BIOPHYSICAL NON-INVASIVE METHODS FOR STRESS DETECTION IN PLANTS

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Approaches to the assessment of the potential effect of environmental stressors have changed substantially in recent years. More attention is given now to the modern modifications of biochemical and biophysical methods, by which incipient stress can be detected well before any visible changes are noticed. Biochemical and molecular alterations are usually the first detectable, quantifiable responses of plants to environmental change. To the most important biochemical markers indicating stress belong stress proteins, stress phytohormones, antioxidative enzymes and substrates. Despite their utility in laboratory, biochemical methods have not been extensively applied in the field research. Instead, rapidly increasing popularity of biophysical approaches can be observed in most research centres. Three of them are discussed in our contribution: *Infra-red analysis of metabolic carbon dioxide exchange as a tool for indicating stress, Application of chlorophyll fluorescence analysis in stress physiology of plants* and *Reflectance techniques for assessment of a plant physiological status.*

The increasing availability of highly sophisticated and easily portable instruments for biophysical, non-invasive analysis of plant responses makes detection of early stress symptoms relatively easy, but not without some danger of misleading interpretation. In selection of the most appropriate instrumentation, specific advantages and disadvantages of the mentioned method and their complementarity (in some respects) should be considered. *Gasometry* of CO_2 exchange is the only source of *absolute* data on changes in carbon metabolism and energy resources in plants under stress. It is the most versatile approach, but, on the other hand, it is rather complicated and time consuming if properly used. Enclosure of plant parts into climatized leaf chamber is usually necessary during measurements, which excludes assessment of photosynthetic responses at larger scales (see GLOSER & GLOSER, 1996). *Fluorimetry* (for an exhaustive review see ROHÁČEK & BARTÁK, 1999) of chlorophyll *in vivo* gives only relative data on changes in photosynthetic apparatus in plants under stress. The photochemical processes in photosystem II are mainly monitored, but some indirect estimation of CO_2 fixation rate is also possible. It is very fast and sensitive method for early stress symptoms detection, but causal analysis of the obtained data is in some cases difficult. Validation of results by some other, more "direct" method (gasometry, biochemical analysis) is much desirable. *Reflectometry* (spectral reflectance of leaves - reviewed by PEÑUELAS & FILELLA, 1998)) provides also only indirect data, which may be used for derivation of their structural or functional changes indicating stress (including water and nutrient deficiency). This method may be easily applied to the stress detection at larger scales (remote sensing of the whole canopy).

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VERTICALLY AND HORIZONTALLY DIVIDED COMPENSATION SOIL LYSIMETERS FOR CONTINUAL MEASUREMENT OF WATER UPTAKE BY DIFFERENT ROOTS AND PART OF ROOTS OF INTACT ROOT SYSTEM

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We designed two types of the soil lysimeters, vertically and horizontally divided. They provided us to measure water uptake continuously and during the whole growing season of maize plants, separately by the seminal roots and nodal roots, and from three (or more) different soil zones. The first type, vertically divided (doubled) compensation lysimeter consist of two separate containers (1.2 m depth, 0.0275 m^3 total volume), make from PVC (Novodur) tube. On top they are connected by a small hole through which mesocotyl diversificated, so the seminal origin roots and nodal vegetative origin adventitious roots of maize plant (for example) developed in separate compartments, and so their water uptake could be separatelly measured. The stable level of underground water is maintained in 1 m depth of soil, and this is comparable with underground water level in the soil around in our conditions. The water and soil are passively aerated through a narrow plastic tubes with holes. The water level is maintained by means of stabilizers connected with water supplying burettes through solenoids that are electronically controlled. Water uptake is here measured volumetrically, daily during season. The second type is compensation lysimeter horizontally divided in three or more compartments representing soil zones, which are isolated one from other by the layers of bee wax applied on gause, to prevent freely water exchange between the compartments. But, growing roots can freely penetrate through the wax from upper into the lower standing compartments. The soil water potential in each of compartment is controlled and could be changed independently, from the field capacity to about permanent wilting point. Soil water potential inside of individual compartments could be daily measured by plaster blocks, which are permanently situated in the middle of each compartment. The plaster blocks are connected with digital equipment showing changes in suction pressure of soil (in kPa). From these changes and portions of added water, minus evaporation loses, is then calculated water uptake by different parts of roots along the intact root system penetrating down through soil zones deep into the soil.

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THE NEW ASPECTS IN STUDY OF XENOBIOTIC EFFECTS ON PLANTS

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Xenobiotics (hazardous or risk substances, contaminants, pollutants) are either organic and inorganic substances, which are not natural components of the ecosystems or in higher natural concentration can act harmful or toxic. For professional and uniform evaluation of the xenobiotic effects on plant organisms, the specific plant toxicity testing (OECD Tests) was recently established. This paper describes the acute and chronic toxicity testing, characterization of both, the most important biomarkers and LC_{50}, EC_{50} and IC_{50} parameters, as well as some of found results concerning the Cd effect on physiological characteristics of medicinal plant *Hypericum perforatum* L.

Cadmium (trace element and toxic metal), being a by-product of many industrial processes, is a major environmental pollutant. It is easily taken up and accumulated by the plants. As its toxic effect on plants is very complex, an accurate distinction between direct and indirect mechanisms of Cd action is complicated. One of the most important reasons for Cd toxicity is an interaction between Cd and Fe. Cd-induced Fe deficiency leads to serious disturbances of the photosynthetic apparatus like inhibition of chlorophyll synthesis, decrease in the pool of Fe-containing electron carriers like cytochromes and ferredoxin, and disorganisation of the chloroplast structure. However, the further Cd effects on different plant organisation level were observed.

The effect of $Cd(NO_3)_2$ on both chloroplasts suspension and young plants of St. John´s Wort (*Hypericum perforatum* L.) were studied. The growth characteristics (root and shoot biomass), photosynthetic and dark respiration rates, content of chlorophyll and total carotenoids as well as fluorescence emission spectra of chloroplasts were estimated. On the basis of found results it could be concluded that *Hypericum perforatum* belongs to the group of plants known as hyperaccumulators. This medicinal plant can be applied not only in phytotherapy (anti-depressant and antiviral effects, dermatitis, burns, rheumatism, gastroenteritis) but it could be also used for remediation of contaminated substrates. There exist two ways: via accumulation of metals into the plant tissues and organs or through the secondary metabolites (biotransformation).

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THE STRESS CONCEPTS AND THE SEARCH FOR OPTIMUM CONDITIONS

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In this contribution, basic definitions and concepts of stress in plants are summarised. Furthermore, the general adaptation syndrome as a response to various types of environmental stress is considered. Finally, plant growth under optimum conditions as opposed to that under stress conditions is evaluated. "Stress is the nonspecific response of the body to any demand"….."Confusion between stress as both an agent and a result can be avoided only by the distinction between 'stress' and 'stressor'." (SELYE, 1973). A stressed plant decreases its activity or growth (OSMOND et al., 1987), but the usefulness of unfavourable conditions for plant survival was generally accepted. For example LARCHER (1987) has stated that "Stress ist ein notwendiger Trainingsanreiz im Leben". In fact, no plant is able to perform its whole living cycle without experiencing multiple unfavourable environmental effects (SEELEY, 1990). In an extensive review, LESHEM & KUIPER (1996) analysed whether there is a general adaptation syndrome response to various types of environmental stress in plants. They dealt with several environmental stress categories, i.e. heat, drought, salinity, chilling, freezing, oxidative stress, desiccation, ozone and UV-B radiation. All these stressors seem to manifest similar endogenous "pleiotropic general adaptation syndrome responses whose final expression includes the concerted production" of oxy-free radical scavengers and anti-oxidants, osmoregulants, abscisic acid, jasmonates, nitric oxide, heat shock proteins and phytochelatins. On the other hand, e.g. PALTA (1990) emphasised the general importance of changes of the membrane properties. According to his measurements, early events responding to unfavourable environmental factors at the cellular level include functional and structural changes in the membrane. Nevertheless, the problem of the general adaptation syndrome response might be more complicated. LAKSO (1990) underlined the importance of the plant organisational level and both morphological and anatomical variations that play an important role in the relationship between the environment including its adverse effects and resulting physiological responses. A plant may react differently according to the hierarchical level of the performed study. Also, plant responses to an environmental stress may be modified by a range of other environmental factors both prior and during the experiment.

In many experiments plants are supposed to be cultivated under no-stress conditions, i.e. under optimum environment. However, it seems to be practically impossible to identify optimum conditions for the whole vegetation of a plant (for review see NÁTR, 1992).

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TISSUE CULTURE AS AN ALTERNATIVE METHOD FOR STUDY OF PLANT RESPONSES TO STRESS.

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Several studies showed that tissue culture system can provide a rapid alternative method for screening of response and tolerance of plants to various natural and anthropic stress factors of the environment (HASEGAWA et al., 1984; STAVAREK, RAINS, 1984; KISHINAMI, WIDHOLM, 1986; LANGEBARTELS, HARMS, 1986; MARÓTI, BOGNÁR, 1985, 1988; BEN-HAYYIM, GOFFER, 1989). The advantage *in vitro* systems is that they are defined, can be controlled and modified. They provide the opportunity to simulate various stress factors which are difficult to regulate under natural conditions (mineral and water stress, extreme temperatures, pH, heavy metals and other chemical compounds). Incubation of plant cells and tissues under stress conditions has enabled to study the adaptive processes, to determined the limits of cell and tissue tolerance for toxicity of stress factors and to ascertain differences in their tolerance. *In vitro* cultures offer possibility for selection and isolation of resistant cell lines and subsequent regeneration of plants tolerant to water stress or to heavy metals. Regenerated tolerant plants themselves represent convenient system for study of the adaptation process to actual stress factor as well as its dependence on the genotype from which the plant was initiated. Callus tissue or cell suspension cultures are considered to be very suitable for studying phytotoxicity of various environmental factors. According to our experience, the shoot cultures can also be used in such studies. The performed experiments confirmed the influence of lead (in the form of $Pb(NO_3)_2$ in concentrations 10^{-4}- 5.10^{-5}) in *Quercus robur* L. as well as pH value of medium (4,0-6,0) in *Vaccinium corymbosum* L. (cv. Berkeley) on multiple shoot of formation.

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THERMODYNAMIC STATE OF WATER AT LIMITS OF PLANT DEHYDRATION

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Dehydration of plants during moderate water stress can cease physiological functions, such as transpiration, photosynthesis, which are mostly restricted by other functions sensitive to water deficit, in this case, by closing stomata. After rehydration, these functions reached the same level. On the contrary, at severe water stress induced by prolonged dehydration of plant tissue, water content can reach the critical value for physiological functions, important for plant viability. After rehydration from this critical value, the physiological activity of some functions is mostly unrecoverable. This critical water content can be reached by primary water stress, occurring during the drought period. It can occur also as a secondary stress acting simultaneously with other stresses e.g. during freezing stress, when the water content in plant cells reaches the critical water content by extracellular freezing of intracellular water. The life strategy of some plant parts such as seeds, pollen, tree buds, and/or resurrection plants is to survive at low water content close to the critical water content. During dehydration of plant tissue the changes of solvent concentration affect kinetics of reaction and the thermodynamic interaction (hydrogen bonding). Since the critical water content occurs at the low water content, it is not easy to measure its thermodynamic activity. Water content, close to the critical water content, is called non-osmotic water volume or unfrozen water or simply bound water. The bound mononolayer of water can be calculated from water vapour isotherms by BET (Brunauer-Emmett-Teller) model. This model defines the bound water thermodynamically, what is important for recognizing various parts of bound water. Thermodynamic characteristics of water activity, characterised by equilibration at relative humidity over different saturated salt solutions of known water activity, or by water potential, are not able to explain fully all limits of dehydration tolerance. The concept of vitrification (from Latin, vitreus, glassy) of cell solution to glasses and rubbers as amorphous solids has been introduced to explain the kinetics of chemical reaction at low water contents. The glass transition temperature of dehydrated samples is mostly measured by differential scanning calorimeters in various modifications. Measurements of vitrification in the plant can help to explain how water activity participates on plant survival in various stresses. Similarly, it can help us to manage experimentally the water status of plants with the aim to store the meristems and seeds in viable state to preserve plant biodiversity.

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STRESS IN RELATION TO CROP PRODUCTION

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Sustainable development of agroproduction and resource conservation is essential for achieving and maintaining food security. That requires better and more comprehensive insights of ecologically sound crop production processes; especially, in fragile environments and resource-poor countries.

Feeding a world population of 8 billion people in 2020 requires strengthening of research; education and technology transfer through partnership among institutions in developed and developing countries. Education is vital to inform producers, consumers and leaders about the full range of challenges and opportunities with advanced technologies in agriculture.

Crop science should be recognised as the key-technology for increasing the production and quality of food, feed and biomass for industrial and energy use. Crop science play a vital role in improving the quality of life for human beings by producing food and renewable resources in a sustainable and safe way.

To foster a greater understanding of environmental impacts, and to promote efficient resource use, knowledge and research tools in crop science should be combined with advances in geospatial information and communication technologies.

There is need of new ideas and data to help:

- identify the economically relevant differences in biophysical conditions around the world (SHERR, 1999)
- analyse their effects on productivity in agriculture and the whole economy (NÁTR, 2000)
- prescribe appropriate responses through agricultural research and government policy (HÚSKA, 1999)

The key topics should include therefore: climate and moisture, soils and nutrients, pests and diseases, crops choice and management, genetics and input response (including genetically modified crops) and food and agricultural policy. In all mentioned areas the stress research plays important role.

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