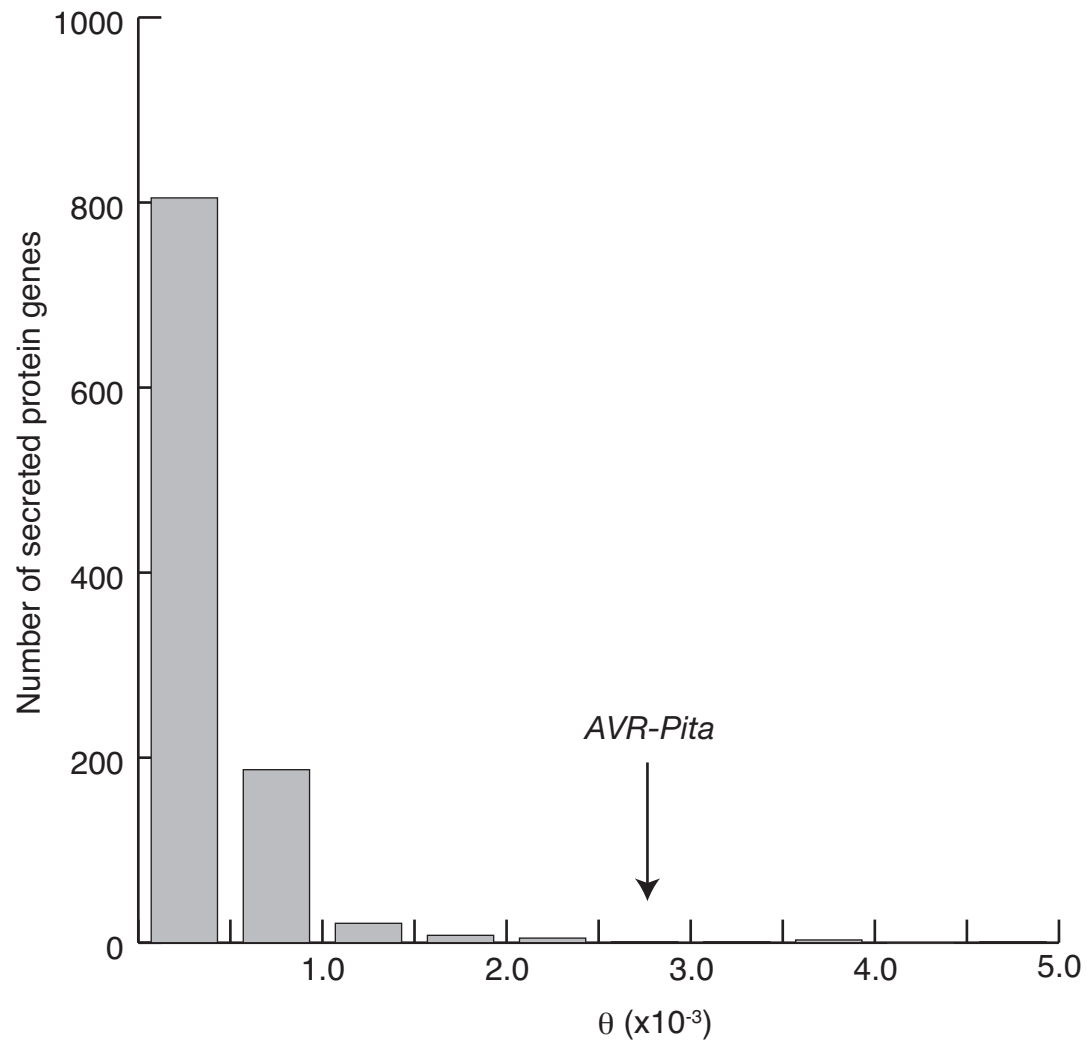
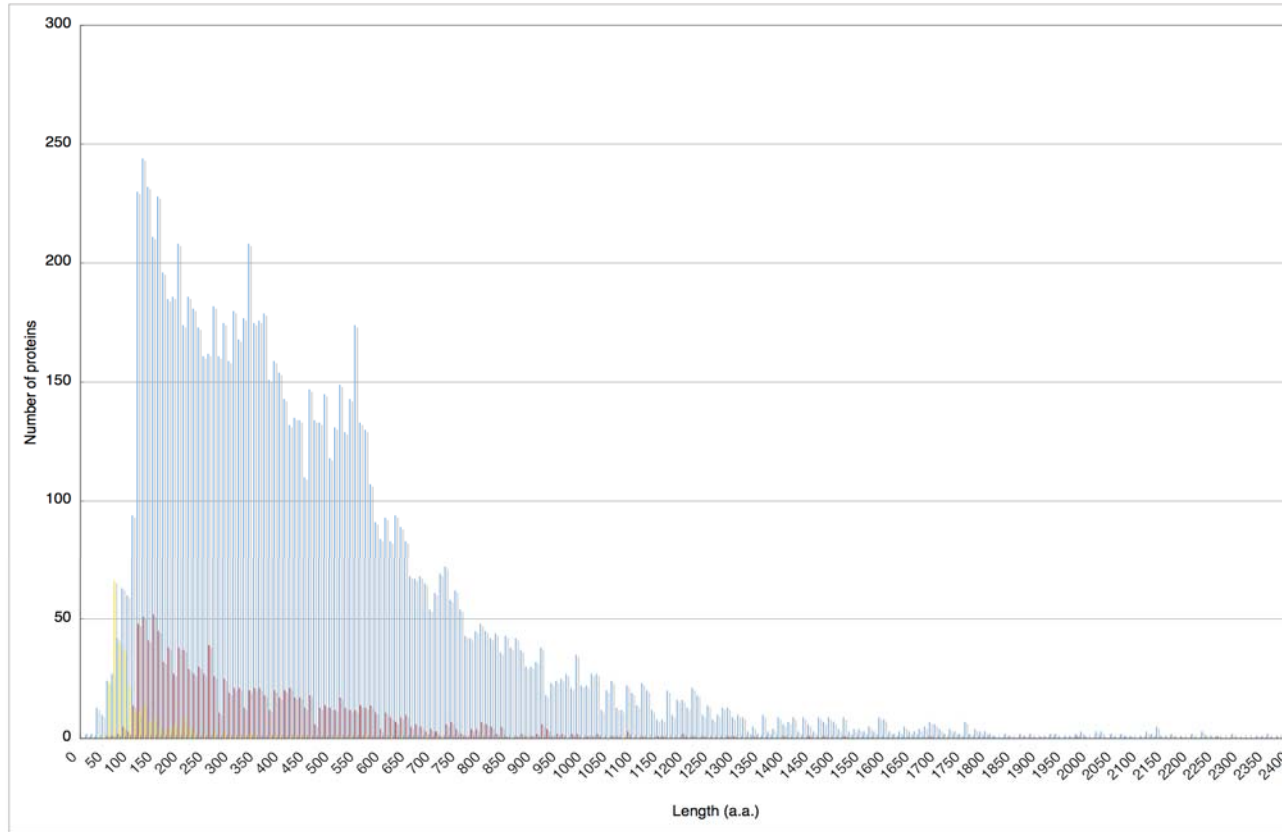


Supplemental Figure 1. Ambiguous symptoms caused by 70-15 isolate infected to various rice cultivars. Each isolate was sprayed onto the leaves of diagnostic rice cultivars differentially harboring eleven known resistance (*R*-) genes, *Pia*, *Pii*, *Pik*, *Pik-m*, *Pik-p*, *Piz*, *Pita*, *Pita2*, *Piz-t*, *Pib*, *Pit* cognate to the *AVR* genes (Kiyosawa, 1986) as well as susceptible cultivars Moukoto and Shin-2, and compatible or incompatible interaction was observed among them. It is notable that in our experiments, the reaction between the isolate 70-15 and these eleven rice *R*-genes could not be precisely defined since the disease symptoms caused by 70-15 were not clear, and we could not assign each reaction as compatible or incompatible. In addition, 70-15-inoculated Moukoto and Shin-2 also showed unclear symptoms. On the contrary, Ina168 induced typical susceptible spindle-shaped lesions on Yahiromochi, K60, Moukoto and Shin-2 leaves, and typical resistance symptoms (few small brown spots) on the leaves of the other cultivars. Photos were taken 10 days after inoculation with 5×10^5 conidia/ml (containing 0.01 % tween 20) of 70-15 or 3×10^5 conidia/ml (containing 0.01 % tween 20) of Ina168.



Supplemental Figure 2. Frequency distribution of nucleotide diversity among 1,032 loci of *M. oryzae* putative secreted protein genes.

Nucleotide diversity (θ ; Watterson 1975) is given in x-axis and number of loci in the given θ range is shown in the y-axis. Out of 1,032 loci, 805 were monomorphic. θ of *AVR-Pita* (MG11081.4) is 0.0029.



Supplemental Figure 3. Size distribution of predicted proteins of *M. oryzae*. Size distribution of 11,109 predicted proteins of Isolate 70-15 (blue), 1,306 predicted secreted proteins of isolate 70-15 (red), and 316 predicted secreted proteins in Ina168-unmapped regions (yellow).

a) pex22

```
ATCGCTTTGC CCTCATTGCA CACAACAACC TCCATTAATA TACATACATA TTTCTATTCC 60
CTTTCATAA CAGTATCCCT ACCCGCCAGC CCCATACACG AAAATTCTCA AAATGCAATT 120
TTCGACAATT TTCATCCCCT TTGCCTTAGC TGCTCTAAAA GTAAGCGCTG CGCCAGCTAG 180
ATTTTGCGTC TATTACGACG GCCACCTTCC CGCGACACGT GTCCTGCTTA TGTACGTTAG 240
AATCGGCACT ACAGCGACTA TTACGGCCCC TGGGCACGAA TTCGAAGTTG AAGCAAAAAGA 300
CCAGAATTGC AAAGTTATTC TCACCAATGG CAAACAAGCA CCGGATTGGC TTGCTGCCGA 360
GCCTTACTAG GTTTAAAACG GAAGAGCAGC AGCTTGCTGG TTAAACTTCA AAATAAACGC 420
CAACTCAGTT ACTACTTACA ATACTATGAT TTCTGCCGAA AATTCCCTCT CCCCCTACCG 480
TACGTGTATA CCTGGGCTTT TACCAGTTT ACTGTTTACG GGAGGAATCA AGTACCTAAA 540
TTTATTTTTT CCTTCAAAT GATTACCAA GTCGGAAACG CCCACAAGGC CTACACCACT 600
CGACGATGAA ACCAGGGTAG GCTTTTGTTA CCTGTGCAAT CATGGCAAAT TATCACTCTT 660
TAGCGAAAGT AAATACAAAC AAACGAGTTT CCAGG 695
```

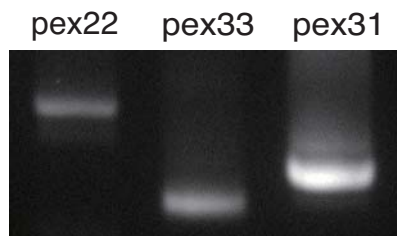
Tag3294
6 counts

b) pex33

```
ATTTTTCATC TAAAACTAA ACATTTAAAT TCAGCTTCAA ATATACCAA ATCCCCTTTT 60
ATTCCTTCCA ATTTACCAA ATGCAACTTT CAAAATTAC TTTCGCTATT GCATTATATG 120
CAATCGGAAT CGCAGCACTT CCCACTCCGG CCAGCCTGAA TGGCAACACT GAGGTCGCAA 180
CCATCTCCGA CGTTAAACTT GAGGCCCGCA GCGACACCAC TTATCATAAA TGCTCCAAAT 240
GCGGTTATGG CAGCGATGAT TCCGACGCGT ATTTTAATCA TAAATGCAAC TAAATCGCATA 300
CAAAGTAAGC TGGATTTTTT CAAACGAGCG GCGGACAGTT ATGGACTATT GCGGGAATTT 360
CAAGGATAGG AAAAGCCAAT TTGAAATATC CGGAATACTT TTTCCAAGCT TAACCATTAA 420
ATAACATGTC TAAATCAAT CTACCTTTCG CGTTTAAA 458
```

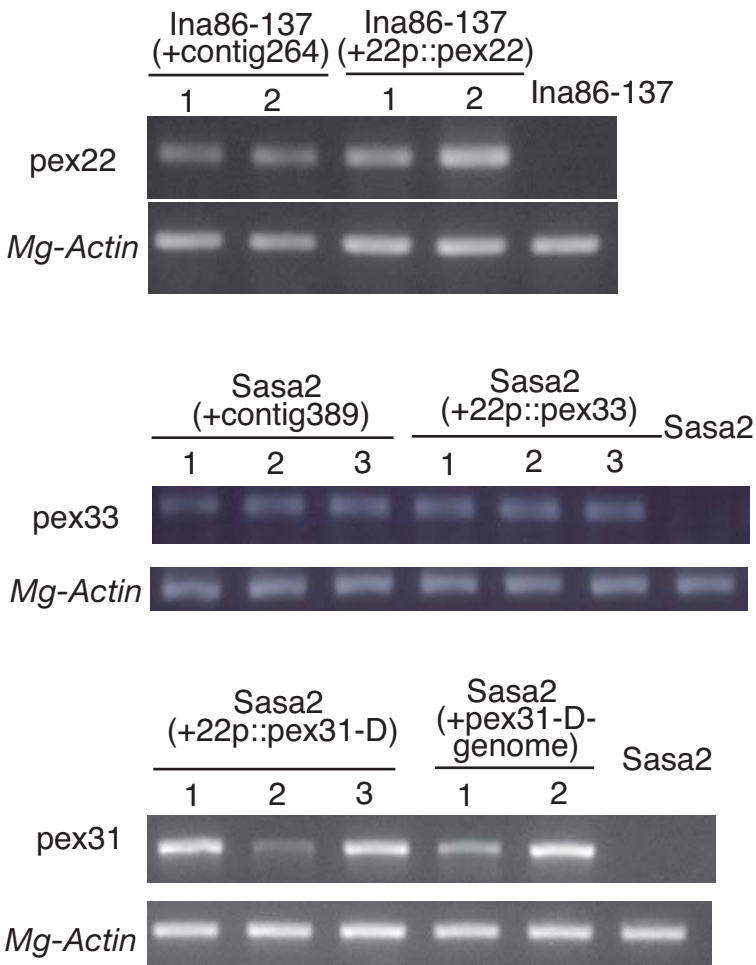
Tag1145
14 counts

c)



Supplemental Figure 4. pex22, pex33, pex31 transcripts are expressed during *M. oryzae* infection of rice leaf sheath as revealed by SuperSAGE and 3'-RACE RT-PCR.





a) DNA sequence of the pex22 gene region. Pex22 ORF is indicated by red color. Location of the SuperSAGE tag Tag3294 is underlined. b) DNA sequence of the pex33 gene region. ORF is indicated by red color. Location of the SuperSAGE tag Tag1145 is underlined. c) 3'-RACE RT-PCR results of pex22, pex33 and pex31.



Supplemental Figure 5. Confirmation of active transcription of pex transgenes by RT-PCR in *M. oryzae* transformants during infection.







a)

O. sativa

<i>M. oryzae</i>	Sasanishiki (<i>Pia</i> ⁺)	Shin-2 (<i>Pia</i> ⁻)
TH68-141		
TH68-141 (+contig264)		













b)

O. sativa

<i>M. oryzae</i>	Kakehashi (<i>Pii</i> ⁺)	Shin-2 (<i>Pii</i> ⁻)
Ina86-137		
Ina86-137 (+contig389)		
Ina86-137 (+22p::pex33)		

c)

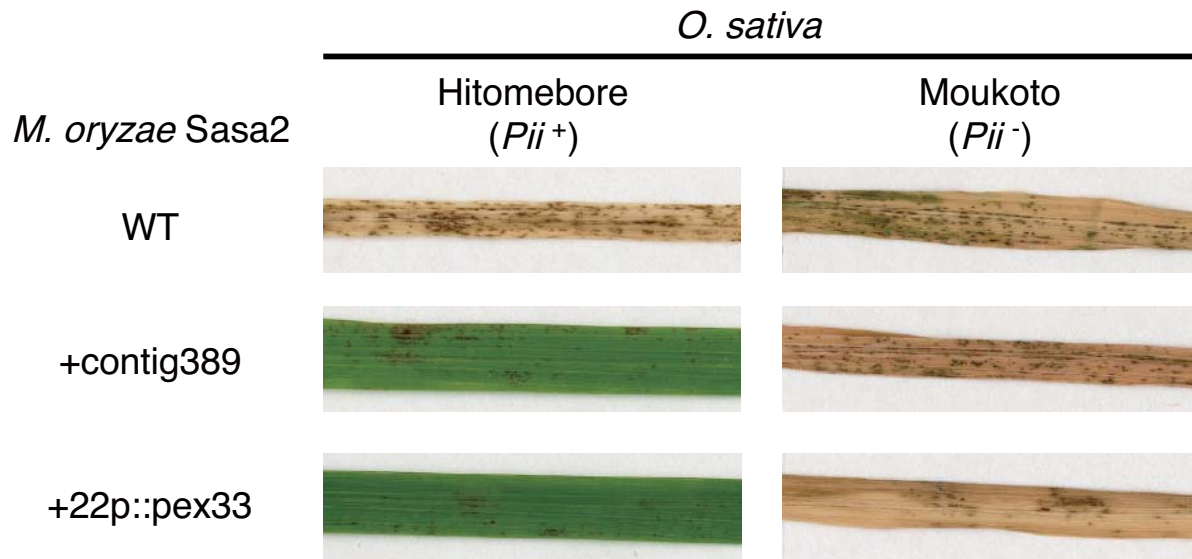
O. sativa

<i>M. oryzae</i>	Kanto51 (<i>Pik</i> ⁺)	Tsuyuake (<i>Pik-m</i> ⁺)	K60 (<i>Pik-p</i> ⁺)	Shin-2 (<i>Pik</i> , <i>Pik-m</i> , <i>Pik-p</i> ⁻)
Ina72				
Ina72 (+pex31-D-genome)				
Ina72 (+22p::pex31-D)				

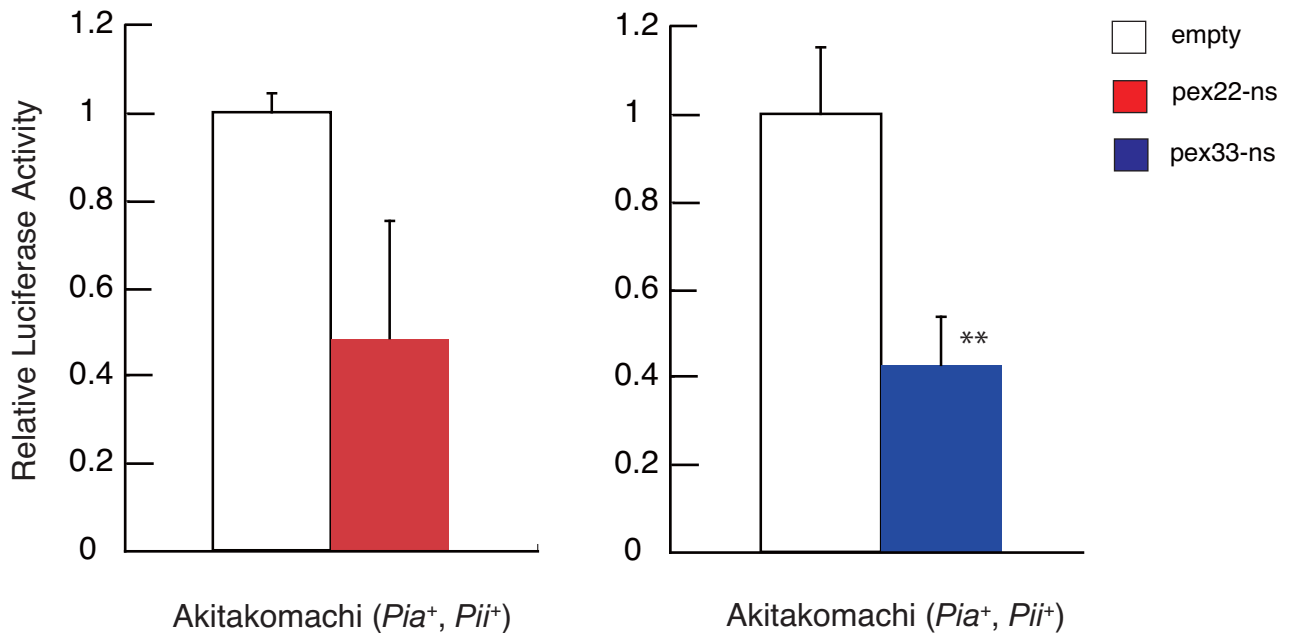
Supplemental Figure 6. Transformation of *M. oryzae* isolates with pex22, pex33 and pex31-D complements *AVR-Pia*, *AVR-Pii* and *AVR-Pik/km/kp*, respectively.

Names of isolates and DNA fragments used for transformation are given in the left of each panel. (a) Transformation of TH68-141 with pex22 conferred the *AVR-Pia* phenotype. (b) Transformation of Ina86-137 with pex33 conferred the *AVR-Pii* phenotype. (c) Transformation of Ina72 with pex31 conferred the *AVR-Pik/km/kp* phenotypes.

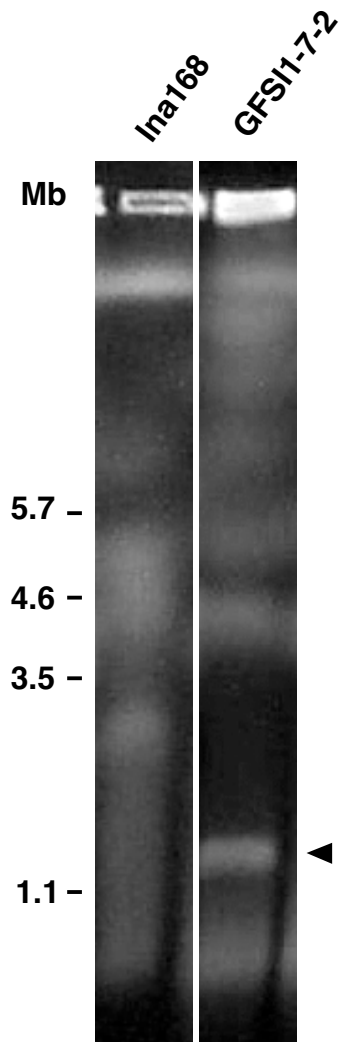
a)



b)



Supplemental Figure 7. Additional results of interactions between *M. oryzae* and rice. a) Results of interaction between *M. oryzae* and rice. The isolate Sasa2 is compatible with a rice cultivar Hitomebore harboring the R-gene *Pii*. Sasa2 transformed with contig389 (+contig389) as well as Sasa2 transformed with a fragment containing pex22 promoter fused with pex33 ORF (+22p::pex33) were incompatible with Hitomebore. Sasa2, [Sasa2(+contig389*)], [Sasa2 (+22p::pex33)] are all compatible with a rice cultivar Moukoto lacking *Pii* suggesting that the effect of transformation with contig389 and 22p::pex33 is *Pii* dependent. Photos were taken 10 days after inoculation with 3×10^5 conidia/ml (containing 0.01 % tween 20) of each strain. b) Interaction between AVR candidates and *P*-genes causes cell death in rice protoplasts. Rice cell viability after



Supplemental Figure 8. Pulsed field gel electrophoresis images of chromosomes of Ina168 and GFSI1-7-2 isolates of *M. oryzae*.
The typical 1.2 Mb supernumerary chromosome of GFSI1-7-2 is indicated by an arrowhead.

Supplemental Table 1.

46 isolates of *M. oryzae* used for EcoTILLING and phylogenetic analysis

Code no.	Code no. in Table1	Isolate	Host	Race	Country
1	2	70-15	<i>Oryza sativa</i>	Unknown	Guiana
2		GUY11	<i>Oryza sativa</i>	137.5	Guiana
3		70-6	<i>Oryza sativa</i>	Unknown	Guiana
4		Br13	<i>Oryza sativa</i>	102	Brazil
5	15	Br18	<i>Oryza sativa</i>	176	Brazil
6	22	Br10	<i>Oryza sativa</i>	403	Brazil
7		Br15	<i>Oryza sativa</i>	507	Brazil
8		VHT6.1	<i>Oryza sativa</i>	002	Vietnam
9		VHG4.5	<i>Oryza sativa</i>	106	Vietnam
10		VTB6.1	<i>Oryza sativa</i>	Unknown	Vietnam
11		VHT3.3	<i>Oryza sativa</i>	Unknown	Vietnam
12		PO-02-7306	<i>Oryza sativa</i>	Unknown	Indonesia
13		PO-02-7501	<i>Oryza sativa</i>	Unknown	Indonesia
14		PO-04-7501	<i>Oryza sativa</i>	Unknown	Indonesia
15		PO-12-7301-2	<i>Oryza sativa</i>	Unknown	Indonesia
16		PO-12-7301	<i>Oryza sativa</i>	Unknown	Indonesia
17		TH3	<i>Oryza sativa</i>	135	Thailand
18		CHNOS60-8-1	<i>Oryza sativa</i>	104.4	China
19	5	Shin85.86	<i>Oryza sativa</i>	001.0	Japan
20		TH68-141	<i>Oryza sativa</i>	003.0	Japan
21		Ken54-04	<i>Oryza sativa</i>	003.0	Japan
22		Ken54-20	<i>Oryza sativa</i>	003.0	Japan
23		0903.4	<i>Oryza sativa</i>	003	Japan
24		TH83-05-15-3	<i>Oryza sativa</i>	003.0	Japan
25		Ina85-182	<i>Oryza sativa</i>	103.0	Japan
26	18	Ina86-137	<i>Oryza sativa</i>	007.0	Japan
27	17	Hoku1	<i>Oryza sativa</i>	007.0	Japan
28	19	2012-1	<i>Oryza sativa</i>	007	Japan

29	20	2403-1	<i>Oryza sativa</i>	007	Japan
30		22-4-1-1	<i>Oryza sativa</i>	107.0	Japan
31		Naga69-150	<i>Oryza sativa</i>	007	Japan
32	16	TH87-20-BII	<i>Oryza sativa</i>	007.2	Japan
33	6	Ina72	<i>Oryza sativa</i>	031.0	Japan
34	9	1836-3	<i>Oryza sativa</i>	033	Japan
35	10	TH68-126	<i>Oryza sativa</i>	033.1	Japan
36	7	TH68-140	<i>Oryza sativa</i>	035.1	Japan
37	11	24-22-1-1	<i>Oryza sativa</i>	037.1	Japan
38	12	9505-3	<i>Oryza sativa</i>	037.1	Japan
39	13	Sasa2	<i>Oryza sativa</i>	037.1	Japan
40	8	TH69-8	<i>Oryza sativa</i>	071.1	Japan
41	1	Ina168	<i>Oryza sativa</i>	101.1	Japan
42		Ken53-33	<i>Oryza sativa</i>	137.1	Japan
43	14	TH78-15	<i>Oryza sativa</i>	177.1	Japan
44	23	P-2b	<i>Oryza sativa</i>	303.1	Japan
45	21	88A	<i>Oryza sativa</i>	433	Japan
46		P2	<i>Oryza sativa</i>	003	Japan

Supplemental Table 2. A list of *M. oryzae* putative secreted proteins possessing the [RK]CxxCxxxxxxxxxxH] motif

>gi_39968261_ref_XP_365521.1_predicted protein [Magnaporthe grisea 70-15]
MQISVSQVLVTLAGLIMGASARLHSAAVCVQNRSYGSTGNGTPQGITYGSYVDYEIDSAATQCACNFYRNR
HTGNNQWDSPCDVCVFDGIQCLSNGWHLGGDEITYYCEKLCNSQGAQAN

>gi_39955784_ref_XP_364190.1_hypothetical protein MG09035.4 [Magnaporthe grisea 70-15]
MQIFNIVQVLGLLAVGASALPTPANVGAVQPVVEGSQLQARSSNFYSAGWTQYPSANSYGPSNSASTYYYYK
CNHCGKHTKEESQKYHQENSHKKGESNYSEVQA

>gi_39946562_ref_XP_362818.1_hypothetical protein MG08230.4 [Magnaporthe grisea 70-15]
MQIFKIVQVLGLLAVGTSALPAPANPAVQPAQGGQLAVHGQPASCPEPCRDVQGAKPGHLQARSRFYAT
SDTGRPLANSGYPTHGYRDAYRCLYCGAVRDDVSAVQDHITYRHSNRGGDTSNYDTTTRVRRDR

>gi_39946240_ref_XP_362657.1_predicted protein [Magnaporthe grisea 70-15]
MHTYKFIQIALLFASVALAIPPPSPNPPPPVQLPNSETKSNRLVSHSCEFCGVVVKPSGPAYLEHYHQN
HREEVWGKLATPSPNPPPPVPTQKVETHAPKTHGCEWCNKVEPSGPAYIKHYKENHEDQVWGKWAGQDAK
ASP

>gi_39945196_ref_XP_362135.1_predicted protein [Magnaporthe grisea 70-15]
MHLSTVSQILALFTAVASSAPTSHA AVRARHVSPEADLLHVVKRGDDTDFSDWQRYSLWNKRVEALSRN
PELVSRYGKCNCSGKGRMDENGMRRHIMFDHYSKDIPEKSAHVNKYIEFRNKGG

>gi_39944940_ref_XP_362007.1_predicted protein [Magnaporthe grisea 70-15]
MKYSKVSFTLALIALGATAAPTDLHKLVARGEVEVCYDPRDPRVPPLQNDPNVEKWIDDKTGHHCFA LMR
GVQPDHPCKACQGHQADLYLDHIHCLAAGHVHANKGDSCEATRQARLD RYKTALKGFRVPYGTDISKLET
GGKGVTFVNDPSPHKLFSGDRSLTQVVKDGNVEFVRPHGKEYRQGEDLYATIQRQWYALKA AKILKAAR
AAKAARTPDASDAGKLEPPAKLATQQRRLDESRS AE

>gi_39973079_ref_XP_367930.1_predicted protein [Magnaporthe grisea 70-15]
MQLHNVCISILALLAAGVFALPAPVNPSEIQARSAADKAKPKPQTERVWMDPEAAQYLS DPRWKLVKKKY
SCGYCDSSSSKVDKINKHRDEVHGT RQAQVDHTIYPGETRLTFERVSGYP

>gi_39972083_ref_XP_367432.1_predicted protein [Magnaporthe grisea 70-15]
MLPLFLVTSFLFTQFLAAAHTENFEIVKGLADKARVKLDLTGQQSYAIYEKFPKAVYNPLDYHMRLIVGH
VECKDDNDCDFAANAFHMAHGGSCERVLGSKTNQHSRNRPWKANHYD KKDIDKPLPQKNRYQWAGPT
RTLSYDEIYALGDKWCGSCFHSRYNKVFNNCHHFVYSLYNKIKQK

>gi_39971991_ref_XP_367386.1_predicted protein [Magnaporthe grisea 70-15]
MRITRALAPLGLLPLPAAAAAVVGIPLQAI DTTATASSAMEPRQAGDGS GGLKQCRDVSLVQSRPGLSL
NDTVSATCHVGTAEYVNTLNLNECLGVDPATLELGWGRAGQLSSFCWGC ELSELETGGPDGHLAVGRVVF
SCVCYDYQGQERATSVRLDDGGIESKDGVLACRNGKRATAIRMNPDAPVRTEPGR CNLGLGDMGYMDCIPK
LYDGKIVPVGERNRYGSVFNDRGD

Supplemental Table 3 A list of plasmids used for the genetic transformation of *M. oryzae*.

Plasmid name	Sources	Description	Construction methods	<i>M. oryzae</i> isolates used for transformation
pCB1004-pex22	pCB1004	2.2 Kb fragment containing pex22	The 2.2 Kb fragment was amplified with the primers NotI-pex22-U1 (5'-ATAAGAATGCGGCCGCTTTCGTGACAGGCACGTCGGG-3'; NotI site is underlined) and XbaI-pex22-L1 (5'-GCTCTAGAACACGAGAATCAAACCTGTA CAGACAGGTGGGTGGGC-3'; XbaI site is underlined) using genomic DNA of Ina168 isolate as template, and inserted into pCB1004.	TH68-141 and Ina86-137 isolates that do not have <i>AVR-Pia</i> locus.
pCB1531-pex22p-EGFP	pCB1531	EGFP fused to pex22 promoter	The 1.4 Kb fragment containing pex22 gene promoter was amplified with the primers NotI-pex22-U1 and XbaI-pex22p-L3 (5'-GCTCTAGAATTTTCGTGTATGGGGCTGGC GGGTAGGG-3'; XbaI site is underlined) using pCB1004-pex22 as template, and inserted into pBAGFP (Kimura et al., 2001).	-
pCB1531-pex22p-pex22	pCB1531	pex22 fused to pex22 promoter	The pex22 ORF was amplified with the primers Xba1-pex22-U2 (5'-GCTCTAGACAAAATGCATTTTTCGACAATTTC-3'; XbaI site is underlined) and BamH1-pex22-L2 (5'-CGGGATCCTAGTAAGGCTCGGCAGCAAGCC-3'; BamH1 site is underlined). EGFP in pCB1531-pex22p-EGFP was replaced with pex22.	Ina86-137 isolate that does not have <i>AVR-Pia</i> locus.
pCB1531-pex33	pCB1531	1.4 Kb fragment containing pex33	The 1.4 Kb fragment was amplified with the primers NotI-pex33-U1 (5'-ATAAGAATGCGGCCGCTTCGCTCTTTTGAT TAAATAC-3'; NotI site is underlined) and XbaI-pex33-L1 (5'-GCTCTAGAAAACAGATTTGGAACCTTTGGT GAAAACCTAGAC-3'; XbaI site is underlined) using genomic DNA of Ina168 isolate as template, and inserted into pCB1531.	Sasa2 and Ina86-137 isolates that do not have <i>AVR-Pii</i> locus.
pCB1531-pex22p-pex33	pCB1531	pex33 fused to pex22 promoter	The pex33 ORF was amplified with the primers pBAFP_kozak_pex33_XbaI_F (5'-GCTCTAGACAAAATGCAACTTCCAAAAT TAC-3'; XbaI site is underlined) and pBAFP_pex33_BamHI_R (5'-CGGGATCCTTAGTTGCATTTATGATTAAATACGC-3'; BamHI site is underlined) using pCB1531-pex33 as template. EGFP in pCB1531-pex22p-EGFP was replaced with pex33.	Sasa2 and Ina86-137 isolates that do not have <i>AVR-Pii</i> locus.
pCB1004-pex31-D	pCB1004	2.2 Kb fragment containing pex31-D	The 2.2 Kb fragment was amplified with the primers NotI-pex31-U1 (5'-ATAAGAATGCGGCCGCAAAAGGAATAAGG CCGACCTCT-3'; NotI site is underlined) and XbaI-pex31-L1 (5'-GCTCTAGATTTAAAAGCCGGGCCTTTTTTTC CCAA-3'; XbaI site is underlined) using genomic DNA of Ina86-137 isolate as template, and inserted into pCB1004.	Sasa2, and Ina72 isolates that do not have <i>AVR-Pik</i> , <i>AVR-Pikm</i> and <i>AVR-Pikp</i> loci.
pCB1531-pex22p-pex31-D	pCB1531	pex31-D fused to pex22 promoter	The pex31-D ORF was amplified with the primers Xba1_kozak_pex31_U1 (5'-GCTCTAGAAAAGTCAATATGCGTGTACCACTTT-3'; XbaI site is underlined) and pBAFP_pex31_BamHI_R (5'-CGGATCCTCGTCAAACCTCCCTACG-3'; BamHI site is underlined) using genomic DNA of Ina86-137 isolate as template. EGFP in pCB1531-pex22p-EGFP was replaced with pex31-D.	Sasa2, and Ina72 isolates that do not have <i>AVR-Pik</i> , <i>AVR-Pikm</i> and <i>AVR-Pikp</i> loci.

Kimura, A, Takano, Y, Furusawa, I, Okuno, T. (2001) Peroxisomal metabolic function is required for appressorium-mediated plant infection by *Colletotrichum lagenarium*. Plant Cell 13: 1945-1957.

Supplemental Table 4. A list of transposons analyzed for the linkage with secreted protein genes

Name	Accession number
DNA transposons	
Pot 2	Z33638.1
Pot 3	U60989.1
Occan	AB074754.1
LTR retrotransposons	
MAGGY	L35053.1
Pyret	AB062507.1
MGLR-3	AF314096
Inago1	AB334124.1
Inago2	AB334125.1
Non-LTR retrotransposons	
MGL (MGR583)	AF018033
Mg-SINE	MGU35313
Other retrotransposons	
Tf2	AY849688.1
MINE-C	EF585237.1
MINE-B	EF585236.1
MINE-A	EF585235.1
MINE	AJ851229.1

Supplemental Table 5. Primers used for plasmid construction and RT-PCR .

Primer name	Sequences (5' - 3')	Description
NotI-pex22-U1	ATAAGAATGCGGCCGCTTTCGTGACAGGCACGTCGGG	Constructions of the plasmids pCB1004-pex22 and pCB1531-pex22p-EGFP
XbaI-pex22-L1	GCTCTAGAACACGAGAATCAAACCTGTACAGACAGGTGGGTGG GC	Constructions of the plasmid pCB1004-pex22
XbaI-pex22p-L3	GCTCTAGAATTTTCGTGTATGGGGCTGGCGGGTAGGG	Constructions of the plasmid pCB1531-pex22p-EGFP
Xba1-pex22-U2	GCTCTAGACAAAATGCATTTTTCGACAATTTTC	Constructions of the plasmid pCB1531-pex22p-pex22
BamH1-pex22-L2	CGGGATCCTAGTAAGGCTCGGCAGCAAGCC	
NotI-pex33-U1	ATAAGAATGCGGCCGCTTCGCTCTTTTGATTAATAC	Constructions of the plasmid pCB1531-pex33
XbaI-pex33-L1	GCTCTAGAAAACAGATTTGGAACCTTGGTGAAAACCTAGAC	
pBAFP_kozak_pex33_XbaI_F	GCTCTAGACCAAATGCAACTTTCCAAAATTAC	Constructions of the plasmid pCB1531-pex22p-pex33
pBAFP_pex33_BamHI_R	CGGGATCCTTAGTTGCATTTATGATTAATACGC	
NotI-pex31-U1	ATAAGAATGCGGCCGCAAAGGAATAAGGCGGACCTCT	Constructions of the plasmid pCB1004-pex31-D
XbaI-pex31-L1	GCTCTAGATTAAGCCGGGCCTTTTTTCCCAA	
Xba1_kozak_pex31_U1	GCTCTAGAAAAGTCAATATGCGTGTTACCACTTT	Constructions of the plasmid pCB1531-pex22p-pex31-D
pBAFP_pex31_BamHI_R	CGGATCCTCGTCAAACCTCCCTACG	
RTpex22-U1	CCGCTCGAGCAAATGGCGCCAGCTAGATTTTGCG	RT-PCR for pex22
RTpex22-L1	CTAGTAAGGCTCGGCAGCAA	
RTpex33-U1	CTTCCCACTCCGGCCAGCCTGAATGGCA	RT-PCR for pex33
RTpex33-L1	ATACTAGTTTAGTTGCATTTATGATTA	
RTpex31-U1	CGACCTAAGTCGAGAGCGAGACCTAAC	RT-PCR for pex31
RTpex31-L1	TAAAAGCCGGGCCTTTTTTCCCAA	
RiceActin-U1	CTGAAGAGCATCCTGTATTG	RT-PCR for rice actin <i>RAc7</i>
RiceActin-L1	GAACCTTTCTGCTCCGATGG	
MgActin-U1	TCGACGTCCGAAAGGATCTGT	RT-PCR for <i>Magnaprothe oryzae</i> Actin
MgActin-L1	ACTCCTGCTTCGAGATCCACATC	