

Scientific Report

BcMtg2 is required for multiple stress tolerance, vegetative development and virulence in *Botrytis cinerea*

Wenyong Shao, Yu Zhang, Jin Wang, Chiyuan Lv and Changjun Chen*

College of Plant Protection, Nanjing Agricultural University,

Nanjing 210095, Jiangsu, China

Corresponding author: Changjun Chen

Phone: 086-25-84395641;

Fax: 086-25-84395641;

E-mail: changjun-chen @njau.edu.cn

Figure S1

Amino acid alignments of *Botrytis cinerea* Mtg2 (BcMtg2) with those of *Neurospora crassa* Mtg2 (NcMtg2), *Candida albicans* Mtg2 (CaMtg2), *Aspergillus nidulans* Mtg2 (AnMtg2), *Magnaporthe oryzae* Mtg2 (MoStr), *Fusarium graminearum* Mtg2 (FgMtg2), *Fusarium oxysporum* (FoMtg2), *Schizosaccharomyces pombe* Mtg2 (SpMtg2), *Saccharomyces cerevisiae* Mtg2 (ScMtg2) and *Sclerotinia sclerotiorum* Mtg2 (SsMtg2). Boxshade program was used to highlight identical (black shading) and similar (grey shading) amino acids. The conserved N-terminal pyridoxal phosphate binding pocket and the C-terminal catalytic residue domain are underlined. Phylogenetic relationship of the Mtg2 gene among *Botrytis cinerea* and other plant pathogens based on amino acid sequence.

10 20 30 40 50 60 70 80 90 100 110 120
 NcMtg2-I-SRCAHSSRLPMPFLYPSLFRASAASVPPATSLA--ARNLRYSTATLEPQHSENDDEARS-LNP-P-DY
 CaMtg2 MIPRLYRSFSISTTLRNELLDTARMLQSSSSNTLNQPKNEIHTTKPTSPESYERIVYEPNYNKPQDETLENSDIPMIKITELSRDLTTGLTTATTISIADYFFGNTTHSHVEVSNLTVL
 AnMtg2-TORTSRLTLSIPLAKRSVCFCSPPNRLSATHPSRGYATDTEPEKESIDAAIAS---DAPSHLNPS-P-DY
 MoMtg2-HSPICACRPQQLALEFLYEFLLRSTAQTPTAAASP---LRQRYYYTETPAADIS---SRLNPS-P-DY
 FoMtg2-M-PRPCPTVSSTFLFPLYESVFLTRHTFGASALR---RRYNSTVNDLPE---SRLNPS-P-DY
 FgMtg2-M-PRCPAMSMSTLLFLYESTSIFLSRETLASTAALAR---RRFNSTINDLPE---SRLNPS-P-DY
 SpMtg2-MLSRLTSISRICCCLIRASSYVTEATQP---
 ScMtg2 ---MSIAWSSVFKEELRLERFLPRVYSTKVPDN-.....-APRAADQWVQLELWVLTITHPEQKKSDHDVS---YTRHINVPLGEVTS--VNLYQRYNKHGS
 SsMtg2-PRCSAVQGTYFLYEFLLSNTSLRVQSPRIHALRQGSTSYEELASESTS---EEFDRLNPNP-P-DY
 BcMtg2-PPRCSTVQGTFLYEFLLSNASRLLIHNRPRIYINAVRRQSTLSEELAESPAA---EEFDRLNPNP-P-DY

130 140 150 160 170 180 190 200 210 220 230 240
 NcMtg2-SNPPDPDKRILKEVAGGGCGCISIPLREFIPECPANGDDGGCAGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGSAACGGRCGDPDIIIVEVGTIVFRIERDPPGERQ
 CaMtg2 KAKRAAKVKNKKRDKLKLIREGCGGCGVSVFREYIPECPGNPGDDGGSGCAGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGSDIDIIIDHICTRVRIDPDFKKFV
 AnMtg2-SRPFEPLCRSTMAGGGCGVSVFREYIPECPGNPGDDGGSGCAGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGSKRCGRCGDIVLIVIVEVGTIVFRIERDPPVAEQQ
 MoMtg2-ASPFEDPLFLTLMAGGGCGVSVFREYIPECPGNPGDDGGSGCAGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGAKKGRCGDIVLIVIVEVGTIVFRIERDPPTEDR
 FoMtg2-ASPFEDPLFLTLMAGGGCGVSVFREYIPECPGNPGDDGGSGCAGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGAKKGRCGDIVLIVIVEVGTIVFRIERDPPVADDE
 FgMtg2-ASPFEDPLFLTLMAGGGCGVSVFREYIPECPGNPGDDGGSGCAGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGAKKGRCGDIVLIVIVEVGTIVFRIERDPPVLDDE
 SpMtg2-RPLPFLRERIQGCGGCSFIFEREPVGFPGGGGCGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGAKKGRCGDIVLIVIVEVGTIVFRIERDPPVLDDE
 ScMtg2-QGNPDRIVKCRSGAGGCGVSVFADAGRSIGFHGGDGGAGCAGVVIYCAVFG-LGSLSMHTTYTAED-CEAGAARQLDGRCGDIVLIVIVEVGTIVFRIERDPPVLDLV
 SsMtg2-SRTIPLDKRILKEVAGGGCGVSVFREYIKAERGPAGFPNGDDGGGCGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGAKKGRCGDIVLIVIVEVGTIVFRIERDPP
 BcMtg2-SRTIPLDKRILKEVAGGGCGVSVFREYIKAERGPAGFPNGDDGGGCGVVIYCAVFG-ETSLHRLAARRHIAEGRGGCGGAKKGRCGDIVLIVIVEVGTIVFRIERDPP

250 260 270 280 290 300 310 320 330 340 350 360
 NcMtg2-L-IDRAKYKRKKRD---FNAALAEAPREEBEGVGETIVBEEPPVNPDLF---FWLILYBGISSSEARRVGA-BLPRLERDRDFQOPPAIYLDINRPPTFPHILLAGGUGGLN
 CaMtg2 S-QREODA---LNDVFMPEFDIDD---RDFIQLNRGGYRPGEGWPKHEDEEYFDRDKPFDQDNEKVTOYDEELLFEEYSNDRPPIVLDCNQVTQRFHLLLGCGGGGLN
 AnMtg2 R-RRALERKMRKKEKAKSGEGEDEDERQALDFPQPIRIDEWVLPYGANFSKTKGQKGDVWVIVEVGTIVFRIERDPPVADDE
 MoMtg2 M-LEKALYGRRRRPA---APAVPAEASVEDAAGE-ELAEAEPLDPLPFR---FWLILYBGMNTTERKRLNDL-PL-RLEKRTTRYSQPAEVLDLSCPTFPHILLAGGUGGLN
 FoMtg2 L DMDKLWRAQQKQKREERENQAALERKQREHEEEEEEQEEEEEEDQDPER---FWLILYBGLSKTDIKQT---VFPFLERKTRRLRQPPPTILDLSPPTFPHILLAGGUGGLN
 FgMtg2 M MDMLWLWRAQQKQKREERMAALERKQREQEENEYEEEEEQEEEEEEDQDPER---FWLILYBGLSKTDIKQT---VFPFLERKTRRLRQPPPTILDLSPPTFPHILLAGGUGGLN
 SpMtg2-QSLEWVQMPGKTPKPLKGQGIVSPVSEATRIGKEVYRTRASS-...-MISGAAEYSELCDTIPQIICVGGVGGGLN
 ScMtg2 ER-EMRKDNNAATLRSILGSTAVNLVSSSGSHRKKIQLYR-...-HEMAWSLFLDKAKAYHENIKDWDLKHOMBEAYDHSLEQSELFDNDQPLACLDINGPPTHVCLLRLGGCGGLN
 SsMtg2-AAIEEBARIQTEAGKLGDHDLDGPYKNDR-...-FWLILYBPAITPEEIAAEFLSPLPKARKSNLAAAQVKGFIISLDSMPERPHILLAGGUGGLN
 BcMtg2-AAIEEDRIMHEAGKLGDHDLDGPYKNDR-...-FWLILYBPAITPEEIAAEFLSPLPKARKSNLAAAQVKGFIISLDSMPERPHILLAGGUGGLN

370 380 390 400 410 420 430 440 450 460 470 480
 NcMtg2-PFPANKERIAPP-FATRGEAATVIRDEELRLIADVGIVGLPNAFKGSTLFLAFTS-...-FVGWPFPTLLEPNTGIVLVIDNNPQ-EVKSYKRIKSDAPVDDPFALTGAPE-EVECRIPTAD
 CaMtg2-M-PEILTKEIKCPPEKGRPGITANFLU-...-GIVGLPGRSS-...-LBRILSARPLTFL-...-FPTLLEPNTGIVLVIDNNPQ-...-KDFPTAD
 AnMtg2-PFWLSRERIPEPFASEGGRERLLE-...-LBRILIAEVGIVG-...-FGRGSTLFLSLS-...-FPTLLEPNTGIVLVIDNNPQ-...-RGEPTAD
 MoMtg2-PFWLSRERIPEPFASTRGERFMSRDELLR-...-LBRILIAEVGIVG-...-FGRGSTLFLSLS-...-FPTLLEPNTGIVLVIDNNPQ-...-RGEPTAD
 FoMtg2-PFPFTSRAFEPF-FATRGGDAVITMRDEELRLIADVGIVGLPNAFKGSTLFLAFTS-...-FVGWPFPTLLEPNTGIVLVIDNNPQ-...-AQLGLP-EEVETEPTAD
 FgMtg2-PFPFTSRAFEPF-FATRGEAATVIRDEELRLIADVGIVGLPNAFKGSTLFLAFTS-...-FVGWPFPTLLEPNTGIVLVIDNNPQ-...-AQLGLP-EAVDTEPTAD
 SpMtg2-VHELSENNRSP-FATRGLTGEQKLLEELK-...-ICEI-GIVGLPGRGSTLFLNCLTSKS-...-FPTLLEPNTGIVLVIDNNPQ-...-SFOVPTAD
 ScMtg2-M-PELTNLIRNPPEPKGRNLGLQHFLFELK-...-AD-...-GIVGLPGRGSTLFLNCLTSKS-...-FPTLLEPNTGIVLVIDNNPQ-...-QDVPTAD
 SsMtg2-PFWTKQIPEPFAATGEAGLRITLEELRLIADVGIVGLPNAFKGSTLFLAFTS-...-FVGWPFPTLLEPNTGIVLVIDNNPQ-...-EPRINPTAD
 BcMtg2-PFWTKQIPEPFAATGEAGLRITLEELRLIADVGIVGLPNAFKGSTLFLAFTS-...-FVGWPFPTLLEPNTGIVLVIDNNPQ-...-EPRINPTAD

490 500 510 520 530 540 550 560 570 580 590 600
 NcMtg2-IEGLIECAHLDGLGCAFLPREFEVAVLAEVWDLDAAGN-...-AVIAALKLWVGLYIAGKREDEEADRERRAARIW-PEAGIDNVSMSGWPASNGMADYPSAAEPAGLSIAAG-...-KPMF
 CaMtg2-IPC-ICRCASONNCGLDFPLRHEIERSGCI-...-FVSL-ESAH-...-FVDFKLLHBEVCG-PRK-EGDN-
 AnMtg2-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-
 MoMtg2-IPC-ICRCASONNCGLDFPLRHEIERSGCI-...-FVSL-ESAH-...-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-
 FoMtg2-IPC-ICRCASONNCGLDFPLRHEIERSGCI-...-FVSL-ESAH-...-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-
 FgMtg2-IPC-ICRCASONNCGLDFPLRHEIERSGCI-...-FVSL-ESAH-...-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-
 SpMtg2-IPC-ICRCASONNCGLDFPLRHEIERSGCI-...-FVSL-ESAH-...-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-
 ScMtg2-IPC-ICRCASONNCGLDFPLRHEIERSGCI-...-FVSL-ESAH-...-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-
 SsMtg2-IPC-ICRCASONNCGLDFPLRHEIERSGCI-...-FVSL-ESAH-...-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-
 BcMtg2-IPC-ICRCASONNCGLDFPLRHEIERSGCI-...-FVSL-ESAH-...-FVDFKLLHBEVCG-PRK-EGDN-...-FVDFKLLHBEVCG-PRK-EGDN-

610 620 630 640 650 660 670
 NcMtg2-VVAAKDLDKDTQANFMEIDYCAAVTRD-DEPHPSGVPGAWIENVEAIFIYV-...-FVDFVWTVCLLDG-
 CaMtg2-VVAAKDLDLSS-GANFWVLRDYIENHHIS-...-EWKIVFVIAPKGEVIERC1KIMSELIAQTKKP-
 AnMtg2-VVAAKDLDLSS-GANFWVLRDYIENHHIS-...-EWKIVFVIAPKGEVIERC1KIMSELIAQTKKP-
 MoMtg2-VVAAKDLDLSS-GANFWVLRDYIENHHIS-...-EWKIVFVIAPKGEVIERC1KIMSELIAQTKKP-
 FoMtg2-VVAAKDLDLSS-GANFWVLRDYIENHHIS-...-EWKIVFVIAPKGEVIERC1KIMSELIAQTKKP-
 FgMtg2-VVAAKDLDLSS-GANFWVLRDYIENHHIS-...-EWKIVFVIAPKGEVIERC1KIMSELIAQTKKP-
 SpMtg2-VVAAKDAAEQD-LLL-KALVERTT-...-VAVI-FVIAPKGEVIERC1KIMSELIAQTKKP-
 ScMtg2-VVAAKDAAEQD-LLL-KALVERTT-...-VAVI-FVIAPKGEVIERC1KIMSELIAQTKKP-
 SsMtg2-VVAAKDAAEQD-LLL-KALVERTT-...-VAVI-FVIAPKGEVIERC1KIMSELIAQTKKP-
 BcMtg2-VVAAKDAAEQD-LLL-KALVERTT-...-VAVI-FVIAPKGEVIERC1KIMSELIAQTKKP-

GTP/Mg²⁺ binding site

Switch region domain

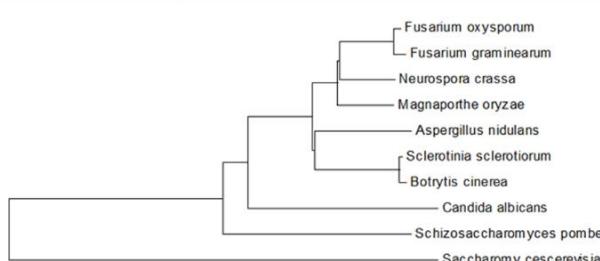


Figure S2

Generation and identification of the BcMtg2 deletion mutant of *Botrytis cinerea*. (A) Gene replacement strategy for BcMtg2. The hygromycin resistance cassette (hph) is denoted by the large grey arrow. Primer (P3–P18) binding sites are indicated by arrows (see Table S1 for the primer sequences). (B) Southern blot hybridization analysis of strains using the 3'-flanking region of BcMtg2 as a probe. Genomic DNA of the wild-type progenitor B05.10, BcMtg2 deletion mutant Δ BcMtg2 and complemented strain Δ BcMtg2C were digested with XbaI. (C) Primer pair P11/P12 was used to specifically amplify the partial BcMtg2. (D) Primer pair P13/P14 was used to validate the selectable marker hph. (E, F) Primer pairs P15/P16 and P17/P18 were used to amplify the two homologous arms with a partial fragment of the connecting area.

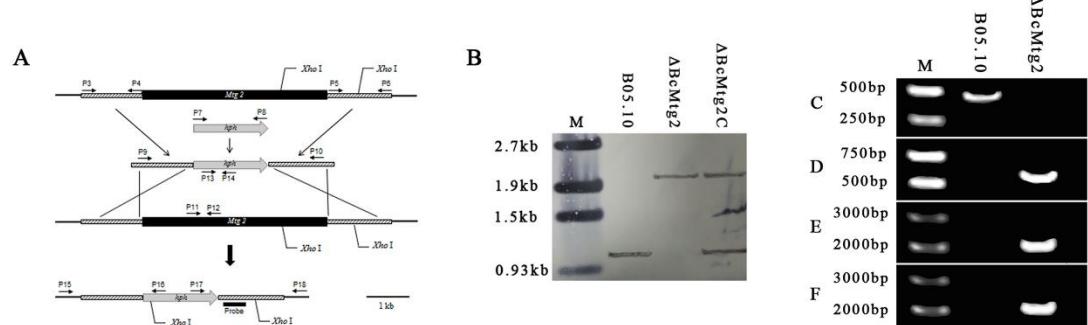


Table S1

Primers used in this study

Primer code	Sequence (5'→3')	Relevant characteristic
P1 ATGCCTCCACGTTGTTCAAC		Amplify the full cDNA sequence of the <i>Mtg2</i> gene
P2 TCATCCATCAAGAAGACCAAC		
P3 TTCGTATGAAGCCGTATGT		Amplify the left homologous arm of the <i>Mtg2</i> gene of <i>B. cinerea</i> (1444 bp)
P4CCACCAGCCAGCCAACAGCTCCCAGCTGTGCGAGCTGGAGA		
P5 CAATACGCAAACCGCCTCTCCCCAGTATTACGGATAGAGGC		Amplify the right homologous arm of the <i>Mtg2</i> gene of <i>B. cinerea</i> (1517 bp)
P6 CAATGAGTTATGGAGGTG		
P7 GGGAGCTGTTGGCTGGCTGGTGG		Amplify the <i>hph</i> gene (1764 bp)
P8 GGGGAGAGGCGGGTTGCGTATTG		
P9 AATGGTGAAGGCTCTGCTGT		Amplify the knockout vector of the <i>Mtg2</i> gene of <i>B. cinerea</i> (4471 bp)
P10 ATGAGTTATGGAGGTGGGT		
P 11 AGTTTACACATTGGAGCCG		Amplify a partial fragment of the <i>Mtg2</i> gene of <i>B. cinerea</i> (437 bp)
P 12 GGGAGATTAGGTGCGAGGG		
P 13 CAAAGCATCAGCTCATCGAGAG		Amplify a partial fragment of the <i>hph</i> gene (503 bp)
P 14 GAAAAGTTCGACAGCGTCTCC		
P 15 GGGTAGTTGGCATGAAAG		Confirm whether the <i>hph</i> genes homologously replaced the <i>Mtg2</i> gene of <i>B. cinerea</i> (2154bp)
P 16 GTACTGCCGATAGTGGAA		
P 17 TCATTGGATGCTGGGTAG		Confirm whether the <i>hph</i> genes homologously replaced the <i>Mtg2</i> gene of <i>B. cinerea</i> (2049bp)

P 18 CTTATGGATCTCGGTAGTGC	
P19 CTGCAGAAccaccatgtggGTCGACAGAAGATGATATTG	Amplify the NEO cassette containing a trpC promoter (1181 bp)
P20 CCGctcgagTCAGAAGAACTCGTCAAGAAGGCG	
P21 TCCcccgggGGAATTGGTTCCGAGGTA	Amplify the <i>Mtg2</i> gene (include the control region of the <i>Mtg2</i> gene) (2614 bp)
P22 GCtctagaGC ATCCAGCGTTCTGTGTA	
P23 TATCTGTCACAAGTGGCGTAT	Amplify a probe for Southern blotting (241 bp)
P24 AAGAATGTTCCCTCCTCCC	
P25 TAGGTGATTGGGACAACAGAG	Amplify the <i>BcBos1</i> gene for quantitative real-time PCR
P26 GTCTCTCAATGGTGCAGATAG	
P27 GAAGGATGGAAGGGTCTGTAG	Amplify the <i>BcSAk</i> gene for quantitative real-time PCR
P28 CTAACTCGGAGAAAGCCTGATG	
P29 GCTACGCCATGGGAAGTAATG	Amplify the <i>BcMkk1</i> gene for quantitative real-time PCR
P30 CTACTTCGCTTCCTCCACTTG	
P31 GGCAAGTTGAAGGAGCAAATC	Amplify the <i>BcGls</i> gene for quantitative real-time PCR
P32 ATCTGGTGCCTGTGTGATAG	
P33 CGTCTGGATTGGTGGTTCTATT	Amplify the reference gene actin for quantitative real-time PCR
P34 ACTCGTCGTACTCTGCTTTG	
P35 CGggatccCGATGCCTCCACGTTGTTCAAC	the full cDNA sequence of the <i>BcMtg2</i> gene for construction of pYES2-BcMtg2
P36 GCtctagaGCTCATCCATCAAGAAGACCAAC	