Characterization of the potential biological control *Acremonium alternatum* using omics approaches

Veronika Berkova, Simona Mensikova, Susann Auer

The excessive use of agrochemicals adversely affects the environment and is not sustainable for the long term. Developing alternative pest and weed control strategies that do not burden the environment and stimulate plants' fitness is inevitable. Endophytic fungi naturally colonize plants and promote growth by facilitating biogenic elements (iron, nitrogen, or phosphorus). It has been described that endophytes can also modulate plant hormone levels. The interaction of *A. alternatum* and potential host plants has previously been studied, but the omics characterization of this promising endophyte is missing. Proteins and metabolites were extracted simultaneously from the Petri dishes cultures. A total of 951 proteins were identified in three biological replicates based on more than 3600 unique peptides. Detailed analysis revealed that more than half of the identified proteins were involved in energy metabolism, translation, and protein folding. Metabolites from the polar fraction were derivatized and analyzed by GC-MS. The most abundant metabolites included sugars and amino acids. Analysis revealed minimal changes in the composition of the metabolites between the older and the younger part of the mycelium. In summary, this work provides a first insight into the molecular composition of *A. alternatum.*

Keywords: **proteome, metabolome, biocontrol, *Acremonium alternatum*, endophytic fungus**

Method for simultaneous detection of *Phytophthora infestans* proteins and DNA in *Solanum tuberosum* samples

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Phytophthora infestans is a model organism for the oomycetes, a distinct lineage of fungus-like eukaryotes. However, *P. infestans* is primarily known as a pathogen that causes the serious potato and tomato disease known as late blight. The means of controlling late blight are limited and require early detection of the pathogen. One of the most reliable method for targeted quantitation is based on qPCR. The amount of sample is often limited, which does not allow the use of other analyses such as proteome analysis by LC-MS. Here, we describe the optimized extraction method for simultaneously extracting the DNA and proteins from an infected leaf of *Solanum tuberosum*. Simultaneous analysis of the amount of *P. infestans* DNA and the proteome composition from the infected leaf opens possibilities for a better understanding of the interaction between *P. infestans* and the plant.

Keywords: **qPCR, proteomics, *Phytophthora infestans*, quantitation**

Identification of powdery mildew (Erysiphales) species on ornamental perennial plants (*Asteraceae*) in the gardens of Mendel University in Brno

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This study is focused on mapping the species representation of powdery mildew (Erysiphales) on ornamental perennial plants of the family *Asteraceae*. During the growing season 2021, the collection of samples was proven in several terms in the Botanical Garden of Mendel University in Brno, in the Academic Garden Horticultural Faculty in Lednice and in the Labyrinth of Nature and Paradise of the Gardens of the Horticultural Faculty of Mendel University in Lednice. A total of 23 plants were collected, which showed signs of powdery mildew infection, of which a specific type of powdery mildew was determined in 15 cases. The samples were subsequently described, processed and specific species of powdery mildew occurring on members of the *Asteraceae* family were identified using microscopic techniques and compared mainly with the Monograph of Erysiphales (Braun and Cook 2012). Subsequently, an inventory of individual species of powdery mildew occurring in the gardens of Mendel University was created and macroscopic and microscopic photographs of these pathogens were taken on the infected plants.

Keywords: **powdery mildew, *Asteraceae*, ornamental perennials**

Short-term application of elevated temperature and drought influences the isotopic composition of winter wheat grains

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The study aimed to determine the differences in carbon (δ13C) and nitrogen (δ15N) stable isotope ratios in grains of three winter wheat varieties grown under optimal and stress conditions. We found that the wheat variety has a significant effect on both δ13C and δ15N isotope ratios. Short-term (nine days) exposure to drought and high temperature during the heading or stem extension development phase significantly enhanced δ13C values, but only high temperature affected δ15N values. Enhanced δ15N values support the assumption that global warming causes a higher representation of the 15N isotope in plants. Moreover, significant interactive effects of temperature and water availability on the values of both isotopes were found implying that C and N metabolisms have been altered under the investigated stress conditions. We conclude that δ13C and δ15N isotope ratios of cereal grains are sensitive indicators of stress conditions, even short-term ones.

Keywords: **wheat, grain, 13C, 15N, drought, high temperature**

Transcript levels of *VRN1*, *PPD-D1*, *PPD-B1* and *PPD-A1* genes during different developmental stages of winter wheat

Nicole Frantova, Pavlina Smutna, Ludmila Holkova

The phenology of winter wheat is largely affected by the expression of the vernalization gene *VRN1* and the *PPD-D1*, *PPD-B1* and *PPD-A1* genes responsible for sensitivity to photoperiod. The transcript levels of these genes were studied in six winter and one facultative wheat variety during growing season 2020. During the development of the shoot apex, the activity of *VRN1* and *PPD-D1* is gradually increased, although some transcript level of the *VRN1* gene was found also in the leaves during the cold weather. In the spring, after vernalization fulfilment and during prolonged daylight, the increased expression of VRN1 and PPD-D1 was related to the change of the developmental stage. From a certain developmental stage, an increase in expression was observed, which corresponds to a change in development. However, the activity of the *PPD-B1* decreases when the development of the shoot apex increases. It is possible that certain combinations of *PPD1* loci alleles can alter head timing by acceleration which can protect sensitive floral meristem during unfavourable weather conditions and therefore, the information about allele combination and regulation conditions of these genes can be used for breeding of new varieties suitable for specific environmental conditions.

Keywords: **wheat, gene, phenology, vernalization, photoperiod**

Atmospheric CO_2 concentration, light intensity, and nitrogen nutrition affect spring barley response to drought and heat stress

Hana Findurova, Barbora Vesela, Emmanuel Opoku, Karel Klem

The aim of this study was to compare physiological responses of two spring barley varieties, differing in their oxidative stress tolerance, to drought and heat stress after pre-treatment under different irradiation regimes, CO_2 concentrations, and nitrogen fertilisation levels. High light intensity, elevated CO_2, and additional UV radiation increased flavonoid accumulation. Moreover, more flavonoids were induced in oxidative stress-sensitive variety Barke. Combined drought and heat stress caused a large decline in CO_2 assimilation, whereas heat stress alone caused only minor changes. Under combined stress, plants grown under low light intensity and no UV irradiation performed the best despite their higher initial water use efficiency and lower flavonoids content.

Keywords: **flavonoids, CO_2 assimilation, water use-efficiency, Barke, Bojos**

Methodology of phenotypes selection of hemp (*Cannabis sativa* L.) for secondary metabolite production

Patrik Schreiber, Nikolas Balog

Hemp is a multipurpose plant which can be used in industry, food production and as a medicine. In medicinal *Cannabis* production, most important factor is content of cannabinoids such as THC (tetrahydrocannabinol), CBD (cannabidiol), CBG (cannabigerol), CBC (cannabichromene), CBL (cannabicyclol) and others. Because medicinal *Cannabis* production is production of pharmaceuticals it is very important to have very standardised product in terms of active compound content. Because of this reason most of medicinal *Cannabis* nowadays is produced from clones of stable selected phenotypes with certain levels of active compounds, mostly cannabinoids. Because it is industry such as any other there are also lots of parameters important for economic model of production facility such as yield, resistance against pests and diseases and many others. During this study we were able to successfully select phenotype dominant in CBG which will be further used for upcoming biological and biotechnological studies under code name G13. Selected phenotype shows unique content of Cannabinoids and was reaching content of CBG 13.57% and THC 0.16%. This selected phenotype may be also used as base material for breeding of hemp variety for Common European Catalogue of agricultural hemp cultivars registered for trading between different European Union countries.

Keywords: *Cannabis*, variety, cannabinoids, breeding

Effects of intermittent-direct-electric-current (IDC) on growth and content on photosynthetic pigments in hemp (*Cannabis sativa* L.)

Nikolas Balog, Tomas Vyhnanek, Petr Kalousek, Patrik Schreiber

Influence of electricity was studied in hemp plants. Impact on plant growth and physiological and yield parameters was investigated. Two different intensities (1500 mA and 2000 mA) was applied for 1 hour a day for total duration of 11 weeks. Height of the plant, stem diameter, biomass production and also yield of flowers was affected by treatment. Treatment had positive effect on content of photosynthetic pigments as chlorophylls and carotenoids.

Keywords: **hemp, electricity, chlorophyll, carotenoids, yield**

Auxin or Sugar? Which has higher impact on bud outgrowth regulation?

Attila Kucsera, Jozef Balla, Stanislav Prochazka

Apical dominance remains important phenomenon in plant physiology with a lot of questions to be answered. In this work we tried to find an answer, whether we should return to the nutrient hypothesis and ascribe a lesser role to auxin in regulation of apical dominance. 7-Day-old decapitated pea plantlets were used as a model system. The effect of sugar and auxin on bud outgrowth was studied by replacing the cotyledon with auxin or sucrose containing paste. Auxin flow was manipulated by wounding or auxin transport inhibitor (2,3,5-triiodobenzoic acid) application. The obtained results indicating that in young plantlets auxin and/or its flow had more pronounced effect on cotyledonary bud outgrowth regulation than the sucrose availability.

Keywords: **apical dominance, phytohormones, auxin, sucrose**

The role of expansins in the control of cell wall properties and root apical meristem development in *Arabidopsis*

Evelina Gahurova, Marketa Samalova, Jan Hejatko

The cell wall (CW) is an important structure of plants as it determines the shape of cells, enables cell connections and acts as a protective barrier against pathogens and the environment. The primary CW consists of polysaccharides cellulose and hemicellulose that provide the basic mechanical strength, and pectins that can alter the viscoelastic properties of the matrix. Expansins (EXPAs) are cell wall-loosening proteins activated during CW acidification triggered by a number of stimuli through the plasma membrane H^+-ATPase proton pump [1]. EXPAs are known to disrupt non-covalent bonds between CW polysaccharides but without structural changes of the CW. EXPAs do not possess a hydrolytic activity but they facilitate the CW loosening by mediating slippage of carbohydrate polymers at load-bearing elements of the CW [2]. However, the molecular mode of action of EXPAs that enables cell wall expansion remains elusive.

We propose that tightly controlled spatiotemporal specificity of expansin expression and hormonal-mediated pH distribution within the root apoplast plays an important regulatory role controlling the root growth and development in *Arabidopsis.* To investigate the possible role of α-expansin 1 (EXPA1), we overexpress *EXPA1* in individual tissues of the root apical meristem (RAM) using the chemically inducible transcription activation system pOp6/LhGR [3]. We follow *in vivo* growth changes using confocal laser scanning microscopy and investigate the role of cell type-specific *EXPA1* expression and pH in the dynamics of root growth and development.

Keywords: –

Natural genetic variability in multistep phosphorelay as a tool to enhance drought tolerance of plants

Katrina Leslie Nicolas, Jan Skalak, Jan Zouhar, Dusan Turek, Jan Hejatko

Facing the climate change associated with severe weather unpredictability and decreasing availability of arable land, securing the food production and resources for the ballooning world population is one of the biggest challenges of humankind to date. Improving plant productivity through the development of climate adaptable varieties via the use of advances in molecular breeding strategies and high-throughput phenotyping technologies is one of the promising avenues to overcome these limitations. Plants as sessile organisms evolved remarkable plasticity controlled by endogenous factors like plant hormones, and external environmental stimuli such as temperature, light and humidity to cope with changing environmental conditions. This study aims to identify natural genetic variants of key regulators which improve~~s~~ the integration of environmental and hormonal (cytokinin) signalling pathways in plants to assess their role as potential targets for developing climate adaptable variety of important crops. One of the key members of such a pathway is Arabidopsis histidine kinases (AHKs). Natural variability in three cytokinin-responsive histidine kinases AHK2, AHK3, AHK4/CRE1 was assayed via search in publicly available resources like 1001 Genomes. E~~e~~cotypes with SNPs found in close proximity with the site of known protein function were selected for further work. The role of the identified genetic variability was studied in relation to the responsiveness of MSP signalling to CKs using root-elongation assay and targeted transcriptomic analysis of CK signalling reporter genes. Accessions with either lower or higher CK sensitivity were identified. Further analysis of the root morphology upon exogenous CK treatment showed stronger reduction of root apical meristem size than the more responsive AHK variants. The size of the root apical meristem, previously shown to be CK-controlled, correlated well with the reduced root length of particular accession, supporting the observed altered CK sensitivity in selected accessions. A ligand-binding assay was also conducted and found no significant changes in the CK affinity to AHK4 variants, harbouring the SNP of ecotypes with increased CK sensitivity. This is implying possible effects of identified SNPs on the ability of AHK4 to transduce the signal down the pathway rather than the CK binding-mediated activation of the sensor. Detailed phenotypic characterization of the ecotypes was also performed to reveal how the plants with SNPs in particular AHK respond to the decreased or enhanced cytokinin signalling and how this affect their response to abiotic stress like drought. The information generated from this study will be used for the targeted improvement of *Brassica* sp. genome resulting in higher yield and better tolerance to different abiotic stress.

Keywords: cytokinin, AHKs, abiotic stress, cytokinin signalling