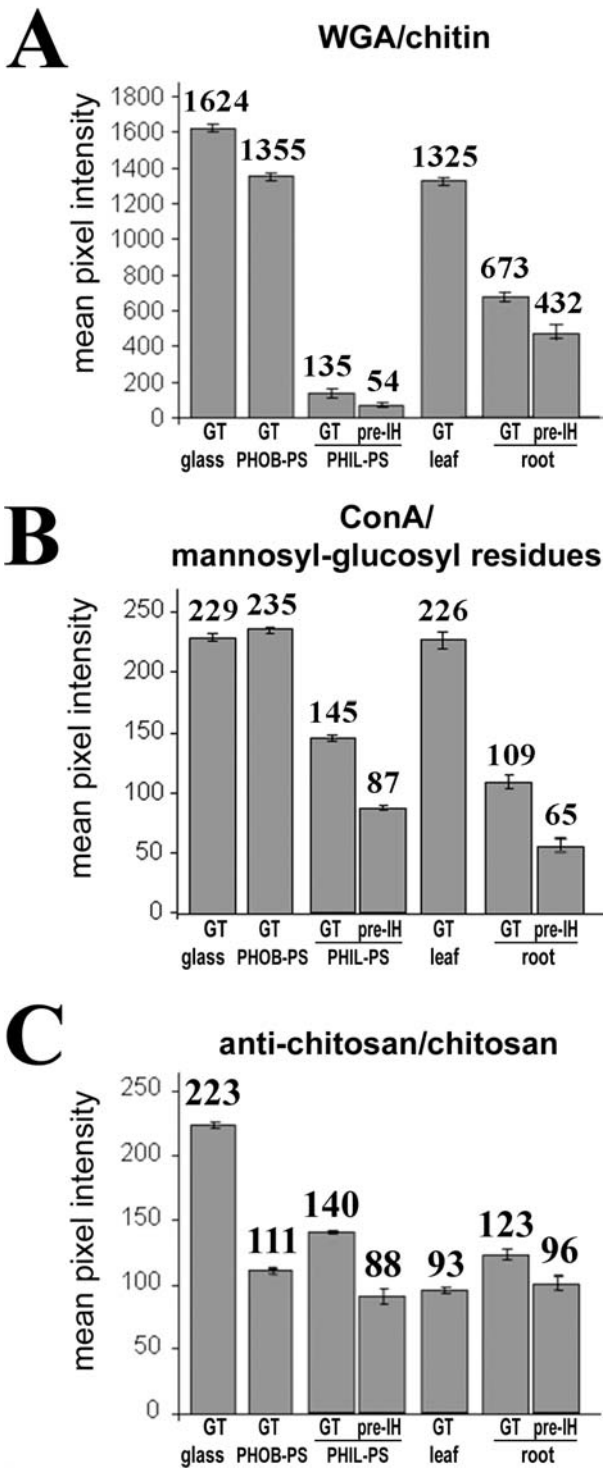
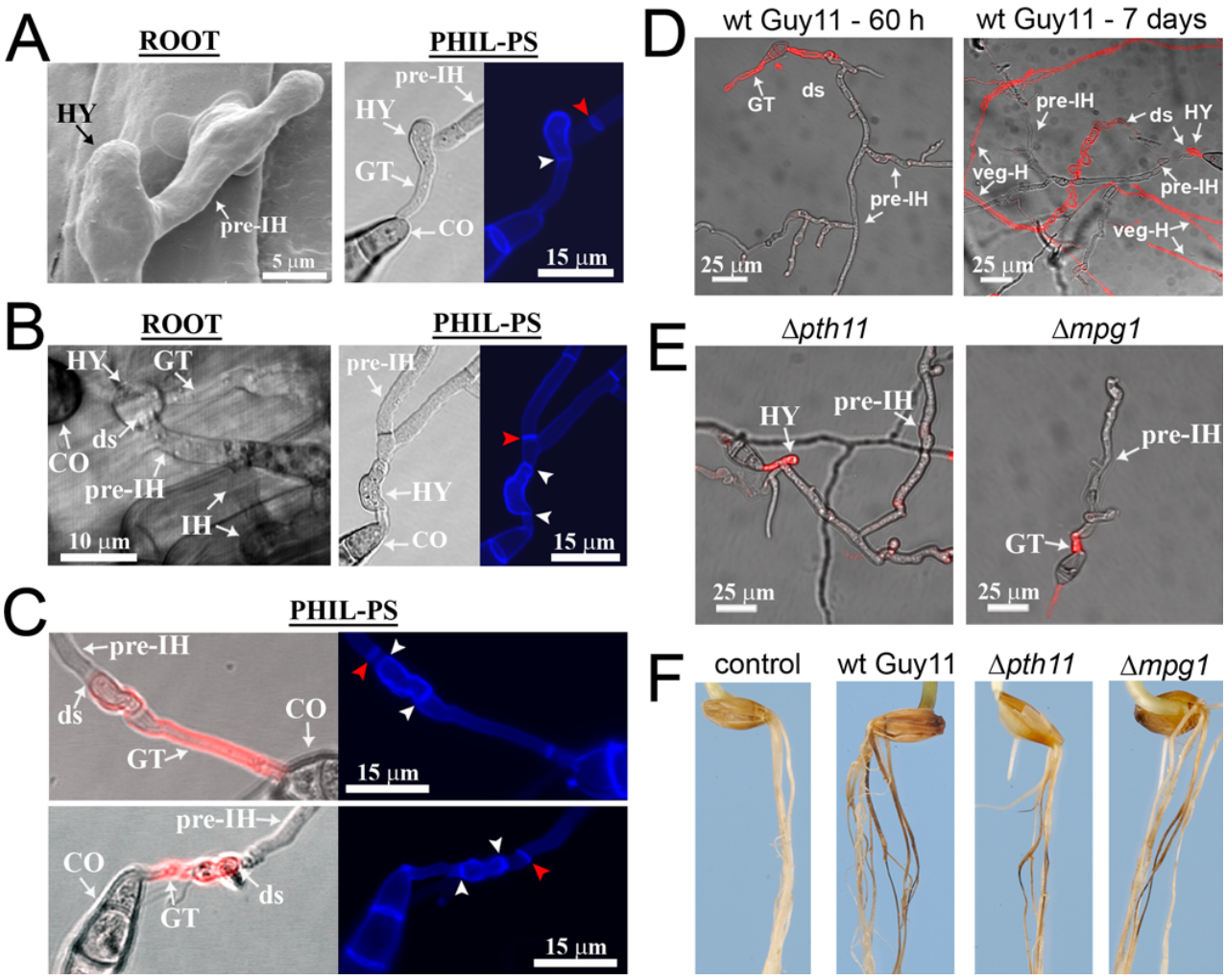
Supplemental Figure 1



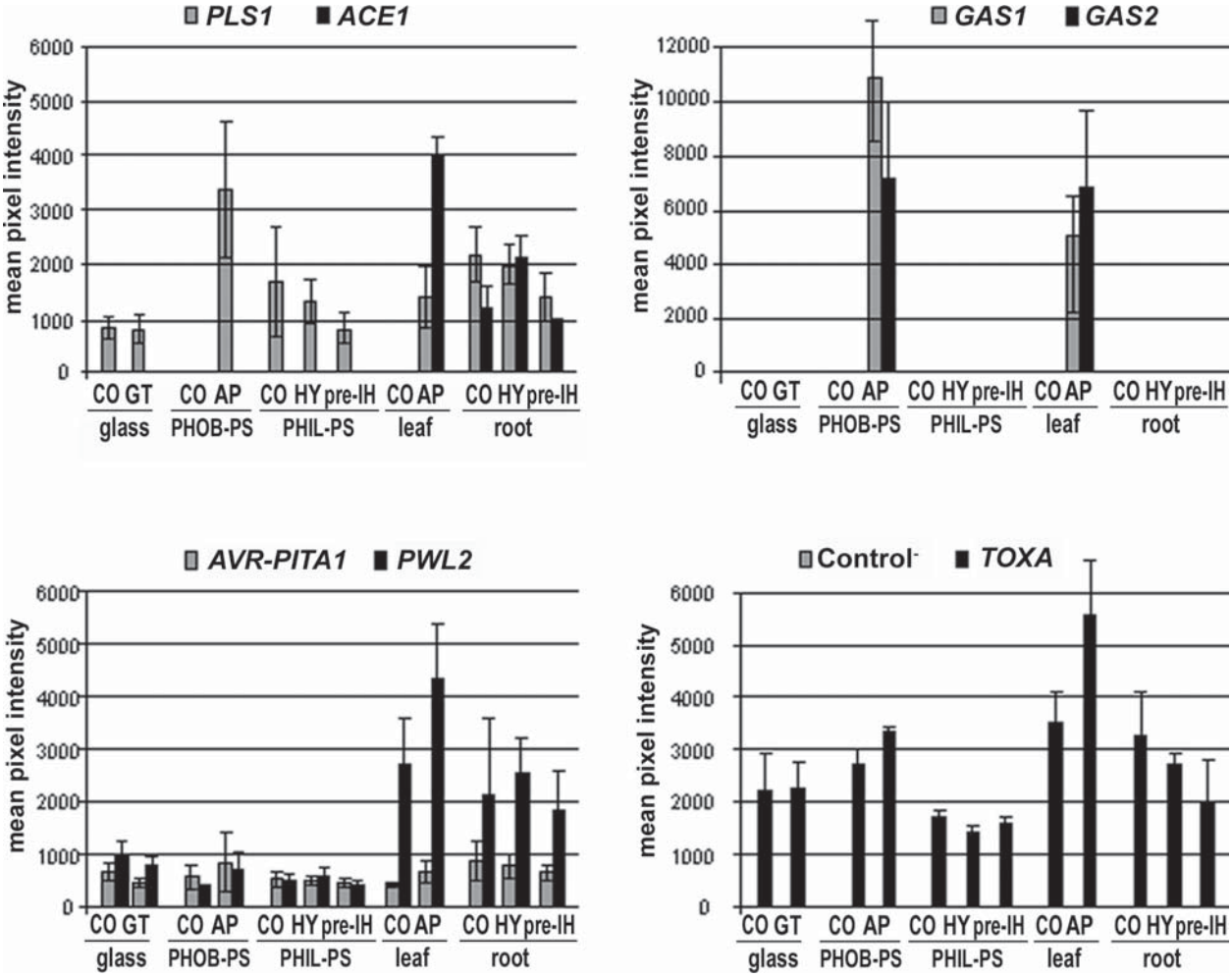
Supplemental Figure 1. Relative fluorescence quantification of carbohydrates and glycoproteins in *M. oryzae* cell wall. Pixel intensity measurements taken from *M. oryzae* germinating conidia at 24 h using **(A)** TRTC-labelled wheat germ agglutinin (WGA, anti-chitin lectin); (**B**) Alexa594-ConA (ConA, anti-mannosyl and anti-glucosyl lectin), or **(C)** anti-chitosan antibodies. Standard errors calculated from three independent experiments. GT: germ tube; pre-IH: pre-invasive hyphae.

Supplemental Figure 2



Supplemental Figure 2. Infection-related development of the wild-type Guy11, Δ*pth11* and Δ*mpg1* mutant strains. (A, B, C) *M. oryzae* Guy11 germinating conidia on roots or on PHIL-PS at 24 h. Germinating conidia (CO) on PHIL-PS were treated with calcofluor white (shown in blue) and TRTC-WGA (shown in red) to visualise fungal cell walls and septa. Calcofluor fluorescence of hyphopodia (HY) cell walls is stronger compared to germ tubes (GT) or pre-IH cell walls, suggesting differences in cell wall composition. **(A)** Pre-IH develops from the bottom of the hyphopodium. On PHIL-PS, the pre-IH forms a septum (red arrowhead) near the hyphopodium. A septum (white arrowhead) at the base of the hyphopodium separates this structure from the germ tube. **(B)** Pre-IH growth is initiated from a side of the hyphopodium. On PHIL-PS, a septum at the beginning and the end of the hyphopodium separates this structure from germ tubes and pre-IH (white arrowheads). Black and white image (left panel) appears in colour in Figure 2A (root)**. (C**) Pre-IH growth initiates from germ tubes developing pre-hyphopodia at their apex. The developmental switch (ds; changes in TRTC-WGA shown in red) occurs in the subcompartment seen at the tip of the germ tube; the strong calcofluor fluorescence suggests that this subcompartment could be a pre-hyphopodium. **(D)** *M. oryzae* Guy11 germinating conidium on PHIL-PS treated with TRTC-WGA after sixty hours or seven days. The red colour indicates fluorescent-labelled WGA (chitin) on the cell wall of germ tubes. Pre-IH cell wall fluorescence remained unaffected after seven days. Vegetative hyphae (veg-H) not in direct contact with the PHIL-PS surface showed strong TRTC-WGA labelling at seven days. **(E)** Germ tubes (GT) of the Δ*pth11* and Δ*mpg1* mutants showing the switch to formation of pre-invasive hyphae (pre-IH) on PHIL-PS. **(F)** Root-infection tests of *M. oryzae* wild type and mutants Δ*pth11* and Δ*mpg1*.

Supplemental Figure 3

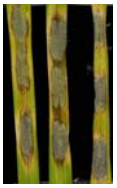
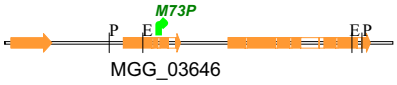
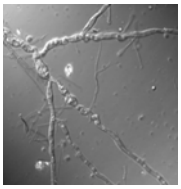
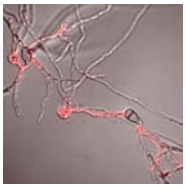
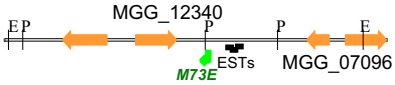


Supplemental Figure 3. Gene-expression analysis of pathogenesis-related genes. Pixel intensity measurements of GFP promoter fusion constructs on germinated conidia at 24 h. Measurements were performed on specific fungal structures. Different scales were used across plots. Standard errors calculated from three independent experiments. CO: conidium; AP: appressorium; HY: hyphopodium; pre-IH: pre-invasive hyphae.

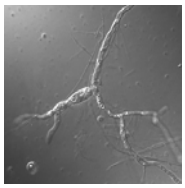
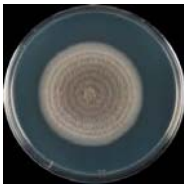
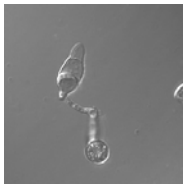
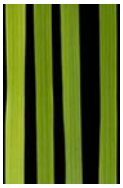
Supplemental Data. Tucker et al. (2010). Plant Cell 10.1105/tpc.109.066340

Supplemental Figure 4. Phenotypes and T-DNA-tagged locations of mutants with altered growth on PHIL-PS.

[a]Green arrows indicate T-DNA insertion
[b]Colony morphology on complete medium (CM)
[c]Morphology of germ tubes and appressoria on PHOB-PS
[d]Morphology of germ tubes and pre-invasive hyphae on PHIL-PS
[e]Infected rice leaves (whole plant infection assays)
[f]Infected barley leaves (detached infection assays - intact leaves)
[g]Infected barley leaves (detached infection assays - wounded leaves/ cuticle was abraded with carborundum powder)
[h]Infected rice roots

Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
Wild-type Guy11 **Intact genome without T-DNA insertion**							
M73a 				No conidia			
M73b 							

Supplemental Data. Tucker et al. (2010). Plant Cell 10.1105/tpc.109.066340

Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M161							
M265							
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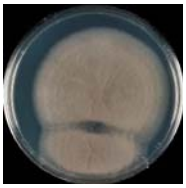
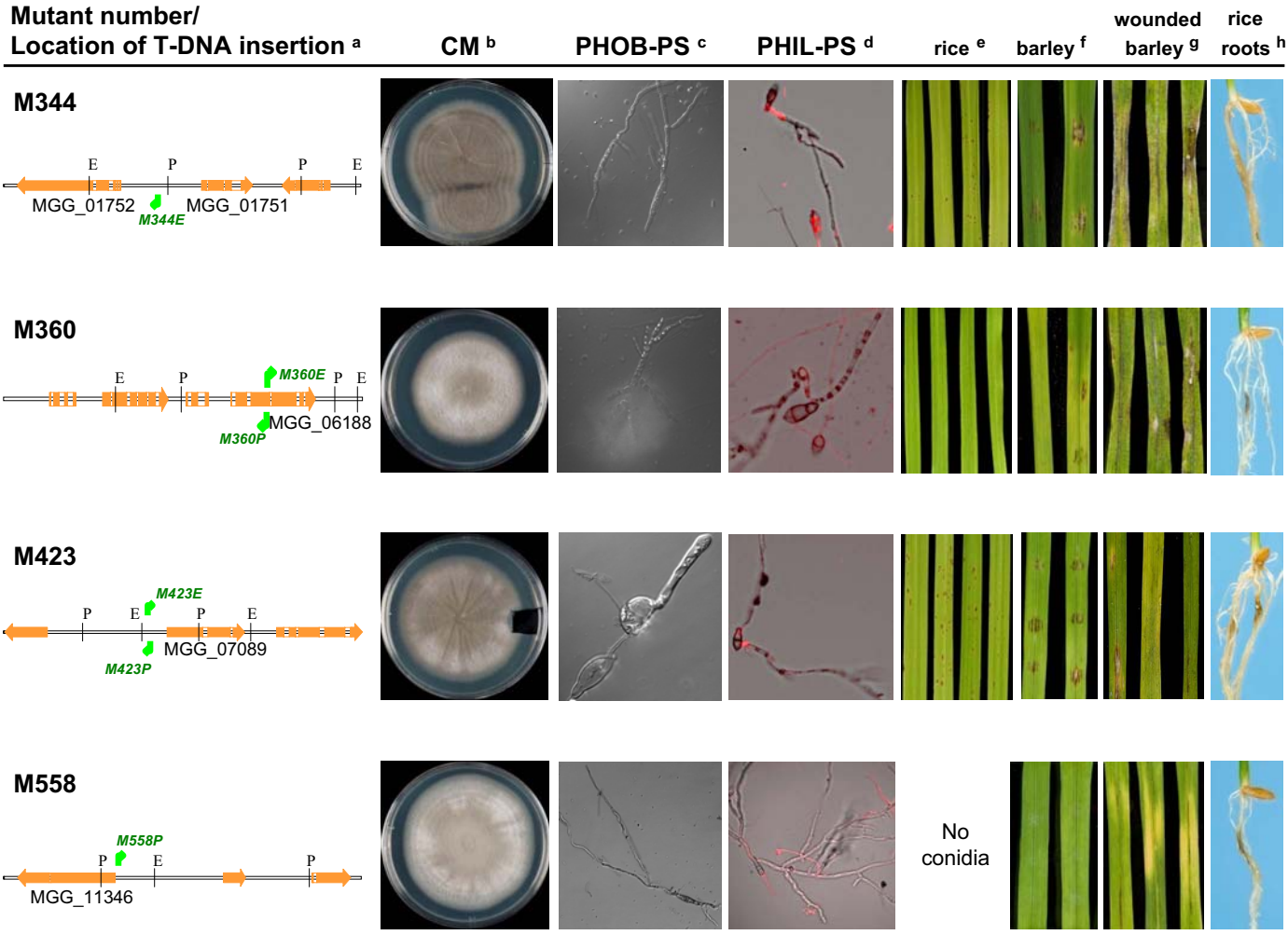
M327							

Supplemental Data. Tucker et al. (2010). Plant Cell 10.1105/tpc.109.066340



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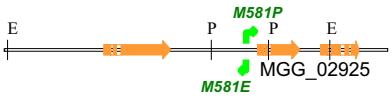
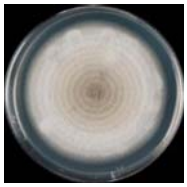
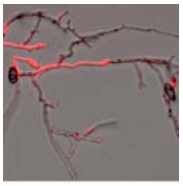
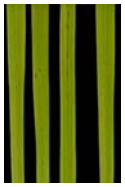
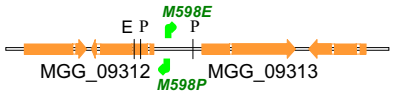
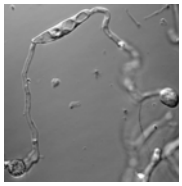
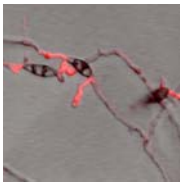
Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M581 							
M598a 							
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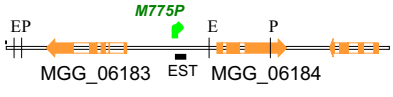
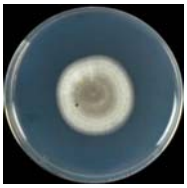
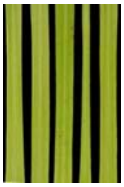
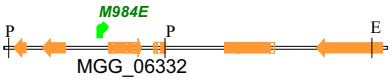
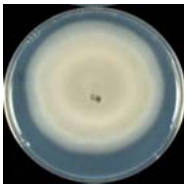
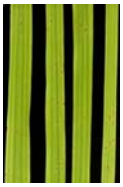
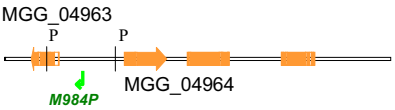
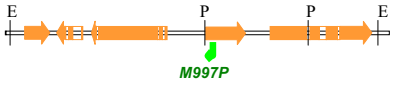
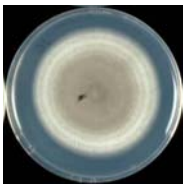
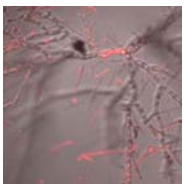
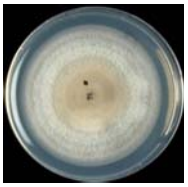
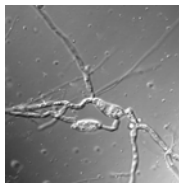
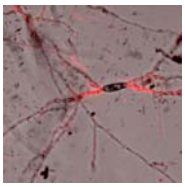
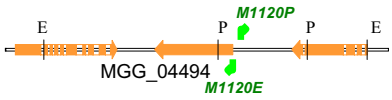
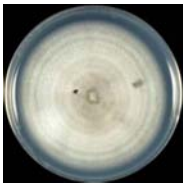
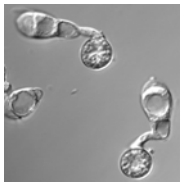
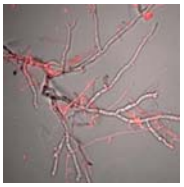
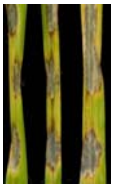
M775 							

Supplemental Data. Tucker et al. (2010). Plant Cell 10.1105/tpc.109.066340

Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M984a 							
M984b 							
M997 							
M1085 **Retrotransposon-like**				No conidia			

Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M1120a 							
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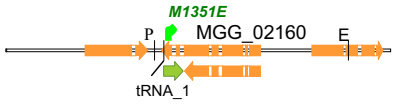
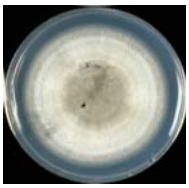
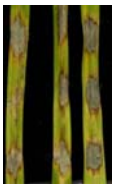
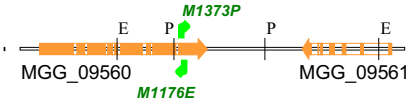
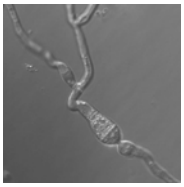
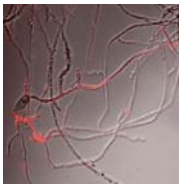
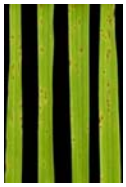
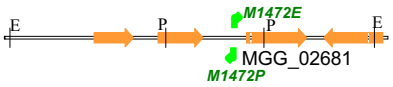
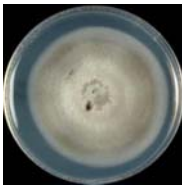
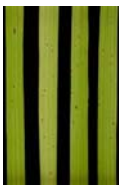
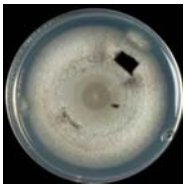
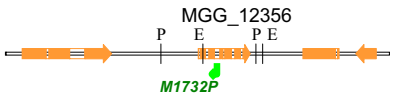
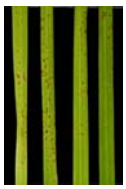
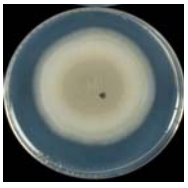
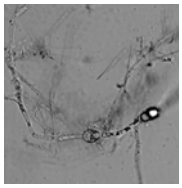
M1351 							
M1373 							

Supplemental Data. Tucker et al. (2010). Plant Cell 10.1105/tpc.109.066340

Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M1472 							
M1623 							
M1732 							
M1854 				No conidia			

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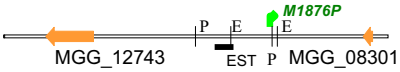
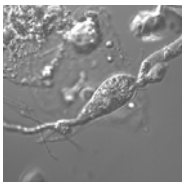
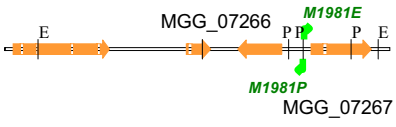
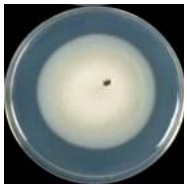
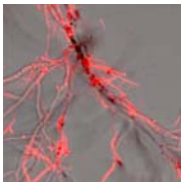
Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M1876 				No conidia			
M1981 				No conidia			
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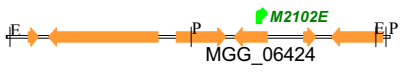
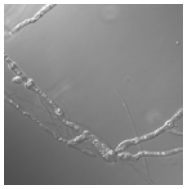
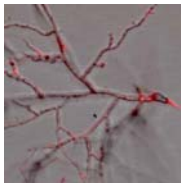
M2102 				No conidia			

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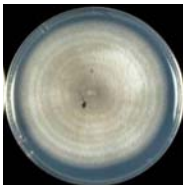
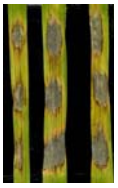
Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M2138a							
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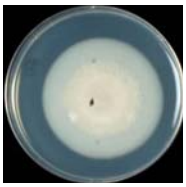
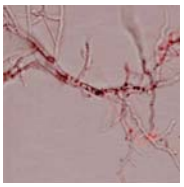
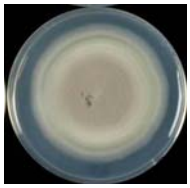
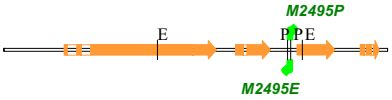
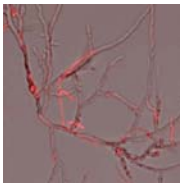
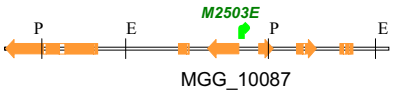
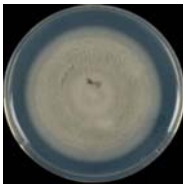
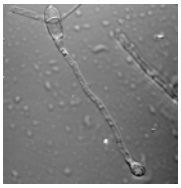
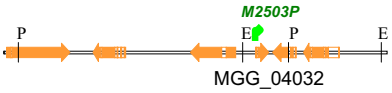
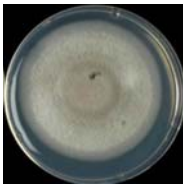
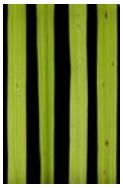
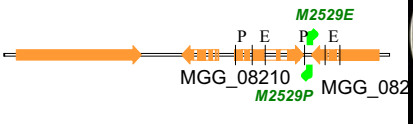
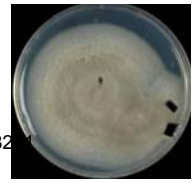
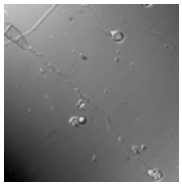
M2140				No conidia			
M2381				No conidia			

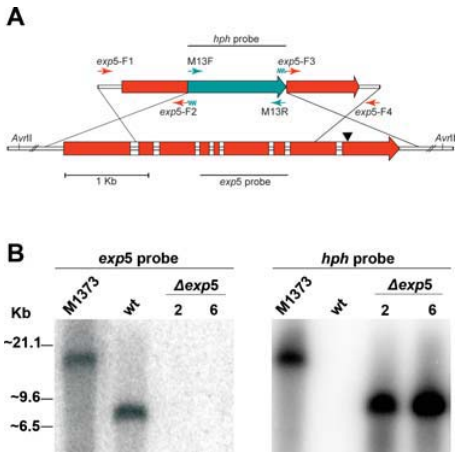
Supplemental Data. Tucker et al. (2010). Plant Cell 10.1105/tpc.109.066340

Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M2495 				No conidia			
M2503a 							
M2503b 							
M2524 							

Supplemental Data. Tucker et al. (2010). Plant Cell 10.1105/tpc.109.066340

Mutant number/ Location of T-DNA insertion [a]	CM [b]	PHOB-PS [c]	PHIL-PS [d]	rice [e]	barley [f]	wounded barley [g]	rice roots [h]
M2529 							

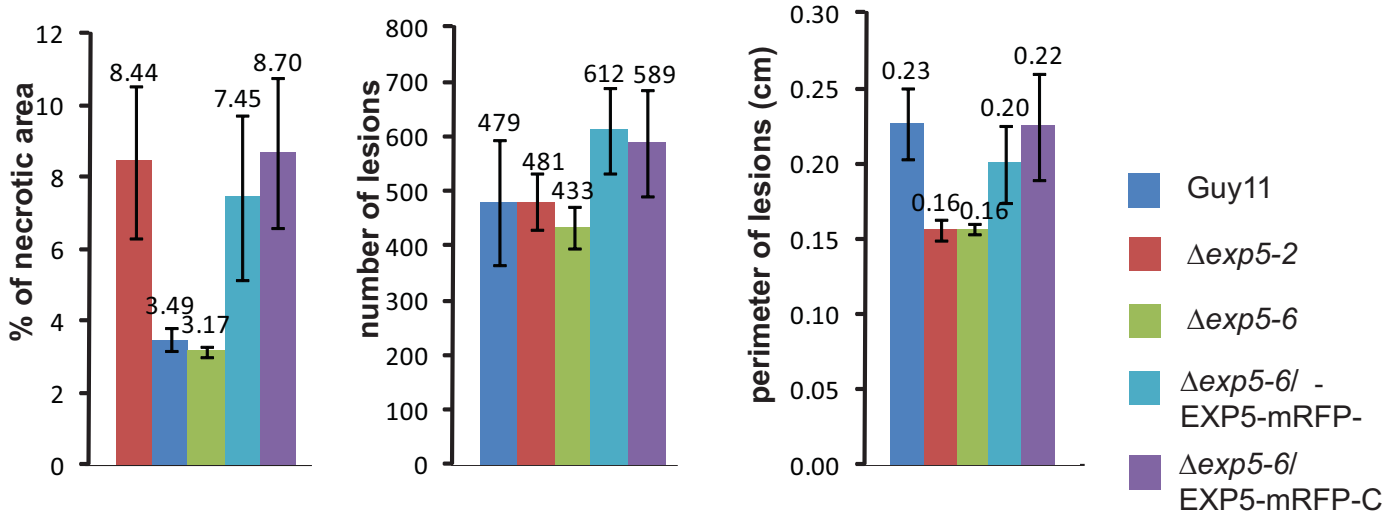
Supplemental Figure 5



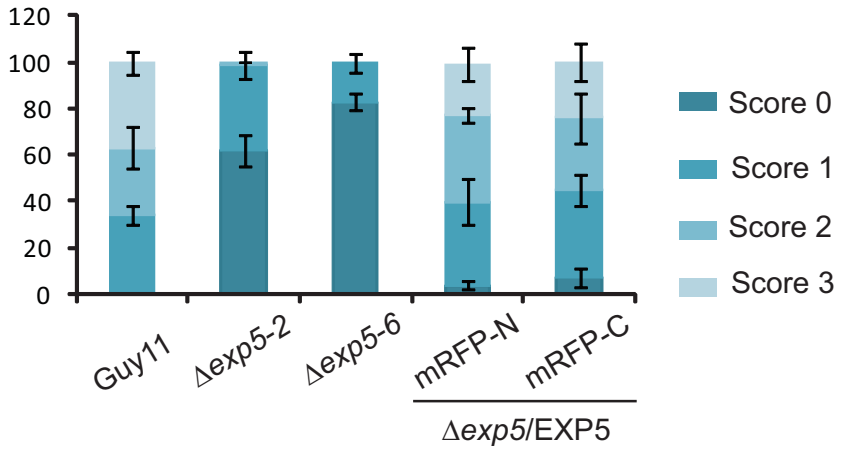
Supplemental Figure 5. Replacement of the *M. oryzae EXP5* gene. (A) Schematic diagram showing the targeted gene deletion strategy. **(B)** Southern Blot analysis of wild type (wt) Guy11 and two independent Δ*exp5* mutant strains. The 6,265 bp band is visible in *Avr*II-digested wt DNA using the *EXP5* gene as a probe (region deleted in the Δ*exp5* mutants). A single band of the expected size is visible in both deletion strains using the 1.6 Kb hygromycin phosphotransferase gene (*hph*) as a probe.

Supplemental Figure 6

A. Leaf infection assays



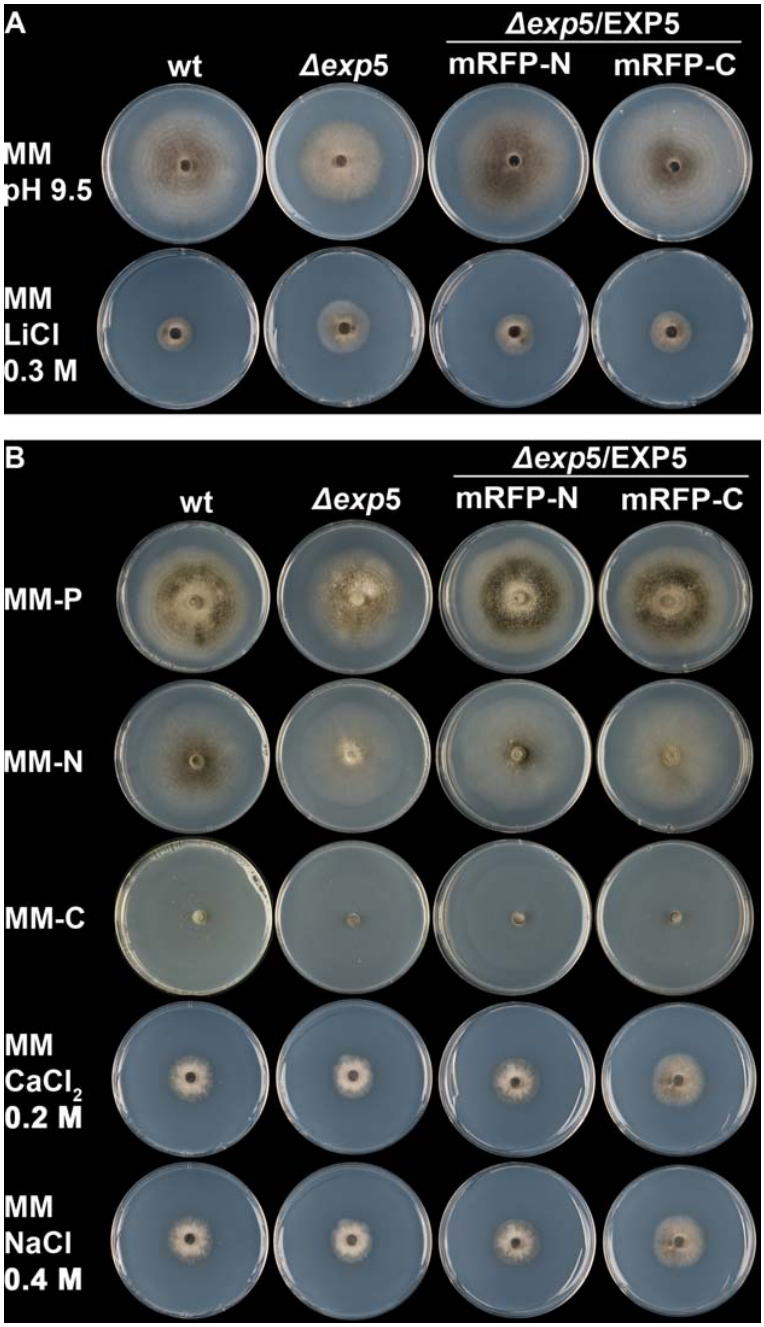
B. Root infection assays



		scored roots/total roots examined (3 experiments)				mean percentage±SE			
	PS-PHIL	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
	Guy11	0/100	34/100	29/100	37/100	0	34±4	29±9	37±5
	Δexp5-2	52/85	32/85	1/85	0/85	62±7	37±6	1	0
	Δexp5-6	77/92	15/92	0/92	0/92	83±4	17±4	0	0
***Δexp5-6*/EXP5-mRFP-N**		4/94	34/94	35/94	21/94	4±2	36±10	37±3	22±7
***Δexp5-6*/EXP5-mRFP-C**		7/95	36/95	30/95	22/95	7±4	38±7	31±11	24±8

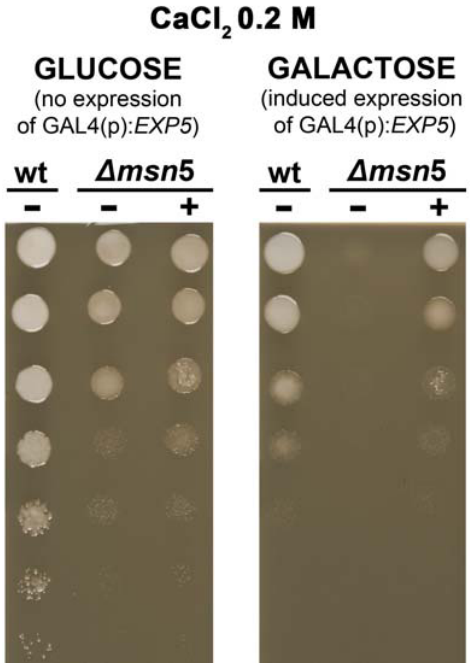
Supplemental Figure 6. Quantification of disease symptoms produced by *M. oryzae* wild type strain Guy11, Δ*exp5-2* and Δ*exp5-6* mutants, and Δ*exp5-6* complemented with EXP5-mRFP-N and EXP5-mRFP-C protein fusions.(A) Whole plant infection assays. Three pots of 10 plants were used per strain and per experiment; standard errors were calculated from three independent experiments. Pictures of all infected leaves were analysed using the image analysis software for disease quantification Assess 2.0 (The American Phytothological Society). **(B)** Scoring of disease symptoms on roots. The table shows total numbers and mean percentages of infected roots with different lesion severity (scoring system: 0 - no symptoms; 1 - strong reduction; 2 - weak reduction; 3 - wild type symptoms). Values used in the chart represent the mean percentage of three experiments (error bars represent standard errors).

Supplemental Figure 7



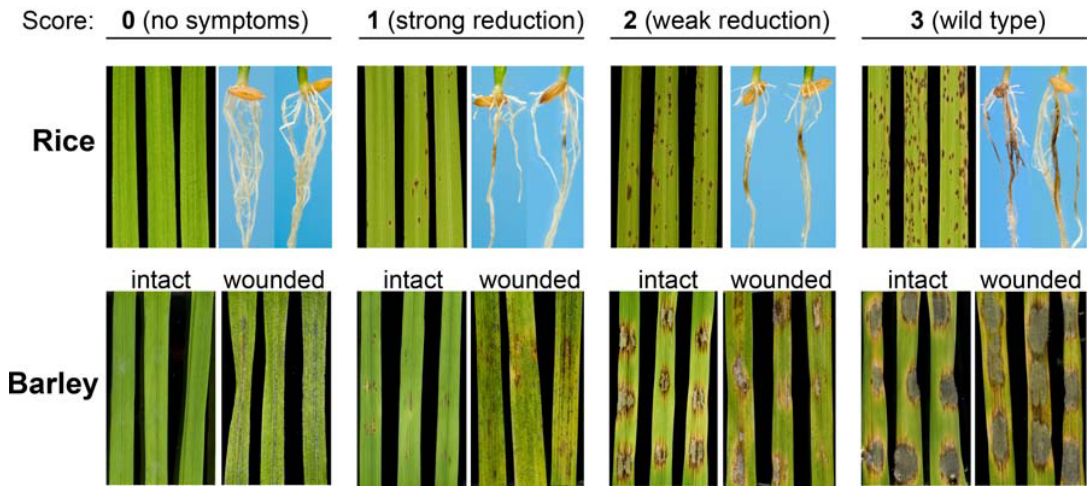
Supplemental Figure 7. **Colony morphology of the wild type, *Δexp*5 and *Δexp*5/EXP5-mRFP strains on various stress-related conditions.** Cultures were incubated for 10 days at 25°C with a 16 h photoperiod. **(A)** Wild type, *Δexp*5, *Δexp*5/EXP5-mRFP-N, and *Δexp*5/EXP5-mRFP-C strains on Minimal Medium (MM) with pH adjusted to 9.5 using NaOH or supplemented with LiCl 0.3 M. **(B)** The same strains on MM without phosphate (MM-P), nitrogen (MM-N) and carbon (MM-C), or MM supplemented with $CaCl_2$ 0.2 M and NaCl 0.4 M.

Supplemental Figure 8



Supplemental Figure 8. Sensitivity of the *S. cerevisiae* Δ*msn*5 mutant to calcium chloride is restored by the *M. oryzae EXP5* gene coding sequence. Ten µl of serial fivefold dilutions from exponentially growing cells in SD minus uracil were spotted on YP/glucose and YP/galactose supplemented with 0.2 M $CaCl_2$. Plates were incubated at 30° C for 2 days. Strains labeled with a minus symbol (-) were transformed with the empty vector, while strains labeled with a plus symbol (+) were transformed with a plasmid containing the *M. oryzae EXP5* cDNA driven by the GAL4 promoter. Reduced growth observed in the wild type strain in the right-hand panel is due to galactose rather than glucose (the preferred substrate) being used as sole carbon source. The *Δmsn*5 mutant shows higher sensitivity to $CaCl_2$ in galactose-containing medium (Alepuz et al., 1999). Under non-inducing conditions (YP/glucose), both *Δmsn*5 (-) and *Δmsn*5 (+) show sensitivity to $CaCl_2$. Under inducing conditions (YP/galactose), *Δmsn*5 (-) fails to grow, while *Δmsn5* (+) grows similarly to the wild type strain.

Supplemental Figure 9



Supplemental Figure 9. Scoring system of rice blast disease symptoms. Visible symptoms observed 5 days after spray inoculation on whole rice plants cv. CO39 or 4 days after drop inoculation on detached leaves of barley cv. Golden Promise (intact or wounded) with *M. oryzae* conidial suspensions. Root infections were carried out with *M. oryzae* mycelial plugs and disease symptoms scored after 15 days. Lesions observed on leaves and roots were scored as 0 (non-pathogenic), 1 (strong symptom reduction), 2 (weak symptom reduction) or 3 (wild-type symptoms) based on colour intensity (for roots), lesion number (for leaves) and lesion extension (for both) of disease symptoms.

Supplemental Table 1. Genes and mutants used in this study.

genes		mutant phenotype	invasive growth*	expression	references
PMK1	MGG_09565; mitogen-activated protein kinase	no appressoria; non-pathogenic	defective	constitutively expressed at low levels in vegetative hyphae, conida and germ tubes; induced expression during appressorium formation and developing conidia	(Xu and Hamer, 1996; Bruno et al., 2004)
MST12	MGG_12958; transcription factor	wild type appressoria;non-pathogenic; defective in microtubule reorganization associated with penetration peg formation	defective	ND	(Park et al., 2002; Park et al., 2004)
CPKA	MGG_06368; catalytic subunit of cAMP-dependent protein kinase A	delayed, smaller and non-fuctional appressoria; reduced virulence; delayed glycogen and lipid mobilization required for turgor generation	wild type	ND	(Xu et al., 1997; Thines et al., 2000)
PTH11	MGG_05871; CFEM-containing G-protein coupled receptor	reduced appressoria; reduced virulence; upstream effector of appressoria differentiation in response to surface cues	wild type	GFP-fusion protein localised in cell membrane and vacuoles during conidia germination and appressoria formation	(DeZwaan et al., 1999)
MPG1	MGG_10315; hydrophobin	reduced conidiation and appressoria; reduced virulence	ND	expressed at early (12 h) and late (72-96 h) time points of infection. Its expression increases during nitrogen and glucose starvation; expression observed during development of aerial hyphae, conidia and appressoria	(Talbot et al., 1993; Kershaw et al., 1998)
CBP1	MGG_12939; putative chitin deacetylase	no appressoria on PHOB; fully pathogenic; involved in recognition of physical factors on artificial hydrophobic surfaces	ND; probably wild type	expressed in germ tubes and repressed completely in vegetative mycelium	(Kamakura et al., 2002)
AVR-Pita1	MGG_11081; fungal effector; metalloprotease	wild type appressoria; *M. oryzae* strains expressing *AVR-Pita1* are non-pathogenic on rice cultivars carrying *Pi-ta* resistant gene	ND	expressed in invasive hyphae at 24 to 32 hr after inoculation and later stages of growth inside the host	(Jia et al., 2000; Orbach et al., 2000)
PWL2	MGG_04301; fungal effector	wild type appressoria; expression of PWL2 on *M. oryzae* rice isolates confers non-pathogenic phenotype toward weeping lovegrass without altering pathogenicity toward rice and barley	ND	specifically expressed during penetration, fungal colonization, and late infection {Fudal, 2007 #1284}	(Sweigard et al., 1995)
GAS1= MAS3	MGG_12337; virulence factor	wild type appressoria; reduced virulence; possibly involved in penetration peg formation and/or function	ND	highly expressed during appressorium formation; expression regulated by PMK1; GAS1 is found preferentially in the vacuole of appressoria; not expressed in invasive hyphae	(Xue et al., 2002)
GAS2= MAS1	MGG_04202; virulence factor	wild type appressoria; reduced virulence; possibly involved in penetration peg formation and/or function	ND	highly expressed during appressorium formation; expression regulated by PMK1; GAS2 is localized in the cytoplasm; not expressed in invasive hyphae	(Xue et al., 2002)
ACE1	MGG_12447; hybrid polyketide synthase/non ribosomal peptide synthetase (PKS-NRPS)	wild type appressoria; the secondary metabolite synthesised by ACE1 confers non-pathogenic phenotype towards rice cultivars carrying *Pi33* resistant gene	ND	expressed in appressoria during fungal penetration; expression is connected to developmental programme that mediates appressorium penetration and does not required host plant signals; expression also detected in infectious hyphae and rapidly disappeared once invasive hyphae began to spread within leaf tissues (~48 h)	(Bohnert et al., 2004; Fudal et al., 2007)
PLS1	MGG_12594; tetraspanin	wild type appressoria; unable to differentiate penetration pegs; non-pathogenic	defective	expressed at similar levels in mycelia, perithecia, spores, appressoria and infected barley leaves; GFP fusion protein localised only in plasma membranes and vacuoles within appressoria	(Clergeot et al., 2001; Lambou et al., 2008)

*: determined by experiments on wounded leaves; ND: not determined

Supplemental Table 2 . Numbers of conidial germ tubes (GT) of *M. oryzae* wild type strain Guy11 and *Δpmk1/Δmst12* mutants developing pre-IH on PHIL-PS and root surfaces.

	conidial GT with pre-IH/total conidia			mean ± SD (three experiments)		
PS-PHIL	**12 hrs**	**24 hrs**	**36 hrs**	**12 hrs**	**24 hrs**	**36 hrs**
Guy11	394/600	462/600	552/600	65.7±6.7	77±9.2	92±2.0
Δmst12	0/600	102/600	176/600	0.0	17±2.0	29.3±2.5
Δpmk1	0/600	4/600	4/600	0.0	0.7±0.6	0.7±1.1

	conidial GT with pre-IH/total conidia			mean ± SD (three experiments)		
ROOTS	**12 hrs**	**24 hrs**	**36 hrs**	**12 hrs**	**24 hrs**	**36 hrs**
Guy11	51/65	66/78	40/43	78.3±3.1	85±4.0	92.7±1.5
Δmst12	0/41	11/72	27/81	0.0	14.7±2.1	33.3±3.5
Δpmk1	0/46	0/43	0/61	0.0	0.0	0.0

Supplemental Table 3. The karyopherin protein family in *M. oryzae*, *S. cerevisiae* and humans. The homology (e value) of *M. oryzae* karyopherins with their corresponding yeast and human protein orthologues is shown. *:essential genes; blue: karyopherins involved in import; orange: karyopherins involved in export; purple: karyopherins that function in both import and export. The function of Kap120 has not yet been determined. Fourteen karyopherins are present in the *M. oryzae* genome; MGG_02927 is the only karyopherin that is closely related to two different yeast karyopherins.

M. oryzae	**Yeast**	**Human**
MGG_09208	kap104; e-85	TNPO1; e-161
MGG_00475	kap122(=Pdr6); e-21	IPO13; e-38
MGG_03668	kap95; e-172	KPNB1; e-152
MGG_05869	kap123; e-106	IPO4; e-45
MGG_03537	kap121*(=Pse1); e 0.0	IPO5; e-175
MGG_01449	kap111(=Mtr10); e-95	TNPO3; e-57
MGG_15072	kap60(kapα=Srp1=Scm1); e-157	KPNA6; e-159
MGG_02927	kap108/kap119 ; e-89/e-119	IPO7/IPO8; e-115/e-103
MGG_11165	kap114; e-59	IPO9; e-61
MGG_00744	kap120; e-71	IPO11; e-78
MGG_09560-***EXP5***	kap142(=Msn5); e-106	exportin-5; XPO5; e-15
MGG_02526	kap124*(=Crm1=Xpo1); e 0.0	XPO1; e-06
MGG_03994	Cse1*(=kapα); e-176	CSE1L; e-127
MGG_10127	Los1; e-66	XPOT; e-87

Supplemental Table 4. Primers used in this study.

Primer name	Sequence
General primers	
2SKF-KpnI	AAAGGTACCAGGGAATAAGGGCGACACGGA
2SKR-KpnI	TATGGTACCTCGCCCTTCCCAACAGTTGCG
M13F	CGCCAGGGTTTTCCCAGTCACGAC
M13R	AGCGGATAACAATTTCACACAGGA
T3	AAATTAACCCTCACTAAAGGGA
AT-RB	GATTGTCGTTTCCCGCCTTCAG
AT-LB2	CCAGTACTAAAATCCAGATCCC
Primers for *EXP5* gene deletion	
exp5-F1	CGCCTTGCTTACACCATTCG
exp5-F2	GTCGTGACTGGGAAAACCCTGGCGAATAATGATGGATGACTCGG
exp5-F3	TCCTGTGTGAAATTGTTATCCGCTATCTGGTCAGGTATCTTGTC
exp5-F4	CTACAGTCCGAAACACCCGC
Primers for EXP5 localisation	
exp5-GW1-3F	GGGGACAACTTTGTATAGAAAAGTTGCTTGGTGAGCCTGCGAGTGGTG
exp5-GW2F	GGGGACAGCTTTCTTGTACAAAGTGGCTATGGCAGCCCTTCCCTCTAA
exp5-GW4F	GGGGACAGCTTTCTTGTACAAAGTGGCTGGCTAGGCGAAGAAGTGCG
exp5-GW1R	GGGGACTGCTTTTTTGTACAAACTTGCGGCGGCCACAAGGACTCG
exp5-GW2-4R	GGGGACAACTTTGTATAATAAAGTTGCTAACGACAGGCATCTTGAAAGGG
exp5-GW3R	GGGGACTGCTTTTTTGTACAAACTTGCTTGTCCGTCAAACAGGTTGGCAAC
Primers for yeast complementation experiments	
EXPrY-F	TATGGTACCATGGCAGCCCTTCCCTCTAA
EXPrY-R	AAAGCGGCCGCCTATTGTCCGTCAAACAGGTTGGC
Primers used for Southern and Northern blot hybridisation with *EXP5* probe	
exp5-S2	GCTACAACAAGAACTCAATGG
exp5-P1	TAGTGTTAGCAAATCGGAGC
Promoter-GFP fusion construct primers	
Nco-5	GCTGTTCTCCAGCCGGTCGCGGAGGC***G***ATG
Nco-3'	CAT***C***GCCTCCGCGACCGGCTGGAGAACAGC
CT74-6	CTTCGGAACCGACTCGATCCTC
5Pr-Gas1	GTCCTATCGATGGACGAATGCTCCAGAAACCAGC
3Pr-Gas1	GTCCT**CCATGG**TTGAAAGAGAAGTGGGGGTGGGT
5Pr-Gas2	GTCCTATCGATGATGATGGCACCTTGGGATGGAGG
3Pr-Gas2	GCTAC**CCATGG**TTGGCGGTTTGAAATTTGTTTTCT
5Pr-PWL2	GTCCTATCGATGGTTACAAACGACCGACAGCTCC
3Pr-PWL2	GTCCT**CCATGG**TTGAAAGTTTTTAATTTTAAAAAGAGATT
5Pr- AVR-Pita	GTCCTATCGATTTGTAAATTTCAAAAGTCAGGGA
3Pr- AVR-Pita	GCTTC**CCATGG**ATTGCAAAAATAATGTTAATTGTGC