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Plenary lecture

PL4.1

Calcium and MAP kinase signaling in PAMP-triggered immunity

D. Scheel

Leibniz Institute of Plant Biochemistry, Department of Stress and Developmental Biology, Germany

Plants detect potential pathogens in their environment *via* pathogen-associated molecular patterns (PAMPs) that are recognized by plant plasma membrane receptors. Typical PAMPs include the bacterial flagellin-derived flg22 peptide, the elf18 peptide of the bacterial elongation factor EF-Tu, bacterial peptidoglycans and lipopolysaccharides, as well as fungal chitin oligomers and glucan fragments from oomycetes. PAMP-binding to their receptors initiates complex signaling networks that activate a multi-component defense response and thereby establish PAMP-triggered immunity. One of the earliest detectable responses after PAMP perception is the activation of ion channels and pumps at the plasma membrane. The resulting ion fluxes, which lead to a transient increase in cytosolic calcium, have been shown to be required for all other downstream responses, such as the activation of mitogen-activated protein kinases (MAPKs), production of reactive oxygen species and defense gene expression. Using a transgenic Arabidopsis line with the calcium reporter, aequorin, and rapid increases in cytosolic calcium levels are detected after PAMP application. To identify regulators of calcium homeostasis, seeds of aequorin-expressing lines were mutagenized and the population screened for mutants with *changed calcium elevation* (*cce*) in response to flg22 treatment. Many mutants in the flg22 receptor, in receptor complex components and in unknown signaling elements were isolated. MAPK cascades are essential not only for controling the defense response but also many developmental processes. The elements that prevent erroneous signaling crosstalk may include expression patterns of the MAPK components, the presence of pathway-specific protein complexes or the MAPK substrate diversity. Different strategies have been employed to isolate MAPK substrates and interacting proteins. Results of the functional analysis of several interacting proteins in the MAPK signal transduction pathway(s) will be presented.

PL4.2

Integrated plant and pathogen 'omics approaches to understanding fungal diseases of wheat

J.J. Rudd, W.-S. Lee, K. Hammond-Kosack, K. Kanyuka

Wheat Pathogenomics Group, Dept of Plant Biology and Crop Science, Rothamsted Research, Harpenden, Herts, UK

Plant infection by pathogenic microbes involves many dynamic changes in molecular communication and adaptation of host and pathogen physiology. Many such interactions form the basis of several devastating diseases of crop plants. We are studying *Zymoseptoria tritici* (*Zt*), also known as *Mycosphaerella graminicola* or *Septoria tritici*, which is a fungal pathogen of wheat leaves and the causal agent of *Septoria tritici* blotch disease. The interaction between *Zt* and wheat is also fast becoming a new model for molecular plant microbe interactions. *Zt* is an intercellular pathogen with a long symptomless period of leaf penetration (biotrophic or endophytic) lasting at least seven days post inoculation, prior to the formation of leaf lesions. We have defined distinct phases of this interaction and used multiple integrated 'omics approaches to study host and pathogen physiology throughout infection. This presentation will describe the interaction between *Zt* and wheat as determined through pathogen genome sequencing, comparative genomics and KO strain generation, though to pathogen and host deep RNA sequencing and functional genomic studies in wheat using virus-induced gene silencing technologies. The integrated output of these studies suggests a remarkable strategy for wheat leaf infection by *Zt*, which can be summarised in a "subterfuge followed by hijack" approach to the temporal manipulation of plant defence responses.

Oral presentation

O4.1

Interaction of plant beneficial and plant pathogenic bacteria with plants An example from *Serratia plymuthica* and *Dickeya solani* story in potato (*Solanum tuberosum* L.)

R. CZAJKOWSKI[1,2], J.M. VAN DER WOLF[2]

[1] Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdańsk, Poland

[2] Plant Research International, Wageningen, The Netherlands

Dickeya and *Pectobacterium* spp. are plant pathogenic bacteria responsible for soft rot and blackleg diseases in potato. In the past, *P. atrosepticum* was considered as a major blackleg and soft rot pathogen in Europe, whereas biovar 1 and 7 *Dickeya* spp. (*D. dianthicola*) strains isolated in Europe were considered of a secondary importance in the blackleg and soft rot etiology. Presently, new *Dickeya* spp. biovar 3 (*D. solani*) are associated with increasing losses in potato production worldwide. *D. solani* possess a higher growth temperature optimum and the elevated virulence in comparison with the *Pectobacterium atrosepticum*. *D. solani* was isolated from seed potatoes in The Netherlands, Finland, Poland, Sweden, France, Belgium, Georgia, Germany and Israel indicating that it is widely spread in Europe. All these isolates were clonal, demonstrating its common origin and possibly one introduction event. Studies on the distribution of a *D. solani* strain in potato tubers indicated that the pathogen was located mainly inside tubers at stolon ends and rarely located in the peel indicating a vascular origin of the pathogen. In line, we found that a GFP-tagged *D. solani* strain systemically colonized progeny tubers via the roots after soil infestation and was able to produce blackleg symptoms. Systemic colonization of plants including roots, stolons and progeny tubers was also found after injection of the GFP strain into potato stems. A biovar 7 *D. dianthicola* was not able to colonize the plants effectively after root or stem inoculation. Potato leaves inoculation with a GFP-tagged *D. solani* strain, showed degradation of the inoculated plant material and spreading of the internal inoculum to the petiole and axil and finally to the main stem. For biological control of *D. solani* in potato we characterized a *Serratia plymuthica* strain A30, an endophyte isolated from rotting potato tuber tissue. Its antagonism is based on antibiosis and requires a direct contact between the pathogen and the control agent. In a potato slice assay, strain A30 eliminated the pathogen and prevented potato tissue maceration by *D. solani* when inoculated in densities at least 100 times higher than the pathogen. To study the interaction between *S. plymuthica* A30 and *D. solani in planta*, fluorescent protein tagged strains were exploited. In repeated greenhouse experiments, a tuber treatment with strain A30 protected potato plants against *D. solani* effectively, resulting in a decrease in the incidence of stem infection of, on average, 97%. Using confocal laser scanning microscopy, the antagonist could be traced in vascular and parenchymatic tissue of tubers, roots and stems at least till 28 days after planting. Results indicated that *S. plymuthica* A30 out-competed *D. solani in planta*. The use of *S. plymuthica* A30 to control *D. solani* in potato has been patented. Overall, our results give an insight on mechanisms by which plant pathogenic and plant beneficial bacteria interact *in planta*.

O4.2

Function of glutathione in *Arabidopsis* immunity and glucosinolate metabolism

M. Piślewska-Bednarek[1], K. Hiruma[2], Y. Takano[3], A. Molina[4], P. Bednarek[1]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[2] Kyoto University, Japan and Max Planck Institute for Plant Breeding Research, Cologne, Germany

[3] Kyoto University, Japan

[4] Centre for Plant Biotechnology and Genomics, Madrid, Spain

Induced defense responses in plants usually involve cell polarization, comprising rearrangement of actin cytoskeleton, directed movement of particular organelles and targeted secretion at the site of pathogen contact. This may also include trafficking and secretion of antimicrobial compounds. Our recent study on the model plant *Arabidopsis thaliana* revealed a novel pathogen triggered metabolism pathway for glucosinolates, amino acid-derived thio-glucosides characteristic for crucifer plants that so far were mainly known as insect deterrents (Bednarek et al., 2009). This pathway requires at least two enzymatic components: CYP81F2 P450 monooxygenase and PEN2-myrosinase. CYP81F2 is essential for the pathogen-induced accumulation of 4-methoxyindol-3-ylmethyl glucosinolate, which in turn is activated by PEN2 for antifungal defense. In addition, our analysis suggested contribution of glutathione to the PEN2/CYP81F2-defence pathway (Bednarek et al., 2009). Here we report on the involvement of glutathione, indole glucosinolates and other tryptophan-derived metabolites to the immunity towards the non-adapted hemibiotrophic pathogen *Colletotrichum gloeosporioides*. In addition, we provide evidence that glutathione transferase constitute an indispensable component of the PEN2 immune pathway.

O4.3

Effects of endogenous signals and *F. oxysporum* on the mechanism regulating genistein synthesis and accumulation in yellow lupine and their impact on plant cell cytoskeleton

I. Morkunas[1], M. Formela[1], Ł. Marczak[2], S. Samardakiewicz[3], A. Kasprowicz[4], P. Wojtaszek[4]

[1] Department of Plant Physiology, Poznan University of Life Sciences, Poznań, Poland

[2] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[3] Laboratory of Electron and Confocal Microscopy, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

[4] Department of Molecular and Cellular Biology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

Sugars such as sucrose, glucose and fructose are not only donors of carbon skeletons for secondary metabolism, including the phenylpropanoid pathway via which genistein is synthesised, but they are also signalling molecules inducing expression of genes coding enzymes involved in isoflavone synthesis. Signal transduction pathways triggered by carbohydrates may be engaged in cross-talk with other signalling pathways, e.g. the salicylic acid or nitric oxide pathways, and enhance defence responses of plants against pathogenic fungi. Thus an increase in the endogenous carbohydrate level enhanced the accumulation of isoflavones, including genistein, in cells of infected embryo axes of *Lupinus luteus* L. cv. Juno. It was found that sucrose may act as an antioxidant, playing an important role of a free radical scavenger in tissues infected with *F. oxysporum*. Observations of actin and tubulin cytoskeletons in cells of infected embryo axes cultured on the medium with sucrose (with an increased genistein level) as well as the medium without sugar showed significant differences in their organisation. In cells of embryo axes cultured at a carbohydrate deficit (with a low level of genistein) a partial breakdown of the actin cytoskeleton was observed in relation to axes with an increased sugar level. The actin cytoskeleton in cells of non-inoculated axes with a high endogenous level of sucrose (+Sn) was formed of long and thick actin microfilament cables surrounding the cells and their branches, creating a dense microfilament meshwork extending in various directions. A particularly high accumulation of actin

was observed in the vicinity of cell nuclei. Additionally, when cells were inoculated with *F. oxysporum* (+Si) bundles of microfilaments were observed to thicken and fluorescence intensity increased in relation to +Sn cells. In turn, in cells inoculated with carbohydrate deficit (–Si) the greatest changes were observed in the actin cytoskeleton, i.e. a shortening of length of all forms of microfilament bundles and the meshwork of microfilament bundles was fragmented. Observations of the tubulin cytoskeleton in –Si also showed numerous disorders, i.e. a decline or a considerable reduction of the number of microtubules, a significant shortening of length (fragmentation) of microtubules and the diffusive character of fluorescence. This study was supported by the Polish Ministry of Science and Higher Education (MNiSW, grant no. N N303 414437).

O4.4

Identification of the sequences undergoing differential regulation during the interaction of *BNYVV*-beet in different rhizomania resistance sources

A. Litwiniec, M. Gośka

Plant Breeding and Acclimatization Institute – National Research Institute Radzików, Research Division in Bydgoszcz, Department of Genetics and Breeding of Root Crops, Laboratory of Biotechnology, Bydgoszcz, Poland

Rhizomania is a disease of beet caused by *Beet necrotic yellow vein virus*. Production and cultivation of resistant cultivars is the only efficient way of protection from disease spread and significant damages to the crop. New resistance sources are identified within wild crop relatives, being especially valuable in face of occurrence of resistance-breaking pathotypes of the virus. The aim of this work was to detect some elements that are differentially regulated in a host-pathogen interaction between different sources of resistance/susceptibility of wild beet accessions. A model population comprising selected gene bank accessions was designed and screened for the presence of hypothetical crucial components of the interaction by PCR. DNA laddering was used to estimate the extent/mode of cell death. Considerable differences were recognized between highly resistant and moderately resistant/susceptible groups, especially in the amount/structure of some suppressor molecules and their cellular targets, as well as in vector-transmission mediating molecules. Concurrently, the DNA laddering experiment suggests the induction of a necrotic phenotype among all the accessions studied, which may be related rather to symptom development than to an active hypersensitivity response. The results thus imply that the major outcome of the host response is determined by interplay between silencing and suppressor mechanisms.

Posters

P4.1

Immunolocalization of extensin in Al-treated pea root nodules

M. Sujkowska-Rybkowska

Department of Botany, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

Apoplast plays the major role in aluminum (Al) tolerance. Pea (*Pisum sativum* L.) root nodules were exposed to Al stress and extensins were localized using immunofluorescence and immunogold labeling with monoclonal antibody (LM3). Root nodule extensins are highly glycosylated plant glycoproteins localized in the extracellular matrix of legume tissues and in the lumen of *Rhizobium*-induced infection threads (IT). Al-treated nodules showed alterations in histological and ultrastructural differentiation. Al inhibited IT growth and development patterns as well as caused disturbances in bacterial release. The extensin epitope was present in the cell walls of nodule cortex, intercellular spaces of nodule parenchyma and also in the lumen of ITs. Al treatment resulted in the abundance of the epitope at the above locations. Presented work shows that Al modifies the composition of cell walls and thus makes them thick and rigid, thereby inhibiting the growth of IT and development of pea root nodules. The possible role of extensin in the plant-mediated control of nodule and infection thread growth was discussed.

P4.2

Tobacco rattle virus (TRV) PSG influence on *Capsicum annuum* and *Nicotiana tabacum* generative organs

K. Otulak, G. Garbaczewska

Faculty of Agriculture and Biology, Department of Botany, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

Transmission of the virus between generations in the seeds takes place in about 20% plant viruses. Transmission involves infection of the embryo and can occur in two ways: directly through the mother plant or indirectly via pollen or the ovule during fertilization. The aim of our study was examination of ultrastructural changes in *Nicotiana tabacum* cv. *Samsun* and *Capsicum annuum* cv. *Y. Wonder* generative organs as a result of TRV infection. We observed tobacco and pepper leaf deformations, systemic necrosis and leaflets chlorosis 15 days after TRV mechanical infection. Infected plants are able to form flowers and fruits, but its amount decreased in comparison to healthy plants. In developing flower buds we observed local necrosis in receptacle. Submicroscopic observations of *Capsicum* and *Nicotiana* flowers revealed sepals and petals parenchyma local necrosis. Electron microscopic examination of flower buds infected with TRV showed necrosis in receptacles vascular bundles, TRV dispersed particles were noticed in phloem (especially sieve elements and phloem parenchyma) and parenchyma. Neither in tobacco nor in capsicum ovules noticed anatomical changes as a result of TRV infection. Ultrastructural ovaries analyses revealed dispersed TRV particles in xylem parenchyma and organized particle inclusions in parenchyma cells of ovaries walls. TRV particles of different lengths were visualized in cytoplasm and vacuole in ovules nucellus and integuments cells in pepper. We didn't observe TRV particles inclusion in embryo sacs and in dividing ovules cells. Much more significant changes were noticed in *Capsicum* anthers. Anatomical stamen investigations indicated a lot of pollen grains in degenerated state. Cells of pepper anther wall were strongly deformated, protoplasts necrotized and virus particles were usually presented in xylem vessels and/or phloem parenchyma. TRV particles of two lengths formed large clusters inside osmophilic masses in the rest of tapetum and were dispersed in endothecium cells in mature anthers. Shortly after microspore release from the tetrad and before mitosis, tapetal cells began to degenerate, which became less distinct between adjacent cells and rupture. On the exine of pollen grains surface virions were very often observed, especially in exine pores areas. The TRV particles were also placed inside pepper and tobacco pollen grains in small vacuoles. Immunogold labeling of TRV PSG capsid protein C-terminal part indicated that CP-TRV was localized inside and on pollen grains. The CLKSYYRRNFEKNF epitope, specific for TRV PSG C-terminal capsid protein region was detected in anther endothecium cells and in the rest of tapetum masses. The observations that pollen grains can be contaminated with TRV and virus particles are presented in ovaries are of interest because it suggested a route by which certain mechanically transmissible viruses may be spread in the natural state.

P4.3

Interference of nematode parasitism by silencing of plant genes

M. Koter[1], M. Matuszkiewicz[1], Ł. Baranowski[2], M. Sobczak[2], A. Wiśniewska[3], J. Dąbrowska-Bronk[1], M. Święcicka[1], W. Skowron[1], M. Filipecki[1]

[1] Department of Plant Genetics Breeding and Biotechnology, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

[2] Department of Botany, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

[3] Department of Plant Physiology, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

Plant cyst nematodes are common pests of many crops causing substantial losses in agriculture. To control Plant Parasitic Nematodes environmentally harmful nematocides or crop rotation is used. A reasonable alternative is silencing of nematode or host plant genes crucial for pathogenesis. From the previously identified 135 tomato genes being up-regulated during *Globodera rostochiensis* migration and syncytium development, we focus on *NIK, NGB* and *NAB.* NIK is a kinase induced by nematodes, NGB is a nucleolar GTP-binding protein and NAB binds auxine.

Transcripts of *NGB* were *in situ* localized only in young syncytia while transcripts of *NAB* were found in 3-14 dpi syncytia and in surrounding cells. The regulatory regions of studied genes were cloned upstream the *uidA* reporter gene and analyzed in tomato and potato roots showing several changes in expression profiles upon infection. Functional analysis was supplemented by the RNAi of selected genes. Silencing of *NAB* or *NGB* genes slightly decreased plant fertility and changed fruit or leaf morphology. This was accompanied by changes in expression of some genes related to auxin and biotic stress signalling. The number of *G. rostochiensis* females was reduced by 57-86% in *in vitro* tests and by 30-46% in pot trials. The observations of the development and ultrastructure of syncytia induced in transgenic lines revealed retarded growth, electron translucent cytoplasm, smaller vacuoles, and reduced number of plastids, mitochondria and ER structures. These results demonstrate that *NGB* and *NAB* genes play an important role in the development of syncytia and link nematode pathogenesis to ribosome biogenesis and auxin function. This work was supported by National Science Centre (grants no. NN302-593938 and 2012/07/ B/NZ9/02027).

P4.4

Combining plant extracts from *in vitro* culture of *Drosera binata* and silver nanoparticles to combat *Staphylococcus aureus*

M. KRYCHOWIAK[1], A. KAWIAK[1,2], R. BANASIUK[1], M. KRAUZE-BARANOWSKA[3], A. KRÓLICKA[1]

[1] Department of Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland

[2] Laboratory of Human Physiology, Medical University of Gdansk, Poland

[3] Department of Pharmacognosy with Medicinal Plant Garden, Medical University of Gdansk, Poland

Drosera binata is a carnivorous plant with an invaluable therapeutic potential in the treatment of infectious diseases due to synthesis of secondary metabolites such as naphtoquinones and flavonoids. Plant tissue of *D. binata* was grown on 1/2 Murashige & Skoog medium with 2% sucrose with or without activated carbon. Biotic (lysate of *Agrobacterium rhizogenes*) and abiotic (jasmonic acid) elicitors were used in order to increase the yield of secondary metabolites in *in vitro* culture of *D. binata*. Chloroform extracts from *D. binata* tissue as well as silver nanoparticles (AgNPs) were used either alone or were combined in order to combat *Staphylococcus aureus*. *S. aureus* is a common gramm positive pathogenic bacterium, whose strains pose a significant problem in nosocomial infections, especially burn wound infections. This organism can penetrate the eschar and invade the intact, underlying subcutaneous tissue to form abscesses of varying sizes. AgNPs are particles of metallic silver of different shapes and sizes (1-100 nm). They exhibit antibacterial, antifungal, and antiviral activity due to their high surface-volume ratio. The aim of this study was to establish the precise proportion of *D. binata* extracts and metallic silver nanoparticles which would prove to be most effective in its antibacterial activity silver. The nanoparticles which are soluble in water are coated with $HS\text{-}(CH_2)_{11}\text{-}N(CH_3)_{3+}$ ligand with average dimension 5.5 and dispersity level 15% were provided by ProChimia Surfaces Co. (Poland). The antibacterial activity tests were conducted on planktonic cultures of *S. aureus* strains: (MRSA 43300 and 703k) obtained from the Laboratory of Microbiology of the Provincial Hospital in Gdansk, Poland, and *S. aureus* Newman strain was used as a reference. The established Minimum Bactericidal Concentration (MBC – concentration which reduces the number of microorganisms by 99.9% or 3 logarithms) enabled the subsequent utilization of the Checkerboard Titration Method. Fractional Bactericidal Index (FBC) was calculated for each combination of the two compounds [FBC index = $A/MBC_A + B/MBC_B$, where MBC_A and MBC_B are the MBCs of AgNPs and the extract separately while A and B are values obtained from the combination of these two compounds]. Based on the obtained FBC the following types of effects can be identified: synergistic (FBC < 0.5), additive (1 > FBC > 0.5), neutral (2 > FBC > 1), or antagonistic (FBC > 2). Our results indicate that combining the *D. binata* extract (containing plumbagin, elagic acid, isorhamnetin and hyperoside in very low concentration) and AgNPs results in their additive (nearly synergistic) mode of action, and significantly reduces the MBC values to 97% and 50% for extract and AgNPs respectively. These results show the potential application of *D. binata* plants grown *in vitro* and AgNPs in the pharmaceutical industry. This work was supported by the LIDER/32/36/L-2/10/NCBiR/2011 grant.

P4.5

Localization of (homo)glutathione in effective and partially effective root nodules of *Medicago truncatula* root nodules

M. Bederska, W. Borucki

Department of Botany, Faculty of Agriculture and Biology, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

In root nodules of leguminous plants such as *Medicago truncatula*, N_2 is fixed by rhizobial bacteroids within infected cells. The nodule is a complex organ consisting of a cortex and an inner central region. The central region of the mature nodules is composed of infected and uninfected cells. The vascular bundles stretch for the length of nodules and reach the death end in nodule meristem. The symbiotic interaction starts with the colonization of the roots of the leguminous host after molecular dialogue between symbionts. In the first steps of the Rhizobium-legume symbiosis different reactive oxygen species (ROS) are generated. The oxidative burst during symbiotic infection is involved in one hand in plant-pathogen defense response, in the other, in expressing bacterial genes which are essential for the nodulation process. GSH (γ-Glu-Cys-Gly) is a low molecular mass thiol tripeptide implicated in the antioxidant defense through the ascorbate/GSH cycle and is able to scavenge ROS directly. It plays a crucial role in plant defense against stresses (biotic and abiotic), heavy metal tolerance and xenobiotic detoxification. One of the characteristics of legumes is the present of hGSH (γ-Glu-Cys-βAla), a homologue of GSH, which is present or/in addition to GSH. In *M. truncatula* hGSH was detected in roots and root nodules whereas GSH through the whole plant. The highest level of hGSH/GSH is observed in the infected zone. The quatitatively and quantitavely study of (h)GSH contributes to the understanding of its role in nodule effectineness. The aim of this work was investigated by histochemical localization of (h)GSH in effective and partially effective root nodules. *Medicago truncatula,* a model plants for legumes, were grown in sterile perlite and inoculated with the suspension of *Sinorhizobium medicae* WSM 419 (fully effective strain) or *Sinorhizobium meliloti* 1021 (partially effective strain). Root nodules were harvested 42 days after inoculation. Fresh longitudinal and cross sections were labeled with MCB (monochlorobimane) and observed under confocal laser scanning microscope (CLSM) to quantify (h)GSH in different types of cells of *M. truncatula*. The observed fluorescence comes from the GSB (glutathione S-bimane conjugate). The sodium azide was added to block the GSB transportation into vacuole. Little is known about how nodule effectiveness is correlated with the level of (homo)glutathione. The investigation facilitated the localization of (h)GSH in different types of nodule cells and even their compartments. This information will be use to discuss possible physiological role of (h)GSH in establishment and maintaining of effective symbiose. It is well understood that (h)GSH plays important role in maintaining of symbiose.

P4.6

The influence of selenium on antifungal activity of extracts of *Codonopsis pilosula* hairy roots

J. Janas, D. Bogusz, M. Wielanek, M. Skłodowska

Department of Plant Physiology and Biochemistry, University of Lodz, Łódź, Poland

Plant secondary metabolites play important role in plant-pathogen interactions. Selenium is not essential for plant existence, but in small amount can increase plant vitality and their pathogen resistance. Transformed root cultures of Asia-derived *Codonopsis pilosula* are characterized by rapid biomass growth and high potential to synthesize antimicrobial secondary metabolites, including polysaccharides, phenolic compounds and saponins. Moreover, because of high sulfur and selenium similarity selenization of plant cultures may lead to the biosynthesis of bioactive metabolites, which normally do not exist in native plants. The aim of this work was to examine the ability of extracts obtained from both selenium-treated and non-treated *C. pilosula* hairy root cultures to inhibit the radial growth of the *Botrytis cinerea, Fusarium culmorum* and *Fusarium avenaceum* mycelia. *C. pilosula* hairy roots were grown in

parallel in the darkness and in the light on the media with standard (1S) and with reduced sulfur (S6+) concentration (1/4S). The roots cultured on the 1/4S medium were treated with the appropriate volume of selenium solution to obtain the final Se6+ concentration of 1 mM. Extracts were obtained by homogenization the roots in methanol, acetone or water, in a ratio of 1:10. In the extracts selenium, polysaccharides, total phenols and saponin contents were determined. The biological activity of the extracts was examined by adding 1 cm^3 of the extract to the medium optimal for mycelia growth. After 7 days, the diameter of the *B. cinerea*, *F. avenaceum* and *F. culmorum* mycelia was measured and the efficiency of the mycelia radial growth inhibition was estimated. The methanol and acetone extracts obtained from both selenium-treated and non-treated hairy roots had the highest concentration of the metabolites that inhibited the growth of *B. cinerea* mycelium. *F. culmorum* and *F. avenaceum* exhibited relatively low level of sensitivity to the extracts examined in the bioassays. Se-containing methanol and acetone extracts obtained from the Se-treated *C. pilosula* root cultures stronger inhibited the radial growth of the mycelia than extracts without selenium. The *C. pilosula* transformed root cultures may be used as a source of natural compounds protecting plants against *Botrytis cinerea*. The treatment of the *C. pilosula* cultures with selenium may increase the biocidal effect of the hairy roots extracts against the fungi.

P4.7

The influence of selenium on antifungal activity of secondary metabolites of transformed root cultures of *Tropaeolum majus, Schkuhria pinnata* and *Saussurea nepalensis*

D. Bogusz, J. Janas, M. Wielanek, M. Skłodowska

Department of Plant Physiology and Biochemistry, University of Lodz, Łódź, Poland

Transformed root cultures of *Tropaeolum majus, Schkuhria pinnata* and *Saussurea nepalensis* synthesize sulphur-containg secondary metabolites, i.e. glucosinolates and thiarubrines. Due to the high physico-chemical similarity, selenium enters the sulfur metabolism pathway which leads to the biosynthesis of metabolites, which normally do not exist in plants, including selenium analogues of the sulfur secondary metabolites. Because of rapid biomass growth and high potential to perform reactions of biosynthesis and biotransformation, transformed roots are a very good form of *in vitro* cultures as a rich source of several bioactive secondary plant metabolites. The aim of this work was to examine the ability of the metabolites synthesized by *T. majus, S. pinnata* and *S. nepalensis* transformed root cultures and their selenium analogues to inhibit the radial growth of *Botrytis cinerea, Fusarium culmorum* and *Fusarium avenaceum* mycelia. The transformed roots were grown in parallel in the darkness and in the light on the media with standard (1S) and with reduced sulfur (S6+) concentration (1/4S). The roots cultured on the 1/4S medium were treated with the appropriate volume of selenium solution to obtain the final Se6+ concentration of 2 mM for the *T. majus* roots and 1 mM for the *S. pinnata* and *S. nepalensis* roots. In order to obtain the methanol, acetone and water extracts 30-day-old roots were homogenized in a ratio of 1:10. In the obtained extracts selenium, glucosinolates and thiarubrines contents were determined. The biological activity of the extracts was examined by adding 0.5 or 1 cm^3 of the extract to the medium optimal for fungi mycelium growth. After 7 days, the diameter of the *B. cinerea*, *F. avenaceum* and *F. culmorum* mycelia was measured and the efficiency of the mycelia radial growth inhibition was estimated. The methanol extracts, obtained from both selenium-treated and non-treated transformed roots, had the highest concentration of the metabolites that inhibited the radial growth of the tested fungi. The strongest inhibition of the growth was found in bioassays on the media containing metabolites from *T. majus* extracts. Se-analogues of the metabolites presented in the extracts from Se-treated *S. nepalensis* and *S. pinnata* transformed roots stronger inhibited the radial growth of the mycelia than the extracts without selenium. The cultures of the transformed roots of the *S. pinnata, S. nepalensis* and *T. majus* may be used as a source of natural compounds, which can protect plants against pathogens, including *Botrytis* and *Fusarium* species. The selenization of the *S. pinnata* and *S. nepalensis* cultures may increase the biocidal effect of the transformed roots extracts against the fungi.

P4.8

Medicago truncatula ABC transporter modulates nodulation efficiency

K. JARZYNIAK[1], J. BANASIAK[2], A. SZEWCZAK[3], M. JASIŃSKI[1,2]

[1] Department of Biochemistry and Biotechnology, Poznań University of Life Sciences, Poland

[2] Department of Natural Products Biochemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[3] Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

Legume plants have a unique capacity to interact symbiotically with nitrogen- xing soil bacteria known as rhizobia. This intimate association results in the formation of root nodules that provide an environment suitable for atmospheric nitrogen conversion into a reduced form readily assimilable by a plant. A key event of the infection process required for nodule organogenesis is activation of the cytokinin signaling pathway in the root cortex, leading to the suppression of polar auxin transport and cortical cell division. However, elucidation of the mechanism that allows localized cytokinin transport/signaling in the inner root tissues remains elusive (Oldroyd et al., 2011). A major function fulfilled by ATP-binding cassette (ABC) proteins is transmembrane translocation of great variety of molecules. Mounting evidence suggests that full-size ABCG transporters could be responsible for transport of signaling molecules, crucial for successful symbiosis between legumes and rhizobia (Sugiyama et al., 2007). Previously, we have identified and classified full-size ABC transporters from the G subfamily in *Medicago truncatula* (Jasinski et al., 2009). Here we present a novel full-size MtABCG20 transporter and we address a question about its putative role in the modulation of nitrogen_fixing symbiosis. The conducted sqRT-PCR analysis revealed that the expression of *MtABCG20* is strongly up-regulated during inoculation with *Sinorhizobium meliloti*. Concomitantly, the *MtABCG20* mRNA accumulated upon cytokinin treatment. As far as nodulation process is concerned, it is noteworthy that *MtABCG20* gene showed organ_specific expression and was found only in the roots. Further investigation revealed *MtABCG20* promoter activity in the root cortex. Interestingly, a similar expression pattern was observed for a *M. truncatula CRE1,* the cytokinin receptor necessary for the initiation of the nodule primordia (Lohar et al., 2006). We observed that silencing of *MtABCG20* expression significantly impaired nodulation efficiency in comparison with wild-type control. The presented data provide a foundation for further studies on the role of MtABCG20 transporter in symbiotic interactions.

P4.9

A full-size ABCG transporter modulates the level of isoflavonoids in *Medicago truncatula*

W. BIAŁA[1,2], J. BANASIAK[2], M. JASIŃSKI[1,2]

[1] Department of Biochemistry and Biotechnology, Poznań University of Life Sciences, Poland

[2] Department of Natural Products Biochemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Full-size ABC proteins belonging to the ABCG subfamily were identified mainly in plants and fungi. They are influencing different physiological processes, and play a particular role in defense response to biotic and abiotic stresses. There is growing number of evidence that ABCG transporters could be responsible for transport of antifungal, antimicrobial secondary metabolites and signaling molecules (Goossens et al., 2003). The ABCG10 from *Medicago truncatula* was proposed as a modulator of isoflavonoid levels during the defense response associated with *de novo* synthesis of medicarpin (Banasiak et al., 2013). Expression analyses revealed that *MtABCG10* transcript is presented in the vascular tissue of different organs and the corresponding protein has been found in the plasma membrane. Treatment of roots with fungal cell wall oligosaccharides (general elicitor) resulted in a strong induction of *MtABCG10* expression together with genes coding enzymes from phenylpropanoid pathway namely: phenylalanine ammonia – lyase (PAL) and isoflavone synthase (IFS). Silencing of *MtABCG10* in Medicago hairy roots resulted in lower accumulation of phenolic compounds, among them were precursors of Medicago phytoalexin medicarpin.

Interestingly exogenous application of such precursors as liquiritigenin and isoliquiritigenin resulted in the induction of *MtABCG10* expression. Loading/transport experiment performed with liquiritigenin and isoliquiritigenin in *MtABCG10* silenced hairy roots has shown a significant differences in the transport efficiency of these compounds between wild type and *MtABCG10* silenced lines. We postulate that MtABCG10 is a transporter of liquiritigenin and isoliquiritgenin free aglycones.

P4.10

Secondary metabolites in the response of model *Brassicaceae* species to *Plectosphaerella cucumerina*

K. Kułak, P. Bednarek

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Plant secondary metabolites are diversified group of compounds, from which many are crucial for plant responses to the environmental stresses, including pathogen attack. Model plant *Arabidopsis thaliana* synthesizes and accumulates constitutively tryptophan (Trp)-derived β_thioglucosides known as indole glucosinolates (IGs). Recent studies revealed that IGs metabolism is triggered by number of fungal and oomycete pathogens, and is essential for the pre-invasive defence of *A. thaliana* (Bednarek, 2012). Furthermore, infection of this model species leads to biosynthesis of other Trp-derived secondary metabolites essential for plant immunity, including camalexin and indole-3-carboxylic acid derivatives. In order to study Trp metabolism conservation between *A. thaliana* and its relatives – *Capsella rubella, Cardamine hirsuta* and *Arabis alpina*, we performed LC/UV/MS metabolite profiling of leaf extracts from plants inoculated with two isolates of the necrotrophic ascomycete pathogen *Plectosphaerella cucumerina* (adapted and non-adapted on *A. thaliana*). We also carried out bioinformatic analysis of accessible genomes of *A. thaliana* relatives, which included identification of putative orthologs of *A. thaliana* genes encoding enzymes involved in pathogen-triggered Trp metabolism. In addition, we corroborated the above mentioned results with RT-PCR transcriptome analysis of selected putative ortholog genes from *C. rubella* genome. Our studies has shown only partial conservation of Trp-metabolism between *A. thaliana* and another tested species, which suggests diversification of Trp secondary metabolism pathways within *Brassicaceae* family.

P4.11

Components of defence strategy induced in *Solanum* species by elicitor from *Phytophthora infestans*

L. Polkowska-Kowalczyk, K. Olszak, J. Tarwacka, J. Szczegielniak, G. Muszyńska, B. Wielgat

Department of Plant Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland

Plants have evolved a complex defence network providing a protection against different stresses. Among components of plant signal transduction pathways, protein kinases and reactive oxygen species (ROS) play a significant role in pathogen defence responses. The ROS produced are assumed to play key role in the integration of diverse strategies leading to disease resistance and may serve as antimicrobial agents. Mitogen-activated protein kinases (MAPKs) cascades and calcium-dependent protein kinases (CDPKs) are crucial components of plants signalling network to defend against numerous potential pathogens. The proteins phosphorylated by protein kinases are involved in gene expression, signalling pathways, ion and water transport through membranes, metabolism and function of cytoskeleton. Changes in MAPKs and CDPKs activities as well as their expression profiles and ROS production were investigated in leaves of *Solanum tuberosum* cv Bzura, *S. tuberosum* clone H-8105 and *S. nigrum* var. *gigantea* that exhibited field resistance, susceptibility and non-host resistance, respectively, in response to the *Phytophthora infestans*, the pathogenic oomycete that causes late blight, the most destructive potato disease. Leaves of *Solanum* species were treated with elicitor (culture filtrate of *P. infestans*, CF). Activities of protein kinases were determined

using "in gel kinase assay". The expression levels of MAPKs and CDPKs were measured by method of RT-PCR. ROS production was estimated using nitroblue tetrazolium. After elicitor treatment, the H-8105 showed the highest increase in the ROS production in comparison with *S. nigrum* var. *gigantea* and Bzura. MAPK and CDPK activities increased in response to elicitor treatment were positively correlated with the level of plant resistance, however varied with respect to intensity and timing. Moreover, we have demonstrated that transcripts of MAPKs and CDPKs are present in all studied *Solanum* species, although only transcript level of CDPKs increased after elicitor treatment. The obtained results widen the knowledge about defence mechanism occurring in *Solanum* species in response to *P. infestans*. This work was partially supported by the National Science Centre, Project 2012/05/B/NZ3/00911.

P4.12

Activity of selected antioxidant enzymes in *Quercus robur* L. leaves infected with *Microsphaera alphitoides*

M. Skwarek, J. Patykowski, A. Nowogórska

Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland

In recent years, the increase in pathogen-induced plant infections has been observed. Trees and bushes are attacked by various species of fungi which cause powdery mildew. This disease was dragged into Europe from North America in the early twentieth century. Among the trees powdery mildew infects particularly oaks, maples and beeches, thus it is well-known to the Polish foresters. Oak powdery mildew caused by a species of fungus – *Microsphaera alphitoides* is the most common. Fungi which cause powdery mildew belong to a division of ascomycetes (*Ascomycota*). Infected plants display white powdery spots on leaves and stems which are characteristic features of the disease. *Microsphaera alphitoides* are obligatory parasites, which can only develop on living organisms. Oak powdery mildew is rarely a problem for large, individual trees but it causes significant damage in forest nurseries. The largest pathological changes were observed on young leaves which grow in early spring. Susceptibility of mature oak leaves to pathogen infection is much lower. The aim of the study was to determine the activities of selected antioxidant enzymes such as peroxidase (POD) and superoxide dismutase (SOD) in the leaves of oak (*Quercus robur* L.) infected with powdery mildew. A significant increase in the activity of POD and SOD was observed in cells of oak leaf, in which the powdery mildew infected area exceeded 50%. Using gel poliacrylamide electrophoresis diversified forms of POD and SOD in comparison to the control leaves were observed. In the tissues infected with the powdery mildew, new forms of POD were indetified. Increase in the activity of POD and SOD indicated that in the cells infected with powdery mildew changes in antioxidant system were initiated. Particularly, increase in SOD activity, an enzyme that catalyzes the formation of hydrogen peroxide toxic to the pathogen, is the evidence of defensive reactions.

P4.13

Involvement of ascorbate, glutathione and protein S-thiolation in benzothiadiazole-inducible defense response of cucumber against *Pseudomonas syringae* pv. *lachrymans*

E. Kuźniak[1], T. Kopczewski[1], R. Głowacki[2], G. Chwatko[2], E. Gajewska[1], M. Wielanek[1], M. Skłodowska[1]

[1] Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland

[2] Department of Environmental Chemistry, Faculty of Chemistry, University of Lodz, Łódź, Poland

Ascorbate- and glutathione-based redox signalling mediates many processes in plant cells, including defence response to biotic stress. Oxidative modifications of redox-sensitive protein cysteines, called protein S-thiolation, have now emerged as a mechanism protecting protein thiols from being oxidized irreversibly, controlling protein function

and regulating the signalling and metabolic pathways. Due to the abundance of glutathione, S-glutathionylation is by far the major form of S-thiolation in plant cells. An inducer of acquired disease resistance in plants, benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), has been shown to enhance plant's defensive capacity only after infection by priming of pathogen-induced genes. However, the mechanism underlying this action remains to be elucidated. We studied the involvement of ascorbate, glutathione and protein S-thiolation in BTH-mediated response of cucumber to *Pseudomonas syringae* pv. *lachrymans* (*Psl*) infection. Hydroponically grown cucumber plants were sprayed with 0.1 mM BTH and after 7 days inoculated with *Psl*. The contents of ascorbate (bipyridyl method), glutathione as well as of cysteine, glutathionylated and cysteinylated proteins (HPLC) were determined in inoculated and systemic (non-inoculated) leaves 2 and 7 days after inoculation. BTH changed the redox state of ascorbate and glutathione pools, and induced accumulation of cystine as well as of glutathionylated and cysteinylated proteins. The most pronounced infection-induced effect observed in BTH-primed plants, both in the inoculated and systemic leaves, was the significantly decreased content of glutathionylated proteins in comparison to non-primed plants. Moreover, BTH-primed and non-primed plants differed with respect to the dynamics of *Psl*-induced changes in the contents of cysteine/cystine as well as glutathionylated and cysteinylated proteins in the inoculated leaves and in the systemic ones. The results suggest that the activation of a priming state in BTH-treated plants could be mediated by redox signalling related to glutathione and protein S-thiolation. Supported by Grant No N N310 302339.

P4.14

Polish strains of *Trichoderma* in cucumber plant growth promotion and induction of defence reaction against *Rhizoctonia solani*

J. Nawrocka[1], U. Małolepsza[1], M. Szczech[2]

[1] Department of Plant Physiology and Biochemistry, University of Lodz, Łódź, Poland

[2] Horticulture Research Institute, Skierniewice, Poland

Trichoderma species includes many agriculturally important strains known as effective biological control agents which may stimulate plant growth and biomass production. The main interest has recently focused around their potential ability to induce defense reactions and systemic resistance in plants; the changes in the activity of enzymes involved in the synthesis of biologically active substances such as phenolic compounds are strongly emphasized in the process. The main objective of this study was to determine the impact of selected Polish *Trichoderma* strains added to the plant growing medium on seed germination and growth of cucumber plants (*Cucumis sativus*) cv. Śremski. Furthermore, the effect of selected strains on induction of plant defense to infection by *Rhizoctonia solani* was studied. Cucumber plants (*Cucumis sativus*) cv. Iwa F1 cultivated in the growing medium containing spores of the selected *Trichoderma* strains were used to biochemical studies. Changes in phenylalanine ammonia lyase enzyme (PAL) activity as well as in total phenolic (TP) and *orto*-dihydroxyphenolic (*o*-DP) compound concentrations in plant leaf tissues were examined. Among the five strains of *Trichoderma* two of them *T. atroviride* TRS 25 and *T. virens* TRS 106 strongly enhanced seed germination and growth of cucumber plants. Moreover, the presence of *Trichoderma* spores in growing medium reduced infection of plants by *R. solani*, paralelly induction of PAL activity and increases in TP and *o*-DP compound concentrations were observed. These results indicate the impact of individual strains of *Trichoderma* on the mobilization of defense mechanisms in the studied plants. The present results encourage further analysis of the selected *Trichoderma* strains as they seem to have a potential to promote plant growth and induce resistance to *R. solani.* Now these strains seem to be a promising source of natural resistance inducers of crop plants, which in the future could be alternative to commonly used chemical pesticides for plant protection in Polish agriculture. This work was supported by grant no: UDA-POIG.01.03.01-00-129/09-05 (EFRR POIG 1.2.1: “Polish strains of *Trichoderma* sp. in biocontrol and to make productive organic waste”).

P4.15

Application of diazotrophic cyanobacteria in plants biomass production

A. Pszczółkowska[1], Z. Romanowska-Duda[1], M. Grzesik[2], W. Pszczółkowski[1]

[1] Department of Ecophysiology and Plant Development, University of Lodz, Łódź, Poland

[2] Institute of Horticulture, Skierniewice, Poland

Cynaobacteria are one of soil primary producers. Due to effective metabolic pathways, they are among the very few groups of organisms that can perform oxygenic photosynthesis and respiration simultaneously in the same compartment. Many cyanobacterial species are able to x nitrogen, and some of them are facultative heterotrophic. Therefore, they can survive and prosper under a wide range of environmental conditions. Cyanobacteria produce wide spectrum of secondary metabolites affecting other organisms e.g. plants. Efficient and cost effective cultivation of fast growing plant species for biomass that can be utilized for energy production is becoming an important element of Polish Energy Policy. The aim of this study was to evaluate the influence and application potential of *Anabaena* sp. PCC7120 in energetic plants cultivation. Preselected strain of diazotrophic cyanobacteria *Anabaena* sp. PCC7120 was cultivated *in vitro* on liquid growth medium. Culture in logarithmic phase of growth was centrifuged and cells were suspended in water. *Sida hermaphrodita* – energetic plant used in this research was cultivated in pots on poor quality soil with recommended dose N:P:K fertilizer. *Anabaena* sp. PCC7120 was applied twice: in the second and fourth week of cultivation. Germination tests were carried out in phytotoxkit boxes (10 seeds per box, single application of cells suspension). During the vegetation plants growth and development, chlorophyll content and photosynthesis intensity were measured. At the end of the season, fresh and dry biomass yield was measured. *Anabaena* sp. PCC7120 significantly accelerated the seedlings development reducing the time required to develop firs leaf. *Anabaena* sp. PCC7120 increased the chlorophyll content, photosynthesis intensity and fresh/dry weight of plants. Applied suspensions increased the height of plants by 217%, fresh weight by 242% and dry weight 229%. The differences in measured parameters between treated and control plans were greater with the time. *Anabaena* sp. PCC7120 produces chemicals that significantly stimulate the growth of *Sida hermaphrodita*. This strain is a fast growing microorganism that might be produce at a large scale for biofertilizer. Research where sponsored by Ministry of Science and Higher Education in Poland, Grant No. N N304 102940.

P4.16

Reactive oxygen species and antioxidant enzymes in the response of common bean to the infection with pathogens with different lifestyles

A. Nowogórska, J. Patykowski, M. Skwarek

Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland

Plants as sessile organisms are permanently exposed to many stress factors, which mainly occur sequentially. Pathogenic common bean diseases such as halo blight and grey mould caused by *Pseudomonas syringae* pv. *phaseolicola* (Psp) and Botrytis cinerea (Bc) respectively, bring about considerable losses in yield. Moreover, these pathogens induce different signalling pathways in plants after infection, so reaction to one can affect the other. It is assumed that oxidative burst is one of the first reactions of plants to biotic stress caused by a pathogen attack. Superoxide anion is generated by addition of one electron to molecular oxygen. Hydrogen peroxide can be produced by two-electron reduction of molecular oxygen or by superoxide anion dismutation. Increase in reactive oxygen species (ROS) production results in higher antioxidant enzyme capacities. Enzymes particularly associated with $O_2^{\bullet -}$ and H_2O_2 are superoxide dismutase, catalase and peroxidase. Specifically cell wall bound peroxidase in apoplastic fraction use hydrogen peroxide as a substrate in cell wall enhancement. The aim of this study was to determine ROS, such as $O_2^{\bullet -}$ and H_2O_2, concentrations and enzymes involved in their scavenging activities in Phaseolus vulgaris cv. Korona after single and sequential infection with selected pathogens. Superoxide anion content in common bean leaves was

measured by nitro blue tetrazolium reduction. Peroxidase assay with phenolic compounds such as ferulic acid (FPOD) and syringaldazine (SPOD) in apoplastic fraction was tested. The analysis of peroxidase isoforms after the ion-exhange DEAE-Sepharose chromatography was also performed. The results showed the significantly increased level of $O_2^{\bullet -}$ 12 and 48 h after Psp infection. High content of H2O2 persisted from 6th h after Psp inoculation to the end of the experiment. Bc infection caused progressive increase in generation of H_2O_2 with maximum values 24 and 48 h after inoculation. The maximum FPOD and SPOD activities were observed as early as 6 h after Psp inoculation, contrary to Bc infection which caused their highest levels in 48th h of experiment. Sequential Bc infection after Psp inoculation did not result in significant differences in the studied parameters in comparison to single bacterial treatment. Earlier generation of $O_2^{\bullet -}$ and H_2O_2 after Psp inoculation was associated with the rapid response preventing the spread of infection. A significant increase in peroxidase activities measured with phenolic compounds correlated with high hydrogen peroxide content demonstrated the intense use of H_2O_2 as a substrate to strengthen cell walls. Such a reaction can be confirmed also by low catalase capacity at the early stages of infection.

P4.17

Localisation of Sec21 protein in *Arabidopsis* root infected with *Heterodera schachtii*

Ł. Baranowski, E. Różańska, M. Sobczak

Department of Botany, Warsaw University of Life Sciences – SGGW, Warszawa, Poland

Plant parasitic cyst-forming nematodes induce a specific feeding structure called a syncytium in host roots. Syncytial elements differ ultrastructurally from typical plant cell, i.e. vacuolar system consists of many small vacuoles and vesicles while the central vacuole is missing. The biogenesis of these vesicles and their trafficking is unknown. Sec21 protein is an essential element of cargo transport from the endoplasmic reticulum to the Golgi apparatus. We analysed spatial and temporal expression patterns of genes encoding Sec21 proteins using semi-quantitative (sq) RT-PCR and immunolocalised their products in Arabidopsis roots containing syncytia induced by *H. schachtii*. Analysis of expression patterns of Sec21 genes in syncytia revealed differential expression of analysed genes. Immunolocalisation confirmed the presence of Sec21 proteins predominantly in syncytia. The presence of Sec21 proteins indicate that small syncytial vesicles are involved in cargo transport between ER-GA.

P4.18

Activities of nitrogen metabolism-related enzymes in cucumber leaves infected with *Pseudomonas syringe* pv. *Lachrymans*

E. Gajewska, E. Kuźniak, M. Naliwajski, S. Sapińska, M. Wielanek, M. Skłodowska

Department of Plant Physiology and Biochemistry, University of Lodz, Łódź, Poland

A bacterial pathogen *Pseudomonas syringae* pv. *lachrymans* (*Psl*) is a causal agent of angular leaf spot, a common disease of *Cucurbitaceae* plants. The disease is especially severe on cucumber and causes heavy losses in its production. Growth and yield of plants depend mainly on proper functioning of the primary metabolism, especially that of nitrogen. Reduction of growth, often observed in plants subjected to stress factors, including pathogenic microorganisms, results mostly from alterations in primary metabolic pathways. Nevertheless, functioning of plants under stress conditions requires changes in metabolism related to increased demand for compounds involved in defense reactions. The objective of this study was to get better insight into the influence of *Psl*-induced infection on the primary nitrogen metabolism in cucumber plants. We focused on the activities of enzymes participating in assimilation of this macroelement. Cucumber (*Cucumis sativus* cv. Polan) plants were grown under controlled conditions in plastic pots filled with mineral wool cubes soaked with a modified Hoagland's medium. Five week-old plants

were inoculated with *Psl* or sterile distilled water (control) using a needleless hypodermic syringe. On the 2nd and 7th days after inoculation (dai) in the 3rd leaf from the bottom the activities of nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH), alanine aminotransferase (AlaAT) and asparagine aminotransferase (AspAT) were estimated. Inoculation of cucumber leaves with *Psl* resulted in significant decline in NR activity, which was observed 7 dai. At this stage of infection a decrease in NR activation state was also found. Functioning of GS-GOGAT cycle was only slightly influenced by *Psl* treatment. The activity of GS increased by about 20% on the 2nd day, while Fd-GOGAT showed about 15% decrease in its activity. Contrary to NADH-GDH activity which exhibited 2-fold increase on the 2nd day, the activities of both aminotransferases were not significantly affected by infection. The obtained results indicate that inoculation with *Psl* leads to alterations in the primary nitrogen metabolism in cucumber plants. Induction of NADH-GDH involved in ammonium utilization and glutamate synthesis might play a defensive role in cucumber response to infection caused by this bacteria. This work was supported by National Science Centre Grant No N N310 302339.

P4.19

Enhancement of accumulation of salicylates and abscisic acid by sugars and *F. oxysporum* infection in yellow lupine

M. Formela[1], K. Drzewiecka[2], T. Chadzinikolau[1], I. Morkunas[1]

[1] Department of Plant Physiology, Poznań University of Life Sciences, Poland

[2] Department of Chemistry, Poznań University of Life Sciences, Poland

Plant resistance to pathogenic fungi is regulated by a complex set of signaling pathways that includes those mediated by the hormones: salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA) and ethylene (ET). It is commonly known that SA promotes resistance against pathogens with a biotrophic lifestyle, whereas JA, ABA and ET act as positive signals in the activation of defense against necrotrophic pathogens. It has also been documented that signal transduction pathways released by carbohydrates converge with hormone-induced pathways. The aim of the presented study was to verify whether the level of soluble carbohydrates, i.e. sucrose, glucose and fructose, affects the accumulation of salicylates and abscisic acids in embryo axes of *Lupinus luteus* L. cv. Juno inoculated with *Fusarium oxysporum* f. sp. *lupini* and cultured in vitro on a medium with sugar or without it. *Fusarium oxysporum* is a facultative parasite having both the biotrophic and necrotrophic phases of feeding. Levels of salicylic acid in both its free form (SA) and that conjugated with a glucoside (SAG), as well aslevels of abscisic acid (ABA) were determined by HPLC. The results of this study revealed a correlation between endogenous levels of soluble carbohydrates and the accumulation of SA and SAG in embryo axes of *Lupinus luteus* L. cv. Juno. Particular attention should be paid to the time point of 48 h after inoculation with the pathogen, where the highest levels of both free and *glucoside-bound* SA were recorded in inoculated embryo axes cultured on a medium with sucrose, glucose or fructose, being higher than in the control, i.e. non-inoculated axes cultured on the medium with sugar. Moreover, a very high accumulation of ABA was observed in embryo axes with high levels of endogenous sugars. Additionally, *F. oxysporum* infection strongly enhances ABA level. The highest accumulation of ABA in tissues with high sugar levels was observed at 48 and 72 h after inoculation. In conclusion, the strong accumulation of salicylates and abscisic acid in yellow lupine embryo axes is the result of amplification of the signal coming from sucrose and infection caused by the pathogenic fungus. This study was supported by the Polish Ministry of Science and Higher Education (MNiSW, grant no. N N303 414437).

P4.20

The influence of BTH and arbutin on PAL activity and phenolic compounds content in cucumber leaves infected with *Pseudomonas syringe* pv. *lachrymans*

M. Skłodowska, M. Wielanek, E. Gajewska, E. Kuźniak
Department of Plant Physiology and Biochemistry, University of Lodz, Łódź, Poland

Stress conditions substantially modify the metabolism of phenolic compounds however it is unclear in which group of these compounds there are changes important for improving plant resistance to infectious diseases. BTH (benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester) at the molecular level has scope of action similar to salicylic acid. BTH treatment can stimulates multiple processes related to the acquisition of systemic acquired resistance, including the increase in the activity of phenylalanine ammonia lyase (PAL), the main enzyme of phenolic compounds pathway. After exogenous supply of arbutin (hydroquinone β-d-glucopyranoside), as a result of plant β-glucosidases action, its conversion to hydroquinone and then to benzoquinone occurs, this may lead to the induction of plant defense reactions. Angular leaf spot, caused by *Pseudomonas syringae* pv. *lachrymans* (*Psl*), is one of the most serious cucumber diseases causing heavy losses in its production. The objective of this work was to study the influence of elicitation with BTH or arbutin on PAL activity as well as total phenolic and flavonoid contents in *Psl*-infected cucumber leaves at the early stage of disease. Cucumber (*C. sativus* cv. Polan) was grown hydroponically in modified Hoagland's medium. Three week-old plants were sprayed with 0.1 mM BTH or 2 mM ARB solution. One week after elicitation three (from the bottom) leaves were inoculated with *Psl* or distilled water (C) using a needle-less hypodermic syringe. Free (SA) and total (SA-T) salicylic acid contents were determined in the 3rd leaf 7 days after elicitation (t_0) as well as 2 (t_2) and 7 (t_7) days after inoculation (dai). Comparing to the non-elicited plants in those treated with BTH for 7 days (t_0) both total phenolic compound and flavonoid contents decreased by about 35 and 40%, respectively. In contrast, PAL activity was increased by 40%. Treatment with arbutin resulted in decrease in flavonoid content and PAL activity by about 40% and 20%. Two days after inoculation Psl infection did not affect total phenolic compound and flavonoid contents both in non-elicited (C+Psl) and elicited plants (ARB+Psl, BTH+Psl) comparing to the respective controls (C, ARB, BTH). At the same time PAL activity was increased in C+Psl and ARB+Psl plants, by about 40 and 60%, respectively, while decreased by about 35% in BTH+Psl plants. After 7 days infection resulted in 25-35% decrease in total phenolic compound and flavonoid contents both in C+Psl, ARB+Psl plants. However PAL activity was enhanced due to Psl infection in the case of C+Psl and BTH+Psl plants, by 100 and 60%, respectively, comparing to the non-inoculated controls. The obtained results indicate that induction of PAL activity and changes in the level of phenolic compounds in plants elicited with BTH or ARB might not play a crucial role in cucumber defense response to *Psl* infection. Supported by National Science Centre Grant No N N310 302339.

P4.21

Involvement of salicylic acid in defense response of BTH- and arbutin-treated *Cucumis sativus* against *Pseudomonas syringe* pv. *lachrymans*

M. Wielanek, E. Gajewska, M. Wagner, E. Kuźniak, M. Skłodowska
Department of Plant Physiology and Biochemistry, University of Lodz, Łódź, Poland

Angular leaf spot, caused by *Pseudomonas syringae* pv. *lachrymans* (*Psl*), is one of the most serious cucumber diseases causing heavy losses in its production. Salicylic acid (SA) is an important signal for the induction of systemic acquired resistance (SAR), is closely associated with a hypersensitivity reaction and the induction of PR protein. Accumulation of SA and its sugar conjugates occurs soon after infection and precedes the expression of resistance

genes. Several molecules can act as elicitors of plant defense reactions. BTH (benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester) is a functional analogue of SA, BTH treatment can elicit many processes related to the acquisition of SAR. After exogenous supply of arbutin (hydroquinone β-d-glucopyranoside), as a result of plant β-glucosidases action, its conversion to hydroquinone and then to benzoquinone occurs, this may lead to the induction of plant defense reactions. The objective of this work was to study the influence of elicitation with BTH or ARB on free and total salicylic acid contents in *Psl*-infected cucumber leaves. Cucumber (*C. sativus* cv. Polan) was grown hydroponically in modified Hoagland's medium. Three week-old plants were sprayed with 0.1 mM BTH or 2 mM ARB solution. One week after elicitation three (from the bottom) leaves were inoculated with *Psl* or distilled water (C) using a needle-less hypodermic syringe. Free (SA) and total (SA-T) salicylic acid contents were determined in the 3rd leaf 7 days after elicitation (t_0) as well as 2 (t_2) and 7 (t_7) days after inoculation (dai). In control (C) plants the highest level of SA and SA-T was detected 2 dai. Comparing to the C plants BTH enhanced both SA and SA-T contents, at t_0, t_2 and t_7 SA-T level increased by about 100, 70 and 300%, respectively, and SA accounted for 33, 90 and 70% of the SA-T. At the same time treatment with Arb resulted in 40, 25 and 80% increase in SA-T, and SA accounted for 28, 41 and 34% of the SA-T, increase in SA content by 185% above C was detected only at t_7. Two dai *Psl* infection decreased both SA and SA-T contents by about 45 and 30%, and SA accounted for 50% of the SA-T, only 7 dai infection resulted in 140 and 165% increase in both SA and SA-T contents, but SA accounted for only 19% of the SA-T. Comparing to the PSL plants in those elicited with BTH or ARB levels of both SA and SA-T were significantly increased. In BTH+Psl plants 2 and 7 dai SA-T content was increased by about 300 and 120%, and SA accounted for 90 and 80% of the SA-T. Arb was weaker than BTH inductor, in ARB+Psl plants 2 and 7 dai SA-T content was increased by about 80 and 12%, and SA accounted for 66 and 45% of the SA-T. The obtained results indicate that induction of salicylic acid biosynthesis and increase in the level of its free form in plants elicited with BTH or ARB might play a defensive role in cucumber response to *Psl* infection. Supported by National Science Centre Grant No N N310 302339.

P4.22

Effect of suboptimal nutrient conditions on nitrogen, photosynthetic and symbiotic status of mycorrhized maize

E. Paluch, W. Polcyn, T. Lehmann, M. Ścierżyńska

Department of Plant Physiology, Adam Mickiewicz University, Poznań, Poland

The benefit of arbuscular mycorrhizal fungi (AMF) to plants is mainly attributed to increased uptake of crucial nutrients, especially phosphorus but also, to some extent, nitrogen. The aim of the present work was investigate the impact of AMF on maize assimilatory capacity at low-nutrient conditions. Maize plants (hybrid Opoka) mycorrhized (AMF1) or not (AMF0) with high quality AMF inoculum (TERI, New Dehli) were grown in phytotron conditions for 7 weeks. Then commercial fertilizer with lowered P content (NPKMg 19:6:20:3 and microelements) was introduced for additional 4 weeks at four dilution (D) levels: 0.125 × D, 0.25 × D, 0.5 × D and 1 × D dose (1 × D = 114 mg N & 36 mg P_2O_5/week/plant). Only the highest fertilization variant revealed no nutrition deficiency symptoms on leaves. In general, AMF colonisation is favoured under low-nutrient (mainly P) conditions. Under applied growth conditions none of fertilization doses suppressed mycorrhization rate since high and similar root colonisation (F%: 92 to 96; M%: 59 to 72), arbuscule frequency (a%: 41 to 60) and sporulation level was observed. This is in contrast to detrimental effects (F% = 32; M% = 1, a% = 0) when 4 × D dose (456 mg N & 144 mg P_2O_5/week/plant) was examined in preliminary experiment. Leaf N status was assessed through fluorometric detection of the chlorophyll/flavonoids ratio called Nitrogen Balance Index (NBI). At 0.125 × D dose plants were ineffective in nitrogen acquisition independently of symbiotic status (NBI ≈ 12). At 0.25 × D efficiency of AMF0 plants did not change, whereas NBI of AMF1 plants was 42% higher. AMF1 plants reached the highest nitrogen status already at 0.5 × D (NBI = 28), whereas AMF0 plants touched this level only at the highest fertilization dose. Influence of suboptimal nutrient conditions on photosynthetic capacity was estimated from chlorophyll *a* fluorescence induction kinetics (Fv/Fm, Fv′/Fm′, Fd/Fs, ΦPSII, qP, NPQ, ETR) and gas exchange parameters measured at saturating light (CER_{max}, g_{CO2}, g_{h2O}, T_{max}, Ci). Parameters of CO_2 exchange at 1xD dose were almost twice better than at 0.5 × D but apparently not dependent on

maize symbiotic status. At 0.5x dose CER_{max} of AMF1 and AMF0 was also similar but lower stomatal conductance and transpiration rate of AMF0 suggests that actual CO_2 fixation capacity of non-mycorrhized plants could be limited due decreased capacity of leaf gas exchange. Fd/Fs – the measure of potential net photosynthesis and Fv/Fm did not differ in relation to symbiotic status under applied growth conditions. Nevertheless, AMF could improve the actual photosynthetic capacity since increased ΦPSII, qP and ETR were observed in mycorrhized plants. Arbuscular mycorrhizal fungi equally colonized maize roots at four suboptimal fertilization levels and were shown to improve the photosynthetic capacity and nitrogen status of maize leaves. The work was supported by grant 2011/01/B/NZ9/00362 from the Polish National Science Centre.

P4.23

Stimulation effect of *Microcystis aeruginosa* MKR 0105 on the metabolic activity and development of willow (*Salix viminalis* L.) plants

M. Grzesik[1], Z. Romanowska-Duda[2], A. Pszczółkowska[2], W. Pszczółkowski[2]

[1] Institute of Horticulture, Skierniewice, Poland

[2] Department of Ecophysiology and Plant Development, University of Lodz, Łódź, Poland

Microcystis aeruginosa MKR 0105 is a species of freshwater *Cyanobacteria*, which are one of the largest group of Gram(–) negative, photosynthetic prokaryotes. The some species of *Cyanobacteria* are able to fix the nitrogen from the air and produce a wide variety of bioactive compounds which can influent plant metabolism. The aim of this study was to investigate the influence of *Microcystis aeruginosa* MKR 0105 on growth, development, health status and metabolic activity of willow (*Salix viminalis* L.) plants, in order to find the possibility to increase the organically grown energy biomass. The woody cuttings were placed in 3 litre pots, filled with standard horticulture soil, in field conditions. During vegetative season, the obtained from these cuttings plants, were sprayed with *M. aeruginosa* MKR 0105, cells water homogenates and lyophilised cells. Height and total shoot length, their health status and metabolic activity was measured every 3-4 weeks during season. Fresh and dry biomass, calorific value and heat of combustion were measured at the end of growing season. The obtained results show that the used of *M. aeruginosa* MKR 0105 improved metabolic activity, growth, development and health status of willow plants. Triple sprayings of plants, with all of the used *M. aeruginosa* monocultures, enlarged plant height and total shoot length. It was associated with the increased $chlorophyll_{a+b}$ content in leaves, photosynthesis (net photosynthesis, stomatal conductance, intercellular CO_2 concentration, transpiration and water use efficiency), higher activity of acid (pH 6,0) and alkaline (pH 7,5) phosphatase, increased total dehydrogenase activity and lower cytomembrane permeability. Treatment with *M. aeruginosa* did not influence the calorific value and heat of combustion, which were similar as in not treated plans. The effectiveness, in the plant development improvements, of treatments with lyophilized cells was similar to the treatments with cells water homogenates. The results indicate that willow plants are sensitive to the metabolites of the used *M. aeruginosa* MKR 0105 monocultures and treatments with this green-blue alga can improve the plant development. Use of *M. aeruginosa* monocultures can be one of the promising directions for the development of organic farming production and increase energy biomass yield with methods friendly to environment. Research indicated also that *M. aeruginosa* MKR 0105 shows a great potential of *Cyanobacteria* as a new source of biostimulating chemicals for energetic crops production. Research where sponsored by National Science Centre in Poland, Grant No N N304 102940.

P4.24

Deprotonated benzothiadiazole-7-carboxylic acid derivatives as a plant systemic acquired resistance inducers

P. Lewandowski[1], R. Kukawka[1], H. Pospieszny[2], M. Smiglak[3]

[1] Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

[2] Institute of Plant Protection, National Research Institute in Poznań, Poland

[3] Poznan Science and Technology Park, Adam Mickiewicz University Foundation, Poznań, Poland

Plant protection against aggressive pathogens such as viruses and bacteria is one of the most challenging tasks facing modern agriculture. Viral plant diseases cause huge losses in quantity and quality of crops thus scientists are undertaking actions to increase the resistance of plants to pathogens. Resistance of plants can be created in many ways. Known and used method is crossing plants or genetic modification. One of the newest research trends is induction of systemic acquired resistance activated by biological or chemical agents. One method of preparation of BTH derivatives is modification of neutral particle into the ion, which may be a base to produce salt. By properly adjusting the active derivative BTH with other biologically active substances (eg, Didecyldimethylammonium anion with antibacterial effect), one can create a bifunctional compound with properties other than the starting components. Obtained compounds will be tested for chemical, physical (dissolution kinetics) and biological (effect on viruses and bacteria and effect of the concentration of the active substance on the growth of crops) activity. A wide range of biologically active agents that can be combined with BTH in bifunctional salt gives wide spectrum for the potential use of such substances in agriculture.

P4.25

Cationic derivatives benzo[1,2,3]thiadiazole-7-carbothioate (ASM) as inducers of plant resistance

R. Kukawka[1], P. Lewandowski[1], H. Pospieszny[2], M. Smiglak[3]

[1] Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

[2] Institute of Plant Protection, National Research Institute in Poznań, Poland

[3] Poznan Science and Technology Park, Adam Mickiewicz University Foundation, Poznań, Poland

Benzo[1,2,3]thiadiazole-7-carbothioate is commercially available compounds acting directly against plant pathogens and, indirectly, stimulating the immune system of plants and reinforcing their resistance against diseases. Especially interesting is possibility of the synthesis of ionic liquids based on cationic or anionic derivatives ASM in correlation with the appropriate ions, which modify the physical properties such as hydrophilicity, hydrophobicity or thermal stability, which allow for the potential use of such products in the agrochemical industry. One of the compounds used to modify the solubility of other biologically active molecules is sodium docusate. Thus it is possible to obtain products with reduced solubility and long-term accumulation of the active substance on the surface of the plant. Another example of modification of properties of the compound is the exchange of anion to salicylate to increase the biological activity of the product. Presented research concerns the synthesis of cationic derivatives of BTH to produce ionic liquids for new and interesting functional properties. The resulting compounds are characterized by examining the physical and chemical properties (solubility kinetics) and biological (plant protection against pathogens, increase plant resistance to pathogens and the effect of the concentration of the active substance on the growth of crops).

P4.26

The reactive nitrogen species affect potato immunity to *Phytophthora infestans*

M. Arasimowicz-Jelonek[1], J. Floryszak-Wieczorek[2], D. Abramowski[2], K. Izbiańska[1]

[1] Department of Plant Ecophysiology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

[2] Department of Plant Physiology, Poznań University of Life Sciences, Poland

Recent research has shown that nitric oxide (NO·) mainly mediates biological function through chemical reactions between spatially controlled accumulation of different reactive nitrogen species (RNS) including i.e. NO·, peroxynitrite ($ONOO^-$) and S-nitrosothiols (RSNOs). To gain insights into NO-dependent mechanism that could control plant immunity to the pathogen we determine the metabolic status of NO governed by the systems involving its positive and negative regulation. An experimental approach involved two cultivars of potato (*Solanum tuberosum* L.) i.e. "Sarpo Mira" – highly resistant and "Bintje" – susceptible to oomycete pathogen *Phytophthora infestans*. Obtained data revealed ca. two-fold increase in $ONOO^-$ content only in the susceptible potato interaction; however, an enhanced level of both parent molecules (NO· and O_2^-) was observed in both potato genotypes. As we found $ONOO^-$ overproduction in susceptible potato was accompanied by a short-time (1-3h) up-regulation of gene coding for thioredoxin peroxidases (TPx). In turn, in the resistant response the induction of TPx gene expression was started to increase at 3rd h and it remained at a relatively high level during the first 24 h after inoculation. Simultaneously, higher level of S-nitrosoglutathione reductase (GSNOR) activity correlated with lower SNOs storage facilitating more potent defense responses. This work was supported by funds from Ministry of Science and Higher Education – project IUVENTUS PLUS no. 2011 000671 and by a grant of The National Science Centre – 2011/01/B/NZ9/00243.

P4.27

Green islands develop in a leaf age-dependent manner during black spot disease of *Brassica juncea*

V.K. Macioszek, K. Rousseau, P. Płudowska, A.K. Kononowicz

Department of Genetics, Plant Molecular Biology and Biotechnology,
Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland

Brassica juncea is a crop susceptible to *Alternaria brassicicola* infection. On its foliar tissues, spreading necrotic lesions develop that can cause even whole plant decay. In our experiments, two cultivars of *B. juncea* Polish one and English wave mustard were infected with *A. brassicicola* spore suspension. It has been found that the younger leaf of the plant the smaller lesion developed. However, green islands formation has been observed only during infection of Polish cultivar and they have been formed only on the oldest leaves, after 48 to 72 hpi. Spreading lesions have been surrounded by chlorotic "halo" and 5 days after infection older leaves (first and second) yellowed, but still green islands could have been observed on them. *Alternaria brassicicola* development on plants with five mature leaves has shown leaf age-dependent tendency. Number of germination tubes and appressoria decreased on younger leaves from early hours of infection to 24 hpi. Decreased contents of chlorophyll a and b have been observed from the younger to the oldest leaves compared to the uninfected samples. The same tendency was observed for polyphenol and carotenoid contents, although up to 72 hpi three oldest leaves showed higher carotenoids concentrations then control ones. This work was supported by grant of National Science Centre 2011/01/B/NZ1/04315.

P4.28

Comparison of *Arabidopsis thaliana* (*col-0*) plants and cell suspension cultures responses to *Alternaria brassicicola* infection

K. Rousseau, V.K. Macioszek, A.K. Kononowicz

Department of Genetics, Palnt Molecular Biology and Biotechnology,
Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland

Alternaria brassicicola is a fungal necrotrophic pathogen, which is able to attack *Cruciferous* plants, developing black spot disease symptoms and causing agricultural wastes every year. *Arabidopsis thaliana* is a model plant, belonging to *Cruciferous* family. *Alternaria brassicicola* is capable to infect Arabidopsis, triggering necrotic lesions and inducing plant's response to fungal infection, including reactive oxygen species (ROS) and production and callose deposition. Incompatible interaction of *A. thaliana col-0* plants with *Alternaria brassicicola* results in limiting of necrotic lesions development to small, non-spreading spots on leaves surface, which evolve only in a site of infection droplet. Also, during the cell suspension culture of Arabidopsis, there is no significant increase in cells mortality after infection. After inoculation by *Alternaria brassicicola* spore suspension, changes in necrosis diameter on leaves (*in planta*) and cell viability (*in vitro*) were measured every 24 hpi to examine influence of fungal infection. Also, the production of reactive oxygen species, as hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet -}$), was analyzed by respectively DAB and NBT staining, and callose deposition was studied by aniline blue staining. Plants were analyzed every 24 hpi and suspension cultures were analyzed every 2 hpi for 12 hours and additionally at 24 hpi.

P4.29

Transcription profiling of *Arabidopsis thaliana* and *Brassica oleracea* var. *capitata* f. *alba* during *Alternaria brassicicola* infection reveals different mode of action of fungus

V.K. Macioszek[1], K. Rousseau[1], A. Żmieńko[2], A.K. Kononowicz[1]

[1] Department of Genetics, Plant Molecular Biology and Biotechnology, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland

[2] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Foliar plant pathogen *Alternaria brassicicola* causes one of the most destructive leaf spot diseases of cultivated crops of *Brassica* genus worldwide. Spreading necrotic lesions lead to the death of infected seedlings or foliar tissues of mature plants and consequently to the host plant decay. *Brassica*'s foliar tissues contain many phenolic compounds and can produce broad spectrum of antimicrobial secondary metabolites e.g. phenolics during pathogen attack, but such defense is insufficient against *A. brassicicola*. The one of the phenolic compounds that is efficient during *A. brassicicola* infection is phytoalexin of *Arabidopsis thaliana* – camalexin. Arabidopsis mutant *pad3* is highly susceptible to *A. brassicicola* otherwise than wild type. From the agricultural point of view, disadvantageous changes in photosynthetic potential appear to be the most important one for plant cultivation. Chloroplasts are the target organelles for *A. brassicicola* and damage of chloroplast structure such as degradation of chloroplast envelope and disturbances of thylakoids during disease development in *Brassica oleracea* var. *capitata* f. *alba* leaf's cells have been occurred. Changes in negatively regulated transcription profiling of infected *B. oleracea* tissues revealed gradual increase (from 12 to 48 hpi) of number of suppressed chloroplast and photosynthesis-related genes. Such events have been not observed during *A. thaliana* infection. This work was supported by grant of Polish Ministry of Science and Higher Education N302 318833 and in part by grant of National Center of Science 2011/01/B/NZ1/04315.