Research Note

Analysis of Genes Expressed During Rice–*Magnaporthe grisea* Interactions

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Expressed sequence tag (EST) analysis was applied to identify rice genes involved in defense responses against infection by the blast fungus *Magnaporthe grisea* and fungal genes involved in growth within the host during a compatible interaction. A total of 511 clones was sequenced from a cDNA library constructed from rice leaves (*Oryza sativa* cv. Nipponbare) infected with *M. grisea* strain 70-15 to generate 296 nonredundant ESTs. The sequences of 293 clones (57.3%) significantly matched National Center for Biotechnology Information database entries; 221 showed homologies with previously identified plant genes and 72 with fungal genes. Among the genes with assigned functions, 32.8% were associated with metabolism, 29.4% with cell/organism defense or pathogenicity, and 18.4% with gene/protein expression. cDNAs encoding a type I metallothionein (*MTs-I*) of rice and a homolog of glucose-repressible gene 1 (*GRG1*) of *Neurospora crassa* were the most abundant representatives of plant and fungal genes, comprising 2.9 and 1.6% of the total clones, respectively. The expression patterns of 10 ESTs, five each from rice and *M. grisea*, were analyzed. Five defense-related genes in rice, including four pathogenesis-related genes and *MTs-I*, were highly expressed during *M. grisea* infection. Expression of five stress-inducible or pathogenicity-related genes of the fungus, including two hydrophobin genes, was also induced during growth within the host. Further characterization of the genes represented in this study would be an aid in unraveling the mechanisms of pathogenicity of *M. grisea* and the defense responses of rice.

Rice blast, caused by *Magnaporthe grisea* (T.T. Hebert) Yaegashi & Udagawa, is one of the most devastating diseases in rice-growing regions worldwide. Controlling this disease has depended on breeding resistant cultivars and applying chemical fungicides. However, the frequent appearance of new races of the pathogen has reduced the effectiveness of resistant cultivars in the field (Ou 1985). Furthermore, environmental concerns have brought about strict regulations on the use of chemical fungicides (de Waard et al. 1993). Understanding the infection mechanisms may contribute to developing new strategies to control this disease. The development of new

strategies for disease control based on a plant's defense mechanisms may also offer the promise of sustainable agricultural production and improvement of environment and human health.

The process of *M. grisea* infection consists of several key steps: conidial attachment to the host surface, germination, appressorium formation, penetration by a penetration peg, and invasive growth in the host plant. Recently, a considerable number of studies have focused on the molecular mechanisms involved in the *M. grisea* pathogenesis, particularly on appressorium elaboration and subsequent infection (Dean 1997; Talbot 1995). Although appressorium development is critical for penetrating the host and is induced by the leaf surface signal, this process appears to depend on the biology of the fungal pathogen, irrespective of the host genotype. After penetration, the fungus encounters an array of preformed or inducible barriers during its attempt to infect the plant. These inducible defense responses are governed by the interaction between plant resistance genes and the corresponding pathogen avirulence genes. In an incompatible interaction, timely recognition of the invading pathogen by the resistance gene product in the host triggers rapid and effective defense responses. These include the hypersensitive response, the production of reactive oxygen species, cell wall fortification, the accumulation of antimicrobial compounds such as phytoalexins, and the induction of pathogenesis-related (PR) proteins (Yang et al. 1997). In a compatible interaction, however, such defense responses occur late, so they fail to restrict ingress of the pathogen. In this respect, the gene products of the pathogen activated in the plant may be putative pathogenicity factors necessary for the establishment and maintenance of plant infection or symptom development. Exploring the pathogen genes required for invasive growth in the host has recently drawn much attention. An ubiquitin-encoding gene (*UEP1*) (McCafferty and Talbot 1998), a gene showing homology to mitochondrial small-subunit rRNA (Talbot and Tongue 1998), and *MPG1*, encoding a class I hydrophobin (Talbot et al. 1993) of *M. grisea*, have been identified and are strongly expressed during colonization of the plant. Null mutants of *M. grisea* in the *ABC1* locus, an ABC transporter-encoding gene, died shortly after penetrating the host's epidermal cells (Urban et al. 1999). Differential hybridization of the genomic library was also used to identify genes expressed in planta in *Phytophthora infestans*, leading to the isolation of nine potential pathogenicity genes whose expression was induced during pathogenesis (Pieterse et al. 1993).

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Rice blast is considered a model system for studying fungal pathogen–plant interactions (Dean 1997; Howard and Valent 1996), not only due to the great economic importance involved but also due to the genetic and molecular-genetic tractability of both the fungus and the host. Rice is also a model cereal species due to its relatively small genome size (Arumuganathan and Earle 1991) and its important syntenic relationships with other cereal species (Gale and Devos 1998). Therefore, extensive genomic studies have been conducted on this crop. Over 40,000 expressed sequence tags (ESTs) have been reported as a result of the rice genome project in Japan (Yamamoto and Sasaki 1997). Furthermore, an international effort to sequence the entire rice genome was begun in 1997 through the International Rice Genome Sequencing Project (Sasaki and Burr 2000). However, studies of defense responses against pathogen attack in rice are rather scarce compared with those in Solanaceous plants. Here, we report EST analysis performed to reveal the genes involved in the interaction between rice and *M. grisea*. A compatible interaction was chosen, since in this system, the fungus expresses genes necessary for survival in the host and the plant expresses defense-related genes.

To construct an *M. grisea* strain 70-15-infected rice cDNA library, rice plants (*Oryza sativa* cv. Nipponbare) at the fourth-leaf stage were spray-inoculated with 2×10^5 spores per ml. Rice leaves on which susceptible type lesions spread through the whole leaves were collected at 84, 96, and 120 h after inoculation. Total RNA was isolated by a single-step RNA isolation procedure using guanidinium thiocyanate (Chomczynski and Sacchi 1987). Poly(A)⁺ RNA was purified through oligo(dexyribosylthymine) cellulose chromatography (Sambrook et al. 1989) and used to construct a cDNA library in the bacteriophage vector λ ZAP XAR following the manufacturer's instructions (Stratagene, La Jolla, CA, U.S.A.). A total of 511 cDNA clones from an *M. grisea*-infected rice cDNA library was sequenced using ABI PRISM BigDye terminator kit (Perkin-Elmer Corp., Norwalk, CT, U.S.A.) on an ABI model 377 DNA sequencer (Applied BioSystems, Foster City, CA, U.S.A.) at The National Instrumentation Center for Environmental Management, Seoul National University, Seoul, Korea. Sequences with an average length of 416 bp after editing were used in database searches. Of these, 293 (57.3%) ESTs showed similarities to National Center for Biotechnology Information (NCBI) database entries, while no significant matches were found in the databases for the rest (218 clones, 42.7%) (Table 1). Analysis of 511 cDNA sequences using ATGC software (Software Development, Tokyo, Japan) generated 296 nonredundant ESTs. A relatively high number of the cDNAs (72 clones comprising 39 nonredundant ESTs) were matched to fungal genes, while the rest of the database matches (221 clones forming 106 nonredundant ESTs) showed similarity to plant genes (Table 2). Among the 39 ESTs that hit fungal genes, 22 nonredundant ESTs were matched to the genes of filamentous fungi, including four *M. grisea* genes, while 17 ESTs showed similarities to yeast genes. It is not surprising that a high percentage of the cDNA was from the pathogen, because a previous study estimated that about 10% of the biomass of infected rice leaves 72 h after inoculation represented *M. grisea* mycelium (Talbot et al. 1993). This assumption proved to be in ESTs from soybean (*Glycine max* (L.) Merr.) infected with *P. sojae*, the causal agent of stem and root rot. Two thirds of the ESTs were estimated to be pathogen

genes through analysis of the G+C content of the sequences (Qutob et al. 2000). Furthermore, an analysis of changes in mRNA abundance in roots of *Arabidopsis thaliana* during an interaction with the sugar beet cyst nematode *Heterodera schachtii* revealed one third of the differentially expressed cDNA clones to be from *H. schachtii* (Hermesmeier et al. 2000). These studies strongly support the hypothesis that analysis of ESTs from a comparable interaction is a way to identify fungal potential pathogenicity genes as well as defense-related genes in the host. Fifty-five of the one hundred six nonredundant ESTs matched plant genes that showed homology to genes stemming from plant species other than rice, and the remaining 51 ESTs matched rice genes best.

Sequences that did not show significant matches to the protein database were also scrutinized against dbEST at NCBI using the BLASTN algorithm (Table 3). Sixteen sequences out of two hundred eighteen were identified as having moderate to high similarity to ESTs from an *M. grisea* appressorium-stage cDNA library (registered by R. A. Dean), while 107 (48.9%) clones were matched to plant ESTs. Four clones hit two ESTs (AW155531 and AW155488) from a mixed library, i.e., a rice blast infection-stage cDNA library that was constructed from rice leaves collected 48 h after inoculation with *M. grisea* strain 70-15. Ninety-one clones, comprising 17.8% of the total clones, were not matched to any database entries.

Among the sequenced cDNAs, 293 (57.3%) for which there were database matches were classified into one of seven functional categories following the Expressed Gene Anatomy Database cellular role classification scheme (White and Kerlavage 1996). cDNAs showing homology to enzymes from ubiquitous metabolic pathways (96 clones, 32.8%) and components of the transcriptional or translational apparatus (54 clones, 18.4%) were prevalent (Table 4). Moreover, a high percentage of cDNAs were functionally assigned to cell/organism defense or pathogenicity for both matches (86 clones, 29.4%). Genes associated with cell division, structure/motility, or signaling/communication were poorly represented in this library. The genes most prevalent among the 72 fungal gene matches were categorized into gene/protein expression, suggesting the occurrence of active protein synthesis during fungal colonization of its host. Nineteen clones, comprising five independent ESTs, were functionally assigned to cell/organism defense or pathogenicity. These included a previously identified pathogenicity gene, *MPG1* of *M. grisea* (Talbot et al. 1993), and a homolog (*MHP1*; *Magnaporthe* hydrophobin gene 1) of the class II hydrophobin gene *CRYP* of *Cryphonectria parasitica* (Carpenter et al. 1992). Several cDNA clones were matched to

Table 1. Sequence similarities between translations of expressed sequence tags and the best match in the NCBI database as determined using BLASTX

Database matches ^a	Number (percentage) of cDNAs		
	Fungal genes	Plant genes	Total
Significant matches	72 (100)	221 (100)	293 (57.3)
High	39 (54.2)	154 (69.8)	193 (37.8)
Moderate or weak	33 (45.8)	67 (30.2)	100 (19.5)
Not significant matches			218 (42.7)
Total			511 (100)

^a The database sequence matches were divided into highly significant ($E \leq e^{-20}$), moderately or weakly significant ($e^{-3} \leq E \leq e^{-19}$), and not significant ($E \geq e^{-2}$) classes according to the expect (E) values.

Table 2. Summary of expressed sequence tags (ESTs) isolated from the *Magnaporthe grisea*-infected rice cDNA library

EST ^a	Accession	Putative identification ^b	Source of comparison ^b	BLASTX ^c
Homologous to fungal gene				
I. Cell division				
mgir1F07	AT003344	Histone H2B	<i>Emericella nidulans</i> (splP23754)	8e-19
II. Cell signaling/cell communication				
III. Cell structure/cytoskeleton				
mgir2H08	AT003354	Actin	<i>Colletotrichum gloeosporioides</i> (AF112537)	7e-07
IV. Cell/organism defense and pathogenesis				
mgir1A10	AT003335	Cryparin	<i>Endothia parasitica</i> (splP52753)	9e-17
mgir1I01	AT003350	Glucose-repressible gene 1	<i>Neurospora crassa</i> (splP22151)	1e-16
mgir3G17	AT003878	BLI-3 protein	<i>Neurospora crassa</i> (splQ01358)	4e-07
mgir3M08	AT003366	Nitrogen starvation-induced gene	<i>Colletotrichum gloeosporioides</i> (U94182)	2e-39
mgir3M23	AT003367	Hydrophobinlike protein (MPG1)	<i>Magnaporthe grisea</i> (splP52751)	8e-29
V. Gene/protein expression				
mgir1A06	AT003334	Ribosomal protein 40S, S12	<i>Blumeria graminis</i> (gil3114615)	3e-40
mgir1B05	AT003339	Ribosomal protein 60S, L34	<i>Saccharomyces cerevisiae</i> (splP04649)	3e-22
mgir1C05	AT003340	Ribosomal protein 40S	<i>Shizosaccharomyces pombe</i> (PIDle1168591)	1e-41
mgir1F09	AT003345	Ribosomal protein 40S, S4	<i>Saccharomyces cerevisiae</i> (splP25443)	2e-41
mgir1F12	AT003346	Proteasome component SUN4	<i>Saccharomyces cerevisiae</i> (splP53616)	9e-13
mgir1F15	AT003482	Elongation factor 1-alpha	<i>Ajellomyces capsulatus</i> (splP40911)	6e-47
mgir1G09	AT003348	Elongation factor 1-alpha	<i>Neurospora crassa</i> (splQ01372)	5e-38
mgir2J04	AT003765	Ribosomal protein S28	<i>Aspergillus niger</i> (AJ249691)	7e-61
mgir3D14	AT003357	Small-subunit ribosomal RNA gene	<i>Magnaporthe grisea</i> (gil3044188)	3e-53
mgir3E04	AT003359	Ribosomal protein 40S, S13	<i>Shizosaccharomyces pombe</i> (splP28189)	7e-14
mgir3G20	AT003361	Ribosomal protein 60S, L18A	<i>Saccharomyces cerevisiae</i> (splP47913)	1e-19
mgir3I09	AT003363	Ribosomal protein S28	<i>Saccharomyces cerevisiae</i> (splP32827)	6e-47
mgir3K11	AT003364	Translation initiation factor 3	<i>Shizosaccharomyces pombe</i> (gnllPIDle1285373)	4e-08
mgir3K15	AT003365	Ribosomal protein L43A	<i>Saccharomyces cerevisiae</i> (refNP015368.1)	4e-11
mgir3P23	AT003369	Ribosomal protein 40S, S28	<i>Neurospora crassa</i> (spl43105)	4e-29
VI. Metabolism				
mgir1A14	AT003337	Cytochrome c oxidase polypeptide	<i>Shizosaccharomyces pombe</i> (gnllPIDle1334472)	4e-18
mgir1D15	AT003341	Guanosine-diphosphatase	<i>Saccharomyces cerevisiae</i> (splP32621)	7e-28
mgir1F19	AT003347	Formate dehydrogenase	<i>Neurospora crassa</i> (splQ07103)	7e-23
mgir1H08	AT003349	Acetyl-CoA acyltransferase	<i>Yarrowia lipolytica</i> (splQ05493)	6e-31
mgir2F14	AT003353	Cytochrome C	<i>Neurospora crassa</i> (splP00048)	1e-11
mgir2J10	AT003355	Succinate-aldehyde dehydrogenase	<i>Shizosaccharomyces pombe</i> (AL136078)	2e-16
mgir3A10	AT003356	Enolase	<i>Cladosporium herbarum</i> (splQ12560)	1e-39
mgir3D17	AT003358	Peroxisomal CoA synthetase	<i>Saccharomyces cerevisiae</i> (splP38137)	9e-07
mgir3G23	AT003757	Inosine-5'-monophosphate dehydrogenase	<i>Saccharomyces cerevisiae</i> (splP50094)	1e-04
mgir3H20	AT003362	C-14 sterol reductase	<i>Neurospora crassa</i> (splP38670)	1e-04
mgir3O03	AT003368	Glutamate synthase (NADPH)	<i>Neurospora crassa</i> (emblCAB92626.1)	1e-44
VII. Unclassified				
mgir1A12	AT003336	Hypothetical 17.5-kDa protein	<i>Saccharomyces cerevisiae</i> (splQ03799)	5e-17
mgir1A22	AT003338	Mg-SINE nuclear element DNA	<i>Magnaporthe grisea</i> (gblU35313)	2e-91
mgir1D19	AT003342	Hypothetical protein	<i>Penicillium chrysogenum</i> (AAF00009)	1e-08
mgir1F05	AT003343	ARP2/3 complex 20 kDa (P20-ARC)	<i>Saccharomyces cerevisiae</i> (splP33204)	3e-53
mgir1H04	AT003483	Hypothetical protein	<i>Neurospora crassa</i> (AL391572)	2e-11
mgir2C17	AT003352	Non-long-terminal-repeat retrotransposon MGL	<i>Magnaporthe grisea</i> (gblAF018033)	2e-06
Homologous to plant gene				
I. Cell division				
mgir1H22	AT003396	Chromatin structural protein Supt5hp	<i>Arabidopsis thaliana</i> (gil3337352)	4e-16
II. Cell signaling/cell communication				
mgir3M15	AT003470	14-3-3-like protein	<i>Oryza sativa</i> (splQ06967)	7e-45
III. Cell structure/cytoskeleton				
IV. Cell/organism defense				
mgir1A04	AT003370	Putative cytochrome P450	<i>Arabidopsis thaliana</i> (gil2760837)	7e-07
mgir1A24	AT003372	Pathogenesis-related protein 1	<i>Zea mays</i> (gil3290004)	3e-61
mgir1B06	AT003373	ABA- and stress-inducible protein	<i>Oryza sativa</i> (gblAF039573)	e-151
mgir1F08	AT003386	Probenazole-inducible protein 1	<i>Oryza sativa</i> (gnldl007947)	3e-78
mgir1G04	AT003389	Salt-inducible-like protein	<i>Arabidopsis thaliana</i> (gil3193306)	1e-04
mgir1G16	AT003391	Glutathione S-transferase	<i>Arabidopsis thaliana</i> (gil3894171)	1e-40
mgir1H23	AT003397	Thaumatococcal protein	<i>Oryza sativa</i> (gil2062387)	1e-08
mgir1I05	AT003399	Superoxide dismutase-2 (Cu-Zn)	<i>Oryza sativa</i> (splP28757)	3e-63
mgir1I16	AT003767	Cysteine proteinase	<i>Zea mays</i> (pirIS60456)	7e-06
mgir1N23	AT003406	GST7 protein	<i>Zea mays</i> (emblCAB38121.1)	2e-13
mgir2I01	AT003420	Metallothioneinlike protein	<i>Hordeum vulgare</i> (dbjID50641)	1e-12
mgir3A09	AT003432	Glutathione S-transferase TSI-1	<i>Aegilops squarrosa</i> (gil2190992)	4e-08
mgir3A19	AT003434	Cinnamyl alcohol dehydrogenase	<i>Aralia cordata</i> (splP42495)	3e-15
mgir3B07	AT003435	Salt-stress-induced protein	<i>Oryza sativa</i> (splP24120)	2e-22
mgir3B21	AT003436	Carbonic anhydrase	<i>Oryza sativa</i> (gnllPIDld1032914)	3e-25
mgir3B23	AT003437	Chitinase (EC 3.2.1.14) 1	<i>Conus tulipa</i> (pirIJE0183)	5e-28
mgir3C18	AT003439	COL2	<i>Arabidopsis thaliana</i> (gil507699)	2e-04
mgir3D04	AT003440	Metallothioneinlike protein	<i>Oryza sativa</i> (gblAF001396)	0.0
mgir3E10	AT003442	Copper homeostasis factor	<i>Arabidopsis thaliana</i> (gil3168840)	7e-05
mgir3F12	AT003446	Carbonic anhydrase	<i>Oryza sativa</i> (dbjIBAA31.953)	6e-53
mgir3G01	AT003447	Amino acid selective channel protein	<i>Hordeum vulgare</i> (emblCAA09867)	4e-54
mgir3G03	AT003448	Metallothioneinlike protein	<i>Oryza sativa</i> (splQ40633)	0.0
mgir3H04	AT003452	Thaumatococcal protein	<i>Oryza sativa</i> (splP31110)	2e-20

(continued on next page)

^a A single representative clone is given for those cases in which multiple cDNAs were identified.^b Putative identification and source of comparison generally indicate the best match to a sequence in the NCBI nonredundant protein database. However, in those cases in which the best match was to an unidentified open reading frame, the NCBI nucleotide sequence is shown.^c Expect values greater than e^{-2} are considered not statistically significant.

Table 2. (continued from preceding page)

EST ^a	Accession	Putative identification ^b	Source of comparison ^b	BLASTX ^c
V. Gene/protein expression	mgir3I17	Pathogenesis-related protein 1	<i>Oryza sativa</i> (gil1888551)	4e-90
	mgir3N05	Metallothioneinlike type 2	<i>Oryza sativa</i> (splP94029)	e-135
V. Gene/protein expression	mgir1B02	Ribosomal protein 40S, S2	<i>Urechis caupo</i> (U30454)	2e-09
	mgir1B09	Ribosomal protein 40S, S3A	<i>Oryza sativa</i> (splP49397)	1e-40
	mgir1E18	MADS transcriptional factor	<i>Solanum tuberosum</i> (gil2735764)	2e-21
	mgir1H24	Ribosomal protein 60S L11B	<i>Arabidopsis thaliana</i> (splP42796)	2e-53
	mgir1K12	Ribosomal protein 60S	<i>Arabidopsis thaliana</i> (splO23290)	2e-10
	mgir2A17	EF-1 alpha	<i>Oryza sativa</i> (D63582)	9e-15
	mgir2C11	Cyclophilin	<i>Arabidopsis thaliana</i> (gblAF192490)	8e-10
	mgir2F17	Cyclophilin 2	<i>Oryza sativa</i> (gblL29470)	8e-04
	mgir2H01	Ribosomal protein 60S	<i>Cicer arietinum</i> (splO65729)	7e-08
	mgir2H18	Translation initiation factor 5A	<i>Oryza sativa</i> (gblAF094773)	1e-06
	mgir2I24	Ubiquitin-conjugating enzyme	<i>Arabidopsis thaliana</i> (gblAC012562)	2e-36
	mgir2J20	26S rRNA 3' end and 18S rRNA	<i>Secale cereale</i> (gblM37231)	e-145
	mgir3A05	EIF-2B-epsilon subunit	<i>Arabidopsis thaliana</i> (gil3033392)	7e-05
	mgir3H11	Ribosomal protein 50S, L31	<i>Medicago sativa</i> (gil3885513)	2e-13
	mgir3I05	Ribosomal protein 40S, S4	<i>Zea mays</i> (spl022424)	5e-30
	mgir3L13	Peptidylprolyl isomerase	<i>Triticum aestivum</i> (splQ43207)	2e-08
	mgir3M22	Ribosomal RNA 40S	<i>Oryza sativa</i> (gblM11585)	e-123
VI. Metabolism	mgir1A11	Chloroplast photosystem I PSK-I	<i>Hordeum vulgare</i> (gblL12707)	e-134
	mgir1B10	Fructose-1,6-bisphosphatase	<i>Oryza sativa</i> (dbjlAB007193)	1e-23
	mgir1B17	Cab1R	<i>Oryza sativa</i> (emblX13908)	0.0
	mgir1D02	23-kDa polypeptide of PS II	<i>Oryza sativa</i> (gil4079798)	4e-47
	mgir1D08	Photosystem I F subunit precursor	<i>Oryza sativa</i> (gil3885892)	4e-59
	mgir1D09	Glucose-6-phosphate dehydrogenase	<i>Spinacea oleracea</i> (gnlle330370)	5e-18
	mgir1D22	Glycolate oxidase	<i>Oryza sativa</i> (gil2570515)	9e-15
	mgir1F01	Chlorophyll alb-binding protein	<i>Oryza sativa</i> (gil3075488)	1e-40
	mgir1F13	Methionine sulfoxide reductase	<i>Arabidopsis thaliana</i> (splP54150)	2e-17
	mgir1G03	Rubisco small-subunit C	<i>Oryza sativa</i> (splP18567)	7e-56
	mgir1G17	Chlorophyll a/b-binding protein	<i>Oryza sativa</i> (gil3789952)	6e-28
	mgir1H14	Farnesyl pyrophosphate synthase	<i>Oryza sativa</i> (gnld1020643)	8e-14
	mgir1J18	Hydroxynitrile lyase	<i>Arabidopsis thaliana</i> (gnlle326849)	6e-29
	mgir1J24	Protein phosphatase 2A	<i>Oryza sativa</i> (gblU49113)	6e-87
	mgir2C08	Cytochrome c (OscC-1) gene	<i>Oryza sativa</i> (gblM63704)	3e-30
	mgir2F08	PS I reaction center subunit XI	<i>Hordeum vulgare</i> (splP23993)	3e-15
	mgir2F19	Glyceraldehyde-3-phosphate dehydrogenase	<i>Oryza sativa</i> (splQ42977)	6e-14
	mgir2K08	NADH-ubiquinone oxidoreductase	<i>Nicotiana tabacum</i> (splO24143)	3e-20
	mgir2L06	ADP-glucose pyrophosphorylase	<i>Cucumis melo</i> (gblAF030383)	1e-08
	mgir2L08	Vacuolar ATP synthase	<i>Oryza sativa</i> (splQ40635)	1e-21
	mgir3A12	Rubisco	<i>Oryza sativa</i> (emblCAA59218)	3e-47
	mgir3C06	CAB apoprotein CP26	<i>Zea mays</i> (gil733454)	3e-24
	mgir3F02	Transaldolase (PotTal 1)	<i>Solanum tuberosum</i> (gil2078350)	1e-47
	mgir3G07	Serine carboxypeptidase isolog	<i>Arabidopsis thaliana</i> (gil1931640)	2e-07
	mgir3G09	Photosystem I chain II precursor	<i>Nicotiana sylvestris</i> (pirilS37380)	2e-13
	mgir3G21	Cab2R	<i>Oryza sativa</i> (emblX13909)	1e-54
	mgir3H05	Sedoheptulose-1,7-bisphosphatase	<i>Triticum aestivum</i> (splP46285)	1e-09
	mgir3H16	Cab26	<i>Oryza sativa</i> (gil3789954)	1e-14
	mgir3K12	PSII 22-kDa protein	<i>Oryza sativa</i> (dbjlD8392)	4e-09
	mgir3K18	Aldolase	<i>Oryza sativa</i> (gil1207279)	3e-19
	mgir3L18	RCABP89	<i>Oryza sativa</i> (gil3126854)	1e-30
	mgir3L22	Malate dehydrogenase	<i>Zea mays</i> (gil2286153)	1e-16
	mgir3M06	PSII 10-kDa polypeptide	<i>Oryza sativa</i> (gblU86018)	1e-64
	mgir3M16	LHCPII type I	<i>Oryza sativa</i> (splP12331)	3e-60
	mgir3O20	Rubisco small subunit	<i>Oryza sativa</i> (splP05347)	1e-21
VII. Unclassified	mgir1D18	N7 protein	<i>Medicago truncatula</i> (PIDle1308274)	2e-08
	mgir1E14	Unknown protein	<i>Arabidopsis thaliana</i> (AB026657)	2e-51
	mgir1E16	NifU-like protein	<i>Arabidopsis thaliana</i> (PIDle1249663)	1e-32
	mgir1G10	PAC clone: P0667A10	<i>Oryza sativa</i> (dbjlAP0667A10)	e-167
	mgir1H11	Unknown protein	<i>Lycopersicon esculentum</i> (AF261140)	4e-19
	mgir1H12	Repetitive element (clone pRG3)	<i>Oryza sativa</i> (gblL36557)	2e-99
	mgir1H16	Receptor kinaselike protein	<i>Oryza longistaminata</i> (gil2586083)	8e-05
	mgir1I14	Hypothetical protein	<i>Arabidopsis thaliana</i> (AL035602)	7e-44
	mgir1L21	NifU-like protein	<i>Arabidopsis thaliana</i> (emblAL021712)	1e-12
	mgir1M05	Ran	<i>Oryza sativa</i> (dbjlAB015287)	0.0
	mgir1P01	Bundle sheath defective protein 2	<i>Zea mays</i> (AF126742)	2e-21
	mgir2B04	Chromosome 1, clone: P0038F12	<i>Oryza sativa</i> (P0038F12)	0.0
	mgir2C24	Aminocyclopropane-1-carboxylate synthase	<i>Nicotiana tabacum</i> (emblX65982)	2e-12
	mgir2E22	Adh1-adh2 region	<i>Oryza sativa</i> (gblAF172282)	1e-05
	mgir2I17	Putative protein	<i>Arabidopsis thaliana</i> (emblAL035523)	9e-09
	mgir2J11	Late embryogenesislike protein	<i>Prunus armeniaca</i> (gil3264769)	5e-27
	mgir3A07	F26F24.9	<i>Arabidopsis thaliana</i> (gblAAF87020.1)	7e-20
	mgir3E17	Protein-induced upon tuberization	<i>Solanum demissum</i> (PIDle247412)	1e-08
	mgir3G06	Putative protein kinase	<i>Arabidopsis thaliana</i> (AC002340)	1e-15
	mgir3H12	Putative protein kinase	<i>Arabidopsis thaliana</i> (gil3763932)	1e-15
	mgir3H18	Hypothetical protein	<i>Arabidopsis thaliana</i> (gnllPIDle248730)	3e-11
	mgir3I23	Sm protein F isolog	<i>Arabidopsis thaliana</i> (gil2281089)	3e-35
	mgir3J23	CPI2	<i>Nicotiana tabacum</i> (gnllPIDle243962)	9e-10
	mgir3L08	Rice (YK426) mRNA	<i>Oryza sativa</i> (dbjlD83527)	0.0
	mgir3N10	Unknown	<i>Arabidopsis thaliana</i> (gil687677)	9e-29
	mgir3O01	p-SINEI-r3 gene, repeat sequence	<i>Oryza sativa</i> (dbjlD10672)	2e-37
	mgir3P10	P54 protein	<i>Pisum sativum</i> (emblY11207)	6e-09

various stress-inducible proteins. The sequence of cDNA clone mgir3G17 showed homology to blue light-inducible gene 3 (*BLI-3*) of *Neurospora crassa* (Eberle and Russo 1994) and was named *PEG1* (in planta expressed gene 1). A homolog (*PEG2*) of a nitrogen starvation-induced gene of *Colletotrichum gloeosporioides* (U94182) and a homolog (*PEG3*) of

glucose-repressible gene 1 (*GRG1*) of *N. crassa* (McNally and Free 1988) were also represented in this library. Nitrogen starvation is reported to strongly induce the expression of a number of in planta-induced genes, such as *ipiB* and *ipiO* of *P. infestans* (Pieterse et al. 1994) and *AVR9* of *Cladosporium fulvum* (van den Ackerveken et al. 1994). Therefore, nitrogen starvation has been widely used to isolate the genes involved in the pathogenicity of phytopathogenic fungi. Recently, a genomic approach was also used to identify potential candidate genes conditioning pathogenicity in *Mycosphaerella graminicola*, the wheat fungal leaf blotch pathogen (Keon et al. 2000). However, nitrogen starvation alone cannot fully mimic the conditions that the fungi might encounter during growth within the host. In fact, *MHP1* transcript was detected only in diseased leaves in Northern blot analysis and could not be found under nutrient starvation conditions. In fact, a set of pectate lyases that were required for full-tissue maceration were identified in *Erwinia chrysanthemi* EC16 and were thought to be expressed only in planta (Kelemu and Collmer 1993). Therefore, the identification of genes expressed in planta should provide insight for better understanding the pathogenicity of a pathogen. The highest proportion of cDNAs (79 clones) among the 221 clones matching plant genes were grouped into metabolism, while only 27 clones were classified into gene/protein expression. Sixty-seven clones comprising twenty-five independent ESTs were functionally assigned to cell/organism defense. Thirteen of these twenty-five ESTs best matched genes from plant species other than rice (Table 2), suggesting novel genes hitherto unidentified in rice. Six different PR proteins, including two each of PR-1 and PR-5 (thaumatinlike protein), PBZ1 (probenazole-inducible gene 1; PR-10), and class III chitinase (PR-8), were represented in this library. Homologs of an acidic PR-1 of maize (mgir1A24) and a class III chitinase of tulip (mgir3B23) were identified for the first time in rice and designated as *PR-1a* (Kim et al. 2001) and *OsChib1*, respectively. Four types of metallothionein were represented, one of which (mgir2I01) showed the best match to that of *Hordeum vulgare*, suggesting the isolation of a novel type of metallothionein in rice.

All cDNA sequences were assembled into a database and compared with each other using the ATGC software (Software Development) to identify overlapping sequences. While 184 clones were present only once, 112 genes were detected from 2 to 15 times. Thus, 296 distinct genes were identified among

Table 3. Summary of dbEST search of not significant matches using BLASTN

Source of expressed sequence tag	Number (percentage) of cDNAs matched to dbEST entries
dbEST matches	127 (58.3)
Plant	107 (49.1)
Fungi ^a	16 (7.4)
Mixed ^b	4 (1.8)
No matches	91 (41.7)
Total	218 (100)

^a dbEST entries that were generated from the *Magnaporthe grisea* apressorium-stage cDNA library (registered by R. A. Dean).

^b dbEST entries that were generated from a rice blast infection-stage cDNA library constructed with rice leaves 48 h after inoculation with *M. grisea* strain 70-15 (registered by R. A. Dean).

Table 4. Classification of expressed sequence tags according to putative biological function

Functional group ^a	Number (percentage) of cDNAs homologous to		Total
	Fungal genes	Plant genes	
Cell division	1 (1.4)	1 (0.4)	2 (0.7)
Cell signaling/ cell communication	0 (0)	1 (0.4)	1 (0.3)
Cell structure/ motility	1 (1.4)	0 (0)	1 (0.3)
Cell/ organism defense	19 (26.4)	67 (30.4)	86 (29.4)
Gene/ protein expression	27 (37.5)	27 (12.2)	54 (18.4)
Metabolism	17 (23.6)	79 (35.7)	96 (32.8)
Unclassified	7 (9.7)	46 (20.8)	53 (18.1)
Total	72 (100)	221 (100)	293 (100)

^a Protein matches from the BLASTX searches were assigned to one of seven functional categories following the Expressed Gene Anatomy Database cellular role classification scheme (White and Kerlavage, 1996).

Table 5. List of redundant cDNA clones occurring more than five times

Expressed sequence tag	Putative identification	Number of cDNAs	% ^a	Origin ^b
mgir3D04	Metallothionein type 1	15	2.9	P
mgir1I01	Glucose repressible gene 1	8	1.6	F
mgir1C20	Unidentified	6	1.2	U
mgir1F08	Probenazole-inducible protein 1	6	1.2	P
mgir3M06	Photosystem II 10-kDa polypeptide	6	1.2	P
mgir3N05	Metallothioneinlike protein type 2	6	1.2	P
mgir3O20	Rubisco (RICRUBPC1)	6	1.2	P
mgir1A24	Pathogenesis-related protein 1	5	1.0	P
mgir3K18	Aldolase	5	1.0	P
mgir3A10	Enolase	5	1.0	F
mgir3A12	Rubisco (RICRUBPC2)	5	1.0	P
mgir3M16	CAB binding protein of LHCII type I	5	1.0	P

^a Percentage was calculated based on 511 cDNA clones analyzed.

^b Putative origin of the expressed sequence tags was indicated based on the best match to a sequence in the NCBI nonredundant database. P = plant gene, F = fungal gene, and U = unidentified.

Fig. 1. Northern blot hybridization of interaction-induced genes. **A**, Plant defense-related genes. *PR-1a*, encoding an acidic PR-1; *PBZ1*, probenazole-inducible protein 1; *OsChib1*, a class III chitinase; *PR-5*, thaumatinlike protein; and *MTs-1*, type I metallothionein. **B**, Fungal stress-inducible or pathogenicity genes. *MHP1*, a class II hydrophobin of *Magnaporthe grisea*; *MPG1*, a class I hydrophobin of *M. grisea*; *PEG1*, a homolog of *BLI-3* of *Neurospora crassa*; *PEG2*, a homolog of the nitrogen starvation-induced gene of *Colletotrichum gloeosporioides*; and *PEG3*, a homolog of *GRG-1* of *N. crassa*. Total RNA was isolated from uninfected rice leaves (lane 1), rice leaves infected with *M. grisea* 120 h after inoculation (lane 2), *M. grisea* mycelia grown in complete medium (lane 3), mycelia grown under nitrogen starvation conditions (lane 4), and mycelia grown under carbon starvation (lane 5). Ten micrograms of total RNA was fractionated in 1.5% formaldehyde gels and transferred to Hybond N+ membranes. The blots were hybridized using the indicated cDNAs as probes.

the 511 cDNA clones analyzed. The ESTs that were detected more than five times are listed in Table 5. The most frequently detected cDNA clone (15 times, 2.9% total clones) encoded the rice type I metallothionein (MTs-1). cDNAs encoding PBZ1 or PR-1, type 2 metallothioneinlike protein, and an unidentified sequence were also sequenced more than five times. Many ESTs appeared to represent various isoforms of plant genes. Four distinct metallothionein sequences, two types of PR-1 and two of PR-5, were represented in this library. Furthermore, various isoforms of genes involved in photosynthesis, including nine isoforms of chlorophyll a/b-binding protein, were detected. Seven plant ribosomal proteins and distinct fungal ribosomal proteins were also represented.

Expression patterns of 10 ESTs showing similarity to defense-related genes and fungal stress-inducible or pathogenicity genes were examined by Northern blot hybridization analysis. The expression of five ESTs showing homology to rice defense-related genes, i.e., *PR-1a*, *OsChib1*, *PBZ1*, *PR-5*, and *MTs-1*, were up-regulated by the pathogen infection, as expected (Fig. 1). The four PR genes were barely detected in uninfected healthy leaves but were strongly expressed after infection with *M. grisea*. *MTs-1* was constitutively expressed in healthy leaves, although its expression was also induced by the infection. Expression of an EST matching *MPG1* of *M. grisea* was induced during growth within the host, as well as in nitrogen-starved medium, as reported earlier (Talbot et al. 1993). *PEG1* was highly expressed during the infection, while nitrogen deprivation down-regulated its expression. *MHP1* was expressed only during invasive growth within the host. *PEG2* was also expressed during the infection but was repressed by nitrogen starvation. The expression of *PEG3* was also strongly induced during growth in planta and by nitrogen starvation (Fig. 1).

In summary, the ESTs generated in this study may, at least in part, represent molecular processes that occur during compatible interaction in the rice-blast pathosystem. Genes originating from both organisms were identified, and a high proportion of these genes were associated with fungal pathogenicity or host defense responses against an invading pathogen. Further characterization of the genes reported here will help unravel the mechanisms of pathogenicity, particularly at the stage of disease progression, and the defense responses of host plants.

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