Additional file 1:

Suppressing a plant-parasitic nematode with fungivorous behavior by fungal transformation of a Bt cry gene

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Methods

**PCR amplifying DNA fragments of cry5Ba3Φ gene with different lengths**

Primers prepared for amplification of cry5Ba3Φ fragments were listed following:

*cry5Ba3Φ* aa 74–698 (1,875bp):

74–698F:
TTCTACCCAAGCATCCAAGATATGAAGGCTTCCATCTCCCTCATC

74–698R:
TCCCCGTCGGCATCTACTGATTTATTGAATCTTTTGGAAACGAATTCA

*cry5Ba3Φ* aa 115–698 (1,758bp):

115–698F:
TTCTACCCAAGCATCCAAGATATGCAACTCTTCAACGCTATCATGG

115–698R:
TCCCCGTCGGCATCTACTGATTTATTGAATCTTTTGGAAACGAATTCA

*cry5Ba3Φ* aa 202–698 (1,497bp):

202–698F:
TTCTACCCAAGCATCCAAGATATGCTACCATCAACGAACTCTACC

202–698R:
TCCCCGTCGGCATCTACTGATTTATTGAATCTTTTGGAAACGAATTCA

*cry5Ba3Φ* aa 1–572 (1,719bp):

1–572F:
TTCTACCCAAGCATCCAAGATATGGCTACCATCAACGAACTCTACC

1–572R:
TCCCCGTCGGCATCTACTGATTTATTGAATCTTTTGGAAACGAATTCA

*cry5Ba3Φ* aa 1–560 (1,683bp):

1–560F:
PCR reaction consisted of 0.2 µg of pTFCM-cry5Ba3Φ template, 2.5 U Pfu DNA polymerase, 5 µl 10× PCR buffer, 4 µl 12.5× dNTPs and 5 µl of 10 µmol/L each primer, adding ddH2O to 50 µl. The cycling conditions were as follows: an initial denaturation of 2 min at 94 °C, followed by 25 cycles of 30 s for denaturation at 94 °C, 30 s for annealing at 55 °C, and 1 min for polymerization at 72 °C, with a final extension of 72 °C for 7 min.

PCR amplifying pTFCM-TRP vector backbone

pTFCM-phiF/-phiR was used as primers (pTFCM-phiF: 5ʹ-TACCTATTCTACCCAGATATGCTACCATCAACGAAGTTTACCTACC-3ʹ; pTFCM-phiR: 5ʹ-TTGGATGCTTGGGTAGAATAGGT-3ʹ) to amplify pTFCM-TRP vector backbone, which resulted in an approximately 11 kb product of pTFCM including trpC promoter and terminator. PCR reaction consisted of 0.2 µg of pTFCM-cry5Ba3Φ template, 2.5 U Pfu DNA polymerase, 5 µl 10× PCR buffer, 4 µl 12.5× dNTPs and 5 µl of 10 µmol/L each primer, adding ddH2O to 50 µl. The cycling conditions were as follows: an initial denaturation of 2 min at 94 °C, followed by 25 cycles of 30 s for denaturation at 94 °C, 30 s for annealing at 55 °C, and 5 min for polymerization at 72 °C, with a final extension of 72 °C for 7 min.
PCR Certification of *B. cinerea* with truncated *cry5Ba3Φ*

Genomic DNAs of the 6 *B. cinerea* transformant strains were extracted for PCR amplification to certify the presence of truncated *cry5Ba3Φ* genes, using primers IDF (5’- ACTAGTCATTGCAGATGAGCTG-3’) and IDR (5’- ACTAGTCATTGCAGATGAGCTGTATCTGGA-3’). PCR reaction consisted of 0.2 μg of DNA template, 1 U Pfu DNA polymerase, 1 μl 10× PCR buffer, 0.8 μl 12.5× dNTPs and 1 μl of 10 μmol/L each primer, adding ddH2O to 10 μl. The cycling conditions were as follows: an initial denaturation of 2 min at 94 °C, followed by 25 cycles of 30 s for denaturation at 94 °C, 30 s for annealing at 55 °C, and 1 min for polymerization at 72 °C, with a final extension of 72 °C for 7 min.
**Figures**

**Figure S1. Phylogenetic analysis of Cry5Ba3 with other homologous cry5 subfamily proteins.** DNA sequences were translated into amino acid data and were outputted for tree construction using the neighbor-joining (NJ) method. Cyt1Aa1 was set as the outgroup to root the phylogeny. The bootstrap test was used to value the relative support for each node with 1000 replicates. GenBank accession numbers were presented after protein names.
Figure S2. Codon modification of *cry5Ba3* to *cry5Ba3Φ*.
Figure S3. Fungal colony morphologies. (a) Wild-type *Botrytis cinerea*. (b) *cry5Ba3Φ*-transgenic *Botrytis cinerea*. (c) The ninth-generation strain of *cry5Ba3Φ*-transgenic *Botrytis cinerea*. 

(a) Wild-type *Botrytis cinerea*. (b) *cry5Ba3Φ*-transgenic *Botrytis cinerea*. (c) The ninth-generation strain of *cry5Ba3Φ*-transgenic *Botrytis cinerea*. 

(a) (b) (c)
Figure S4. Fungal colony morphologies of *Botrytis cinerea* transformants with different lengths of cry5Ba3Φ. (a) *Botrytis cinerea* (pTFCM-cry5Ba3Φ aa 74–698). (b) *Botrytis cinerea* (pTFCM-cry5Ba3Φ aa 115–698). (c) *Botrytis cinerea* (pTFCM-cry5Ba3Φ aa 202–698). (d) *Botrytis cinerea* (pTFCM-cry5Ba3Φ aa 1–572). (e) *Botrytis cinerea* (pTFCM-cry5Ba3Φ aa 1–560). (f) *Botrytis cinerea* (pTFCM-cry5Ba3Φ aa 74–572).