Calpains are involved in asexual and sexual development, cell wall integrity and pathogenicity of the rice blast fungus

Xiao-Hong Liu, Guo-Ao Ning, Lu-Yao Huang, Ya-Hui Zhao, Bo Dong, Jian-Ping Lu, Fu-Cheng Lin

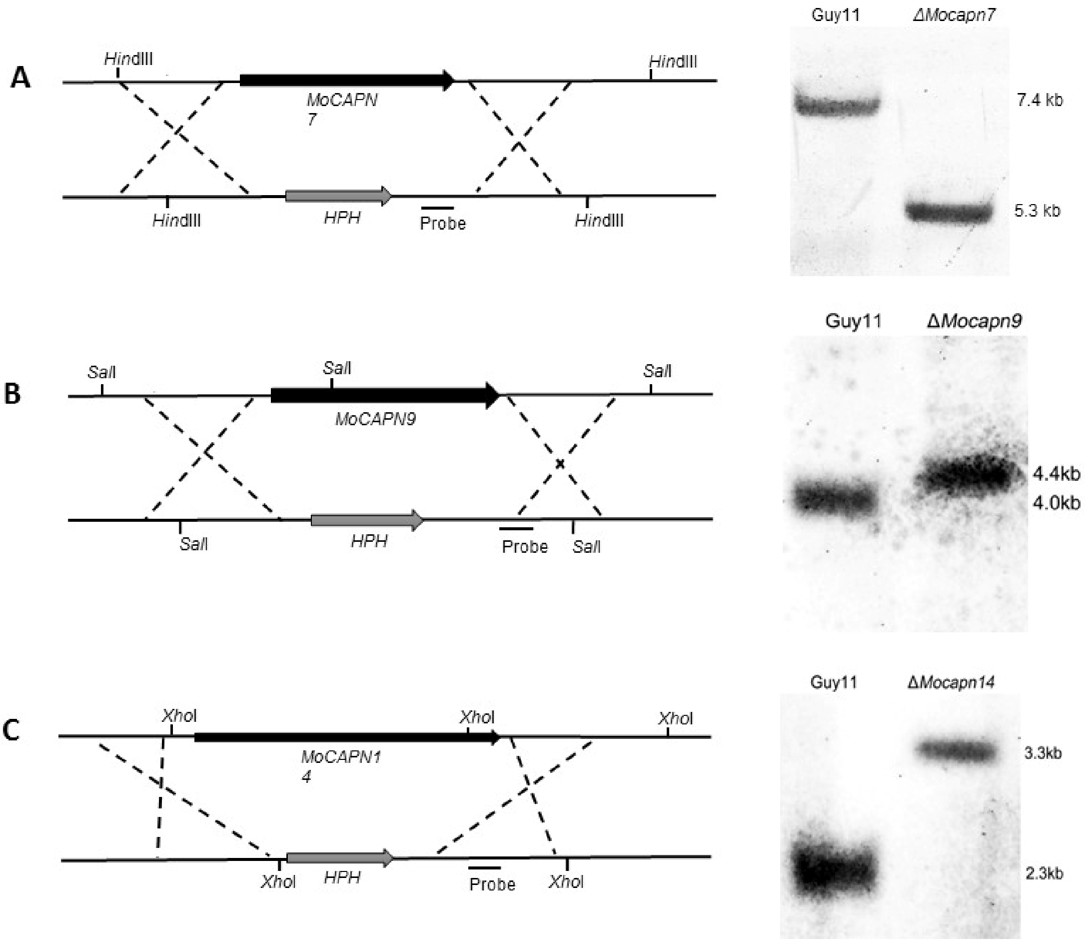

Figure S1 Targeted gene replacement of *MoCAPN7*, *MoCAPN9* and *MoCAPN14* and Southern blot analysis of the mutants. The gene deletion vectors were constructed using pKO1B as described in the Experimental procedures. The mutants were verified by Southern blot analysis. Restriction enzymes and probes for the Southern blot are labeled in the diagram of the gene replacement vectors. (A) *Hin*dIII-digested genomic DNA was hybridized with the 0.5 kb probe amplified with primers MGG_06335probe-F/R. A single band was shifted from 7.4 kb in Guy11 to 5.3 kb in Δ*Mocapn7*. A similar strategy was performed to verify deletion of *MoCAPN9* (B) and *MoCAPN14* (C).

Table S1 Identification of calpains in *M. oryzae*.

Calpains in *Homo sapiens*	**Homologues of calpains in *M. oryzae***	**Designated Name in *M. oryzae***	**Predicted Number of Amino Acids/Mass[a]**	**Sequence Identity/Similarity With Human Calpains (%)**	**Domains (Start-End)[b]**	**E-value[b]**	**Reference**
Calpain1	MGG_15810	MoCapn1	1223/135.14	34/47	Cyspc (165-502)	8.98e-48	Khan *et al.*, (2014)
					PepC2 (173-292)	3.9e-20	
					PepC2 (310-490)	5.7e-47	
Calpain2	MGG_08526	MoCapn2	575/65.16	23/41	Cyspc (205-574)	1e-165	this study[c]
					PepC2 (363-574)	2.4e-13	
Calpain3	MGG_14872	MoCapn3	798/90.4	30/42	Cyspc (98-148)	9.72e-44	Khan *et al.*, (2014)
					PepC2 (98-407)	7.9e-69	
Calpain4	MGG_04818	MoCapn4	257/29.52	33/53	EFh (91-119)	2e-10	Khan *et al.*, (2014)
					EFh (128-156)	6e-12	
					EFh (158-186)	4e-11	
					EFh (194-222)	1e-9	
					EFh (224-251)	7e-10	
Calpain7	MGG_06335	MoCapn7/PalB	899/98.21	28/43	Cyspc (98-443)	1.89e-55	this study
					CalpainIII (594-727)	3.2e-9	
					PepC2 (115-443)	1.7e-32	
Calpain9	MGG_01072	MoCapn9	678/73.26	34/59	EFh (24-52)	3e-11	this study
					EFh (62-90)	1e-10	
					EFh (93-121)	4e-12	
					EFh (129-157)	1e-13	

					Mito_carr (357-468)	7.8e-20	
					Mito_carr (475-571)	5.9e-22	
					Mito_carr (583-677)	2.4e-26	
Calpain14	MGG_07573	MoCapn14	1060/118.39	32/44	Cyspc (116-482)	1.98e-26	this study
					PepC2 (159-472)	1.3e-58	
Calpain1	MGG_16201	MoCapn1B	150/17	27/52	EFh (12-40)	5.96-e	this study[c]
					EFh (48-76)	1.19-e	
Calpain3				26/46	EFh (85-113)	1.37-e	
					EFh (121-149)	6.39-e	

a. Mass is in kilo-daltons (KD).
b. The domains and the E-value was predicted using simple modular architecture research tool (SMART).
c. The null mutant was not got in this study.

Table S2 List of primers for the flank fragments of the knockout vectors

Name	**Sequence(5’-3’)**
MGG_06335upF	GCTGTACAAGTAAGAGCTCGGTACCCGGGGATCGAGCGATGGAGACATGATTAA
MGG_06335upR	ACCAAAATAGGCATTCATTGTTGACCTCCACTAAAAGCACCCAAAGAAGAGTAG
MGG_06335downF	CACTCGTCCGAGGGCAAAGGAATAGAGTAGATGTTCTGTTGAGATGGCTTGTTT
MGG_06335downR	TTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCAGGCATGGGCATGGTAATAGTC
MGG_07573upF	GCTGTACAAGTAAGAGCTCGGTACCCGGGGATCTAAGACGCAGCGGGCTTGGAG
MGG_07573upR	ACCAAAATAGGCATTCATTGTTGACCTCCACTAATGGGCGGGAGAAATTAGGAA
MGG_07573downF	CACTCGTCCGAGGGCAAAGGAATAGAGTAGATGAAGATCGCTCGTTAAGACCCG
MGG07573downR	TTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCATGACACTGTTTGCATCCTCGT
MGG_01072up1F	GCTGTACAAGTAAGAGCTCGGTACCCGGGGATCACAAGGGTTGGGTTCGTG
MGG_01072up1R	CCAAAAAGTGCTCCTTCAATATCATCTTCTCGGATTACTGCGGGTTCT
MGG_01072down1F	CCGTCCGGTCCTGCCCGTCACCGAGATTTAGAGTTATACGGCTACTATGACCT
MGG_01072down1R	TTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCAAGTGCCACAGAAGCAATAC
MGG_04818upF	GCTGTACAAGTAAGAGCTCGGTACCCGGGGATCTTAGCATACCCATAACCAAA
MGG_04818upR	CCAAAAAGTGCTCCTTCAATATCATCTTCTAATAAGAAGTAGAAACCGAGAC
MGG_04818downF	CCGTCCGGTCCTGCCCGTCACCGAGATTTAGTGGCGACCATTTCAAGAC
MGG_04818downR	TTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCAGCTCGGTGGCAACATCCT
MGG_01072upF	GCTGTACAAGTAAGAGCTCGGTACCCGGGGATCCCAGGACGTGGTCGTCATTAG
MGG_01072upR	ACCAAAATAGGCATTCATTGTTGACCTCCACTAAGTTGTCGCAGGTAGGTAGGC

MGG_01072downF	CACTCGTCCGAGGGCAAAGGAATAGAGTAGATGGGCTCGTTCTCCCATTGTAGT
MGG_01072downR	TTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACCTCGACGCACCGCCAAAGTA
MGG_16201upF	GCTGTACAAGTAAGAGCTCGGTACCCGGGGATCGCGGGAGAAGTAGTGGAGTTT
MGG_16201upR	ACCAAAATAGGCATTCATTGTTGACCTCCACTATTCGTCTTGTTGGGATGGTTG
MGG_16201downF	CACTCGTCCGAGGGCAAAGGAATAGAGTAGATGGGCGGTTCTTTGATGCCTTTA
MGG_16201downR	TTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCATACCTTTCTGGGCGTTTCTGC
MGG_08526upF	GGTACCCGGGGATCCTCTAGAGTGTTCCTTTGCCCAGTG
MGG_08526upR	CTCCTTCAATATCATCTTCTCTTTCGTCTTTGCCTTCT
MGG_08526downF	TGCCCGTCACCGAGATTTAGGTTGATGGATGGTATTTGGGTA
MGG_08526downR	ACGACGGCCAGTGCCAAGCTTGGGTTTGTTCTGGGCTGAC
HPHF	TAGTGGAGGTCAACAATGAAT
HPHR	CATCTACTCTATTCCTTTGCC
BARKOF	AGAAGATGATATTGAAGGAGCA
BARKOR	CTAAATCTCGGTGACGGGCA

Table S3 List of primers for identification of the gene-deleted mutants

Name	**Sequence(5’-3’)**
MGG_06335up-hphF	AGAGCGTAGCATTGACCTTGA
MGG_06335up-hphR	ACGGTGGATGAACCATTGACT
MGG_06335innerF	CTGGGCAGTTAGCATCGT
MGG_06335innerR	TGGAGCAGCAACATAGGC
MGG_07573up-hphF	ATGTTATTGTTGTCGTCGTTG
MGG_07573up-hphR	CTACTCTATTCCTTTGCCCTC
MGG_07573innerF	TCGCACGCAAACTACCTC
MGG_07573innerR	CGTCCGTCTTCGCATCTT
MGG_01072inner1F	AGGAATGGCGGTAGGTTG
MGG_01072inner1R	GTTTGGCATTTGTGAGGC
MGG_01072up-barF	ATACAGCAGTGGCAAGTCG
MGG_01072uP-barR	TGAATCTGAAATAAAGGGAGG
MGG_04818innerF	CGAGCCCGAATCAGTAAG
MGG_04818innerR	GAAACCAATGGTGCCAGA
MGG_04818up-barF	AAGGTGGTTGGTTAGTGGT
MGG_04818up-barR	ATCTGAAATAAAGGGAGGAA
MGG_01072up-hphF	AGGCAGGACACGCTCTATTCA
MGG_01072up-hphR	GACAAACGCACCAAGTTATCG
MGG_01072innerF	AGGAATGGCGGTAGGTTG
MGG_01072inner	GTTTGGCATTTGTGAGGC
MGG_16201up-hphF	TTGACGGTTCGGTAAAGTTGT
MGG_16201up-hphR	CAGTTCGGTTTCAGGCAGGTC
MGG_16201innerF	ATGGTTCCCACAGGCTGATTC
MGG_16201innerR	TCGTTGTCTACCGCACCTACT
MGG_08526up-barF	GCAGCGATGCTTTACCCG
MGG_08526up_barR	AGCCCGATGACAGCGACCAC
MGG_08526innerF	GCAAGGCGGAGCGGTTTA
MGG_08526innerR	GAAGCGGTTGTAGGCGGTA

Table S4 List of primers for complementation and the probes of Southern blot

Name	**Sequence(5’-3’)**
MGG_06335comF	CACAATGGCCGGATCCATGGAAGCAAAATCATTGG
MGG_06335comR	TGCTCACCATCCCGGGGTCATCGTTGTACTCCCACTG
MGG_07573comF	CACAATGGCCGGATCCATGTTTGCACCAAGGGGCAAGG
MGG_07573comR	TGCTCACCATCCCGGGTGATTTGTCCTCTCCGTCC
MGG_01072comF	CACAATGGCCGGATCCATGAAGATCACAGATGTGGTC
MGG_01072comR	TGCTCACCATCCCGGGCTGTAGATTGAGGAACTTC
MGG_04818comF	CACAATGGCCGGATCCATGGCGTACAACCGACCCTAC
MGG_04818comR	TGCTCACCATCCCGGGCTTGAGCTGCTTCAGAATCTC
MGG_06335probeF	CTGACCAAGCACCAAGCG
MGG_06335probeR	CCCTGCGGGTATCCTATC
MGG_07573probeF	ATCAGGGCACAGATTCCA
MGG_07573probeR	ATCGGGCAAACTCTACAA
MGG_01072probeF	TGACAAGTGTATCAAGCGAAGT
MGG_01072probeR	GTGCCAGTGCCACAGAAG

Table S5 List of primers for quantitative RT-PCR

Name	**Sequence(5’-3’)**
MGG_06335RTF	CTGTTATGGATGTGCGAGTAGAG
MGG_06335RTR	TGCTGGAGAGTTTGGTGTC
MGG_07573RTF	TCAAACTTCGTAACCCCTGG
MGG_07573RTR	ACGTGATCCAAAAGACCGAG
MGG_01072RTF	AGAAAACCAAGCCCTCCAAG
MGG_01072RTR	GTTCTGGCTTTTGTGTTGACG
MGG_04818RTF	AACCAGAAACTTCCGTCTCC
MGG_04818RTR	TTCTCATATCGGCTTTGTGGG
MGG_14872RTF	CCGCTTTTGATTTTCGTGAGG
MGG_14872RTR	CATTAGAGGGAGCCAGGTTTC
MGG_15810RTF	GCCAAGGGAGACAAAACAATG
MGG_15810RTR	TTGTAGCCATACTCGGTTGC
MGG_08526RTF	CGGTTTACGCAAAAGGACATG
MGG_08526RTR	CCACCTTGACCTTGATGTTG
MGG_16201RTF	GAGCAAGTTTCCGAGTTCAAG
MGG_16201RTR	CGACCTCGTTGATCATATCCTG
HPHRTF	CGATAACTTGGTGCGTTTGTC
HPHRTR	TTGGATGCTTGGGTAGAATAGG

BARRTF	CGATAACTTGGTGCGTTTGTC
BARRTF	GGCTCATGGTTACTTCCTAATCG
β-tubulinRTF	ACAACTTCGTCTTCGGTCAG
β-tubulinRTF	GTGATCTGGAAACCCTGGAG

Table S6 Growth-inhibiting rate (%)*

Growth media	**Guy11**	**Δ*Mocapn4***	**Δ*Mocapn9***	**Δ*Mocapn14***	**Δ*Mocapn7***	**Δ*Mocapn4*Δ*Mocapn7***	**Δ*Mocapn9*Δ*Mocapn7***
CM	0	0	0	0	0	0	0
CM+200μg/ml Congo red	70.45±7.04^{C**}	80.75±1.90^{B}	93.86±2.14^{A}	92.86±1.17^{A}	80.75±0.74^{B}	72.67±0.69^{C}	84.99±0.26^{B}
CM+100μg/ml CFW	71.57±2.97^{C}	82.65±0.61^{B}	85.97±2.21^{A}	87.60±2.52^{A}	82.41±1.51^{B}	73.05±1.03^{C}	88.74±1.17^{A}
CM+0.01%SDS	44.29±0.85^{B}	44.34±0.60^{B}	43.61±1.11^{C}	44.03±1.25^{B}	47.07±2.05^{A}	43.58±0.80^{C}	47.73±0.89^{A}

*Growth-inhibition rate of strains on CM plates supplemented with 100μg/ml CFW, 0.01%SDS and 200μg/ml Congo red at 28 °C in dark for 6 days. Growth-inhibition rates were calculated as described in Experimental procedures.

** Different letters indicate a statistically significant difference ($P<0.01$).