Some Genetic, Biochemical and Morphological Analysis of Selected Powdery Mildew Strains at the Beginning of Sporulation on Barley

Elena HLINKOVÁ[1], Milan BOBÁK[2], Vladena BAUEROVÁ-HLINKOVÁ[3], Ján RAFAY[4]

[1]Comenius University Bratislava, Faculty of Natural Sciences, Institute of Cell Biology,
Mlynská dolina G-1, 84215 Bratislava, Slovakia; hlinkova@fns.uniba.sk
[2]Faculty of Natural Sciences, Department of Plant Physiology, Mlynská dolina B-2, 84215 Bratislava, Slovakia
[3]Institute of Molecular Biology SAS, Dubravská cesta 21, 845 51 Bratislava, Slovakia
[4]Research Institute of Animal Production, Hlohovecká cesta 2, 949 92 Nitra, Slovakia

Abstract

The present work analyzes some characteristics of four powdery mildew pathotypes, 'RU-3', 'Sk-5/11', 'Sk-12/1' and 'A-4/0', selected from the wild strains of BGH from Central European regions. Our results showed that the studied BGH strains differ in the virulence and avirulence genes in their genomes, in the prolongation of their asexual phase of the growth and also in their morphological and biochemical characteristics. Protein analysis confirmed the genetic differences between the studied powdery mildew pathotypes. Abundant acid glucanases in all studied BHG pathotypes were found between molecular weights Mr ≈ 25-35 kDa and 11-22kDa. Races 'RU-3' and 'A-4/0' also contained low molecular weight glucanases with Mr ≈ 9-14kDa. Immunological analyses showed higher specificity of pathogen chitinases to plant antibody compared to barley cultivars carrying different dominant/semidominant resistance genes. Rabbit antibody prepared against the plant interacellular acid chitinase Chi 14.4 (PR-4) gave the positive signal for two powdery mildew races, 'Sk-5/11' and 'A-4/0'. These pathotypes were more aggressive compared to races 'Sk-12/1' and 'RU-3'. Their genomes contained more virulence genes and the asexual phase of the growth was shorter. Ultrastructural analyses of BGH body in the sensitive barley cultivar cells, showed presence of virus like particles, which probably play a role in the synthesis of some PR-proteins with hydrolytic function. Genetic and biochemical analyses indicate that some powdery mildew pathotypes contain genes in their genome which are orthological to those in their hosts, thus making them suitable subjects for the future as a source of new resistance genes for plant breeding.

Keywords: barley, powdery mildew, β-1,3-glucanase, chitinase, PR-proteins, immunodetection

Abbreviations: Glu-β-1,3-glucanase, Chi-chitinase, BGH-*Blumeria graminis* (DC) Speer f.sp. *hordei*, PR-pathogen related, cv-cultivar, rg-resistance gene.

Introduction

Blumeria graminis (DC) Speer f. sp. *hordei* (BGH) is one of the most widespread pathogen influencing the growth processes of cereals and, in the later phases, the quality and quantity of grains. BGH has a very flexible genome which can be very effectively modified by environmental factors as well as low concentration doses of fungicides used in fields conditions for the plant protection. The genome of BGH is not very wide and contains approximately 10 000 genes (Carlil and Watkinson, 1995). The asexual phase of the powdery mildew development has a relatively short life-cycle which causes fast reproduction of this pathogen (Hartleb *et al*., 1997). Moreover, the genome flexibility of this pathogen causes its fast adaptation to changing environmental conditions. The pathogenicity of BGH is manifested in many virulent strains which overcome the resistance genes of the host plant. Resistance genes protect the host plants from to the pathogens and

their proteins are very often incorporated into their cell walls or create a group of transmembrane proteins (Lyon and Newton, 2005; Hartleb *et al*., 1997). Proteins of R-genes not only protect the host plants against the pathogens but they enable completing the growth phase of the host by an incompatible and hypersensitive reaction. The host plant R-genes, which were overcome or fungicide protection which was ineffective, cause death of infected plants in 20-80% of cases (Dreiseitl and Jorgensen, 2000). In the cases when the ontogenetic cycle of the infected host plant was finished, the grains in spikes showed poor development and the quantity and quality of the storage proteins was lower compared to the control grains from the healthy plants. The amount of glutenins and hordeins in the grains decreased (Hlinková, unpublished results). Research has been primary directed toward the taxonomy, morphology, physiology and genetics of the interactions BGH with barley from the early phases of infection (Hatleb *et al*., 1997; Kunoh, 1982; Bushnell and Rowell,

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1981). Much less is known about their molecular, genetic, biochemical characteristics and ultrastructural analysis from the later stages of asexual phases of BGH development. These facts led us to the study of some genetic, molecular and ultrastructural changes for selected pathotypes of BGH in the latest phase of infection as a source of genes for future possibilities of increasing resistance of barley genomes against powdery mildew infections.

Materials and methods

Three powdery mildew pathotypes 'Sk-5/11', 'Sk-12/1' and 'A-4/0' were selected from the wild strains of Central Europe BGH isolates in west Slovakia and north Austria, respectively. Pathotype 'RU-3' was received from the collection of isolates of TU München Saint-Stephen (Germany). Genetic and ontogenetic analyses were done on the leaf segments of barley near the isogenic line cv. 'Pallas' carrying different resistance genes from *Ml-a* locus: P02 (*Ml-a3*); P04B (*Ml-a7*); P10 (*Ml-a12*). As a control barley genotype, the sensitive cultivar 'Dvoran' without resistance genes from the *Ml-a* locus was used. Leaf segments were prepared from eight day old aseptically cultivated barley plants. Segments, spread with BGH conidium-spores, were cultivated in Petri dishes on 0.6% agar medium supplemented with 2.5 mg.l^{-1} benzimidazol at 8°C and illumination of 32 µmol.m^2.s^{-1}.

Morphological and ultrastructural analyses

Conidium-spores of BGH at the beginning of the sporulation phase of their growth, were resuspended in a sterile solution of 2.5% saccharose and immediately photographed with a Nikon (Japan) light microscope at a magnification of 320x. Morphological analyses of the early and late phases of powdery mildew development were done with Nikon light microscope at 320x magnification on fixed and stained samples. Fixation of samples (fixation solution: 19 ml of 96% ethanol, 6 ml chloroform, 250 µl

TCA) took 24 hours at 6°C. Colour-less leaf segments were stained with Coommassie brilliant blue R-250 solution (0.01%-our modification) of the Wolf Frič (1981) method.

Transmission electron microscopy (TEM) was performed on a semi-thin section prepared according to Bobák *et al.* (1998). Ultrathin sections were stained with uranyl acetate and lead citrate according to Reynolds (1963) and Bobák *et al.* (1995). Results were obtained with a Jeol 2000 FX electron microscope.

Molecular and biochemical analyses

Intracellular proteins were extracted from 25 µl conidia-spores homogenized in 0.1 M Na-phosphate buffer pH 7 (Hlinková *et al.*, 1995). The quantitative content of soluble proteins was determined according to Bradford (1976). Separation of native and denatured proteins was done on 12.5% discontinue polyacrylamide gel. Protein samples and A-PAGE solutions in the non-denaturing conditions did not contain SDS and β-mercaptoethanol. The concentration of separated protein samples per lane for A- PAGE was 2.5µg. Hemoglobin (Mr ≈ 68.52; 30; 15.5 kDa), albumin (Mr ≈ 66.7; 66.2 kDa) and lysozyme (Mr ≈ 14.4 kDa; Serva, Germany) were used as a molecular mass standards. Electrophoresis was done at 6°C by U = 4 V cm^{-1}. Acid β-1,3-glucanases were detected after A-PAGE directly on the slab gels according to Pan *et al.* (1991). Primary polyclonal rabbit antibodies were prepared against barley PR-4 (Chi 14.4 kDa extracellular) and PR-2 (Glu 24.4 kDa membrane) proteins synthesized in the host plant after infection with BGH, strains Sk5/11 and 'RU-3', respectively. PR-4 was isolated from the infection site of the host plant into 0.1 M phosphate buffer, pH7. PR-2 protein was obtained from the leaf tissue distant from the infection site into 0.01 M phosphate buffer pH4, containing 0.5 mM MgCl$_2$, 10% Triton X-100, 2 M urea and 25% glycerol. For the rabbit immunization, both proteins were isolated after 1D-A-PAGE into 0.1 M phos-

A   B   C   D   E

Fig. 1 Panel A: Conidium-spore of powdery mildew, race 'RU-3', in the period of pathogen sporulation-144 hours after inoculation; Bar: 10µm; Magnification: 380 x; Panel B: Early stage of BGH germinating on sensitive cultivar 'Dvoran'. Pathotype 'Sk-5/11' had a lateral type of germination and its asexual phase of development finished after 90 hours-24 hours after inoculation, A-apressorium; p- papilla; Magnification: 380x. Panel C:Incompatible interaction between host and pathogen. Pathotype 'Sk-12/1' growing on the isogenic barley line P02 (r.g.: Ml-a3) - 36 hours after inoculation-incompatible interaction. Transmission electron microscopy was done according to Bobák *et al.*(1998); bar: 10µm. Panel D: Compatible interaction between BGH and host plant. Pathotype 'Sk-5/11' germinating on the sensitive cultivar barley with well developed haustorium (H) body- 48 hours after inoculation; Panel E: The end of asexual phase of the BGH growth. Well developed air mycelium of BGH-race 'RU-3'- 120 hours after inoculation; Magnification: 240x

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phate buffer pH7. Antibodies to barley PR-2 and PR-4 proteins and slot-blot immunological reactions were done according to Repka (1997).

Results and discussion

Studied powdery mildew races were selected from wild Central European strains of BGH. Strains were cleaned in *in vitro* conditions during three subsequent transfers on aseptic barley segments prepared from the primary leaves of cv. 'Dvoran'.

Morphological analyses showed that the selected strains of BGH differ in their diameter and length of conidium-spores. In the period of sporulation, the smallest diameter of conidium-spores was found for race 'RU-3' (30 µm, Fig. 1A). This strain was used as a standard. BGH strains selected from the West Slovakia wild races, 'Sk-5/11' and 'Sk-12/1', had diameters from 40 to 50 µm. The duration of the asexual phase of BGH was the shortest for race 'RU-3' (96 h), for other races these phases lasted from 110-136 hours. The longest asexual phase of the growth was found for the pathotype 'Sk-5/11'. This pathotype of BGH also differed in the colour of their air-mycelia. Race 'RU-3' and 'Sk-12/1' were white, 'Sk-5/11' was slightly pink and 'A-4/0' had a light brown color. On the eighth day after inoculation of the barley leaf segments with BGH, the surface under air-mycelia of BGH on the leaf segments of cv. 'Dvoran' did not contain "green islands". The appearance of "green islands" beneath powdery mildew air-mycelia, in the infected leaf (only by compatible interactions) in the latest asexual phase of BGH infection, indicates on a connection with growth regulators during the pathogenesis, preferentially with cytokinins (Hartleb *et al.*, 1997) and appears only for some strains of BGH. Conidium-spores races of 'RU-3' and 'A-4/0' germinated apically and 'Sk-5/11' and Sk-12 /1 laterally (Fig. 1B). Haustorium body of BGH- race 'Sk-5/11' was fully developed 48 hours after inoculation (Fig. 1C). Fully developed air-mycelliun of BGH -race 'RU-3' (Fig. 1E) on the sensitive barley cultivar 'Dvoran' was differentiated 120 hours after inoculation. Fig. 1C showed incompatible reaction of BGH race 'Sk-12/1' with barley isogenic line P02(r.g. *Ml-a3*).Transmission electron microscopy (TEM) showed fully destroyed germinating conidium-spore BGH 36 h after inoculation. Virulence analysis and reaction type of powdery mildew races studied on the isogenic barley lines carrying different resistance genes (*Ml-a3, Ml-a7 and Ml-a12*) showed that 'A-4/0' is the most virulent strain (Tab. 1). MMinimal virulence ability was exhibited by strain 'Sk-12/1'. From the studied powdery mildew races for the following analysis were selected strains 'RU-3' and 'Sk-5/11' as the strains with a widespectrum of effects to different resistance genes of host barley plants. Incompatible reactions were detected on barley genotypes P02 (r.g. *Ml-a3*) for 'RU-3' and 'Sk-12/1'; hypersensitive reactions were found for P04B (r.g. *Ml-a7*) with races 'Sk-12/1' and 'Sk-5/11'.

The reaction type of BGH was designated according to its phenotype on the host genotype (Schwarzbach and Fischbeck, 1981):

0-no visible changes/incompatible reaction

II-necrotic spots with weak pathogen sporulation

III-necrotic spots with strong pathogen sporulation

IV-strong pathogen sporulation without necrotic spots

HR-hypersensitive reaction

The presence of the virulence/avirulence genes in the genome of analyzed BGH strains is represented in Tab. 2. For these analyses the following isogenic barley lines cv. 'Pallas' were used: P02(*Ml-a3*), P04B(*Ml-a7*), P08B(*Ml-a9*), P10(*Ml-a12*), P11(*Ml-a13*). Cultivar 'Salome' was used only for the test on the overcomming of *mlo* gene for BGH-race 'Sk-5/11'. Overcoming of resistance genes and basic resistance of the host plants led to the compatible interactions of BGH with the host barley genotypes. The most resistant barley genotypes against the analysed BGH

Tab. 1. Virulence analysis and reaction types of used powdery mildew races

Pathogen	Barley genotype/R-gene			
	'Dvoran'	P02/*Mla-3*	P04B/*Ml-a7*	P10/*Ml-a12*
'RU-3'	III-IV	0	III	IV
'A-4/0'	III	IV	IV	IV
'Sk-12/1'	IV	0	HR	HR
'Sk-5/11'	IV	HR	HR	III

races were isogenic line P02(r.g. *Ml-a3*) and P04B(r.g. *Ml-a7*).

Studies which include ultrastructural analysis of developing haustorium BGH and virulence analyses are rare. Ultrastructural analyses done by Kunoh (1982) which are unique, are preferentially concentrated on the central body of haustorium and nucleus of BGH in the early phases of infection (from 24-48 h). Ultrastructural analysis of haustorium "fingers" in the later phase of powdery mildew infection had not been published yet. We found that these "fingers" are covered with double membrane, which completely separate haustorium and its "fingers" from the intracellular space of the host cell (Fig. 2). Fingers contain virus like particles (VLP) in larger amount as

Tab. 2. Virulence genes identified in four barley powdery mildew races

Race	Virulence genes
'RU-3'	*avrMl-a3; virMl-a7;virMl-a9;virMl-a12;virMl-a13*
'A-4/0'	*virMl-a3;virMl-a7; vir Ml-a12*
'Sk-12/1'	*virMl-a3; avrMl-a7; avrMl-a12*
'Sk-5/11'	*avrMla3*; avrMl-a7*; virMl-12; virml-o*

vir; avr- virulence/avirulence genes present in the genome of studied powdery mildew pathotypes; *-Hypersensitive reaction; Analysis were done on barley isogenic lines cv. 'Pallas' with dominant and semidominant specific resistance genes (R)

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central body of haustorium. VLP, as we can see in EHM (extra haustorium membrane), are transported via endocytosis to the extracellular space of the haustorium (intracellular space of infected epidermal host cell). Surface of VLP is structured and with high probability it contains glycoprotein molecules. Such particles were identified by ultrastructural analysis of *Streptoccocus aureus* intracellular space (Pastorek, personal communication).VLP with high probability contains dsRNA, while iRNA probably participates in the synthesis of ribosomal inactivating proteins (RIPs) during the defense reaction of the host (Hartleb *et al.,* 1997; Hlinková *et al.*, 2005). These proteins have a relativly small molecular mass and were found preferentially in the cathodic protein patterns (C-PAGE). Their amount is highest in the infection site and it is independent from the host genotype. They have a strong hydrolytic function (Hlinková *et al.*, 2005; 2008).

In the late phase of infection, BGH hydrolytic enzymes and, probably, RIPs fully destroyed membrane system of intracellular structures of infected host cell (Fig. 2). Nucleus, vacuoles, mitochondria and other subcellular structures were hydrolyzed. Infected epidermal cell contained only membrane debris and BGH haustorium. Membrane debris (D) had a size from 2 to 25 nm (Fig. 2). Morphological and genetic differences of BGH studied races were represented in the protein patterns. Denaturated protein patterns of BGH-race 'Sk-5/11' is shown in Fig. 3. Load per lane was 0.8 µg crude protein extract. Gel was stained with AgNO$_3$, according to Beňová *et al.* (2005), as a pro-

tein ladder, wide spectrum protein molecular mass standard from Fermentas Life Sciences was used. Asterix indicated the presence of Chi 14.4 in the protein pattern of a pathogen whose presence of native form was confirmed by slot-blot immunoblot (Fig. 3 and 4).

The numerical values of non-denaturated acid protein compared to acid protein pattern BGH race 'RU-3' are shown in Tab. 3. They differ through the entire interval of Mr of acid proteins separated on 12.5% PAAG. The presence of proteins with Mr ≈ 34.4 and 38 kDa (mitotic activated proteins kinases-(Mészárosz *et al.,* 2000)) indicate on the presence of mitotic cycles in more slowly developed strains of BGH, 'A-4/0' and 'Sk-5/11'.

Other identified proteins are connected with the metabolic pathways characteristic for the early phases of powdery mildew sporulation (last stage of asexual phase of development). Biochemical analyses showed the presence of acid β-1,3-glucanases (Glu) in the protein patterns (Tab. 4) which were synthesized in an abundant amount. Differences in Glu with high molecular weight indicate on a various activity of *glu* constitutional genes connected with early phases of BGH sporulation. Glu with Mr < 40 kDa are presented in all patterns. Their presence is probably connected with an early stage of ascospores differentiation. Very low Mr glucanases have their protein analogs in the barley cells infected with BGH by compatible interactions (Hlinková *et al*., 2002). Glucanases with identical Mr were also found in transgenic potato plants (Moravčíková *et al*., 2004).
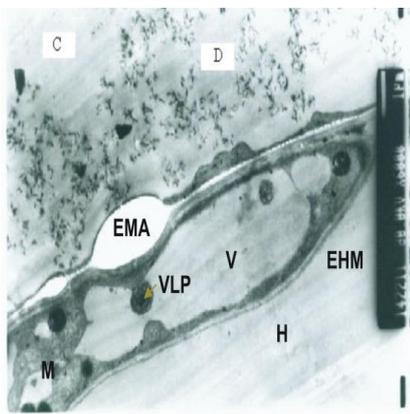

Fig. 2. Longitudinal ultra-thin section of infected epidermal barley leaf cell with well developed "finger" of BGH/'RU-3' haustorium in the sensitive barley cultivar 'Dvoran'.

72 hours after inoculation.V-vacuole; M-mitochondria; VLP-virus like particles; H-haustorium finger; EHM-extracellular haustorium membrane; EMA-extracellular matrix of haustorium; D- debris (2-25nm); M-mitochondria; C-intracellular space of infected cell; bar: 1 µm
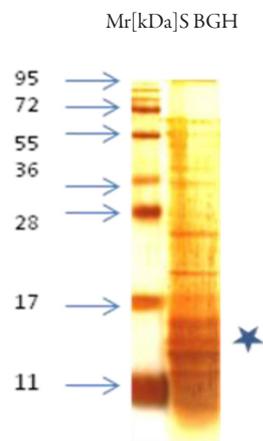

Fig. 3. SDS-PAGE protein patern of intracellular extract isolated from BGH conidium-spores race 'Sk-5/11' in the end of asexual phase of the growth.

Load per lane was 0.8µg crude protein extract. Gel was stained with AgNO3 according to Beňová *et al.* (2005); as a protein ladder, wide spectrum protein molecular mass standard from Fermentas Life Sciences was used. Asterix indicate the presence of protein/polypeptide with chitinase activity - Chi 14.4.
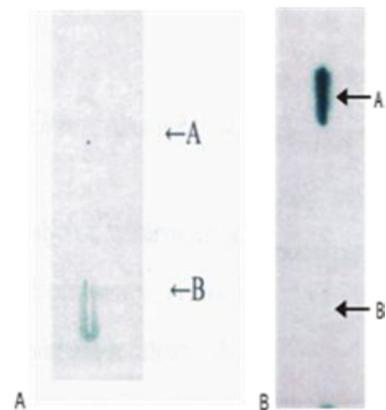

Fig. 4. Slot-blot immunoblot with partial specific antibodies prepared against the acidic extracellular barley Chi 14.4 (A) and acid membrane Glu 24.4 (B) with different powdery mildew protein extracts.

SDS-PAGE protein patern of intracellular extract isolated from BGH conidium-spores race 'Sk-5/11' in the end of asexual phase of the growth Load per lane was 0.8µg crude protein extract. Gel was stained with AgNO$_3$ according to Beňová *et al.* (2005); as a protein ladder, wide spectrum protein molecular mass standard from Fermentas Life Sciences was used. Asterix indicate the presence of protein/polypeptide with chitinase activity - Chi 14.4.

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The second group of hydrolytic enzymes which we can find in the BGH protein patterns in this development phases are chitinases (Chi). During pathogen infection their role probably consists in decreasing host defence reactions-immobilization of DR-proteins and destruction of cell wall of the host by compatible interaction as well as probably on the participation of papilla creation in the early phases (Chi with high molecular masses). The latest

Tab. 3. Differences in the acid protein patterns of BGH races compared to 'RU-3'

Race	Proteins Mr (kDa)
'A-4/0'	220; 150; 120; 84; 74; 50; 46; 42; 34; 17; 15; 14.5; 12; 10; 9
'Sk-12/1'	250; 220; 120; 50; 48; 45; 42; 31; 25; 21; 15; 14; 17; 12; 11; 10
'Sk-5/11'	250; 120; 74; 66; 45; 34; 38; 36; 26; 21; 17; 14.4; 16

Analyses were performed for silver stained gels (Beňová *et al.* 2005). The load was 0.9 μg of proteins per lane.

phases of infection are connected with higher amount of Chi with lower masses. In the infected plant cells by compatible interaction, chitinases were presented in greater abundance in the intracellular space (Hlinková *et al.*, 2002; 2004). Differences in the protein patterns Chi indicate on the same conclusion. Differences in the Mr can more over depend on the developmental phases and and genes connected with this process as well as on the amount of β-1,4-

Tab. 4. The presence of acid intracellular β-1,3-glucanases in the protein extracts of powdery mildew

Race	Acid β-1, 3- glucanases, Mr (kDa)
'RU-3'	200; 80; 42; 28; 24.5; 11; 8
'A-4/0'	250; 150; 100; 80; 40; 31; 28; 12; 10; 9
'Sk-12/1'	250; 120; 40; 31; 10;
'Sk-5/11'	250; 40; 31; 28; 21; 12; 10

Glucanases were detected according to Pan *et al.* (1989). The load was 12 μg protein per lane. Laminarin was used as a substrate.

N-acetyl glucosoamine radicals (Collinge *et al.*, 1993).

The presence of low molecular chitinases synthesized in the infected barley plant cells and in the conidium-spores of BGH at the end of asexual phase pathogen development, led us to find out if antibody prepared against the barley intercellular acid Chi 14.4 is serologically related to some powdery mildew Chi proteins. Slot-blot immunological analysis of BGH extracts for 'RU-3' and 'Sk-5/11' extracts with polyclonal plant antibody prepared for plant PR-2 and PR-4 proteins showed that some BGH strains give apositive immunological signal to them (Fig. 4). The positive serological signal indicated on the presence of the active orthological genes in the genome BGH- race 'Sk-5/11' during this phase of development (Fig. 3). The protein/ polypeptide with chitinase activity and identical Mr is designated with asterix in the pattern. This fact indicates

the posibility to use some BGH gene to active protection of barley genotypes.

Conclusions

Results obtained from our experiments showed that studied races of BGH differed on morphological, molecular and genetic levels as well as in the duration of the asexual phase of development. Biochemical and molecular changes showed that they strongly depend on the recognition of the specific resistance gene products of the host plants as well as on the efficiency of the avirulence/virulence genes of the pathogens. Immunological reactions showed that some strains of BGH contain serologically related active genes to intercellular barley Chi 14.4 and membrane Glu 24.4. Proteomic analyses of these proteins are continued.

References

Bobák, M., B. Blehová, J. Krištín, M. Ovečka and J. Šamaj (1995). Direct plant regeneration from leaf explants of Drosera rotundifolia cultured in vitro. Plant Cell Org. Tissue Cul. 43:43-49.

Bobák, M., A. Hlavačka, M. Ovečka and J. Šamaj (1998). Effect of trifluralin and colchicine on the extracellular matrix surface networks during early stages of direct somatic embryogenesis of *Drosera rotundifolia* L. J. Plant Physiol. 155:387-392.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein dye binding. Annal. Biochem. 72:248-254.

Beňová, A., E. Hlinková and A. Šimonovičová (2005). Changes in the protein patterns of microscopic fungi *Aspergilus niger* TIEG and *Aspergilus clavatus* DESM by stress conditions Phytopedon (Bratislava) 4:19-27.

Bushnell, W. R. and J. B. Rowell (1981). Supressors of defense reaction: A model for roles in specificity. Phytopathology 71:1012-1014.

Carlil, M. J. and S. C. Watkinson (1995). The Fungi, A P, London.

Dreiseitl, A. and J. H. Jorgensen (2000). Powdery midew resistance in Czech and Slovak barley cultivars. Plant Breeding 119:203-209.

Fehér, M., T. P. Pasternak and D. Dudits (2003). Transition of somatic plant cells to an embryogenic state. Plant Cell Tiss. Org. Cult. 74:201-228.

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Freialdenhoven, A. B, K. Scherag, D. B. Hollricher, H. Collinge, H. Thordal-Christensen and P. Schulze-Lefert (1994). Nar-1 and Nar-2, two loci required for Mla-12-specified race specific resistance to powdery mildew in barley. Plant Cell 6:983-994.

Hartleb, H., R. Heitefuss and H. H. Hoppe (1997). Resistance of crop plants against fungi, (Ed. H. Hartleb), G. Fischer, Jena-Stutgart-Lübeck-Ulm.

Hlinková, E., M. Sýkora and Z. Šubr (1995). Content of soluble proteins in barley leaves after powdery mildew (*Erysiphe graminis* f.sp. *hordei* Marchal) infection. Agriculture 41:597-613.

Hlinková, E., M. Bobák and P. Illéš (2002). Chitinases and endoglucanases synthesized in the infected barley leaves in the powdery mildew period sporulation. Plant Protect. Scien. 38(2):469-473.

Hlinková, E., D. Perečko, J. Rafay, M. Bobák, Z. Kutarňová and Z. Kováčová (2005). Cathodic PR and DR-intercellular proteins synthesized in the infected barley leaves-their biochemical function. J. App. Biomed. (Suppl.) 3:17-18.

Hlinková, E., M. Bobák, V. Repka, J. Rafay, Z. Kováčová and M. Hudecová (2004). Biochemical changes in the cell wall and membranes infected barley leaves carrying various resistance genes in the period pathogen sporulation, pp. 40-44. In: M. Užík (Eds.). New knowledge from genetics and plant breeding, Proceedings from the 11-th International conference Piešťany November 24-25, 2004, SCAR Piešťany (in Slovak).

Hlinková, E., V. Bauerová-Hlinková, M. Bobák and D. Valková (2008). Some molecular and biochemical characteristics of PR-4 protein synthesized by host-pathogen interaction with different genetic background, pp. 108-111. In: Šudyová V. and Gregová E. (Eds.). New knowledge from genetics and plant breeding, Proceedings from the 15-th International conference Piešťany, November 12-13, 2008, SCAR Piešťany (in Slovak).

Kunoh, H. (1982). Plant infection. Jap. Scie. Soc., Springer Verlag, Tokyo, Berlin.

Lyon, G. D. and A. C. Newton (2005). Disease resistance-related proteins in barley http://www.scri.sari.ac.uk/SCRI/Web/Site/...logy/Cereals

Mészárosz, T., P. Miskolczi, F. Ayaydin, A. Pettkó-Szandtner, A. Peres, Z. Madyar, G. V. Horváth, L. Bakó, A. Fehér and D. Dudits (2000). Multiplay cyclin-dependent kinase complexes and phosphatase control G2/M progression in alfa alfa cells. Plant Mol. Biol. 43:595-605.

Moravčíková, J., I. Matušíková, J. Libantová, M. Bauer and L. Mlynárová (2004). Expression of cucumber class III chitinase and Nicotiana plumbaginfolia I glucanase genes in transgenic potato plants. Plant Cell Tiss. Org. Cult. 79:161-168.

Pan, Q. S., S. Ye and J. Kuç (1991). A technics for detection of chitinases , β-1,3-glucanases and protein patterns after a single separation using polyacrylamide gel electrophoresis and isoelectrofocusation. Phytopathology 81:970-974.

Repka, V. (1997). Intra and extracellular isoforms of PR-3 class chitinase in virus-infected cucumber plants. Acta Virol. 41:71-75.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-213.

Schwarzbach, E. and G. Fischbeck (1981). Die Mehltauresist-enzfaktoren von Sommer und Winter Sorten des Gernstens in der Bundes Republik Deutschland. Z. Pfl. Zücht. 87:309-318.

Wolf, G. and F. Frič (1981). A rapid staining method for *Erysiphe graminis* f.sp. *hordei* in and on whole barley leaves with protein-specific dye. Phytopathology 71:596-998.