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REVIEW

Plant physiology, genetics, biotechnology and pathology in the Institute of Experimental Botany of the Czechoslovak Academy of Sciences (1962-1992)

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Abstract

The main scientific results achieved in individual departments of the Institute of Experimental Botany during 30 years of its existence are briefly summarized. They include methods of studying photosynthesis, ontogenetic changes of photosynthetic characteristics, stress factors affecting photosynthetic activities, photosynthesis of transgenic plants and during *in vitro* cultivation, roles of auxins and cytokinins in plant growth and development, development and testing of new plant growth regulators, models of organogenesis *in vitro*, metabolic and mutagenic activities of phenolic substances, hormonal regulation of flowering, activities of promutagens (nitrosamines, 7,12-dimethylbenzanthracene), model systems of genetic damage, repair synthesis and post-replication repair, developmental pollen biology and biotechnology, extracellular nucleolytic activity of pollen, selection of apple scab immune cultivars of apple tree, chemotaxonomy of *Fabaceae* and *Allium* species, selection pressures in embryoids, somatic embryogenesis and nuclear genome changes in plant cell and callus cultures, discoveries of new plant viruses, virus spread and persistence in crops, development of polyclonal and monoclonal antibodies, role of oxidative pentosephosphate cycle in biosynthesis of viral RNA, and virus diseases of forest trees.

In 1891, the Czech Academy of Sciences and Arts was founded in Prague thanks to the efforts of Josef Hlávka. On its basis, the Czechoslovak Academy of Sciences was set up in 1952. One of the research institutes of the Academy was the Biological Institute. In 1962, the Biological Institute was divided into several institutes. One of

them, the Institute of Experimental Botany, which originated from the departments of physiology, genetics and pathology of plants, now celebrates 30 years of its scientific activity. The Institute publishes the journals Biologia Plantarum (from 1959) and Photosynthetica (from 1967) and in its laboratories the bibliographic annuals Photosynthesis Bibliography (Šesták and Čatský 1974-1992) and Water in Plants Bibliography (Pospíšilová and Solárová 1977-1992) are prepared. The Institute concentrates on research in plant physiology (photosynthesis, plant growth and development), genetics, biotechnology and pathology. In this review we present the main findings achieved by researchers in individual departments of the Institute during its existence.

In the **Department of Physiology of Photosynthesis**, important results have been achieved in four research directions:

(*1*) Among inventions and improvements of methods for studying photosynthesis, an elaboration of a field method based on potentiometric or colorimetric CO_2 determination, the first use of the differential mode of infrared gas analysers in gas exchange measuring systems, the construction of new assimilation chambers, improvements of colorimetric, spectrophotometric and paper and thin layer chromatographic methods of chlorophyll determination, should be mentioned. A new method for the measurement of water saturation deficit was also developed. These studies resulted in the publication of four successful methodological manuals (Slavík 1965, 1974, Šesták and Čatský 1966, Šesták *et al.* 1971). Later, methods of chloroplast immobilization and photochemical activities of immobilized chloroplast isolated from leaves of various ages and water deficit were tested (Synková *et al.* 1990, Synková and Šesták 1991).

(*2*) Comprehensive studies of the ontogenetic changes in leaf characteristics began with the papers of Šesták and Čatský (1962) and Slavík (1963). These studies compared changes in leaf anatomy, chloroplast ultrastructure, conductances for CO_2 and water vapour transfer, gas exchange, photosystem and photophosphorylation activities and activities of carboxylases as well as of water relations during leaf development, in leaves of different insertion level and in different positions on the leaf blade, and evaluated their possible role in limiting photosynthetic productivity (Čatský 1965). In just unfolded and senescent leaves, net photosynthetic rate was limited mostly by the activities of photochemical and biochemical processes of photosynthesis, whereas in photosynthetically mature leaves the main limitation was CO_2 transfer inside the leaf (Čatský *et al.* 1976). New terms were introduced (chlorophyll compensation point, photosynthetic maturity - Šesták 1966). Summary of these studies lead to a comprehensive explanation of photosynthetic ontogeny in a book edited by Šesták (1985).

(*3*) The experimental results concerning the short-term as well as long-term effects of CO_2 concentration in air, irradiance and leaf water relations and their interactions with the above mentioned photosynthetic characteristics and transpiration rate were used for the development of mathematical models of way in which photosynthesis is affected by air humidity, of the localization of the water vapourization places in leaf tissue, of the extrastomatal and transmesophyllar water vapour transfer, and of stomatal and nonstomatal limitation of photosynthesis under stress (Slavík 1975,

Šantrůček and Slavík 1990, Sekerka *et al.* 1991). The differences in sensitivity of stomata on adaxial and abaxial epidermes to environmental factors leading to differences in adaxial and abaxial transpiration and photosynthesis rates were found to be very important for plant water use efficiency (Václavík 1984, Pospíšilová and Solárová 1987).

(*4*) In studies of photosynthetic characteristics of transgenic plants produced by introduction of plasmids carrying either only the resistance to antibiotics or affecting also the content of growth regulators and thus the growth and morphology of plantlets, our results are among the first in the world (Tichá *et al.* 1988, Šiffel *et al.* 1988). During micropropagation of plants (the first acclimation to very special *in vitro* conditions and the second one to *ex vitro* conditions - Pospíšilová *et al.* 1988) the limitation of photosynthesis by low CO_2 concentration during the light period was found in plantlets cultivated *in vitro* (Solárová 1989, Solárová *et al.* 1989). The method for increasing their photosynthesis by increasing CO_2 concentration has found application in practice.

The research activity of the **Department of Plant Growth and Development** has up to now focused on investigation of hormonal control of plant growth and development.

A pioneering work was done by studying contribution of indolepyruvate and indoleacetamide pathways to indol-3-yl-acetic acid (IAA) pool in tobacco tissues transformed by T_i plasmid of *Agrobacterium tumefaciens* in comparison with the IAA metabolism in *A. tumefaciens* itself (Kutáček and Rovenská 1991). Also, a series of original studies was devoted to characterization of enzymes of IAA metabolism and to establishing their control of free IAA level (Kutáček 1988). IAA-binding site was characterized in the particulate fraction of tobacco suspension culture and its role as tentative receptor was investigated with respect to culture growth (Zažímalová *et al.* 1988). Multiphasic character of dose responses including auxin effects was established in kale explants. In was explained as originating due to concentration changes within the cell solute and not to multiphasic uptake (Luštinec 1988).

An original contribution was made in studying the regulatory role of exogenous cytokinins and auxins in cytokinin biosynthesis and degradation. The stimulatory effect of N^6-benzyladenine (BAP) on synthesis and excretion of natural cytokinins by immobilized tobacco cells was established (Vaňková *et al.* 1987). Moreover, exogenous cytokinins including purinyl and urea-type derivatives were found to enhance the activity of cytokinin oxidase in tobacco calli (Motyka and Kamínek 1990). Genotypic variation of cytokinin oxidase forms in *Phaseolus* calli was described (Kamínek and Armstrong 1990) for the first time. These findings suggest a multiple control mechanism in hormonal regulation of cytokinin metabolism and provide ways to overcome the barrier of responsiveness to phytohormones in explant regenerating systems.

A new cytokinin exhibiting high biological activity - N^6-(*m*-hydroxybenzyl) adenosine was developed in cooperation with the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences, and its use in agriculture

and horticulture was successfully tested (Kamínek *et al.* 1987). A highly specific radioimmunoassay for cytokinins was developed (Březinová *et al.* 1992).

Non-embryonic and embryonic suspension cultures of *Medicago sativa* were characterized by increased phenylalanine ammonia-lyase activity, ethylene production and changes in phenolic acids content. Such differences may serve as markers of embryogenic capacity of genotypes used in biotechnologies (Cvikrová *et al.* 1991).

Another important finding is that oxidation products of phenolic substances may possess antimutagenic activity (Pospíšil *et al.* 1988).

A generally applicable model system using the short-day plant *Chenopodium rubrum* has been developed to investigate the hormonal regulation of flowering (Ullmann *et al.* 1985). The effects of exogenous phytohormones which either enhance or inhibit flowering were found to be strictly timed, organ localized and often correlative in nature (Krekule 1979). Some of these effects were explained in terms of changing the rates and directions of cell growth within the apical meristem (Seidlová 1989). We were among the first in an attempt to obtain biochemical markers of the developmental state of the apical meristem (Teltscherová 1962). The changes in the levels of IAA, cytokinins and ethylene found during photoperiodic flower induction in both short-day *C. rubrum* and long-day *C. murale* in all plant organs with the exception of apical buds reflected photoperiodic conditions without any obvious correlation to flower induction (Krekule *et al.* 1989). However, changes in the cytokinin levels in the apical buds seem to reflect the induced state (Macháčková *et al.* 1992). Direct electric current applied during the inductive darkness inhibits flowering in *C. rubrum*, possibly due to interference with the transport of floral stimulus (Macháčková *et al.* 1990), and hence the endogenous rhythm of flowering is caused by changing sensitivity of the apical meristem (Macháčková and Krekule 1991).

Techniques of plant histochemistry became a useful tool in studying morphogenesis. Thus, Beneš *et al.* (1989) demonstrated that the enzyme pattern within root meristems did not reflect the processes of histogenesis but the differentiation within particular histogenes.

In the **Department of Genetics**, Velemínský and Gichner (1968) first reported that a higher plant *Arabidopsis thaliana* could be mutated with promutagenic nitrosamines having a methyl group and suggested that plants had the enzymatic capacity to activate promutagens. The studies with mammalian monooxygenase activity inhibitors (*e.g.*, diethyldithiocarbamate, CO, 9-hydroxyellipticine, organic solvents) suggested the involvement of cytochrome P-450 dependent hydroxylation in the activation pathway of nitrosamine promutagens in the seeds of *Arabidopsis* (Gichner and Velemínský 1984). The activation of the promutagen 7,12-dimethylbenzanthracene by cell free extracts of tulips was demonstrated *in vitro* (Pánková *et al.* 1986).

A model system was developed in barley which enabled through storage of alkylating agents treated seeds to regulate the yield of induced genetic damage, *e.g.* chromosome aberrations and chlorophyll mutations (Gichner and Gaul 1971). With

the help of this system the pre-replication repair of DNA single strand breaks induced by alkylating agents was demonstrated for the first time in higher plants (Velemínský *et al.* 1973). Repair synthesis and post-replication repair were observed in barley embryos treated with the alkylating agent N-methyl-N-nitrosourea (Velemínský *et al.* 1977, 1980a), AP- and UV-endonuclease were isolated from barley leaves and chloroplasts (Švachulová *et al.* 1978, Velemínský *et al.* 1980b) and the repair of DNA double strand breaks induced by bleomycine was reported (Angelis *et al.* 1989).

The ontogeny of tobacco pollen in the anther was shown to be characterized by specific events in gene activity and cell metabolism which could be defined as terminal cell differentiation resulting in a functional gametophyte and as maturation leading to pollen dormancy required for pollen dispersal (Tupý 1982, Tupý *et al.* 1983). This concept led to the development of a method for production of dormant fertile pollen from isolated microspores *in vitro*, providing new opportunities for basic and applied research (Tupý *et al.* 1991). In normally developing tobacco pollen the cycle of the vegetative nucleus is arrested at G_1 (= G_0) and the release of this arrest is involved in induction of pollen embryogenesis by starvation treatment (Žárský *et al.* 1990).

Studies on the functional phase of tobacco pollen development including the requirements of pollen tubes for bivalent cations, carbon source, organic nitrogen and cytoplasmic regulation of pH through proton extrusion resulted in a new method of pollen culture enabling continuous pollen tube growth for days to a length of several cm (Tupý and Říhová 1984). The elongation of pollen tubes requires the synthesis of specific proteins and is characterized by increasing amounts of a 69 kDa glycoprotein in non-covalent binding to cell walls. This glycoprotein is not expressed during pollen development in the anther, but in pollen tubes it is the most intensely synthesized protein and its synthesis occurs on preformed mRNA, independently of transcription (Čapková et al. 1987, 1988). The *in vitro* produced pollen tubes can be used for *in vitro* fertilization and seed production (Balatková *et al.* 1977).

An extracellular nucleolytic activity of pollen against native DNA was reported for the first time in suspension cultures of tobacco pollen (Tupý *et al.* 1980). The activity is released from pollen grains within the first minutes of their contact with a germination medium (Matoušek and Tupý 1983). This phenomenon appears to be a general characteristic of pollen (Matoušek and Tupý 1985). The enzyme is a plant sugar nonspecific endonuclease (nuclease I, EC 3.1.30) with preference for single-stranded molecules (Matoušek and Tupý 1984). The nucleases from various pollen species vary in electrophoretic mobility and number of molecular forms, but exhibit similar substrate specificities (Matoušek and Tupý 1985).

Several apple selections carrying the "Malus floribunda" (Vf) type of immunity to apple scab [*Venturia inaequalis* (Cke)] have been released as commercial cultivars or for advanced testing.

In chemotaxonomic studies focused on protein markers in *Fabaceae* and *Allium* species, taxons, cultivars and genotypes were characterized by different levels of individual seed proteins. Immunochemical isoenzyme analyses clarified some taxonomic problems such as the classification of the polyphyletic genus *Phaseolus* (Kloz and Klozová 1968, 1974, Kloz 1971).

In the **Department of Plant Biotechnology**, the main research program was concentrated on *in vitro* manipulation in higher plants. The genetic analysis of anther cultures of *Nicotiana tabacum* mutants demonstrated for the first time the existence of selection pressure in a system of haploid embryoids developing in the anther, the stimulation effect of lower concentrations of chemomutagens on homozygous materials and heterosis at the anther level (Vagera *et al.* 1976). In pollen and somatic embryogenesis (*Nicotiana*, *Datura*, *Daucus*, *Capsicum*, *Medicago* and *Triticum*), the morphoregulatory effect of iron ions was discovered (Havránek and Vagera 1979, Vagera 1990). On the basis of these and other findings, fundamental changes in culture media composition have been proposed (Vagera and Jílek 1984). In Czechoslovak materials of barley and wheat, one-step androgenesis was realized for the first time, and fertile spontaneously polyhaploid plants were obtained.

Somatic embryogenesis was induced also in callus cultures of *Vicia faba* (Kubaláková and Griga 1989). The study of induced somatic embryogenesis in different lines and cultivars of maize demonstrated the possibility to transfer embryogenic responsivity also to non-responsive materials. Highly embryogenic cell and protoplast cultures of alfalfa have been derived showing a high degree of ploidy level stability (Binarová and Doležel 1988). A system of *in vitro* selection for resistance to many biotic and abiotic stress factors was developed. Resistant cell lines and regenerated plants were cytologically and biochemically characterized (Binarová *et al.* 1990). The most efficient selections were made for resistance to toxic filtrates of the plant pathogen *Fusarium* spp. Immunocytochemical methods for visualization of plant cytoskeleton and detection of DNA synthesis were developed or adapted for plant cells, especially as concerns somatic embryo development. In sugar beet the stability of cytoplasmic male sterility in regenerants from long-term callus culture was demonstrated.

Within the research of nuclear genome changes during *in vitro* culture of plant cells (Doležel and Novák 1985, Doležel and Binarová 1989, Doležel 1991), a procedure for the preparation of plant chromosome suspensions was developed. Isolated chromosomes are suitable for high resolution analysis including background-free *in situ* hybridization. The suspensions are used also for flow cytometric analysis and sorting of single chromosome types for construction of chromosome-specific gene libraries and for gene mapping.

For easy and quick identification of a new cytokinins family (Strnad *et al.* 1990) containing an aromatic nucleus in the side chain (ARCK) an entirely new analytical strategy was prepared, based on testing the immunoreactivity in HPLC fractionated extracts. This could elucidate the metabolic pathway (selectively labelled ARCK with tritium) and its role in the regulation of growth and development of plants. Metabolites of N^6-benzyladenine and its ortho- and meta-hydroxylated derivatives were identified for the first time as new phytohormones.

The most important results achieved in the **Department of Plant Pathology** came from the studies of diseases and their causal agents with virus and mycoplasma-like etiology (Blattný 1969, Ulrychová and Petrů 1980, Polák 1985). The main aim was to minimize the possibilities of outbreak of epidemics in agroecosystems and crop losses due to these pathogens. More than fifteen newly described virus species were

discovered by scientists of the Department (Blattný *et al.* 1965, Slykhuis and Polák 1971, Polák and Slykhuis 1972, Čech *et al.* 1980, Pozděna *et al.* 1980). Many economically important viruses were shown to be present in Central Europe and on the Czechoslovak territory. Orientation on the studies of persistence of such viruses in biennial and perennial wild plants and in weeds, together with the studies on insect-plant virus interrelations and vector efficiency, elucidated problems of circulation of particular viruses in the open and stressed the significance of natural foci of virus infection for dissemination and spread of pathogens into cultivated susceptible crops (Brčák 1971, 1979, Polák 1985). Results achieved in attempts to improve knowledge of chemical composition of viruses, their morphology, architecture, physico-chemical properties, stability during isolation and purification procedures and replication mechanisms had played a decisive role in development of precise diagnostic methods, both biological and serological, on the basis of polyclonal and monoclonal antibodies (Čech *et al.* 1980, Pozděna *et al.* 1980, Filigarová 1982, Čeřovská *et al.* 1991).

Studies in pathophysiology revealed the role of the oxidative pentosephosphate cycle in biosynthesis of viral RNA and enzymes activities in connection with resistance and susceptibility of the host to virus infection (Šindelář 1986a,b, Šindelář and Šindelářová 1987a,b).

Since 1985 ecological studies in virology have been oriented towards virus diseases of forest trees and forest ecosystems and on the mechanism of persistence of infectious virions in surface waters (Polák *et al.* 1990, Polák and Branišová 1991).

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