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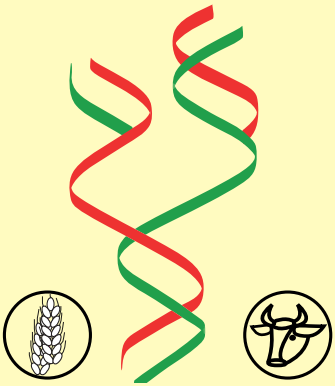
of XXIVth Genetic Days 2010



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International Conference

XXIVth GENETIC DAYS

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"Functional genomics and proteomics for sustainable agriculture"

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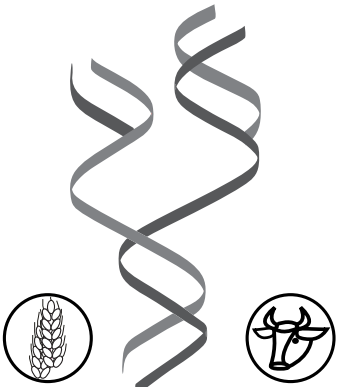

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Content

Invited Speaker Abstracts

Genome based technologies for identifying genes important for swine production

Dan Nonneman*

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Functional genomics in dissecting livestock production traits

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Extensive genomic studies of domestic animals have started almost twenty years ago. At the beginning projects on marker genome mapping were established (PigMap - 1991, BovMap – 1992, DogMap – 1993 etc) and presently dense marker maps are available. The next step was focused on genome sequencing. This goal was already also achieved for major domestic animal species (cattle, pig, horse, chicken, dog and cat). The use of both tools, including comparative genomics approach, along with SNP microarrays facilitated detection of numerous QTL regions for production traits. Unfortunately, rather small number of functional polymorphisms was identified. In the present decade functional genomics (transcriptomics, proteomics, metabolomics, nutrigenomics etc) and epigenomics (RNA interference, genome imprinting, histone modifications and nucleus architecture) have received a special attention. The use of expression microarrays and quantitative RT-PCR revealed extensive differences in terms of transcription level. This was observed for numerous genes, while tissues collected at different stages of individual development and/or from animals representing different breeds or performance type were studied. Genome imprinting is also considered as an important mechanism which may cause phenotypic effects. Progress in the field of microRNAs revealed that posttranscriptional gene expression control may also effects on variation of quantitative traits. It is foreseen that functional genomics will indicate new candidate genes which can be responsible for phenotypic variation of production and functional traits.

Transcriptomics and pig meat quality

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Pig meat quality traits are quantitative complex characteristics of considerable importance to the producers, consumers and processing industry and are influenced by a large number of factors. Genetic effects play a crucial role in designing pig carcass and meat quality and the heritability values indicate a degree of genetic control on phenotypic variability. The lack of knowledge both on genes involved in the control of meat quality and on the quantitative effect of the single genes and their interactions, represent a limit for the complete exploitation of the opportunities of a selection plan. Recent technological advances in DNA and RNA analysis have created new opportunities to study the complex field of meat quality traits in pigs considering a more holistic view of the biological system under study with respect to meat traits. The process of detecting and localizing causative genes can be supported in a considerably way taking into account not only the knowledge of DNA sequence variation and its association with variation in phenotypes but also the molecular phenotypes such as transcript abundance that can be considered like an intermediate trait in the chain of causation from gene variation to phenotypic variation. The omics science that considers and analyses gene expression on a genome wide level is called transcriptomics. High throughput transcriptomic approaches including microarrays have been widely used in pig genetics to identify genes influencing meat quality traits and allowing to analyze the expression level of series of genes in different stages or genetic background.

Estimation of genetic effects as a prerequisite for selection

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The success of selection depends strongly on both the quality of the available data and on the computational methodology used for exploiting them. Reliable breeding value estimation or estimation of genetic effects is of crucial importance. And for the estimation of breeding values relationship matrices play the major role. In the first part of our presentation we will discuss breeding value estimation using classical pedigree based relationship matrices on the one hand and based on known QTL genotypes on the other hand. A further aspect is marker assisted breeding value estimation, when QTL genotype probabilities must be estimated from marker data. In the second part because of its topicality we deal with realised relationship matrices and breeding value estimation for genomic selection. Along with increasingly cheaper procedures for the extraction of marker especially SNP information out of the genome of farm animals the opportunity arises to replace the previously used merely pedigree-based relationship matrices with actually realised ones. In the literature various kinds of realised relationship matrices were considered, which will be discussed in this presentation. Moreover, some approaches for the solution of technical problems in connection with calculations using such matrices will be addressed. Finally, we will present and discuss some methods described in literature for a unified estimation of breeding values for genomic selection, which are based on a relationship matrix incorporating pedigree information and genomic information as well.

Genomic Selection: Promise, Experience, Expectation

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Accurate Mass Spectrometry Based Proteomics Reveals Protein Isoforms Distinguish Plant Phenotypes of *Solanum tuberosum*

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We have developed a method called Mass Accuracy Precursor Alignment that allows the comparison of unprecedented numbers of shotgun proteomics analyses on a personal computer in a matter of hours. We applied it to 183 LC-MS analyses and 2 million MS/MS spectra and discriminated all of the proteomic phenotypes of field grown tubers of 12 tetraploid cultivars of the crop plant Solanum tuberosum. Expressed polymorphism in major gene families such as lipoxygenases and cystein protease inhibitors that regulate tuberization and development was the primary source of variability between the cultivars suggesting that protein isoforms may determine the different plant phenotypes to a high degree. Peptides unique to these isoforms were used to classify peptides with common abundance profiles showing that quantity ratios of highly similar proteins can be inferred with reasonable accuracy when proteins are digested into peptides prior to analysis. We identified nearly 4000 proteins which we used for quantitative functional annotation making this the most extensive study of the tuber proteome to date.

Use of the Conditional Overexpression System to identify novel regulatory genes in environmental stress responses and ABA signaling

László Szabados[1*], Mary Prathiba Joseph[1], Imma Perez Salamó[1], Csaba Koncz[2], Csaba Papdi[1]

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The adaptation of plants to extreme environments is controlled by a well-balanced, genetically determined signalling system. The identification and characterization of plant genes which control responses to environmental stresses is an essential step to elucidate this complex regulatory network.

To perform genetic screens for identification of novel plant genes involved in the control of abiotic stress responses, cDNA expression libraries from Arabidopsis and the halophyte Thellungiella were created in a Gateway version of estradiol-inducible XVE binary vector (Controlled cDNA Overexpression Systems, COS). The COS systems were employed in genetic screens by selecting for ABA insensitivity, salt tolerance and activation of a stress-responsive reporter gene (ADH1-LUC). More than fifty cDNAs conferring dominant, estradiol-dependent phenotypes, were identified by PCR amplification and sequence analysis. Characterization of a cDNA conferring insensitivity to ABA in germination assays has identified a heat-shock protein, a novel zinc finger transcription factor or genes controlling sugar metabolism. Screening for enhanced salt tolerance in germination and seedling growth assays, cDNAs encoding a 2-alkenal reductase (2AER), a heat shock transcription factor, or a novel S1 domain protein were identified. Screening for transcriptional activation of stress- and ABA-inducible ADH1-LUC reporter gene has identified the ERF/AP2-type transcription factor RAP2.12, which enhanced ADH1-LUC bioluminescence and increased ADH enzyme activity in the presence of estradiol.

Our data illustrate that cDNA expression libraries can be used for efficient genetic identification and characterization of novel regulators of abiotic stress responses.

This work was supported by OTKA Grants 68226, 81765, EU project FP6-020232-2.

Transcriptome and proteome analysis to understand the clubroot disease of *Brassicaceae*

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Plasmodiophora brassicae is an obligate biotrophic protist causing the clubroot disease of Brassicaceae. The disease is among the most devastating within this plant family and important economic crops such as oilseed rape and cabbage are among the hosts. Infected roots show gall formation, which is accompanied by smaller upper parts of the plants, yield loss due to assimilate redirection and finally water and nutrient depletion. We use the model plant *Arabidopsis thaliana* to dissect the molecular mechanisms leading to the disease development. Transcriptome analysis of the host plant revealed target genes, which have been used in mutant and transgenic approaches to elucidate their roles for the development of the root galls. Among them are genes encoding enzymes for plant hormone synthesis and metabolism, sugar metabolism and redistribution. Interestingly, defense genes were not strongly upregulated in that biotrophic interaction. Since the pathogen can not be cultivated without its host and not much sequence information is available for *Plasmodiophora brassicae*, we have started a proteome approach to better understand the life cycle of the pathogen. So far it was possible to extract proteins from resting spores, which are the overseasoning structures of the pathogen and identify several proteins by mass spectrometry. Taken together, transcriptome and proteome analysis can help to understand obligate biotrophic interactions and may lead to targets for disease control in the future.

Putative splicing factor PX1 regulaters vascular patterning

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Understanding of molecular processes regulating plant vascular formation belongs to most crucial tasks of modern biotechnology. Research during past years has established plant hormone cytokinin as a crucial regulator of model plant *Arabidopsis* vascular development. Protein AHP6, a member of cytokinin transduction cascade, plays a critical role during protoxylem formation. In order to decipher molecular mechanisms linking plant hormone pathways to protoxylem formation, we performed a genetic screen, using *AHP6::GFP* as marker of cytokinin activity in protoxylem. Among others, *px1* mutant has been isolated. Besides it aberrant protoxylem formation, *px1* shows a pleiotropic phenotype, such as defective gravitropism, abnormal cotyledonal development or altered hormonal responses. Rough mapping and whole genome sequencing revealed that *PX1* codes for a weak allele of an embryonic lethal gene, presumably involved in RNA processing. Based on its orthologs from animal systems, we explore role of alternative splicing in vascular development.

Transgenesis in Pea (*Pisum sativum* L.) for Virus Resistance

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Pea Enation Mosaic Virus (PEMV) and Pea Seed-borne Mosaic Virus (PSbMV) cause serious damages on peas resulting in significant decrease of seed yield. As the available pea germplasm exhibiting PEMV and/or PSbMV resistance is limited, the genetic engineering is an alternative to generate pea plants with desired characteristics. The used strategy was the introgression of the whole sequence of viral coat protein gene (*cp*) inducing Coat Protein Mediated Resistance (CPMR) and introduction of a short sense and antisense *cp* fragment thus inducing the resistance mediated by Post-Transcriptional Gene Silencing (PTGS). Here, the data on successful induction of PTGS will be presented and discussed. The pGreenII based plasmid containing 309 bp length *cp* PSbMV fragment in sense and antisense orientation, *35S::uidA* reporter gene, and *nos::bar* gene was used for transformation of pea cv. Raman. The insertion of the transgene was confirmed by PCR, and positive transgenic plants (T_0) were selected. After PSbMV mechanical inoculation, virus replication was evaluated by quantitative RT-PCR and symptom development was recorded. T_1 perspective lines showing no symptoms of infection, comparable with healthy controls in growth and seed production were selected. In non-symptomatic GM plants the detected virus replication was significantly lower as compared to non-GM controls. The ability to resist the infection was successfully propagated to the next T_2 and T_3 generations. The T_4 generation GM plants were released into the environment (field) and are evaluated for agronomic performance in 2010.

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Session 1 - Animal molecular genetics, genomics and proteomics

Association analyses of *RETN*, *UBL5*, *CDF* and *INSR* genes within QTL for meat quality on q arm of SSC2

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Several quantitative trait loci (QTL) for different meat quality traits have been localized on the q arm of porcine chromosome 2 at position 55 – 78 cM. Association analyses were performed in a commercial Landrace x Chinese-European (LCE) crossbred population (N = 438) slaughtered at approximately 127 kg and an average age of 198 days with records for performance (growth, fat and meat accretion), meat quality (intramuscular fat - IMF, Minolta L*, Minolta a*, Minolta b*) and backfat fatty acid composition traits. Polymorphisms within positional candidate genes cloned from homologous regions on HSA19 - *UBL5* (AM950288:g.543G>A), *RETN* (AM157180:g.1473A >G causing substitution Ala36Thr), *INSR* (AM950289:g.537T>C) and *CFD* (AM950287:g. 306C>T) were located at positions 62.1, 64.0, 68.0 and 70.7 cM, respectively, on the current USDA USMARC map of SSC2 and had the following allele frequencies in the LCE: *UBL5* 543G – 0.57, *RETN* 1473G – 0.84, *INSR* 537C – 0.70 and *CFD* 306C – 0.73. The effects of alleles within the candidate genes on recorded traits were estimated using an animal model. Significant effects ($P < 0.05$) were found for IMF (*RETN*) and Minolta L* (*RETN*, *CFD*). Significant allele substitution effects were 0.47 SD for IMF (*RETN*) and 0.68 SD for Minolta L* (*RETN*, *CFD*). Suggestive effects ($P < 0.10$) on IMF (UBL5, CFD), Minolta a* (INSR, CFD) and Minolta b* were also observed. Our results support localization of QTL for meat quality traits in this region but a higher number of gene-tagged markers with known gene order are needed for detailed linkage disequilibrium mapping of these QTL. (Supported by the Czech Science Foundation P502/10/1216)

Analysis of polymorphism *CSN3* gene in cattle by PCR-SSCP method

Martina Miluchova[*], Anna Trakovicka, Michal Gabor

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The work was oriented to identification of κ-casein gene (*CSN3*) polymorphism in population of Slovak Pinzgau cattle. *CSN3* is localized in bovine chromosome 6. *CSN3* genotype *AA* is mostly associated with higher yield of milk, proteins and fat, opposite to *BB* genotype which is bind with higher percentage of proteins and fat content in cow milk. *B* allele of κ-casein gene (*CSN3*) is associated with better technological properties of the milk (decreasing of coagulation time, forming of harder and thicker curd, higher cheese production). The material involved 93 cattle. Bovine genomic DNA was isolated from samples of blood by using fenol-chlorophorm deprotenization and ethanol precipitation and used in order to estimate *CSN3* genotypes by means of PCR-SSCP method. In the population of Slovak Pinzgau cattle included in this study were detected dominance of homozygous genotype *AA* above heterozygous genotype *AB*. The homozygous genotype BB was represented the least, therefore allele *A* dominated above the allele *B*. Sources of research financing: project VEGA no. 1/0061/10, project VEGA no. 1/0046/10.

Detection of polymorphism for calpastatin gene (*CAST*) in Slovak Pinzgau cattle and Charolais by PCR-SSCP method

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The work was oriented to identification of polymorphism of bovine calpastatin (*CAST*) gene and analysis of genotype structure in population of Slovak Pinzgau cattle and Charolais. The calpastatin gene is the member of the calpain-calpastatin proteolytic system and inhibitor activity of the calpains which are important in tenderization process. The *CAST* gene is important candidate gene of meat tenderness in cattle. The polymorphism of *CAST* gene was studied in a group of 207 animals (130 Slovak pinzgau cattle and 77 Charolais). Bovine genomic DNA was isolated from samples of blood by using purification kit from Macherrey Nagel. The polymorphism of *CAST* gene for marker *UoG-CAST* was analyzed by PCR-SSCP method. In the population of Slovak Pinzgau cattle included in this study were detected the following frequency of alleles and genotypes for marker *UoG-CAST*. Frequencies of allele *G* and preferable allele *C* were 0.7538 and 0.2462 and frequencies of genotypes were 0.5692 (genotype *CC*), 0.3693 (genotype *CG*) and 0.0615 (genotype *GG*). Frequencie of allele *G* and preferable allele *C* in population of Charolais were 0.3701 and 0.6299 and frequencies of genotypes were 0.4156 (genotype *CC*), 0.4286 (genotype *CG*) and 0.1558 (genotype *GG*).

This work was supported by the Slovak Research and Development Agency under the contract No. LPP-0220-09.

Porcine *NAMPT*, *PDK4* and *PRKAR2B* genes in association with meat performance traits in pigs

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NAMPT is an essential enzyme in the NAD biosynthetic pathway. Findings obtained in rodents and humans suggest a role of visfatin in glucose metabolism and pathogenesis of type 2 diabetes. PDK4 is one of the four isoforms of mitochondria genes and its regulation may be the most important mechanism for controlling the activity of pyruvate dehydrogenase complex that catalyzes the oxidation of pyruvate to acetyl-CoA. PRKAR2B is one of the regulatory subunits of cAMP-dependent Protein Kinase that regulates a number of cellular processes including proliferation, ion transport and gene transcription. Knockout studies in mice suggest that this subunit may play an important role in regulating energy balance and adiposity.

Association analyses were carried out between already reported SNP *AM999341:g.669T>C* detected in intron 9 of the *NAMPT* gene, new SNP *EU880536.1:g.1135G>A* in intron 10 of the *PDK4* gene, new SNP *FN995222:g.917G>T* in 3´UTR of *PRKAR2B* gene and backfat thickness (BF), average daily gain (ADG) and lean meat content (LM) in Czech Large White (CLW) (n = 215), Black Pied Prestice (BPP) (n = 96) and Landrace (L) (n = 105). Higher BF was associated with *NAMPT TT* genotype in CLW ($P \leq 0.05$), *NAMPT CC* genotype in BPP and *PDK4 GG* genotype in BPP ($P \leq 0.01$). LM was higher in animals with *NAMPT CT* genotype in L ($P \leq 0.01$), *PRKAR2B TG* genotype in BPP and *PDK4 TG* genotype in BPP ($P \leq 0.05$). ADG was associated only with *PRKAR2B GG* genotype in CLW ($P \leq 0.05$).

This work was supported by the Czech Science Foundation (523/07/0353).

Structural and functional bases for gene network organization

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Background: The main aim of the molecular-genetic marker using was to reveal the common and particular traits of population-genetic structures for reconstruction of their divergence ways and to search of genetic tags of artificial and natural selection in domesticated and closely related wild species of animals. The comparative analysis of polymorphism of 102 loci (ISSR-PCR markers) was carried out.

Results: The obtaining data testified the nonrandomness of distribution of the DNA fragments, flanking by invert repeats of microsatellites, in genomes. Revealed conservatism agreed well with considerations of A.Lima de Faria about nonrandom distribution of tandem repeats across chromosomes and participation of some of them in structural and functional organization of linear eukaryotic chromosomes ("chromosome phenotype"). It was supposed as the structural base to organization of synexpression groups of the genes having the general laws in expression and simultaneously participating in one general metabolic process. Estimation of organ-specific gene expression profiles using DNA microarrays was used for searching the synexpression gene groups. Complexity in the analysis of gene expression profiles in relation of external regulation signals and also in connection with cross hybridization of some probe to different genes (cross of homology) was shown.

Conclusion: The problems connected with development of the theory of gene network structural organization and conserved and dynamic parts of them were considered. Universal properties of the network organization and the mutual relations between them, interactions between genetic and ecological factors were discussed.

Inverted repeats and their role in nuclear mechanisms

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To improve use of genetic resources breeders need testing systems which allow reliable identification of genofonds' features. One solution is an analysis of polymorphism of inverted repeats (ISSR-PCR). To estimate their informational value a comparative genetic analysis of cattle, sheep breeds; bisons and aurochs by using of inverted repeats as PCR primers was carried out. Data about specie and breed specific features of combinations of DNA fragments of various lengths were collected. Most of them were revealed by using of purine/pyrimidine tracks which can take part in triplex DNA structures formation and gene expression regulation. Genbank search with using BLAST algorithms allowed localize some of these tracks in genes of II class MHC, moreover microsatellite $(GA)_9C$ is observed both in sheep and cattle exons, this fact may describe its higher conservatism of fragments lengths. At the same time GAGAG sequence is consensual for the GAF transcription regulation factor, and $(GA)_n$ is a target of binding CTCF which plays key role in chromatin structuring and its changes when another gene expression program is enabled.

Data acquired gives evidence about dependence between length polymorphism of DNA fragments flanked by inverted repeats and nucleotide sequence of such loci, another fact points to higher polymorphism levels of those which flanks belong to purine/pyrimidine tracks, among which it's lower for GA containing sequences. Conservatism of the latter may be caused by involving of GA repeats into binding proteins, which take part in gene transcription programs regulation, in particular by changing chromatin structure.

Correlations between phenotypic variability and allele distribution on molecular-genetic markers in sheep breed creation

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Background: Creation of new Siberian type of meet-wool sheep was carried out on the base of complex interbred crosses between Altay tine-wool breed and two meet-wool breeds - Lincoln and Romni-march. The variability in productivity traits and the distribution in allele and genotype frequencies in different variants of interbreed crosses were evaluated.

Results: The genetic interaction with the use of genetic distance on the base of allele variants in 30 loci between different sheep crossing and initial parent breeds was estimated. It was analyzed the dynamics of genetic structure from initial stages of two- and three breed crossings before its consolidation and the statement, and also the consequent reproduction in the process of new meet-wool sheep breed creation. The locus-specific features of genetic mutual relations between generations of new breed and initial parental breeds were revealed. The obtained data demonstrated the population-genetic consolidation in generations of intermedial sheep cross-bred groups and also moving genetic structure of them to sheep of new breed group. Formation general cluster with initial Altay sheep breed testified the moving of genetic structure of investigated sheep generation to genetic structure of indigenous breed highly adapted for local conditions.

Conclusion: It was obvious that in generations, obtaining in process of new breed creation, the evolution of the genetic structure, connected, apparently, with the population-genetic answer to varying parities of artificial and natural selections, were realized. The presence of close relationships between different levels of variability of quantitative productivity traits and molecular-genetic markers was revealed.

The origin of the Old Kladruber Horses verified via Mitochondrial DNA

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D-loop of the mitochondrial DNA from 170 Old Kladrubian horses representing 23 maternal lines from the original stud at Kladruby nad Labem and some private farms was used for DNA sequencing. The sequencing analysis of the 293 bp long fragment of the upstream part of the D-loop region revealed 16 haplotypes.

Upstream part of the D-loop region (378 bp), which is considered as one of the most variable region within the mammalian mitochondrial DNA, was sequenced. 293 bp of this fragment were used for the sequence analysis.

According to 48 polymorphic sites, the 23 maternal lines were grouped into 16 distinct mitochondrial haplotypes. The Old Kladrubian sequences differ each other from one to twelve loci. 9 lines have their own distinct haplotypes. 8 lines are clustered in 3 haplogroups. Line Almerina, according to pedigree data splitted into 6 sublines, in fact consists of two distinct haplotypes (subline Almerina-Aluta and the rest of the sublines), though differing only in one locus. A comparison of Old Kladrubian haplotypes with 23 maternal lines (according to the pedigrees) showed a disagreement of biological parentage with pedigree data for 2 horses (i.e. 1.2 %) of the sample set.

Detection of bovine mastitis pathogens using real-time polymerase chain reaction assay

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Mastitis, an intramammary infection, is economically the most important and the most frequently occurring infectious disease in dairy cattle. Mastitis can bring about significant reduction of milk production, nutritional quality and workability of the milk. The most common causes of this infection are microbial pathogens, especially *Staphylococcus aureus*, *Streptococcus agalastiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. The aim of this study is to validate real-time PCR (polymerase chain reaction) assay, a new method that identifies 11 major pathogen species and a gene coding for staphylococcal β-lactamase production (penicillin resistance).

For detection of bovine mastitis causing pathogens PathoProof mastitis PCR assay (Finnzymes Diagnostics) was used. This method is based on real-time PCR. During 4 hours it is possible to detect and quantify 11 main agents and penicillin resistance gene. Raw, frozen or bronopol preserved milk can be used for this analysis. The real-time PCR was carried out using Bio-Rad Chromo4 Real-Time PCR Detection System. Results were evaluated by Northern Lab Mastitis Studio General Edition 1.03c.

The real-time PCR assay shows perfect analytical exactness. This method covers more than 99% of pathogens responsible for bovine mastitis. This exact and fast identification brings advantages for breeders and for veterinarian, too. Pathogens are detected earlier compared to bacterial cultivation that's why treatment of infected cows can come earlier. This study provides better and more exact results in bovine mastitis pathogens detection comparing to classical bacterial culture method.

This study was supported by Ministry of Agriculture of the Czech Republic (project No. 2B0837).

New SNP in *MITF* gene associated with white coat colour and white spotting in German Boxer dogs

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Breed standard defines two acceptable coat colours in German Boxer, fawn and brindle with or without white markings (flashy and solid coloured respectively). The extreme white coat colour is prohibited from breeding. White spotting in Boxer dogs is a monogenic and semidominant trait. *MITF* gene was identified as the candidate gene for white spotting in some dog breeds, including Boxer. We studied *MITF* gene in 88 of German Boxer dogs of different coat colour (35 white, 30 brindle and 31 fawn) and we found a new single nucleotide polymorphism - SNP in the intron 3 of this gene (c.478-242T>C). SNP was analyzed by PCR-RFLP test using *Msp*I. Two alleles, *A* (682-bp fragment) and *a* (278- and 404-bp fragments) were detected. This SPN shows association with white coat colour in German Boxers. Extreme white animals were aa, fawn and brindle dogs were *AA* or *Aa*. Parents with genotype *Aa* have got white coat colour puppies in litter. We suggest that this SNP can be use for prediction of coat colour in German Boxer litters.

Congenital disorders in the cattle population of the Czech Republic

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Background: Congenital disorders do not occur often. Involving both hereditarily caused defects and malformations suffered during pregnancy, the definition is rather vague from the genetics point of view. In the Czech Republic, a surveillance programme has been established to check the genetic health of cattle. Here, sires born between 1986 and 2001 were analysed.

Results: The offspring of 474 sires - 215 Czech Simmental, 236 Holstein, and 23 beef - were diagnosed with congenital disorders which were unevenly distributed in that only 18 occurred in the progeny of 10 and more sires, in contrast to the 88 occurring in the progeny of 1 sire only. Umbilical hernia was the most frequently noted disorder, and 136 sires fathered progeny with limb anomalies. The most frequent gestational accident was schistosomus reflexus, the results suggesting a familial burden. Three sires fathering offspring with afflicted spinal column and limbs were heterozygous for Complex Vertebral Malformation (CVM), though they had not been reported as such. Foetal defects and stillbirth were quite frequent, and the calves affected were fathered by 56 sires.

Conclusions: The genetic analysis of rarely occurring disorders is difficult, when over a long term period only a few cases are reported, and even then the discrimination between inherited and acquired defects is troublesome. Nevertheless, sires fathering affected offspring should not be used widely in artifical insemination programmes, but disqualified for fathering of stock bulls or even definitively culled, as the possible spread of inherited recessive disorders may result in a negative impact on the economics of the cattle industry

Microsatellite null alleles in animal parentage testing

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Background: DNA microsatellites are one of the most extensively used markers for parentage testing because they are codominant and typically have large numbers of alleles, but there are some limits, of course. One potential drawback is the presence of null alleles.

By definition, a microsatellite null allele is any allele at a microsatellite locus that consistently fails to amplify to detected levels via the polymerase chain reaction (PCR). Point mutation in the primer annealing sites in such species may lead to the occurrence of null alleles. Null alleles are found to a varying degree in paternity and identity verification microsatellite panels in all species.

Results and Conclusions: Over twelve years of DNA microsatellite genotyping in Immunogenetics laboratory presence of null alleles was revealed in: HTG10, HMS3 and HMS7 loci in horses; BM2113, TGLA122 and INRA023 loci in bovine, OarAE129 locus in ovine and SW353 locus in swine.

The frequency and occurrence of null alleles is in all species breed-specific. For example: high HTG10 null allele frequency is typical for thoroughbred horse, presence of null allele at the bovine INRA023 is breed-specific for Blonde D´Aquitaine.

The occurrences of null alleles create false homozygotes and may be the basis for erroneous parentity exclusion, especially in the computer evaluation of the question of parentage.

On the other side, null allele is an allele with codominant inheritance and may be (in the hands of an expert) useful for parentity verification too.

Session 2 - Animal genetics, breeding and diversity

Performance test evaluation of the Old Kladruby stallions´ offspring

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The aim of my work was evaluation of 413 offspring of 31 Old Kladruby horse sires with regard to performance test. The data were collected within the period 1995 - 2007. These traits were included in the analysis: type and sex expression, body lines, fundament, general harmony, dressage, general impression, walk, trot, canter, marathon, dressage test, obstacle driving test, first pull, second pull and third pull. The overall means, standard deviations and variation coefficients were calculated for the whole breed and offspring of individual stallions having 5 or more offspring. Following fixed effects were considered: sire, sex, colour variety, breed and age. There were included 6 interactions. The highly significant differences were found in type and sex expression, body lines, fundament, general harmony, general impression, walk, marathon, dressage test and obstacle driving test. The significant differences were found in dressage and trot. The highly significant interaction between sex and sire was found in obstacle driving test. The significant interactions between colour variety and sire were found in body lines and general harmony. The significant or highly significant interactions indicate changing the order. The results evaluate Old Kladruby sire on basis of offspring's results in performance test.

Old Kladruber horse as the gene resource in CR

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The Old Kladruber horse is the only Czech domestic original horse breed and an important genetic resource of horses not only on the scale of the Czech Republic and Europe but also worldwide. The today existing breed is a warmblooder created on the basis of the Old Spanish and Old Italian horses formely declared as a gala-carrossier, nowadays used for carriage driving sports, dressage and recreational riding. According to the FAO this breed can be characterized as endangered breed, despite continuously growing population (in 1993 N_e = 114, in 2009 N_e = 204). The breed was closed against gene immigration in 1992. The population consists of 5 grey lines and 5 black lines. The analysis of inbreeding showed in the last decade the trend of decreasing (mares from 7.75% to 4.88%), no tendency caused by inbreeding depression was found in the fertility rate. The great attention is payed to the body conformation; in 1995 a linear description system of type traits for 32 traits was introduced in Old Kladruber breed. The analyses using GLM procedures (SAS, 2005) revealed specific conformation properties of grey and black population usable in breeding and conservation of genetic diversity within the breed. The estimated heritabilities for type traits showed the possibility of reduction of traits described – since 2008 is described 26 traits. Further studies were carried out on coat colour and its properties in the Old Kladruber grey population.

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Growth models in Rabbits

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The potential of rabbits can be utilized to address the problems of food scarcity. Only limited studies have been conducted in rabbits of tropical countries, where climate, diet, management and stock resources can differ markedly from those in temperate countries.

The experiment on growth rate in rabbits was carried out in a total of two hundred and thirty five rabbits belonging to Grey giant, Soviet Chinchilla, White Giant and their crossbreds maintained under identical conditions at the University Rabbit Farm of The Kerala Agricultural University, Kerala, India. The animals were housed in individual wire meshed cages and put on ration of ad libtum green fodder and up to two hundred gram concentrate feed of fifteen to twenty percent crude protein per day depending on their age and size and given access to drinking water round the clock.

Different growth models were fitted to the data pertaining to body weights from birth to fourteen weeks of age. The models fitted were linear, quadratic, cubic, compound, growth, exponential and logistic curves. Adjusted R^2 were computed for each model. Growth curves were fitted for males and females of each breed. Higher R^2 values were obtained for linear, quadratic and cubic models. Best model for each category was identified. By using these models live weights at later ages could be predicted from early partial live weight data.

Application of genetic markers in conservation genetics of peregrine falcon (*Falco peregrinus*) populations

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The critically endangered *Falco peregrinus* seems to increase the establishment not only in the Czech Republic but also in Europe. The main goal of this study was to find and test specific raptors markers, which could help with monitoring of breeding places free – living falcons and improve the point of view the genetic markers in conservation biology. A total of 30 individuals were divided into two groups according to their origin, all samples were originated from the Czech Republic. The dataset was 15 individuals of falcons living in a captivity and 15 individuals were free – living. We used 10 microsatellites (NVH fp13, NVH fp89, NVH fp86-2, NVH fp5, NVH fp31, NVH fp92-1, NVH fp79-4, NVH fp107, NVH fp54, NVH fp79-1) to estimate genetic variance of these two groups. AMOVA revealed variance of 3.11 % among populations (captivity and wild), 14.50 % variance among individuals within populations and 82.39 % variance within individuals.

It is necessary to know sex structure, if you study free living population of *Falco peregrinus*. That is why we tried to evaluate the sex testing based on the chromodomain helicase DNA binding gene (CHD), which is located on the avian sex chromosomes. There are two alleles of the gene, CHD – W is unique for females, because it is located on the W chromosome, and CHD – Z, which is typical for both sexes. All these markers suggested being a new possibility how to research the populations of Falco peregrinus.

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Factors effecting on the calving interval in Population of Slovak Spotted Breed

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Fertility is important factor for profitability breeding of cattle. The aim this work was points the interesting effects on the calving internal dairy cows in population of the Slovak spotted breed. This data were analysed using the SAS version 9.3.1 and linear model with fixed effects of herd-years-season of calving, breed-type, number of lactation and sire on the calving interval of Slovak spotted breed in period 1990 and 2004.

Average at calving interval was 408.52 ± 86.71 days and average milk production after first, second and third lactation was 3447.28 kg, 3880.67 kg and 4110.95 kg, respectively. The linear model to represent coefficient determination R^2 = 0.0871 % (P<0,001) for calving interval with all fixed effects. The analyses by the effect was the highest effect of herd-years-season of calving R^2 = 0.0529 %, than effect of sire R^2 = 0.0226 %. These effects were statistically high significant (P<0,001).

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Phenotypic variability of PRCD expression in different dog breeds

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Progressive rod-cone degeneration (*PRCD*) is canine late onset autosomal photoreceptor degeneration with great variation of disease development from 3 to 13 years of age. The time of onset and the disease progression varies between dog breeds as well as in individuals. PRCD in canine is one form of retinitis pigmentosa (RP) in human due to its clinical similarity and identical causative mutation in a novel retinal gene. *PRCD* gene was mapped to the centromeric region of canine chromosome 9 (CFA9).

We report here population studies of 699 dogs of the following breeds and frequencies of the disease causing mutation: American Cocker Spaniel (0.09), English Cocker Spaniel (0.34), Chesapeake Bay Retriever (0.14), Nova Scotia Duck Tolling Retriever (0.44), Labrador Retriever (0.07), Poodle Toy (0.45), Poodle Miniature (0.20), Poodle Medium (0.05), Chinese Crested Dog (0.02), and Shiperke (0.06). No disease causing mutation was observed in English Springer Spaniel, Welsh Springer Spaniel, Flat Coated Retriever, Golden Retriever, Poodle Standard, and Australian Cattle Dog.

The disease resulting complete blindness in affected individual in almost every case. A modifier gene is likely to segregate in genomic proximity to *PRCD* gene phenotypic expression. In four Chinese Crested Dogs and one Poodle Toy we have observed complete development of progressive retinal atrophy even when all of them were genetically homozygous healthy in *PRCD* gene. It seems to be due to other disease causing mutation in the different gene.

Genetic diversity and pedigree analysis in the Czech draft horse population

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The objective of this work was the analysis of cold blooded horse population in Czech Republic. In this work we found out differences in amount of the inbreeding coefficient, generation interval and effective population size in the Silesian Noric (SN), Noric (N) and Czech-Moravian Belgian horse (CMB) with using pedigree records from foals (917 in SN, 1986 N and 2323 in CMB) born in period 1990-2007. The average value of coefficient inbreeding there was 3.39%, 1.56% and 2.88%, respectively. During the whole period, the inbreeding increased from 2.01% to 4.32% in SN. In CMB, on the start of this period there were inbreeding 2.50% and in the end of this period there were value of 4.52%. Similar situation was in N too. On the beginning these period the average value was 1.20% and in the end 2.41%. Trend was rising in both sexes. Generation interval between parents and their offspring were 8.5 years in SN, 8.6 years in CMB and 8.9 years in N. Longer interval was found between fathers and their offspring than between mothers and offspring. Effective population size was calculated with respecting generation interval and changing value of inbreeding coefficient, smallest effective population size was in SN (43.1), bigger was in CMB (52.2) and the biggest size was found in N (79.1). The results show, these populations are endangered and there is desirable rescue program to improve.

Variability of gene *H-FABP* in a group of pigs Czech Large White breed

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Gene *H-FABP* (Heart fatty acid-binding protein) belongs to the *FABP* family and plays an essential role in long-chain fatty acid uptake. This gene is located on the 6th chromosome of pig and is one of candidate genes of intramuscular fat (IMF). IMF is one of the main factors affecting sensory meat quality pork meat. The aim of this work was analyze two single nucleotide polymorphisms (SNP) of *H-FABP* and for experiment we tested 53 pigs Czech Large White breed. First polymorphism is localized at the X98558:g.1321G>C, exon 1 and second is at Y16180:g.1878 A>T in intron 2. Both polymorphisms were detected by PCR-RFLP and the PCR products were digested with *Hinf*I and *Hae*III. The frequencies of allele *H* and *h* of polymorphism of *H-FABP* determined by *Hinf*I are 0.32 and 0.68, respectively. The second polymorphisms of *H-FABP* determined by *Hae*III have frequencies of allele *D* and *d*, 0.39 and 0.61, respectively.

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Perspectives of the Cattle Genetic Evaluation Systems in the Slovak Republic

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An actual implementation and upcoming models for analyzing milk yields, somatic cell count, type traits, fertility traits and longevity traits in the Slovak Republic were described for Holstein, Simmental and Pinzgau cattle population. The latest international genetic evaluation for dairy cattle production traits and an impact of the evaluation on the national genetic evaluation system is discussed. New opportunities and new breeding goals in the area of cattle genomic evaluation and genomic selection on the base specific Interbull services for countries without own genome programe or small size of the reference population are presented. Simulated genomic data and official estimated breeding values for 744 Holstein and for 425 Simmental bulls were analyzed together on the different number of single nucleotide polymorphisms.

Development of sires´ breeding values for live weight at 210 days of age by 2007 compared with their value in 1997

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The objective of this work was to evaluate the stability of breeding value (BV) during years 1997-2007 in Aberdeen Angus sires born before 1994. The estimation of the BV for live weight at 210 days of age was made using SAS 9.1 and BLUP F90 software. The sires were divided into 3 groups according to their BV in 1997. Generally, those sires with an above-average BV for direct effect (DE) in 1997 remained at this level during subsequent years and sires with an average BV, respectively below-average BV, remained in the same groups as well. However, intra-group variability changed during observed period. Standard deviations ranged from 1.99 to 8.34 kg in 1997 and increased during the evaluated years. The BV´s variability also increased. A low level of balance in the BV for ME was determined among groups in accordance with division of the dataset into groups according to the achieved BV for DE, because an extremely low proportion of animals with the best BV for ME was evaluated in relation to negative genetic correlation between DE and ME. The lowest correlations, r = 0.19, respectively r = 0.14, were calculated in bulls with an average BV for DE, respectively ME, between 1997 and 2007. Funded by MSMT 6046070901 and MZe 0002701404.

Stability of breeding values in Aberdeen Angus population born in the Czech Republic in 1997

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The objective of this work was to evaluate the progress of breeding value (BV) from 1997 to 2007 in Aberdeen Angus bred in the Czech Republic and born in 1997. The estimate was taken for the BV of live weight at 210 days of age in 361 bulls and 260 cows using SAS 9.1. and BLUP F90. The average BV for direct effect (DE) in the bulls evaluated ranged from +1.92 kg in 2002 to +4.05 kg in 1998, while in the cows this BV was in a range from +2.5 kg in 2002 to +3.22 kg in 2005. The BV for maternal effect (ME) varied in the bulls in accordance with negative genetic correlation from -2.18 kg in 2001 to +0.17 kg in 2005. This BV in the cows represented a range from -3.12 kg in 1999 to 0.35 kg in 2006. Correlations of the bulls´ BVs between individual years ranged from +0.69 between 1997 and 2007 to +0.98 between 2006 and 2007. The lowest correlation of the BV for ME in cows, r = +0.45, was detected between 1997 and 2007, while the highest, r = +0.99, between 2006 and 2007. These results documented close relationships among BVs and a high level of reliability of the BV for DE and ME. Funded by MSMT 6046070901 and MZe 0002701404.

The development of breeding values in Charolais in the Czech Republic between 1997 and 2007

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Longevity is an important trait in beef cattle as well as growth and reproduction traits. In accordance with longevity, the breeding value (BV) stability has an effect on the productiveness of the selection system. The estimate of BV was calculated for live weight at 210 days of age. The model equation for prediction of the BV included the following features: sex, peers, animal, mother, age of the mother, permanent environment of the mother, and random error. The BV estimation for the direct (DE) and maternal effect (ME) was performed using SAS 9.1 and BLUP F90. An increase in the number of animals evaluated, as well as an increase of the average BVs for DE, was detected during subsequent years of the observed period. The lowest BV for DE, +1.11, was calculated in 1748 animals in 1997, while the highest average BV for DE, +5.59, was reached in 20873 animals in 2007. Average BV for ME ranged from -0.48 in 1997 to +1.3 in 2006. The number of animals in whole population and in observation increased significantly between 2006 and 2007, and therefore the average BV for ME declined to 0.55. Also, the variability between BVs for DE and ME increased during period observed. Funded by MSMT 6046070901 and MZe 0002701404.

The stability of the breeding values of Charolais bulls and cows between 1997 and 2007

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Bulls and cows are used in mating with different intensities and therefore the effect of their genetic potential on the quality of their progeny differs. Breeders try to improve the growth resulsts of beef cattle while maintaining longevity. Live weight at weaning defines the market value per head - the weaned calf - and is the most important trait. The calculation was performed for breeding value (BV) for growth ability up to 210 days in 482 Charolais bulls and 1266 cows born before 1997. The BV estimates for direct effect (DE) and maternal effect (ME) were calculated using SAS 9.1. and BLUP F90. The lowest average BV for DE in bulls, +1.13, was determined in 2001. The highest average BV for DE in bulls, +4.57, was estimated in 2007. In cows, the BV for DE ranged from -0.92 in 2001 to 1.92 in 2007. The lowest average BV for ME in bulls, -1.6, was recorded in 1999, while the highest value, +1.55, in 2006. The range of average BVs for ME in cows was from 0.39 in 1999 to 0.57 in 2004. During the course of the monitored period a positive development of the BVs for DE and ME was reached. Funded by MSMT 6046070901 and MZe 0002701404.

The course of breeding values in Aberdeen Angus sires used in mating from 1997 to 2007

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The breeding values (BVs) for direct (DE) and maternal effects (ME) for live weight at 210 days of age were estimated using SAS 9.1. and BLUP F90 in relation to the growth of sires´ progeny in the Aberdeen Angus during the period 1997-2007. The dataset of bulls born before 1994 was divided into 2 groups; group 1 - 46 bulls with more than 40 calves in Performance recording in 2007, and group 2 - 825 bulls with less than 40 calves. The average BVs for DE in group 1 during the years evaluated ranged from +4.06 kg in 1999 to +5.77 kg in 1997, while in group 2, from +1.16 kg in 1999 to +2.22 kg in 1997. The BVs for DE had a similar development during observed period. The average BVs for ME varied from -4.15 kg in 2001 to -0.15 kg in 2006 in group 1, and from -1.65 kg in 1997 to 1.11 kg in 2006 in group 2. A tendency of low improvement in the BVs for DE and ME was detected during evaluated years. The correlation coefficients of the BVs were from r = +0.3 to +1.0 in accordance with lower, respectively longer, difference between the individual years of the BV estimation. Funded by MSMT6046070901 and MZe0002701404.

Effect of the country of origin on the growth of progeny and correlations among breeding values

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The objective of work was to evaluate the breeding values (BV) of Charolais sires (n = 2848) bred in the Czech Republic from 1990 to 2009. The effect of 5 countries (Germany, Austria, France, the Slovak and Czech Republic) was evaluated. The live weight (LW) at birth of the sires´ progeny ranged from 39.6 kg to 44.2 kg and statistically differed (P<0.05) in France (44.2 kg) and Germany (43.9 kg) only. The LW at 210 days of age varied from 286.9 kg to 336.7 kg (P<0.05). A high level of Pearson´s correlation coefficients, r = 0.5 to 0.01 (P<0.05), was calculated between the BVs for DE and ME of LW at 120, 210, and 365 days of age. The closest relationships was detected between the BVs for ME at 120 and 210 days of age, while the lowest was determined between the BVs for ME for LW at 120 and 365 days of age. Medium correlations from 0.11 to 0.27 (P<0.05) were calculated between the BVs of DE and ME for LW at birth and difficulty of calving. Negative correlations, r = 0.32-0.05 (P<0.05) were found between the BVs of DE and ME for LW at birth, 120 days of age, the relative BV of muscling, height at the withers, body capacity, and LW gain during the test. Funded by MSMT6046070901.

Association analysis of genetic markers with piroplasma infection in a donkey breed from Italy

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Piroplasmosis is a tick-borne disease caused by blood protozoans, in donkeys mainly by Theileria equi and Babesia caballi. Genetic resistance to protozoan parasites was reported in humans and animals. Here, a pilot study of a donkey population (breed Asino di Martina Franca) exposed to infection with both parasites in the Apulia region, Italy, is presented. The presence of parasites in the peripheral blood was assessed by species specific polymerase chain reaction (PCR) of 18S rRNA gene and was adopted as the criterion of infection. Donkeys were classified as positive or negative for the presence of at least one parasite species. A combined approach was used for the association analysis. Twelve microsatellites out of 17-plex PCR of microsatellites used for parentage testing in horses and 5 single nucleotide polymorphisms (SNPs) located in 4 candidate immunity-related genes (IL12A, IL12B, IL12RB2, IL17A) were used as markers for this purpose. Statistical associations were determined by the Chi-square test or by Fisher exact test with Bonneferoni corrections for the numbers of comparisons. The microsatellites AHT004 and ASB023 were associated with ´resistance´ to infection (Pcorr<0.01 and Pcorr<0.05, respectively). The microsatellite HTG010 was marginally associated with ´susceptibility´ (Pcorr<0.06). No significant associations were found for SNPs in candidate genes, only a marginal association (Puncorr<0.07) for the gene IL17A could be observed. The candidate chromosome regions surrounding the two microsatellite markers merit to be further investigated.

Genetical and environment components of micronuclei test

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Background: Modern problems of increase in pollution by ecotoxicants leads to necessity of search the optimal methods for monitoring of biological consequences of their accumulation. It leads to necessity of working out the bioindication methods of the general ecotoxic effects with the use of mammalian bioindicator species that is especially important for the estimation of safety of foodstuff production conditions. As such bioindicators widely use the farm animal species as they contact to the same environment conditions, as the human and, besides, are sources of food resources. The most convenient method to revealing of the gene toxic effects is the variants of the micronuclei test in peripheral blood cells. However application of such tests demands of the information accumulation on the spontaneous levels of micronuclei, and also genetic factors influencing on this test.

Results: We revealed the differences in micronuclei test results between the cattle breeds differentiated by productivity directions. The comparative micronuclei test analysis in cattle, yaks and interspecies hybrids between them was carried out for studying the influences of interspecies hybridization on characteristics of the micronuclei test. The greatest frequency of cytogenetic anomalies was revealed in peripheral blood cells of interspecies hybrids.

Conclusion: The obtained data testified the presence of genetic components in variability of estimations of the genetic damages that it was necessary to consider under bioindication of ecotoxic pollution with the use of peripheral blood cells of farm animal species.

Inbreeding trends and pedigree completeness analysis in active Pinzgau bulls` population

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The objective of the study was to determine completeness, inbreeding levels and characteristics based on probabilities of gene origin. There were evaluated 26 bulls in reference population, pedigree file consisted of 379 individuals. Genetic diversity was evaluated by software Endog 4.6. The average inbreeding coefficient of evaluated bulls was 0.32 ± 0.93 % and average individual increase of inbreeding was ΔF = 0.12 ± 0.37 %. The average individual relatedness coefficient was 0.98 ± 0.53 %. The maximum number of generations traced was 4.39 ± 0.94, the average number of fully traced generations was 2.077 ± 0.744, the equivalent of complete generations was 3.011 ± 0.547, effective number of founders was 99 and effective number of ancestors 23. The number of ancestors explaining 50 % of diversity was 10. Bull LOH 001 was the most frequently used in reproduction of population, producing 93 offspring. Results will be used in breeding management of Pinzgau population and monitoring of parameters characterizing genetic diversity and its development, as well.

Inbreeding and Melanoma in Old Kladruber Horses

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The Old Kladruber horse is original Czech horse breed, currently kept in two colour varieties – grey and black. The grey coat colour is a result of the progressive greying - the loss of pigment in hairs. Older grey horses often develop dermal melanoma which occurs most frequently on the bottom of the tail or at the anal and perineal area. The data collection was carried out in grey variety of Old Kladruber breed in stud Kladruby nad Labem, Benice stud and others. Data were collected after detailed inspecting and measuring 376 horses repeatedly during four consecutive years. The greying level was measured using Spectrophotometer Minolta 2500D. Melanoma was detected visually and by palpation on every horse and classified using 5 grade scale. Inbreeding coefficients (F_x) were estimated as the coancestry coefficients using INBREED procedure of SAS package. The fully informative pedigrees with 5 generations of ancestors were used. The GLM model (SAS, 2004) was used to examine the influence of effects age, line, sex, year of evaluation, stud, greying level and F_x on melanoma status. The incidence of melanoma in Old Kladruber grey horses was confirmed. The occurrence and grade of melanoma is strictly progressive with the age of the horse. The statistical significance for effect line suggests the impact of heredity on melanoma prevalence. Although F_x is also statistical significant effect in the model used, very low value of regression coefficient ($b_{x/y}$ = 0.02) shows negligible change of melanoma status with the increase of F_x.

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Genetic diversity of brown bear (*Ursus arctos*) in Slovakia

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The genetic information provides valuable data on the population history, phylogenetic relationships within groups, as well as ethology and reproductive biology of the species. This information should be essential for the proper management of endangered species and wild animals. The rapid development of molecular genetic methods in the recent years has resulted in the application of genetic research to the rescue of the low numbered populations and species with limited gene reserves. Genetic characteristics were used to map the population of the cryptic species and also the populations of the brown bear. This work focuses the genetic structure of populations of brown bear in Slovakia. The species were similarly affected as the other species by the rapid decline of the suitable habitats and by the over-hunting. The brown bear is indigenous species in Slovakia. In the western Slovakia the species disappeared at the turn of the 19th and 20th century. In the second decade of the 20th century the west-carpathian population was separated from east-carpathian population. Since 1932 the bear is protected by law and so the number of individuals grows. In the present the estimated number of bear is 700 to 800 individuals. However, the GREEN REPORT informs over 1900 bears in Slovakia. This discrepancy could possibly be the result of the distortion or possible duplication.

This study presents the results of the analysis of the genetic variability in the contemporary populations of brown bears in Slovakia. For this purpose we have analyzed over 200 samples from several parts of Slovakia using 13 microsatellite markers. Relatively high degree of genetic variability was found in the whole population. The level of genetic differentiation suggests a similarity of each subpopulation in Slovakia. These results are the basis for determining the population size by capture-recapture research using the molecular genetic methods and non-invasive way of collecting samples.

Polymorphism of *IGF1* Gene and its Association with Phenotypic Traits in Ross 308 Broiler Chickens

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The chicken insulin-like growth factor 1 gene (*IGF1*) is a biological candidate gene to investigate growth, body composition, metabolic and skeletal traits and also positional candidate gene for growth and fat deposition in chickens. The broiler population Ross 308 was used to study relations between *IGF1* gene polymorphism and phenotypic traits. The chickens were individually marked by wing markers. During the experiment, there was measured individual body weight of chickens at hatch and every week up to 6 wk of age. The blood samples were collected in EDTA-treated tubes before slaughter for identification the *IGF1* genotypes. There were evaluated slaughter characteristics after slaughter and also the drip loss was determined and the texture of raw meat. A single nucleotide polymorphism (SNP) was identified by using PCR-RFLP method. Genotypical frequencies were for genotype *AA*: 0.83, *AC*: 0.17, and *CC*: 0.00. Higher average body weight and gain at the all control days were found in chicken with *AA* genotype. The highest WPC had homozygous males (2951.4 g). Also higher liver weight and liver weight percentage of weight of poultry carcass with giblets, and the breast muscle weight were found in *AA* genotype in the comparison of broiler heterozygous. Higher leg muscle weight had chicken with genotype *AC*, but no differences were significant.

CORD 1 (Cone-Rod Dystrophy 1) in Czech Republic Dachshunds

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Cone-rod dystrophy 1 (cord1) is a reccesive condition that occurs naturally in miniature longhaired daschhunds (MLHDs). It´s an 44-bp insertion in exon 2 retinitis pigmentosa GTPase regulator-interacting protein 1 (*RPGIP1*) gene. The reading frame of exon2/exon3 is changed and a premature stop codon is introduced. Cord1 is homologous by human Leber congenital amaurosis. First signs appear at age of 6 months and causing a blinding of affected individual.

We have analysed 600 samples of group of standard, and group of miniature and rabbit Dachshunds. Both groups were divided by type of the coat – short-, wire- and long-haired. Frequnce of the disease allele is calculated. Because varieties are bred together disease alel is observed in the whole population of Dachshunds.

Realized effect of different selection criteria applied in breeding herds on performance in multiplier herds

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Different breeding management as well as higher selection pressure on the performance traits has been applied on population of Large White sows. The sows were divided into two subpopulations (hyperprolific or normal). The hypothesis that progeny of sows from hyperprolific subpopulation breed in multiplier herds have higher litter size traits was tested. The effect on the growth performance traits are reported so. About 1933 farrowings of 614 Czech Large White sows were included. The number of functional nipples increased remarkably in the sows of hyperprolific subpopulation although the selection pressure on this trait was small. Likewise, both studied growth performance traits reacted positively on the selection pressure and the differences between populations were highly significant. Surprisingly, no significant differences in litter size traits were found either in the first or in the first to fifth parity. The results outlined that the selection criteria applied in the breeding herds can efficiently increase the traits with middle or high heritability coefficients in multiplier herds. However, the selection seems rather non effective as far as the litter size traits are concerned as heritability coefficients of these is low and hence are influenced by crossbreeding. Using of auxiliary selection traits should be therefore considered for improvement of economic efficiency of multiplier herds.

Variability of *LPIN1* and *MC4R* in Czech Large White pig breed

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The aim of this work was *MC4R* and *LPIN1* allele frequencies determination in Czech Large White pig breed in the Czech Republic. *MC4R* (Melanocortin 4 receptor) gene has an influence i.a. to regulation of metabolism, food intake and obesity. *LPIN1* (Lipin1) is candidate gene for human lipodystrophy. It plays role in lipid metabolism and adipose tissue development. In total 54 Czech Large White pigs were tested for C/T polymorphism in *LPIN1* and G/A polymorphism in *MC4R* by PCR-RFLP. Both polymorphisms were detected by *Taq*I restriction endonuclease. In *LPIN1*, 40 animals were homozygous *CC*, 14 heterozygous *CT*, no genotype *TT* was detected. Relative frequency of allele *C* was 0.87. In *MC4R*, 4 pigs were found homozygous *GG*, 23 heterozygous *GA* and 27 homozygous *AA*. Relative frequency of allele *A* was 0.71.

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Genetic diversity among pinzgauer cattle population in Slovak Republic.

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DNA-based molecular markers such as microsatellites have a very high level of polymorphism and have been successfully used to estimate genetic variation in breeding programs and to access the level of genetic conservation schemes (Almeida et al., 2000). The aim of the present study was to provide information on the genetic structure in Pinzgauer cattle breed in Slovakia based on polymorphism at 11 DNA microsatellite loci (*BM1824, BM2113, ETH3, ETH10, ETH225, INRA23, SPS115, TGLA53, TGLA122, TGLA126, TGLA227*).

Our study was investigated in the Slovak population of 80 Pinzgauer cattle. DNA was amplified in one multiplex PCR for amplifying *BM1824, BM2113, ETH3, ETH10, ETH225, INRA23, SPS115, TGLA53, TGLA122, TGLA126, TGLA227*) microsatellites using StockMark for Cattle (AB). The analysis of PCR fragments was performed using an ABI 310 sequencer. Size of analyzed DNA fragments was determined in base pairs using computer package GeneScan v.3.7 (AB). The amount of variation in Pinzgauer population was measured with statistical parameters as the average number of alleles per locus and observed heterozygosity (H_o), expected heterozygosity (H_e) by Nei, 1987 and PIC value (Botstein et al., 1980). The statistical parameters were calculated using Powermarker v.3.23 software (Liu and Muse, 2003).

Total 93 alleles were identified in the Pinzgauer breed. The number of alleles per each locus ranged from 5 (*BM1824, TGLA126*) to 12 (*TGLA53, TGLA122*). The average value of H_e was 0.753 and H_o was 0.767. Mean PIC value was 0.719. The Power of Exlusion ranged from 0.373 (*BM1824*) to 0.720 (*TGLA53, ETH3*). The values of PE confirmed the usefulness of this set of microsatellite markers in parentage testing of Pinzgauer cattle in Slovakia.

Polymorphism of *ABCG2* and *FASN* by Czech Pied and Czech Holstein sires and German Holstein sires

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Genetic research in farm animals focuses mainly on the identification of genes influencing economically important traits that could be useful in breeding programs. In the present work, the polymorphisms located in the *ABCG2* (ATP binding cassette, subfamily G, member 2) by Czech Pied (350 individuals) and Czech Holstein Sires (250 individuals) and German Holstein Sires (350 individuals) and in the *FASN* (fatty acid synthase) by German Holstein Sires (266 individuals) were studied. Fatty acid synthase (FASN) is a multifunctional protein that carries out the synthesis of fatty acids so it plays a central role in de novo lipogenesis in mammals. A single nucleotide change (A/C) in exon 14 is capable of encoding a substitution of tyrosine 581 to serine (Y581S) in the *ABCG2* (ATP binding cassette, subfamily G, member 2) gene and affects milk production traits. The $ABCG2^A$ allele decreases milk yield and increases protein and fat concentration. Genotypes were identified using PCR-RFLP. The frequency of allele *A* of *ABCG2* locus was high in all our populations, $ABCG2^A$ was predominant. The population was monomorphic in this locus. In the German Holstein Sires, the frequency of allele G = 0,522 was found. The frequencies of genotypes were: *AA* 0,218, *AG* 0,518 and *GG* 0,263. The observed and theoretical expected genotype frequencies at *FASN* loci were in agreement with Hardy-Weinberg equilibrium in the German Holstein Sires.

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Impact of different mating strategies on management of inbreeding in Pinzgau population

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Hodnotili sme priamy efekt štruktúry populácie v šľachtiteľskom programe a miery intenzity selekcie na prírastok inbrídingu za generáciu. Vzhľadom na existujúcu štruktúru populácie sme pomocou stochastickej simulácie Monte Carlo analyzovali vplyv zmeny stratégie a intenzity selekcie, resp. zmeny pripárovacej stratégie s cieľom minimalizovania prírastku inbrídingu. V práci bolo porovnávané náhodné pripárovanie so selekciou podľa fenotypových resp. BLUP plemenných hodnôt. Selekcia podľa BLUP plemenných hodnôt má za následok vyšší prírastok inbrídingu vo všetkých sledovaných alternatívach. Prírastok inbrídingu bol štatisticky preukazne vyšší v alternatíve so zaradením 4 otcov býkov (dif = 1,2696+) resp. pri zaradení 5 otcov býkov (dif = 0,8665+). Pri zaradení 2 resp. 3 otcov býkov rozdiel nebol štatisticky významný. Vnútropopulačná diverzita je dôležitou zložkou globálnej biodiverzity nielen voľne žijúcich ale aj hospodárskych zvierat. Kritickou hodnotou pre meranie straty genetickej diverzity v populácii je prírastok inbrídingu. Pre zachovanie genetickej diverzity a zabránenie straty je nevyhnutné minimalizovať prírastok inbrídingu v malých populáciách akými sú aj ohrozené plemená. Klasické riešenie vyžadovalo vyrovnanie počtu otcov a matiek v pripárovacom pláne. Naopak vo väčšine komerčných populácií sa využíva nízky počet plemenných zvierat s vysokým pripárovacím podielom. Problémom je tiež udržiavať vysoký počet dospelých plemenných samcov. Na podklade teórie dlhodobých genetických príspevkov je možné modelovať viacgeneračný vývoj v rodokmeňoch. Tento predpoklad sa potvrdil stochastickou simuláciou. Ďalší výskum bude zameraný na porovnanie efektu selekcie podľa BLUP plemenných hodnôt pri použití zámerného pripárovania.
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Selection indices in Slovak cattle populations

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The objective of the study was to evaluate the genetic trends in various indicators of breeding values for editing existing selection indices in the cattle population in the Slovac Republic. We calculated the genetic trends estimated breeding values for milk yield, somatic cells count and breeding values of indicators of the type traits of the cattle population. In the evaluation were included only bulls, with minimum 20 daughters were born from 1980 to 2003. The most significant positive genetic trend was in the evaluation of the stature (BB) = 82 points and dairy strength (DS) = 78 points. Separately we evaluated the difference in average breeding values aggregated traits of black and red Holstein breed variety. Was found a real difference for the stature (+0,39), for the dairy strength (+0,35), for the feet and legs (+0,31), for the udder (+0,37), for the type (+0,52), for the condition (-0,14), for the Slovac Production Index (+1334) and for the Slovac Holstein Index (+60). For the purpose of genetic evaluation is a point system of evaluation body condicion 1-5 transformed on the point system 1-9. And dairy strength is at the 78 points. In the holstein breed populations found positive genetic trend for the Slovac Production Index SPI (+190,932), breeding value of milk PH of milk (+ 46,207), for protein breeding value PH of protein (+ 1, 381) and for transformed somatic cells SB (- 0,003). Somatic cells have in this case the positive direction and thus decrease the proven positive genetic trend should be included in the new index by a selection of our recommendations.

Detection of *Paenibacillus larvae* from the honey samples by RTQ PCR

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The aim of this study was testing an effective diagnostics of *Paenibacillus larvae* from honey samples. *P. larvae* is a G+ bacteria that causes American Foulbrood. Method was divided into 3 parts: cultivation on MYPGP agar, elementary tests and RTQ PCR. Honey sample was inoculated on MYPGP agar plates. After cultivation and re-inoculation each isolate was analysed in term of catalase activity and G+ confirmation. We isolated DNA from 24 h cultures by mixture with 100 µl distilled water, incubation at 95 °C for 15 min and centrifugation for 10 min. Supernatant (2 µl) was used for RTQ PCR that was realized next isolation DNA. Temperature profile of RTQ PCR was following: initial denaturation at 95 °C for 2 min and 40 cycles (comprised of 3 steps) – denaturation at 95 °C for 20 s, annealing at 50 °C for 20 s and polymerisation at 72 °C for 20 s. Length of fragments from RTQ PCR was controlled by electrophoresis in 2 % agarose gel. We analysed 20 honey samples. We obtained 11 isolates originated from these samples. All isolates were G+ and 3 of 11 were catalase negative. None or deficient catalase activity is one of P. larvae characteristics. Results of RTQ PCR point out, that fragment was formed in all 11 isolates. But after electrophoreses we found, that fragment with specific length was not formed. Additional optimalization of RTQ PCR for *Paenibacillus larvae* is necessary.

Harmonisation of the overall type traits by fleckvieh populations in Europe

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Breeding values and genetic parameters were estimated for overall type traits of udder and feet & legs by Czech fleckvieh cattle. Both overall traits were computed from single type traits in two ways: on the basis of current calculation of overall type traits and the second method was based on international harmonization of type traits by EVF. Each individual type trait used for construction of overall trait by the second method was rescaled from 1-9 scale to scale, which was optimized by cubic regression to the best development of trait having regard to longevity (Vicario 2009, Krogmeier 2010).

Heritability was estimated for individual and overall type by REML procedure. Breeding values were estimated for overall udder, feet and legs and for all type traits, which were used for overall type traits. The heritabilities of overall udder were 0.23 for the first method and 0.21 for the second method. Coefficient of heritability for overall feet and legs was 0.10, resp. 0.07. Correlation of breeding values between the first and the second method was 0.74 for udder, resp. 0.72 for feet and legs.

Heritabilities coefficients for feet and legs were similar to literature; overall udder heritability was in both cases lower. Correlations between both methods were relatively strong (0.74 overall udder, resp. 0.72 overall feet and legs).

Effect of candidate genes pigs 6th chromosome on qualitative traits

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In this study we focused to identify polymorphism *H-FABP*(*Hinf*I) and *RYR1* (*Hin*6I) gene and evaluate chosen qualitative traits. We used 55 young sows of hybrid combination Large White and Landrace for our experiment. Genotyping of pigs was performed by the PCR – RFLP method. Consequently we identified three genotypes *HH* (7), *Hh* (24), *hh* (24) of *H-FABP* and two genotypes of *RYR1*, genotype *NN* (45) and genotype *Nn* (10), we observed absence of genotype *nn*. In the population it was revealed *h* allele as the allele with a higher frequency (0.6546) in comparison with *H* allele (0.3454). Frequency of allele *N* of sows was 0.9090 and frequency of allele *n* was only 0.0910. In this group of sows the *HH* genotype expressed in the percentage of the lean meat (LM), where it reached the highest average amount - 56.96 % (*HH* > *hh* > *Hh*) and at the back fat's thickness with the lowest average amount - 15.38 mm (*HH* < *hh* < *Hh*). Dominant homozygote *NN* has been shown mainly in the percentage of LM where it reached the lowest average amount of 55.48 % and back fat's thickness where it caused the highest average amount of 17.09 mm. We recommend to use these results for comparison with other hybrid combinations.

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Genetic Evaluation of Daily Gains in Dual-Purpose Bulls

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Estimation of genetic preposition of animal with as high accuracy as possible and therewith the prediction of genetic potential, which will be devolved to the offspring, is reflected both in the sphere of animal breeding and economic aspects of animal husbandry.

The average daily gains of more than 7000 Czech Pied bulls from 7 rearing stations were analyzed using multi-trait animal model and random-regression models with the Legendre polynomials (LP) of the 2nd to the 4th degree. The effects of HYS-class (fixed), animal (random), permanent environment (random) and residuum were included in the models.

Rank correlations between the estimated breeding values of various animals group were calculated and a variety of top-lists were analyzed. In general, models with LP of the 3rd and 4th degree showed higher rank correlations with MTM in comparison to model with LP of the 2nd degree. Further, with regard to the number of common animals in the 1% and 10% top-lists, the models with higher polynomial degree are to be preferred for the estimation of breeding value.

Future research activity should be focused on the validation of methodology by evaluation of independent data sets which prolong the observed period to a higher age of the animals, and which test other types of functions.

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Polymorphism of the human Leptin gene

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The aim of our work was screening polymorphism of the leptin gene. Leptin is a product of obese (ob) gene expression that plays a role in energy metabolism and body weight. The human leptin gene is located in the 17 chromosome. The restriction site is located at the position 2549 bp (C→A). The present study included 12 sportsmen with a average age of 21 years. The average value of BMI was estimate on 23.15 and cholesterol reached 4.17 mmol/l. For amplification of specific fragments for LEP gene detection the following oligonucleotide primers forward 5´-TTTCCTGAATTTTCCCGTGAG-3´ and reverse 5´-AAAGCAAAGACAGGCATAAAAA-3´. The size of amplified PCR product is 242bp. Subsequently we will use the specific restriction enzyme *Cfo*I and expected length of fragments is 181+61 bp in the homozygote *CC*, 242+181+61 bp in the heterozygote *AC* and 242 bp in the homozygote *AA*. We assume that this mutation has connection with human obesity level.

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Criteria of selecting SNPs when estimating haplotype frequencies for Polish dairy cattle

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Background: The possibility of rapid genotyping of huge numbers of markers has become affordable for dairy cattle. When dealing with this large number of SNPs, association studies become very computationally demanding and time consuming. Each marker brings different quantity of information about considered trait. SNPs with large effects on trait of economic importance may be very useful for indentifying regions of the genome for further analysis.

The aim of this study was to performe, investigate and compare few methods of SNPs selection. Different subsets of markers are needed depending on the type of analysis. Statistically independent SNPs are required in case of considering each marker's effect separately. The reason of that is necessity of avoidance of duplications of the same information source in the statistical model. When haplotyping, in accordance with haplotype definition, the subset of rather strongly dependent SNPs has to be used.

Material and Methods: The data set of biallelic genotypes was used. Different SNP selection criteria can be found in the literature. The most popular and commonly used are MAF (minor allel frequency) and LD (linkage disequilibrium). MAF is defined as a frequency of less frequent allel of the marker. LD is a measure of the dependence of pairs of markers. Some ratios for each considered chromosome were also computed to investigate effects of genotypes and an entropy-based method for selecting SNPs involved.

Results and Conclusions: The study showed that the higher LD between markers, the more similar MAF of these markers. Using haplotypes instead of single SNPs leads to avoidance of high dimensions of matrices in the statistical model and enables not to waste LD information.

Inbreeding calculation in the european breeding aardvark *Orycterous afer* (pallas, 1766)

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Aardvark *Orycteropus afer* are endemic species with separated fylogenetic evolution to Africa south of the Sahara. The Aardvark was considered to be difficult to maintain in captivity with respect to monofagy and high demands. The main problem in captivity is small number of reproducible individuals with the result that narrow option to maintenance of genetic variability. The four individual lines arose in European Zoos during last century. These lines blend together within a period of 40 years. The objective of this work was analysis of European Aardvark *Orycteropus afer* breeding (GenoPro 2009) and calculation of inbreeding coefficient (SAS/STAT Inbreed Procedure). The data were used from European Studbook for the Aardvark *Orycteropus afer*. The first study Inbreeding Coefficients of Individuals was focused on F_x for 162 animals registered since 1910 in European Studbook. In all population were only 3 individuals with F_x 0.250, rest of population (162 individuals) had F_x 0.000. The second study Inbreeding Coefficients of Mating was focused on F_x for 140 possible offspring of living Aardvark (10 males and 14 females). 24 parents combinations have the highest F_x (0.250), 91 parents combinations have the variable F_x (0.0313; 0.0625; 0.0781; 0.1250; 0.1563; 0.1875), 49 parents combinations have the F_x 0.000. It is necessity to have regard for these results because lot of consanguinity (incl. twins), are used in European Aardvark breeding.

Use of the *FecB* (Booroola) gene for establishment of prolific line of Merinolandschaf sheep

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The aim of the study was to detect possible presence of *FecB* mutation in Merinolandschaf sheep population in the Czech Republic and its linkage with prolificacy, lamb loses and growth intensity. DNA samples (blood or saliva) were collected from animals with high breeding values for litter size or with Booroola rams in their pedigree. The presence of *FecB* (Booroola) high prolificacy major gene in the Merinolandschaf population was confirmed by the genotypization on the *BMPR-1R* locus. Totally 65 heterozygous and 5 homozygous carriers of the $FecB^B$ allele have been found until now in Merinolandschaf population. The Booroola major gene carriers are closely related to each other. Mean litter size of the $FecB^B$/- ewes was 2.46 lambs/lambing, while the population mean for ML sheep breed in the CR was 1.40 lambs/lambing. Mortality of lambs was slightly higher in the progeny of $FecB^B$ carriers (18.6 %) compared to non-carriers (15.1 %). Average live weight of lambs at the age of 100 days was higher in non-carriers group (33.9 kg) than in group of lambs with $FecB^B$ allele. Animals with majorgene *FecB* are used for breeding of prolific line of Merinolandschaf sheep.

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Genetic variability of Shagya – Arabian horse derived from pedigree information

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The aim of the paper was to analyze genetic diversity of Shagya - Arabian horse population based on description of probability of identity by gene descent and origin. Reference population created 107 living Shagya-Arabian horses (15 stallions, 92 mares) registered in Shagya-Arabian studbook Slovakia from 2002 to 2007, exploited in provincial breeding. The pedigree data contained information of 1952 animals. The pedigree completeness of reference population was evaluated for each animal by the number of fully traced generations (5.59), maximum number of generations traced (34.18) and equivalent complete generations 9.54. The effective number of founders was 167 and the effective number of ancestors 21. Only 8 ancestors were necessary to explain 50 % of total genetic variability. The average values of inbreeding as well as relatedness in reference population were 4.08 %, respectively 3.03 % and the average increase of individual inbreeding intensity was 0.47 %.

Detection of *Salmonella* spp., *Salmonella enterica* ser. *typhimurium* and *enteritidis* by step one real time PCR in ready-to-eat food

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The aim of this study was follow the contamination of live chickens with *Salmonella* spp. by using the Step One real-time PCR. Detection of food-borne pathogens using conventional culture techniques takes up to 5 days to get a result. This includes primary and secondary enrichment and serological confirmation of colonies grown on agar plates. We used the PrepSEQ Rapid Spin Sample Preparation Kit for isolation of DNA and MicroSEQ® *Salmonella* spp. Detection Kit for pursuance with the real time PCR (Applied Biosystems). The samples were obtained by taking swabs from body of live chickens. In the investigated samples before incubation we could detect strain of *Salmonella* spp. in six out of eight samples, as well as internal positive control (IPC), which was positive in all samples. In the samples after 16 hours incubation we could detect strain of Salmonella spp. in samples, as well as internal positive control (IPC), which was positive in all samples. This Step One real-time PCR assay is extremely useful for any laboratory in possession of a real time PCR. It is a fast, reproducible, simple, specific and sensitive way to detect nucleic acids, which could be used in clinical diagnostic tests in the future.

Procedures of evaluation of genomic breeding value

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The evaluation of an animal is based on highly reliable production records. It is usually in two steps. In the first step the BLUP procedures are used for adjustment of non-genetic systematic effects. In the second step the pseudo-data, like daughter-yield-deviations are used as inputs. Evaluation is by BLUP-AM with random effects of remaining polygenes, with relationship matrix between animals (A), and effects of particular markers. Genomic breeding value (GEBV) is then selection index with combination of polygenic and marker's parts. Including large number of SNP markers (50K) increase the reliability of evaluation of young animals of dairy cattle from 0.30 if only the pedigree value is used to 0.60 when the genomic breeding value is applied. Information about genetic markers can be gathered in realised genomic relationship matrix (G). Similar result of GEBV is then achieved by using in second step only effect of animal, without genetic markers, and substituting G for A. In real population has two step procedure disadvantages, because it is difficult compare genotyped and ungenotyped animals, and second step needs input parameters known without error. Therefore one-step procedure is used, which evaluates both genotyped and ungenotyped animals at the same run, and produces one common ranking of all animals in a whole population. Principal part of one-step procedure is augmented pedigree-genomic relationship matrix. This procedure allows working with usually used models of animal evaluation on national scale.

SSCP detection of rabbit candidate gene

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The aim of this work was to optimise SSCP method to analyse polymorphism in rabbit *MSTN* gene, which is candidate gene responsible for double muscling of different animals (cattle, sheep, poultry). In 2008 was sequenced rabbit *MSTN* gene and there was find one SNP, which seems to be regulation factor of rabbit muscle growth (intron 2, possition 34, C→T). For genotypization was used PCR–RFLP. We optimised silver – stained SSCP method for mutation identification, which is cheaper in a compared to PCR-RFLP.

For optimization was used 12%, 14%, and 16% nondenaturing polyacrylamide gel and running time 5, 7, 9 hours. Because target sequence of *MSTN* gene was only 80bp long, the best concentration of the gel was 14%, running time was 7 hours at 20ºC temperature. It was necessary to use also 5% glycerol to adjust pH. With this method we detected all of three genotypes (*CC*, *CT* and *TT*). Gel was stained with silver and there was needed to change time of staining and fixing solution treatment from 3 minutes as is ussual for this method, to only a few seconds (less than 45 seconds). Results of this detection were verified with PCR – RFLP analyse.

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Genetic parameters between reproductive traits in mink stock

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The aim of the study was to evaluate genetic parameters between reproductive traits in minks. Coefficients of heritability and repeatability for litter size and the number of reared young minks did not exceed 0.1. Thus, it could be concluded that there was a minimum effect of genotype on the fluctuation of the number of born and reared young minks. The next analysed trait that was connected with reproduction, namely the pregnancy duration was also characterized by low values of coefficients of heritability and repeatability, which amounted 0.17 and 0.18, respectively.

Positive correlations, both genetic and phenotypic, between the number of reared young minks and all analysed reproductive traits were found. The largest phenotypic correlations (0.91) between litter size and the number of reared young minks were proved. Positive and large phenotypic correlations between the number of reared young minks and pregnancy duration were also stated and it amounted to 0.63. The negative phenotypic correlations, however, between the number of born minks and pregnancy duration (-0.17) were determined. The genetic correlations between the traits were also negative and amounted to -0.73. The largest genetic correlations between the number of born and reared young minks were found. Genetic correlations between the traits amounted to 0.68. Also positive, but relatively low genetic correlations (0.08) between the number of reared young minks and pregnancy duration were proved. Low values of heritability and repeatability correlations between analysed reproductive traits indicated larger effect of environmental factors on the traits.

Analysis of genetic parameters for conformation characters in mink population

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Coefficients of heritability for animal size and fur quality in minks (colour purity, colour type, fur quality and total estimation) as well as genetic and phentotypic correlations bewteen the characters were determined in the paper. The analysis was carried out on two mink stocks in 8-year period (approximately 2000 animals). The largest coefficients of heritability (0.33) for colour purity of mink fur were found, whereas mink size was characterized by lower coefficients, which amounted to 0.23. The coefficients of heritability for total estimation were similar to that for mink size and it amounted to 0.19, while heritability for fur quality was 0.13. The lowest coefficients of heritability (0.11) for mink body weight were proved.

Phenotypic correlations between total sum of points and animal size, colour purity, fur quality amounted to 0.51, 0.54 and 0.70, respectively. Similar genetic correlations between the characters were found and they amounted to 0.55, 0.60 and 0.49, respectively. Relatively low phenotypic correlations, which amounted to 0.18, between body weight and total sum of points were stated. Genotypic correlations, however, between the characters were considerably higher (0.51). Low and negative genetic correlations between colour purity and fur quality as well as between colour purity and mink body weight were proved and they amounted to -0.07, -0.01 and -0.11, respectively. Moreover, negative and very low correlations between animal size and fur quality were also found. The phenotypic correlations for the characters amounted to -0.01 and the genetic correlations -0.06. The genetic correlations between body weight and its size in points (0.74) were the highest of all, whereas the phenotypic correlations between the characters amounted to 0.21.

Genetic and phenotypic trends in reproductive traits and conformation in mink population

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Phenotypic and genetic trends for reproductive traits and conformation characters in mink population in 9-year period were estimated. The trends were assumed to be changes in time unit, namely in one year. The trend line for the number of born young minks had a decreasing tendency, whereas for the number of reared minks had an increasing tendency. Contrary to phenotypic trends, which were characterized by relatively straight lines, genetic trends for reproductive traits and conformation characters had a step course. Despite the fluctuations of breeding value for conformation characters in minks, lines of genetic trends for analysed traits (animal size, colour purity, colour type, fur quality, total sum of points) had an increasing tendency. The most favourable changes for the total estimation were found, which could evidence good quality of breeding material in general with regard to the conformation characters in mniks (animal size and fur quality). Lines of genetic trends for reproductive traits also showed an increasing tendency. Particularly favourable changes refered to the number of born young minks. In the beginning negative breeding values of the trait was found, however in the last year of the studies positive values were proved. Compared to the breeding values for litter size, slighter differences for the number of reared young minks between the first and the last year of the study were stated. To sum up, it could be admitted that the analysed animals were good breeding material and selection in mink prolificacy and in their conformation characters were conducted in the proper direction.

Model for estimating breeding values of milking speed

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Pro odhad PH dojitelnosti byl navrhnut nový jednoznakový animal model nahrazující CC-test, který je již dnes překonanou metodou výpočtu. Dojitelnost se v ČR posuzuje jako průměrný minutový výdojek. Dle metodiky se v ČR měří dojitelnost pouze na první laktaci jedenkrát za život krávy, to vede k tomu, že použitá dědivost je pouze 0,19. Data obsahovala 72.106 měření pro plemeno C, 69.522 pro plemeno H. Data byla upravována programem SAS 9.1 a genetické parametry odhadnuté programem AIREMLF90. Takto bylo testováno celkem devět modelů. Výsledné model byl testován programem BLUPF90. Výsledky byly opět vyhodnoceny v SAS 9.1.

Nejdůležitějším efektem bylo stádo, rok, období. Jako další efekty v modelu byly zahrnuty: efekt věku při prvním otelení lineární regresí, den laktace, jako kvadratická regrese a nádoj v době zkoušky dojitelnosti také jako kvadratická regrese.

Modely byly testovány i při použití genetických skupin neznámých rodičů. Skupiny byly sestaveny dle země původu zvířete, ročníku narození, podílu krve plemene a plemenného využití. Genetické skupiny zlepšily variační rozpětí PH, a snížily reziduální rozptyl. Byla shrnuta doporučení pro úpravu kontroly dojitelnosti. Plemenné hodnoty se doporučuje publikovat jen pro býky.

Divergent selection for shape of the growth curve and technological quality of eggs

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Our previous study revealed that the HG and LG lines of Japanese quail divergently selected for shape of the growth curve more than 30 generations significantly differed in production, size and composition of eggs. The aim of present study was to verify whether these inter-line differences were also associated with differences in the technological quality of eggs, that is in the egg shape index, albumen index, Haugh units, yolk index, colour of yolk and strength, thickness or deformability of the shell. Similarly as in the previous study, the analysed eggs came from 55 quail of each line that were the progeny of generation 32 obtained from a single hatch. The husbandry of quail was similar to that provided during selection. Technological quality of eggs was estimated during the first 5 months of lay (73-100 eggs/month/line). The results showed that a lower production of egg mass (lower production and size of eggs) noted in HG versus LG quail was not accompanied by a lower technological quality of eggs. In most of measured traits (egg shape index, albumen index, yolk index, colour of yolk and strength, thickness as well as deformability of shell) the eggs of both lines were essentially the same. In addition, significant inter-line differences in favour of the HG line were revealed in Haugh units.

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Selection indexes in cattle breeding

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Background: Selection index is used to evaluate the traits of animals important from breeding aspects in a complex method. Animals for breeding are for all time selected on a multi-trait basis. While sometimes the breeder's intent is to change one trait only, changes in the complex of traits occur because the traits are mutually conditioned.

Results: Several types of indexes exist for dairy cattle:

1. Indexes for selection of parents of successive generation
2. Indexes for selection of the economically most fitting individuals (during their lifetime)
3. Partial indexes for groups of evaluated traits

Known values of performance of traits after adjustment for distorting effects of farm environment, i.e. breeding values, are a pre-condition for the evaluation of animals. The foundations of the construction of selection indexes in animal breeding laid Hazel (1943). Cunningham (1969, 1975) elaborated detailed methods for the construction of indexes to calculate relative weights of traits. An overview of the methods of index construction with constraints was presented by Brascamp (1984). Population-genetic parameters and economic weights of traits are basic input parameters for the construction of indexes.

Conclusions: This paper presents methodology and development of construction of the selection indexes in cattle breeding. The constructed indexes are substantially influenced by population genetic and economic parameters.

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Genetic parameters for SEUROP carcass traits in Czech beef cattle

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The aim of this study was to estimate genetic parameters for SEUROP carcass traits in beef cattle in Czech Republic by using multiple trait animal linear and linear-threshold model, where carcass weight (CW) was taken as linear and carcass conformation class (CC) a carcass fatness class (CF) as threshold traits. Field data on 4276 animals, which were slaughtered between the ages of 252 and 730 days, were analyses. Effects of HYS (herd, year, season, abbatoir), breed, sex, classificator, linear regression of the heterosis coefficient and Legendre polynomial regression of the age at slaughter were statistically significant. The effect of HYS was considered as random. In addition we used fixed regresion on CW in model for estimation of genetic parameters for CC and CF by using two trait model. REMLF90 and THRGIBBS1F90 programmes were used to estimate genetic parameters.

The estimated heritability of CW was 0.295 from linear model and 0.306 from linear-threshold model, for CC 0.187 and 0.237 and for CF 0.089 and 0.146. The estimated heritabilities were higher from linear-threshold model than from linear model. There was high genetic correlation between CW and CC (0.829 and 0.959), medial genetic correlation between CW and CF (0.332 and 0.328) and very low genetic correlation between CC and CF (0.071 and 0.053).

There was negative correlation between CC and CF (-0.430) and markedly lower heritability for CC (0.077) when carcass weight was used as fixed effect in model.

The evaluation of exterior of dairy cattle

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Genetic evaluation is central point in the course of breeding and improving yield of animals. The most important parameter of genetic evaluation of cattle is estimation of breeding value, which has complex character. The estimate of breeding value is generally made on a base of more use properties.

The most used methods for estimate of breeding value are methods known as BLUP – animal models. The principle of these methods is best linear unbiased prediction.

In the Czech Republic nowadays is currently estimated breeding value of production (primary) traits. The secondary traits, including for example exterior, aren't estimated in the Czech Republic either ever or badly – based on the old and unsatisfactory models. It´s important to improve the models for better estimate of these traits.

Assessment of inbreeding depression for trait of linear type description in Czech draft horse

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Genetic parameters, breeding values and inbreeding depression for 22 traits of linear type description and of 4 body measurements were evaluated in 1,744 horses of three original Czech draft breeds in a period of 18 years (1990–2007). The model included fixed effects: sex, year of description, breed, classifier and with random effect: animal. Heritability coefficients for the particular traits were in the range of 0.11–0.55 and genetic correlations ranged from -0.63 to 0.97. Inbreeding depression, expressed as coefficients of regression on one percent of inbreeding, was in the range from -0.0992 to 0.0242 points in the particular traits. The inclusion of inbreeding depression in the model resulted in a moderate change in h2 in a third of traits. The value of rG increased or decreased in two-thirds of traits by 0.01. Standard deviations of breeding values of linear description traits were in the range of 0.30–0.72 and 0.62–6.18 in the traits of body measurements. The values of Spearman's rank correlation coefficient among breeding values estimated by a model without inbreeding depression and by a model with inbreeding depression in the particular traits were 0.916 – 0.999, 0.710 – 0.992 and 0.827 – 0.998 for the sample of all horses and for subgroups of 10% of the best and 10% of the worst horses, respectively. If the average value of inbreeding coefficient is low (0.03), it is not necessary to include the influence of inbreeding depression in the genetic evaluation of individuals of original Czech draft horses.

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Comparison of health during test by two hybrids lines broiler rabbit HYLA

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Aim of this analysis was health during test by two hybrids lines broiler rabbits HYLA (REG 8000 × 3070 and REG 3160 × 3070) (n=130). Rabbits from the multiple litters of one commercial farm were weaned at the age of 34 – 35 days. After adaptation period from 35 to 42 days of age were rabbits fattened during the interval from 42 to 84 days of age. Effect of hybrid lines on the health was analyzed using linear model, applying logistic link function. The value 0 was assigned for rabbits dead during the test or for rabbits with diarrhea. The value 1 was assigned for other animals. During the test was fed a complete granulated fattening mixture which contained herbs anticoccidica EMANOX. During the test were found significant differences between the two genotypes. For animals of the hybrids lines REG 8000 × 3070 was estimated 98% probability for the mean survival to the end of the test. For animals of the hybrids lines REG 3160 × 3070 was estimated 78% probability for the mean survival to the end of the test. The estimation of the chances showed that hybrids lines REG 3160 × 3070 in comparison with the hybrids lines REG 8000 × 3070 had above 0.161 times lower chance for survival to the end of the test. No difference between hybrids lines was found for the incidence of the diarrhea. The results demonstrated that the hybrids lines REG 8000 × 3070 had better reaction to herbs anticoccidica EMANOX.

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The development of microsatellite panel for genetic resource nutria (*Myocastor coypus*) in the Czech Republic

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The objective of this work was to develop microsatellite panel for nutria, genetic resource in the Czech Republic. The nutria or coypu (*Myocastor coypus*), the only member of the family *Myocastoridae*, is a large semi-aquatic herbivore rodent native to South America that has been introduced worldwide since the early 1900´s with the intent of fur farming. In contrast of other farm animal species breeding of coypu has not a long tradition – domestication of animals started at the beginning of 20th century in their original habitat and the first import of nutrias in Czechoslovakia has been realised in 1925. Nutria in the Czech Republic falls into genetic resources (by the law no.130/2006) which are protected by National Program Preservation and Utilisation of Genetic Resources of Farm Animals which includes all 3 colour forms of nutria (Standard, Moravian silver, Prestice multicolour). Biological samples (hairs or blood) of 15 unrelated animals (N = 5 for each colour form) were collected, used for DNA isolation and 11 microsatellite markers analysis by multiplex PCR and by multicolored capillary electrophoresis on ABI PRISM 310 (Applied Biosystems). This panel of 11 microsatellite markers will be usable as a tool for species characterisation of nutria in the Czech Republic, for genetic diversity revealing, determination of inbreeding level and also for monitoring of changes in this small population.

Association analysis of *SERPINE1* gene in different pig breeds

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The *SERPINE1* gene encodes endothelial plasminogen activator inhibitor-1 (PAI1). We detected SNPs FN396538:g.566G>A in intron 3 and FN396538:g.154A>G in exon 3 causing amino acid substitution Ile159Val. The polymorphism 566G>A was detected with *Mbi*I PCR-RFLP and SNP 154A>G with allele-specific PCR assay. The SERPINE1 gene was mapped to the proximal end of SSC3. The SNPs were genotyped on 565 animals of 12th–15th generation of the Meishan × Large White (M×LW) cross (PIC, Hendersonville, USA) with records for weight (test end), life daily gain, testtime daily gain, loin depth, backfat depth and on 333 animals of Wild Boar × Meishan (W×M) population with 46 trait records (growth, fattening, fat, muscling, meat quality, stress resistance and body conformation). Frequencies of alleles 566G and 154A were 0.59 and 0.54, respectively, in the M×LW population. In the W×M population frequencies of alleles 566G and 154A were 0.49 and 0.49, respectively. In the whole population M×LW no associations between traits and the SNPs were revealed. However association analysis done within sexes showed that the SNPs 566G>A and 154A>G were associated with loin depth (P = 0.01) only for females (N = 239). The alleles 566G and 154A were associated with higher loin depth. The reason of results differences between males and females was not obvious. In the W×M population, where a large extent of linkage disequilibrium is expected, the loci are associated with many traits; the most significant of them are connected with growth. Supported by the Czech Science Foundation (523/07/0353).

Session 3 - Plant molecular genetics, genomics and proteomics

Opposing regulation of hypocotyl elongation by cytokinins at low intensity of white and red light

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Cytokinins (CKs) were reported not to affect hypocotyl elongation in *Arabidopsis* seedlings grown in the white 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 μmol m^{-2} s^{-1}), a pronounced stimulation of hypocotyl elongation by CKs was found when the seedlings were cultivated at a decreased white light intensity (20 μmol m^{-2} 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 cytokinin receptors AHK2 and AHK3. This CK action was independent of ethylene signaling and partially inhibited by IAA. In contrary, inhibition of hypocotyl elongation by CKs was observed at a decreased red light intensity (20 μmol m^{-2} s^{-1}). Mutant analysis revealed that this effect is mediated by AHK3. Supported by grant Nos. LC06034, 1M06030, AV0Z50040507 and AV0Z50040702.

Expression of ELIPs at different light conditions in *Arabidopsis thaliana* with elevated cytokinin levels

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Cytokinins (CKs) are plant hormones that play important roles during plant growth and development. In particular, they influence chloroplast development, nutrient mobilization, senescence and the cell cycle. Light quality and intensity are important factors that affect a range of plant processes. Early light induced proteins (ELIPs) belong to the superfamily of Chlorophyll a-binding proteins (CABs). They are expressed and accumulated during early phase of deetiolation and stress conditions. It is assumed that ELIPs play a photoprotective function under high light intensity. Some effects of CKs and light are identical. This fact led us to set up our experiments, whose aim is to identify changes at ELIP protein level in plants with elevated CK levels grown under different light intensities (20, 100 and 330 μmol m^{-2} s^{-1}). In our work we revealed positive effect of CKs on ELIP accumulation in light dependent manner. ELIP accumulation was elevated in CK treated plants under all three light conditions. We suppose, CKs and light action is additive.

Prospects and limits of senescence-induced *ipt* gene expression for enhancement of plant productivity

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Cytokinins in cooperation with some other plant hormones, namely auxin, control number of developmental processes which are essential for plant productivity involving shoot branching, uptake of nutrients, plastid development, suppression of leaf senescence and seed (grain) formation. Auto-regulative expression of cytokinin biosynthetic *ipt* gene under control of senescence-induced SAG12 promoter has appeared as promising tool for delay of leaf senescence and extension of period of active photosynthesis favorable for production of assimilates. We shall report that actual efficiency of senescence-induced formation of cytokinins differs in different crop plants depending on their developmental strategy and cropping plant parts. It is also influenced by different ability of different plant species to maintain cytokinin homeostasis by metabolic degradation and inactivation of accumulated cytokinins.

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Transgenic Pea Lines With *gm-SPI-2* Gene

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Construct EHA105:pWell11 bearing an inhibitor of proteases *gmSPI-2* (*Galleria mellonela* Silk Protein Inhibitor) with antibacterial and antifungal activities was utilised for genetic transformation of several field pea cultivars. The aim of the research work was development of transgenic pea lines with enhanced resistance to fungal pathogens and insect pests.

Plantlets regenerated after transformation procedure were transferred *ex vitro* and grown to seeds. Pea lines positively tested for reporter genes were multiplied and searched in a glasshouse test for resistance to insects. In 2010 three different tests aimed at three different insect pests were made. In the first trial the levels of resistance of transgenic and non-transgenic plants to adults and larvae of pea leaf weevils (*Sitona lineatus* L.) were compared. The levels of leaf and stipule damage caused by adults were not different on transgenic and non-transgenic plants. But the levels of damage of *rhizobium* nodes caused by larvae located on the main radix were significantly lower in transgenic compared to non-transgenic plants. The second trial was aimed at testing plant susceptibility to thrips *Kakothrips pisivorus*. Significant differences between the transgenic and non-transgenic plants in relation to the pest were not proved. The third trial comparing mortality of eggs and larvae of pea weevils (*Bruchus pisorum* L.) on transgenic and non-transgenic plants is in progress at the time of writing this abstract.

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Agrobacterium-mediated transformation of linseed (*Linum usitatissimum* L.) with *αMT*, *CP* and *SPI2* genes proved by molecular methods

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Flax/linseed is an important multipurpose crop for food and industrial utilization. The commercial value of this crop can be still improved through the insertion of genes of interest (GOI) by genetic engineering approaches. Here we present the *Agrobacterium*-mediated transformation of linseed with three GOI, namely (1) metal-binding mammalian metallothionein α-MT, (2) short synthetic metal-binding peptide CP, and (3) proteinase inhibitor from silk of waxmoth (*Galleria mellonella* L.). First two GOI (α-MT, *CP*) were translationally fused to *uidA* reporter gene, the third one (*gmspi2*) was fused to the sequence for GFP (green fluorescent protein); all vectors contained *nptII* selectable marker gene (kanamycin resistance) under the control of 35S CaMV promoter.

Hypocotyl segments of linseed lines AGT-917 and AGT-0583 were transformed with three GOI. The presence of *αMT*, *CP* and *gmspi2* transgenes was confirmed by PCR and Southern blot in kanamycin-selected putative T_0 transformants and their T_1 and T_2 seed progenies. Southern blot analyses have shown that GM lines contained usually 1 to 2 copies of transgenes. GM lines with *αMT* transgene were multiplied in the field conditions (T_1, T_2). GM lines with stably inherited transgenes for respective GOI will be tested for Cd tolerance/ accumulation (*αMT*, *CP*), and for resistance to linseed fungal pathogens and insect pests in the greenhouse and field conditions.

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Designing recombinant maize β-glucosidase Zm-p60.1: Novel enzymes for modulating cytokinin metabolism

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Maize β-glucosidase Zm-p60.1 is one of many enzymes which are important for plant development. It liberates free zeatin from its transport or/and storage from zeatin-*O*-β-glucoside. Using an adapted site specific non-saturated random mutagenesis approach we prepared five multi-site mutants in and around the active site (W373K/P372T/M376L, W373K/P372S/M376L, W373K/M376L, W373K/P372T and W373K/P372S) derived from the single mutant W373K to study the effect(s) of amino-acid changes on substrate specificity towards artificial (4-nitrophenyl-*O*-β-D-glucopyranoside and 4-methylumbelliferyl *O*-β-D-glucopyranoside) and natural (*trans*-zeatin-*O*-β-D-glucopyranoside, *cis*-zeatin-*O*-β-D-glucopyranoside, *trans*-zeatin-*N7*-β-D-glucopyranoside and *trans*-zeatin-*N9*-β-D-glucopyranoside) substrates. Kinetic and substrate specificity studies confirmed large differences among set of enzymes. All enzymes surprisingly preferred *cis*-zeatin-*O*-β-D-glucopyranoside over *trans*-zeatin-*O*-β-D-glucopyranoside, whereas differences in hydrolytic efficiencies are considerable. Quantitative thin layer chromatography confirmed the best hydrolysis (9.1-fold) towards *cis*-zeatin-*O*-β-D-glucopyranoside in the triple mutant W373K/P372T/M376L. Moreover, it was also confirmed that only wild-type hydrolyzed *trans*-zeatin-*N9*-β-D-glucopyranoside. No known plant β-glucosidase hydrolyze this substrate. Hydrolysis of *trans*-zeatin-*N7*-β-D-glucopyranoside was not observed at all.

Intensity of white light modulates phosphoproteome dynamics in response to cytokinin treatment in *Arabidopsis*

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In plants, cytokinins (CKs) have been implicated in many developmental processes and responses to environmental cues. Light and 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 quantity of white light at the phosphoproteomic level, we employed phosphoproteome isolation followed by 2-DE and image analysis to compare changes in phosphoproteome dynamics in *Arabidopsis thaliana* seedlings in response to CK treatment at standard (90 μmol m^{-2} s^{-1}) and decreased (20 μmol m^{-2} s^{-1}) white light intensities. We followed phosphoproteomic changes in course of 2, 6 and 15 minutes intervals of CK treatment. 34 differentially expressed phosphoprotein spots (representing about 8 % of detected spots) were found. Out of the 34 phosphoprotein spots, 5 were regulated in a comparable fashion at both light intensities, while opposing regulation was found for 15 phosphoprotein spots at the two light intensities. 3 phosphoprotein spots were differentially regulated at only standard or decreased light intensity, respectively.

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Identification of plant proteins by the combination of electromigration techniques and MS

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A protein study by MS demands proper purification and separation steps prior to MS analysis. The method of Divergent Flow Isoelectric Focusing (DF IEF) has shown an improvement of the sample preparation in proteomic studies. Proteins from rough extracts are concentrated during IEF according to their pI values into the collected fractions while salts and impurities are removed. DF IEF was carried out in a separation channel of polyester non-woven web. Both device design and composition of carrier-buffers were newly redesigned regarding to higher separation resolution needed for the study of post-translational modifications. The number of collected fractions from the channel was increased to twenty. DC voltage (800 V) was brought to two pairs of electrodes situated on the channel sides. The pI values of proteins in fractions were monitored visually by colored pI markers. Separated fractions were collected in twenty micro vials and further analyzed. The further analysis involved SDS page followed by MS analysis of peptides and MS analysis of intact proteins. As an experimental source of plant proteins were selected leaves of young plants of *Arabidopsis thaliana*, and barley grain and malt (*Hordeum vulgare* L.).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. The increase of glycated protein modifications during technological process of brewing was confirmed by MS. This work was supported by the Ministry of Education, Youth and Sports, Czech Republic (No. 1M06030 and No. 1M0570), by the Grant Agency of the Academy of Sciences of the Czech Republic, (No. IAAX00310701 and IAA600040701), and from the Institutional Research Plan AV0Z40310501.

Expression of photosynthesis- and pathogenesis-related genes in tobacco with activable endogenous production of cytokinins

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Cytokinins are plant hormones implicated in a number of growth, developmental and physiological processes in plants. Further, we suppose that cytokinins are implicated in oxidative stress. We employed ipt activable system to increase endogenous cytokinin level in tobacco. The activation and subsequent elevation of cytokinin level lead to occurrence of necrotic lessions and other marks of oxidative stress. Two independent lines with different degree of severity in the phenotype were compared. Using real-time RT-PCR we investigated *ipt* transcript level, transcript level of some genes implicated in photosynthesis (*CAB*, *FNR*, *VDE*) and pathogene response (*PR-1b*, *PR-Q*). Real-time RT-PCR analysis showed strong down-regulation of *CAB*, *FNR* and *VDE* and up-regulation of genes included in pathogen response in activated plants. In photosynthesis-related genes the decline of transcript level showed the same pattern in older expanded as well in young leaves in plants with increased endogenous cytokinins. A difference between old and young leaves emerged in pathogenesis-related genes – while PR-1 was strongly induced in both, old as well as young leaves, PR-Q was induced only in old leaves. Comparing the both lines, the degree of up/down-regulation of monitored genes was in accordance with the degree of the phenotype severity. To find a new tool for studying of cytokinins in tobacco we further examined some potential transcriptional markers for cytokinins. No potential transcriptional marker of cytokinins showed useful properties. Taken together, the damage emerging after activation of cytokinin biosynthesis gene *ipt* in tobacco is accompanied by dramatic changes in gene expression suggesting changes in photosynthesis and induction of pathogen response.

Cytokinins induce HR-like cell death

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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 effects of *ipt* expression in tobacco in detail. We show that hypersensitive response like cell death in expanded tobacco leaves proceeds shortly after *ipt* induction – the first lesions accompanied with huge wilting being observed in app. 60 hours after induction of *ipt* expression, and lesions can spread over the entire leaf area within 4 days after induction. Formation of visible lesions was preceded by increase in reactive oxygen species and membrane damage. Further, we demonstrate that lesion formation is a light-dependent process as it is prevented by shading. The level of key redox regulator glutathione was also diminished after *ipt* expression. Furthermore we show that expression of *ipt* is followed by stomata closure but resulting decrease in pore conductivity couldn't fully explain extent of damage to leaves as revealed by application of lanolin on surface of the leaves.

Comparative analysis of the two contrasting flax cultivars upon cadmium exposure

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Phytoremediation represents an effective low-cost approach for removing pollutants from contaminated soil and water. Cadmium (Cd) is a wide-spread and serious pollutant due to its high toxicity and carcinogenicity. Flax (*Linum usitatissimum* L.) is a crop with a high phytoremediation potential. However, significant differences in Cd tolerance were previously detected among commercial flax cultivars. Notably, cv. Jitka showed substantially higher tolerance to Cd in soil and in suspension cultures than cv. Tábor. In our study, significant differences were revealed in physiological and biochemical parameters between Cd-treated plantlets of cv. Jitka and cv. Tábor. Higher levels of glutathione were detected in cv. Jitka than in cv. Tábor. In contrast to cv. Tábor, no significant increase in malondialdehyde level was observed in roots of cv. Jitka exposed to the highest Cd concentration. Comparative proteomic analysis was carried out to detect changes in protein expression in these two contrasting flax cultivars. Differentially expressed proteins were identified by two-dimensional gel electrophoresis followed by mass spectrometry. Analysis of the root proteome revealed three proteins selectively up-regulated in cv. Jitka. In contrast, no significant differences in response to Cd were found in the shoot proteome. 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 necessary for phytoremediation.

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Effect of decreased light intensity on Arabidopsis responsiveness to increased levels of endogenous cytokinins – 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 quantity of white light at the proteomic level, we used 2-DE followed by image analysis and MALDI-TOF/TOF MS to compare changes in steady-state protein levels in Arabidopsis thaliana seedlings with increased content of endogenous CKs cultivated at standard (90 μmol m^{-2} s^{-1}) and decreased (20 μmol m^{-2} s^{-1}) white light intensities. After activation of the CK-biosynthetic gene ipt, we followed proteomic changes during subsequent 5 days of cultivation. 61 differentially expressed protein spots (representing about 12% of detected spots) were found. Out of the 61 protein spots, 36 were regulated in a comparable fashion at both light intensities, and 2 and 23 were differentially regulated at only standard or decreased light intensity, respectively. Till now 56 proteins have been identified, and can be classified as proteins involved in seed germination, photosynthesis, carbon and nitrogen metabolism and metabolism of xenobiotics.

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Constructs for induction of virus resistance in pea

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Viral diseases are one of the serious problems that can cause great economical loss during pea cultivation. The most frequently detected viruses in the Czech Republic in samples collected from inspected pea fields were Pea enation mosaic virus (PEMV) and Pea seed-borne mosaic virus (PSbMV). Here we present results of testing of an alternative approach – the use of genetic modification to achieve the induction of resistance to viral diseases by inverted repeats post-transcriptional gene silencing (IR-PTGS). We are using the vector system pHannibal for preparation of hairpin (hp)RNA. For both PEMV and PSbMV we cloned sense and antisense CP cDNA between the 35S promoter and CAMV terminator of pHannibal that should result in hairpin RNA formation. In a next step this 35S sense/antisense cassette is inserted into the T-DNA of pGREEN vector system that already includes reporter (*uidA*) and selection (*bar*) genes. The final step of cloning is the transformation of *Agrobacterium tumefaciens* strains with the binary plasmid (our construct and pSoup) via electroporation. Further we are using tobacco leaf disc transformation for testing of the function of our constructs. The aim of this work is to prepare transgenic pea plants resistant to PEMV and PSbMV.

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Testing of modified plants bearing a protease inhibitor gene *spi-2* to antimicrobial activity

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Plant pathogens cause yearly enormous losses in quality and yield of cultivated crops. Insertion of genes encoding proteins with antimicrobial activity into genome of a number of cultivated plant species represents a promising strategy for boasting genetic disease resistance against a broad range of plant pathogens. In this study, the *spi-2* gene (silk proteinase inhibitor 2) isolated from Wax Moth, *Galleria mellonella* was introduced into tobacco plants cv. Petit Havana, in order to regenerate transgenic plants, resistant to bacterial and fungal diseases. The *spi-2* gene sequence was fused with a modified *m-gfp5* signal gene of pacific jellyfish *Aequorea victoria*. Transformation was performed using a binary *Agrobacterium tumefaciens* based vector carrying plasmid pPRD400-fusion15 construct (O. Navrátil, Institute of Experimental Botany AS CR, Prague) with the *nptII* selectable marker gene. Several transgenic clones resistant to kanamycin were obtained. The presence of *spi-2:gfp* gene sequences in plants was confirmed by a polymerase chain reaction (PCR) method. Reverse transcriptase PCR analysis confirmed the expression of the inserted genes. However, western blot analysis didn't fully confirm the presence of the appropriate proteins. Six transgenic clones were tested *in vitro* and *in vivo* for disease resistance. Antibacterial activity of regenerated plants was determined using dish tests with two bacterial pathogens - *Pseudomonas syringae* pv. *tomato* and *Clavibacter michiganensis* sbsp. *michiganensis*. Antifungal activity was evaluated after inoculation of whole plants or plant segments with a few fungal pathogens e.g. *Botrytis cinerea*, *Fusarium solani*, *Fusarium alternaria*. To assess and quantify possible host plant response to viral attack, a Turnip mosaic virus (TuMV) isolate was used. (Project No. 1M06030 of MEYS of the Czech Republic)

New direction of research the enzyme Zm-p60.1 β-glucosidase from *Zea mays*

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Background: Development and growth of the plant is mostly controlled by plant hormones (phytohormones), whose concentration is regulated by enzymes. One of the enzymes that are involved in this regulation is the maize Zm-p60.1 β-glucosidase. Protein engineering offers a means to change the substrate specificity of this enzyme. Changes in substrate specificity of enzymes can be achieved by mutations at the DNA level. Introduced mutation may have destructive side effects on protein structure. One of the ways to avoid these negative impacts of mutation is to increase the structural strength of the enzyme by adding new hydrogen bonds, whose side effect is an increase in thermostability of the enzyme.

Results: The aim was to study available informations on the Glycoside Hydrolase 1 family (at www.CAZy.org), to subjugate selected group of enzymes to bioinformatic analysis and on that basis to propose another possible direction of research, the enzyme Zm-p60.1 β-glucosidase (EC 3.2.1.21) from maize (*Zea mays*). Based on the analysis of publications and informations contained in the CAZy database this work proposes to increase enzyme thermostability Zm-p60.1 by introducing single point mutations D112K, which should lead to new hydrogen bonds, which would contribute to the stabilization of the protein. New, more stable enzyme Zm-p60.1 should be the default option for the preparation of the next generation of enzymes with modified substrate specificity towards cytokines conjugates.

Conclusions: Bioinformatics analysis of wide set of enzymes in Glycoside Hydrolase 1 family discover new possibility direction of research the enzyme Zm-p60.1 β-glucosidase (EC 3.2.1.21) from maize (*Zea mays*).

Novel links between temperature, calcium and cytokinin signaling identified by proteome- and phosphoproteome-wide profiling in *Arabidopsis*

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Cytokinins (CKs) regulate diverse developmental and physiological processes in plants whose molecular mechanisms of action are being intensely researched. Previously, we identified a number of early cytokinin response proteins and phosphoproteins of Arabidopsis thaliana. They indicated novel links between temperature and cytokinin signaling, and an involvement of calcium ions in cytokinin signaling. Here, we present results of further proteomic experiments aimed to bring a closer insight into these cross-talks. Using sub-proteome and phosphoproteome profiling, we identified early cytokinin response proteins and phosphoproteins whose regulation by cytokinins is abolished in the presence of calcium signaling inhibitors and/or altered under cold or heat shock.

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In vivo analysis of protein-protein interactions within the two-component signaling network in *Arabidopsis*

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Two-component systems (TCS) belong to important sensing/response mechanisms in higher plants. This mechanism is used to perceive and transduce the signal of cytokinin, plant hormone regulating many processes of growth and development. Ethylene receptors also belong to the family of two-component receptors. However, the role of their two-component signaling activity was not clarified yet and ligands for several other receptors of this family were not discovered. In the model plant *Arabidopsis thaliana* TCS are composed of hybrid histidine kinases (AHKs, 8 genes), histidine-containting phosphotransfer proteins (AHPs, 6 genes) and response regulators (ARRs, 24 genes) that are biochemically linked by His-to-Asp phosphorelay. The specific interactions between AHPs and the receiver domains of AHKs could represent a key to specific cellular responses to different signals mediated by two-component signaling network. In this study the interactions between all receiver domains of AHKs and all six AHPs were analyzed *in vivo*. Using the modified Matchmaker™ yeast two-hybrid system (Clontech), we were able to evaluate interaction specificity between these proteins. As an independent method for the results verification, Bimolecular Fluorescence Complementation analysis (BiFC) was carried out in transiently transformed tobacco leaves. Moreover, quantification of the fluorescence intensity in this analysis has reflected the different affinities between AHPs interacting with the receiver domains. We revealed that receiver domains of AHKs interact with a specific set of AHPs. AHP5 interacts with each of the receiver domains, other AHPs interact more specifically. Receiver domains of non-cytokin receptors interact with the AHPs with higher affinity then the cytokinin receptors.

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Possibility of plant virus detection using antibodies against recombinant plant viral structural and non-structural proteins

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With the development of molecular biology techniques cloning and expressing of plant viral genes coding for structural and non-structural proteins has become an important strategy for obtaining large amounts of antigens with uniform concentration and stable properties. We report here the strategy for production of polyclonal antibodies against recombinant proteins of viruses which infect potatoes, namely antibodies against coat proteins (CP) of *Potato virus A* (PVA), *Potato virus Y* (PVY), *Potato mop-top virus* (PMTV), *Potato virus X* (PVX) and *Potato leafroll virus* (PLRV), and also against non-structural proteins of PMTV and PLRV. At present we are working on antibodies for safety detection of *Potato virus M* (PVM). The obtained sera and antibodies were tested for the detection of mentioned pathogens in laboratory hosts (tobacco species) and natural host *Solanum tuberosum*. The obtained antisera have been successfully used for plant virus detection by Western blot analysis 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. Nevertheless, the obtained polyclonal antibodies could be a very good tool not only for virus detection but also for the study of their life cycle.(Project No. 1M06030 of MEYS of the Czech Republic)

The variability of SSR markers in wheat donors of colored grain

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Genetic variability of 24 genotypes of wheat (*Triticum aestivum* L., 2n = 6x = 42 chromosomes, BBAADD) and one genotype *Thinopyrum ponticum* (Podp.) Barkworth & D. R. Dewey (2n = 10x = 70 chromosomes, $JJJJJJ^{s}J^{s}J^{s}J^{s}$) was studied using the SSR method. We analysed wheat donors with purple pericarp, blue aleurone, yellow grain and red grain. SSR markers localized on chromosomes of A and B genome were chosen from the literature for the analysis. We detected 44 alleles from 7 markers with an average of 6.3 alleles per locus (ranging from 4 to 8 per locus). The calculated average polymorphic information content was 0.55 and ranged between 0.44 and 0.65. Based on 7 SSR markers a dendrogram was calculated, which highly significantly differentiates the *Thinopyrum ponticum* from the other analysed genotypes that form 2 subclasters. Each subcluster can be separated into 2 groups, within the one group was identified a high genetic similarity of isogenic lines of wheat Novosibirskaya 67 with red grain and purple pericarp. Used SSR markers can be used for the study of the level of variability and purity of the analysed samples in breeding programs (IGA MENDELU No. IP 1/2010).

Analysis of proteome in wheat substitution lines during long-term cold acclimation

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Winter wheat must experience vernalization before flowering occurs. The transition from vegetative phase into reproductive phase of the plant individual development is generally associated with a decline in plant potential to resist the impacts of cold and frost. The aim of our research is to bring a new insight into plant vernalization process using quantitative proteomics method two-dimensional difference gel electrophoresis (2D-DIGE).

Crowns of winter wheat cultivar Mironovskaya 808 were used to study the quantitative changes in crown proteome during long-term cold acclimation (CA; 0, 21, 84 days at 6 °C). In total, 15 % of 2013 quantified spots were found to be the spots of interest (i.e., differentially expressed polypeptides up- and down regulated; absolute abundance variation of at least 1.5-fold, p, 0.05). All spots of interest were analyzed by MALDI-TOF/TOF and identified as proteins by searching against the Viridiplantae protein database or wheat EST database using a MASCOT server. In total, 22 % of the identified spots of interest belong to known stress-associated proteins as HSPs, dehydrins, cold shock protein domain, ascorbate peroxidase, etc. In comparison with 21-day cold-acclimated plants, most proteins showed decrease in accumulation in crowns after 84 days of CA. However, other proteins (e.g., chopper chaperone) showed increase in accumulation after 84 days compared with 21 days of CA. Moreover, we were able to distinguish not only non-acclimated plants vs. cold acclimated ones, but also the partially vernalized plants (21 days of CA) plants from the plants with saturated vernalization requirement (84 days of CA).

GFP-target Gene Fusion in Gene Expression Studies of Serine Protease Inhibitor in Plants

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Genetic modifications (GM) of plants have offered a new challenge to basic research as well as to its applications in agriculture, forestry, horticulture, pharmaceutical industry, protection of the environment, phytoremediation and other areas. Since the first introduction of GM crops to fields in 1996 and their next broad scale cultivation worldwide, new generations of engineered plants based on qualitatively new principles are intensively developed. In agriculture, modifications based on the introduction of foreign/modified gene(s), offer an efficient way to improve numerous, mainly qualitative, traits of crops like their resistance/ tolerance to biotic- (e.g. pests, diseases) and abiotic stress (herbicides, drought, salinity, etc.). The study of potentially useful candidate genes is simplified by their linkage to suitable reporter (marker) sequences. Different variants of the green fluorescent protein (GFP) are widely used to trace transgene expression and are known as a convenient and efficient marker of successful plant transformation. In this study we report on the expression in plants of modified gene *gmspi-2*, which encodes silk proteinase inhibitor 2 of the wax moth *Galleria mellonella*, either alone or fused in different ways to the modified *m-gfp5-ER* signal gene (for GFP) of the pacific jelly fish *Aquorea victoria*. The purpose of our effort is the design of a proper vector for plant transformations focused on the enhancement of crop resistance to pathogenic organisms. The fused *m-gfp5-ER* sequence could potentially help not only in tracing expression of the target gene and detection of its protein product of low M.R. that is uneasy to detect directly, but it can also stabilize protein conformation and thereby enhance its activity. Our contribution gives an overview of data obtained by various molecular methods (vector construction, PAGE, Western blot), expression systems (*E. coli*, tobacco) and in situ studies (fluorescence). The results are discussed in respect of future proteinase inhibitor variants and their possible use in crop protection. (Project No. 1M06030 of MEYS of the Czech Republic)

Genetically modified tobaccos for enhanced phytoremediation of PCBs and toluene

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Phytoremediation can provide a useful, cheap and effective tool for decontamination of our environment. The aim of this work is to construct genetically modified plants with increased capabilities to degrade organic pollutants such as PCBs and toluene.

Environmentally important genes of bacterial dioxygenases – *bphC* gene and *todC1C2* genes were chosen to clone into plant of *Nicotiana tabacum*. First chosen *bphC* gene encodes 2,3-dihydroxybiphenyl-1,2-dioxygenase which cleaves the aromatic ring of dihydroxybiphenyl and was cloned in fusion with the gene for β-glucuronidase (*GUS*), luciferase (*LUC*) or with a histidine tail. The *todC1C2* genes were chosen to clone into plants to produce oxygenase ISP_{TOL} (with

histidine tail), a component of bacterial toluene-2,3-dioxygenase that can oxidize toluene and other organic pollutants. Several genetic constructs were designed and prepared and the possible expression of desired proteins in tobacco plants was studied by transient expression. Expressed dioxygenases BphC/His, BphC/GUS, BphC/LUC and ISP_{TOL}/6×His were then detected by Western blot or histochemically. Genetic constructs successfully expressing dioxygenase's genes were used for preparation of transgenic tobacco plants. The presence of transgenic DNA, mRNA and desired protein was determined in parental and first filial generation of transgenic plants with *bphC* gene. The ability to remove the toxic substrate 2,3-dihydroxybiphenyl from media was studied with selected transgenic lines. Transgenic line H3 (harboring *bphC* gene with histidine tail) showed 95 % higher decrease of the substrate content in medium than nontransgenic plants. Properties of transgenic plants will be further studied.

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met region from plasmid pA81 from bacterium *Achromobacter xylosoxidans* A8 and associated phenotypical effects in *Escherichia coli*

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Gram-negative soil bacterium *Achromobacter xylosoxidans* A8 hosts a 92,5-kb plasmid pA81. The analysis of nucleotide sequence revealed that the plasmid harbors a *met* region consisting of seven genes *metTDYRAB*. The homology searches suggested identities of encoded gene products as (i) putative membrane protein from Pb^{2+}/Fe^{2+} family of transporters (*metT* gene), (ii) transporter of Cation-Diffusion Facilitator family (*metD*), (iii) steroldesaturase (*metY*), (iv) member of MerR family of metal-responsive transcriptional activators/repressors (*metR*), (v) efflux P1-ATPase (*metA*), (vi) putative membrane lipoprotein (*metB*) along with its cognate (vii) prolipoprotein signal peptidase (*metC*). The contribution of *met* region to the resistance of *A. xylosoxidans* to Cd^{2+} was confirmed. In order to study the metalloresistance phenotype, which would be determined by the individual *met* genes, vectors based on pBla were constructed. These allowed constitutive expression of the individual met genes in *E. coli* GG48. The clones expressing the *metA* gene showed increased metalloresistance, as compared with a strain harboring pBla vector alone. While the growth of control cells was inhibited by 50% (IC_{50}) at 1 μM Cd^{2+} and 40 μM Zn^{2+}, metA cells showed IC_{50} values of 45 μM Cd^{2+} and 150 μM Zn^{2+}. Moreover, expression of *metA* reduced the accumulation of Cd^{2+} and Zn^{2+} by (37 ± 15)% and (20 ± 4)%, respectively. Taken together, these data attest that MetA is a functional transporter of CPx-ATPase subfamily. When expressed in *E. coli*, remaining met genes did not exert any phenotype that would suggest their functionality in metalloresistance.

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Preparation of transgenic flax with yeast *HisCUP* gene under the RbcS promoter for increasing heavy metal accumulation

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In nature exist plants able to accumulate heavy metals in high concentration. These plants have very often slow growth, produce small amount biomass and therefore they are not suitable for phytoremediation. This problem could be solved by preparation of transgenic plants with appropriate properties. The yeast *CUP* gene encoding yeast protein metallothionein has high affinity to heavy metals and therefore it was chosen for cloning into plant genome of flax (*Linum usitatissimum*). Plasmid pNOV2819 (*Syngenta*) containing gene for enzyme phosphomannose isomerase (for selection of transgenic plants) was chosen as plant cloning vector. Cassette RbcS with promoter (P-RbcS) and terminator (T-RbcS) of the RUBISCO enzyme was first cleaved out of plasmid pIV and then inserted into plasmid pNOV2819. *HisCUP* gene (CUP with histidine tail) was amplified by PCR from plasmid pTrc*HisCUP*, formerly used for preparation of transgenic tobacco plants. *HisCUP* gene was then cloned into prepared plasmid pNOV2819/RbcS. Afterwards, the engineered plasmid pNOV2819/RbcS/*HisCUP* was transferred to bacteria *Agrobacterium tumefaciens* C58-C1 (pCH32). The possible expression of *HisCUP* gene in plant of *Nicotiana tabacum* was studied by transient expression using *A. tumefaciens* C58-C1 (pCH32) harboring pNOV2819/RbcS/*HisCUP*. Metallothionein with histidine tail was isolated from plant tissue by affinity chromatography and then it was detected immunochemically by commercial antibody against histidine tail. Finally the transformation of flax was performed via agrobacterial infection. Regenerated plants are nowadays growing on selective medium with mannose as a selective agent. Acknowledgement: The work on this project was supported by the grants 1M06030 and MSM 6046137305, Z40550506, MSMT No. 21/2010.

Brassinosteroid-binding proteins: searching for new exemplars and their relation to brassinosteroids

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Brassinosteroids are group of phytohormones, plant endogenous signal molecules affecting differentiation, growth, photomorphogenesis, senescence and stress tolerance. The mechanism of their action is based on interaction of brassinosteroids and system of membrane receptors.

We prepared a range of affinity columns with different types of brassinosteroid molecules immobilised on polymeric matrices. Extracts from 4-weeks-old tobacco (*Nicotiana tabacum*) callus, young leaves of New Zealand spinach (*Tetragonia tetragonioides*) and young shoots of flax (*Linum usitatissimum*) were applied to the columns. All proteins with affinity to brassinosteroids were screened on SDS-PAGE gels and the most abundant proteins were analysed by *N*-terminal sequencing or LC/MS/MS.

Here we report on the isolation and identification of three plant proteins with affinity to the synthetic analogues of naturally occurring brassinosteroids. The first one (from tobacco callus) was identified as the osmotin-like protein, an already known pathogenesis-related protein from tobacco, connected with plant adaptation to stress factors as drying, wounding or attack of pathogens, revealing also antifungal activity. The second one (from flax and from New Zealand spinach)

was identified as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which is the key photosynthetic enzyme converting inorganic carbon into organic compounds. The last one (from New Zealand spinach, too) was recognized as plant glutathione-*S*-transferase, enzyme with known role in plant stress tolerance. The relationship between brassinosteroids and all these proteins is now under investigation; nevertheless the first results indicate that e. g. the brassinosteroids are important to increasing the RuBisCO activity.

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Session 4 - Plant genetics, breeding and diversity

Resistance to *M. graminicola* in the Czech old wheat cultivars

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Septoria tritici blotch (STB) caused by fungal pathogen *Mycosphaerella graminicola* ((Fuckel) J.Schröt.) is the one of the significant foliar diseases of wheat crops worldwide. In the Czech Republic importance of this disease also has been gradually increasing during the last 25 years. In favorable conditions, such as high rainfall and moderate temperature, this disease may reduce grain yield by 50 %. The cultivation of resistant wheat cultivars might provide an effective and economical way of STB controlling. The isolate specific resistance of wheat to STB follows the gene-for-gen relationship. In our study we tried to detect specific genes in collection of the Czech old wheat cultivars. Selected resistant varieties could be applied into the breeding as alternative resistance sources. 183 wheat varieties which were bred and cultivated in the period 1900 - 1960 were tested. 15 % of these cultivars were specifically resistant to at least one of eight *M. graminicola* isolates. The selected *M. graminicola* isolates were originated from the Czech natural populations. It was necessary to verify characteristics of resistant varieties using molecular biology methods. We used published microsatellite markers (SSRs). However, predicted length of amplified fragments was different from our amplified fragments in some cases. In this case it was not possible to asses which cultivars carried resistance genes. The different geographical origin of tested material seems to be a reason for malfunction of used markers. Future investigation is necessary to design and optimize molecular markers available for biological material used in this work. (This work was supported by grants of Ministry of Agriculture of the Czech Republic No.: QH81284 and Ministry of Education of the Czech Republic No.: 6046070901)

Dehydrins as markers of low-temperature tolerance in wheat and barley

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Cold and frost limit agronomic productivity of wheat and barley. In cold-treated plants, complex physiological processes become induced which are aimed at enhancement of plant tolerance to low temperatures and they are generally termed cold acclimation (CA). The process of CA in wheat and barley is associated with accumulation of hydrophilic LEA-II proteins called dehydrins. We have focused on cold-inducible WCS120 dehydrins in wheat and their orthologue DHN5 in barley. When dehydrin accumulation in fully cold-acclimated plants was evaluated, a correlation between dehydrin accumulation and the level of acquired frost tolerance (FT) was found both in wheat and barley. During a long-term CA, differences in DHN5 level as well as acquired FT level were observed between barley doubled-haploid (DH) lines derived from a cross between a spring and a winter genotype. The transition from a vegetative stage to a reproductive stage was accompanied by a decrease in DHN5 level in both spring and winter genotypes whereas the acquired FT level remained high in vernalized winter lines under continuous CA treatment. When wheat and barley cultivars were grown at different temperatures, cultivars with a higher acquired FT began accumulating WCS120 (common wheat) or DHN5 (barley) at higher levels than the less tolerant cultivars not only under cold, but also at mild temperatures. The experiments have proven that WCS120 proteins in common wheat and DHN5 in barley can be considered reliable markers of FT not only when plants are exposed to cold, but even when they are grown at mild temperatures.

Diversity and Agronomic Utility of Leaf Types in Pea (*Pisum sativum* L.)

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Pea (*Pisum sativum* L.) is an economically important food crop. Mendel adopted it for his set of seminal genetic experiments that established the underlying principles of heredity. Like other genera of the tribe Fabeae, the pea leaf has a compound structure consisting of a pair of large stipules, a rachis, two or three pairs of leaflets and an odd number of tendrils. The developmental regulation of leaflets and tendrils is under the control of two independent genes, *af* (afila), located on linkage group I, and tl (tendril-less or acacia), located on linkage group V. The normal leaf type phenotype having both leaflets and tendrils has the genotype *AfAf TlTl*. In genotypes with the structure *afaf TlTl*, all leaflets are transformed into tendrils which confer superior standing ability compared to normal leaf types and have significantly contributed to the increased grain yields, becoming popular around the world under the names of *afila* or *semi-leafless* peas. On the other hand, genotypes with *AfAf tltl* structure where all tendrils are transformed into leaflets are rather more prone to lodging but may be interesting for forage production. Further study of the interaction of afila and tendril-less with other foliar and plant stature mutants coupled with seeking of novel allelic variation at these loci is helping to continue to bring about improved agronomic performance and yields.

The stability of phenotype in genetically modified potatoes

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Cold-stored potato tubers convert subsequently starch to reducing sugars (so called low temperature sweetening). The phenomenon was partially solved by introduction of the bacterial gene *Lbpfk* coding for cold-tolerant phosphofructokinase. Two of Czech potato cultivars were transformed by *Lbpfk* and the transgenic lines were verified for the presence of the transgene and its expression. The transgenic potato plants were tested for three years in field trials i.e. under natural conditions. The harvested tubers were cold-stored for more than three months and reducing sugar content and frying colour of chips were quantified. The transgenic plants derived from both cultivars displayed already after one year cultivation lower reducing sugar content in tubers, as well as improved frying colour of potato chips compared to values of nontransformed control plants. These values vary from year to year probably due to different climatic conditions. Nevertheless, the moiety of the transgenic lines retain desirable values for both reducing sugar content and frying colour. The transgenic lines of the cultivar Vladan seem more stable than the transgenic lines from the other cultivar VE C 70/2 concerning values after prolonged cold-storage and subsequent reconditioning of the tubers. We simultaneously measured sucrose content in the stored tubers. The transgenic lines of Vladan again displayed better stability concerning the total amount of all three sugars measured (sucrose, glucose, and fructose).

This work was supported by the grant No. 1M06030 of the Ministry of education, Youth and Sports of the Czech Republic.

Marker assisted pea breeding: *eIF4Ee* and *eiIF4Eiso* allele specific markers to *Pea seed-borne mosaic virus* (PSbMV) resistance.

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Traditional plant breeding relies upon crosses and subsequent selection of genotypes to meet desirable traits. The incorporation of marker-assisted selection (MAS) into breeding strategies would result in a reduction in the number of offspring to be propagated, selected and tested. In the case of pea (*Pisum sativum* L.), the testing of resistance to viral pathogens such as *Pea seed-borne mosaic virus* (PSbMV) is included in the breeding process. Resistance to the common strains of PSbMV is conferred by a single recessive gene (*eIF4E*), localized on LG VI (*sbm-1* locus). We have analyzed for variation in the *eIF4E* genomic sequences from 43 pea varieties and breeding lines, reported as donors of resistance. Furthemore, initial analysis of second *sbm-2* locus confering resistance to L1 strains of PSbMV with homologous *eIF4Eiso* gene was performed. This enabled a comprehensive investigation of the *eIF4E*/ *eIF4Eiso* genes structure and identification of mutations responsible for PSbMV resistance. Subsequently, gene-specific single nucleotide polymorphism (SNP) and co-dominant amplicon length polymorphism markers were developed. All markers were reproducibly amplified across a broad spectra of pea varieties and breeding lines. These were found to be 100% accurate in detecting the presence of the virus when compared to symptomology and ELISA testing . Hence, these molecular will substantially speed-up PSbMV diagnosis and resistance breeding processes. The work was supported by the Czech Ministry of Agriculture 91A229 project.

Identification of alleles in *Glu-A1*, *Glu-B1*, and *Glu-D1* locus in selected genotypes of triticale

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The allele-specific PCR (AS-PCR) was used to identify the allele composition of loci *Glu-A1*, *Glu-B1* and *Glu-D1* of high-molecular-weight (HMW) glutenin subunits in 15 triticale genotypes (varieties and breeding lines). Amplified DNA fragments of HMW glutenin alleles were separated by agarose slab-gel electrophoresis. Differences among all the three alleles at the locus Glu-A1 [*Glu-A1a* (encoding Ax1), *1b* (Ax2*) and *1c* (AxNull)] were found. Most frequently we detected the allele *Glu-A1b* (86.7 %). The allele *Glu-A1a* was detected in the variety Pawo and the allele *Glu-A1c* in the variety Leontino. We optimised PCR reaction (annealing) of a specific marker for the allele *Glu-A1b* in PS3 primer combination. In the variety Palomino the alleles *Glu-A1b* and *Glu-A1c* were detected from different DNA isolation. DNA was isolated from a mixed sample and so it was not possible to distinguish whether it was an admixture in the seed sample or a heterozygous constitution of the genotype. Five allelic combinations were detected at the locus *Glu-B1* [*Glu-B1-1a+2o* (encoding Bx7+By8*), *Glu-B1-1a+2s* (Bx7+By18*), *Glu-B1-1a+2z* (Bx7+By20*), *Glu-B1-1b+2a* (Bx7*+By8), and *Glu-B1-1b+2o* (Bx7*+By8*)] by primers PS4-PS7. The allele *Glu-D1d* (HMW subunits 5+10) was detected in breeding lines that carried translocations of segments of wheat chromosome 1D to rye chromosome 1R only. We would like to optimise allele-specific PCRs for detection of alleles for good bread-making quality because identification of wheat Glu-1 alleles using DNA markers can be used for early marker-assisted selection (MAS) of triticale genotypes with good bread-making quality (MEYS, Czech Republic, project MSM2532885901).

The Stability of Microsatellite Markers in Pea (*Pisum sativum* L.)

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Microsatellites, or simple sequence repeats (SSRs) are commonly used for mapping and diversity studies in crop plants. Moreover, SSRs are recommended as additional markers in the system of Distinctness, Uniformity and Stability (DUS) testing by The International Union for the Protection of New Varieties of Plants and their utility for variety discrimination was published for numerous plants including pea. Despite the extensive and undeniable usefulness of SSRs their mutation dynamics is still not well understood. Reported microsatellite mutation rates are significantly higher than base substitution rates. In our study we focused on stability of three microsatellite loci located in intergenic, LTR retrotransposon and expressed gene regions. Single seed origin individuals from seven accessions of dry seed pea (*Pisum sativum* subsp. *sativum* var. *sativum* cv. Sponsor, Merkur, Jackpot, Hardy, Zekon, Menhir and Grana) were cultivated for ten subsequent generations (2004-2010) in glasshouse conditions. Analysis of alleles in three SSR loci have shown that all selected SSR markers kept the same fragment length. Furthemore sequencing analysis of selected samples was made to exclude homolasy. The results support the use of microsatellite markers for pea genetic diversity studies, trait mapping and variety identification. This work was supported by Ministry of Education of Czech Republic MSM2678424601 project.

Powerful tool to transform barley (*Hordeum vulgare* L.)

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Spring barley (*Hordeum vulgare* L. conv. *distichon* var. *nici*) is one of the most agronomically important crops in Europe, and in the Czech Republic it is the second most widely grown crop. About seventy percent of the total harvest is used for feed, the production of malt representing around thirty percent. Breeding programs of barley are focused on improving agronomical trails, and on malting and feeding qualities. During the last ten years, modern genetics and biotechnology has provided a number of tools that can be used to improve the nutritional value of barley. Two techniques, *Agrobacterium*-mediated transformation and particle bombardment, were used for transform immature embryos of spring barley cv. Golden Promise. Two different constructs were used bearing agronomically important genes: *dapA* for higher lysine content, and *phyA* for eliminating the anti-nutritional effect of endogenous phytase in the grain of barley. Transgenic barley plants were evaluated by PCR, Real-Time PCR and Western blot. A high efficiency of barley transformation was achieved using the *Agrobacterium*-mediated technique: from 150 transformed immature embryos with plasmid pBract214::sdapA, 158 putative transgenic plants were regenerated; 22% of these plants produced the desired protein. This work was supported by project 1M06030 MEYS Czech Republic.

Evaluation of protein quality of winter triticale cultivars

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Hexaploid triticale (x *Triticosecale* Wittmack) is a cereal created by crossing between wheat and rye. In comparison with wheat, triticale has a many significant properties, because it has lately become interesting subject of study. Despite the fact that some hexaploid forms of triticale have low baking properties, this species can be considered as a culture with genetic potential, and also as a source of commercially important genes, including those for disease resistance. The aim of our work was to detect protein quality in winter triticale. Samples comes from 9 countries, predominantly from Europe and the rest from USA. Biochemical analysis as determination of overall nitrogen by Kjeldahl, determination of fraction composition by Golenkov and determination of protein nitrogen by Barstein were performed. The protein content in samples varied between 8.48 - 13.6 %. Generally, we can allege that the highest content of overall nitrogen as well as content of crude protein achieved relatively new cultivars from USA. The protein fractional composition decides first of all about the food and the technological quality of grain. Content of albumins, globulins, prolamins and glutenins in the triticale seeds ranged comparably to common wheat. The cultivar identification and the specification of some its commercially important properties is possible to define for a consideration of the electrophoretical spectrum of the grain storage proteins. Our work deals with evaluation of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) by vertical electrophoretic separation in SDS-PAGE. Separation of storage proteins was performed by internationally valid ISTA methodology (Wrigley, 1992). An average content of HMW-GS achieved 11.47 %. The highest content of HMW-GS reached cultivar Terrelland 22 originated from USA. The highest score of LMW-GS was detected in german cultivar Trimmer 69.23 %. At french cultivar Aprim the highest score of albumins and globulins 43.34 % was detected. The quality of triticale cultivars was approaching to that of the common winter wheat. This work was supported by a VEGA project No.1/0471/09.

Cytokinins and polar transport of auxin in axillary pea buds

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The influence of cytokinin on auxin transport during release of axillary buds from apical dominance was studied. Expression of auxin-carrier coding genes *PsAUX1* (*AUXIN RESISTANT 1*) and *PsPIN1* (*PIN-FORMED 1*) was explored in axillary buds of the 2nd node of 7-day pea plants (*Pisum sativum* L.) cv. Vladan after decapitation or after exogenous application of benzyladenine (6-benzylaminopurine) onto axillary buds of intact plants. Localization of the PsPIN1 protein, the key factor for polar transport of auxin in axillary buds, was visualised by immunohistochemistry. After exogenous application of cytokinin the expression of *PsAUX1* and *PsPIN1* rapidly increased with a simultaneous rapid decrease in PsDRM1 and *PsAD1* expression - genes related to bud dormancy. The same changes in expression were observed after decapitation, however they were markedly slower. The PsPIN1 auxin efflux carrier in the inhibited axillary buds of intact plants was localised in a non-polar manner. After exogenous application of cytokinin gradual polarisation of the PsPIN1 protein occurred on the basal pole of polar auxin transport competent cells. Despite the fact that direct auxin application to buds of intact plants led to an increase in *PsAUX1* and *PsPIN1* expression, the buds remained dormant (non-growing) what was accompanied by persistent expression of the dormancy markers *PsDRM1* and *PsAD1*. The results indicate a possible effect of cytokinins on biosynthesis, and/or transport of auxin in axillary buds and they highlight the importance of auxin-cytokinin crosstalk in the regulation of bud outgrowth after breaking of apical dominance.

The impact of trinitrotoluene on *Arabidopsis* transcriptome in shoots and roots

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Tritol (2,4,6-trinitrotoluene; TNT) one of most common explosive was spread into the environment predominantly from the place of manufacturing, packing and using. Phytoremediation could be a good technology for solving of the problem of contamination of large areas by organic pollutants, but the real applications require deep understanding to detoxification process in plants. In our experiments we studied the effect of TNT on gene expression in *Arabidopsis* shoots and roots by microarrays. Seven-day exposure to TNT resulted in 170 up- and 122 down-regulated genes in the shoots and 61 up- and 51 down-regulated genes in the roots (expression difference >1.5 fold; p[t-test] < 0.05). TNT concentration, 5µg/ml, was selected according to the dose response analysis and study of TNT uptake from liquid media. Only small overlap of TNT effects on transcriptome was observed between shoots and roots. The shoots exhibited induction of several genes associated with toxin metabolism, such as UDP glycosyltransferases and ABC family transporters. Another up-regulated gene encoded nitrate reductase 1, potential candidate for reduction of nitro groups on TNT ring. In roots, the genes coding for enzymes involved in the cell wall modifications were abundantly up-regulated. Microarray data indicated that after relatively long incubation with TNT (seven days), metabolism of this xenobiotic proceeded mainly in aerial parts, while its translocation into cell walls still took place in the roots. Results obtained by microarray hybridization were validated by quantitative real-time RT PCR. This study was supported by projecs NPVII no. 2B06187, 2B08058 and 1MPO6030.

Phylogeny, phylogeography and genetic diversity of *Pisum* genus

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Tribe Fabeae (formerly *Vicieae*) contains some of humanity's most important grain legume crops, namely *Lathyrus* (grasspea) (about 160 species); *Lens* (lentils) (4 species); *Pisum* (peas) (3 species); *Vicia* (vetches) (about 140 species) and the monotypic genus *Vavilovia*. The tribe contains several economically important members, with *Pisum* the third most important grain legume. Reconstructing its phylogenetic relationship is essential for understanding the origin and diversification of this economically and ecologically important group. Our study based on molecular data, have positioned *Pisum* between *Vicia* and *Lathyrus* and being closely allied to *Vavilovia*. Study of phylogeography using combination of plastid and nuclear markers suggested the spread of wild pea from centre of origin (Middle East), eastwards (till Caucassus, Iran and Afghanistan) and westwards to Mediterranean region (till Spain, Italy and northern Africa). To allow direct data comparison, the *Pisum* genus germplasm collections, we performed model-based Bayesian analysis of the population structure of retrotransposon-based markers using BAPS software on the composed set of 4,429 accessions from three large world germplasm collections. This analysis of genetic diversity identified separate

clusters containing wild material, distinguishing *P. fulvum* from *P. elatius* and *P. abyssinicum*, supporting the view of separate species or subspecies. In addition to genetic relationships these results provided also insight into geographical and phylogenetical partitioning of genetic diversity. This study provides the framework for defining world *Pisum* germplasm diversity well as suggested model for the domestication. P.S. acknowledges financial support from the Ministry of Education of Czech Republic, MSM 2678424601, LA08011 and Bioversity International AEGIS 10/048 projects.

Session 5 - Vision for animal breeding and genetics in research and education

The phenotype trends for Large White and Landrace pigs after introducing M BLUP Animal Model in genetic evaluations in years 2000-2009 in Slovakia

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In this work with the analysis of dam pig breeds Large White (LW) and Landrace (L) over the years 2000-2009 in relation to the introduction of M BLUP animal model into the genetic evaluation of pigs in the Slovak Republic focusing on the phenotype trends in years 2000-2009. The average daily gain of the overall population average during 2000-2009 was found in L 809.7 g, LW 779.3 g. The tendency in the last years 2000-2009 is declining. The best daily gain was reached in the year 2001 LW + 27.7 g and L +45.3 g. As far as the feed consumption per 1 kg of live weight is concerned, the overall population average was reached in LW – 2.88 kg and L 2.86 kg. The tendency to reduce feed consumption was slightly increasing. The analysis of slaughter parameters over the years 2000-2009 showed the increasing tendency concerning fat thickness in LW, namely from 18.30 (2000) to 12.40 mm (2009), which is the reduction by 5.9 mm; in L from 16.40 mm (2000) to 12.10 mm (2009), which is the reduction by 4.30 mm. There was also a positive tendency in the percentage of carcass lean meat in a slaughter body. The overall population average for the years 2000-2009 was as follows: LW –54.36 % and L 54.61 %. The highest values of meatiness in 2000-2009 were achieved in LW +1.59 % and L +2.92 %. Apart from the daily gains in all parameters, there were also found both highly significant statistical differences and interactions between a period and a breed.

Applications of genetic markers in animal breeding and diversity protection: a project supported by the European Regional Development Fund

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The contribution is focused on promotion of a project "Applications of genetic markers in animal breeding and diversity protection" which has been supported by the European Regional Development Fund, Operational Program for Research and Development. The project is coordinated by the Animal Production Research Centre and Constantine the Philosopher University in Nitra as a partner. The aims of the project are (1) to find new knowledge about genetic markers for health, production traits and origin of selected animal species; (2) to develop methods of genetic markers analysis and direct diagnostic guidelines for breeding applications; and (3) to apply the knowledge and methods in animal breeding in cooperation with breeders and breeding companies. The project has a potential to bring innovations and improvements in animal genetic research and technologies. Especially new techniques, optimized methods and diagnostic guidelines could be applicable in animal breeding practice with an impact on competitiveness and effectiveness of animal production. Finally, an implementation of new research findings into study programs has a power to improve educational process at the University. The contribution was supported by the project No. 26220220033, approved within the OPVaV-2008/2.2/01-SORO.

Application of SSR markers in the subject Genetics at MENDELU

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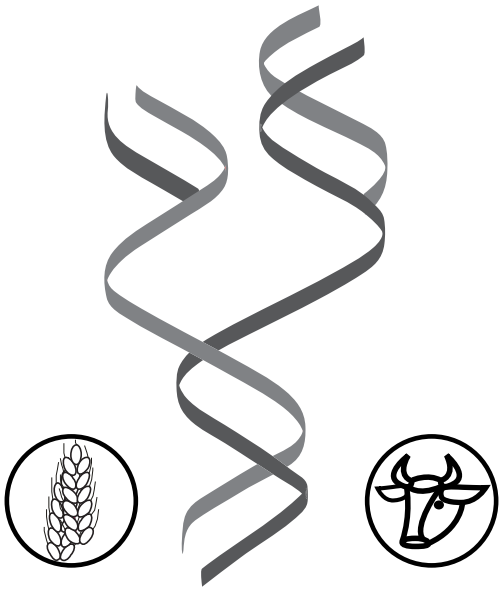
Methods of molecular genetics are extensively used in scientific work and in education nowadays. A polymerase-chain reaction based method was introduced into the Genetics course in bachelor studies at Mendel University in Brno (Department of Plant Biology). As the best method detection of variability of microsatellite markers (SSRs) was chosen. This markers were applied in selected genotypes of triticale (x *Triticosecale* Wittmack, 2n = 6x = 42 chromosomes, BBAARR). Genomic DNA was isolated from young plants (6 days old) using the DNeasy Plant Mini Kit isolation kit (Qiagen, GE). Wheat and rye SSR markers selected and analysed in accordance with literature. The amplified SSR products were visualized on 8% non-denaturating polyacrylamid (PAA) gels in TBE buffer (300 V) followed by colouring with silver (0.2% $AgNO_3$). The resulting electrophoreograms were converted to binary matrices represented by the presence (1) or absence (0) of the alleles and then evaluated by means of statistical software FreeTree version 9.1 using the UPGMA construction method and similarity coefficients. The software TreeView version 1.6 was used for the graphical expression of the matrix. The diversity index (DI), the probabilities of identity (PI) and the polymorphic information contents (PIC) of SSR markers were calculated. A manual containing detailed description of the method and statistical evaluation is available on web site the Department of Plant Biology

MEYS, Czech Republic, FRVŠ project no. 6/2010/F4a



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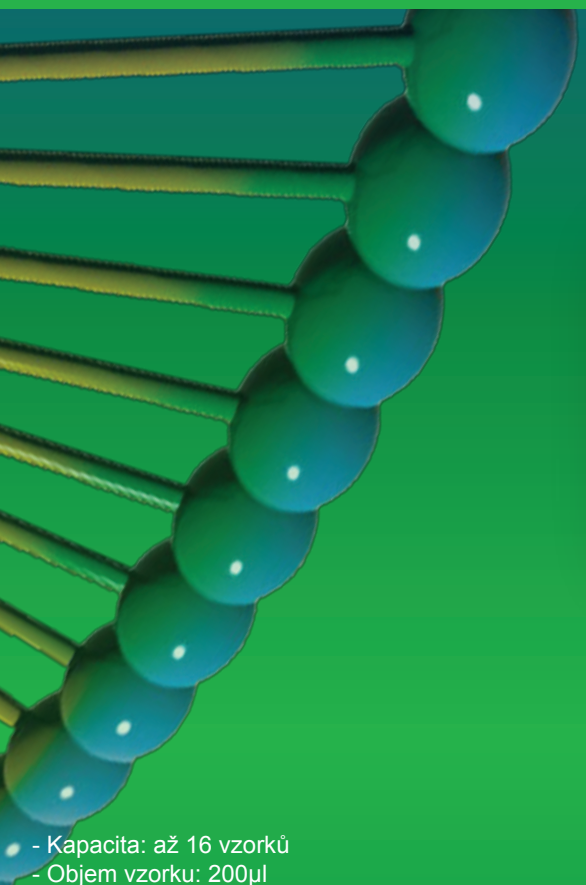

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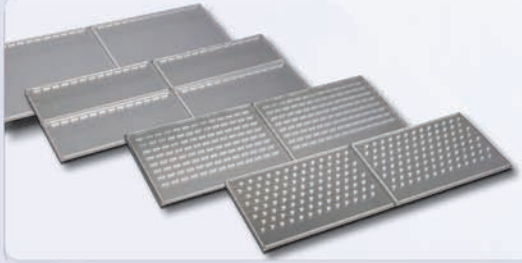

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