SZENT ISTVÁN UNIVERSITY

PROTEINS ASSOCIATED WITH EARLY BASAL RESISTANCE AND THEIR USE TO IDENTIFY THE RESPECTIVE BACTERIAL ELICITORS

Thesis of doctoral dissertation

Gabriella Varga

Institute of Viticulture and Enology

Kecskemét, 2006

Denomination of doctoral school:

Plant Science

sub program:

Plant Breeding Through Methods of Genetics and Biotechnology

Head of school:

Dr. Ferenc Virányi
Doctor of the Hungarian Academy of Sciences
SIU, Faculty of Agricultural and Environmental Sciences, Department of Plant Protection

Supervisors:

Dr. Zoltán Klement†
Research Professor, Member of the Hungarian Academy of Sciences
Plant Protection Institute, Hungarian Academy of Sciences

Dr. Péter Ott
Senior Scientist
Plant Protection Institute, Hungarian Academy of Sciences

....

Approval of Head of the School Approval of the Supervisor

INTRODUCTION AND MAIN OBJECTIVES

Induced resistance that – along with preformed defences – successfully stops growth of non-pathogenic and non-plant pathogenic bacteria in the plant intercellular spaces of plants and contributes to the defence mechanisms against compatible and incompatible bacteria (Ott et al., 2006) has been referred to as basal resistance (BR). Its activation is based on recognition of general elicitors, (i.e. essential bacterial cell components, so-called pathogen associated molecular patterns „PAMPs"), rather than of pathogen-specific elicitors (such as avirulence proteins) (Gomez-Gomez and Boller, 2002). Hitherto known bacterial PAMPs are the cell surface-related flagellin (flg), lipopolysaccharides (LPSs) or peptidoglycan and the cytosolic cold shock proteins (CSPs) or translation elongation factor (EF-Tu) (Zipfel and Felix, 2005). The early basal resistance (EBR) of plants is a locally induced defence mechanism which, due to its very short development time (3-6 hours), is the first line of resistance against bacteria (Klement et al., 2003). The symptomless EBR is void of cell death characteristic for HR or other visible symptoms even at submicroscopical level (Ott et al., 1998). Bacteria possessing general elicitors, such as *hrp* (HR and pathogenicity) mutant and heat killed compatible or incompatible bacteria are unable to avoid recognition by the plant surveillance system. The live virulent (compatible) bacteria can suppress/tolerate the specific and non-specific defence mechanisms (Espinosa and Alfano, 2004; Ott et al., 2006) and it has to be noted that killed *Pseudomonas tabaci* cells in tobacco induce BR instead of disease (Burgyán and Klement, 1979). Bacterial multiplication and the *hrp* system of the incompatible bacteria are

inhibited in the EBR-protected plant tissue (Klement et al., 2003). A visible sign of the latter is that the programmed cell death fails to occur after a subsequent infection. This HR-preventing capacity serves as a reliable method to demonstrate operation of EBR. Besides, there are many physiological and molecular changes indicating EBR. The high number of genes upregulated show the complexity of EBR. Among them are genes associated with cell wall fortification, counteraction of active oxygen species and synthesis of antimicrobial compounds (e.c. pathogenesis-related (PR) proteins) (Szatmári et al., 2006). We still do not know what factors of the BR-protected tissue affect bacteria directly. Ott (2002) pointed out changes in the protein composition of intercellular fluid of tobacco. The aim of this study was to continue that 'pioneer' work. Our objectives were elaborated on the basis of the mentioned dissertation and on the more accented importance and research directions of EBR:

1. Confirm the relation of previously revealed extracellular proteins to EBR and designate more proteins to examination. Specify further the induction properties of the EBR-related proteins with special regard to PR-protein inducing treatments.
2. Identify proteins strongly correlated with EBR, based on their amino-acid sequence. Assess, if possible, function of this proteins. Substantiate application of EBR-related proteins as molecular markers of the resistance, key part of which is the quantitative analysis of expression profiles of the marker-proteins. Identify general bacterial elicitors of EBR using the EBR molecular markers.

3. Functional analysis of EBR-related proteins to reinforce their role in bacteriostatic activity of the resistance.
4. The crown gall disease in viticulture is one of the serious grapevine diseases without effective control measures, except prevention. Examining possibilities of application of EBR against this important pathogen (*Agrobacterium*) is our further objective.

MATERIALS AND METHODS

Tobacco plants (*Nicotiana tabacum* L. cv Samsun) grown in a greenhouse and with 4-8 fully expanded leaves were used for experiments. NahG-10 plants were kindly provided by NOVARTIS, Agricultural Biotechnology Research, Research Triangle Park, NC, USA. Bacterium strains were cultured in King's Medium B broth (King et al., 1954). Besides live bacteria heat killed *Pseudomonas tabaci, P.* s. pv. *phaseolicola* were used. The live or killed bacterial suspension (5×10^8 cells/ml) were injected into leaves (Klement, 1990). Proteins in the intercellular washing fluid of tobacco (Klement, 1965) were separated under discontinuous, native, Ornstein-Davis buffer system (pH 6.8 for stacking gels, pH 8.8 for resolving gels, and pH 8.3 for running buffer, Ornstein and Davis, 1964) and stained with silver (Heukeshofen and Dernik, 1985). Identification of amino-acid sequence of proteins were carried out in collaboration with Proteomics Research Group of Biological Research Center, Hungarian Academy of Sciences. Chitinase activity was detected in an in-gel assay (Trudel and Asselin, 1989). Here, overlay gels contained 0.04 % glycol-

chitin and were stained with Fluorescent Brightener 28 (Sigma), visualized with UV transilluminator. Proteins with chitinolytic activity appeared as nonfluorescent bands. Proteins in the intercellular washing fluid were isoelectrically focused in liquid phase. Lysozyme activity (Selsted and Martinez, 1980) was measured in fractions containing EBR-related or other chitinases. EBR-induction by bacteria or elicitor epitopes were verified with detection of HR-preventive capacity of functional EBR (Klement et al., 1999). Georg Felix (Zürich-Basel Plant Science Center, Botanisches Institut der Universitat Basel, Switzerland) kindly provided the following elicitors: 22 aminoacid-long flagellin peptides isolated from *P. avenae*, 22 aminoacid-long CSP peptides from *Micrococcus lisodeicticus*, 26 aminoacid-long EF-Tu peptide from *Escherichia coli*. Peptidoglycan was from Fluka, LPS preparations were from Institut für Pflanzenpathologie und Pflanzenschutz der Georg-August-Universität, Göttingen, Germany. Expression of *chto3D9* gene was examined by Real Time PCR analysis with the primers 5' CAA CCA TTC GAG CCA 3', 5' TAA GCC TCA CAA CCG TG 3'.

RESULTS

Proteins in the intercellular fluid of tobacco during biotic stress conditions correlate with EBR

Injection of heat-killed *P. s.* pv. *phaseolicola* into tobacco leaves induced the symptomless EBR within 3-6 hours. Appearance of at least two protein bands in a region below 30 kDa molecular weight were detected parallel

with HR-preventive feature of EBR. Expression of the ’215’ and ’250’ proteins (Ott, 2002) were observable in all of the EBR-inducing bacterial treatments such as live, non-plant pathogenic *Escherichia coli*, Gram-positive *Micrococcus lisodeicticus,* saprophytic *P. fluorescens*, live *hrp* mutant of plant pathogenic *P. syringae* pv. *syringae hrpJ*, genetically modified *Agrobacterium tumefaciens* lacking T-DNA or heat killed virulent (compatible) *P. tabaci*. EBR is not as effective against compatible pathogens as for example against saprophytes. It may not inhibit the disease completely but reduces multiplication rate of the bacteria (Ott et al., 2006). Compatible bacteria are able to block manifestation of EBR and in correlation with this appearance of the ’215’ and ’250’ extracellular proteins failed as well. Only a slight induction was detected during *P. tabaci* infection in case of high density inoculum (10^9 cells/ml).

We simulated abiotic stresses by injection of different solutions into tobacco leaves: osmotic stress (0.4 M mannitol), salt stress (0.2 M NaCl) and oxidative stress (40 μM paraquat or 0.3 M hydrogen peroxide). These compounds are also known as inducers of several PR proteins. Six hours after injection a minor appearance of the ’215’ and ’250’ protein bands was observed only after mannitol and salt treatments. At later sampling (12 and 24 hours post inoculation (hpi)) the proteins disappeared. Some molecules that participate in the signalling events of plant defense mechanisms, such as jasmonic acid, ethylene or salicylic acid (SA), individually induce a broad set of PR-proteins (Van Loon, 1983). Methyl-jasmonate (100 μM) and 1-amino-ciclopropane-carboxilic-acid (precursor of ethylene) (1 mM) did not induce the ’215’ and ’250’ proteins. Salicylic acid (0.6 mM) induced only a slight expression of these proteins in the

intercellular fluid and the HR inhibition was not detected in the injected areas pretreated with salicylic acid or with the other signal molecules. In NahG plants that are unable to accumulate SA, HR prevention and expression of the ’215’ and ’250’ proteins were at the same level as in wild type ’Samsun’ plants. Benzo-thiadiazol (BTH, Bion®) treatment is accompanied by production of PR-proteins (Burketova et al., 1999), however our results showed that the above proteins correlating with EBR did not coincide with the defense stimulated by BTH.

Identification of EBR215/250 proteins and qualifying them as markers of the resistance

According to results of mass spectrometrical analysis the determined sequences of the’215’ and ’250’ proteins are similar to conservative regions of plant chitinases. In addition, these proteins, which appear to be unknown, resemble each other very much. The in-gel chitinase activity assay was suitable not only for the selective detection of chitinases but also for quantitative evaluation of proteins. The lytic zones showed the chitinase activity and the molecular weight and their sizes were proportionate with the amount of proteins. The chitinases did not appear after *P. tabaci* treatment, as expected. We have not identified the whole amino-acid sequence yet. The molecular mass of the known sequence is 10 663.9 Da, isoelectric point is pH 4.82. There was a conserved glycosid-hydrolase domain distinguishable in the known region of EBR215/250 proteins which is characteristic of family 19 chitinases. Family 19 chitinases are similar to several lysozymes. The EBR215/250 chitinases migrated to the isoelectric fraction of pH 3.8-4.4. The fraction, and especially the ’215’ and ’250’

proteins showed lysozyme activity towards *M. lisodeicticus* cells. Measurements of *chto3D9* gene activity, which codes for EBR215/250 proteins (AJ880384, Ott et al., 2006) suggested transcriptional regulation of these EBR marker proteins. Measuring gene and/or protein expression of EBR markers allowed to test and compare some general bacterial elicitors, which is instrumental in understanding signal transduction pathways underlying the BR as well as in substituting whole bacterial cells as elicitors.

EBR induction by pathogen associated molecular patterns (PAMPs)

The number of known PAMPs which trigger defense reactions in plants has increased recently. (Felix et al., 1999; Dow et al., 2000; Felix and Boller, 2003; Kuntze et al., 2004). The known responses are medium alkalinization, oxidative burst in cell cultures, ethylene production, PR-gene expression and growth inhibition of intact plants (Felix et al., 1999; Gomez-Gomez et al., 1999). The flagella play a role in motility of bacteria. The 15 aminoacid-long highly conserved motif of flagellin, the building block of eubacterial flagellae, at any of the time courses under survey (6, 24, 48, 72 hpi) induced neither the EBR215/250 chitinases nor the HR inhibition. LPS from *P. s.* pv. *coriandricola* triggered the marker-proteins and a slight HR-prevention only around 48-72 hpi. The EBR215/250 chitinases and partially induced HR-inhibition after peptidoglycan treatment were detected at 12 hpi. The 22 amino-acid long flagellin peptide (flg22) elicits all known EBR features. The *chto3D9* gene expression reaches maximum level at 3 hpi, the EBR-related proteins and the HR

inhibition were detected at 6 hpi. The level of gene activation and protein expression is exceeded that induced by 5 x 10^8 cells/ml bacteria.

The 22 amino-acid long conserved motif of bacterial cold shock proteins (CSPs) is located inside the bacterial cell. This PAMP triggered a broad set of defense responses in *Solanaceae* plants (Felix and Boller, 2003). At 6 and 12 hours after injection of tobacco leaves with csp22 only low amount of EBR related chitinases were observable. At 24 hpi the physiological and molecular markers of EBR became more intense. A 26 amino acid-long peptide on the N-terminus of the elongation factor EF-Tu, a highly conserved and the most abundant bacterial protein, also acts as a PAMP in *Cruciferae* plants. The induced responses were medium alkalinisation, ethylene production, oxidative burst, but they were not observed in tobacco (Kuntze et al., 2004). Injection of elf26 peptide to tobacco leaves results weak but increasing expression of EBR215/250 proteins and the pretreated leaves showed HR prevention at 19 hpi.

Limitation of crown gall disease by flagellin-induced plant defence

The most effective plant protection method against crown gall disease caused by *Agrobacterium* species is the prevention. Whereas flg22 considerably induced all of the EBR features (see above) it seems to be a suitable elicitor to examine effect of BR to *Agrobacterium* infection. Leaves of *in vitro* tobacco plants were injected with flg22 solution and 6 or 24 hours later infected with *Agrobacteria*. The bacteria were killed 14 or 24 hours later. In experiments when 6 hour-long flg22 pretreatment continued by 14 hours co-cultivation time (the time while bacteria have

opportunity transform the T-DNA to plant cell), we observed lower amount of tumors. When 24 hpi pretreatment followed by 24 hours long co-cultivation reduction of tumors were not significant. This combination in some cases results in increased tumor formation.

New scientific results

1. We proved that EBR215/250 proteins separated from intercellular fluid of tobacco are in close and quantitative correlation with operation of early basal resistance (EBR). Treatments with bacteria which triggered the EBR also induced the proteins, before or concurrent with EBR. The compatible *P. tabaci* can not avoid recognition of its PAMPs by the plant surveillance system, but suppress the plant response, in this case the EBR215/250 chitinases.
2. The EBR215/250 proteins were induced slightly or not at all after osmotic, salt or oxidative stresses. These proteins differ from most of the known PR-proteins that they did not appear in the intercellular fluid after injection with salicylic acid, methyl-jasmonate or ethylene. The EBR-related proteins presumably do not play an important role in the defense mechanism stimulated by Bion®.
3. Based on their amino-acid sequence the proteins are hitherto unidentified chitinases (EBR215/250).
4. We concluded that a 15 amino-acid long flagellin peptide and lipopolysaccharide from *P. s.* pv. *coriandricola* were not elicitors

of the EBR as shown by the HR-inhibition test, nor of the EBR215/250 chitinases.

5. Peptidoglycan, a 22 amino-acid long cold shock and the 26 amino-acid long elongation factor (EF-Tu) peptide from conserved region of the proteins induced partial EBR and weakly elicited the EBR-marker proteins.
6. The 22 amino-acid long flagellin peptide strongly induced the EBR215/250 proteins and the EBR.
7. The EBR215/250 chitinases have lysozyme activity.
8. Defense response due to 6 hours long flg22 pretreatment had inhibitory effect to infection by *Agrobacterium tumefaciens*. However, the 24 hours long flg22 pretreatment failed in this respect.

CONCLUSIONS AND SUGGESTIONS

Blocking of protein synthesis in tobacco leaf by cycloheximide or heat shock prevented the EBR (Bozsó et al., 1999). In concert with these phenomena, vigorous changes in protein composition of intercellular fluid of tobacco indicated an active response mechanism during EBR (Ott, 2002). Our main goals were to determine the correlation between the protein induction and defence reaction induced by bacteria; the extracellular proteins characterizing this response; the possible roles of these proteins in EBR; the general bacterial elicitors of EBR; and whether resistance induced by this manner is effective against several types of bacterial diseases. Since the EBR215/250 proteins were detectable by all of the examined bacterial treatments except the compatible *P. tabaci*, they

presumably have a role in the course of the resistance and this was confirmed by the fact that the compatible pathogens aspire to inhibit theirs expression. Their correlation with EBR was reinforced by their early appearance after induction (recognition of bacteria). Treatments triggering known PR-proteins aroused attention to their specific induction properties. Their presence in the apoplastic fluid was characteristic to biotic stress, i.e. bacteria. Injection into leaves of signal molecules, such as salicylic acid, ethylene or jasmonic acid, triggered neither the EBR215/250 proteins nor the EBR itself, thus this resistance mechanism does not seem to be a consequence of these hormones. Moreover, the EBR seems to develop through salicylic acid-independent pathways as it was not influenced in NahG plants. In addition, the EBR215/250 proteins were not detectable during the defence mechanism induced by BTH.

An important question is what functions may be conceivable in the machinery of EBR. Proteins with lysozyme activity may have a direct effect on bacterial cells. The chitinase/lysozymes may damage or degrade the peptidoglycan layer of the bacterial cell wall. PR-proteins with known enzymatic activity and EBR215/250 chitinases were obtainable in the range 3.8-4.4 pH fraction. The known PR-proteins did not show lytic activity towards *M. lisodeicticus* cells, so the lysozyme activity of this fraction could be assigned to the EBR215/250 chitinases. Lytic activity may play an important role in the defence at least in two respects. Direct inhibitory effect against bacteria and/or indirect release of hidden or embedded elicitors.

Induction of EBR with heat-killed bacterial suspension probably involved recognition of several elicitors. Whether the whole EBR

mechanism develops as a response to individual PAMPs or its physiological and molecular events are combined effects of all elicitors? To answer this question requires specifying the individual PAMPs. Identifying the elicitor molecules is also important in experiments planned to improve resistance against important pathogens using *in vitro* cultures. The delayed response to LPS rather indicates elicitor activity for the late basal resistance which operates from 24-48 hours after inoculation. Peptidoglycan did not induce EBR to the extent induced by heat-killed cells, as measured by any of the assays (EBR215/250 proteins, HR-prevention). Nevertheless, the rapid appearance of EBR-related proteins and their maximal intensity at 12 hours after injection, which was similar to the response induced by heat killed bacteria, refers to a potential to alert the plant surveillance system. The flg22 peptide triggered the molecular and physiological markers of EBR as well. This is the first demonstration of HR-preventing elicitor capacity of flg22 peptide. Expression of EBR215/250 proteins was slight but fast after elicitation with csp22, they appeared in the intercellular fluid by six hours after injection. Activity of chitinases continuously increased and their amount at 24 hours after injection reached a level which coincided with the HR-inhibition feature. From this we concluded that a positive correlation exists between the amount of chitinases and HR-inhibition. A possible reason of the failure of HR-prevention beside low protein level at six hours may be that EBR consists of wide spectra of responses the chitinases of which represent only one segment. Up to the present it was known that peptide elf26 induces defence responses only in *Cruciferae* plants (Kuntze et al., 2004). A response was detected in tobacco with our

bioassays, as expression of EBR215/250 chitinases and HR-inhibition suggest recognition of this peptide in plants outside *Brassicaceae* family.

A response induced by flg22, probably in the frame of the EBR, reduced the tumor formation on tobacco leaf segments. To comprehend more of the results by the longer pretreatment is one of the objectives of the nearest future. Our conclusion that EBR may be used against important plant pathogens is reinforced by a recent study where a PAMP-mediated response had inhibitory effect against *A. tumefaciens* infection (Zipfel et al., 2006). Further examination of induction, specificity and role of EBR-related chitinases in EBR (effect of gene silencing to EBR) may lead us to develop more resistant plants due to enhancement or acceleration of expression of the contributing genes. As in these plants the genetical modification would be based on controlling their own proteins it shall be less misgiving to environmental protection.

REFERENCES

Bozsó, Z., Ott , P. G., Kecskés, M. L. and Klement Z. (1999) Effect of heat and cycloheximide treatment of tobacco on the ability of *Pseudomonas syringae* pv. *syringae* 61 *hrp/hrmA* mutants to cause HR. Phys. Mol. Plant Pathol., 55: 215-223.

Burgyán, J. and Klement, Z. (1979) Early induced selective inhibition of incompatible bacteria in tobacco plants. Phytopathol. Med., 18: 153–161.

Burketova, L., Sindelarova, M. and Sindelarov, L. (1999) Benzothiadiazol as an inducer of β-1,3-glucanase and chitinase isozymes in sugar beet. Biol. Plant., 42: 423-430.

Dow, M., Newman, M. A. and von Roepenack, E. (2000) The induction and modulation of plant defense responses by bacterial lipopolysaccharides. Annu. Rev. Phytopathol., 38: 241-261.

Espinosa, A. and Alfano, J. R. (2004) Disabling surveillance: bacterial type III secretion system effectors that suppress innate immunity. Cell. Microbiol., 6: 1027-1040.

Gomez-Gomez, L. and Boller, T. (2002) Flagellin perception: a paradigm for innate immunity. Trends Plant Sci., 7: 251-256.

Gomez-Gomez, L., Felix, G. and Boller, T. (1999) A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. Plant J., 18: 277-284.

Felix, G. and Boller, T. (2003) Molecular sensing of bacteria in plants. The highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. J. Biol. Chem. 278: 6201-6208.

Felix, G., Duran, J. D., Volko, S. and Boller, T. (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J., 18: 265-276.

Heukeshofen, J. and Dernik, R. (1985) Simplified method for silver staining of proteins in polyacrilamide gels and the mechanism of silver staining. Electrophoresis, 6: 103-112.

King, E. O., Ward, M. K. and Raney, D. E. (1954) Two simple media for the demonstration of pyocianin and fluorescin. J. Bacteriol. 170: 4748-4756.

Klement, Z. (1965) Method of obtaining fluid from the intercellular spaces of foliage and the fluids merit as substrate for phytobacterial pathogens. Phytopathology 55: 1033-1034.

Klement, Z. (1990) Inoculation of plant tissues. In: Klement, Z., Rudolph, L., Sands, D. C. Methods in phytobacteriology. Budapest, Akadémiai Kiadó 96-121.

Klement, Z., Bozsó, Z., Kecskés, M. L., Besenyei, E., Czelleng, A., and Ott, P. G. (2003) Local early induced resistance of plants as the first line of defence against bacteria. Pest. Manag. Sci., 59: 465-474

Klement, Z., Bozsó, Z., Ott, P. G., Kecskés, M. L. and Rudolph, K. (1999) Symptomless resistant response instead of the hypersensitive reaction in tobacco after infiltration of heterologous pathovars of *Pseudomonas Syringae.* J. Phytopathol. 12: 479-489.

Kuntze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T. and Felix, G. (2004) The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants. Plant Cell, 16: 3496-3507.

Ornstein, L. and Davis, B. J. (1964) Disc electrophoresis. I. Background and theory. Ann. New York Acad. Sci. 121: 32-49.

Ott P. G. (2002) A korai indukált rezisztencia (EIR) és a hiperszenzitív reakció (HR) növényben lezajló folyamatainak és kölcsönhatásainak jellemzése. Doktori értekezés, Szent István Egyetem Kertészettudományi Kar.

Ott, P. G., Szabó, L., Klement, Z. and Balázs, E. (1998) Submicroscopical evidence of bacterially induced resistance in tobacco leaves. Acta. Phytopathol. Acad. Sci. Hung., 32. 265-280.

Ott, P. G., Varga, G. J., Szatmári, Á., Bozsó, Z., Klement, É., Medzihradszky, K. F., Besenyei, E., Czelleng, A. and Klement, Z. (2006) Novel extracellular chitinases rapidly and specifically induced by general bacterial elicitors and suppressed by virulent bacteria as a marker of early basal resistance in tobacco. Mol. Plant-Microbe Interact., 19: 161-172.

Selsted, M. E. and Martinez, R. J. (1980) A simple and ultrasensitive enzymatic assay fot the quantitative determination of lysozyme in the picogram range. Anal. Biochem., 109: 67-70.

Szatmári, Á., Ott, P. G., Varga, G. J., Besenyei, E., Czelleng, A., Klement, Z. and Bozsó, Z. (2006) Characterisation of basal resistance (BR) by expression

patterns of newly isolated representative genes in tobacco. Plant Cell Rep., 25: 728-740.

Trudel, J. and Asselin, A. (1989) Detection of chitinase activity after polyacrylamide gel electrophoresis. Anal. Biochem. 178: 362-366.

Van Loon, L. C. (1983) The induction of pathogenesis-related proteins by pathogens and specific chemicals. Neth. J. Plant Pathol., 89: 265-273.

Zipfel, C. and Felix, G. (2005) Plants and animals: different taste for microbes? Curr. Opin. Plant Biol., 8: 353-360.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D. G., Boller, T. and Felix, G. (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. Cell, 125: 749-760.

SCIENTIFIC PUBLICATIONS

Articles

1. Czelleng, A., Bozsó, Z., Ott, P. G., Besenyei, E., **Varga, G. J**., Szatmári, Á., Hafez, Y. M. and Klement, Z. (2004) Isolation of *in planta*-induced genes of *Pseudomonas viridiflava*. ***Acta Phytopathologica et Entomologica Hungarica***, 39: 361-375.

2. **Varga G. J**., Ott P. G., Klement É., Medzihradszky K. F. és Klement Z. (2005) A korai általános rezisztenciával kapcsolatos új kitinázok dohányban. ***Növényvédelem***, 7: 287-296.

3. Bozsó, Z., Ott, P. G., Szamári, Á., Czelleng, A., **Varga, G. J**., Besenyei, E., Sárdi, É., Bányai, É. and Klement, Z. (2005) Early detection of bacterium induced basal resistance in tobacco leaves with diamino-benzidine (DAB) and dichlorofluorescein diacetate (DCFH-DA). ***Journal of Phytopathology***, 153: 596-607. If: 0,575.

4. Czelleng, A., Bozsó, Z., Ott, P. G., Besenyei, E., **Varga, G. J.,** Szatmári, Á., Király, L. and Klement, Z. (2005) Identification of virulence-associated genes of *Pseudomonas viridiflava* activated during infection by use of a novel IVET promoter probing plasmid. ***Current Microbiology***, 52: 282-286. If: 1,15.

5. Besenyei, E., Ott, P. G., Bozsó, Z., Czelleng, A., Szatmári, Á., **Varga, G. J.** and Klement, Z. (2005) Low temperature delay and inhibition of a plant defence mechanism: early basal resistance in tobacco. ***Acta Phytopathologica et Entomologica Hungarica,*** 40: 323-332.

6. Ott, P. G., **Varga, G. J**., Szatmári, Á., Bozsó, Z., Klement, É., Medzihradszky, K. F., Besenyi, E., Czelleng, A. and Klement, Z. (2006) Novel extracellular chitinases rapidly and specifically induced by general bacterial elicitors and suppressed by virulent bacteria as a marker of early basal resistance in tobacco. ***Molecular Plant-Microbe Interaction***, 19: 161-172. If: 3,580.

7. Szatmári, Á., Ott, P. G., **Varga, G. J.,** Besenyei, E., Czelleng, A., Klement, Z. and Bozsó, Z. (2006) Characterisation of basal resistance (BR) by expression patterns of newly isolated representative genes in tobacco. ***Plant Cell Reports***, 25: 728-740. If: 1,457.

8. **Varga, G. J**., Szegedi, E., Ott, P. G. and Szabó, E. (2006) Early basal resistance inhibits tumor induction by *Agrobacterium tumefaciens*. ***Cereal Resarch Communication***, 34: 97-100. If: 0,157

9. Szabó, E., Klement, É., Medzihradszky, K. F., **Varga, G. J.**, Besenyei, E., Ott, P. G. (2006) Changes of apoplast protein composition during early basal resistance (EBR) in tobacco. ***Cereal Research Communication***, 34: 677-680. If: 0,157.

10. Ott, P. G., **Varga, G. J**., Szatmári, Á., Bozsó, Z., Besenyi, E., Czelleng, A. and Szabó, E. (2006) Basal resistance of plants: from discovery to molecular characterisation. ***Acta Phytopathologica et Entomologica Hungarica***, 41: 37-46.

11. **Varga, G. J**. et al. előkészületben (2006) Induction of early basal resistance (EBR) in tobacco plants with general bacterial elicitors (PAMPs).

Abstracts

12. Ott, P. G., **Varga, G. J**., Szatmári, Á., Bozsó, Z., Klement, É., Medzihradszky, K. F and Klement, Z. (2003) Characterisation of apoplastic proteins associated with the early induced resistance (EIR). 11th International Congress on Molecular Plant-Microbe Interactions (pp.130) St.-Petersburg, Russia July 18-26.

13. **Varga, G. J**., Ott, P. G., Besenyei, E., Bozsó, Z., Czelleng, A., Szatmári, Á., and Klement, Z. (2004) Novel Bacterially Induced Chitinases in tobacco with expression features different from most part of other known chitinases. International Joint Workshop on PR-proteins and Induced Resistance (pp. 96) Elsinor, Denmark May 5-9.

14. Szatmári, Á., Bozsó, Z., Besenyei, E., **Varga, G. J**., Ott, P. G., Czelleng, A. and Klement, Z. (2004) Cloning and analysis of genes related to the early form of general defence response of plants against bacteria by subtractive hybridisation and quantitative PCR. The 14th FESPB Congress (pp.265) Cracow, Poland August 23-27.

15. Besenyei, E., Ott, P. G., Bozsó, Z., Szatmári, Á., **Varga, G. J**., Czelleng, A. and Klement, Z. (2004) Inhibition of plant defence responses by low temperatures. 7th Conference of the European Foundation for Plant Pathology and British Society for Plant Pathology Presidential Meeting (pp. 31) University of Aberdeen, UK September 5-10.

Popular scientific articles

16. **Varga G. J**. (2005) Növényvédelem – önerőből. Élet és Tudomány, 32: 1001-1003. OTKA-Élet és Tudomány Kutatásismertető Cikkpályázat 2004, III. díj.

Lectures

17. **Varga G. J**., Ott P. G., Besenyei E., Bozsó Z., Czelleng A., Szatmári Á., Klement É., Medzihradszky K. F. and Klement Z. (2004) Korai indukált rezisztenciához kapcsolható új kitinázok dohányban. 50. Növényvédelmi Tudományos Napok (pp. 105) Budapest Február 24-25.

18. **Varga G. J**., Ott P.G. és Klement Z. (2005) A baktériumok árulkodó jelei: Mi alapján ismerik fel a növények a baktériumokat? 51. Növényvédelmi Tudományos Napok (pp. 38) Budapest, Február 22-23.