature. The reaction was different in winter and spring-type cultivar. In winter cultivar PSII excitation pressure (1-q<sub>p</sub>) which is consider as parameter connected with temperature sensing (HUNER et al. 1996) was higher in plants dehardened under cold-day conditions. But in the case of spring cultivar, which promoted flowering during the first days of deacclimation. In the following weeks 1-q<sub>p</sub> increases in all cases excluding plants dehardened at continuous 12 °C - which starts to flower in the later term. It may be concluded that promotion of intensive shoot elongation during dehardening may disturb the functioning of PSII-dependend temperature sensing.

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# HISTOCHEMICAL STUDY ON PHENOLIC COMPOUNDS IN LEAF TISSUES OF LACTUCA SPP. INFECTED BY BREMIA LACTUCAE

## Michaela SEDLÁŘOVÁ\*, Aleš LEBEDA\*, Pavla BINAROVÁ\*

Injuries of plant cells or tissues are associated with changes in metabolism of phenolic compounds (BENNETT et al., 1996). These contribute to the resistance response, i.e. alterations pass off at a faster rate in plants with hypersensitive reaction where the pathogen growth ceases with the development of necrosis. To investigate the events taking course in *Lactuca* spp. cells infected by race NL 16 of *Bremia lactucae*, staining methods with fast blue BB, vanilin, aniline sulphate (for light microscopy) and aniline blue (for fluorescence microscopy) were used. Leaf tissues of nine genotypes already involved in previous experiments (SEDLÁŘOVÁ et al., 1999) have been studied 24 and 48 hours after inoculation (hai) as these stages are crucial for the host-pathogen recognition and development of infection process. In resistant genotypes some phenols were detected 24 hai associated with oxidative processes at the beginning of HR which cause browning of the infected cells (cv. Mariska) and adjacent tissue (genotypes of *L. virosa*). In susceptible ones the staining for phenolic compounds was negative and HR occurred very rarely. Callose deposits were situated in the site of penetration, on the neck between primary and secondary vesicles, around haustoria in both susceptible and resistant genotypes.

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# INFLUENCE OF INDUCED ROOT HYPOXIA ON THE BIOMASS PRODUCTION OF TWO GRASS SPECIES

## Karel SUCHÝ\*

Some of the grassland communities occuring in wet rivers floodplains represent a renewable agricultural resource, providing feed or, potentially organic matter for combustion and energy yield. In these communities most important species from agricultural viewpoint are reed canarygrass (Phalaris arundinacea L.) and, especially, meadow foxtail (Alopecurus pratensis L.) (KVĚT et al., 1996). Commonly affected stress factor in these growing conditions is often long-term flooding or waterlogging inducing oxygen deficiency in soil (ARMSTRONG, 1991; ARMSTRONG, BRÄNDLE, JACKSON, 1994). This deficiency, called hypoxia, in root environment cause mostly negative changes in growth and production both underground and aboveground biomass (DREW, STOLZY, 1991). Because the hypoxic conditions in natural soils are relatively wide oscillating reflecting large number of environmental factors and their exact measurement is connected with some difficulties, the plant seedlings were cultivated in liquid nutrient solution on the thin layer of chemicaly inactive substrate (agroperlit). In four experiments where each consist off the control and hypoxic treatment the plants were cultivated one month in plastic pots (volume 4 l for Alopecurus or 10 l for Phalaris) under defined temperature and light conditions in phytotron. Hypoxia was reached with regular lactic acid amendement as organic carbon source (DUNBABIN, POKORNÝ, BOWMER, 1988) and redox potential and content of dissolved oxygen in liquid nutrient solution were measured. After finishing of experiments the roots and aboveground biomass were dried, weighed and subsequently recalculated as dry mass. m<sup>-2</sup>. R/S (root/shoot) ratio was also determined. The pH, O<sub>2</sub> content and redox potential mV value courses were expresed in the graphs. The level of hypoxia gained from lactic acid was moderately or medium reduced. In all experiments aboveground and underground biomass yield was significantly lower under hypoxia, depending on its intensity.

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# CHANGES IN ACTIVITY OF SOME ANTIOXIDATIVE ENZYMES DURING COLD TREATMENT

Tomáš TAKÁČ\*

A main object of our study was to elucidate the potential role of antioxidant enzymes in protecting maize (*Zea mays* L.) seedlings from chilling injury. For this purpose two hybrids with differences in chilling tolerance (chilling-tolerant ULTRA and chilling-sensitive X0954D) were exposed to severe chilling temperature (6/6 °C) for different period (2 and 5 days) and then transferred to optimal temperatures as recovery treatment. Some morphological changes (length and branching of roots) and activity of scavenging enzymes (catalase, ascorbate peroxidase, glutathione reductase) were examined. Analyses were performed in leaves, mesocotyl and roots. Chilling stress caused differences in length and branching of roots. There were different responses of the two hybrids.

Activities of antioxidant enzymes were also affected by chilling stress. Leaf ascorbate peroxidase activity increased in both hybrids especially after 5 days of chilling stress and decreased during the postchilling period. On the other hand, the activity of APX in roots declined. No significant differences between cold resistant and cold sensitive hybrids were obtained. Activity of catalase was much higher in mesocotyl and in leaves then in the roots. Increase in activity became significant after five days of exposure to chilling temperatures. We observed a stronger increase of its activity in the chilling-sensitive hybrid. In leaves after temporary increase a strong decline in catalase activity was observed. Chilling induced also increasing of glutathione reductase activity in mesocotyl. There were no differences in leaves.

These results indicate the real contribution of antioxidative enzymes on cold tolerance of maize. The cold resistant hybrid maintains a better defence against activated oxygen species than the cold sensitive one during low temperature treatment.

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# RATE OF SOIL HYGIENE AROUND THE METALLURGICAL FACTORY

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The conditions of soil hygiene were evaluated according to total, realisable, mobile and acceptable forms of heavy metals in casual xexus whith the composition of solid emissions from the metallurgical works Kovohuty Krompachy and deposited wastes and with the geochemical anomalies in this regions. Experimental localities were selected in relation to the operative emission source of the matallurgical plant, which has been operating since 1920 (production iron, later copper). The chemical composition of solid light ashes containing heavy metals is different, depending especially on their origin. Light ashes emitted from shaft furnaces have a high content of zinc and lead, while those of flame furnaces and conventons contain a high level of copper, iron and arsenic. The soil samples from the experimental localities (Krompachy, Richnava, Klulnavy and Slovinky) were collected by the general rules of sampling valid for the basic network of monitoring agricultural soils. Also the determination methods of heavy metals in soil were the same as those used for monitoring heavy metals in agricultural land. The most significant excess of the indication C values (for sanitation) was detected in copper, and arsenic. Furthermore, the evaluated soil was also found contaminated by zinc, lead, nickel and cadmium. Based on this result, 379 ha of agricultural land (of which 17 % being temporarily uncultivated) have been biologically and chemically recultivated since 1995. The obtained results of an occurrence of heavy metals in soil are of the antropogenic origin, except one ground plot, where a combination of the emission effects and geochemical anomalies was relevant. Extra high contents of mobile and mobilisable forms of copper and cadmium were determinated in shallow cambisols on crystaline rocks of heavy and mediumheavy soils and in gley fluvisols.

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# EFFECT OF STRESS FACTORS ON NORWAY SPRUCE EMBRYOGENIC CULTURE INDUCTION

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Somatic embryogenesis is one of the results of plant survival strategy in stress conditions blocking sexual reproduction. The effect of the duration of the oxygen deficit and cold pre-treatment of zygotic embryos was the first object under study. Optimal pre-treatment took about 5 days a longer period decreased the number of achieved embryogenic cultures. Pre-treatment of 7 days totally blocked the germination potential of seeds. 2,4-D is one of the main stress factors inducing somatic embryogenesis on Norway spruce zygotic embryos. It could combine with injury of embryos. <sup>14</sup>C-2,4-D was used to study the effect of longitudinal bisection on its uptake and metabolism in zygotic embryos. In injured embryos the maximum was reached by 8 hours earlier than in intact ones. On the contrary, the following decrease of the 2,4-D level was much deeper than in intact embryos. Injury also decreased the metabolic activity. The total number of embryogenic cultures achieved on injured embryos was higher than on intact ones.

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# ARRANGEMENT OF SOIL REACTION IN RELATIONSHIP TO HEAVY METAL CONTENTS IN BIOMASS OF SOME AGRICULTURAL PLANTS

## Alena VOLLMANNOVÁ\*, Ladislav LAHUČKÝ\*, Monika HALÁSOVÁ\*

The short time laboratory biological test was realised in defined temperature and moisture conditions. The extremly acid soil from emission region Krompachy with following characteristics was used: pH / KCl = 4,07; humus /Tjurin/ = 4,18 %; HA = 43.31 mmol.kg<sup>-1</sup>; S = 94,10 mmol.kg<sup>-1</sup>; T = 137,41 mmol.kg<sup>-1</sup>; V = 68,48 %; N-NH<sub>4</sub><sup>+</sup> = 19,66 mg.kg<sup>-1</sup>; N-NO<sub>3</sub><sup>-</sup> = 44,38 mg.kg<sup>-1</sup>; P /Egner/ = 24,75 mg.kg<sup>-1</sup>; K /Schachtschabel/ = 64,5 mg.kg<sup>-1</sup>; Mg /Schachtschabel/ = 90,4 mg.kg<sup>-1</sup>.

On one pot /700 g of soil/ 0,66 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0,66 g of superphosphate a 0,26 g of 60 % KCl in unlimed variants was applied and in limed variants also 3 g CaCO<sub>3</sub> was added. Tested plants were: maize, sunflower, bean, oats a lucerne. Oats and maize were collected after 36 days, another three plants after 45 days from formation of experiment. After mineralisation of plant samples heavy metal contents (Fe, Mn, Zn, Cu, Co, Ni, Pb, Cr and Cd) were determined by AAS method. In most of samples (88,9 % of all samples) positive influence of CaCO<sub>3</sub> application was observed. The most significant influence of CaCO<sub>3</sub> showed sunflower by determination of Mn content (only 14,66 % of Mn was determined in limed variant in comparison with its content in unlimed variant). In some samples (11,1 % of all samples) no influence of liming was observed, even heavy metal content in limed variant (e.g. Fe content in limed variant of lucerne was 173,82 % of its content in unlimed variant).

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# THE EXTENT OF ATTACK OF SELECTED GRASS SPECIES BY THE FUSARIUM FUNGI

## Bohumila VOŽENÍLKOVÁ\*, František KLIMEŠ\*

Our observations concentrated on a study of the spread and harmfulness of pathogenic fungi (the genus *Fusarium*) found on the dominant grass species (*Deschampsia caespitosa, Festuca rubra, Holcus mollis*) growing in a permanent grass growth in the central part of the Bohemian Forest (location Zhůří; elevation 1050 - 1080m.). The following three variants of cultivation were observed at the experiment site: (1) mulched growth (mulched only once), (2) unharvested growth (fallow land), and (3) mown growth (mown once). Plant pathology evaluation was carried out during the vegetation period on three dates. Our observations showed the greatest development of the *Fusarium* fungi on the unharvested growth. On the other hand the least extent of attack was found on the mown growth. The least harmful attack by the fungi was found on *Deschampsia caespitosa* and the most serious damage was found on *Festuca rubra*. As far as the time dinamics of the *Fusarium* fungi development is concerned the greatest development of disease was noticed at the beginning of the second decade in May.

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# LOW TEMPERATURE AFTER EFFECT ON RAPE (BRASSICA NAPUS L.) CALLUS RESPONSE TO SECONDARY METABOLITES OF PHOMA LINGAM (TODE EX FR.) DESM.

## Iwona ŻUR<sup>1</sup>, Katarzyna HURA<sup>2</sup>

Plants react to both biotic and abiotic stress factors, like pathogen infection or low temperature by activating sophisticated defence mechanisms. Induction of defence responses demands additional energy what in the early events should change the rate of cell metabolism. We study if low-temperature hardening influence the metabolism and resistance of *Brassica napus* (L.) calli treated with secondary m metabolites of *Phoma lingam* (Tode ex. Fr.) Desm.

Calli were exposed to low temperature stress (two weeks at  $2^{\circ}$ C) before treatment with fungal elicitor. The measurements of respiration, heat emission and tissue viability were performed after 0, 1, 2, 3 and 9 days of elicitor treatment.

Compared with the control, hardened tissue showed considerably lower respiration rate during first 48 h after elicitation, then the enhancement of the process to 135% of control was noted. Heat evolution was not significantly affected by hardening, although in general elicitor treated calli produced heat at lower rate. The analyse of tissue viability indicate increased metabolic activity (at about 20 %) of hardened calli treated with fungus metabolites, whereas unhardened calli decreased its viability at about 50 %.

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# **RESPONSE TO CADMIUM IN BRASSICA NAPUS (L.) PROTOPLAST CULTURES**

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There are controversial data if protoplasts could serve as a biological model for stress studies. The main disadvantage of such system is the stresogenic isolation procedure which could replete defence system to such extent that it become insensible to any other stressors. There is also possibility that protoplasts lack some substantial unit of signal transduction system. From the other hand there are some data indicating that defence reaction characteristic for plants and suspension cultures are still present in protoplasts. In the experiment protoplast cultures of *Brassica napus* have been used as a possible model system for the study of Cd-toxity in plants.

Protoplast cultures were exposed to 2.5, 12.5 and 25  $\mu$ M·dm<sup>-3</sup> Cd during 3-day culture and examined by light microscopy determining protoplasts viability and possible changes in their morphology (size, shape, structure alterations).

Cadmium influenced viability of protoplasts just 24 h after treatment, increasing number of damaged cells to even 250 % as compared with the control. Toxic effect increased with increasing level of Cd and length of exposure. The highest Cd concentration resulted also in decreased protoplasts size.

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