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Proteome analysis of rice leaf tissue in response to blast disease (*Magnaporthe grisea*) pathogen

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Abstract

Blast disease, caused by *Magnaporthe grisea* (Hebert) Barr, is often an important impediment in the production of rice in temperate and tropical areas. Blast is considered as the most injurious disease of rice in Iran, resulting in severe loss especially to susceptible rice varieties. In order to we used two dimensional gel electrophoresis (2-DE) to investigate the response of rice leave tissue against blast disease at seedling stage two local varieties were grown in growth chamber under controlled condition. In this study, two rice varieties *viz.* Khazar and Hashemi, was used according to the relatively large area of cultivation in the north of Iran. 20 days old seedlings were inoculated with fungi isolated Gill 804, supplied from rice research institute. 48 hour after inoculation, by blast fungus proteins were extracted from rice leaves tissue and two-dimensional gels obtained after the imaging, with PDQuest software compared and expression levels of each proteins were analyzed and also ten days after inoculation different characteristics such as number of spots, spot size, infection type and leaf length were evaluated. Results indicated Five proteins in Susceptible varieties and five proteins in Resistant varieties were induced or increased in the inoculated leaves. In susceptible varieties (Hashemi) proteins that were differentially expression including (β -1,3- glucanase (Glu1), (β -1,3-glucanase (Glu2), Putative transketolase, Manganese-superoxide dismutase and Isoflavoneoid reductase. In Resistant varieties (Khazar) proteins that were differentially expression including, β -1,3-glucanase 2 (Glu2), Glutathione S-transferase (GST), Peroxidase (POX), Putative malate dehydrogenase and Putative catalase. These results suggest that in resistant varieties 3 proteins (Glu2, POX and GST) had more expression change to blast disease that caused different response in plant antifungal activity, and antioxidant activity to disease. Results for morphological traits indicated that significant differences exist between the two varieties with respect to disease resistant. Khazar and Hashemi were resistant and susceptible to blast disease infection, respectively.

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Introduction

Rice is Iran's second most important crop, providing food for the majority of its population (Javan-Nikkhah, 2001). Every year, various hazardous factors including pests, diseases, weeds, drought, and also during harvest and storing period damages, etc. inflict heavy losses to rice. Blast with the causal agent of *Magnaporthe grisea* (Hebert) Barr, is considered to be the most important disease of rice in Iran resulting in severe losses to susceptible rice varieties (Javan-Nikkhah, 2001). Despite the availability of resistant varieties to blast and their increased cultivation in recent years, Iranian farmers still prefer the susceptible local varieties, that being due to their more superior qualities. The *M. grisea*-rice interaction is a model system for understanding plant disease, largely because of its great economic importance, but also because of the genetic and molecular-genetic tractability of the fungus (Valent and Chumley, 1991). The defense responses are often activated by the action of a host resistance gene and a pathogen a virulence gene, as proposed by the gene-for-gene hypothesis (Flor, 1971). The *M. grisea* interaction with rice causes the rice blast disease, one of the most serious fungal diseases in rice, and has been known to cause a great loss of rice production (Talbot, 1995). During rice-rice blast fungus interactions, it has been reported that resistant and susceptible responses between rice varieties and pathogenic races of the rice blast fungus are largely determined genetically, based on the gene-for-gene hypothesis (Flor, 1971; Valent and Chumley, 1991). In plants with a resistant phenotype, early recognition of the pathogen by the resistance gene product in the host triggers rapid and effective defense responses, such as generation of reactive oxygen species, a localized hypersensitive response, accumulation of phytoalexins, and expression of pathogenesis-related proteins (Dixon *et al.*, 1990; Staskawicz *et al.*, 1995). In a susceptible interaction such defense responses often occur so late that the host fails to restrict ingress of the pathogen. Molecular identification of host genes involved in defense responses to *M. grisea* has previously been carried out using suppression subtractive hybridization (Xiong *et al.*, 2001) and by

differential display (Kim *et al.*, 2000). In general, an interaction is incompatible when the rice plant recognizes the invading pathogen early enough through activation of a host resistance gene, resulting in a hypersensitive response (HR) and the triggering of rapid and effective defense responses, including the production of pathogenesis-related (PR) proteins, oxidative enzymes, and phytoalexins (Dixon *et al.*, 1990). In contrast, an interaction is compatible when the rice plant responds too late to restricting of the pathogen. In the post-genomic era, proteomics is becoming increasingly important because proteins are directly related to function (Pandey and Mann, 2000). The high resolution of 2-DE is a powerful tool for separating complex protein mixtures, and has been employed to analyze proteins in response to environmental changes (Celis and Olsen, 1994). Very few proteomic analyses of the whole plant-pathogen interactions have been reported. The first proteomics study was reported in the 2003 using the SCCs system (Kim *et al.*, 2003). Fourteen spots encoded six different family protein were identified at 24 and 48 h after inoculation. Most of the identified proteins were related to pathogen or defense responses, including PBZ1, SaT, RLK, β -glucosidase, OsIRL, and OsPR10. Another study in this field was performed by kim *et al.* (2004), in order to analysis of pathogen-responsive proteins from rice leaves that induced by rice blast fungus. In this study rice leaf was infected with compatible (KJ 301) and incompatible (KJ 401) fungus. Matrix-assisted laser desorption/ionization-time of flight analysis of these differentially displayed proteins, showed them to be two receptor-like protein kinases (RLK), two β -1,3-Glucanases (Glu1, Glu2), Thaumatin-like protein (TLP), Peroxidase (POX), probenazole-inducible protein (PBZ1), and rice pathogenesis-related protein 10 (OsPR-10). Among these proteins, RLK, TLP, PBZ, and OsPR 10 proteins were induced more in the incompatible interactions than in compatible ones. This results suggesting that those proteins may be very important for rice defense system. In this study we want to investigate Proteome of rice leaf tissue in response to blast disease.

Materials and methods

Plant materials

Two local rice varieties, Hashemi and Khazar which were susceptible and resistant to blast disease respectively, were used in the present study. The experiment was conducted in Laboratory conditions at Faculty of Agriculture, University of Tabriz. Fourth leaf stage rice seedlings 20 days old were used for inoculation of whole plants with blast fungus. In order to maximize the infection rate of varieties to blast disease high Nitrogen fertilizer was used. Inoculated plants were kept in a humidity chamber at (24-26°C) and harvested at 48 hours after inoculation. Inoculated plants harvested at, 48 hours after inoculation for protein extraction. About 10 days after inoculation stress-related traits were measured for all the plants and then was sprayed onto the leaves using an air sprayer since the appearance of first blast lesions on rice seedlings, the blast severity measurement started the infection type and final severity of blast identified.

Preparation of the fungal conidia

Conidia were spread on rice polish agar medium (25 gr of rice polish and 20 gr of agar/L) and grown in the dark at plates were incubated at 26°C for 5–7 days under fluorescent light after removing the aerial mycelia with a sterilized loop. Conidia were collected from 7-day-old cultures by agitating the cultures with distilled H₂O containing 0.02% Tween 20, and filtering them through two layers of kimwipe to remove aerial mycelia and cell debris. The suspension was washed twice in distilled H₂O containing Tween 20 by centrifugation. The conidia were diluted with distilled H₂O containing Tween 20. Final concentration used for inoculation was 1×10⁵ conidia/mL.

Protein extraction and 2-DE analyses

Proteins were extracted from rice leaves tissue 48 hours after inoculation the blast fungus. 2-DE was performed according to the method of Toorchi *et al.*, 2009. The IEF gel mixture consisted of a 4.5% w/v acrylamide solution, 9.5 M urea, 2% v/v NP-40, and 2.5% v/v ampholytes (pH 3–10 and pH 5–8). Each sample (500 mg of protein for CBB staining) was

mixed with sample buffer and then loaded. IEF was performed at 200 V for 0.5 h, 400 V for 16 h, and then 600 V for 1h. Each focused gel was put into a 20 mL screw-cap tube with 5 mL of an equilibration buffer that contained 10% v/v glycerol, 2.5% w/v SDS, 125 mM Tris-HCl (pH 6.8), 5% v/v β-mercaptoethanol, and 0.1 mg/mL bromophenol blue. It was then agitated gently at room temperature for 30 min. SDS-PAGE in the second dimension was carried out as described by Laemmli (1970). The 2-DE gels were CBB-stained by incubation of the gels in 0.2% w/v Coomassie R-250, 50% v/v methanol, and 10% v/v glacial acetic acid followed by destaining in 10% v/v glycerol and 50% v/v methanol (Toorchi *et al.*, 2009).

Image acquisition and data analysis

The intensities of induced protein spots 48 h after treatment were scanned by a high-resolution scanner (GS-800 Calibrated Imaging Densitometer; Bio-Rad) at a resolution of 300 dpi and 24-bit color. The scanned gels were saved as TIF images for subsequent analysis using PDQuest software (version 8.0, Bio-Rad). Spot detection, spot measurement, background subtraction and spot matching were performed. Following automated spot detection, gel images were carefully edited. Three well-separated gels of each treatment were used to create “replicate groups”. Statistic, quantitative and qualitative “analysis sets” were created between each control group and corresponding treated group. The quantity of the protein spot was expressed as the volume of that spot, defined as the sum of the intensities of all the pixels that made up the spot. The Molecular Weight of the proteins on gels was determined based on standard protein markers and pI was determined by the migration of protein spots along the 11cm IEF (3-10 linear).

Database searching

Protein identification using matching the pI and MW values of the changed spots with those from the protein databanks, searching program against the NCBI *O.sativa* protein database.

Results

Rice plants inoculated with either an incompatible (Khazar) or compatible (Hashemi) varieties were used to identify pathogen-induced proteins. About 10 days after inoculation stress-related traits were measured for all the plants. Statistic analysis of resistance components data such as infection type, number of spots, mean size of sporulated lesions (mm²) and leaf length also showed significant difference at 1% probability level. The interaction between varieties and stress was significant for only infection type, number of spot and mean size of sporulated lesions (mm²). Hashemi varieties with infection type (3-5)

was susceptible and Khazar varieties with infection type (1-2) were resistant to blast disease. Sporulated lesions number in Hashemi varieties was 4/63 spot in any leaf, and in Khazar varieties was 2/15 spot in any leaf. Length of leaf in Hashemi varieties was and in Khazar varieties was that are shown in table 1. Evaluation of these two varieties conformed the susceptibility and resistance behavior of Hashemi and Khazar receptively (Figure 1). Proteome analysis was performed by 2-Dimensional electrophoresis and Commasie Brilliant Blue staining, lead to the detection of 118 protein spots.

Table 1. Univariate Analysis of Variance for traits related to blast disease in two varieties Hashemi and Khazar.

Source	df	Mean square			
		Infection type	Number of spot	Men size of spot	Length leaf
Stress	1	20/988**	34/578**	1259/725**	56/117**
Varieties	1	1/833**	4/60**	139/742**	137/025**
Stress×Varieties	1	1/883**	4/60**	139/742**	1/591 ^{ns}
Error	8	0/099	0/084	7/553	1/357
C.V (%)		17/11	26/82	23/80	5/00

DE analysis of proteins induced by *M.grisea*

Proteome analysis was performed by 2-Dimensional electrophoresis and Commasie Brilliant Blue staining, lead to the detection of 118 protein spots in both varieties. In Hashemi varieties gels on base of induction factor 28 common protein spot significantly varied from control that by t-test caused identified 5 spots have differentially expressed that spots number A, B, C, D and E increased in expression during the blast disease stress that are shown in gel electrophoresis (Fig. 2 and 3). Thus in Khazar spot were detected on 2-DE gels that base of induction

factor 25 common protein spot significantly varied from control that by t-test caused identified 5 spots have differentially expressed that spots number D, G, H, I and J are shown in gel electrophoresis (Fig. 4 and 5). Matching the pI and MW values of the changed spots with those from the protein databanks NCBI/*Oryzae sativa*, 5 protein spots in Hashemi and 5 protein spots in Khazar varieties were recognized probably. All of protein spots have differentially induced in rice leaves treated with rice blast fungus in tables 2 and 3 are shown.

Table 2. Identification of differentially induced proteins from rice leaves treated with rice blast fungus in Hashemi varieties.

Spot name	Protein name	Accession number	NCBI pI/Mr (kDa)	Theoretical pI/Mr (kDa)	Experimental
A	Putative transketolase 1		XP-550612	5.43/ 68.88	5.2 / 68.71
B	β -1, 3- glucanase (Glu1)		BBA77783	4.6 / 34	5.18 / 36
C	Manganese-superoxidedismutase		BAA86897	6.5 / 24.99	6.98 / 24.45
D	β -1, 3-glucanase (Glu2)		BBA77785	7.0 / 35	7.33 / 35.63
E	Isoflavoneoid reductase		AY071920	6.44 / 41	6.64 / 41.82

Table 3. Identification of differentially induced proteins from rice leaves treated with rice blast fungus in Khazar varieties.

Spot name	Protein name	NCBI Accession number	Theoretical pI/Mr (kDa)	Experimental pI/Mr (kDa)
D	β -1, 3-glucanase (Glu2)	BBA77785	7.0 / 35	7.2 / 35.63
G	peroxidase (POX)	AAC49818	5.8 / 33	6.56 / 36.03
H	Glutathione S-transferase	AAS86422	7.04 / 30.73	7.5 / 29.58
I	Putativemalate dehydrogenase	BAD13225	6.96 / 47.01	7.21 / 47.57
J	Putative catalase	BAD0771	6.25 / 56.7	6/83 / 60.9

Discussion

Identification of pathogen-responsive proteins

Defense proteins

Protein spots B and D in Hashemi and thus D and G in Khazar probably are associated with processes of pathogen-responsive Proteins in plant. Significant increase in expression of protein spot B (β -1, 3-glucanase (Glu1) and D (β -1, 3-glucanase Glu2) in Hashemi and Khazar varieties indicating the increase of plant antifungal activity in response to blast disease. In addition to significant increase in expression of β -1, 3-glucanase 2 in Khazar varieties probably is one of the resistant to blast disease. The β -1,3-glucanases have been reported to be induced in many other plants in response to various pathogens and during developmental events (Hennig *et al.*, 1993). Members of these families are induced when exposed to pathogens and have antifungal activity (VanLoon *et al.*, 1999). It was reported that a transgenic plant harboring the rice β -1,3 Glu gene (Gns1) showed a lesion mimic phenotype and exhibited enhanced resistance against the rice blast fungus (Nishizawa *et al.*, 2003). A comprehensive analysis of β -1, 3-glucanase family genes was performed. Among 27 analyzed rice β -1, 3-glucanases, 22 are highly regulated by *M. Oryzae e* infection in rice leaves, suggesting the β -1, 3-glucanase association with *M. Oryzae e* defense mechanism (Hwang *et al.*, 2007). Thus significant increase in expression of protein spot G (peroxidase) in Khazar varieties confer its involvement in controlling the invasion of pathogen by the production of reactive oxygen species and probably are associated resistant to blast disease. It has been reported that peroxidase expression correlates with resistance in many plant species, including barley, tobacco, wheat, and rice. In general, it has been proposed that pathogenesis-related peroxidases are involved in controlling the invasion of pathogen by the production of reactive oxygen species and by the reinforcement of physical barriers that prevent pathogen penetration of cell walls (Kim *et al.*, 2003). In a previous study, one of the rice POX proteins, the POX gene in rice infected with *Xanthomonas Oryzae e* pv. *Oryzae e* was isolated and characterized (Agrawal, 2002).

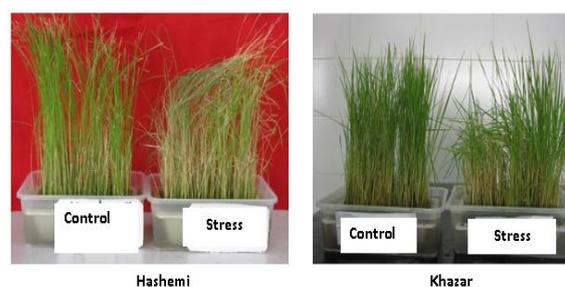


Fig. 1. About 10 days after inoculation stress-related traits infection type, number of spots, spots size and leaf length in rice leave inoculated with the compatible (Hashemi) and incompatible (Khazar).

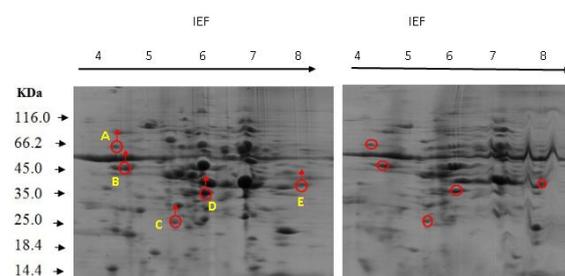


Fig. 2. 2DE analysis of Hashemi varieties proteins induced by rice blast fungus in rice leaves. Protein sample (500 μ g) in 2-DE gels (pI 4-8), and CBB-stained. Pathogen-induced proteins were in the gel (A, B, C, D, and E). A total of five proteins were induced by rice blast fungus (gill 804). Arrows indicate proteins induced over control. The relative MW rare indicated on the left side in kDa. The number of each protein spot (1-5) corresponds to its listing in Table 2.

Molecule biosynthesis and metabolism proteins

Significant increase in expression of protein spot E (Isoflavenoid reductase) indicating increase of in enzyme activity produced by isoflavenoid phytoalexins and their accumulation in plant in response to blast disease. Thus increase of expression spot A (Putative transketolas) in Hashemi varieties and spot I (Putative malate dehydrogenase) in Khazar varieties indicating the increase of plant biosynthesis and metabolism of molecules in response to blast disease.

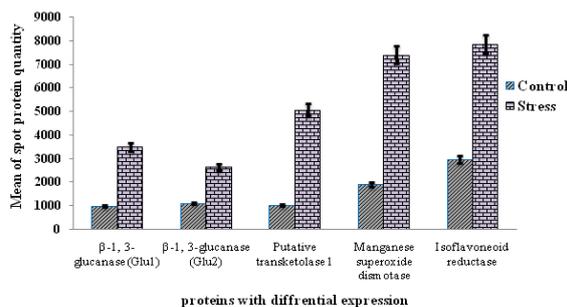


Fig. 3. The expression levels of β-1, 3-glucanase 1, β-1, 3-glucanase 2, Putative transketolase, manganese superoxide dismutase, and Isoflavonoid reductase compared to those of controls in *Hashemi* varieties. The intensities of five induced protein spots were measured using a densitometer (Bio-Rad) and compared to those of the controls. The average values of relative induction levels of three replicate samples are shown in the histograms.

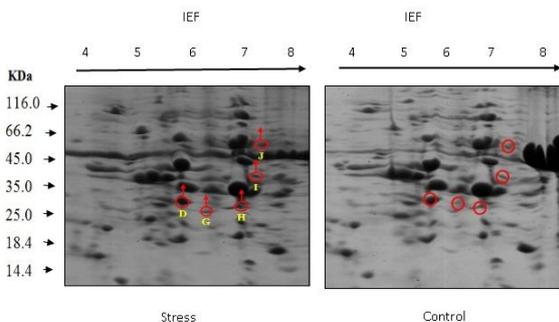


Fig. 4. 2-DE analysis of *Khazar* varieties proteins induced by rice blast fungus in rice leaves. Protein sample (500 μg) in 2-DE gels (pI 4-8), and CBB-stained. Pathogen-induced proteins were in the gel (A, B, C, D, E, and F). A total of five proteins were induced by rice blast fungus (gill 804). Arrows indicate proteins induced over control. The relative MW rare indicated on the left side in kDa. The number of each protein spot (1–5) corresponds to its listing in Table 3.

ROS-related proteins

Significant increase in expression of protein spot C (Manganese-superoxide dismutase), in *Hashemi* varieties and protein spot H (Glutathion S-transferase) and spot J (Putative catalase) in *Khazar* varieties suggest the oxidative burst and the cognate redox signaling may play a central role in integration and coordination of the multitude of plant defense responses and these are an important groups of enzymes that remove reactive oxygen species in plants (Lamb and Dixon, 1997). One of the most rapid

plant responses occurring after pathogen recognition is the oxidative burst, which involves ROS production, primarily superoxide (O_2^-) and hydrogen peroxide (H_2O_2) at the site of the attempted invasion (Wojtaszek *et al.*, 2002; Grant, 1997). A final result of the induced oxidative burst may be its participation in the HR and PCD (Uckelhoven and Kogel, 2003; Levine *et al.*, 1994). SOD, GST and CAT belong to an important group of enzymes that remove active oxygen species in plants. SOD acts as the first line of defences response against ROS, dismutating superoxide to H_2O_2 (Alscher *et al.*, 2002). Agrawal *et al.* (2002) observed that Mn-SOD intensity increased after rice leaves were treated with O_3 . Following treatment with crude extracts of rice blast fungus, the SOD gene was dramatically induced in suspension-cultured rice cells (Matsumura *et al.*, 2003). GST is the enzyme responsible for detoxifying xenobiotic by catalysing their conjugation with tripeptide glutathione (Edwards *et al.*, 2000). GST is also known to be elicitor-induced and acts as an antioxidant during an incompatible interaction between plants and their pathogens (Mullineaux *et al.*, 2000; Dixon *et al.*, 2002). The oxidative burst and induction of GST were used as markers for induction of the pathogen defence response when *Arabidopsis* cell suspension cultures were treated with an elicitor (Ndimba *et al.*, 2003). GST was induced in maize embryos infected with the fungus *Fusarium verticillioides* (Campo *et al.*, 2004). In our study, up-regulation of SOD may be indicating a rapid oxidative burst in rice, which was required for activation and establishment of signalling cascades. GST is also known to be elicitor-induced and acts as an antioxidant during an incompatible interaction between plants and their pathogens (Mullineaux *et al.*, 2000; Dixon *et al.*, 2002).

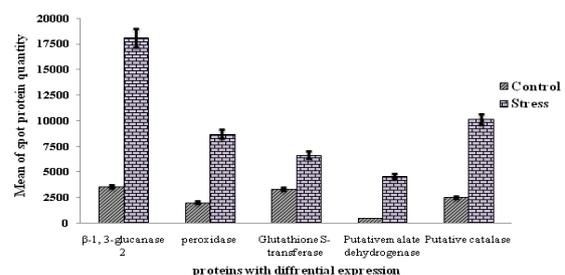


Fig. 5. The expression levels of β-1, 3-glucanase 2, peroxidase, Glutathion S-transtransferase, Putative

malate dehydrogenase and putative catalase compared to those of controls in Khazar varieties. The intensities of five induced protein spots were measured using a densitometer (Bio-Rad) and compared to those of the controls. The average values of relative induction levels of three replicate samples are shown in the histogram.

Concluding remarks

The proteomic analysis is a very useful tool for providing complex information about differences in the plant proteome during abiotic and biotic stresses. In this research proteomic approaches were carried out to identify differentially expressed proteins from rice leaf responsive to rice blast fungus, *M. grisea*. The differential display the proteome of pathogen-responsive proteins between rice varieties Hashemi and Khazar that are susceptible and resistant to blast disease, were also studied. In Hashemi varieties 5 protein spot increased in expression during the blast disease, including Two β -1, 3-glucanase (Glu1 and Glu2), Isoflavenoid reductase, Putative transketolas, Putative malate dehydrogenase, SOD (Manganese-superoxide dismutase were induced in the inoculated leaf. In Khazar varieties 5 protein increased including β -1, 3-glucanase (Glu1), POX (peroxidase), GST (Glutathion S-transferase) and Putative Catalase were expression during the blast disease. For the system to operate efficiently, a wide variety of proteins must work together to build multiplex signal transduction pathways. Although these data make a significant contribution to our understanding of plant defences, additional studies will be necessary to fully elucidate the complexity of the response network. These temporal and quantitative differences in expression may provide the host cell with a leading edge in its defense against pathogens.

References

Agrawal GK, Rakwal R, Yonekura M, Kubo A, Saji H. 2002. Proteome analysis of differentially displayed proteins as a tool for investigating ozone stress in rice (*Oryza sativa* L.) seedlings. *Proteomics* **2**, 947-959.

Agrawal GK, Rakwal R, Yonekura M, Kubo A, Saji H. 2002. Chitosan activates defence /stress response(s) in the leaves of *Oryza sativa* seedlings. *Plant Physiology and Biochemistry*, **40**, 1061-1069. [http://dx.doi.org/10.1016/S0981-9428mn\(02\)01471-7](http://dx.doi.org/10.1016/S0981-9428mn(02)01471-7)

Alscher GR, Neval E, Lenwood SH. 2002. Role of superoxide dismutase (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany* **53**, 1331-1341.

<http://dx.doi.org/10.1093/jexbot/53.372.133>

Campo S, Carrascal M, Coca M, Abian J. 2004. The defence response of germinating maize embryos against fungal infection: a proteomics approach. *Proteomics* **4**, 383-396.

<http://dx.doi.org/10.1002/pmic.200300657>.

Celis JE, Olsen E. 1994. A qualitative and quantitative protein database approach identifies individual and groups of functionally related proteins that are differentially regulated in simian virus 40 (SV40) transformed human keratinocytes: An overview of the functional changes associated with the transformed phenotype. *Electrophoresis* **15**, 309-344.

<http://dx.doi.org/10.1002/elps.1150150153>.

Dixon RA, Lamb CJ. 1990. Molecular Communication in Interactions between Plants and Microbial Pathogens. *Annu review. Plant Physiology. Plant Molecular Biology* **41**, 339-367.

<http://dx.doi.org/10.1146/annurev.pp.41.060190.002011>.

Dixon DP, Laphorn A, Edwards R. 2002 Plant glutathione transferases. *Genome Biology* **3**, 3004.1-3004.10.

<http://genomebiology.com/2002/3/3/reviews/3004>

Edwards R, Dixon DP, Walbot V. 2000. Plant glutathione S-transferases, enzymes with multiple functions in sickness and in health. *Trends Plant Science* **5**, 193-198.

[http://dx.doi.org/10.1016/S1360-1385\(00\)01601-0](http://dx.doi.org/10.1016/S1360-1385(00)01601-0).

- Flor HH.** 1971. Current Status of the Gene-For-Gene Concept. *Annu Review. Phytopathology* **9**, 275-296. <http://dx.doi.org/10.1146/annurev.py.09.090171.001423>.
- Grant JJ.** 1997. Role of reactive oxygen intermediates and cognate redox signalling in disease resistance. *Plant Physiology* **124**, 21-29. <http://dx.doi.org/10.1104/p.124.1.21>.
- Hennig J, Dewey RE, Cutt JR, Klessig DF.** 1993. Pathogen, salicylic acid and developmental dependent expression of a β -1, 3-glucanase/GUS gene fusion in transgenic tobacco plants. *Plant Journal* **4**, 481-493. <http://dx.doi.org/10.1046/j.1365313X.1993.04030481.x>.
- Hwang DH, Kim ST, Kim SG, Kang KY.** 2007. Comprehensive analysis of the expression of twenty-seven beta-1, 3-glucanase genes in rice (*Oryza sativa* L.). *Molecular Cells* **23**, 207-214.
- Javan-Nikkhah M.** 2001. Study on Genetic Diversity of Rice Blast Causal Agent, *Magnaporthe grisea* (Hebert) Barr, Based on Determination of VCGs, Pathogenicity Test and Molecular Approach in Guilan Province. PhD. Thesis, Plant Protection Department, Faculty of Agriculture, Tehran University, Tehran, Iran.
- Kim CY, Lee SH, Park HC, Bae CG.** 2000. Identification of rice blast fungal elicitor-responsive genes by differential display analysis. *Molecular Plant Microbe Interact* **13**, 470-474.
- Kim ST, Cho KS, Yu S, Kim SG, Hong JC, Han C, Bae DW, Nam MH, kang KY.** 2003. Proteomics analysis of differentially expressed protein induced by rice blast fungus and elicitor in suspension-cultured rice cells. *Proteomics* **3**, 2368-2378. <http://dx.doi.org/10.1002/pmic.200300577>.
- Kim ST, Kim SG, Hwang DH, Kang SY, Kim HJ, Lee BH, Lee JJ, Kang KY.** 2004. Proteomics analysis of pathogen-responsive proteins from rice leaves induced by rice blast fungus, *Magnaporthe grisea*. *Proteomics* **4**, 3569-2578. <http://dx.doi.org/10.1002/pmic.200400999>.
- Lamb C, Dixon RA.** 1997. The oxidative burst in plant disease resistance. *Ann Review Plant Physiology. Plant Molecular Biology* **48**, 251-271. <http://dx.doi.org/10.1146/annurev.arplant.48.1.251>
- Laemmli UK.** 1970. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* **227**, 680-685. <http://dx.doi.org/10.1038/227680a0>.
- Levine A, Tenhaken R, Dixon RA, Lamb C.** 1994. H₂O₂ from the oxidative burst orchestrates the plant hypersensitive response. *Cell* **79**, 583-593. [http://dx.doi.org/10.1016/0092-8674\(94\)90544-4](http://dx.doi.org/10.1016/0092-8674(94)90544-4).
- Matsumura H, Nirasawa S, Kiba A, Urasaki N.** 2003. Over expression of Box inhibitor suppresses the fungal elicitor-induced cell death in rice (*Oryza sativa* L.) cells. *Plant Journal* **33**, 425-434. <http://dx.doi.org/10.1046/j.1365313X.2003.01639.x>.
- Mullineaux P, Ball L, Escobar C, Karpinska B.** 2000. Are diverse signalling pathways integrated in the regulation of Arabidopsis antioxidant defence gene expression in response to excess excitation energy?. *Philos Trans R Soc Lond B Biology Science* **355**, 1531-1540.
- Nishizawa Y, Saruta M, Nakazono K, Nishio Z.** 2003. Characterization of transgenic rice plants over-expressing the stress-inducible β -glucanase gene Gns1. *Plant Molecular Biology* **51**, 143-152. <http://dx.doi.org/10.1023/A:1020714426540>.
- Ndimba B, Chivasa S, Richard S, Simon JW.** 2003. Proteomic analysis of changes in the extracellular matrix of Arabidopsis cell suspension cultures induced by fungal elicitors. *Proteomics* **3**, 1047-1059. <http://dx.doi.org/10.1002/pmic.200300413>.

- Pandey A, Mann M.** 2000. Review article Proteomics to study genes and genomes. *Nature* **405**, 837-846.
<http://dx.doi.org/10.1038/35015709>
- Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JD.** 1995. Molecular genetics of plant disease resistance. *Science* **268**, 661-667.
- Talbot NJ.** 1995. Having a blast: exploring the pathogenicity of *Magnaporthe grisea*. *Trends Microbiology* **3**, 9-16.
[http://dx.doi.org/10.1016/S0966-842X\(00\)88862-9](http://dx.doi.org/10.1016/S0966-842X(00)88862-9)
- Toorchi M, Yukawa K, Nouri MZ, Komatsu S.** 2009. Proteomics approach for identifying osmotic-stress-related protein in soybean roots. *Peptide* **30**, 2108-2117.
<http://dx.doi.org/10.1016/j.peptides.2009.09.006>
- Uckelhoven R, Kogel KH.** 2003. Reactive oxygen intermediates in plant-microbe interactions: who is who in powdery mildew resistance. *Planta* **216**, 891-902.
<http://dx.doi.org/10.1007/s00425-003-0973-z>
- Van Loon LC, van strien EA.** 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of pr-1 type proteins. *Physiological and Molecular Plant Pathology* **55**, 85-97.
- Valent B, Chumley FG.** 1991. Molecular Genetic Analysis of the Rice Blast Fungus, *Magnaporthe grisea*. *Annual Review of Phytopathology* **29**, 443-467.
<http://dx.doi.org/10.1146/annurev.py.29.090191.002303>
- Wojtaszek P.** 1997. Oxidative burst: an early plant response to pathogen infection. *Biochemistry Journal* **322**, 681-692.
- Xiong L, Lee MW, Qi M, Yang Y.** 2001. Identification of Defence-Related Rice Genes by Suppression Subtractive Hybridization and Differential Screening. *Molecular Plant-Microbe Interaction* **14**, 685-692.
<http://dx.doi.org/10.1094/MPMI.2001.14.5>