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A novel *Magnaporthe oryzae* gene *MCG1*, encoding an extracellular globular protein, affects conidial germination and appressorial formation

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Hai-Jiao Li, Jian-Ping Lu, Irshad Ali Khan, Li-Lin Zhang, Rong-Lin He and Fu-Cheng Lin (2011) A novel *Magnaporthe oryzae* gene *MCG1*, encoding an extracellular globular protein, affects conidial germination and appressorial formation. Journal of Agricultural Technology 7(6): 1647-1660.

We cloned and characterized *MCG1*, a novel gene encoding an extracellular globular protein, from the rice blast fungus *Magnaporthe oryzae*. The Mcg1 gene (MGG_00339.7) is 1368-bp in length and encodes a protein of 456 amino acids containing a hydrophobic N-terminal signal peptide. The function of *MCG1* gene was studied using *MCG1* gene targeted-deleted mutant. Results obtained from Real Time PCR showed that the *MCG1* gene was primarily expressed during appressorial formation. Reduced conidial germination and appressorial formation showed by mutant Δ*mcg1* imply that this gene plays an important role in conidial germination and appressorial formation.

Key Words: *Magnaporthe oryzae*, extracellular globular protein, conidial germination, appressorium

Introduction

Rice blast disease, caused by ascomycete fungus *Magnaporthe oryzae*, is one of the most severe and widespread diseases of rice worldwide (Ou, 1985). Primarily found in China, over 3.8×10^6 hm^2 of rice have been exposed to this disease yearly since 1990. The rice blast fungus has been used as a primary model system for elucidating various aspects of host plant-fungal pathogen interactions (Valent, 1990; Dean *et al*., 2005). The cell membrane or cell wall of the pathogen acts as an interface between the plant cells and the pathogen during host penetration by fungal growth. The signals outside the cell, imported through the cell membrane activate fungal developmental events which result in cell differentiation or growth (Gronover *et al*., 2001; Dean *et al*., 2005).

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Previous studies demonstrated that Pth11, a cell-surface integral membrane protein, activates appressorium differentiation of *M. oryzae* via G-protein-coupled receptors in response to inductive surface cues (DeZwaan *et al*., 1999; Kulkarni *et al*., 2005) and Mpg1, a hydrophobin located at the cell membrane, is also responsible for signal transduction during conidiation and appressorial formation (Talbot *et al*., 1993; Talbot *et al*., 1996). Recently, MoMsb2 were found to sense signals from surface hydrophobicity and cutin monomers, and MoSho1 to recognize signals from rice leaf waxes (Liu *et al*., 2011). Mhp1, another hydrophobin, is required for fungal development and plant colonization (Kim *et al*., 2005). Similarly, the recognition sites of a resistant variety of plant are located at the cell surface of the pathogen. Despite significant progress made in understanding signal transduction pathways across the cell membranes in appressorial formation of *M. oryzae*, many extracellular gene products involved in signal transduction have not been fully characterized. Little is known about the functional and pathological significance of the Mcg1 protein in the rice blast fungus or its homologs in other filamentous fungi. In this paper, we report the cloning and characterization of an extracellular globular protein encoding gene *MCG1* from *M. oryzae* and molecular structure, expression and functional analysis of this gene by gene targeted-deleted method.

Materials and methods

Fungal isolates and growth conditions

M. oryzae wild-type strain Guy-11, mutant strain and rescued transformant were cultured on complete medium (CM) plates and incubated at 25^{o}C for 9-12 days with a 14-h-light and 10-h-dark cycle using fluorescent lights (Talbot *et al*., 1993). Crossing with strain 2539 was conducted on oat meal agar (OMA) medium (30 g oat in 1000 ml distilled water). The CM media and OCM media (CM media with 20% sucrose) were supplemented with 200 μg ml^{-1} hygromycin B (Roche Diagnostics, Basel, Switzerland) or 250 μg ml^{-1} glufosinate ammonium for initial screening of resistant transformants.

Cloning and sequencing of the MCG1 gene

An EST (ESTs214, GenBank Accession No. CN121336) was isolated from the subtracted appressorium cDNA library of *M. oryzae* strain Guy-11 (Lu *et al*., 2005a) and the genomic locus (MGG_00339.7) in the *M. oryzae* strain 70-15 was identified through homology search of *M. oryzae* genome database (http://www.broad.mit.edu).

PCR-amplified cDNA fragment containing the full coding sequence (CDS) from an appressorium cDNA library of *M. oryzae* (Lu *et al*., 2005b) using s214-cDNA1p1 (5'-CTCGCCCATTGTTGCCATTCTCCT-3') and s214-cDNA1p2 (5'-TGACTTCCACCAGCGTTTTGTAGC-3') as primers, was cloned into pUCm-T vector (Sangon Ltd, Shanghai, China) and sequenced on an ABI377 DNA sequencer (PE Applied Biosystems, Foster City, USA).

The cDNA and protein sequences were subjected to blastn and blastp searches against the GenBank database (Altschul *et al*., 1997). The protein sequence was predicted on the basis of a signal peptide using SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/) (Bendtsen *et al*., 2004), for protein structure using PredictProtein (http://www.predictprotein.org/) (Rost *et al*., 2004), for membrane helix using WoLFPSORT (http://wolfpsort.seq.cbrc.jp/) (Horton *et al*., 2006) and TargetP 1.1 (http://www.cbs.dtu.dk/services/TargetP-1.1/) (Bendtsen *et al*., 2004). Sequences of the Mcg1 protein and homologous protein were aligned using CLUSTAL X software (Thompson *et al*., 1997). A phylogenetic tree was generated using the neighbor-joining method (Saitou *et al*., 1987), using the MEGA4 program (Tamura *et al*., 2007).

The differential expression of *MCG1* at mycelia/conidia and appressoria of the rice blast fungus was detected by Real Time PCR, using primers S214-qRTF (5'- CTTGGCGTCTCTAGGCTTAAC -3')/ S214-qRTR (5'-CGACGTATGAGATGGTGAGC -3') and Tubulin-qPCRF (5'-ATTGTTCACCTTCAGACCGG-3')/ Tubulin-qPCRR (5'-TTGAAGTAGACGCTCATACGC-3') for MCG1 and β-tubulin (control) respectively. RNA isolation from mycelia, germinating conidia or apprressoria and Real Time PCR were performed as described previously (Lu *et al*., 2007; Liu *et al*., 2008).

Construction of targeted gene disruption vector

An interim vector, pBS-HPH1-s214-Dn constructed by inserting a 1.46-kb fragment of the downstream flanking sequence was amplified from genomic DNA of the wild-type Guy-11, using primers s214-Dn-P1 (5'-GGaagcttCGTCGCATTGGAGCTGGTTGAA-3') and s214-Dn-P2 (5'-GGtctagaGCGGGCGGATCTACGGTGCTTTGT-3') and cloned into the *Hin*dIII/*Xba*I sites of pBS-HPH1 containing a hygromycin resistance cassette (*HPH*) (Lu *et al*., 2007). A 1.42-kb fragment of the upstream flanking sequence amplified from genomic DNA of the wild-type Guy-11, using primers s214-Up-P1 (5'- TTctcgagTTTTGGGGATGGTATGGTGTTGAA-3') and s214-Up-P2 (5'- TTgtcgacTCGGCTGCCTGGATGATGG-3') was then inserted into the *Xho*I/*Sal*I sites of pBS-HPH1-s214-Dn to produce the gene deletion vector, pBS-MCG1. The vector pBS-MCG1 was *Xho*I-linearized, purified by gel

electrophoresis and transformed to the protoplasts of the wild-type strain Guy-11 using 200 μg mL^{-1} hygromycin B (Roche Diagnostics, Basel, Switzerland) as a selective marker as described previously (Lu *et al*., 2007).

Screening and identification of transformants

Transformants were screened on OCM media or CM media containing 200 μg mL^{-1} hygromycin B. Knock-out hygromycin-resistant transformants were initially confirmed by PCR using primers s214-check-p1 (5'-GCCGGCGAATTCTGCGTCTACTC-3) and s214-check-p2 (5'-CCCTAATCTTGGCCCCTCGCTTGGTC-3') internal to *MCG1*. Knock-out mutants were purified by single conidia isolation and finally confirmed by Southern blot analysis. For southern blot analysis, genomic DNA of mutant Δ*mcg1* and wild-type strain Guy-11 was isolated, digested with *Hin*dIII, separated on 0.7 % agarose gel and transferred to a positively charged nylon membrane. The 1.46-kb fragment of the downstream flanking sequence of the *MCG1* gene, amplified from genomic DNA using primers s214-low-p1 and s214-low-p2, was labeled as a probe. The targeted gene deletion event was confirmed by the detection of a 2.6-kb fragment in the transformants compared to a 4.4-kb fragment in the wild-type strain Guy-11.

Complementation of Δmcg1 mutants

For complementation, a 3.4-kb fragment containing a 1.4- and a 0.5-kb upstream and downstream sequences amplified from the genomic DNA of the wild-type strain Guy-11 with primers s214-hb-p1 (5'-TTgcggccgcAACTCCGGTCGCCATTTGATTTG-3') and s214-hb-p2 (5'-AAatcgatAGTGTTTGGCCGCGTCTCTGC-3') was cloned into pGEM-T vector (Promega, USA) and inserted into the *Not*1 and *Cla*1 sites of pBarKS1 to generate pBARHB-MCG1. The 3.4-kb pBARHB-MCG1 vector was linearzied and transformed into the mutant Δ*mcg1* using glufosinate ammonium (250 μg mL^{-1}) as a selective marker for the mutant complementation (Lu *et al*., 2007). Complementation was also confirmed by southern blot analysis and RT-PCR.

Assays for phenotypic defects of the mutant Δmcg1

To study mycelial growth characteristics, mutant Δ*mcg1* and wild-type strain Guy-11 were grown on CM medium, CM-C medium (CM medium without the carbon source) and CM-N medium (CM medium without the nitrogen source) and hypertonic medium (CM medium with 1 M NaCl) at 25^0C

for 9 days using the procedure described previously (Talbot *et al*., 1993). This assay was repeated 3 times with 5 replications each independently. Assays for conidiogenesis, conidial germination and appressorial formation were carried out as described previously (Lu *et al*., 2007). To find conidiation three 1-cm mycelial block of mutant Mcg1 and wild-type strain Guy-11 was suspended in 3 ml sterile distilled water and 10 µl conidial suspension was used to determine the conidial concentration by using a haemocytometer. For conidial germination and appressorial formation, a 20 µl drop of conidial suspension (10^6 conidia ml^{-1}) was placed on plastic coverslip and incubated in a humid chamber at 25^0C for 2, 4, 6 and 24 h. Each assay was repeated 3 times with over 300 conidia for each of 5 replications.

Appressorium turgor was estimated using incipient cytorrhysis (cell collapse) assays (Howard *et al*., 1991). At 24 h post-incubation (hpi), the surface water of conidium drops was removed carefully and replaced with an equal volume (20 µl) of a glycerol solution series varying in concentration from 0.5 M to 4.0 M. The number of appressoria that had collapsed after 10 min was recorded microscopically. The experiments were all replicated three times and more than 300 appressoria were recorded in each.

Pathogenicity assays

Inoculation of 14-day-old rice seedlings (*Oryzae sativa* Cv. CO-39) for pathogenicity assay was conducted as described previously (Balhadère *et al*., 1999). 14-day-old rice seedlings were sprayed evenly with 10 ml conidial suspension (10^5 conidia ml^{-1}) of the *M. oryzae* strain Guy-11 and mutant Mcg1 using an artist's airbrush (Lu *et al*., 2007). Inoculated rice seedlings were kept in a dew chamber at 25°C for 48 h in the dark, and incubated in a growth chamber for 5 days with a 14-h-light and 10-h-dark cycle using fluorescent lights. Disease severity was monitored over a period of 7 days dpi according to the disease rating scale proposed by Bonman *et al*., (1986).

Results

Isolation and characterization of MCG1 gene

A 1.5-kb cDNA fragment containing the full *MCG1* CDS was PCR-amplified from an appressorium cDNA library of *M. oryzae* Guy-11 (Lu *et al*., 2005), cloned into TA vector and the gene was named *MCG1* for *M. oryzae* conidial germination correlative gene 1 after confirmation by sequence analysis (GenBank Accession no. EF544024). The cDNA fragment contained a 1368-bp open reading frame (ORF) encoding 456 amino acids. The locus

(MGG_00339.7) of the *MCG1* gene in the genomic DNA was identified through a homology search of the *M. oryzae* genome database (Dean *et al*., 2005). It was found that the *MCG1* gene (MGG_00339.7) was located on chromosome VI of *M. oryzae* strain 70-15. Furthermore, the *MCG1* gene was mainly expressed during the appressorium formation stage rather than during mycelium stage after confirmation by Real Time PCR (Fig. 1).

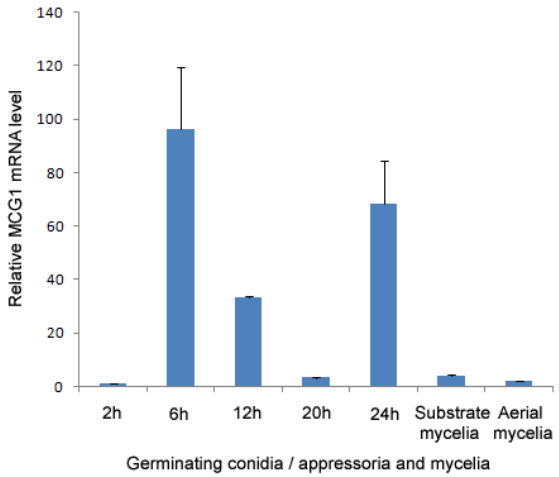

Fig. 1. *MCG1* expression in germinating conidia / appressoria and mycelia in the rice blast fungus. Real Time PCR was performed for quantification of *MCG1* expression in the wild-type Guy-11. RNA was isolated from germinating conidia / appressoria incubated on hydrophobic membrane at 25 °C for 2 h, 6 h, 12 h, 20 h and 24 h, substrate mycelia grown in liquid CM media and aerial mycelia grown on solid CM media. The relative *MCG1* mRNA level in germinating conidia incubated for 2 h (0.019, β-tubulin as control) is as 1 (unit). The results showed the *MCG1* expression was highly up-regulated in conidia/appressoria incubated for 6h and 24h. Error bar is standard deviation.

Using *MCG1* cDNA sequence, Blastn and Blastp searches resulted in 4 homologous EST sequences and 100 homologous protein sequences with *E*-vlaue < e-03 and < e-10, belonging to hypothetical proteins in GeneBank database. A phylogenetic tree based on the alignment of a Mcg1 protein and its homologues in different fungi were shown in Fig. 2. The gene (*MCG1*) did not show any significant homology with any other protein of known function using BLAST searches.

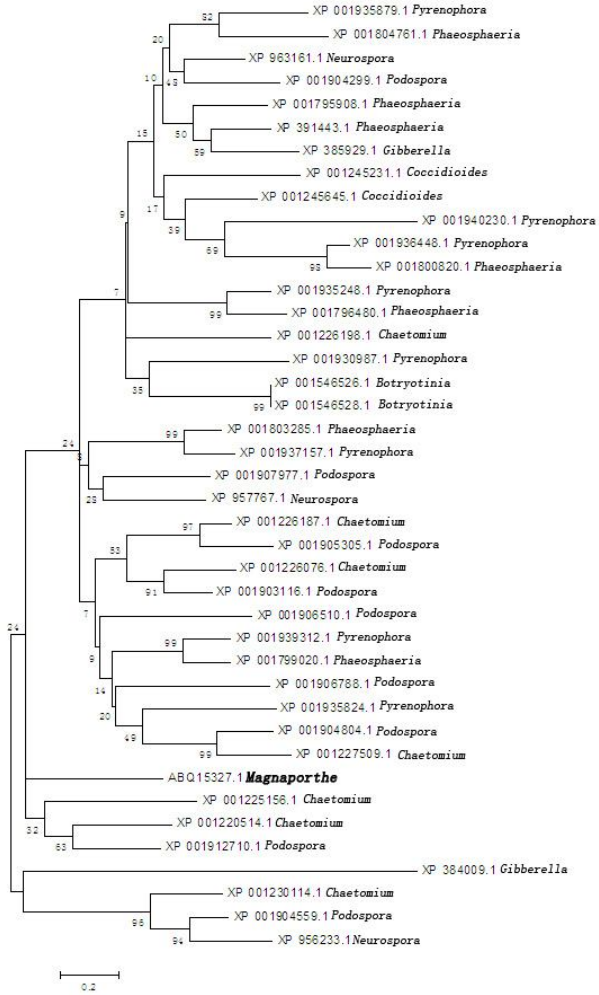

Fig. 2. Comparative sequences analysis of the Mcg1 protein and homologous proteins from GenBank. Bootstrap analysis was based on 500 trials.

Predictprotein program (Rost *et al*., 1996) predicted that the Mcg1 protein (GenBank_Accession: EF544024) did not contain transmembrane helices (by PHD predictions), and is a globular protein (by GLOBE prediction). WoLFPSORT program (Horton *et al*., 2006) predicted that Mcg1 was a secretory protein. TargetP 1.1 program (Emanuelsson *et al*., 2000) also predicted that Mcg1 was a secretory protein containing a signal peptide. And the NN and HMM models of SignalP 3.0 (Bendtsen *et al*., 2004) predicted that Mcg1 contains a hydrophobic N-terminal signal peptide, with a cleavage site between position 25 (serine) and 26 (serine). Mcg1 also contained two potential N-glycosylation sites at positions 90–93 (NASN) and positions 356 to 359

(NLTW) (Bairoch *et al*., 1997). These predicted results show that Mcg1 is an extracellular globular protein.

Disruption and complementation of MCG1 gene

To determine the function of *MCG1* gene, we constructed a knock-out vector pBS-MCG1 bestowing resistance to hygromycin B (Fig. 3a). The 7.8-kb *Xho*I fragment of pBS- MCG1 was transformed to the protoplasts of the wild-type Guy-11 to delete the ORF of *MCG1* gene. Eight transformants resistant to hygromycin were obtained and inititially screened by PCR with the primers internal to *MCG1*. One transformant, s214-8, out of eight was finally confirmed by southern blot analysis, purified by single conidia isolation and then used for phenotypic analysis. In southern blot analysis, *Xho*1-digested s214-8 transformant and wild-type Guy-11 genomic DNA showed a 2.6- and a 4.4-kb band respectively using a 1.46-kb fragment of the downstream flanking sequence of the *MCG1* gene as a probe (Fig. 3b). Band shift from 2.6- to 4.4-kb confirmed that a gene disruption event had deleted the *MCG1* gene by homologous recombination in transformant s214-8.

For complementation, the mutant Δ*mcg1* was transformed with *MCG1* gene containing its native promoter and full length gene sequence. In southern blot analysis, two bands 2.6- and 3.5-kb were detected in the rescued transformant s214HB1. The appearance of one additional band (3.5-kb) implied that a new *MCG1* gene had transformed into the rescued transformant (Fig. 3b). The RT-PCR results also showed *MCG1* expression lacked in *MCG1*-deleted mutant s214-8, but appeared in rescued transformant s214HB1 again (Fig. 3c).

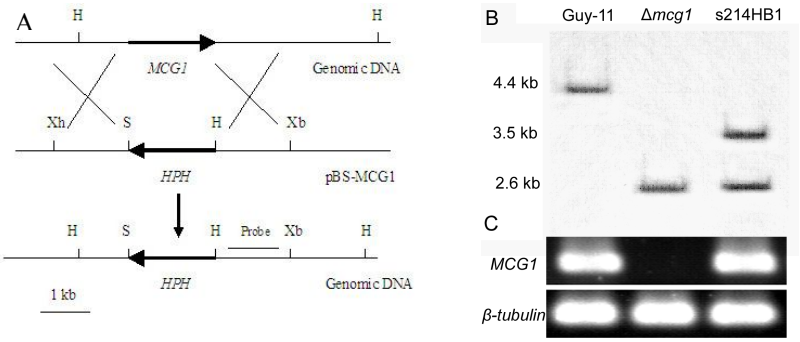

Fig. 3. Disruption of the *MCG1* gene. Map of the targeted gene disruption vector pBS-MCG1 of *MCG1* gene. (A) The gene knock-out vector pBS-MCG1 was Xho1-linearized, purified by gel electrophoresis and transformed to the protoplasts of *M. oryzae* strain Guy-11. H, *Hin*dIII; S, *Sal*I; Xh, *Xho*I; Xb, *Xba*I; Probe, DNA gel blot probe. (B) Southern blot analysis of Δ*mcg1*, complementation strain (s214HB1) and wild-type strain Guy-11 DNA digested with *Hind*III, separated by 0.7 % agarose gels and probed with the 1.4-kb *MCG1* gene downstream fragment (Fig. 3B). The mutant Δ*mcg1* showed an expected 2.6-kb band in contrast to a 4.4-kb band in the wild-type Guy-11, while in the rescued-transformant, two (2.6- and 3.5-kb) bands were detected. (C) RT-PCR analysis for *MCG1* expressed in Δ*mcg1*, s214HB1 and Guy-11, β-tubulin as a control. *MCG1* expressed in wild-type strain Guy-11 and complementation strain (s214HB1), not in mutant Δ*mcg1*.

Assay for mycelial growth and conidiation of mutant Δmcg1

A mycelial growth assay was conducted for mutant Δ*mcg1* and wild-type Guy-11 on CM, CM-C, CM-N and CM-hypertonic medium. On all the media tested, the mycelial growth rate of the mutant Δ*mcg1* was a little higher than that of the wild-type Guy-11 and conidiogenesis on CM medium was comparable ($p \leq 0.05$) (Table 1). The aerial hyphae produced by the mutant Δmcg1 were well developed similar with those of the wild-type Guy11. When compared with the wild-type Guy-11, the mutant Δ*mcg1* on CM medium, showed similar colony morphology (Fig 4). The mutant Δ*mcg1* after mating with strain 2539 produced normal perithecia with viable ascospores, as those of the wild-type strain Guy-11.

Table 1. Mycelial growth of mutant Δ*mcg1* and wild-type Guy-11

Strains	**Mycelium growth (cm)**				**Conidiation ($\times 10^5$n/cm^2)**
	CM	CM-C	CM-N	CM-NaCl	
Guy-11	6.4±0.1b	3.5±0.1b	5.3±0.1b	2.8±0.2b	7.4±0.4 a
Δ*mcg1*	6.6±0.1a	3.8±0.1a	5.5±0.1a	3.1±0.1a	6.9±0.6 a
s214HB1	6.4±0.1b	3.6±0.1b	5.1±0.1b	2.8±0.1b	7.1±0.5 a

An mycelial plug (0.5 cm in diameter) of Δ*mcg1,* wild-type Guy-11 and complementation strain (s214HB1) was placed on CM, CM-C, CM-N and CM-NaCl media and cultured at 25ºC for 9 days. The diameter (cm) of mycelium was measured 9 days post-inoculation. Conidiation by mutant and Guy-11 on CM plate was assessed after suspending three 1-cm mycelial plugs of *Δmcg1*, Guy-11 and complementation strain with 3 ml sterile distilled water using a haemocytometer. Average values with the same lowercase letters in each column are not significantly different, as estimated by Duncan's test ($p \leq 0.05$).

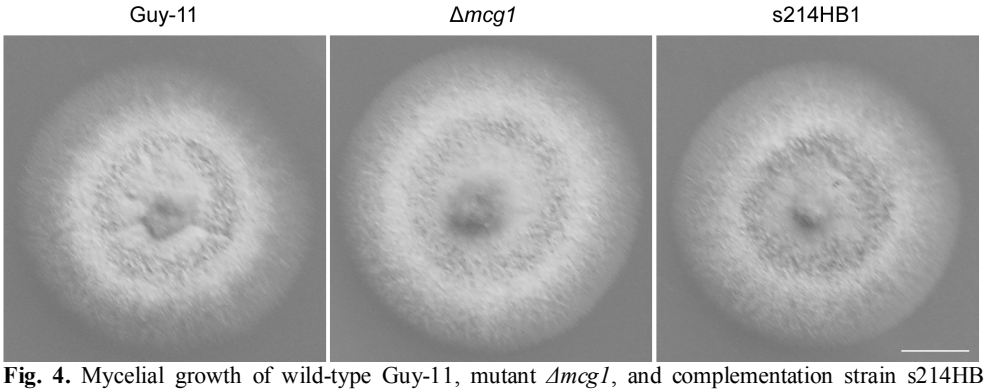

Fig. 4. Mycelial growth of wild-type Guy-11, mutant *Δmcg1*, and complementation strain s214HB1. Wild-type Guy-11, mutant Δ*mcg1*, and complementation strain s214HB1 were grown on complete medium at 25^{o}C for 9 days. Pictures were taken 9 days post-inoculation. Bar: 1 cm.

MCG1 affects conidial germination and appressorial formation

In the assay of conidial germination, conidia of Δ*mcg1* mutants were slower to germinate than those of the wild-type strain Guy-11 (Table 2). At 2 and 4 h post-inoculation (hpi), the germinated conidia of the mutant Δ*mcg1* on plastic coverslips, were 45.9% and 92.2 % compared with 90.2% and 98.5 % of the wild-type Guy-11 ($p \leq 0.05$).

In appressorial formation assay, the rate of appressoria formation of the mutant Δ*mcg1* was slower than that of the wild-type Guy-11 (Table 2). At 6 hpi, the mutant Δ*mcg1* and wild-type Guy-11 produced 75.5% and 86.6 % appressoria respectively (Table 2). In the mutant Δ*mcg1*, 86.2 % conidia examined produced appressoria compared with 99.3 % in the wild-type Guy-11 at 24 hpi.

The mutant Δ*mcg1* showed an unaffected rate of collapsed appressoria (23.7±0.6%) in 2 M glycerol solution at 24 hpi as almost the same that of the wild-type Guy-11 (23.1±2.1 %) ($p \leq 0.05$), implying that the turgor pressure in the appressoria is not affected by the disruption of *MCG1* gene. After the *MCG1* gene was re-introduced into the mutant Δ*mcg1*, the delay in germinated conidia and appressorial formation was restored (Table 2).

Table 2. Conidial germination and appressorial formation of mutant *Δmcg1* and Guy-11

Strain	Conidial germination[a] %		Appressorium formation[b] %	
	2 h	4 h	6 h	24 h
Guy-11	90.2±1.9 a	98.5±0.2 a	86.6±5.2 a	99.3±0.4 a
Δ*mcg1*	45.9±1.8 b	92.2±2.6 b	75.5±4.0 b	86.2±5.7 b
s214HB1	88.6±0.6 a	97.5±0.7 a	85.2±1.2 a	98.3±0.8 a

[a] Data for the percentage of conidial germination was analyzed after incubating a drop (20 ul each) of conidial suspension (1×10^5 conidia mL^{-1}) of mutant Δ*mcg1*, complementation strain (rescued) and Guy-11 on plastic coverslip at 25°C for 2 and 4 h.

[b]The percentage of appressorial formation was measured after incubation of 20 ul conidial suspension (1×10^5 conidia mL^{-1}) of mutant Δ*mcg1*, complementation strain (s214HB1) and Guy-11 on plastic coverslip at 25°C for 6 and 24 hr. Average values with the same lowercase letters in each column are not significantly different, as estimated by Duncan's test ($p \leq 0.05$). Data are the mean of three replications with over 300 conidia examined each time.

Pathogenicity assays of mutant Δmcg1

Infection assay on blast susceptible rice cultivar (Cv. CO-39) was carried out to assess the role of *MCG1* gene in pathogenicity. Upon spray inoculation of the rice seedlings with the conidial suspension, the mutant Δ*mcg1* showed reduced pathogenicity with smaller lesions than those of the wild-type Guy-11 but the mutant did not lose its pathogenicity completely (Fig. 5).

Fig. 5. Pathogenicity assay of mutant Δ*mcg1*, wild-type Guy-11 and control (0.2 % gelatin) on blast succeptible rice cultivar (Cv. CO-39) seedlings. Rice seedlings were spray inoculated with conidial suspension (1 $\times 10^5$ conidia mL^{-1}) of mutant Δ*mcg1*, wild-type Guy-11 and 0.2 % gelatin (control). Seedlings were photographed 7 days post-inoculation. This assay was repeated twice using 20 seedlings per experiment for each strain of Δ*mcg1* and wild-type Guy-11.

Discussion

After the genome of *M. oryzae* sequenced (Dean *et al*., 2005), the principal task faced by researchers was to elucidate the function of the identified genes. The fungus regulates its developmental events which are sensed by the proteins located at cell membrane (Gronover *et al*., 2001; Dean *et al*., 2005; Bahn *et al*., 2007). In this study, we used a targeted gene disruption method to describe the function of the *MCG1* gene, encoding an extracellular globular protein in the development of *M. oryzae* by the mutant Δ*mcg1*.

The conidial germination and appressorial formation were delayed in the Δ*mcg1* mutant while the mycelium growth and pathogenicity was less affected by the deletion of *MCG1*. Furthermore, conidiation and turgor pressure in the appressoria were unaffected by the Δ*mcg1* mutant. These data implied that *MCG1* gene played roles during conidial germination and appressorial formation. However, delay in appressorial formation might be due to delayed conidial germination. The fact that *MCG1* gene primarily expressed during appressorial formation, was consistent with its role in conidial germination and appressorial formation. Several genes have been identified to be involved in conidial germination or appressorial formation by *M. oryzae*. In fungi, conidial germination contains several different stages such as recognition of surface cues, adhesion to surface, signaling pathways (Ca^{2+} signaling pathway, RAS-

MAPK pathway and cAMP-PKA pathway), initiation of protein synthesis and later conidial germination (Osherov and May 2002). In *M. oryzae*, cAMP signaling pathways regulate conidial germination and appressorial formation (Lee and Dean, 1993; Choi and Dean 1997; Adachi and Hamer 1998), MAP kinase pathways regulate appressorial formation (Xu, 2000), Ca^{2+} signaling pathways regulate appressorial formation and conidial germination (Lee and Lee 1998; Wang *et al*., 2003). The endogenous nutrient-related genes also affect the efficiency of conidial germination and appressorial formation, such as *MoATG5* (Lu *et al*., 2009) and *MoATG8* in *M. oryzae* (Veneault-Fourrey, *et al*., 2006) and *AoATG8* in *Aspergillus oryzae* (Kikuma *et al*., 2006). The environmental signals imported through the cell membrane, could activate conidial germination or appressorial differentiation through transmembrane proteins or extrinsic proteins (Talbot *et al*., 1993; DeZwaan *et al*., 1999; Gronover *et al*., 2001; Liu *et al*., 2011). Some transmembrane proteins in *M. oryzae* are related to conidial germination and appressorial formation such as Pth11, a transmembrane protein, senses host surface recognition signals and activates appressorium differentiation in response to inductive surface cues, or represses differentiation on poorly inductive surfaces (DeZwaan *et al*., 1999). Mpg1 and Mhp1 are also required for conidial germination and appressorium elaboration *M. oryzae* (Talbot *et al*., 1993; Kim *et al*., 2005). Mpg1 and Mhp1 sense hydrophobic surfaces which are required for the pathogenicity of *M. oryzae*. Extracellular matrix protein 1 (Emp1), containing a hydrophobic C-terminal sequence, required for appressorial formation of *M. oryzae*, has no role in conidial germination (Ahn *et al*., 2004). The effect of Mcg1, a secreted globular protein, on conidial germination and appressorial formation is weaker than that of Pth11, Mpg1 or Mhp1.

The phenotypes showed by the mutant Δ*mcg1* indicate that the *MCG1* gene is involved in conidial germination, appressorial formation of the rice blast fungus. These data show that *MCG1* is likely to affect conidial germination and appressorial formation by sensing environmental cues.

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