



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

XXXIXth Annual Meeting
of the European Society
for New Methods in Agricultural Research
25th - 29th August 2009, Brno, Czech Republic

Book of Abstracts

Conference focus:

“ Genomics and Proteomics in Plant and Animal Breeding ”

Held on the occasion of the 90th anniversary of the foundation
of the Mendel University of Agriculture and Forestry in Brno

Local Organizer

Mendel University of Agriculture and Forestry
in Brno

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- Department of Plant Biology
- Department of Molecular Biology and Radiobiology
- Department of Morphology, Physiology and Animal Genetics

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Conference Program

WEDNESDAY - 26.08.09

07:30 – 09:00 REGISTRATION

09:00 – 09:30 OPENING CEREMONY

PLENARY SESSION I

09:30 – 10:20 M ARC VAN M ONTAGU: THE IMPORTANCE OF PLANT BIOTECHNOLOGY FOR SOCIETY AND ENVIRONMENT

10:20 – 11:10 COLIN TURNBULL: BUILDING BETTER PLANTS: SHOOT ARCHITECTURE TRAITS FOR CROP IMPROVEMENT

11:10 – 11:40 COFFEE BREAK

11:40 – 12:30 ANN DEPICKER: GENETICS AND EPIGENETICS OF TRANSGENE LOCI

12:30 – 13:20 WENDY HARWOOD: PERFECTING CROP TRANSFORMATION

13:30 LUNCH

WORKING GROUPS SESSIONS

WG2

14:40 – 15:00 KECHEVA R. Z.: MATHEMATICAL DESCRIPTION OF THE DOSE-EFFECT PHENOMENON ON THE PITUITARY GLAND-ADRENAL GLANDS AXIS AFTER EXTERNAL GAMMA IRRADIATION

15:00 – 15:20 KORNAS S.: PSEUDOINFECTION OF FOALS WITH INTESTINAL PARASITES

15:20 – 15:40 KORNAS S.: DIVERSITY OF THE STRONGYLIDAE SPECIES IN HORSES PRELIMINARY STUDY

15:40 – 16:00 POPOV B.: RADIOPROTECTION FROM GENETIC DAMAGES BY HABERLEA RHODOPENSIS. IN VIVO/IN VITRO STUDY WITH RABBITS.

16:00 – 16:20 SECHMAN A.: EFFECT OF TRIIODOTHYRONINE ON LH- AND 8-BR-CAMP-STIMULATED STEROIDS SECRETION BY OVARIAN FOLLICLES OF THE HEN (GALLUS DOMESTICUS)

16:20 – 16:40 GLAZKO V.I.: DNA TECHNOLOGIES IN INVESTIGATION OF CHROMOSOME AND CELL PHENOTYPES

WG3

14:40 – 15:00 BUDOJ G.: METHOD TO RELEASE AND STUDY NITROGEN DEFICIENCY IN DIFFERENT PLANT SPECIES

15:00 – 15:20 BUDOJ G.: EXPERIMENTAL OPTIMAL RATES OF FERTILIZERS, EXPOR, IN GRAPE-VINE: SCIENCE AND SCIENTIFIC DILETTANTISM

15:20 – 15:40 CORSINI A.: BIOLOGICAL MOBILIZATION OF ARSENIC IN A PYRITE CINDERS POLLUTED SOIL

15:40 – 16:00 FILIPPOV M. F.: INVESTIGATION OF URANIUM AND CAESIUM SORPTION BY SOIL FROM THE REGION OF LOCATION OF RADIOACTIVE WASTE REPOSITORY IN BULGARIA

16:00 – 16:20 GERTSISA.: USING A WASTE PRODUCT FOR SOIL IMPROVEMENT

16:20 – 16:40 PÖSCHL M.: RECENT RADIO-CONTAMINATION OF SOILS IN THE CZECH REPUBLIC WITH RADIOCAESIUM

WG4

- 14:40 – 14:55 MACEK T.: PHYTOECDYSTEROIDS MAY HAVE A NEW ROLE – REGULATION OF PLANT ENZYME ACTIVITIES
- 14:55 – 15:10 PODLIPNÁ R.: ACCUMULATION OF HEAVY METALS AND DEGRADATION OF EXPLOSIVES BY SUSPENSION CULTURES OF TRANSGENIC FLAX
- 15:10 – 15:25 HRADILOVÁ J.: COMPARATIVE ANALYSIS OF PROTEOME CHANGES IN CONTRASTING FLAX CULTIVARS UPON CADMIUM EXPOSURE
- 15:25 – 15:40 VRBOVÁ M.: AGROBACTERIUM-MEDIATED TRANSFORMATION OF FLAX - A FUNCTIONAL METODOLOGY
- 15:40 – 15:55 ŠVÁBOVÁ L.: GENETIC MODIFICATION EXPERIMENTS ON PEA WITH CONSTRUCTS CONTAINING GMSPI-2 GENE AIMED AT IMPROVED PATHOGEN AND INSECT RESISTANCE
- 15:55 – 16:10 RAKOUSKÝ S.: ASSESSMENT OF A FUSED SERIN PROTEASE INHIBITOR SPI2:GFP GENE EXPRESSION IN TRANSGENIC PLANTS USING BIOTESTS WITH PLANT PATHOGENES
- 16:10 – 16:25 LEBEDEV V.G.: LONG-TERM STABILITY OF TRANSGENE EXPRESSION IN PEAR PLANTS IN THE GREENHOUSE AND IN THE FIELD
- 16:25 – 16:40 BALLA J.: COMPETITIVE CANALIZATION OF PIN-DEPENDENT AUXIN FLOW FROM TWO SOURCES
- 16:40 COFFEE BREAK

WG1

- 17:20 – 17:40 VITOROVIC G.: RADIOECOLOGICAL INVESTIGATION IN THE ANIMAL PRODUCTS FROM THE FOOD CHAIN IN THE BELGRADE ENVIRONMENT
- 17:40 – 18:00 JACKOWSKA-TRACZ A.: EFFECT OF HIGH PRESSURE TREATMENT ON SURVIVAL OF CAMPYLOBACTER JEJUNI IN MEAT
- 18:00 – 18:20 SZCZAWINSKI J.: GROWTH OF SALMONELLA ENTERITIDIS REDUCED BY MICROWAVE OR CONVENTIONAL HEATING

WG3

- 17:20 – 17:40 PUTYATIN Y.V.: INDIVIDUAL OPTIMUMS OF SOIL CATION COMPOSITION FOR CROPS TO MINIMIZE ^{137}CS AND ^{90}SR FLOWS IN FOODCHAINS
- 17:40 – 18:00 BUDOI G.: TABLES, NOMOGRAMS AND MATHEMATICAL FORMULAS CONCERNING THE PH VARIATION WITH THE CONCENTRATION OF FERTILIZERS SOLUTIONS AS FOLIAR FEEDING: I. SULFATES OF MACRONUTRIENTS
- 18:00 – 18:20 BUDOI G.: TABLES, NOMOGRAMS AND MATHEMATICAL FORMULAS CONCERNING THE PH VARIATION WITH THE CONCENTRATION OF FERTILIZERS SOLUTIONS AS FOLIAR FEEDING: II. SULFATES OF MICRONUTRIENTS

WG4

- 17:20 – 17:35 CERNÝ M.: EARLY CYTOKININ RESPONSE PROTEINS AND PHOSPHO-PROTEINS OF ARABIDOPSIS THALIANA IDENTIFIED BY PROTEOME AND PHOSPHOPROTEOME PROFILING
- 17:35 – 17:50 NOVÁK J.: OXIDATIVE STRESS IN NICOTIANA TABACUM WITH ELEVATED LEVEL OF CYTOKININS
- 17:50 – 18:05 REKOVÁ A.: CYTOKININS CAN STIMULATE ARABIDOPSIS HYPOCOTYL ELONGATION AT DECREASED LIGHT INTENSITY
- 18:05 – 18:20 SÝKOROVÁ B.: DIFFERENT SENESCENCE-SUPPRESSION ACTIVITIES OF ISOPRENOID AND AROMATIC CYTOKININS IN OAT AND WHEAT LEAVES REFLECT DIFFERENCES IN CYTOKININ UPTAKE AND METABOLISM IN THE TWO PLANT SPECIES
- 18:20 – 18:35 ŽIŽKOVÁ E.: CYTOKININ N-GLUCOSIDES: THEIR APPARENT BIOLOGICAL INACTIVITY RECONSIDERED
- 18:30 – 19:30 ESNA COMMITTEE MEETING
- 19:30 GET-TOGETHER PARTY

THURSDAY - 27.08.09

PLENARY SESSION II

09:30 – 10:20 KLAUS PALME: TOWARDS SYSTEMS ANALYSIS OF PLANT HORMONE ACTION
10:20 – 11:10 ALEXANDER HEYL: IDENTIFICATION OF PROTEIN INTERACTIONS WITHIN THE
CYTOKININ SIGNAL TRANSDUCTION PATHWAY AND BEYOND

11:10 – 11:40 COFFEE BREAK

11:40 – 12:30 BRUNO MÜLLER: FOLLOWING THE TRACES OF CYTOKININ DURING PLANT
DEVELOPMENT

12:30 – 13:20 ONDREJ NOVÁK: NEW PURIFICATION AND MS APPROACH FOR CYTOKININ
ANALYSIS

13:30 LUNCH

WORKING GROUPS SESSIONS

WG3

14:40 – 15:00 LICINA V.: A NEW TECHNOLOGICAL APPROACH IN SANDY COALMINE
DEPOSOL AMELIORATION MEASURES

15:00 – 15:20 OGUT M.: THE SELECTION OF GRAFTED MAHALEB CULTIVARS (PRUNUS
MAHALEB L.) THAT ARE RESISTANT TO HIGH CaCO₃ CONTENTS IN SOIL

15:20 – 15:40 OGUT M.: PHOSPHATE SOLUBILIZATION POTENTIALS OF ACINETOBACTER
STRAINS

15:40 – 16:00 VOLKOGON M.V.: IAA AND ZEATIN BALANCE IN SOYBEAN PLANTS
INOCULATED WITH BRADYRHIZOBIUM JAPONICUM STRAINS

16:00 – 16:20 GREGO S.: SOIL PROTEOMIC: A TOOL TO STUDY SOIL FUNCTIONAL
BIODIVERSITY

16:20 – 16:40 MACKOVÁ M.: STABLE ISOTOPE PROBING IN BIOREMEDIATION –
IDENTIFICATION OF (POLYCHLORINATED) BIPHENYL-METABOLIZING
RHIZOSPHERIC BACTERIA IN CONTAMINATED SOIL

WG4

14:40 – 14:55 KIRAKOSYAN D.: APPLICATION OF BIOTECHNOLOGY METHODS IN CUCUMBER
HETEROSIS BREEDING

14:55 – 15:10 SMETANSKA I.: PERSPECTIVES OF USING CELL, HAIRY ROOT AND SPROUT
CULTURES IN FOOD TECHNOLOGY

15:10 – 15:25 STEHLIK L.: UTILIZATION OF IN SITU RNA HYBRIDISATION TECHNIQUE IN
STUDY OF EUPHORBIA PULCHERRIMA FLOWER ABSCISSION

15:25 – 15:40 KABASHNIKOVA L.F.: THE STATE OF XANTOPHYLL PIGMENTS IN ETIOLATED
SEEDLINGS OF HEXAPLOID TRITICALE WITH DIFFERENT TYPES OF
INTERGENOMIC CHROMOSOME SUBSTITUTIONS UNDER HEAT STRESS

15:40 – 15:55 HOVHANNISYAN N.A.: RAPD MARKERS ASSOCIATED WITH SALT TOLERANCE IN
WILD TRITICACEAE: AN EXAMPLE OF TOOLS FOR EVALUATION OF BREEDING
POTENTIAL IN CROP WILD RELATIVES

15:55 – 16:10 ZLATI C.: HISTOLOGICAL INVESTIGATION ON GRAFT FORMATION OF SOME
PEAR VARIETIES GRAFTED ON QUINCE

16:10 – 16:25 GRADINARIU G.: INVESTIGATION ON ANATOMICAL AND HISTOLOGICAL
STRUCTURE OF GRAFT UNION IN PLUM

WG5

- 14:40 – 15:00 ANTOCE A.O.: DIFFERENTIATION OF FETEASCA NEAGRA WINES FROM VARIOUS AGRO-ECOSYSTEMS BY THE USE OF AN ELECTRONIC NOSE
- 15:00 – 15:20 NAMOLOSANU I.: THE INFLUENCE OF THE AGRO-ECOSYSTEM ON THE QUALITY OF FETEASCA NEAGRA RED WINES IN THREE ROMANIAN VITICULTURAL CENTRES
- 15:20 – 15:40 DASCALU D.M.: LANDSCAPING IN THE AGRICULTURAL ENVIRONMENT
- 15:40 – 16:00 OPREA C.: ENVIRONMENTAL POLLUTION ASSESSMENT ON AGROECOSYSTEM IN AN OIL INDUSTRY AREA: A MULTIDISCIPLINARY APPROACH
- 16:00 – 16:20 OPREA C.: EVALUATION OF SOME QUALITY ATTRIBUTES OF RADISH IN THE SOUTH OF ROMANIA
- 16:20 – 16:40 ZÁHORA J.: UNWANTED DELETERIOUS IMPACTS OF THE NITROGEN SURPLUS FROM AGRICULTURE AND INDUSTRY

WG6

- 14:40 – 15:00 LUCINI L.: QUECHERS FOLLOWED BY TANDEM MASS SPECTROMETRY METHODS FOR THE ANALYSIS OF PESTICIDE RESIDUES IN CROPS
- 15:00 – 15:20 TUNCBILEK, A.S.: THE EFFECT OF STORAGE TEMPERATURE ON LARVAL AND ADULT STAGES OF BRACON BEBETOR (HYMENOPTERA: BRACONIDAE)
- 15:20 – 15:40 SEHNAL F.: PERFORMANCE OF LEPTINOTARSA DECEMLINEATA ON GM POTATOES EXPRESSING GALANTHUS NIVALS AGGLUTININ (GNA)
- 15:40 – 16:00 CEROVSKÁ N.: ANTIBODIES AGAINST RECOMBINANT PLANT VIRAL STRUCTURAL AND NON-STRUCTURAL PROTEINS AND THEIR USE FOR VIRUS DETECTION
- 16:00 – 16:20 MACEK T.: TRANSGENIC PLANTS EMITTING INSECT PHEROMONES
- 16:40 COFFEE BREAK
- 17:00 – 18:30 POSTER SESSIONS
- 19:45 GALA DINNER

FRIDAY - 28.08.09

PLENARY SESSION III

09:00 – 11:00 EVERY WG WILL NOMINATE 1 PRESENTATION FOR THIS PART OF PLENARY SESSION III

11:00 – 11:20 COFFEE BREAK

11:20 – 12:10 PETR HORÍN: BIOLOGY OF ANIMAL BREEDING: FROM GENOMES TO CONSERVATION GENETICS

12:10 – 13:00 ANTONÍN STRATIL: GENOMICS IN ANIMAL BREEDING TO IMPROVE PRODUCTION

13:00 – 13:30 CLOSING CEREMONY

13:30 LUNCH

15:00 – 17:00 GUIDED TOUR TO THE MENDEL MUSEUM

Plenary Sessions

THE IMPORTANCE OF PLANT BIOTECHNOLOGY FOR SOCIETY AND ENVIRONMENT

VAN MONTAGU MARC

Institute Plant Biotechnology for Developing Countries (IPBO), Gent University, Belgium,
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A 35 years ago, when studying crown gall inductions by *Agrobacterium tumefaciens*, we became aware of the existence of many type of galls: teratoma crown galls, witches broom, leafy galls, genetic tumours, insect galls etc. This subject keeps fascinating me and I wonder if they indeed are all due to changes in the concentration and ratio of auxine/cytokinin, as was thought in those days? As soon as we realised that crown gall induction was a natural genetic engineering event, attention focused on altering the Ti-plasmid in a way that it would become a vector for the introduction of novel genes into plants. In view of the recent discoveries of many classes of small regulatory RNA's, often processed from large transcripts and of many hundreds of small peptides, some involved in auxine "potentiation", one may wonder if a deeper study of these T-DNA's should not merit better attention. *Agrobacterium* mediated gene transfer became a potent method in fundamental plant molecular genetic research. It became also the base for engineering novel and important traits into crop plants. Today more the hundred million hectares of transgenic crops are grown annually and the number steadily increases. Remarkably however the GM's commercialised limit themselves too a few crops (corn, soy, cotton and some rapeseed) and too two traits, insect tolerance and herbicide resistance. Is the pipeline clogged? Yes it is, hundreds of new prototype plants were constructed by public and private sector scientists in the developed and developing world. Few received authorisation for field trials, none for commercialisation. It is the duty of public sector scientist to explain why the GM technology can help the plant breeders to construct the novel plants which society and our environment badly need. With the ongoing population growth in the 3rd World countries. The knowledge that today already 1 Bi persons are undernourished and half of the world population has to live with less than 2 € a day, forces us to develop a high yielding agriculture. To double the output of our harvests, will have to be done on the same amount of arable land. For this we need higher yielding crops, with a better uptake of nutrients, with lower losses to biotic and abiotic stresses. On this same surface we also will also have to grow plants as raw material for industry, since our chemical end plastic industry cannot perform with the high petroleum prices experienced in 2008. Together with being high yielding our agriculture and industry will have to be less polluting, otherwise the loss of biodiversity will be irreversible. Plant biotechnologists do not only have the challenging task of engineering these plants, but they also will have to explain to society, why this is needed and what the enormous benefits for environment can be.

BUILDING BETTER PLANTS: SHOOT ARCHITECTURE TRAITS FOR CROP IMPROVEMENT

TURNBULL COLIN

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The vegetative shoot of plants functions essentially as a set of solar energy collectors, supported by a scaffold of stems and connected to the rest of the plant by vascular systems. At some point in most life cycles, the shoot also supports reproductive structures. The spatial configuration of all these structural elements comprises the shoot's architecture, and can be described quantitatively. Architectural diversity is genetically based but is substantially influenced by environment, especially light quality and quantity, and nutrient and water availability. In many limiting environments, the full developmental potential of the individual plant may not be achieved, and this will impact on crop yield at the population level. In this paper, I will review some notable successes in crop improvement over recent decades that resulted from altered shoot architecture, and will then address strategies for addressing future requirements for increased yield under changing and unpredictable environments. Discussion will include how to translate discoveries in models such as *Arabidopsis* into crop species. As an illustration, genes that affect stem number will be examined in experiments on light and nutrient limitation. The case will be made, first, that traits are broadly conserved across species with diverse architectures, and second, that new insights can be derived from conducting experiments at both the individual plant and population levels.

GENETICS AND EPIGENETICS OF TRANSGENE LOCI IN PLANTS

DEPICKER ANNA¹, VERMEERSCH L.¹, DE BUCK S.¹, BLEYS A.¹, BEDRICHOVA J.², KRIZOVA K.², KHAITOVA L.², FOJTOVA M.², KOVARIK A.²

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Transgenic plants are most commonly obtained via *Agrobacterium* mediated transformation vector systems. A designed T-DNA sequence flanked by the T-DNA right and left border repeats is integrated via illegitimate recombination at semi random positions in the plant genome. These insertions are generally stably maintained and transmitted to the progeny as Mendelian loci.

In the first part of my presentation, I will discuss the phenomenon that different transformants containing the same T-DNA show very variable expression profiles of the T-DNA encoded transgenes. This variability illustrates the epigenetic susceptibility of transgenes to sequence structure and context. Originally, all transgene expression variation was attributed to position effects, ie. the influence of neighbouring regulatory elements. Later, in the beginning of the nineties, a correlation was seen between the locus structure and the transgene expression: low expressing transformants contained generally inverted repeated T-DNA copies or many clustered copies, while high expressing transformants contained single, tandem or unlinked T-DNA copies. Upon analysis at the transcriptional level, it could be concluded that most of the transgenes driven by a strong promoter such as the 35S were silenced by a post-transcriptional silencing mechanism while most of the weak promoter driven transgenes were silenced via a transcriptional silencing system. Many studies later confirmed that especially the locus structure, then the dosage effect and only in last instance the position are responsible for the downregulation of the transgene expression. The property that posttranscriptionally silenced T-DNA loci not only downregulate their own transgenes, but also homologous transgenes and plant endogenes with homology in the transcribed region has strongly stimulated research in this area because of the obvious many applications resulting from the targeted phenotypic inactivation of wild type endogenes. Some examples illustrating the above summarised observations will be documented with examples of the group in Gent of A. Depicker.

In a second part, I will focus on the epigenetic stability of 2 transgene loci in tobacco and most of this work has been done in the group in Brno of A. Kovarik. A first transgene locus (Lo1) contains 2 identical 35S driven neomycin phosphotransferase (*nptII*) transgenes in inverted repeat resulting in convergent transcription and subsequent silencing of the *nptII* transgene; the second transgene locus (Lo2) contains the same transgene inserted as a single copy. It was observed that the single copy locus (Lo2) was not methylated and expressing the transgene at high levels during several generations and also in derived calli. On the other hand, in Lo1, the center of the inverted repeat and the 3' half of the *nptII* genes (but not the 35S promoters) were densely methylated, and the inverted repeated transgenes were posttranscriptionally silenced resulting in low accumulation levels of the encoded protein in the plant leaves as well as in derived callus. In Lo1Lo2 hybrids, the Lo1 locus silences the locus 2 in trans and the target *nptII* gene in Lo2 becomes heavily methylated as is expected on the basis of RNA directed DNA methylation. After segregation of the Lo1 locus, the *nptII* gene in Lo2 resumed high expression, but unexpectedly retained its dense methylation pattern for at least two generations, and these Lo2 epialleles with more than 60% of CG methylation in the coding region expressed as well as the original unmethylated Lo2 alleles.

Another dimension to the work was given by the surprising observation that especially during callus culture the inverted repeated locus was epigenetically unstable. Along with the time of tissue culturing, gradual epigenetic programming occurred as could be monitored by increased methylation of the 35S promoter region and a switch from posttranscriptional to transcriptional silencing. Plants regenerated from the callus retained the new epigenetic state (designated Lo1E, transcriptionally silenced, no in trans silencing of lo2 and strong promoter methylation) not only in the differentiated tissues but also in subsequent generations. In contrast to the epigenetic instability of the inverted repeat containing Lo1, the single copy transgene in Lo2 remained unmethylated and stably expressing in the same callus culture conditions.

PERFECTING CROP TRANSFORMATION

HARWOOD WENDY

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Crop transformation is a vital research tool for the determination of gene function as well as a valuable technology contributing to the development of new crop varieties. Although for most crops transformation is now considered routine, there is still a need for improvement in a number of areas. These areas fall into two groups; those concerned with the actual transformation process, including efficiency and genotype dependence, and those concerned with the quality of the transformed plants produced, including control of transgene expression and integration into the plant genome. In barley, the efficiency of *Agrobacterium*-mediated transformation has been greatly increased by making a few simple changes to the transformation procedure. In terms of the quality of the transformation event, transgene expression has been controlled using intron-mediated enhancement. Detailed examination of transgene insertion in barley has provided new information on the transgene insertion site that is of value in shaping future strategies for using crop transformation as a functional genomics tool.

TOWARDS SYSTEMS ANALYSIS OF PLANT HORMONE ACTION

PALME KLAUS

FRIAS – Freiburg Institute of Advances Studies, Centre for Systems Biology, Centre for Biological Signalling Studies, Centre for Applied Biosciences, Institute of Biology, Faculty of Biology, University of Freiburg (Germany), Email: klaus.palme@biologie.uni-freiburg.de

Hormones have been at the centre of plant physiology research for more than a century. Research into plant hormones has at times been considered as a rather vague subject, but the systematic application of genetic and molecular techniques has led to key insights that have revitalized the field. In this talk I will focus on the plant hormone auxin and its action, highlight recent mutagenesis and molecular studies, which have delineated the pathways of auxin transport, perception and signal transduction, and which together define the roles of auxin in controlling growth and patterning. Particular emphasis will be given to the temporal and spatial dynamics of auxin-regulated gene expression. Our systems biology analysis provides a roadmap for auxin-dependent processes underpinning the concept of an ‘auxin code’: a tissue-specific fingerprint of gene expression that initiates specific developmental processes.

IDENTIFICATION OF PROTEIN INTERACTIONS WITHIN THE CYTOKININ SIGNAL TRANSDUCTION PATHWAY AND BEYOND

HEYL ALEXANDER

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Many developmental processes and physiological responses to environmental change are mediated by the phytohormone cytokinin. Cytokinin is perceived and its signal subsequently transduced by a multi-step two-component system. In *Arabidopsis thaliana* the two-component system consists of three receptors and 26 signaling proteins (phospho-transmitters and response regulators) that relay the signal from the plasma membrane to the nucleus and modulate the cellular response. While the basic flow of the signal within the two-component system is well understood, it is unclear how this pathway is integrated into the cellular proteome. Knowledge about the interactions of cytokinin signaling proteins with other proteins should contribute to our understanding of the signaling chains beyond this pathway. Therefore, we conducted medium-scale yeast two-hybrid screens. In total, the screening of more than 6.3×10^7 transformants resulted in the identification of 162 different protein interactions, 138 of which were novel. Most of the interacting proteins belonged to the functional categories of signal transduction and protein metabolism. In most cases interacting protein pairs co-localized, a prerequisite to being of biological relevance. The resulting interaction network map revealed large differences in the connectivity within the proteome.

This study is the first systematic protein-protein interaction experimental approach for a plant signal system and provides numerous starting points for further analysis of the molecular mechanisms used to convert the signal carried by the TCS into biological processes.

FOLLOWING THE TRACES OF CYTOKININ DURING PLANT DEVELOPMENT

MÜLLER BRUNO

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Development of a multicellular organism involves coordinated determination of cells from an undifferentiated state. Typically, initial asymmetries are established by localized signals, which then initiate gene cascades that lead to a refinement of the pattern. Both animals and plants use a limited set of signaling systems to provide positional cues for a myriad of processes. The plant hormones represent such signals. As small organic molecules, they are hard to detect in situ. Therefore, reporter genes to visualize the cellular response triggered by plant hormones are vital tools. The auxin-sensitive reporter DR5 has been instrumental to uncover the function of localized auxin signaling. In contrast, the role of cytokinin signaling in pattern formation is less understood. I have designed a sensitive sensor, TCS, which labels the cells in planta that respond to cytokinin signaling. This novel tool has allowed me to uncover a transient antagonism between cytokinin and auxin signaling, which is essential for setting up the root stem-cell system during embryogenesis. Further improvement of TCS has allowed addressing the function of cytokinin in female gametophyte development.

NEW PURIFICATION AND MS APPROACH FOR CYTOKININ ANALYSIS

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The identification and quantification of plant hormones in plant tissues are necessary for physiological studies of their metabolism and mode of action. The major problem associated with plant hormone analysis is that the amount of phytohormones present endogenously in plant tissues is very low, usually in the range of fmol to pmol/g fresh weight. Development of simple purification of real samples by batch immunoextraction (Hauserova et al., 2005) and application of new analytical approaches based on UPLC separation (Novak et al., 2008) makes possible a new direction in plant hormone research. A fast chromatography technique, the ultra performance liquid chromatography (Acquity™ UPLC, Waters) was coupled to triple quadrupole mass spectrometer (Xevo™ TQ MS, Waters) equipped with an electrospray interface (ESI) and the unique performance of collision cell – ScanWave™. Small amount (1 mg) samples of 10-day-old *A. thaliana* plants were purified by stop-to-go-microextraction follow by an immunoaffinity step and process was completed by fast chromatographic analysis of naturally occurring cytokinins (bases, ribosides, O- and N-glucosides, and nucleotides) in 5 minutes. In multiple reaction monitoring mode, the detection limit for most of cytokinins was close to 50 amol and achieved linear range was at least five orders of magnitude. The method provides substantial improvements in terms of robustness, sensitivity, selectivity, convenience, through-put and cost-effectiveness over previous methods published. In conclusion, we believe that UPLC-ESI(+)-MS/MS technology can be used for fast and sensitive quantitative analysis showing reproducibility in the plant hormones profiling (cytokinins, auxins, abscisic acid, gibberellins, brassinosteroids etc.) in different plant extracts.

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BIOLOGY OF ANIMAL BREEDING: FROM GENOMES TO CONSERVATION GENETICS

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Efficient improvement of production and other desirable traits in domestic animals must be based on understanding biological mechanisms of these often complex traits. Current genomic techniques are based on the knowledge of whole genome sequences in many important domestic animal species. Their structural and functional annotation, as well as analysis of individual variation is underway. The results allow analyzing the extent of genetic diversity in domestic, captive and natural populations and effects of natural and artificial selection. Molecular dissection of important complex traits based on the individual genome variation and on comparative genomics leads to identification of their biological mechanisms. The knowledge of genes involved can then be used for identification of markers of genetic diversity, production and/or disease resistance. They can serve as efficient tools for estimating breeding value, for monitoring effects of intensive selection on genetic variation of domestic animal populations, and for analyzing genetic diversity of endangered breeds, captive and/or natural populations. Genomic analysis of host and pathogen interactions underlying genetic resistance to disease will be used as an example illustrating the potential of genomics in domestic animal breeding.

GENOMICS IN ANIMAL BREEDING TO IMPROVE PRODUCTION

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Most production traits of farm animals are controlled by many genes, called quantitative trait loci (QTLs), and environmental factors. Mapping and characterization of QTLs and identification of underlying causative genes and mutations are primarily aimed at improving the efficiency of production. Substantial structural genomic resources have been developed for several species, which include genetic, physical and comparative maps, EST and SNP collections, etc. The genetic maps are the basis for QTL mapping. Hundreds of QTLs have been identified in each species and these have been utilized for search for causative and candidate genes. Several causative genes and mutations affecting production traits were identified and numerous candidate genes have been studied. The genomic resources have facilitated whole-genome sequencing, which is near completion for some species. The information from structural genomics is employed in functional genomics, which is aimed at the application of global experimental approaches to assess gene functions. Together with proteomics it will help to better understand physiology of the organisms and to use the knowledge for improvement of animal production.

Work Group 1:

Food preservation and safety

Working group N°1 (oral)

EFFECT OF HIGH PRESSURE TREATMENT ON SURVIVAL OF CAMPYLOBACTER JEJUNI IN MEAT

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C. jejuni is the leading cause of bacterial food-borne gastroenteritis in industrialized countries. When animals infected with *Campylobacters* are slaughtered, bacteria can be transferred from the intestines to the meat. High hydrostatic pressure treatment is a new safe and effective food preservation technique. The objective of the study was to investigate the effect of high hydrostatic pressure on the survival of *C. jejuni* in beef. The decontaminated samples of raw beef were placed in polyamide-polyethylene pouches and inoculated with stationary-phase cells of *C. jejuni*. The inoculated samples were sealed under the vacuum and subjected to treatments consisted of combinations of pressure (200, 300, 400 MPa) and time (5, 10, 15 min) at 4°C. The number of surviving *C. jejuni* per gram was determined by the ten-fold dilution method. The bacterial counts were transformed to logarithms and D-10 values were calculated using linear regression method. The results indicate that *C. jejuni* is more pressure sensitive than other food-borne pathogens. The pressure treatment parameters which are proposed for food preservation should be sufficient to kill *C. jejuni* in raw beef as well.

Working group N°1 (oral)

GROWTH OF SALMONELLA ENTERITIDIS REDUCED BY MICROWAVE OR CONVENTIONAL HEATING

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The aims of the study were: (a) subjecting *Salmonella enteritidis* to heat or microwave treatment to obtain bacterial populations reduced by 4, 5 and 6 logarithmic units, (b) determining and comparing the effects of conventional heating in water bath and microwaving on the growth of *Salmonella enteritidis* in nutrient broth incubated at 10°C for 432 hours using the parameters of growth curve calculated from Gompertz equation as well as parameters calculated from Baranyi model. It was found that in the most cases untreated *salmonellae* had a shorter generation time, higher exponential growth rate and higher maximum growth rate than the heated and microwaved bacteria. Growth of bacteria populations subjected to microwave treatment was more dynamic than bacteria subjected to conventional heating. It was expressed by statistically significant differences in generation time and exponential growth rate calculated from Gompertz equation as well as maximum growth rate calculated from Baranyi model for both compared groups of *salmonellae*.

Working group N°1 (oral)

RADIOECOLOGICAL INVESTIGATION IN THE ANIMAL PRODUCTS FROM THE FOOD CHAIN IN THE BELGRADE ENVIRONMENT

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The activity concentrations of ^{40}K , ^{238}U , ^{232}Th and ^{137}Cs were measured using gamma spectrometric method in different food chain samples from environment of six regions around the Belgrade, during the period 2007 - 2008 years. These are the areas in which animal-origined food is manufactured and from where it gets to the Belgrade markets everyday. Relatively high activities of ^{40}K and ^{137}Cs were detected in the soil. These results indicate that ^{137}Cs is present in Belgrade environment even 20 years after nuclear accident in Chernobyl. However, in the samples of foodstuffs (Alfaalfa, maize), animal products (meat, milk, cheese and eggs) and bioindicators (meat of wild animals and fish), activity concentrations of primordial radionuclides and ^{137}Cs were low and below the detection limits. On the basis of the obtained results it can be observed that animal products of food chain from the natural environment around Belgrade, are radioactively safe.

Working group N°1 (poster)

FACTOR EFFECTING PRIMARY CHOICE OF CONSUMER FOOD PURCHASING, ESTIMATES USING MULTINOMIAL LOGIT MODEL

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Willingness to pay for pesticide free tomatoes were calculated and its effects of socio-demographic and risk variables were measured. The data were obtained from 666 consumers by survey method in May 2008. The surveys are conducted in Ankara and Tokat Provinces at same time. Ordered probit model was used to determine the probability of WTP for pesticide free tomatoes. The findings showed that Turkish consumers had willingness to pay not exceed 30% of regular tomatoes prices.

THE EFFECT OF THE VEGETATION PHASE OF SORGHUM-MULTICUTTING HYBRID " SWEET VIRGINIA" ON THE NUTRIENT VALUE

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The silages of Sorghum are an alternative, a supplement to the feed based on maize silages. The ensiled matter should be harvested before heading or at the beginning of heading. Sorghum is characterized by a lower content of fiber and higher organic matter digestibility. The main source of energy are the soluble sugars. The sugars positively affect the sappiness and dry matter intake by the animals. The nutrient composition was monitored from 1th July till 3th September, in 3-5 daily periods. The highest content of the crude protein and soluble sugars was at the beginning of July (216,7;95,8 g/kg dry matter), in following months was the content of these nutrients lower. The dry matter (150,1 g/kg DM) and content of fiber (incl. ADF, NDF fraction) were gradually higher (213,7; 237,7; 398,4 g/kg DM).

Work Group 2:

Advanced methods in animal sciences

Working group N°2 (oral)

MATHEMATICAL DESCRIPTION OF THE DOSE-EFFECT PHENOMENON ON THE PITUITARY GLAND-ADRENAL GLANDS AXIS AFTER EXTERNAL GAMMA IRRADIATION

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Ionized radiation has both quantitative and qualitative effects on morphological and functional systemic structures, including the pituitary-adrenal axis. Depending on the dose, the normal relationships along the pituitary-adrenal axis are altered although at a various extent. In order to establish these changes after external gamma irradiation and their description by a mathematical model, experiments with irradiation of pigs within the dose range of 2.2 – 4.5 Gy have been performed. ACTH and cortisol blood concentrations were analyzed up to the 30th day after the exposure. On the basis of obtained results, four time zones were established as followed: between hours 1–12; hour 24 – day 3; days 5–7 and days 10–30. The mathematical model required normalization of data for each phase and each period and parameter. The time course of occurring processes along the pituitary-adrenal axis was satisfied by a third-degree polynomial function. The equation had a definite prognostic significance for all phases of post radiation events occurring in pigs within the dose range 2.2- 4.5 Gy and could be possibly extrapolated.

Working group N°2 (oral)

DNA TECHNOLOGIES IN INVESTIGATION OF CHROMOSOME AND CELL PHENOTYPES

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The comparative analysis of polymorphism of DNA fragments, flanking by the inverted repeats of decanucleotides (RAPD), microsatellites (ISSR) and terminal sites of retrotransposones (IRAP) in genomes of domestic and closely related wild species (*Bos taurus*, *Bison bonasus*, *Bison bison*, *Ovis aries* L., *Ovis nivicola borealis*) was carried out. DNA regions with the high conservatives on the length were observed. Possible connection of such conservatives with the belonging of DNA flank fragments to purin/pirimidin tracts was discussed. It was coordinated well with the hypothesis of Lima de Faria about the "chromosomal fields" about close relations between nucleotide sequences and chromosome morphology. With the use of DNA arrays the comparative analysis of profiles of the gene expression of two pigs organs, liver and kidney was carried out. 40 genes were revealed, which expressions were essentially above in kidney, than in liver. The basic differences had appeared connected with the genes supervising ionic exchange, and also mechanisms of cellular division. It was corresponded well with dominating participation of kidneys in maintenance of ionic balance in blood and also with lowered activity of cytokinesis in a liver (polyploidy of hepatocytes). It was demonstrated the possibilities to use of the short DNA fragments for in-depth studies of "chromosome phenotype", genetic-biochemical mechanisms of cellular and tissue phenotype formation.

Working group N°2 (oral)

PSEUDOINFECTION OF FOALS WITH INTESTINAL PARASITES

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The aim of this study was to detect pseudoinfection with nematodes of foals connected with coprophagy. The research was conducted in 2007 on 187 Thoroughbreds foals from large stud farm from southern Poland. During the study the foals were dewormed with Grovermina (1% ivermectin) when they reached 1.5 month of its life, next every 2-3 months till 1 year of life. Faecal samples were gathered from the rectum and examined by concentration Mc Master method (gravity=1.28). The larval cultures were done to made the nematodes differentiation into cyathostomes (Cyathostominae) or large strongyles (Strongylinae). Protozoan (*Eimeria leuckarti*) and nematodes: roundworm (*Parascaris equorum*), cyathostomes (Cyathostominae) (based on larval cultures), were found. Oocysts *E. leuckarti* were detected from 14 to 47 day old foals, which indicates the patent infection on the basis on length of prepatent period of this parasite. Based on age and/or ivermectin treatment as well as the prepatent period, it was suggested that most of infections with *P. equorum* and cyathostomes were pseudoinfections, probably due to the ingestion of infected faeces of their mares or some other horses.

Working group N°2 (oral)

DIVERSITY OF THE STRONGYLIDAE SPECIES IN HORSES PRELIMINARY STUDY

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The aim of the study was to evaluate the diversity of the Strongylidae species, including large strongyles (Strongylinae) and cyathostomes (Cyathostominae) in horses. The research was conducted in 2008 on 14 horses from riding club. The level of Strongylidae infection was determined based on concentration Mc Master method, after that the horses were dewormed with ivermectin. Fecal samples (about 2kg), were collected 24, 48 and 74 hours after treatment. A total of 4760 cyathostomes as well as 21 large strongyles were found in the fecal samples and identified to species. Based on the results the relative abundance - number of large strongyles and cyathostomes / % of individual species within total population was determined. The study revealed the infection of examined horses with 5 species of large strongyles and 17 of cyathostomes. The number of different cyathostomes species found in one host ranged from 3 to 15. Four species, i.e. *C. catinatum* (43.42%), *C. longibursatus* (12.1%), *C. nassatus* (10.06%) and *C. pateratum* (8.66%) constituted the highest relative abundance.

Working group N°2 (oral)

THE USE OF DIFFERENT ADSORBENTS IN THE CASE OF ALIMENTARY CONTAMINATION BROILER CHICKEN WITH ^{137}Cs

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Increased radiocontamination and unequal distribution of radioactive substances in the environment have led numerous researchers to find an adequate way to protect animals and animal derived food from radioactive contamination. The objective of this study was to investigate the binding efficiency of AFCF and clinoptilolite, mixed to the feed and administered orally using gastric tube to chronically ^{137}Cs alimentary contaminated broiler chicks. With AFCF as a cesium binder, on day 13 of measuring the ^{137}Cs activity in dark meat was 83-85% lower than that in the control group, 80-83% in white meat and 89 % in liver. Natural clinoptilolite demonstrated lower binding efficiency. On day 13 of measuring the ^{137}Cs activity in dark meat was 58-70%, in white meat was 53-69% lower than that in the control group and 67-70% in liver.

Working group N°2 (oral)

RADIOPROTECTION FROM GENETIC DAMAGES BY HABERLEA RHODOPENSIS IN VIVO/IN VITRO STUDY WITH RABBITS

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The radioprotective effect of plant extract (*Haberlea rhodopensis*) was investigated in blood lymphocytes from rabbits. Peripheral blood samples were collected from the ear marginal vein 2 hours after single injection of different concentrations of extract (*Haberlea rhodopensis*). One hour later the whole blood was exposed to 200 cGy of ^{60}Co gamma irradiation. Specimens of irradiated blood from rabbits were cultured for 50 hours by the method of Hungerford. Lymphocytes were analyzed in their first mitotic division for chromosome structural abnormalities. The yield of chromosome aberrations in lymphocytes of treated with HR and untreated rabbits were compared. The results showed decreasing in chromosome aberrations in pretreated sampled compared to controls (only irradiated).

Working group N°2 (oral)

EFFECT OF TRIIODOTHYRONINE ON LH- AND 8-BR-CAMP-STIMULATED STEROIDS SECRETION BY OVARIAN FOLLICLES OF THE HEN (*GALLUS DOMESTICUS*)

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In order to investigate the effect of triiodothyronine (T3) on sex steroids (progesterone [P4] and estradiol [E2]) secretion by a chicken ovary, white non-hierarchical (1 4, 4 6, 6 8 mm; WFs) and yellow preovulatory (F3-F1) follicles were isolated 22.5 h before ovulation. WFs and fragments of theca (TL) and granulosa (GL) layers of F3 F1 follicles were incubated for 24 h in 6 groups in medium supplemented with: a) control, LH (10 ng/ml), T3 (10 ng/ml), and LH+T3 (1, 10, 100 ng/ml); b) control, LH (10 ng/ml), T3 (10 ng/ml), 8 Br cAMP (0.5 mM; the cell-permeable cAMP analog), LH+T3 and 8-Br-cAMP+T3. P4 and E2 in culture media were determined by RIA. Inhibitory effect of T3 on LH and 8 Br cAMP-stimulated E2 secretion by WFs and TL of F3-F2 follicles and stimulatory one on P4 secretion by GL of F3-F1 follicles were found. We conclude that T3 affects steroidogenesis in chicken ovarian follicles by modulation of cAMP/protein kinase A signalling system. Moreover, these results support our earlier assumption concerning involvement of T3 in the process of WFs selection into preovulatory hierarchy and its engagement in follicular growth and maturation. Supported by grant NN311006436

Working group N°2 (poster)

ANALYSIS OF MICROSATELLITE MARKERS IN THE PEREGRINE FALCON (*FALCO PEREGRINUS*) AND THE SAKER FALCON (*FALCO CHERRUG*) POPULATIONS

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Analyses of the DNA variability play an important role in studying population genetics. The main aim of this study was the estimation of five microsatellite markers in the peregrine falcon (*Falco peregrinus*) and the saker falcon (*Falco cherrug*). The dataset of 21 individuals was divided into two populations according to their species. The sample sizes were 14 individuals of the peregrine falcon and 7 of the saker falcons living in the Czech Republic. DNA was extracted from feathers. Detection of alleles was based on the multiplex PCR reaction and the fragmentation analysis. We detected 38 different alleles for all five loci and 9 unique alleles of the microsatellites for the peregrine falcons NVH fp13 (92), NVH fp89 (115,151) and the saker falcons NVH fp31 (142,146), NVH fp79-4 (138), NVH fp89 (117,119,141). We investigated genetic structure of populations and evaluated the standard statistical values (number of allele, allele frequencies, polymorphism information content, theoretical heterozygosity, observed heterozygosity, F statistics,...). These results are very important for our following research.

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Working group N°2 (poster)

COMMON PARASITES OF RODENTS FROM PET SHOPS PRELIMINARY STUDIES

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This study was aimed to evaluate species composition and incidence of intestinal parasites of rodents originating from three pet shops. A total of 41 animals were sampled, i.e. 12 hamsters, 4 mice, 7 rats, 6 guinea pigs, 2 gerbils, 5 degus and 5 chinchillas. Coproscopic examination was performed by means of quantitative test tube flotation technique. Cellophane tape test method was used for founding pinworms in 26 samples from mice, rats and hamster. *Eimeria* sp. was recorded in 12.2% of animals. Prevalence of coccidia infection in several hosts equaled 28.6% (in rats), 20.0% (chinchillas and degus), and 16.7% (guinea pigs). As regards nematodes, *Trichuris* sp. prevalence reached 33.3%, *Syphacia mesocriceti* 16.7% (in hamsters), *Aspiculus tetraptera* 75.0% (mice) and *Dentostomella translucida* was noted during coproscopic examination in gerbils. Two animals (one mouse and one hamster) were infected by *Hymenolepis diminuta* tapeworm. *S. mesocriceti* (in 10% of hamsters) and *S. muris* (in 60% of rats) was recorded by cellophane tape test method. Various groups of parasites were recorded in different shops. Only *Syphacia* spp. and *A. tetraptera* was observed simultaneously in two of them.

Working group N°2 (poster)

HERBS USAGE IN PORK PRODUCTION

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Herbs and their products are source of medical properties human medicine worldwide as well as in animal production. In view of ban of large antibiotic usage in feedstuffs in European union was herbs additives focuses as potential alternative. The goal is to improve animal production and ensure quality and safety of animal products (food). In our experiments anethole plant oils were evaluated. Authors of scientific papers describe anti-oxidative, anti-biotic, fyto-estrogenic, sialogenic and other properties in human or animal science. The essential oils from anise (*Pimpinella anisum* 90 % anethole content) and fennel (*Foeniculum vulgare* 60 % of anethole content) was fed to pigs on level of 0,1 % in feed mixture. Balance experiments showed slightly improving of organic matter digestibility in pigs (0,5 % with anise treatment). In growth experiment with 114 piglets the different level of anise oil was used (0,05 0,15 % in feed mixture) and the experiment show th at these treatments do not affect the growth intensity by any way (average daily gain of control 578 g vs. 567 and 561 g, not statistically significant differences).

Working group N°2 (poster)

PORCINE INSULIN RECEPTOR SUBSTRATE 4 (IRS4) GENE: CLONING, POLYMORPHISM AND ASSOCIATION STUDY

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The insulin receptor substrate 4 is cytoplasmic protein that contains many potential tyrosine and serine/threonine phosphorylation sites phosphorylated by the insulin receptor tyrosine kinase upon receptor stimulation. On the basis of human IRS4 gene sequence and combinations of comparative PCR and IPCR methods complete sequence of porcine IRS4 (FN424076) has been cloned. Putative porcine protein is composed of 1311 amino acids; the homology is 84% for human and 71% for mouse protein. Three SNPs have been detected: FN424076:g.96C>T, FN424076:g.1829T>C and FN424076:g.3956C>T. RH mapping showed the most significant linkage to microsatellites SW1426 (0.24 R, LOD =16.6) and SW2048 (0.75 R, LOD = 5.02). Linkage mapping placed the gene on current USDA –USMARC linkage map at position 82 cM on SSCX. The FN424076:g.1829T>C was genotyped in M x LW composite population (n = 554) with records for weight at the end of test, lifetime daily gain, testtime daily gain, loin depth and backfat depth. Association analyses performed by the GLM (SAS) showed highly significant association with backfat when tested across sexes and in boars (P =<0.0001) but not in gilts. (GACR 523/07/0353)

Working group N°2 (poster)

HELMINTHS OF PIGS SLAUGHTERED IN A LOCAL ABATTOIR (SOUTH POLAND)

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The study was aimed to assess the level of nematode infection in fatteners and defective sows supplied to slaughter, as well as to analyse the zoonotic risk linked to the presence of swine pathogens. Throughout 2008, a total of 312 animals (296 fatteners and 16 sows) originated from 48 small farms were subjected to examination. Postmortem, local lesions caused by roundworm larvae were seen in the livers of 26 fatteners, and in 3 cases hydatid cysts of *Echinococcus granulosus* were detected. No *Trichinella spiralis* infective larvae were recorded in diaphragma processed by the pepsin-HCl digestion method. Individual faecal samples were collected per rectum and analysed by means of the concentration McMaster technique. Old sows were mainly infected with *Oesophagostomum* spp. (prevalence 87.5%, mean intensity 911 eggs per gram of faeces – EPG). Singular animals harboured *Ascaris suum*, *Trichuris suis* and *Strongyloides ransomi* (20-40 EPG). Among fatteners, the prevalence of nematode infection reached 72.2%. *Oesophagostomum* spp. (52.2%, 940 EPG) and *A. suum* (44.4%, 930 EPG) prevailed, whereas *T. suis* (mean 186 EPG) and *S. ransomi* (51 EPG) affected only 10.2 and 11.5% of pigs, respectively.

Working group N°2 (poster)

OCCURENCE OF FASCIOLA HEPATICA IN SLAUGHTERED CATTLE

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The aim of the study was to estimate the percentage of livers changed by flukes, prevalence and intensity of infection of *Fasciola hepatica* and *Dicrocoelium dendriticum* in slaughtered cattle. The necropsies were conducted from March to December 2008 in slaughterhouse located in southern Poland. A total of 845 animals from 2 to 15 year old were studied. Among that number of animals 179 livers were rejected by the meat inspectors and collected. Than livers were examined in the laboratory to look for the changes caused by liver flukes or to find out the presence of *Fasciola hepatica* or *Dicrocoelium dendriticum*. The pathological changes characteristic for fascioliasis were detected in 176 animals (20.8%) among 845 heads, mainly in thick bile ducts, while in 3 (1.7%) from 179 examined the observed changes did not show the consequences of fascioliasis. The specimens of *Fasciola hepatica* were found in 99 animals (prevalence of infection 56.2 %), with the mean intensity reaching 2.3 flukes per one host. *Dicrocoelium dendriticum* was detected four times (2.3% of animals) in smaller bile ducts with the mean intensity 180.5 flukes per host.

Working group N°2 (poster)

RELIABILITY AND SENSITIVITY OF THE IN VIVO MEASUREMENT OF RADIOCAESIUM ACTIVITY IN AQUARIUM FISH.

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The model environment in an aquarium with definable conditions can provide new information about the biological accumulation of ^{137}Cs in the vegetation and in living organisms, and about the transfer indicators between the individual compartments of the aquatic environment. This work was focused on the development of a rapid method for *in vivo* monitoring of ^{137}Cs in small aquarium fish using the gamma-spectrometric system with a pure germanium (HPGe) detector. Standardization of two simple geometric arrangements in the system was tested: 100 ml or 10 ml, respectively, of cultivating water with 5 - 10 fishes in a pot placed on the window of the detector. Radiocaesium activity concentration in observed artificially contaminated fishes (*Poecilia reticulata*, 0.5 - 0.6 g) ranged from 1.8 to 3.5 Bq g⁻¹. Within a measuring time of 15 - 30 minutes the used spectrometric system and the two geometric arrangements enabled *in vivo* measurement of ^{137}Cs in small fishes with very high reliability (MDA < 0.24 Bq g⁻¹ or 0.13 Bq g⁻¹, respectively) and sensitivity (MSA < 0.097 Bq g⁻¹ or 0.054 Bq g⁻¹, respectively).

Working group N°2 (poster)

INTESTINAL PARASITE INFECTION IN CATTLE FROM SMALL FARMS

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The aim of this study was to estimate the level of intestinal parasite infection in cattle from small farms. The researches were conducted during the pasture season 2008 on 160 heads of cattle in different age originating from 9 places situated in 2 districts. In the farms cattle were not dewormed. During the study cattle were fed only with pasture grass. Faecal samples collected individually from cattle were examined by concentration McMaster method. *Eimeria* spp., *Fasciola hepatica*, *Trichostrongylidae* and occasionally *Moniezia* sp. was found in the examined cattle. The level of parasites infection depends on management system and age of animals. In the first district prevalence of *Eimeria* spp. infection was 20-100% with oocysts output 50-600 OPG and *Trichostrongylidae* 25-100% and 50-850 EPG respectively. In the second district 9% of examined cattle was infected with *Fasciola hepatica*, 7% with *Eimeria* spp. and 2% with *Trichostrongylidae*, but in some of examined farms the prevalence of *Fasciola hepatica* reached even 30-45%, *Eimeria* spp. 10-25%, and *Trichostrongylidae* up to 20%.

Working group N°2 (poster)

POLYMORPHISMS OF PORCINE SERPINE1 GENE

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SERPINE1 gene encodes for plasminogen activator inhibitor-1 (PAI-1) which plays a key role in incidence of thrombosis and atherosclerosis in human. The plasma level of PAI-1 is elevated with obesity and type 2 diabetes because of synthesis of this protein by adipose tissue and PAI-1 is an important predictor of the onset of the disease. Porcine SERPINE1 or PAI-1 gene is localised on SSC3 close to QTL for fatness. In our study fragment of SERPINE1 gene was PCR cloned and comparative sequenced. The polymorphisms in intron 3 of this gene were detected among different pig breeds (Meishan, Wild Boar, Pietrain). The total of fifteen polymorphisms was detected (14 SNPs and 1 indel). For next study FN396538:g.566G>A was chosen. The allele A in this polymorphism was observed in Wild Boar and Pietrain and allele G was observed in Meishan. This SNP was genotyped in Czech Large White pigs (n = 98) by MbiI PCR-RFLP where frequencies of allele A and G were 0.66 and 0.34, respectively. These results may be used for linkage mapping of porcine chromosome 3 and detected polymorphisms will be used for association study with fat deposition in pigs.

Working group N°2 (poster)

THE EFFECTS OF SEX AND BODY WEIGHT ON MUSCLE FIBRE CHARACTERISTICS OF PIG LONGISSIMUS LUMBORUM MUSCLE

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This study evaluates the effects of sex and slaughter weight on muscle fibre composition of m. longissimus lumborum in fatteners. 60 Polish Large White pigs were slaughtered at 60, 90, 120, 150, 180 and 210 days of age. Muscle fibres were identified as I, IIA and IIB types based upon their NADH dehydrogenase activity. The results obtained indicated that sex had no effect on muscle fibre type percentage but had a significant effect on muscle fibre size. In pigs aged between 150 and 210 days females had larger size of type IIB fibres than castrated males. Moreover, in both sexes studied, we found a significant increase in the percentage of type IIB muscle fibres paralleled by a decrease in the percentage of type IIA and I muscle fibres in pigs aged between 60 and 90 days. Thereafter, the proportion of type IIB fibres, opposite to type IIA and I, kept increasing but at a much slower rate. Independent on sex of animals, the highest increase in muscle fibre diameter was found in pigs aged between 60 and 120 days. After 150 days of breeding the rate of muscle fibre growth was slower and not significant between 180 and 210 days.

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Working group N°2 (poster)

LOCALIZATION AND ACTIVITY OF 3 β -HYDROXYSTEROID DEHYDROGENASE IN OVARIAN FOLLICLES OF THE DOMESTIC HEN (GALLUS DOMESTICUS) DURING THE OVULATORY CYCLE

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The aim of the study was to determine the activity and localization of 3 β HSD dehydrogenase in the ovarian follicles during their growth and development, and to examine changes in the activity of this enzyme in ovarian follicles during the ovulatory cycle. 25 Hy-Line Brown laying hens aged 40 weeks were decapitated at five stages of the ovulatory cycle (0.5, 6, 12, 18 and 24 h before ovulation). A stroma and the following follicles were isolated from the ovary: white follicles (1-4 and 4-8mm) and the three largest preovulatory follicles (F1, F2, F3). The activity and localization of 3 β HSD in the ovarian follicles were detected using the histochemical method. The results obtained indicate that localization of 3 β -HSD changes from the theca layer of stromal and white follicles to the granulosa layer of yellow preovulatory follicles. The stage of the ovulatory cycle has no effect on 3 β -HSD activity in follicles that have not entered the hierarchy but has a significant effect on increasing the enzymatic activity of the granulosa in the largest preovulatory follicles (F1) 6 and 0.5 h before ovulation and on reducing the enzymatic activity of the internal theca 6 h before ovulation.

Working group N°2 (poster)

STRUCTURE AND STEROIDOGENIC FUNCTION OF THE GRANULAR LAYER OF THE PREOVULATORY OVARIAN FOLLICLES F1 OF THE HEN.

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The study was performed to determine the structure and steroidogenic activity of the granular cells derived from the germinal disc region, proximal and distal region of the largest preovulatory ovarian follicles F1 of the hen. The studies were carried out on 26 forty-weeks-old egg-laying Hy-Line Brown hens. The morphology of the granular cells was studied by histological assessment and scanning electron microscopy. Moreover, the steroidogenic activity of the granular cells (3 β -HSD activity) has been shown using histochemical assay. The current findings indicate that morphology and function of the granular layer in hen's preovulatory ovarian follicles F1 depend on region of the follicle. In the germinal disc region the granular cells form a multilayer, while in the proximal and distal regions granular cells form a monolayer. Moreover, steroidogenic activity of the granular cells increases significantly, during increasing the distance from the germinal disc. Additionally, in all examined regions of follicle F1, using SEM microscopy, we observed projection of the granular cells into the oocyte.

Working group N°2 (poster)

EFFECT OF HYPOTHALAMIC NEUROPEPTIDES (CRH, AVP AND OXY) ON IN VITRO CATECHOLAMINES RELEASE BY SHEEP ADRENAL GLAND

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Corticoliberin (CRH), vasopressin (AVP) and oxytocin (OXY) are produced locally by chromaffin cells (CC) of the adrenal medulla, and are accepted to be the most important factors in mediating the stress response on the hypothalamic level. The aim of this study was to investigate in vitro adrenal medulla activity following CRH, AVP and OXY treatment by adrenaline (A) and noradrenaline (NA) release from CC. Adrenal medullas isolated from 3-years-old female sheep (out of reproductive period) were cut into small pieces and incubated in 1 ml of medium without hormone or supplemented with CRH, AVP and OXY (10⁻⁷, 10⁻⁸ and 10⁻⁹ M). The media were changed every 30 min, and A and NA were determined by RIA method in collected supernatants. CRH supported A and NA release from CC. The prompting AVP influence on A release were visible after application of the two lower doses of this neuropeptide. On the other hand, AVP reduced NA release from CC. Strong, inhibitory OXY effect on catecholamine release was observed, regardless of employed doses of this hormone. Our results indicate attenuating role of OXY on adrenal gland activity and better coping with stress.

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Work Group 3:
Soil plant relationships

Working group N°3 (oral)

TABLES, NOMOGRAMS AND MATHEMATICAL FORMULAS CONCERNING THE pH VARIATION WITH THE CONCENTRATION OF FERTILIZERS SOLUTIONS AS FOLIAR FEEDING: I. SULFATES OF MACRONUTRIENTS

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This is the first paper from a series. In some fertilizers, such as sulfates, the pH decreases with the increase of the fertilizer's concentration, producing burnings of the leaves and, in some cases, precipitations of some nutrients, such as P, Mo, eventually presented in solution if complex solutions have to be prepared. The obtained data tables, nomograms and formulas can be used to evaluate the pH of the solution as related on the needed concentration for a given plant species and substantiate the amount of a given base of a given concentration to be added to the fertilizer's solution in order to correct and bring the pH into the optimal area, generally slightly acid. This paper presents the research results for $(\text{NH}_4)_2\text{SO}_4$ (with concentrations from 0 to 3 %), K_2SO_4 (0 ÷ 2.5 %) and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0 ÷ 6 %), both for fresh solutions in current water (CW) with pH 7.18 and in distilled water (DW), for comparisons. In $(\text{NH}_4)_2\text{SO}_4$, the pH decreases from 7.18 to 6.63 in CW and reaches 5.33 in DW. In K_2SO_4 , the pH decreases to 2.71 both in CW and DW. In $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, the pH decreases to 6.5 in CW and reaches 5.0 in DW. In all the three sulfates, the pH in CW is higher than in DW.

Working group N°3 (oral)

TABLES, NOMOGRAMS AND MATHEMATICAL FORMULAS CONCERNING THE PH VARIATION WITH THE CONCENTRATION OF FERTILIZERS SOLUTIONS AS FOLIAR FEEDING: II. SULFATES OF MICRONUTRIENTS

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This second paper from the series presents the research results carried out for $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4 \cdot \text{FeSO}_4 \cdot 6\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, both for solutions in current/common water (CW) with pH 7.18 and in distilled water (DW), for comparisons. In $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ (with concentrations from 0 to 4 %), the pH decreases from 7.18 to 4.38 (very strongly acid) in CW and reaches 3.98 (extremely acid) in DW. In $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0 to 5 %), fresh solutions, the pH decreases from 7.18 to 3.96 in CW and reaches 3.52 in DW, so becomes extremely acid. In $(\text{NH}_4)_2\text{SO}_4 \cdot \text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ (0 to 3 %), the pH decreases from 7.18 to 3.74 (extremely acid) in CW and reaches 3.51 (extremely acid) in DW. In $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (concentrations from 0 to 3 %), the pH decreases from 7.18 to only 6.22 (slightly acid) in CW, but reaches 3.60 (extremely acid) in DW. In $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (concentrations from 0 to 1 %), the pH decreases from 7.18 to 6.15 (slightly acid) in CW and reaches 5.43 (strongly acid) in DW. In all sulfates, the pH in DW is less than in CW, but differences diminishes with the increase of the concentration.

Working group N°3 (oral)

METHOD TO RELEASE AND STUDY NITROGEN DEFICIENCY IN DIFFERENT PLANT SPECIES

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The concept of gradually exhaustion of the accessible nitrogen from a small volume of soil has been used in order to release N deficiency, in conditions of repeated soil fertilization with a complete Hoagland solution, but without N. Verification of this concept has been done on 34 plant species: 7 field crops, 4 fodder, 6 medicinal and aromatic, 17 vegetables. One variant fertilized with complete Hoagland solution (including N) existed for each species as control. The plants have been cultivated in small pots in vegetable house, on a mollic brown-reddish soil, with pH 6 (slow acid), degree of base saturation 85% and soil nitrogen index 1.8. Plants cultivation on soil presented a great advantage vis a vis the American researchers method, with plant cultivation in nutritional solutions, because the plants were not submitted to an immediately nutritional shock, but gradually, as it happens in natural conditions. The experiment gave excellent results, typical N deficiency symptoms, in all species, confirming the righteousness of the experimental concept. Original images with visual symptoms and detailed descriptions in all species have been obtained.

Working group N°3 (oral)

EXPERIMENTAL OPTIMAL RATES OF FERTILIZERS, EXPOR, IN GRAPE-VINE: SCIENCE AND SCIENTIFIC DILETTANTISM

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In Romania, during 1981-1987, the official system of N, P, K mineral fertilization in grape-vine was that of ExpOR, elaborated by Borlan et al. In 1987, Budoï et al. substantiated the system of economically optimal rates, EOR, which was a progress; this became the official system. In 2007, Budoï brought essential improvements to EOR system and substantiated the technically optimal rates. In 1996, the Research-Development Institute for Viticulture and Winemaking Valea Calugareasca (IC-DVV) promoted, through a "scientific" paper, fertilization in ExpOR in Romanian viticulture, but the authors conception is an unacceptable mixtum compositum between ExpOR and EOR systems, based on a severe confusion, generatrix of great calculating errors: the authors consider the c coefficients from the exponents of the ExpOR models as actions coefficients, C_e , variable with the expected yield level, Y_e , which is valid for EOR, but false for ExpOR. The model modified by IC-DVV, with $C_e = f(Y_e)$, gives erroneous overestimated rates, till double N rates, potentially pollutants. Although in 2007 Budoï demonstrated the IC-DVV's errors, IC-DVV is refractory both the observations and the recent improvements.

Working group N°3 (oral)

BIOLOGICAL MOBILIZATION OF ARSENIC IN A PYRITE CINDERS POLLUTED SOIL

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Bioavailability of arsenic (As) in soil is governed by chemical and biological factors, in particular the redox condition. The objective of this research was to study (1) the effect of plant material decomposition on As mobilization in a contaminated soil under flooded and not flooded conditions; (2) As uptake and translocation in *Salix purpurea* L. The experimental soil was a mixture (1:1 w/w) of a mineral unpolluted soil and pyrite cinders, contaminated by several metals and As. 1-year old cuttings of *Salix* were transplanted in pots containing 1 kg of soil amended with dry alfalfa. During two months of growth two environmental conditions were tested by maintaining soil in submersion or at 70% of the water holding capacity. Control pots without alfalfa and in not submerged conditions were also included in the experimental design. Samples of soil solution were weekly collected by Rhyzon syringes and pH, redox potential, Fe, total As, As(V) and As(III) were determined. Leaves of *Salix* were sampled at day 0 and day 56 and As and Fe concentrations were determined. The weakly reductive conditions induced by organic matter decomposition in submersion resulted in a fast arsenic mobilization.

Working group N°3 (oral)

INVESTIGATION OF URANIUM AND CAESIUM SORPTION BY SOIL FROM THE REGION OF LOCATION OF RADIOACTIVE WASTE REPOSITORY IN BULGARIA

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The global nuclear fall-out connected with test weapon in atmosphere, radioactive waste and accidents are the main sources of hit for radioactive nuclides in soils. The purpose present work was the determination of the uranium (VI) and caesium (I) distribution between aqueous solutions and brown forest soil from the region of the radioactive waste repository in Sofia region (Bulgaria). At investigation of sorption on soil samples from aqueous solution was used ^{237}U ($T_{1/2}=6.75$ d), and ^{132}I ($T_{1/2}=6.479$ d) radioactive nuclides, which were produced in the photonuclear reactions of the $^{238}\text{U}(\gamma, n)$ and $^{238}\text{U}(\gamma, f)$ [1] at an electron accelerator MT-25 microtron of the FLNR, JINR. Maximum energy of the bremsstrahlung was 23.5 MeV. The electron current was 15 μA . The measurement of gamma-spectra were carried out using HP Ge-detector by the 30 cm^3 volume and resolution of 2.5 keV on line 1.333 MeV (^{60}Co). The distribution coefficients (K_d) of the uranium and caesium between soil and aqueous solutions were determined. In the range pH 1 to pH 7. The U(VI) speciation in the solution at various uranium concentrations ($1 \cdot 10^{-7}$ - $1 \cdot 10^{-2}$ mol/l) in the range pH 1 - 7 was calculated using the programme "Speciation" [2, 3]. The sorption characteristics obtained for uranium (VI) and caesium (I) in the system soil-solution can be use to predict the migration behavior of uranium and caesium in brown forest soil at accidents and acid rains.

Working group N°3 (oral)

USING A WASTE PRODUCT FOR SOIL IMPROVEMENT

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The paper industry faces the problem of disposing the residual paper pulp (solid and liquid waste). Landfilling, incineration and lately soil application are the ways to dispose most of this material. Due to diverse property ranges of the material coming from different sources and treated under various methods, each type deserves unique evaluation and results from various studies on soil application range in their conclusions. To evaluate the potential use as a soil improving material, a four-years study is conducted in Hellas (Greece) using residual recycled paper pulp (RRPP). The study is established under two different soil types (a clay loam and a sandy loam texture) with variable climatic conditions in each site. A number of crop species are also under evaluation at each cycle following an intensive 12-month growth cycle and there are 3 rates of the RRPP (1%, 2% and 4% v/v calculated for the top 0.15 m soil depth) and applied cumulatively to the same plots every year. Low Input Sustainable Agriculture (LISA) practices were used, with minimum fertilizer and irrigation inputs applied and almost no other agrochemical was used. The results of the first two years indicated positive effect to soil's organic matter content, water holding capacity, and other important soil physical and chemical properties, affecting crop growth and yield. The yield analysis of the crop species indicated that increased yield was caused by the RRPP in some of the cases.

Working group N°3 (oral)

IMPACT OF PRESOWING NODULATION ON SOYBEAN TOLERANCE TO NITROGEN SUPPLY AS EUSTRESS OR DISTRESS FACTOR

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In present work efficiency of presowing Soybean treatment with nodulating bacteria for plant's resistance to N-fertilizer as eu- or distress factor (30 and 180 kgN/ha accordingly) has been studied. N-fertilizer resulted dose-dependent rising of malonic dialdehyde (MDA) content in leaves of Soybean at phase of flowering. Although, plants that were undergone by presowing nodulation, accumulated MDA in leaves less intensively. The highest content of MDA in Soybean leaves at the bean-filling phase has been noticed at plants that was presowing treated with nodulating bacteria and additionally supplied with 180 kgN/ha. Decreasing of sulfolipid SQDG content in researched samples without presowing treatment at both phases of development has been observed. However, its content at plants with presowing nodulation at the phase of flowering was doubled by 30 kgN/ha and increased on 40% by 180 kgN/ha. Dose-dependent rising of SQDG at the bean-filling phase was defined. Thus, presowing treatment of Soybean seeds with nodulating bacteria apartly and with additional supply Soybean during cultivation with nitrogen fertilizer (30 kgN/ha) has shown eustress effect for plant organism during vegetation.

Working group N°3 (oral)

SOIL PROTEOMIC: A TOOL TO STUDY SOIL FUNCTIONAL BIODIVERSITY

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Current soil enzyme methods measure potential enzyme activities, which are indicative of overall enzyme concentrations. The same enzyme can have different location in soil matrix being intracellular, free in soil solution, adsorbed on a substrate or on soil colloids. The measurement of enzyme activity in soil is considered as a sensitive indicator of functional diversity in soil. However, the information is incomplete and no indication can be obtained on microbial community composition or on its change by soil management or land use. Community genomic that analyse genomic DNA directly from environmental sample, are providing opportunities to genetically characterise microbial diversity in soil ecosystems. Apart the nucleic acid approach, the new growing soil proteomic has the potential to provide a high resolution representation of genotypic and phenotypic characters of distinct community members. However, soil proteomic is still on his infancy and it was applied to characterise soil enzymes present in dissolved organic matter. Recently we find a non-homogenous distribution in the granulometric soil fractions of several enzymes linked to the bigeochemical cycles of nutrients. We believe that this could facilitate the progress of soil proteomic. The extraction of the proteins from the single inorganic fractions could simplify the extraction of enzymes from soil and help to link the functional biodiversity to microbial diversity, allowing a better understanding of microbial cell-environment interactions, such as responses of soil microorganisms to stress, land use and pollution.

Working group N°3 (oral)

A NEW TECHNOLOGICAL APPROACH IN SANDY COALMINE DEPOSOL AMELIORATION MEASURES

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This investigation, based on pot experimets with three different cultures (Zea maise, Medicago sativa, Brassica rapa), was aimed to asses the best amelioration practice for sandy coal mine tailing soils (deposols). The improvement of the phisico-chemical and nutritional status of investigated deposols (the application of solid and slow realize fertilizer, combined with different organo-mineral materials - coal dust, coal ash and cattle manure) were evaluated by testing growth parameters and plant productivity in 17 treatments per used culture. The high level of some heavy metals (cca. 50 mg/kg Ni) in investigated deposlos was found, indicated that the special attention should be given to the accumulation of heavy metals in above ground plant's parts. Beside the effect on plant's growth, the use of deferent organo-mineral mixtures and fertilizer treatments, also induced changes between treatments, so, the mineral content of analyzed plants were also dissected. The obtained results indicate the problem of high Ni accumulation in plant's root (>5-34,67mg/kg Zea maise, >5-15,92 Brassica rapa), where the question of Cr mobility within substrates and plants stayed open.

Working group N°3 (oral)

THE SELECTION OF GRAFTED MAHALEB CULTIVARS (PRUNUS MAHALEB L.) THAT ARE RESISTANT TO HIGH CaCO₃ CONTENTS IN SOIL

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Grafted M106, M11, M26, M9 and non-grafted mahaleb cultivars, which were commonly used in Konya, Karaman, and Cumra regions were employed in this research. The plantlets were grown in 30 cm plastic growth pouches, which contained soil with varying CaCO₃ contents: low (%0-1), low-medium (%1-5), medium (%5-15), high (%15-25) and very high (%25). The chlorophyll contents, macro and micro elements, plant height, plant radius development were measured in the plants to determine the effects of CaCO₃. The experimental soils have clayey contents, slightly alkaline, and low in macro and micro elements. In terms of plant height development, M 106, M11, and M9 types are resistant to CaCO₃, and the rest of them are sensitive to CaCO₃. The mineral contents of leaves of mahaleb plantlets grown in soils with varying CaCO₃ contents: N, 2.07-3.42 %; P, 0.18-0.49 %; K, 0.78-2.92 %; Ca, 0.79-1.13 %; Mg, 0.26-0.60 %, S, 0.37-1.15 %; Fe, 211-516 ppm; Zn, 9-75 ppm; B, 27-48 ppm; Cu, 0.05-0.16 ppm; Mn, 71-249 ppm. As the CaCO₃ content increased, chlorophyll content decreased dramatically.

Working group N°3 (oral)

PHOSPHATE SOLUBILIZATION POTENTIALS OF ACINETOBACTER STRAINS

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Phosphate solubilizing bacteria can be used as soil or seed inoculum to increase soil phosphorus (P) availability for agricultural purposes. A total of 21 phosphate solubilizers were isolated from the rhizosphere of wheat and maize grown in the pots, which contained soil samples. The isolates were distributed among the genera, *Acinetobacter* (7), *Pseudomonas* (7), *Enterobacter* (2), *Enterococcus* (1), *Pantoea* (1), *Bacillus* (1), and uncultured bacterium (2) as determined by the 16S rRNA gene sequence analysis. *Acinetobacter* strains were the only pyrroloquinoline-quinone (PQQ) independent and most effective P-solubilizers in Pikovskaya's agar. The mean phosphorus dissolved in liquid cultures of *Acinetobacter* strains in a five day incubation ranged between 167 and 888 ppm P. The initial pH of 7.8 dropped below 4.7 in six isolates with a gluconic acid production in the concentrations ranging between 27.5 and 37.5 mM. There was a linear regression between soluble-P and gluconic acid concentrations in the bacterial cultures. Inoculation with only one out of seven *Acinetobacter* strains significantly (P<0.05) increased both plant phosphorus content at 15 days after emergence (DAE) and dry matter accumulation at 30 DAE in wheat (*Triticum aestivum* L.). The plant P-content in inoculated plants had a linear relationship with soluble-P in the bacterial cultures. Consequently, this study showed that gluconic acid production directly affects phosphate solubilization *in vitro*, which in turn influences plant P-content in inoculated plants.

Working group N°3 (oral)

RECENT RADIO-CONTAMINATION OF SOILS IN THE CZECH REPUBLIC WITH RADIOCAESIUM

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The radioactive atmospheric fallout followed after nuclear weapons tests in the past and first of all after the accident of NPP Chernobyl caused contamination of soils also in the Czech Republic (CR). The radiocesium (^{137}Cs) content in superficial soils of CR was measured in the work. Samples of arable and other soils were collected according to standard method on 21 localities in 2008. Gamma-spectrometric measuring system with a pure germanium detector calibrated with certificated standards was used for determination of the ^{137}Cs activity concentrations in the samples. The concentrations in soils reached values in range from 4.86 Bq kg⁻¹ to 72.66 Bq kg⁻¹. Most of the concentrations were significantly lower compared to values of ^{137}Cs in soils sampled in the same localities in the past. The decrease was caused by both the loss of activities due to radioactive decay, and the method of the soil use. Summarized data confirmed the regional distribution rate of the ^{137}Cs contamination of CR due to wet deposition in the first days after the accident of NPP Chernobyl in 1986.

Working group N°3 (oral)

INDIVIDUAL OPTIMUMS OF SOIL CATION COMPOSITION FOR CROPS TO MINIMIZE ^{137}Cs AND ^{90}Sr FLOWS IN FOOD CHAINS

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Accident in the Chernobyl nuclear power plant (ChNPP) resulted in a large radioactive contamination of territories. In view of high prevalence of ^{90}Sr and ^{137}Cs in soils these radionuclides are considered as the most radiological important radionuclides presented in agro-ecosystems of Belarus. Dealing with rehabilitation of radiopolluted territories means that the remediation activities are mostly aimed to ensure an application of rational agriculture practices in adverse ecological conditions. As a consequence, large-scale countermeasures in agriculture of the affected territories have appeared to be necessary. Countermeasures applied after ChNPP accident on the contaminated agricultural lands have changed essentially the soil agrochemical properties and radionuclide transfer factors from soil to plants. The main task of investigation was the development of comprehensive approaches for preventing of radionuclides migration in biological chains. The studies performed for the sod-podzolic loamy sand soils (Podzoluvisol) contaminated by ^{137}Cs and ^{90}Sr radionuclides testified to the individual optimums of soil physicochemical properties as $\text{pH}_{(\text{KCl})}$, potassium and calcium for each type of crops. For the main agricultural crops (winter rye, spring wheat, barley, spring rapeseed, red clover, and potatoes) the minimal biological availability of ^{137}Cs and ^{90}Sr is registered for $\text{pH}_{(\text{KCl})}$ within 6.1-7.2, the exchangeable Ca content within 830-1400 mg kg⁻¹, the exchangeable Mg content within 190-250 and moveable potassium (K_2O) content 370-460 mg kg⁻¹ of soil. The efficiency of soil saturation with calcium, magnesium and potassium in radionuclide discrimination is much higher for soils with a low supply with these cations. The data of agroecological optimums is evidence of attainment and keeping at a high level of fertility of contaminated soils than in “clean” agricultural lands to minimize radionuclide flows from soil to plants. On the other hand, lime and potassium fertilizers applications in extra rates will not be effective measures to reduce radionuclides transfer to crops on soils having pH and cations concentrations higher than values of agroecological optimums. The received data are to define long-term strategy of countermeasure application in plant production of contaminated lands.

Working group N°3 (oral)

STABLE ISOTOPE PROBING IN BIOREMEDIATION IDENTIFICATION OF (POLYCHLORINATED) BIPHENYL-METABOLIZING RHIZOSPHERIC BACTERIA IN CONTAMINATED SOIL

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The knowledge of bacterial diversity had been extended by possibility to study besides cultured also the yet uncultivable bacteria. Introduction of DNA-based stable isotope probing allowed linking community structure with function. Active PCB-metabolizing bacteria in non-vegetated and horseradish-vegetated soil contaminated by PCB were identified. 16S rRNA gene clone libraries constructed from ¹³C-DNA following incubation with ¹³C-biphenyl showed the main players catabolising biphenyl. In silico digestion of the sequences in the clone libraries was used to predict terminal-restriction fragments that were matched to the peaks in the T-RFLP profiles in order to indicate the relative abundance of taxa. Our results indicate how plants affect the spectrum of bacteria and confirm the importance of SIP for revealing truly active members of the community. Uhlik et al. Sci Total Env 407, 2009, 3611-3619. Work was supported by grant 2B08031, 1M06030, MSM 6046137305, Z40550506

Working group N°3 (oral)

IAA AND ZEATIN BALANCE IN SOYBEAN PLANTS INOCULATED WITH BRADYRHIZOBIUM JAPONICUM STRAINS

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Specificity of the legume-rhizobial symbiotic systems requires high control levels with phytohormones playing an important regulatory role their. Implementation of transposonic mutagenesis means resulted in the selection of rhizobial bacteria with changed characteristics can help to extend present-day understanding of hormonal reaction of plants to the inoculation with named strains. In our experiments we had studied changes in auxin (IAA) and cytokinins (zeatin) contents in soybean plants under the inoculation with strains (646, 604) and Tn5-mutants (21-2, 9-1, 107, 113) of Bradyrhizobium japonicum and revealed correlations between strains virulence, nitrogenase activity, symbiosis efficiency and phytohormonal balance. The inoculation of soybean with strains and Tn5-mutants of B. japonicum had resulted in very certain plants hormones behavior. The inverse proportion was observed between IAA pool and nitrogenase activity of root nodules at 35 day after coming-up. Studying of cytokinins contents changes during plants vegetation had revealed that zeatin contents in root nodules had direct relation to their nitrogen fixing activity as well as to accumulation of above ground mass.

Working group N°3 (poster)

THE USE OF PHOSPHATE MOBILIZING BACTERIA BY CULTIVATE OF CEREALS
IN CONDITIONS OF SOUTH UKRAINE

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Microbe preparations are important element of contemporary agricultural, which improve of mineral nutrition of plants and promoted of their yield. In our researches was revealed the influence of microbe preparations on the base of phosphate mobilizing bacteria (PMB) on biological activity of soils in rhizosphere of winter wheat (cultivar Fantasy), spring barley (cultivar Stalker) and their yield in field experiments. Our experiments were carried out in climate conditions of south Ukraine: wheat was cultivated in Crimea (south chernozem), barley – in Kherson region (dark-chestnut soil). In experiments were conducted next backgrounds of fertilizer: barley – without fertilizer (I), N30P30(II), N57,3(III); wheat - P30(IV), P60(V), P90(VI). Pre-sowing inoculation of the seeds was supplied by preparations phosphoenterin, albobacterin, polymyxobacterin, control – without inoculation. The positive effect of PMB on the activity CO₂ to assign (of) (by) soils in rhizosphere of cereals has been established. Better results of this showing in rhizosphere of barley by phase cresting on the backgrounds II and III were marked: phosphoenterin increased twice, polymyxobacterin – treble in comparison.

Working group N°3 (poster)

EVALUATION OF SOIL METALS BIOAVAILABILITY AND LEAF MICRONUTRIENT
CONCENTRATION IN APPLE ORCHARDS UNDER CONVENTIONAL AND ORGANIC
MANAGEMENT

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A comparative study of organic and conventional apple orchard management systems was conducted in order to compares Fe, Mn, Cu and Zn availability in soil as well as to evaluate the concentration of micronutrients in the leaves of apple trees. The two orchards were nearby located in order to exclude possible pedoclimatic influences on the measured variables. The soil in both orchards was characterized as a clay loam – clay soil and was very uniform in morphological and physical properties suggesting that any differences in the measured soil parameters may be attributed to the management system and not to soil heterogeneity. Soil samples from the conventional orchard exhibited higher concentrations of Cu and Zn probably due to the application of various agrochemicals like pesticides and synthetic fertilizers in the conventionally managed soils. Significant differences among leaf micronutrient concentrations in the two management systems were observed. Leaf analysis revealed higher concentrations of Mn and Zn in conventionally grown trees while the opposite stood for Cu due probably to the extent use of many copper-containing fungicides in organic orchards in Greece.

Working group N°3 (poster)

INFLUENCE OF SOIL PROPERTIES AND PLANT POTASSIUM CONTENT ON Cs ACCUMULATION BY TRIFOLIUM PRATENSE PLANTS

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A greenhouse pot experiment was carried out to investigate the soil properties that influence Cs uptake by *Trifolium pratense* plants and to determine Cs and K interactions in the plant shoots. Four soil types were selected and soil in each pot was contaminated with 40 mg kg⁻¹ of Cs in the form of CsCl. Soils were sowed with *Trifolium pratense* seeds at 60, 240 and 420 days after contamination (treatments). Two cuts were performed on *Trifolium* shoots. Concerning the effect of soil type, the results showed that the cation exchange capacity to the exchangeable soil potassium ratio at the end of each treatment influences Cs uptake by plants in both cuts. As this ratio increased, Cs concentration in plants decreased, indicating that the particle size fractions as well as the K content in soils play a predominant role on Cs availability to plants. A significantly negative correlation between Cs concentration and K content in the shoots of *Trifolium* plants in both cuts was observed. The estimated discrimination factor (DF), often used to evaluate a plant's efficiency to absorb soil nutrients was in all treatments below unity, suggesting a preferential uptake of K over Cs.

Working group N°3 (poster)

SPORE GERMINATION OF TWO DIFFERENT ISOLATES OF ARBUSCULAR MYCORRHIZAL FUNGI

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Arbuscular mycorrhizae are formed between more than 80% of vascular plants and arbuscular mycorrhizal fungi (belonging to the phylum Glomeromycota). Arbuscular mycorrhizal fungi are considered as obligate biotrophs that they need to their growth host plant. Spores of arbuscular mycorrhizal fungi are able to germinate but they germinate very badly or never. The aim of the work was to establish the optimal method and conditions for germination of arbuscular mycorrhizal fungi in non-sterile conditions. We used spores of arbuscular mycorrhizal fungi *Gigaspora gigantea* (isolate NC150) and *Glomus intraradices* (isolate SW101). Based on our results we could recommend for the next experiments the *Gigaspora gigantea*.

Working group N°3 (poster)

INFLUENCE OF CLAY MINERALS ON BRADYRHIZOBIUM JAPONICUM GROWTH AND LEGUME-RHIZOBIA SYMBIOSIS FORMATION

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Clay minerals are able to influence on growth activity, motility, chemotaxis and survival rate of bacteria at superoptimal temperatures. It was shown that composition of the minerals with soil bacteria can enhance productivity of vegetable plants. The effect of three types of clay minerals (bentonite, montmorillonite and palygorskite) on growth activity of *B. japonicum* 634b and *B.japonicum* 10k as well as on *B. japonicum*-soybean symbiosis formation was examined. The minerals caused increase of microorganisms number during different time periods as a result of their addition into the bacterial growth medium. At the same time bentonite possessed more essential stimulatory effect on the growth of both rhizobial strains. Bentonite had promoted soybean-*B. japonicum* 634b symbiosis formation at seeds inoculation with binary bacterial cultures, consisting of rhizobia and nonrhizobial rhizospheric bacteria. Our findings suggest possibility of clay minerals usage in order to increase rate of soil bacteria with useful properties in plant root zone and efficiency of symbiotic plant-microbe interactions.

Working group N°3 (poster)

ACTIVITY DYNAMICS OF ASCORBATE- AND GUAIACOL PEROXIDASES UNDER FORMATION OF SYMBIOTIC SYSTEMS DIFFERENT BY THEIR ACTIVITY

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Enhancement of the oxidation processes intensity is one of the mechanisms of nodulation control in legume plants by rhizobia. Two enzymes – ascorbateperoxidase (AP) and guaiacolperoxidase (GP) are considered to play an important role in regulation of oxidation processes of symbiotical apparatus of legumes. In our work we have studied activity of AP and GP during soybean symbiotic apparatus early formation and functioning period under presowing seeds inoculation with strains and Tn5-mutants of *B. japonicum* with various symbiotic characteristics. Activity dynamics of the studied enzymes had testified their functional differences during formation and functioning of the symbiotic structures. Thus, GP plays an important role during nodules formation while its activity decreases with ending of legume plants symbiotic apparatus formation. AP, instead, is involved during nitrogen fixation by nodules. Thereby, production of active oxygen forms is an important component of cells response to the infection with symbiotic bacteria. At this host-plant controls their level due to the antioxidant system which includes number of enzymes, and peroxidases in particular.

Working group N°3 (poster)

HEAVY METALS CONCENTRATIONS IN URBAN AND RURAL SOILS OF AN INDUSTRIAL AREA IN GREECE

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A project was designed to monitor heavy metals distributions in urban and rural soils of the Thriasian plain, a heavily industrialized area in Attica, Greece. In this paper the preliminary results for the top soils of Eleusina, one of the Thriasian's major towns, are presented. Grid sampling was chosen and 40 soil samples (0-5 cm depth), equally representing the urban and the rural soils of the town, were collected. Total Pb, Zn, Cu, Cr and Ni concentrations in the soil samples were determined by aqua regia extraction. Since sampling close to site pollution sources carefully avoided, the total metal concentrations were not extremely high but in most cases were found above the target values. A clear, but not significant, trend for increased metal loadings in the rural soils was observed. Significant correlations between the total metal concentrations were found, suggesting common origin and similar metals dispersion and redistribution in the studied area. The performed cluster analysis showed that Pb, Cu, Zn and Cr in urban soils primarily associate to soil carbonates and Ni to clay fraction, while in rural soils metals associate to organic matter.

Working group N°3 (poster)

THE INFLUENCE OF SOIL CONDITIONERS ON THE QUALITY OF GROWING MEDIA AND NURSERY PRODUCTION

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The aim of this study was to minimize the stress factors affecting the quality of nursery production. There were evaluated the influence of synthetic soil conditioners and two irrigation regimes on the quality of ornamental nursery production grown in containers. The effect of soil conditioners on temperature, moisture and chemical characteristics of growing medium was measured in this study. This trial was held in experimental field located at the Horticultural Faculty of Mendel University (Brno) in Lednice. Weigela x hybrida 'Viktorie' has been chosen as a model plant. As soil conditioners there were used Hydrogel, TerraCottem and Agrosil LR, which were incorporated in the growing medium RKS II. During vegetative period there were observed physiological parameters (stomatal conductance, fluorescence of chlorophyll and chlorophyll content) as well as morphological parameters (plants length).

LABILE ORGANIC CARBON CONTENT AND ITS RELATIONSHIP WITH LABILE FORMS OF SELECTED TRACE ELEMENTS

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Organic carbon undergoes short and long term transformation in the soil. Under a dynamic equilibrium a portion of organic carbon is mineralized, and the same portion is newly formed. This portion of carbon is considered as active (= labile) organic carbon. We followed total carbon content, labile carbon content and labile trace elements content in selected arable soils and grassland (Haplic Cambisol, Gleyic Luvisol, Haplic Stagnosol and Haplic Luvisol). Total organic carbon content was determined by oxidimetric titration. Labile organic carbon was determined by hot water extraction method. Humus quality was assessed by HA/FA ratio and by absorption in UV-VIS spectral range. Humus fractionation was made by short fractionation method. Labile forms of trace elements (Zn, Cd, Cu, Co, Pb, Mo and Se) were determined by electrothermal atomic absorption spectrometry after extraction of the soil samples in the solution of 0.01M CaCl₂. Labile carbon content varied from 340 – 2,212mg/kg. Higher values were determined in grassland. Humus quality in grassland was lower to compare with arable soils. Correlation between total carbon content, HS sum and HA sum and labile Zn and Cd was determined. Correlation between labile organic carbon and labile Zn was found. Keywords: Labile soil carbon, labile trace elements, humus fractionation. This work was supported by the project MŠMT No. 2B08039.

Work Group 4:

Plants science and biotechnology

Working group N°4 (oral)

COMPETITIVE CANALIZATION OF PIN-DEPENDENT AUXIN FLOW FROM TWO SOURCES

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Shoot branching is one of the major determinants of plant architecture. It is known that transport of phytohormone auxin in the stem is necessary for the control of shoot branching by a dominant apex. Here we show that axillary buds of pea (*Pisum sativum* L.) establish the directional auxin export by subcellular polarization of PIN auxin transporters following decapitation. Similar PIN polarization and canalization of laterally applied auxin on the decapitated stem is prevented by apical auxin application. These results support a model, that the apical and lateral auxin sources compete for primary channels of auxin transport in the stem to control release of axillary buds from dormancy. In the presented work we also studied the possible role of strigolactone (GR24) as a modulator of the auxin transport capacity. This work was supported by grants of the Czech Ministry of Education (LC06034 and MSM0021622415), Czech Science Foundation (522/02/D137), the Grant Agency of the Academy of Sciences of the Czech Republic (IAA601630703), and IGA Nr. 5/2009.

Working group N°4 (oral)

ASSESSMENT OF A FUSED SERINE PROTEASE INHIBITOR SPI2:GFP GENE EXPRESSION IN TRANSGENIC PLANTS USING BIOTESTS WITH PLANT PATHOGENES

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Genetic modification is an efficient way to improve numerous, mainly qualitative, traits of crops like their resistance/ tolerance to biotic- (e.g. pests, diseases) and abiotic stresses (herbicides, drought, salinity, etc.). An aim of this study was to test performance of transgenic tobacco plants modified by a fused gene sequences of *gmspi2* (*Galleria mellonella* silk proteinase inhibitor 2) isolated from Wax Moth *Galleria mellonella* and of modified *m-gfp5-ER* signal gene of pacific jelly fish *Aequorea victoria* to the attack of various plant pathogens. Using a binary *Agrobacterium tumefaciens*-based vector bearing plasmid pPRD400-fusion15 construct (O. Navrátil, Institute of Experimental Botany AS CR, Prague) with plant selectable marker *nptII* the *in vitro* plants were recovered and selfed to avoid of testing chimaeras and related problems. Presence of target- and selectable genes in plants of T₀ and T₁ generations was verified by polymerase chain reaction (PCR) and other methods. Evaluation of plant resistance among various transgenic individuals was performed using, both *in vitro* and *in planta* tests. Special attention was given to fungal pathogens, of which, e.g. *Fusarium oxysporum* f. sp. *lini* is the causal agents of the most serious damage of flax crop in the Czech Republic. On the other hand, *F. oxysporum* strains have been shown as an extremely aggressive pathogene and so difficult to direct use in *in vitro* tests of plant resistance. Due to it the attention was focused to other important pathogens showing slower growth on half-synthetic media (*Ascochyta pisi*, *Botrytis cinerea*, *Phoma* sp. (*fabae*). To assess and quantify possible host plant response to viral attack a Turnip yellow mosaic virus (TYMV) isolate was used because one of its mechanisms of plant cell defence suppression involves the synthesis of serine protease. Results obtained up to now in our studies will be referred to and discussed in respect to, especially the strategy of transgenesis and assessment methods used. This study was supported by project No. 1M06030 of Ministry of Education, Youth and Sports of the Czech Republic.

Working group N°4 (oral)

EARLY CYTOKININ RESPONSE PROTEINS AND PHOSPHOPROTEINS OF ARABIDOPSIS THALIANA IDENTIFIED BY PROTEOME AND PHOSPHOPROTEOME PROFILING

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Cytokinins (CKs) regulate diverse developmental processes in plants. To get an insight into CK regulated molecular events at the proteome level, we employed 2-DE followed by image analysis and MALDI-TOF-TOF MS to analyze early changes in steady-state protein levels and phosphorylation status of the proteome in CK treated Arabidopsis seedlings. Effects of four principal CKs, t-Z, iP, BA and TDZ were compared. We observed over 160 and 90 differently expressed proteins in proteome and phosphoproteome maps, respectively, which represent about 20% of detected protein spots. Out of them, 102 proteins were identified. They represent a snapshot of early links involved in CK regulated signaling circuits and cellular processes including light signaling and photosynthesis, nitrogen metabolism, ethylene biosynthesis, CLAVATA pathway, and protein and gene expression regulation which are in line with previously described CK functions. Furthermore, our results point to a link between temperature and CK signaling. Supported by grants IAA600040701, LC06034, 1M06030, 206/09/2062.

Working group N°4 (oral)

INVESTIGATION ON ANATOMICAL AND HISTOLOGICAL STRUCTURE OF GRAFT UNION IN PLUM

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Fruit tree species rootstock selection is a most important problem for fruit growing sector which is distinguished by relatively short period of life and premature perishing of many trees. Varieties affinity to different rootstocks determinates the rate of succesful union between scion and rootstock and is the basis for the longevity of grafted trees. During grafting phenolic compounds from the cut surface cells oxidize and produce necrotic layer isolating the surfaces. Young xylem of which don't specialize from the cambium, callus cells formed from the xylem medular ray and seconder shell cells destroy the necrotic layers on the cut surface. Then, the cavity between rootstock and scion is filled and reestablish the connection between them. After this stage, the transport of water and nutrients through the grafting area occurs. So, it is important for a succesful grafting to know the anatomical development between tissue of scion and rootstock after grafting. This research was aimed to determine the anatomical structure of graft union in some graft combinations of plum using chip-budding grafting technique. The study was carried out in USAMV Iasi experimental farm in 2008. In the study there were used four scion varieties and Prunus cerasifera as rootstock. Tissue samples were taken from graft unions one year after grafting and fixed in formalin/glacial acetic acid/alcohol solution. It was determined that necrotic layers which are scattered and get brown as a result of enzymatic reaction are localiyed in the joining area tissues. By observing the development of vascular tissues we may say wether the combination is compatible or not and graft combination process take place in good condition. The study will make contribution, as a pattern, to nursery plant production to observe graft combination process which may directly affect plant production performance on new rootstock selections.

Working group N°4 (oral)

RAPD MARKERS ASSOCIATED WITH SALT TOLERANCE IN WILD TRITICACEAE: AN EXAMPLE OF TOOLS FOR EVALUATION OF BREEDING POTENTIAL IN CROP WILD RELATIVES

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Overcoming abiotic and biotic stresses are some of the major challenges facing plant breeders addressing sustainable wheat production in the 21st century. The aim of this study was to identify markers associated with salt tolerance in wild accessions of *Triticum boeoticum* and *Triticum urartu* by using both in vitro salt tolerance evaluation tests and RAPD marker analyses. 60 accessions of *T. boeoticum* and *T. urartu* were screened for salt tolerance under in vitro conditions. DNA from selected 10 tolerant and 6 non-tolerant genotypes of each species were extracted and formed into two contrasting bulks, and interrogated with 30 10-mer RAPD markers. Primer P10 and P15 produced the bands specific for tolerant genotypes, and primer P22 – a band specific for non tolerant ones. The results obtained suggests, that RAPD analyses can be recommended as cheap options to identify markers in bulked analyses of wheat and can be used efficiently in pre-breeding programs. Finally, accessions of *T. boeoticum* and *T. urartu* can be useful sources of genes for salt tolerance which will contribute to prevent wheat production losses and improve livelihood and food security of people.

Working group N°4 (oral)

COMPARATIVE ANALYSIS OF PROTEOME CHANGES IN CONTRASTING FLAX CULTIVARS UPON CADMIUM EXPOSURE

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Cadmium (Cd) is classified as a serious pollutant due to its high toxicity, high carcinogenicity, and widespread presence in the environment. Phytoremediation represents an effective low-cost approach for removing pollutants from contaminated soils, and a crop with significant phytoremediation potential is flax. However, significant differences in Cd accumulation and tolerance were previously found among commercial flax cultivars. Notably, cv. Jitka showed substantially higher tolerance to elevated Cd levels in soil and plant tissues than cv. Tábor. Here, significant changes in the expression of 14 proteins (related to disease/defense, metabolism, protein destination and storage, signal transduction, energy and cell structure) were detected by image and mass spectrometric analysis of two-dimensionally separated proteins extracted from Cd-treated cell suspension cultures derived from these contrasting cultivars. Further, two proteins, ferritin and glutamine synthetase (a key enzyme in glutathione biosynthesis), were only up-regulated by Cd in cv. Jitka, indicating that Cd tolerance mechanisms in this cultivar may include maintenance of low Cd levels at sensitive sites by ferritin and low-molecular weight thiol peptides binding Cd. The identified changes could facilitate marker-assisted breeding for Cd tolerance and the development of transgenic flax lines with enhanced Cd tolerance and accumulation capacities for phytoremediating Cd-contaminated soils. Acknowledgements: This work was supported by grants 1M06030 and MSM 2678424601 (Ministry of Education of the Czech Republic), and AV0Z50040507, AV0Z50040702 and AV0Z40310501 (Academy of Sciences of the Czech Republic).

Working group N°4 (oral)

THE STATE OF XANTOPHYLL PIGMENTS IN ETIOLATED SEEDLINGS OF HEXAPLOID TRITICALE WITH DIFFERENT TYPES OF INTERGENOMIC CHROMOSOME SUBSTITUTIONS UNDER HEAT STRESS

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Changes in carotenoid state were investigated under heat shock (HS, 42 °C, 3 h) in the leaves of the etiolated seedlings of hexaploid triticale with different types of intergenomic substitutions of chromosomes (substituted hexaploid wheat-rye amphidiploids, WRAHS). New data on changes in xanthophylls content in etiolated seedlings under heat stress were obtained. The metabolic heterogeneity of the violaxanthin (Vx) pool and different character of stress response of seedlings has been detected. The investigated genotypes could be divided into two groups according to the initial content of Vx as well as to the degree of Vx de-epoxidation. The lines WRAHS with one or two groups of replacements of chromosomes was characterized by lower relative content of Vx in the controls combined with a higher degree of its de-epoxidation under HS. For the lines WRAHS with three groups of replacements of chromosomes, there was a higher initial content of Vx but approximately lower degree of de-epoxidation under HS. The quantitation of carotenoid interconversion is discussed as selection criterion of sensitivity of triticale seedlings under HS.

Working group N°4 (oral)

APPLICATION OF BIOTECHNOLOGY METHODS IN CUCUMBER HETEROSIS BREEDING

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Biotechnology has the potential to provide the answers to most intractable problems concerning agriculture. Application of biotechnology methods in breeding is giving opportunities to develop new varieties and hybrids. Research program is conducted with application of in vitro methods, for obtaining regenerant lines, which have been used as parent for the creation of new varieties and hybrids. The purpose of the research was to obtain initial material by using callus culture in vitro methods for cucumber heterosis breeding, and especially creation new improved heterosic cucumber hybrids. Regenerants were produced during the process of long cultivation of regenerating callus tissues. As a result of regenerants study were chosen 67 somaclones from which 7 regenerants with significant breeding characteristics. 5 regenerant plants were involved in the crossing for producing heterosic parthenocarpic cucumber hybrids. Several hybrid F1 combinations were obtained as result. From them have been chosen parthenocarpic greenhouse cucumber hybrid Zovaspyur F1, as a more high-yielding, early maturity, high quality fruits. Today Zovaspyur F1 is being cultivated in the greenhouses of Armenia.

Working group N°4 (oral)

LONG-TERM STABILITY OF TRANSGENE EXPRESSION IN PEAR PLANTS IN THE GREENHOUSE AND IN THE FIELD

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High stability of foreign gene expression is essential for commercialization of transgenic trees. We evaluated stability of marker gene expression in pear clonal rootstock plants during several years. Nineteen transgenic lines expressing GUS gene with and without intron were analyzed since 1999 under greenhouse conditions and since 2001 under field conditions. Fluorometric assay of GUS activity demonstrated differences between various lines but we did not observe gene silencing in any line. Intron enhanced expression of GUS gene during all years by stable manner. Transgene expression in flowers, seeds and fruit flesh was detected by histochemical assay. All transgenic pear plants did not show any morphological abnormalities. Our results confirm possibility of stable transgene expression in fruit trees after long-term cultivation under natural conditions.

Working group N°4 (oral)

PHYTOECDYSTEROIDS MAY HAVE A NEW ROLE REGULATION OF PLANT ENZYME ACTIVITIES

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Bioaffinity chromatography on immobilized steroid ligands revealed a number of plant proteins exhibiting affinity to oxysterols, and that these compounds influence biological activity of some proteins. We found activity increase of ribulose 1,5-bisphosphate carboxylase/oxygenase by effect of ecdysteroids and brassinosteroids. Insect hormones ecdysteroids are synthesized in many plant species and their function has not yet been fully understood, can increase the carboxylase activity of isolated RuBisCO/ /by more than 10 %. We tested more analogues, all with similar effect. This protein is considered to be the most abundant protein in the world - it makes up 30-50 % of the soluble proteins in leaves. RuBisCO is accepted as the limiting step in photosynthesis. Thus increase of its activity might be very important. Computer modeling and crystallization experiments in presence of oxysterol ligands are underway, as well as analysis of synergistic effects of other known effectors or inhibitors of RuBisCO. Also other plant proteins revealed similar affinity to oxysterols, and are currently investigated. Uhlík et al., Steroids, 73, 1433-1440 (2008). The support to projects 1M06030, Z40550506, MSM6046137305 is highly acknowledged.

Working group N°4 (oral)

OXIDATIVE STRESS IN NICOTIANA TABACUM WITH ELEVATED LEVEL OF CYTOKININS

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Cytokinins (CKs) can, among others, positively regulate shoot development and delay onset of senescence. However, recently opposite effects of CK action, namely promotion of programmed cell death, and cytotoxic effects of over-expression of the CK-biosynthetic gene *ipt* in tobacco, were recognized. Here we investigated the cytotoxic effects of *ipt* expression in tobacco in detail. We show that lesion formation in expanded tobacco leaves proceeds shortly after *ipt* induction – the first lesions being observed in app. 60 hours after induction of *ipt* expression, and lesions can spread over the entire leaf area within 5 days after induction. Formation of visible lesions was preceded by increase in reactive oxygen species (ROS) as indicated by DAB and 2',7'-dichlorofluorescein diacetate staining despite increase in a key ROS scavenging enzyme – APX. Further, we demonstrate that lesion formation is a light-dependent process as it is prevented by shading. Concomitantly with increase in ROS levels, transcript levels of genes involved in light phase of photosynthesis (*FNR1* and *CAB*), and xanthophyll metabolism (*VDE*) were markedly down regulated as revealed by RT-qPCR analysis. (Supported by 1M06030)

Working group N°4 (oral)

ACCUMULATION OF HEAVY METALS AND DEGRADATION OF EXPLOSIVES BY SUSPENSION CULTURES OF TRANSGENIC FLAX

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Genetic engineering is powerful methods for enhancing natural phytoremediation capabilities of crop plants. In the cooperation with Agritec, Ltd., we obtained potentially transformed flax explants of two cv.Norlin and cv.Venica. The explants were transformed using *A. tumefaciens* strain EHA 105 containing transformation vector pBI-aMT with a-domain of mammalian metallothionein or vector pBICP encoding CP peptide known to bind heavy metal ions. This plasmids contain the *uidA(gus)* gene. Primary callus was replanting on solid MS medium supplemented by 2,4 - dichlorophenoxyacetic acid, kinetin and selection marker kanamycine. Before next subcultivation one part of the callus was tested to GUS activity using histochemical analysis and only the calluses with GUS positive part were subcultivated. Suspension cultures derived from these calluses were tested to their possibility to degrade nitrocompounds and accumulate heavy metals. The concentrations of nitrocompounds were measured in nutrient medium and in acetone extracts of cells via HPLC analysis. The concentrations of the metals were determined by AAS measurement.

Working group N°4 (oral)

CYTOKININS CAN STIMULATE ARABIDOPSIS HYPOCOTYL ELONGATION AT DECREASED LIGHT INTENSITY

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Cytokinins (CKs) were reported not to affect hypocotyl elongation in Arabidopsis seedlings grown in the light, and CK reportedly promoted hypocotyl elongation in the white light when ethylene action or auxin transport was blocked. Here we re-investigated the effects of CKs on hypocotyl elongation in Arabidopsis. While only a marginal effect of CKs on hypocotyl length was observed at a standard white light intensity ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$), a pronounced stimulation of hypocotyl elongation by CKs was found when the seedlings were cultivated at a decreased white light intensity ($20 \mu\text{mol m}^{-2} \text{s}^{-1}$). The CK effect on hypocotyl length was due to cell elongation. The stimulation of hypocotyl elongation was observed for all four principal CKs (t-Z, iP, BA, TDZ) and was dose-dependent in the nanomolar range. The stimulatory effect of the CKs was antagonized by PI-55. Mutant and transgenic plant analysis indicated that the canonical two-component response pathway is necessary for this process with prevailing contribution of AHK2 and AHK3 receptors. This CK action was independent of ethylene signaling and partially inhibited by IAA. Supported by grant Nos. LC06034, 1M06030.

Working group N°4 (oral)

PERSPECTIVES OF USING CELL, HAIRY ROOT AND SPROUT CULTURES IN FOOD TECHNOLOGY

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Our study focuses on obtaining health related compounds that can be used as components of functional food or nutraceuticals. We compare the efficiency of plants and in vitro cultures as source of metabolites. In vitro cultures can be cultivated independent on season; especially if the initial plants are rare or have specific growth requirements. As in vitro we use cell, hairy root, and shoot cultures. The optimal choice of production system depends on the plant species, cultivation conditions and methods of extraction.

Working group N°4 (oral)

UTILIZATION OF IN SITU RNA HYBRIDISATION TECHNIQUE IN STUDY OF EUPHORBIA PULCHERRIMA FLOWER ABSCISSION

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In situ RNA hybridization techniques allow specific nucleic acid sequences to be detected in morphologically preserved chromosomes, cells or tissue sections. In combination with immunocytochemistry, in situ RNA hybridization can show the relationship of microscopic topological information to gene activity at the mRNA level. The premature abscission of flowers leads to severe losses in crop value. Usually, abscission of flower buds in (*Euphorbia pulcherrima*) poinsettia occurs over approximately 2 to 3 weeks. Here we investigate gene D2-122 which is expressed during abscission. Flowers pedicels of mature plants were removed on day 6 and 7 (D6, D7) to use as a plant material for experimentation. Insert D2-122 was transfected into pCR4-TOPO cells in *E. Coli* cultures. In vitro transcription has been used to produce DIG labeled RNA probes. The highest identified expression was noted in distal parts of longitudinal parts of tissues, above abscission zones (AZs). D2-122 is expressed in dying cells or in cells after flowering. Results were obtained during BIO 350 course at UMB in Norway in January 2009.

Working group N°4 (oral)

GENETIC MODIFICATION EXPERIMENTS ON PEA WITH CONSTRUCTS CONTAINING GMSPI-2 GENE AIMED AT IMPROVED PATHOGEN AND INSECT RESISTANCE

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Some types of protease inhibitors isolated from insect digestive tract may cause – if expressed in plants - increased resistance to fungal pathogens and insects. A small inhibitor (36 aminoacids) with antibacterial and antifungal activities was isolated from silk fibres of *Galleria mellonella*. Optimised constructs pWell09 and pWell11 bearing gene gmSPI-2 (*Galleria mellonella* Silk Proteinase Inhibitor 2) were prepared by cloning of the fusion protein sequence into the pGREENII 0229 plasmid (John Innes Center, UK), which includes the selection gene nos::bar and also the 35S::uidA reporter gene. Method of *Agrobacterium*-mediated genetic transformation of pea was applied on several regeneration systems in vitro (organogenesis from axillary and apical meristems of mature seeds, organogenesis from embryo axis of immature embryos), and in „non-tissue“ approach (trimmed imbibed seeds). The selection of potential transformants proceeded in all three regeneration protocols on regeneration media supplemented with 10 mg phosphinothricin. All regenerated plants were tested histochemically for uidA gene and by PCR for presence of gmSPI-2 transgene, the effectiveness of transformation ranged between 1-5% from initially established explants. Progeny of T1 plants will be further tested in glasshouse and field tests for resistance to necrotrophic fungal pathogens and insect pests.

Working group N°4 (oral)

DIFFERENT SENESCENCE-SUPPRESSION ACTIVITIES OF ISOPRENOID AND AROMATIC CYTOKININS IN OAT AND WHEAT LEAVES REFLECT DIFFERENCES IN CYTOKININ UPTAKE AND METABOLISM IN THE TWO PLANT SPECIES

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Isoprenoid and aromatic cytokinins exhibit different senescence-suppressing activities when applied to detached wheat and oat leaves. We demonstrate that isoprenoid trans-zeatin riboside (t-ZR) and aromatic 3-hydroxybenzyl adenosine (meta-topolin riboside, mTR) differ in their uptake rates, effects on induction of cytokinin oxidase/dehydrogenase (CKX) activity and accumulation of different endogenous cytokinins in leaves of the two plant species. While the wheat leaf apices are rapidly saturated with both applied [³H]mT and [³H]t-Z, the uptake of the two cytokinins by oat leaf apices is gradual and reaches saturation as late as after 16 h of incubation. Application of exogenous t-ZR and mTR dramatically enhances CKX activity, namely in oat leaf apices. Higher increase in CKX activity is most probably responsible for lower senescence-suppression activity of t-ZR (substrate of CKX) as compared to CKX-resistant 3OHBAR in oat leaf apices. Results suggest that different cytokinins can exhibit different biological activities even in closely-related plant species depending on their uptake and metabolism of endogenous cytokinins.

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Working group N°4 (oral)

AGROBACTERIUM-MEDIATED TRANSFORMATION OF FLAX - A FUNCTIONAL METODOLOGY

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Transgenic plants of flax (*Linum usitatissimum* L.) cvs. AGT-0917 have been obtained by *Agrobacterium*-mediated transformation. *A. tumefaciens* strain EHA150 carrying binary vector with gene of interest (GOI), respectively pBI-alpha-MT with alpha-domain of metal-binding mammalian metallothionein alpha-mt, which can be responsible for heavy metal binding and detoxication, was used for transformation. The insertion of a MT gene into flax genome has been confirmed by PCR-analysis and GUS assay. Various modifications of *Agrobacterium*-mediated transformation protocol were studied in order to improve transformation efficiency. Partial removal of hypocotyl epidermal tissue increased transient transformation efficiency, 100 mg/l acetosyringone and 200 mg/l cellulase in cocultivation medium increased transient transformation efficiency. The GUS/PCR confirmed transformation efficiency was 1.6 % in cv. Jitka and 4.4 % in line AGT-0917. Transgenic plants were successfully transferred to soil for further evaluation. Cd content in roots, stem and capsules of GM T0 plants (after harvest of T1 seeds) grown in the soil with natural Cd background was higher as compared to non-transformed controls. Acknowledgement: 1M06030

Working group N°4 (oral)

CYTOKININ N-GLUCOSIDES: THEIR APPARENT BIOLOGICAL INACTIVITY RECONSIDERED

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Cytokinin (CK)-N7- and N9-glucosides represent apparently final deactivation products of CKs in plant metabolism. Although covering up to 90% of total CKs in some plant species, they are considered to exhibit no activities in bioassays. Despite of this, we revealed pronounced inhibitory effects of N9-glucosides of isoprenoid and aromatic CKs (10^{-4} M and 5×10^{-4} M) on the dark-induced chlorophyll degradation in detached oat leaves. Based on the high stability in tested solutions, a possible spontaneous conversion of CK-N9-glucosides to corresponding bioactive bases was excluded. In detached oat leaves, however, no antisenescent effects were found for corresponding CK-N7-glucosides applied at the same concentrations. As the LC-MS analysis revealed no variation in endogenous CK levels and profiles between N7- and N9-glucoside-treated plants it is presumed that distinct antisenescent effects of CK-N7- and N9-glucosides might be attributed to differences in their signalling, transport etc. rather than in biosynthesis and metabolism. Besides, remarkable cell division effects were surprisingly found for dihydrozeatin-N7-glucoside in tobacco callus bioassay. Supported by GA ASCR 600380701.

Working group N°4 (oral)

HISTOLOGICAL INVESTIGATION ON GRAFT FORMATION OF SOME PEAR VARIETIESGRAFTED ON QUINCE

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It is known that success of grafting in higher plants depends on two essential factors: the physiological compatibility between the two graft partners, scion and rootstock and the proper alignment of like tissues. Vascular tissue formation is the last stage of the successful grafting. Formation of a strong union depends on differentiation and amount of new vascular elements formed after grafting. Histological evaluation on graft sections gives us first information about compatibility or incompatibility of combination in short time. This research was aimed to determine the anatomical structure of graft union in some heterograft combinations of pear/quince. The study was carried out in USAMV Iasi Experimental Farm in 2008. In the study there were used four scion varieties and quince as rootstock. Tissue samples were taken from graft unions one year after grafting and fixed in formalin/glacial acetic acid/alcohol solution. Cambium differentiating, necrotic layers, cambial continuity and vascular tissues formation were analyzed. It was observed from the anatomical structure of graft union area that new cambium, xylem and phloem tissues were formed and there was needed longer time for continuous cambial merging. Some abnormalities were seen at graft union area at some combinations and it was suggested that there could be a not very good compatibility for these graft combinations. The applicability of this study could be the possibility to apply an early selection method that could predict the future of a determinate combination long before the external symptoms can be observed.

Working group N°4 (poster)

PROTEOMIC ANALYSIS OF A. THALIANA INFLUENCED BY LIGHT AND CYTOKININS

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Proteome analysis is able to reveal differences of protein expression caused for example by influences of environmental factors (light, cold, starvation) or effects of various substances (hormones, chemicals). Proteome analysis includes protein isolation, 2D gel electrophoresis, image analysis and mass spectrometry for identification of proteins with changed expression. We used this method for studying the differences between two light intensities with simultaneously increased endogenous cytokinin levels in Arabidopsis plants. The aim of our work is to reveal differences and/or similarities between the action of light and cytokinins because some of their effects are seemingly identical (eg. chloroplast development).

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Working group N°4 (poster)

EPIGENETIC CHANGES IN GRAPEVINE AFTER IN VITRO CULTIVATION AND IN VITRO THERMOTHERAPY

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The molecular basis of the stress-induced somaclonal variability is not precisely known, but it is understood to be generated via a combination of genetic and/or epigenetic changes. Epigenetic changes are among others caused by reversible methylation of DNA sequences. MSAP analysis using a pair of isoschizomers, HpaII and MspI, which are differentially sensitive to cytosine methylation at the CCGG sites, was used to analyse DNA-methylation alterations in stressed grapevine plants. Our aim was to evaluate the potential changes under stressing conditions copying as far as possible the situation prevailing in commercial breeding if *in vitro* micropropagation via nodal segments or *in vitro* thermotherapy for virus elimination is used. Original approach for data processing allowed us to evaluate the impact of different factors on the frequency of DNA methylation changes. Generally, it seems that impact of used stresses on character of DNA methylation changes in regenerants is relatively weak, often less than effect of initially used maternal plants.

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Working group N°4 (poster)

DETERMINATION AND EXTRACTION OF PHENOLIC ACIDS IN ROSEMARY (ROSMARINUS PROSTRATUS) WITH STRESS INDUCTION

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The objective of this research was to determine the concentration and to extract phenolic acids in rosemary (*Rosmarinus prostratus*). These acids have a high importance for the human health and for the food industry because of antioxidative, antibacterial and anti-inflammatory properties. We have determined caffeic, vanillic, chlorogenic and iso-chlorogenic acid in leaves of rosemary. We have seen that the last compound is present in higher quantity in comparison with the other acids. To increase the amount of phenolic acids we try to stress the plants by using high hydrostatic pressure system (50, 70, 100 MPa), but we had the opposite effect. We are applying other stress factors as UV-B radiation and pulsed electric field to see their effect on the phenolic acids production.

Working group N°4 (poster)

ETHYLENE PRODUCTION AND THE NUMBER OF FLOWERING BUDS OF APRICOTS AS THE MARKERS OF EXIT FROM ENDOGENOUS DORMANCY

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Production of ethylene and the number of flowering buds were observed for two years for four selected apricot variants – ‘Sundrop’, ‘Stark Early Orang’ (SEO), ‘Vestar’ and ‘Betinka’. Fifty percent of flower buds flowered early in the laboratory condition during the experiment year 2007/2008, with the latest variant ‘Stark Early Orange’ flowering from 17th January 2008. The ‘Stark Early Orange’ variant flowered two weeks later (14 days) in the experimental year 2008/2009 – 2nd February 2008. The respective order of flowering for the given variants was the same in both experimental years. The production of ethylene in the experimental period, when in laboratory conditions flowered 50% of flower buds until mid February, had also a similar trend. The increasing production of ethylene during initial and full flowering stages is also dependent on the ambient temperature before the sampling of the twigs. Low temperatures lead to increased ethylene production, whereas higher ones result in decreased amounts of ethylene produced.

Working group N°4 (poster)

ANALYSIS OF APRICOT CULTIVARS BY SSR PRIMERS

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Apricot is one of the most important species of stone fruit. Studies of genetic diversity and genetic relatedness assisted by molecular markers can improve the use of different genotypes in breeding programs and the design of new crosses. The genetic analysis of apricots and closely related species were performed using SSR. The total of 95 cultivars maintained in the gene resources collection of Horticultural Faculty in Lednice, Department of Fruit Growing, were analyzed using 9 microsatellite primers. These primers produced from 14 to 28 alleles per locus. The dendrogram of genetic similarity was constructed, based on the Jaccard coefficient. The analyzed cultivars have been successfully distinguished into the clusters. One of clusters contains the group of interspecific hybrids and botanic species. Another cluster is consisted mainly from Asian cultivars and also from hybrids of crossing between European and Chinese cultivars. American cultivars are situated in another cluster. Remaining part of dendrogram include cultivars from different eco-geographic groups. In this collection there was also situated the group of genotypes with supposed relatedness to 'Velkopavlovická'.

Working group N°4 (poster)

GENETIC EROSION: THE REAL DANGER FOR GENE BANK ACCESSIONS!

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The main purpose of ex situ genebanks is to maintain integrity and functionality of stored seed samples. The long-term conservation of entire genetic spectra is required together with maintenance of sufficient seed amount for users. Periodical regeneration of samples is unfortunately performed mostly on limited space with small number of individuals, which both increase the risk of small population size, leading to decrease or even loss of diversity. We focused on the possibility of genetic erosion (in broader sense) – drift or shift during conservation process of the pea (*Pisum sativum* L.) genetic resources. Also the effectiveness of different marker methods used (morphological characterization according to UPOV standards and microsatellites) in monitoring the genetic stability/erosion during conservation process will be discussed.

Working group N°4 (poster)

THE LEVEL OF CYTOKININ IN CADMIUM-SENSITIVE AND RESISTANT CULTIVARS OF FLAX TREATED WITH CADMIUM(II) IONS

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Phytohormones are compounds involved in the regulation of physiological and developmental processes including the modulation of plant stress responses. It was found that plants under stress conditions produce various phytohormones relating to the type of the stress and its duration. In this study, we aim at monitoring the levels of zeatine cytokinins (CK) in flax plants (*Linum sativum*) exposed to cadmium(II) ions (0, 10 and 100 μmol). To test our experimental design, we selected three cultivars of flax with different accumulation capacity and tolerance to cadmium(II) ions. It was found that the levels of CK in the cultivars of interest varied from ten to hundreds $\text{pg}\cdot\text{g}^{-1}$ of fresh weight. Other finding was that their concentration was generally higher in the root compared to above ground part. In the case of 10 $\mu\text{mol}\cdot\text{l}^{-1}$ cadmium(II) ions, the content of CK was higher in sensitive cultivar compared to control plants only. The other three cultivars had lower content of these substances. In contrast, the highest concentration of cadmium(II) ions was reported to stimulate synthesis of CK instead of the sensitive cultivar, where the decrease was detected. Acknowledgement (1M06030)

Working group N°4 (poster)

QUANTIFICATION OF CYTOKININS BY USING OF SCREEN PRINTED ELECTRODES

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Cytokinins are a large group of structurally related phytohormones, which are intensively studied in the context of their physiological functions in plants. Therefore it is necessary to have a method for their detection. In some model experiments the need for a simple determination of cytokinins exceeds exact quantification and speciation of each of them. On the contrary, there are preferred cheap and rapid methods such as immunological determination. We have developed competitive Enzyme-Linked Immunosorbent Assay (ELISA) method for the determination of zeatine cytokinins via electroactive substrate using miniaturized voltammetric sensors. We optimized electrochemical detection steps such as the effect of frequency on the signal height and measured the linear dose-response dependence. Under the optimal conditions it is possible to quantify the substrate from 360 ng / ml , which was sufficient for the implementation of ELISA.

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Working group N°4 (poster)

THE DIFFERENCES IN THE DYNAMICS OF FORMATION OF STRESS-INDUCED-THIOLS IN EIGHTEEN CULTIVARS OF FLAX

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Binding of metal ions via free –SH moieties is one the most important process in metal ions metabolism in plants. SH moieties is present in large number of biomolecules, but apply only to those who are present in large quantities and have high affinity for metals, or are precursors for the formation of strong metal binding. These conditions meet cysteine, glutathione and phytochelatins. Quantitative synthesis of these substances determines the capacity of metals, which can plant be up-taken by a plant without toxic symptoms. Description of the dynamics of molecular mechanisms is valuable information for phytoremediation technologies and also the aim of this study. We used eighteen cultivars of flax for our purposes. We determined cysteine (Cys), reduced (GSH) and oxidized (GSSG) glutathione and phytochelatins (PCs 2-4). We found that the highest content of Cys and GSH produced cultivar Tabor (1.822 mmol/g fresh weight) and PC 2-4 cultivar Bonet (0.027 mmol/g fresh weight). Cultivar Super synthesized the lowest content of thiols. The financial support from grants GACR 522/07/0692 and 1M06030 is greatly acknowledged.

Working group N°4 (poster)

BARLEY AS A SOURCE OF SUBSTANCES BENEFICIAL TO HEALTH

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Production of spring barley in the Czech Republic is important mainly as a raw material for production of malt. In addition, it is also necessary to mention production of so-called functional foodstuffs or nutraceuticals, these are phytonutrients from barley caryopses that are suitable for nutrition of people and at the same time they are beneficial to health, particularly in preventive nutrition in relation to civilization diseases. We determined tocopherols content (i.e. isomers of vitamin E), arabinoxylans, beta-glucans, ferulic acid and superoxide dismutase activity at twelve spring barley varieties/lines in the three-year monitoring (2005, 2007-08). Content of tocopherols was affected by the varieties, locations and chemical treatment. Superoxide dismutase activity was not statistically significantly affected by chemical treatment but similarly to tocopherols content it was affected by the varieties and locations. Based on our study, the line KM 1057 with high contents of tocopherols, arabinoxylans and the highest activity superoxide dismutase and the line KM 2084 with high beta-glucans content were selected, when their breeding is completed, these lines will be suitable varieties for direct food use.

Acknowledgement: Study was supported by the M. of Education, Youth & Sports, project 1M0570

Working group N°4 (poster)

INTERACTION BETWEEN BARLEY CARYOPSES DORMANCY AND MALT QUALITY

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Dormancy (post harvest maturation) of barley caryopses is an important factor affecting their biological and malting values. Intensity of dormancy of six malting barley varieties (Bojos, Jersey, Malz, Prestige, Sebastian, and Tolar) was tested in laboratory experiments. The depth of dormancy was assessed according to physiological parameters (production of ethylene, ethane, carbon dioxide and consumption of oxygen) determined at germination of caryopses in the terms immediately after harvest, 3 and 6 weeks after harvest. Malt yield was also assessed. The selected malt quality parameters- β -glucan content, α -amylase activity, homogeneity and modification by the Carlsberg method were correlated with the physiological parameters. Statistical stepwise regression was used to determine significant dependencies between ethylene production and malt yield and during barley steeping and germination on β -glucan content, homogeneity and modification of malt. Ethylene production at the beginning of germination is a highly significant indicator of alpha-amylase activity. Production of CO₂ is an indicator of malt homogeneity and modification. Quantity of the consumed oxygen after the first steep refers to malt yield and future malt modification. Study was supported by the Ministry of Education, Youth and Physical Training of the Czech Republic.1M0570, Research Center for Study of Extract Compounds in Barley and Hop.

Working group N°4 (poster)

USE OF TRANSGENOSIS FOR THE INDUCTION OF PLANT RESISTANCE TO INSECT PESTS

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Insect pests are one of the serious problems that can cause great economical loss during pea cultivation. Integrated plant protection is a modern trend against pest and diseases, and includes also creation of resistant varieties by biotechnological tools, especially transgenesis. For preparation of pea plants resistant to insect pests the strategy of expression of an insect protease inhibitor driven by a constitutive promoter was used. The protease inhibitor GmSPI2 was isolated by Nirmala et al. (2001: Eur. J. Biochem., 268: 2064-2073) from *Galleria mellonella*. To avoid difficulties with the localization of protein expression a functional fusion p35S::GmSPI2-GFP was prepared in the binary vector pGreenII (John Innes Center, UK). The transformation efficiency of the construct was tested by the tobacco leaf disc transformation. The GFP expression in regenerated plants was monitored by confocal laser scanning microscopy. Pea plants were transformed by the *in vivo* transformation method (Švábová et al., 2005: Biol. Plant., 49: 361-370). Transgenic lines have been prepared for testing of transgene expression and insect resistance.

This work was supported by a grant 1M06030.

Working group N°4 (poster)

REVERSIBLE GLUCOSYLATION IN THE HOMEOSTASIS OF ZEATIN-TYPE CYTOKININ THE ROLE OF SUBCELLULAR COMPARTMENTATION

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The maize beta-glucosidase Zm-p60.1 catalyzes the only known deglycosylation of cytokinin (CK) glucosides, releasing free CK from O- and N3-glucosides. Reversible glucosylation is important in the homeostasis of active CK forms in the cell and the sub-cellular location of this conversion is proposed to affect final CK levels. We have used Zm-p60.1 expressed in different subcellular compartments as a molecular tool to address this question. We have shown that over-expression of Zm-p60.1 disrupts the zeatin metabolic network during early seedling development that the vacuole is indeed the storage organelle for ZOG. We have investigated the phenotypes of the progeny of crosses with plants over-expressing the glucosyltransferase ZOG1, and the progeny of crosses between a couple of the subcellular variants of Zm-p60.1. The molecular and physiological characterization of the phenotypes will be presented.

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Working group N°4

INTERACTION BETWEEN 2,4-D, ACC AND ETHYLENE IN GROWTH OF TOBACCO BY-2 SUSPENSION

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We studied the correlations between accumulation of 2,4-D and ACC in cells of BY-2 suspension culture and their ethylene production during 6-day-period of cultivation with 0.1, 1, 10 and 100 μ M 2,4-D in the medium. The highest viability of BY-2 cells during cultivation on the medium with 1 μ M 2,4-D was observed. The increased total cell number was statistically significant at the 2nd day of cultivation. Medium with 100 μ M 2,4-D decreased the total cell number and viability after 3 days of cultivation. Higher concentration of 2,4-D in the medium caused accumulation of 2,4-D in the cells. Content of ACC in cells was higher at the beginning of cultivation. High concentration of 2,4-D in the medium maintained ACC level to the end of cultivation. Production of ethylene was the highest at the beginning and the end of cultivation. During cultivation on 100 μ M 2,4-D production of ethylene was not elevated after 4th day of cultivation. Concentration of 2,4-D in the medium affect viability and growth of cells by increasing synthesis and accumulation of ACC. Viable and growing suspension accumulated less ACC and produced more ethylene.

Working group N°4 (poster)

THE LEVEL OF ABSCISIC ACID, CYTOKININS AND ETHYLENE PRODUCTION DURING TUBER FORMATION OF POTATO (*SOLANUM TUBEROSUM* L.) IN VITRO

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The aim of this study was to determine the level of abscisic acid, cytokinins, ethylene and CO₂ production during potato microtuber formation in vitro. Stem nodal segments of potato plants were cultivated in vitro for 8 weeks on Murashige-Skoog medium containing modified level of inorganic nitrogen (10-12 µM or 65-70 µM), 80 g.l⁻¹ sucrose and 10 mg.l⁻¹ benzylaminopurine. Cultures with low nitrogen level in induction medium showed higher frequency of tuber formation, but high content of nitrogen increased the fresh weight of tubers. During induction of tuber formation (first two weeks of cultivation) nodal segments produced ethylene and increased level of cytokinins. Later ethylene production inhibited the growth of stolons and tuber formation. Higher level of abscisic acid was determined in leaves, later in stolons and tubers on nodal segments cultivated on induction medium with low level of nitrogen.

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Working group N°4 (poster)

METABOLOME OF SUNFLOWER PLANTS

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The aim of this study was to suggest and optimize several methods for detection of enzymes closely related with protection of an organism against free radicals. As an experimental model, we selected various cultivars of sunflower plants treated with cadmium(II) ions. We optimized easy-to-use and automatic detection of several enzymes involved in free radicals scavenging and related biochemical pathways. Particularly, we aimed at phytochelatin synthase, glutathione transferase, glutathione reductase, glutathione peroxidase, catalase, superoxid dismutase, ascorbate peroxidase, urease, aspartate aminotransferase, alanine aminotransferase and others. Moreover, we compared the altered activity of the above mentioned enzymes with the level of low molecular mass thiols determined by liquid chromatography with electrochemical detection. Multi-instrumental approach for evaluation of phytoremediation potential of several sunflower cultivars can bring new interesting point of view on plant metabolome affected by heavy metal ions.

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Working group N°4 (poster)

INFLUENCE OF CADMIUM(II) IONS ON TWENTY TWO CULTIVARS OF FLAX (LINUM USITATISSIMUM) PLANTS FOR PHYTOREMEDIATION

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One of technologies for decontamination and remediation of the polluted environment by using environment-friendly technologies is called phytoremediation. To consider whether the specific plant specie and its cultivar is able to or is not able to remediate the polluted environment, complex analysis of protective mechanisms, enzymatic system and others markers is needed. In this study we investigated the influence of cadmium(II) ions on twenty two cultivars of flax (*Linum usitatissimum*). Seven day old flax seedlings were treated with cadmium(II) ions (0 and 80 μ M) for five days. We determined activities of alanine transaminase and aspartate transaminase, total content of proteins and thiols, and particular concentration of phytochelatins (desGlyPC, PC2, PC3, PC4 and PC5) and reduced and oxidized glutathione. We compared all obtained results and calculated "Factor of Phytoremediation Suitability". This factor includes all determined markers. A cultivar called "Agata" has one of the highest factors.

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Working group N°4 (poster)

BIOINDICATION OF ENVIRONMENTAL STRESS

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Polycyclic aromatic hydrocarbons (PAHs) participate in a considerable extent of increasing anthropogenic pollution in the environment. Plants have ability for their bioconcentration and bioconversion. An attention is paid to molecular, biochemical and physiological indicators of the exposure to organic compounds which could precede the visible injury of plants. The aim of this study was to use the biochemical and physiological parameters of pea plants (*Pisum sativum* L.) as early indicators of phytotoxic effect of PAH fluorathene (FLT). Pea seeds germination and seedlings growth were evaluated after 3 and 7 days, respectively. Plants were cultivated in Reid-York nutrient solution with FLT (0, 0.1, 1 and 5 mg/l) for 20 and 27 days. FLT presented both in the environment and plant tissues caused changes not only on subcellular level (PSII, photosynthetic pigments, GSH) but also on level of whole plant organs (biomass production). Changes of values of chlorophyll fluorescence parameters and GSH content show an influence of FLT on structure and function of membranes accompanied by free radicals formation. This work was financed by Czech Science Foundation (GACR 522/09/0239).

Working group N°4 (poster)

CHANGES OF BIOLOGICAL ACTIVITY OF WHEAT GERM AGGLUTININ UNDER AFFECTING OF ITS HAPTEN

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Plant lectins are molecules exhibiting a wide range of biological activities. Carbohydrate specificity is a fundamental characteristic of lectins. Interaction haptens with active centers of lectin can reduce or completely block the agglutinin effects. Modulation of the effect of wheat germ agglutinin (WGA) with haptens, N-acetyl-D-glucosamine (GlcNAc), in case of pre-sowing treatment of spring wheat seeds, on biological components of the system "plant-soil-microorganisms" was studied. WGA stimulated the root and green mass formation, chlorophyll content, wheat grain productivity as well as the amount of soil nitrogen-fixing microorganisms, their nitrogenase activity and the growth-activation ability of rhizosphere soil. Haptens GlcNAc binding the active centers of WGA inhibited its effect in relation to the functioning of nitrogen-fixing rhizospheric microorganisms and development of plants.

Working group N°4 (poster)

CONTENT OF ALOE VERA L. ACTIVE CONSTITUENTS AS A FUNCTION OF CULTIVAR AND GROWTH CONDITIONS

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Aloe vera L. is a plant containing many substances (mostly anthraquinones and polysaccharides) with beneficial effects to human health. The polysaccharides are nutritional supplements which seem to prevent diabetes and arteriosclerosis, and to affect positively the immunity system. Anthraquinones (aloin and its aglycone aloin) have bactericidal, laxative, anti-inflammatory and anti-tumoral activity. Aloins are easily oxidized by light and air, and their content seems to vary upon several factors. The aim of this study was to evaluate Aloe vera active constituents in plants having different cultivars, age and growth conditions. Anthraquinones were determined by liquid chromatography followed by tandem mass spectrometry with electrospray ionization source in negative mode (LC-ESI/MS/MS) and quantified by the external standard method. Polysaccharides were instead determined colorimetrically at 540 nm after binding with Congo red dye. The results evidenced that aloin is the anthraquinone relevant in Aloe vera, detectable at a concentration level ranging from 280 mg/kg to 700 mg/kg, depending on cultivar considered, leaves age and growth conditions (glass or plastic houses).

Working group N°4 (poster)

MODULATION OF CYTOKININ ACTION BY DECREASED INTENSITY OF WHITE LIGHT IN ARABIDOPSIS - A PROTEOMIC ANALYSIS

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Light and cytokinin (CK) signaling are intertwined at several levels, and the underlying molecular mechanisms are being actively researched. To get an insight into the modulation of CK action by decreased light intensity at the proteomic level, we used 2-DE followed by image analysis and MALDI-TOF-TOF MS to analyze changes in steady-state protein levels in *Arabidopsis thaliana* seedlings with increased content of endogenous CKs cultivated at standard ($90 \mu\text{mol m}^{-2} \text{s}^{-1}$) and decreased ($20 \mu\text{mol m}^{-2} \text{s}^{-1}$) white light intensities. After activation of the CK-biosynthetic gene *ipt*, we observed about 75 differentially expressed protein spots (representing about 12% of detected spots). Out of the 75 protein spots, 22 were regulated in a comparable fashion at both light intensities, and 8 and 45 were differentially regulated at only standard or decreased light intensity, respectively. Till now more than 40 proteins have been identified, and can be classified as proteins involved in seed germination, photosynthesis, carbon and nitrogen metabolism and metabolism of xenobiotics. (Supported by grants IAA600040701, LC06034 and 1M06030.)

Working group N°4 (poster)

PURIFICATION AND CHARACTERIZATION OF PROTEINS FROM PLANT MATERIAL USING DIVERGENT FLOW ISOELECTRIC FOCUSING FOLLOWED BY MALDI MS

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A protein study by MS demands proper purification and separation steps prior to MS analysis. The goal of this work was to separate and prepare the proteins from the extremely crude extracts from plant materials for further analysis. The extracts from the leaves of *Arabidopsis thaliana*, and barely grain were used as model sources of studied proteins. The method of Divergent Flow Isoelectric Focusing (DF IEF) was carried out in a separation channel with increasing width. Separated fractions were collected in 20 micro vials and further analyzed by MALDI-TOF MS. The results confirmed that proteins are separated and concentrated during IEF according to their pI values while salts and impurities are removed, which significantly improved the quality of MS spectra of intact proteins. Moreover the proteins were concentrated in collected fractions which simplified and enhanced the subsequent study of occurred post-translational modifications by other techniques including SDS-PAGE, in-gel and in-solution digestions.

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Working group N°4 (poster)

CYTOKININ SPECIFIC MAIZE β -GLUCOSIDASE ZM.P60.1. PROTEIN DESIGN AND DIRECTED EVOLUTION IN UNDERSTANDING STRUCTURE-FUNCTION RELATIONSHIPS

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Our model enzyme, the maize β -glucosidase Zm-p60.1, is important for the regulation of plant development due to its role in the targeted release of active phytohormones (cytokinins) from their inactive storage forms, cytokinin-O-glucosides. Among the glucosidases involved in plant hormonal metabolism, Zm-p60.1 is the best described. β -glucosidases comprise a highly diverse group of proteins in terms of homologous enzymes, which enables bioinformatics as well as the elucidation of their biological significance. Methods of rational design in protein engineering are the foundation of our work. By changing amino acid residues at the important spots we study the determination of substrate specificity and influence(s) of particular residue on kinetics and structure of the enzyme. That approach, however, has several limitations. Therefore we have adopted a strategy of random site directed mutagenesis followed by directed evolution to investigate the functional relationships between amino acid residues. Acknowledgements: This project was supported by grants LC06034, 1M06030, LC06010, MSM0021622415 and MSM0021622412 from the Ministry of Education, Youth and Sports of the Czech Republic, and AV0Z50040507 and AV0Z50040702 from the Academy of Sciences of the Czech Republic.

Working group N°4 (poster)

DIFFERENT EFFECTS OF PLANT HORMONES ON THE GROWTH AND GLUCOSINOLATE ACCUMULATION OF BRASSICACEOUS HAIRY ROOT CULTURES

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Terrestrial plants are an extremely diverse source of secondary metabolites. Several compounds are known to have health-promoting effects to human. Since the mid 1980s several laboratories realized the potential of using transformed roots by *Agrobacterium rhizogenes* for biosynthesis of certain secondary metabolites. The advantage of usually fast growing hairy root cultures(HRC) is that they are easy to cultivate in defined media and conditions. Characteristic secondary metabolites of Brassicaceae are the glucosinolate (GS). Some GS exhibit protective activities against cancer. The goal of our study was to establish HRC of Brassicaceae and increase accumulation of GS and biomass. *Brassica botrytis*, *Raphanus sativus*, *Sinapis alba*, and *Brassica rapa* f. *teltowiensis* were transformed with *A. rhizogenes* LBA 9402. Weight increases of HRC within four weeks in MS medium and final GS contents were determined. Bio mass increase was significantly induced in *B. botrytis*, *R. sativus*, and *S. alba* by adding a plant hormone mixture including 2,4-D. The dominant metabolites in the HRC were indole GS. Plant hormone mixture induced an eight-fold GS increase in *B. botrytis* and a two-fold in *R. sativus*.

Working group N°4 (poster)

THE TRANSGENE EXPRESSION MODIFYING THE SUGAR CONTENT IN POTATO TUBERS

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We addressed the phenomenon of low temperature sweetening in cold-stored potato tubers by engineering the glycolytic pathway. Subsequently three different Czech cultivars were transformed by Lbpfk producing cold-tolerant phosphofructokinase. The transgenic potato plants were tested in field trials and their tubers cold-stored for more than three months to verify the effect of Lbpfk under natural conditions. Reducing sugar content and frying colour of chips were measured. The transgenic plants derived from all three cultivars displayed lower reducing sugar content, as well as improved frying colour compared to nontransformed control plants. Some transgenic lines from the last cultivar (VE C 70/2) were subjected to more detailed analysis concerning the transgene expression. The strongest expression on the transcription level was found just under skin compared to other parts of tuber. The values are similar round the tuber except the stolon protruding part. The expression slightly varies among tubers of the same transgenic line, nevertheless, the different lines display different level of the transgene transcription. We plan to study the stability of the transgene expression. Acknowledgement: 1M06030.

Working group N°4 (poster)

IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH RESISTANCE TO HEAVY METALS IN THE GENUS *SILENE*

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There are big problems with contamination of soils caused by heavy metals in many countries. High levels of heavy metals in soil have a negative effect on ecosystems because they can enter food through agricultural products. We can use plants to remove these pollutants from the environment using phytoremediation. Determination of resistance genes associated with tolerance and accumulation of heavy metals, as well as understanding of their biological functions are of primary importance resulting in improvement of phytoremediation techniques. The first evidence that plant can resist and accumulate heavy metals was observed in genus *Silene* (1934). Although many plant populations within this genus grow in heavy metal polluted areas almost nothing is known about genetic and physiological mechanisms involved in heavy metal metabolism in these species. Recently, we have suggested metallothioneins (MTs) as candidate genes responsible for this resistance in *Silene* species. Searching cDNA databases of several *Silene* species, we found two genes belonging to MTs, eventually. To amplify these genes, we designed gene specific primers. This work was supported by IGA AF MZLU, DP3/2009.

Working group N°4 (poster)

INTRODUCTION OF BACTERIAL GENES FOR DIOXYGENASES INTO PLANT GENOME TO INCREASE PHYTOREMEDIATION ABILITIES OF AROMATIC POLLUTANTS

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Exploitation of transgenic plants in phytoremediation can provide an effective improvement. We constructed genetically modified plants with increased capabilities to degrade organic pollutants such as PCB, toluene, TCE etc. Bacterial genes coding bacterial dioxygenases – bphC gene and todC1C2 were cloned into tobacco. BphC gene encodes 2,3-dihydroxybiphenyl-1,2-dioxygenase cleaving the aromatic ring of dihydroxybiphenyl (cloned in fusion with GUS, LUC or polyhistidine), todC1C2 produces enzyme oxidizing pollutants, both genes under CaMV 35S or RbcS promoter. Several constructs were prepared and expression of desired proteins in tobacco plants was studied by transient expression via agrobacterial infiltration. Expressed oxygenases His/BphC, BphC/GUS, BphC/LUC and His/ISPTOL were detected by Western blot or histochemically. BphC gene was transferred into *Nicotiana tabacum* genome by agroinfection. The presence of transgenic DNA and expressed proteins was studied using several techniques. Novakova et al. *Biotechnik. Bioeng.* 102, 2009, 29-37. Acknowledgement: grants MSMT 1M06030, ME-09024, MSM 6046137305, Z40550506

Working group N°4 (poster)

EXPRESSION OF FUNGAL PHYTASE GENE IN BARLEY

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Seeds of cereals, oil crops and legumes are major nutritional components for monogastric animals, and bread and bakery products are included in the basic foodstuffs. Phytic acid, myo-inositolhexakisphosphate (IP6) is the ester of myo-inositol and phosphoric acid, produces salts, with ions of iron, manganese, magnesium, zinc and calcium which are bound to phytic acid and are difficult to digest. These complexes negatively affect the usability of phosphorus and bound cations in human and animals foodstuff. An effective way of eliminating the antinutritional effect is overproduction of endogenous phytase in grain of cereals. Zygotic immature embryos of spring barley cv. Golden Promise were co-transformed by particle bombardment with three combinations of plasmids pAHC25/pAMFIT (containing the gene *phyA* from *Aspergillus niger*, from prof. C. Fogher), pDB1/pAMFIT and pAL51/pAMFIT. Two PCR-positive tetraploid lines T0(HH3E and HH1A from JIC, Norwich) were used to decrease ploidy level by anther culture. The regenerated plants were evaluated by PCR and RT-PCR methods. This work supported by project 1M060 of the Ministry of Education, Youth and Sports of the Czech Republic.

TRANSFORMATION OF BARLEY WITH *dapA* GENE

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Nutritional quality of human and animal foodstuffs is determined by the content of essential amino acids. In many bacteria and in higher plants, lysine, threonine, methionine and isoleucine are synthesized by the aspartate biosynthetic pathway, which is predominantly regulated by feed-back inhibition of aspartate kinase (AK) and dihydrodipicolinate synthase (DHPS). DHPS is the key enzyme in the regulatory step for lysine biosynthesis. The bacterial enzyme is much less sensitive (50-fold) to feedback inhibition by lysine than its plant counterparts. Cereal grains such as wheat, barley and maize contain insufficient levels of lysine. By development of transformation technology, it is possible to express bacterial DHPS in crops of agronomic importance and increase free-lysine content. Two constructs pBract214::sTPdapA and pBract214::mdapA containing the *dapA* gene from *Escherichia coli* coding for the bacterial DHPS were used for transformation of barley. The vector pBract214::sTPdapA in addition includes the transit peptide Rubisco *Hordeum vulgare* ribulose-1,5-bisphosphate carboxylase small subunit, Genbank U43493. An *Agrobacterium*-mediated technique was used for transformation of immature embryos of spring barley cv. Golden Promise. Transgenic barley plants of the T₀ generation were evaluated by PCR and Real-Time PCR. This work was supported by project 1M06030 of the Ministry of Education, Youth and Sports of the Czech Republic.

PHYSIOLOGICAL STATE OF LILAC PLANTS UNDER ALLELOPATHIC STRESS

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The lilac (*Syringa vulgaris* L.) is a valuable ornamental plant, a source of essential oil. Long-term cultivation of lilac leads to reduction of its growth. The aim of the research was to study the physiological changes in lilac seedlings in response to allelochemicals which come into soil as the products of plant residues decay. Allelopathic stress was modelled under greenhouse conditions. Lilac seedlings were grown for 15 months in grey forest fallow soil mixed with lilac cultivars residues (a mixture of roots, fallen flowers and leaves; 2% to soil weight). Application of lilac residues reduced photosynthetic pigments content in leaves. In general, chlorophyll A is found to be the most susceptible to the allelopathic influence. Free proline amount in leaves of seedlings grown under decay of plant residues was 1.2-6.6 times higher than that of control depending on sample collection term, cultivar characteristics of lilac residues. Increase in proline content was plant response to stress caused by allelochemicals. Inhibition of growth of the seedlings grown in soil with lilac residues was observed. Thus, decay products of lilac cultivars residues are phytotoxic to the seedlings.

Working group N°4 (poster)

NMR ANALYSES OF THE RECEIVER DOMAIN OF CKI1 HISTIDINE KINASE AND ITS INTERACTION WITH AHP PROTEINS

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In a multistep phosphorelay, the receiver domain of sensor histidine kinases is supposed to be involved in protein-protein interactions with its downstream signalling partners, the AHP proteins. Here we show that CKI1_{RD} interacts with AHP2, 3, and 5 with different affinities. To understand protein-protein interactions on the molecular level, the structure of CKI1_{RD} in solution has been studied in details by nuclear magnetic resonance (NMR). Effects of magnesium ions (Mg²⁺) and phosphate analogue beryllium fluoride on chemical shift changes of CKI1_{RD} have been studied in a series of titration experiments and the most significantly affected residues were identified. Observed chemical shift changes were mapped on a solved crystallographic structure of the protein. Molecular motions were investigated by NMR relaxation experiments with free, Mg²⁺-bound, and berylliofluorinated CKI1_{RD}. Based on these data and in combination with bioinformatics approach, we determined four regions that might be responsible for the observed specificity of protein-protein interactions between CKI1_{RD} and individual AHP proteins. (Supported by 1M06030, LC06034, MSM0021622413, MSM0021622415, 204/08/H054.)

Working group N°4 (poster)

CYTOKININS MODULATE AUXIN-INDUCED ORGANOGENESIS IN PLANTS VIA REGULATION OF THE AUXIN EFFLUX

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Cytokinins (CKs) and auxins were shown to be the principal regulators of organogenic response in plants. However, the molecular mechanisms of the auxin/CKs interaction are still largely unknown. Here we show that auxin is able to induce organogenic response in hypocotyl explants, while CK modulates the auxin-induced organogenesis via regulation of intercellular auxin distribution. De novo organogenesis is accompanied with production of endogenous CKs and tissue-specific activation of CK signalling. The CK-mediated modulation of organogenesis could be partially simulated by the polar auxin transport inhibitor. Further, CKs reduce auxin efflux from cultured BY-2 tobacco cells and regulate the expression of auxin efflux carriers from the PIN family. Finally, we show that endogenous CKs are necessary to maintain proper auxin distribution and PINs expression in *Arabidopsis* roots. Based on these findings we propose a model, in which auxin acts as a trigger of the organogenic processes, whose output is modulated by the endogenously produced CKs via regulation of expression of auxin efflux carriers. (Supported by LC06034, MSM0021622415.)

Working group N°4 (poster)

MEMBRANE PERMEABILISATION AS A METHOD FOR OBTAINING ANTHOCYANINS FROM RASPBERRY PLANTS

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High concentration of anthocyanins in raspberries gives the great benefit for breeders, food and pharmaceutical industry. Our research concerns the development of methods for receiving anthocyanins from raspberries. Raspberries in aqueous solution are treated with Pulsed electric fields (PEF), which enhances anthocyanin synthesis and release. High pressure (HP) treatment at 50 to 100 MP resulted in the immediate increase of anthocyanin synthesis. PEF and HP acts as a physical elicitor, triggering metabolic pathways may be an alternative strategy leading to the induction or enhancement of secondary metabolite synthesis in plant cells. There was a significant increase in anthocyanins from Raspberries treated with high pressure at 50 MP for 3 mins and also in treatment with PEF at 0,5 KV/cm 50 pulses.

Working group N°4 (poster)

TARGETING OF STEVIOSIDES BIOSYNTHESIS IN CELL, HAIRY ROOTS AND SPROUTS CULTURE OF STEVIA REBAUDIANA BERTONNI

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The one of the most well established natural sweeteners are steviosides that are synthesized in the leaves of in *Stevia rebaudiana* Bertoni. We have established the in vitro sprout culture of *Stevia* and developed the adjusted nutrient medium based on MS-formulation. The statistical comparison of the results suggested that biomass gain of the *Stevia rebaudiana* in sprout culture can be 3-4 times higher than that one in meristem culture and even higher in comparison with any other cultivation system (cell and hairy root culture). The quantitative and qualitative composition of the extracts from cell, meristem, hairy root and sprout culture proved to be a subject of considerable (statistically significant) impact of the cultivation systems themselves.

Working group N°4 (poster)

CHANGES IN GROWTH OF CELL SUSPENSION CULTURE INDUCED BY POLYCYCLIC AROMATIC HYDROCARBON

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We studied effect of fluoranthene (FLT; 0, 0.5, 5 and 15 μ M) on the physiological processes, viability and growth of BY-2 tobacco cells suspension culture. With increasing concentration of FLT in medium viability and the number of cells decreased (up to three times on the medium with 15 μ M of FLT). Production of ethane and CO₂ increased especially in exponential growth phase on the highest concentration of FLT. In contrary, production of ethylene at 15 μ M FLT decreased. Peak of 1-aminocyclopropane-1-carboxylic acid (ACC) concentration in control cells forewent the ethylene peak. The content of 2,4-D decreased less on the medium with FLT than on the control. FLT did not affect significantly the content of abscisic acid.

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Working group N°4 (poster)

SCHISANDRA CHINENSIS EMBRYOGENIC CULTURE AND DIBENZOCYCLOOCTADIENE LIGNAN PRODUCTION

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Dibenzo[a,c]cyclooctadiene lignans display many significant biological effects and recently three lignans of *Schisandra chinensis* have found as potent P-glycoprotein inhibitors. This is very important for overcome cancer multidrug resistance. Biotechnological lignan production by plant cell culture may be one of the possibilities of the effective supply of these compounds. The aim of our work is to study ability of embryogenic culture of *S. chinensis* to produce selected dibenzocyclooctadiene lignans under effect of adsorption from media *in vitro*. We studied two polymeric resins amberlite XAD-2 and XAD-7. We have observed selectivity of absorption of individual lignans from culture media. This fact may facilitate the isolation of lignans adsorbed onto resins. The amount of lignans produced by culture increased several time under amberlite influence. Addition of polymeric adsorbents to culture medium seems to be useful tool for achievement of lignans of special interest from embryogenic *in vitro* culture of *S. chinensis*.

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IMPROVING OF BREAD-MAKING QUALITY IN TRITICALE BY CHROMOSOME 1R TRANSLOCATIONS

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A breeding programme is presented whose aim is to develop new cultivars of triticale suitable for yeast-leavened dough products. Triticale donors with translocations of chromosome 1R, which were developed by A. J. Lukaszewski, are used. A segment of chromosome 1D with high-molecular glutenin allele Glu-D1d and locus Gli-D1, replacing some secalin loci, were transferred to 1R. The breeding is based on selection methods using molecular markers or electrophoretic separation of proteins that allow detecting Glu-D1d and Gli-D1. Donors with lower α -amylase activity and with short stem are used. *In vitro* techniques are used to develop double-haploids. Results of grain quality of selected lines with the confirmed presence of Glu-D1d and Gli-D1 are given. Their quality parameters are close to those of wheat classified into B category (bread quality). Differences between breeding programmes in the Czech Republic (Kromeríž) and Poland (Borowo) are discussed. The improvement of bread-making quality in triticale will lead toward cheaper and healthy production of food cereals (increasing lysine content), enrichment of food spectrum, and extending the area planted with triticale. Acknowledgement: grant MSM2532885901.

Work Group 5:
Quality of the agro-ecosystem

Working group N°5 (oral)

DIFFERENTIATION OF FETEASCA NEAGRA WINES FROM VARIOUS AGRO-ECOSYSTEMS BY THE USE OF AN ELECTRONIC NOSE

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Feteasca neagra is a Romanian grape variety that gives quality red wines, with a specific sensory profile. Although easily recognizable from other varietal wines, Feteasca neagra has a higher variability than other grape varieties, adapting itself to the specific agro-ecosystem, so that its sensory expression depends highly on the culture conditions. While it is generally agreed that Feteasca's tipicity is represented by some sensory attributes it was found that they are not displayed in all agro-systems. By the use of an electronic nose we obtained the volatile profile of the wines produced in three different viticultural-ecosystems. The study included wines from Pietroasa, Valea Calugareasca and Odobesti, vinified in 5 technological variants for each centre. The results showed that the influence of the agro-ecosystem is more decisive than the technology applied for this variety. There was no exception to this rule, even in the case of wines obtained with a special oak aromatization practice. In many wines the varietal aroma is overwhelmed by the oak aroma and the wines lose their personality, but in this case the volatile profile was not so much affected, due to its complexity.

Working group N°5 (oral)

LANDSCAPING IN THE AGRICULTURAL ENVIRONMENT

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Even since the 70's - 80's the artist Hundertwasser underlined the importance of agricultural plantations in the urban environment. His ideas seemed, that time, childish utopia. Today, in the context of ecological disasters that threaten the planet, these ideas are not very useful but saving solutions, waiting to be put into practice. How can we sustain through correct proposals the aesthetic aspect of agricultural urban plantations, without diminishing the multifunctional effects, helpful on multiple levels - hygienic-sanitary, ecologic, economic, urban functional, social? In the history of landscape architecture creations there weren't many compositions connected to the agricultural terrains or even agricultural landscape. Only in the past few decades, the beauty of the features and particularities of the agricultural landscape arouse the interest of some daring landscape designers. It is not about the well known utilitarian decorative plantations but creations that tend towards a visionary symbolism. Why this delayed interest regarding the agricultural environment? Which would be the virtues and qualities of the agricultural landscape from the landscape architecture point of view.

Working group N°5 (oral)

THE INFLUENCE OF THE AGRO-ECOSYSTEM ON THE QUALITY OF FETEASCA NEAGRA RED WINES IN THREE ROMANIAN VITICULTURAL CENTRES

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Feteasca neagra is a Romanian indigenous variety that is recognized as highly representative for our red wine assortment. The study took into account a diversity of Feteasca neagra wines produced in different regions and assessed the differences imposed by the agro-ecosystem. Wines made of this variety in three viticultural centres were screened for differences in composition and sensory attributes. The favourability of the ecosystems was also evaluated from ecological and geographical viewpoints, the climatic data being calculated as means for ten year periods. The physico-chemical analyses and the sensory evaluation of the wines produced in 5 technological variants for each centre, with three repetition for each variant, show that the influence of the agro-ecosystem is more decisive than the winemaking technology applied for this variety. In all wines the colour (intensity, hue, CIELab parameters) was influenced by the ecosystem more than by the technological variations. The study shows that Feteasca neagra variety has great flexibility and exhibits in various degrees some or other of its oenological qualities in accordance to the specific ecosystem in which it is cultivated.

Working group N°5 (oral)

ENVIRONMENTAL POLLUTION ASSESSMENT ON AGROECOSYSTEM IN AN OIL INDUSTRY AREA: A MULTIDISCIPLINARY APPROACH

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The oil industry impacts on croplands were monitored by different environmental media. In the developed approach, trace element concentrations in soil, water, epiphytic mosses and crops were determined. All the sampling locations based on a regular monitoring grid network were selected. To all samples collected in area of the oil refinery (20x20 km²), one to three locations has to be contributing to each sampling location. The spots were further categorized by groups in relation with the downwind distance from the oil smelter complex. Nuclear and atomic techniques as well as statistical procedures to interpret the analytical data were used. The analyses were carried out on the grain fractions < 2 mm by using the photon gamma activation analysis and X-ray fluorescence method. The survey showed that in the monitored area bordering the oil refinery complex there are zones with significantly elevated concentrations of Ni, Pb and V and other heavy metals as As, Cr, Cu, Fe, Sb and Zn linked to anthropogenic activities in the oil and hard industry. Samples could be considered contaminated by Cr, Cu, Hg, Ni, Pb, V and Zn, with concentrations, in general, close or higher than the maximum permissible values established by the Romanian legislation. The approach used in this article can be employed for food crops quality control, since it permits the quantification of microelements and environmental conditions in a simultaneous measurement.

Working group N°5 (oral)

EVALUATION OF SOME QUALITY ATTRIBUTES OF RADISH IN THE SOUTH OF ROMANIA

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Radish is popularly used in dietary alimentation of population in Romania, but there is limited information about some attributes of this agricultural crop. Radish material was processed from two popular varieties and evaluated for chemical concentrations of inorganic constituents. The analytical evaluation of the radish samples was carried out by X-ray fluorescence method. Black radish has better quality and environmental attributes and could be recommended to radish consuming areas.

Working group N°5 (oral)

UNWANTED DELETERIOUS IMPACTS OF THE NITROGEN SURPLUS FROM AGRICULTURE AND INDUSTRY

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To be competitive enough press farmers to the use of synthetic nitrogen fertilizer, which is prevailing source of anthropogenically produced N. According to Galloway et al. (2008) much anthropogenic N is lost to air, water, and land to cause a cascade of environmental problems. The aim of the contribution is to describe the similarity of the consequences of the N surplus to three different natural or seminatural ecosystems; to the heathland, mountain forest and alpine meadow. The consequences are more or less the same – destabilization of the current plant communities. Moreover, we can distinguish two different periods of the soil N availability during the year. The first one is the period of vegetation dormancy and early spring with the N luxury coming from atmospheric depositions and the second one is the following vegetation period with the minor soil N availability. At some point the ecosystems compartments and mainly the soil become unable to retain more N, and the plant composition will start to change or the ecosystems will start to decline. It is necessary to seek ways to increase food production while minimizing N loss and its subsequent deleterious impact to environment.

Working group N°5 (poster)

REASERCH CONCERNING DOUBLE PROTECTION ON VEGETABLES, GROWN IN POLYETHYLENE TUNNELS

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In Europe the main ways to obtain earlier yields are: plastic tunnels, low plastic tunnels or directly temporary protection using different cover crops. This way plants have better conditions to grow so the productions are earlier. The climatically conditions in Transylvania are less favorable for obtaining early vegetable production than in the South or the West of Romania. In the South and the West of Romania vegetable farmers obtain one or two weeks earlier vegetable crops than in Transylvania, the Middle and the North of Romania. Due to this reason, vegetable growers from our region (Transylvania) try to find some technological arrangements that allow them to obtain earlier crops. In our research we followed the influence of plant protection on vegetables cultivated in polyethylene tunnels. Two varieties and two different densities have been used. It has also been studied the effect of simple and double plant protection on early production. It is known that lettuce, chicory, spinach, beetroot and carrots are the vegetables with the highest risk of accumulating nitrates and nitrites. In this experiment the level of nitrates, nitrites and C vitamin in lettuce have been studied. It has been noticed that the double protection of lettuce in polyethylene tunnels assured a better growth of plants and so an earlier production. The nitrates and nitrites content in lettuce cultivated in the polyethylene tunnels of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, were low. In our experiment, the values of nitrates were between 995,2 mg/kg and 1544 mg/kg and nitrites between 15,4 and 25,3 mg/kg. The ascorbic acid (Vitamin C) values were not influenced by the plant protection type. The double protection variants accumulated the highest content in nitrates and nitrites.

Working group N°5 (poster)

RESEARCH CONCERNING BROCCOLI (BRASSICA OLERACEA L. CONVAR. BOTRYTIS ALEF. VAR. ITALICA) CULTIVATION IN POLYETHYLENE TUNNELS IN TRANSYLVANIA

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Broccoli is a very valuable vegetable. It's a good source of vitamins (A, B2, C) minerals as calcium, potassium and it has therapeutically properties too. Broccoli doesn't have a long history in Romania. It's cultivated on small parcels by small gardeners for their own consumption and broccoli that we, romanians , find in supermarkets are imported from other countries as Italy, Spain, Turkey etc. and it's quite expensive. Broccoli is very good against cancer and it is used a lot in alternative medicine against cancer. Because in Romania, and not only, cancer rate is growing every year, people should start eating much more broccoli. Romanians begun more and more to eat broccoli. Broccoli became in the last years ones of the most appreciate and looking for vegetable in Romania. Broccoli technology of cultivation in Romania is not very well established, that's why we tried in our research to find the best way to produce broccoli. In our researches we tested three hybrids: Fiesta F1, Marathon F1 and Belstar F1, using different densities and different ways to set the crop in the field (with two rows or three rows). We started our crop with seedlings produced in heated green houses. The sowing date was 16 January 2008 then we transplanted them in first of February and we planted in the polyethylene tunnel in 12 of March 2008. We used the technologies recommended by special literature. Harvesting started in 15 May 2008 with the main stalk (shoot) and finished in 11 June 2008 with secondary stalks. The differences between yields were quite big, the highest yield was obtained at Marathon hybrid at a density of fifty thousand pl/ha, a yield of 18,30 t/ha, followed by the same hybrid at a density of fourty thousand pl/ha with a yield of 16,09 t/ha. The lowest yield was obtained at Belstar hybrid with 12,18 t/ha and 10,25 t/ha at densities of fifty thousand pl/ha and fourty thousand pl/ha.

Working group N°5 (poster)

THE CULTIVAR INFLUENCE ON THE PRODUCTIVE POTENTIAL AND BIOCHEMICAL CONTENT OF CYNARA SSP. INFLORESCENCES

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The artichoke and cardon are little known and cultivated in Romania. Although there are no organized growers to cultivate these plants of the genus *Cynara* ssp., in Romania, lately, due to their nutritional properties, these vegetables are sold on the market but they are imported. This study conducted in the south of Romania, where the artichoke and cardon find proper conditions for growth and development, presents the results of the experiments that had as aim the behaviour of these plants as vegetables. There were taken in study: the cardon, two varieties of artichoke (artichokes green and red) and 3 hybrids (L3, 02, 03), on which there were made determinations of the total mass of the inflorescences and of the edible part, and biochemical analysis (sugar content, dry substance, acidity and nitrogen content). The results show that for cardon, the total mass of the inflorescences and the mass of the edible parts had the highest value, and in case of artichokes, L3 hybrid recorded the smallest total weight of the inflorescences and the hybrid 03 the lowest value of the edible part weight.

Working group N°5 (poster)

UTILIZATION OF SEWAGE SLUDGE FOR SWEET CORN PRODUCTION

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Recommendations for industrial sludge application rates in agriculture are based on the fertilizer values and on the concentrations of trace metals present in the sludge and soil. The aim of our work was to determine the possibility of application of sludge in the plant production through the analysis of essential and toxic element uptake by sweet corn. The experimental plots were conducted in 4 replications using randomized block design (24 plots, sized 1 m² of each, with 8 plants per plot). The plots were treated with different doses (1, 2, 3, 4, 5 t ha⁻¹) of sewage sludge before the sowing. Macronutrient and toxic element content of sewage sludge was in mg kg⁻¹: N: 52700, P: 5550, K: 5270, Pb: <7,50, Ni: 7,02, Cr: 34,47, Cd: <1,00. Soil and plant samples were collected during the harvest time. The soils were characterised by high P and K content and low to medium organic content. Heavy metal contents of soil and yield were analysed for the same component like in the case of sewage sludge. The results showed that the P and K uptake by sweet corn was generally higher at treated plots than the control, but the differences were not significant.

Working group N°5 (poster)

RESEARCHES REGARDING THE INFLUENCE OF MUTAGENESIS INDUCTION METOD ON THE BIOCHEMICAL AND CHEMICAL CHARACTERISTICS OF APPLE TREES CULTIVARS

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The surface of apple culture is on all continents and in temperate zone, north and in the south hemisphere. In fruits crops, apple represents the 4th place on the globe with 56 millions tones annual (FAO, 2006). Nutritive value of apples are well known and represents variable content of sugar, proteins, ascorbic acid, and mineral substances. Consume of fresh fruits or juice, alimentary pastes, jellies, gems assure on entire year the vitamins for a better life. Apples are a part in all food diets and therapeutically value is well known in different illness (determine the absorption of gastric secretions, the elimination of toxins, diuretically effect). The aims of the research is to test ten apple cultivars from the point of view of glucoses, water content, proteins, acidity, vitamin C and also mineral elements: N, P, K, Ca, Mg, Fe. Results obtain for glucose shows a higher content of sugar between 9.53 and 12.34%. The higher sugar content was registered at H3/73 cultivar (12.34%). Total acidity (%) is between 0.127 and 0.345%, a small content registered at all cultivars and determine a good quality of fruits and a good taste. The highest value is obtained at Jonathan apple cultivar. Vitamin C varied between 25.75 and 77 mg/100g, Mutzu and Jonathan cultivar registered the highest value for C vitamin. Mineral elements N, P and K varied with the apple cultivar. The values for Ca varied between 2.5, 7.8 mg/100g, and Fe between 0.2 and 0.28 mg/100g. The accumulation of sugar and vitamin C in high quantity shows an unpolluted atmosphere and soil in the zone of apple culture and there is any risk of food contamination.

Working group N°5 (poster)

WAYS OF IMPROVING THE EFFICIENCY OF MULTIPLICATION IN SOME SPECIES OF ORNAMENTAL PLANTS

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The aim of the researches was to stimulate seeds germination and seedlings rhizogenesis through the use of special treatments, non-conventional, applied to biological material from flower species grown in field or in protected areas. In most of the species that were studied the proposed treatments (magnetic field, biostimulators) had a favorable effect.

Working group N°5 (poster)

POTENTIAL RESPIRATION OF CHERNOZEMS IN BRECLAV REGION

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The potential soil respiration were estimated in the period of time from 2006 till 2007. The aim of this work was to evaluate microbiological respiration in selected Chernozems in Breclav region. The new method using apparatus Vaisala GMT220 and new accesories were applied. Soil samples were enriched with mineral and organic substances (ammonia sulphate – sample „N“, glucose – sample „G“ and sample with ammonia sulphate and glucose – „NG“) and the original sample was used as a control (a basal sample „B“). We came to the conclusion that the higher deficit of substances in the original sample was caused by higher intensity of respiration.

Working group N°5 (oral)

DIGITAL PRECISION AGRICULTURE® - A SOFTWARE TO OPTIMIZE CROP PRODUCTION AND SOIL SUSTAINABILITY

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Precision Agriculture (PA) is a set of management practices and cutting edge technologies used to identify soil and crop variability in space and provide optimum crop and soil management practices per uniform unit areas established (management zones). Current use of technologies is rather complicated and expensive for use by single farmers or even small groups. Latest developments in technology and friendlier use of equipment (sensors and dataloggers) enable the use of PA techniques more end users. Educational aspects of this approach are also emphasized and case studies are presented in this paper to demonstrate that simple technologies can be used efficiently enough to provide better management of crops and soils, using the principles of PA. DIGITAL PRECISION AGRICULTURE® version 1.0 is a software developed under a 2-years research program in Hellas (Greece) with the objective to provide valuable integrated information and guidance to farmers, consultants, policy makers, researchers, students and other end-users for efficient and sustainable soil and crop management. The software receives inputs on fundamental but easily measured soil properties affecting crop growth and yield, taken in a substantial number of points per farm site, and outputs digital maps of relative homogeneous Management Zones (MZ) to be used for optimization of Input Use Efficiency (IUE) and therefore provide higher income and cause less adverse environmental effects.

Working group N°5 (poster)

THE COROLOGY, ECOLOGY AND PHYTOSOCIOLOGY OF THE RHODODENDRO MYRTIFOLII-PINETUM MUGO COLDEA 1985 (SYN. PINETUM MUGO CARPATICUM (SOÓ 1930) SZAFER, PAWL. KULCZ. 1931) PLANT COMMUNITY IN THE CAPATÂNII MOUNTAINS (CARPATHIANS MOUNTAINS)

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Various physical and geographical conditions in Capatânie Mountains are conspicuously reflected in the structure of the vegetable carpet which, in this area, is one of a rare complexity. Because of that reason this area represents one of the most interesting natural areas of the country from a scientific and practical point of view. Juniper trees vegetate at the subalpine level, on the superior limit of the common spruces, on low slopes but on steep mountainsides as well. To be noticed is that in the Capatânie Mountains, the enlightened phytocoenoses of the *Pinus mugo* are to be seen even at the superior mountain level, going down under the level of common spruce forest by the altitude of 1.400 metres. This thing was observed in the summer of 2003 in a place called "Izvorul Sec". In the observed area the enlightened phytocoenoses of *Pinus mugo* fill long areas at "Sua Funicelu", at "Izvorul Sec" and under Balota Peak and Capatâna Peak. In the last decades, in order to extend grazing areas, a lot of settings on fire and deforestation of the juniper trees were made in the Capatânie Mountains.

Working group N°5 (poster)

CONTRIBUTIONS REGARDING THE STUDY OF THE COLONISING CLINOPODIO VULGARIS - PTERIDIETUM AQUILINII DIHORU 1975 PLANT COMMUNITIES (SYN. PTERIDIETUM AQUILINI JOUANNE ET CHOUARD 1929) IN THE CAPATÂNII MOUNTAINS

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The Capatânie Mountains are part of the Parâng Mountains, the researched area lying between: to the East the Oltet rivulet, to the West the Olt River, to the South the subCarpathian area of Oltenia, to the North the Latoritei Mountains, the Valley of Lotru and the Depression of Loviste. The fern *Pteridium aquilinum* (L.) Kuhn, known under the names "wolf's clothes" and "field fern", is an example of a plant very well adapted, which ensures its being widespread as compared to other cormophytes. Wolf's clothes is a colonising and invasive plant which fits very well to agro-ecosystems and natural, degraded ecosystems, being almost impossible to combat. Although it is a native plant, because it stretches over large areas in our country, more particularly in the hill and lower mountain areas, due to the inefficient methods of combat, we think that it should be paid special attention to. The extension of the invaded areas underlies three main causes: agricultural desertion, the reduction of pasturing and deforesting. The effects are: economic, ecological (the reduction of biodiversity) and they also affect the health of both the animals and man.

Working group N°5 (poster)

SOIL MICROBIAL ACTIVITIES IN THE SOUTH OF SPAIN ALONG A PLUVIOMETRIC GRADIENT

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Concerning the regional scale spatial variability, results of experimental field work conducted along a climatic transect, from the Mediterranean climate to the arid zone in the south of Spain, show that: (1) the soil respiration were tightly coupled with the carbon compounds available in soil (2) the in situ ammonification was nearly the same along a pluviometric gradient; (3) the nitrification was increasing with aridity identically in control soils, and after the addition of cellulose and raw silk; (4) the contact time of the water with the soil matrix was sufficient to retain NH_4^+ , but insufficient for a retention of NO_3^- ; (5) the key factor influencing the movement of nitrate and thereby promoting the losses of base cations was the frequency and intensity of precipitation not only a soil-internal N surplus.

Working group N°5 (poster)

THE INFLUENCE OF THE "TERROIR" CONCERNING THE QUANTITY AND QUALITY GRAPES PRODUCTIONS AT WHITES GRAPEVINE VARIETIES GROWING IN THE IASI VINEYARD

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A good management of the vineyard need to master this concept and understanding its different influences on wines' quality allows the choice on the most appropriate technical methods. Romania is conscious of its viticultural potential for a long time and the complex interactions of its terroir deserve to be analyzed for each area of production. In an international context characterized by a strong competition, quality and typicity are the key notions to emphasize the wine. Being a traditional occupation in our country, Romanian viticulture has already established the most favourable areas in order to obtain high quality wines from determined regions, but the purpose of this study is strict delimitation- at lot level-of high quality areas from a controlled denomination of origin region. The researchs was carried out during 2005-2008 an they evince the dependence of quality at grapes and wines with genetical, ecological and technological factors its variable and complex. This primary study was to characterize the terroir of Iasi-Copou's vineyard to get some general information about the behavior of two varieties of vines: Muscat Ottonel and Fetească regală. In accordance with the research topic proposed, the observations and determinations were focused on influence of soil characteristics on several biochemical and productive parameters of Muscat Ottonel and Fetească regală (soluble glucides content, total acidity, berries weight and average vine production), in a well - known viticultural center from northen part of Romania. The results of this study can be used for qualitative and quantitative differentiation – within the precincts of controlled denomination of origin area - of high favourable lots for obtaining grape in order to produce high quality wines.

Work Group 6:

Plant crop protection and biotechnology

Working group N°6 (oral)

ANTIBODIES AGAINST RECOMBINANT PLANT VIRAL STRUCTURAL AND NON-STRUCTURAL PROTEINS AND THEIR USE FOR VIRUS DETECTION

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The use of recombinant structural and non-structural plant viral proteins is an attractive strategy for the production of antibodies against viruses, which are present in low concentrations in infected plants, or are difficult to purify. We concentrated on preparation of antibodies against the main viruses infecting potatoes. We raised polyclonal antibodies to recombinant coat proteins of Potato virus A (PVA), Potato virus Y (PVY^N-W), Potato virus X (PVX), Potato mop-top virus (PMTV) and to a recombinant non-structural protein TGBp1 of PMTV. Further we work on antibodies for detection of Potato leafroll virus (PLRV) and its non-structural protein PLRV ORF2. The obtained sera and antibodies were tested for the detection of mentioned pathogens in laboratory hosts (tobacco species) and natural host *Solanum tuberosum* and have been successfully used for virus detection by Western blot and indirect PTA ELISA, but they have failed in DAS ELISA. Our antibodies did not recognize native epitopes, but only epitopes which were affected by some denaturation steps.

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Working group N°6 (oral)

PERFORMANCE OF LEPTINOTARSA DECEMLINEATA ON GM POTATOES EXPRESSING GALANTHUS NIVALIS AGGLUTININ (GNA)

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The repertoire of defense mechanisms against herbivores includes in some plants sugar-binding peptides (lectins) that interfere with digestion. The genes encoding lectins have therefore been transferred into crops with the aim to render them resistant to insect pests. GNA lectin from the snowdrop (*Gallanthus nivalis* agglutinin) has been expressed in several plant species and was reported to reduce performance of the sucking insects, such as aphids. We explored GNA action on the Colorado potato beetle (CPB), *Leptinotarsa decemlineata*. In the initial tests we fed newly hatched CPB larvae artificial diet containing 20, 40, 100, 300, 400, 500, and 1000 ppm of GNA, respectively. The concentrations from 300 to 1000 ppm GNA caused 100 % larval mortality. Mortality was reduced to zero with 20 ppm concentration. The concentrations of 40 and 100 ppm extended larval development but most insects reached the adult stage. Similar extension of larval development and somewhat reduced body size were also observed in larvae that were grown on the genetically modified potato plants expressing GNA. Adults obtained from larvae exposed to GNA either in artificial diet or in the potato leaves exhibited similar reduction of fecundity and egg hatchability. Progeny reared again on potatoes expressing GNA yielded very small number of beetles of the next generation. The results show that GNA is toxic to CPB only in high doses but hardly noticeable effects of low doses cause within 3 generations severe population reduction.

Working group N°6 (oral)

QUECHERS FOLLOWED BY TANDEM MASS SPECTROMETRY METHODS FOR THE ANALYSIS OF PESTICIDE RESIDUES IN CROPS

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Liquid chromatography followed by tandem mass spectrometry has become, during recent years, the best analytical technique to determine pesticide residues due to its great sensitivity, specificity even in complex matrices, and linearity of response. The most common limit, observable when clean up of the extracts is not conducted, is the variability in the response, ascribable to ionic suppression or enhancement at the interface. QuEChERS is an emerging clean up technique based on dispersive SPE, which is excellently applied for pesticide residue analysis, in particular when chromatography is followed by mass spectrometry detection. Extracts purification according to QuEChERS principles, followed by liquid chromatography tandem mass spectrometry (LC-MM/MS/MS) was applied to several crops to determine residues of some insecticide, fungicide, herbicide and plant growth regulators. The mean recovery ranged from 74% to 109%, and Relative Standard Deviation was 14% as maximum. The Limit of Quantification was 0.01 mg/kg for each target analyte, in all matrices considered, and the linear dynamic range was verified ($R^2 > 0.99$) over two orders of magnitude.

Working group N°6 (oral)

TRANSGENIC PLANTS EMITTING INSECT PHEROMONES

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Transgenic plants of environmental benefit typically consist of plants that either reduce the input of agrochemicals into the environment or make the biological remediation of contaminated areas more efficient. We prepared GM plants that produce and emit insect pheromones. This can represent one of the roles the next generation of GM plants might play in preventing and reducing chemical contamination. Grown close to, or around, a field of food crops requiring protection, for example, this type of GM plant emits a pheromone attracting male moth pests, thereby reducing their ability to mate effectively. In such cases, the protected crop does not itself need to be transgenic. We constructed tobacco plants that produce an insect sexual pheromone from their own fatty acid pool. This was achieved by inserting the gene encoding acyl-CoA- Δ^{11} -(-/Z)-desaturase (from cabbage looper moth), which is responsible for the production of the sexual pheromone in female moths. It was proved that these plants emit the pheromone.

Nesnerova et al., Green Chem. 6, 2004, 305-307, Macek et al. Trends Biotechnol. 26, 2008, 146-152.

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Working group N°6 (oral)

THE EFFECT OF STORAGE TEMPERATURE ON LARVAL AND ADULT STAGES OF
BRACON BEBETOR (HYMENOPTERA: BRACONIDAE)

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The ectoparasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae) is a gregarious parasitoid that attacks a variety of important lepidopterous pests of stored product and in the field. Nowadays the parasitoid is of interest for preventing infestations of pyralid moths in flourmills. Laboratory study was conducted to evaluate the efficacy of storage temperature on larval and adult stages. Either larvae or adults were exposed to low temperatures between 4 and 12°C. Larval stage lived up to 14 days at 4°C, but not reached to adult stage. On the other hand, when larvae stored at 8°C, it reached to adult stage. Adult stage lived up to 14 days at 4°C, but not laid eggs. When adults stored at 12°C, it lived and laid eggs as much as untreated control. Our results show that egg production and adult emergence decreased with decreasing storage temperatures and increasing storage time.

Working group N°6 (poster)

SOME PEACH AND NECTARIN CULTIVARS STORAGE CAPACITY RELATED TO
THE POST HARVEST PHYSICAL TREATMENTS

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The most important link in the postharvest technology is constituted by the physical treatment during the storage period. In the present paper we want to demonstrate the influence of the post-harvest storage conditions on the quality and shelf life of the peach and nectarine fruits. The experiment has been organized at the Research Institute of Fruit Growing-Constanta and at the Faculty of Horticulture Science of Bucharest with four peach and nectarine varieties: Cora, Delta, Southland and Cardinal. The lower moisture percent and loss weight, for all varieties has been registered at variant were the fruits are stored under modified and refrigerated atmosphere. In the case of fruits stored at 2°C and 85-90% relative humidity under modified atmosphere, the fruit storage capacity was better and the qualitative characteristics were higher as compared with the untreated control (stored in room conditions).

Working group N°6 (poster)

POPULATION DYNAMICS OF CACOPSYLLA MELANONEURA (HOMOPTERA: PSYLLIDAE) IN BANEASA-BUCHAREST AREA

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Population dynamics of the hawthorn psyllid, *Cacopsylla melanoneura*, were monitored on apple trees orchards and on hawthorn trees near apple trees orchards in Baneasa-Bucharest area in 2009, using the yellow sticky traps and beat trays methods. Our investigations have showed that *C. melanoneura* have one generation per year. The first overwintering adults were collected at the end of February on hawthorn and at the beginning of March on apple trees. The springtime generation adults were observed from the beginning of May to first decade of June. The most abundant populations of psyllid were registered on hawthorn trees, their primary host. Based on these results a continuous survey of the psyllid species present on hawthorn trees and apple trees orchards will be carried out in next time in order to detect the presence of the phytoplasma in insects and plants and to establish the possible role of psyllid as apple proliferation vector.

Working group N°6 (poster)

INSECT PESTS MANAGEMENT USING PHEROMONES IN ROMANIA

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The paper presents a synthesis of the main results of the trials carried out using pheromones in Romania. Research Institute for Chemistry Cluj-Napoca has formulated synthetic pheromones of the main insect pests over the last 40 years and the scientists from different institutes and universities have found wide application in the fields of agriculture, forestry and urban management. The most important application is to detect the presence of the insects and monitoring their populations in order to warn the treatments: in orchards for main pests (codling moth, leaf miners, summer fruit moth, plum fruit moth, oriental fruit moth, the peach twig borer), in field crops (the Western corn rootworm, the European corn borer), in green spaces (the horse chestnut leaf miner), management of stored grain pests (Indian meal moth). The second major application of pheromones is to control the pests and reduce the damages using different products and methods: mass trapping, mating disruption, attract & kill. In the last years specific products to use in Attract & Kill method against codling moth, summer fruit moth and grape moth were manufactured and the favourable results were reported.

Working group N°6 (poster)

COMPARATIVE ANALYSIS OF SEROLOGICAL AND MOLECULAR METHODS FOR THE DETECTION OF BEAN YELLOW MOSAIC VIRUS IN GLADIOLUS

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Four methods were compared for the detection of Bean yellow mosaic virus (BYMV) in both gladiolus leaves and corms: ELISA, one step-RT-PCR, real time RT-PCR and IC-RT-PCR. The initial screening of samples was done by DAS-ELISA to eliminate a high percentage of virus-negative samples; considerably reducing the number of plants to analyze further by one step RT-PCR, real time RT-PCR and IC-RT-PCR were used. ELISA was more reliable method to detect BYMV in leaves but in corms it fails to detect. RNA was extracted from leaves and corms or cormlets were used for the detection of BYMV by one step RT-PCR, real time RT-PCR and IC-RT-PCR method. The one step RT-PCR method was more sensitive and it was able to detect BYMV at very low concentration in leaves but in corms or cormlets it amplified very weak signals. In real time RT-PCR, it able to detect BYMV both on leaves and corms or cormlets tissues. Similarly, IC-RT-PCR using the PABs and specific primers in the region of the coat protein (CP) gene, this assays detected their respective target virus (BYMV) at low concentrations and this methods showed more reliable for the detection of BYMV in comparison with other two methods.

Working group N°6 (poster)

SPECIES DIVERSITY OF PLANT-INHABITING PHYTOSEIID MITES (PARASITIFORMES, PHYTOSEIIDAE) IN BOTANICAL GARDEN OF KARASIN NATIONAL UNIVERSITY OF KHARKOV (UKRAINE)

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The specific structure of phytoseiids in a decorative and recreational plantings particularly in plant communities of botanical gardens where introduced plants make an considerable part of phytocenosis insufficient investigated. The drift of new predatory species to artificial plant associations can cause not only quantitative changes aboriginal mite associations, supplementing their new species, but also qualitatively and numerically change efficiency of their functioning, in particular, to raise a degree of security of plants from the pests. The species diversity and distribution of plant-inhabiting phytoseiid mites (Parasitiformes, Phytoseiidae) in botanical garden of Karasin National University of Kharkov (Ukraine) were studied. Eighteen phytoseiid species were found. Species occurrence phytoseiid mites and relative fidelity of them to plant life were installed. Complexes on plants predatory mites of family Phytoseiidae can give a basis for expansion of searching of effective predators and their further using in a biological control of plant pest.

Working group N°6 (poster)

THE STRUCTURE, DYNAMICS AND ABUNDANCE OF CARABIDAE SPECIES FROM APPLE ORCHARDS

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Observations were made in 2007 in a plantation of apple. Collection of material was done with soil type traps Barber. Inside the traps was a solution of formalin 3-4% concentration, for killing captured insects. Traps were installed in May and worked until October. Periodically the biological material was collected from traps and formalin solution was supplemented or replaced if necessary. Of the material collected were selected carabidae species, which were then determined up to a series of indices, such as structure, dynamics, frequency, abundance etc. The species most commonly collected were *Pseudophonus rufipes*, *Anisodactylus signatus*, *Amara aenea*, *Harpalus tardus*, *Calathus tardus*, *Calathus fusipes*, *Dalichus chalensis*, *Amara crenata*, *Pterostichus vulgaris*.

Working group N°6 (poster)

OBSERVATIONS REGARDING THE FLORISTIC AND ENTOMOFAUNISTIC BIODIVERSITY FROM SOME PRATICOL SYSTEMS

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The observations were made in year 2008 in natural pastures from the department of Iassy and department of Vaslui, north-east of Romania. The pasture flora from the studied plots has 117 species of vascular plants, belonging to 84 genera and 24 families. In the researched pastures, the most common are the hemicryptophyte species, 20% are hemiterophyte species, 20% are therophyte species, the majority of the identified species belong to the eurasian element and a few are invasive species. The most common invasive species are: *Xanthium italicum*, *X. spinosum*, *Elaeagnus angustifolia*, *Lepidium draba*, etc. *Lepidium draba* L. (Brassicaceae), originary from Europe, became a problem plant in many agricultural ecosystems from our country. Following the observations regarding the entomofaunistic biodiversity from some praticol ecosystems, the conclusion is that the species belong to Class Insecta (8 orders) and to Class Arachnida (Acarina and Araneae orders). From all this species, the potential biological agents of *Lepidium draba*, with a high degree of control are *Ceutorhynchus cardariae*, *Psylliodes wrasei* and *Aceria draba*.

Working group N°6 (poster)

OBSERVATIONS ON THE BIODIVERSITY OF COLEOPTERS SPECIES FROM SOME AGRICULTURAL ECOSYSTEMS

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In 2007 and 2008, in some agricultural ecosystems (orchards, vineyards, vegetable cultures, pastures) observations on the structure, dynamics and abundance of the coleopteres were carried out. For this, wet Barber soil traps with a formalin solution, 3-4% were used. In each ecosystem were used 6 traps and the material has been collected regularly from May until October. The collected coleopters species were then determined. Following calculations it was found that the most common species collected were: *Abax ater* Duft., *Otiorrhynchus raucus* F., *Cicindela germanica* L., *Coccinella 7punctata* L., *Ophonus puncticollis* Payk., *Amara aenea* Dejean., *Pseudophonus rufipes* Mull., *Carabus violaceus* L.

Working group N°6 (poster)

COMPARISON OF ANTIOXIDANT ENZYMES OF IRRADIATED WITH NON-IRRADIATED *Bracon hebetor* (HYMENOPTERA: BRACONIDAE)

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Adult parasitoids of *Bracon hebetor* were exposed to gamma radiation ranging from 0 to 200 Gy to measure antioxidant enzymes, glutathione-S-transferase (GST) and catalase (CAT). Levels of activity of GST and CAT were measured in whole body extract of *B. hebetor* with an objective of finding any clue for the important larval parasitoid's capacity to tolerate different doses of radiation. The activity of GST dramatically increased for both male and female parasitoids irradiated with 100 Gy when compared with untreated controls. Maximum CAT activity was measured at 200 Gy and 100 Gy for female and male parasitoids, respectively. Our results confirm the hypothesis that radiation increases the level of oxidative stress in *B. hebetor* adults.

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Working group N°1 (poster)

DETERMINATION OF SOME PHYSICAL, MECHANICAL AND CHEMICAL PROPERTIES DURING PHYSIOLOGICAL MATURITY AND RIPENING PERIODS OF KIWIFRUIT (CV. HAYWARD)

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Physical, mechanical and chemical properties during physiological maturity and ripening periods of kiwifruit were determined in this study. The average length, width, thickness, and fruit mass ranged from 64.1 to 63.2 mm; 52.0 to 51.5 mm; 46.8 to 46.3 mm and 91.4 to 89.2 g, during physiological maturity and ripening periods of kiwifruit, respectively. The sphericity, surface area, the bulk and fruit densities and porosity during physiological maturity and ripening periods of kiwifruit ranged from 83.6 to 83.9%; 90.2 to 88.2 cm²; 374.5 to 397.7 kg/m³; 1014.6 to 1047.8 kg/m³ and 63.2 to 61.1%, respectively. The static coefficients of friction during physiological maturity and ripening periods of kiwifruit on various surfaces, namely, galvanized metal, chipboard, glass, plywood and rubber ranged from 0.28 to 0.39; from 0.32 to 0.42. The rubber surface offered the maximum friction followed by chipboard, plywood, galvanized metal and glass for physiological maturity and ripening periods of kiwifruit. The total soluble solid content, titratable acidity and pH of kiwifruits changed from 7.43 to 14.67%; 1.84 to 1.73%, and 3.16 to 3.27; during physiological maturity and ripening periods, respectively.

Working group N°1 (poster)

THE PHYSICAL, MECHANICAL AND CHEMICAL PROPERTIES OF PERSIMMON FRUIT (CV. FUYU)

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In this study, the physical, mechanical and chemical properties of persimmon fruit (cv. Fuyu) were determined. The physical properties such as length, width, geometric mean diameter, sphericity, surface area, fruit mass, fruit density, bulk density, porosity, colour characteristics (L, a, b), mechanical characteristics namely static coefficient of friction, skin firmness, and chemical properties such as the total soluble solid content, titratable acidity, and pH were determined for persimmon fruits. The mean values of length, width, geometric mean diameter, and fruit mass were 49.75 mm, 72.15 mm, 63.47 mm, 146.11 g, respectively. The average sphericity, surface area, the bulk and fruit densities, and porosity of persimmon fruits 1.28; 127.05 mm²; 540.82 kg/m³; 932.68 kg/m³, and 41.48%, respectively. The static coefficients of friction on various surfaces, namely, galvanized metal, glass, and rubber were 0.299, 0.278, and 0.308, respectively. The rubber surface offered the maximum friction followed by galvanized metal and glass. The total soluble solid content, titratable acidity, and pH of persimmon fruit were 11.53%, 0.012 g/100 g and 5.55, respectively. The skin fruits colour characteristics such as L, a, b were 91.80, 39.84 and 89.33, respectively.