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|---|--|-----|
| BAS2 (<i>C. gloeosporioides</i> (<i>H. brasiliensis</i>)) | MVRI . TLF T T L A L A N T A F A Q I K P N N A G A S K V G K G D G S C F I I T G G C V S D A D C S | 50 |
| ELA38255.1 (<i>C. gloeosporioides</i> Nara gc5) | MVRI . TLF T T L A L A N T A F A Q I K P N N A G A S K V G K G D G S C F I I T G G C V S D A D C S | 50 |
| XP_018155336.1 (<i>C. higginsianum</i> IMI 349063) | MVRV . T L L A T L A F A A T A F A Q I T P N K A G S S N V G K G D G S C F I I T G G C V D D S D C S | 50 |
| XP_008095160.1 (<i>C. graminicola</i> M1.001) | MVRI . T L F A T I A F A A T A F S . L S P N N A G A R N V G K G D G S C F I I T G G C V D N A D C S | 49 |
| XP_003717663.1 (<i>M. oryzae</i> 70-15) | MVRV S T F A A I L A M A L S V T A N V T P N D A G A K N V G T G N G C C F I I T G G C V N G T D C Q | 51 |
| CDP29900.1 (<i>P. anserina</i> S mat+) | MVRI . T V T A L L A F V V T A M A Q I T P N N A G A R N V G C G N G S C F I I T G G C V N N A D C A | 50 |
| | | |
| BAS2 (<i>C. gloeosporioides</i> (<i>H. brasiliensis</i>)) | S A C C A . N A S G V G V C S A E A A C F C N G K N G C G F D D P N A A A T I A A A C A Q A K K C G | 99 |
| ELA38255.1 (<i>C. gloeosporioides</i> Nara gc5) | S A C C A . N A S G V G V C S A E A A C F C N G K N G C G F D D P N A A A T I A A A C A Q A K K C G | 99 |
| XP_018155336.1 (<i>C. higginsianum</i> IMI 349063) | S A C C A . D A S G V G V C S A E A A C F C N G K N G C N F V D P N R E A T I A A A C A Q A E K C G | 99 |
| XP_008095160.1 (<i>C. graminicola</i> M1.001) | S A C C A . N L S G V G I C S A E A A C F C N G K K G C N F V D P N K D A T I A A A K A Q V K K C G | 98 |
| XP_003717663.1 (<i>M. oryzae</i> 70-15) | S R C C A G N G E N K G V C S N E V A A N C N G K T G C G F E D P N K A C T V K E A K E Q V K K C G | 101 |
| CDP29900.1 (<i>P. anserina</i> S mat+) | S G C C A . D A S G V G V C S A E A A C F C N G K N G C G F V D P N A C C T I A A A C A Q V A R C G | 99 |

Figure S1. Alignment of amino acid sequences of BAS2 of *C. gloeosporioides* from *H. brasiliensis*, *C. gloeosporioides* Nara gc5, *C. higginsianum*, *C. graminicola*, *M. oryzae*, and *P. anserina*.

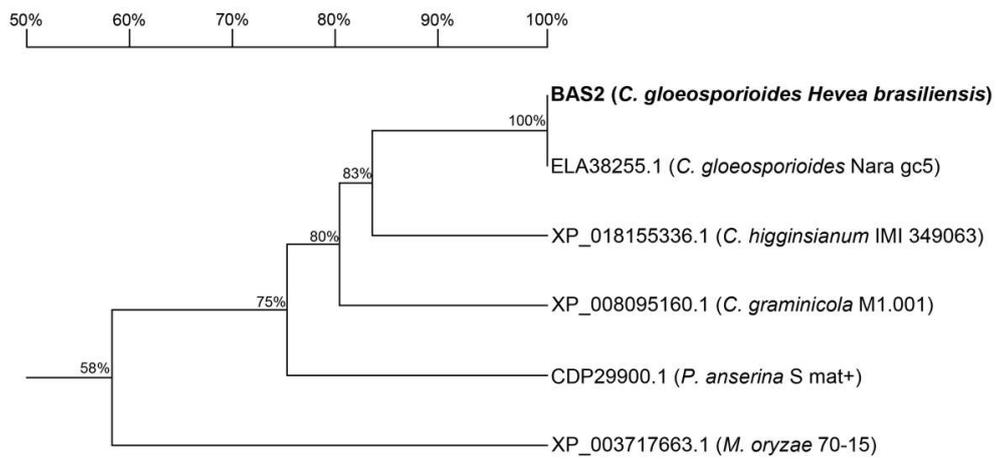


Figure S2. Phylogenetic analysis of BAS2 proteins. The neighbor joining phylogenetic tree was constructed by MEGA 7.0 according to the evolutionary relationship between BAS2 proteins in *C. gloeosporioides* from *H. brasiliensis*, *C. gloeosporioides* Nara gc5, *C. higginsianum*, *C. graminicola*, *M. oryzae*, and *P. anserina*.

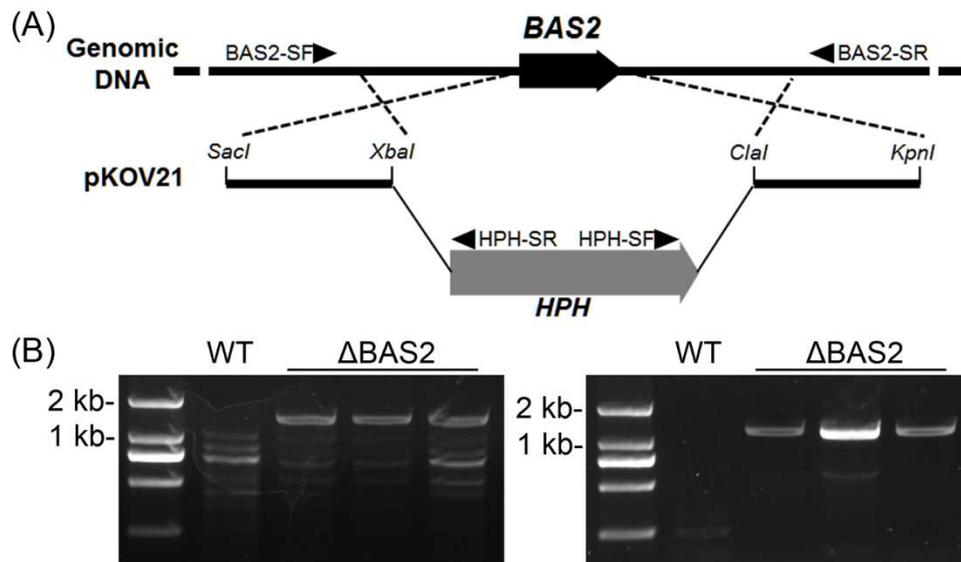


Figure S3. (A) The gene deletion strategy. Putative mutants were screened with diagnostic primers, indicated by black triangles. (B) Confirmation of the correct recombination of deletion cassettes with gene loci by Southern blot.

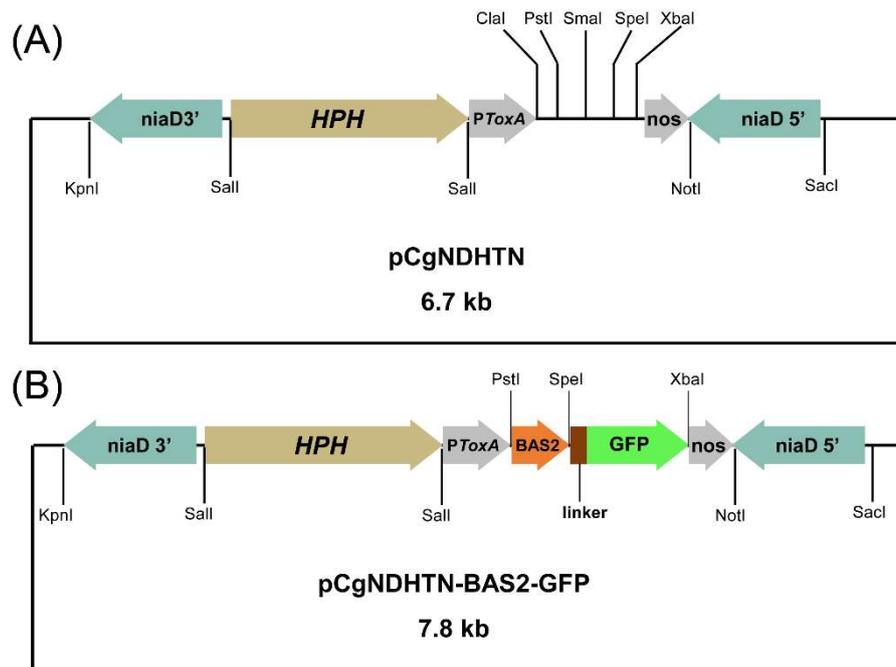


Figure S4. Strategy for generation of BAS2-GFP fusion overexpressing mutants. (A) Expression system using the gene loci of nitrate reductase (*niaD*). Promoter of ToxA and terminator *nos* were used for the gene expression; the Hygromycin phosphotransferase gene (*HPH*) was used for transformant selection. (B) The open reading frame of *BAS2* and the coding sequence of GFP with an N-terminal linker were linked together to construct the fusion expressing vector.

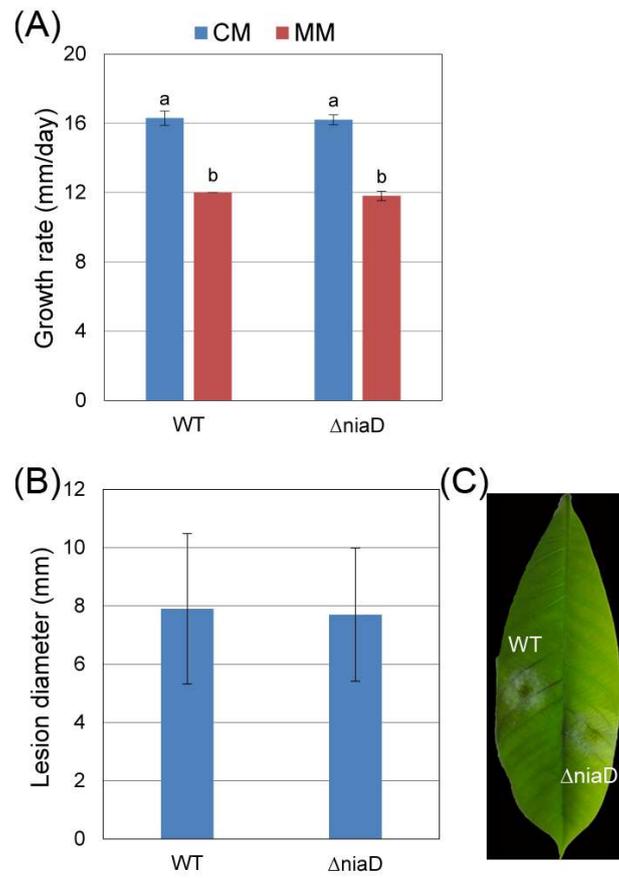


Figure S5. Phenotype assays of Δ niaD. **(A)** Growth rate assay of WT and Δ niaD cultured on complete medium (CM) and minimal medium (MM). **(B)** Mean lesion diameters after inoculation with conidia for 3 days. Bars represent standard deviation (SD). **(C)** Disease symptoms of rubber-tree leaves after inoculation with conidia suspension for 3 days.

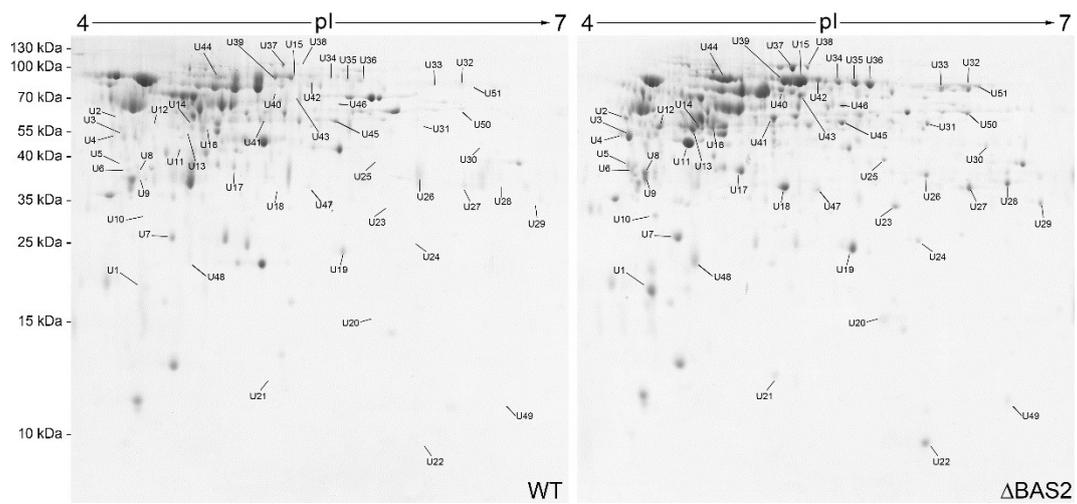


Figure S6. Two-dimensional patterns of extracellular proteomes of WT and Δ BAS2. Arrows indicate protein spots that were upregulated in abundance more than 1.5-fold between WT and Δ BAS2.

Table S1. PCR primers used in this study. Lowercase letters indicate induced restriction sites.

| Number | Primer | Sequence (5'→3') | Application |
|--------|-----------|---|--|
| 1 | BAS2-5F | cgagctcTCGCAAAAATGTTCCAG | BAS2 deletion |
| 2 | BAS2-5R | gctctagaTTTCGCGGTAGTTGAGTG | BAS2 deletion |
| 3 | BAS2-3F | ccatcgatGCTGGAAAATGCAGAAACT | BAS2 deletion |
| 4 | BAS2-3R | ggggtaccAACATGGGGCAGGAGAC | BAS2 deletion |
| 5 | BAS2-JC5F | GCGCATTCTTTGAGGTTTCTTG | ΔBAS2 diagnosis |
| 6 | HYG-JCR | TGAGTTCAGGCTTTTTTCATTTGG | ΔBAS2 diagnosis |
| 7 | HYG-JCF | ACAGCGGTCATTGACTGGAGCGA | ΔBAS2 diagnosis |
| 8 | BAS2-JC3R | AAGGGCGGCGACAGTGAAGAGG | ΔBAS2 diagnosis |
| 9 | niaD-5F | gagctcAAGGAGTCCCGTTTGT | <i>niaD</i> deletion, expression system |
| 10 | niaD-5R | gcggccgcACTGACGACTGGCTTGTC | <i>niaD</i> deletion, expression system |
| 11 | niaD-3F | gtcgacACGAGCTGCCGTTTTTAG | <i>niaD</i> deletion, expression system |
| 12 | niaD-3R | ggtaccCGGTCACGACGCTGTAA | <i>niaD</i> deletion, expression system |
| 13 | niaD-JC5F | TGCCAGTAGCGTGGTTTAGGTC | ΔniaD diagnosis |
| 14 | niaD-JC5R | tctagaAATTTCCCGATCGTTC | ΔniaD diagnosis |
| 15 | niaD-JC3F | ACAGCGGTCATTGACTGGAGCGA | ΔniaD diagnosis |
| 16 | niaD-JC3R | AGTGTCACAGATGTCGTGTTGC | ΔniaD diagnosis |
| 17 | Ptoxa-F | gtcgacTGGAATGCATGGAGGAG | Expression system of <i>C. gloeosporioides</i> |
| 18 | Ptoxa-R | atcgatGACCTATATTCATTCAT | Expression system of <i>C. gloeosporioides</i> |
| 19 | Tnos-F | tctagaAATTTCCCGATCGTTC | Expression system of <i>C. gloeosporioides</i> |
| 20 | Tnos-R | gcggccgcCCGATCTAGTAACATAG | Expression system of <i>C. gloeosporioides</i> |
| 21 | HPH-F | gtcgacAACTGATATTGAAGGAG | Expression system of <i>C. gloeosporioides</i> |
| 22 | HPH-R | gtcgacAACTGGTTCCCGGTCGG | Expression system of <i>C. gloeosporioides</i> |
| 23 | cBAS2-F1 | ctgcagATGGTCCGCATCACTCT | BAS2-GFP fusion expressing mutant |
| 24 | cBAS2-R1 | actagtGAAACCTTGCTTCTTGG | BAS2-GFP fusion expressing mutant |
| 25 | GFPlink-F | actagtGGAGCTGGTGCAGGCGCTGGAGC CGGTGCCATGGTGAGCAAGGGCGA | BAS2-GFP fusion expressing mutant |

| | | | |
|----|----------|--------------------------|---|
| 26 | GFP-R | tctagaTTACTTGTACAGCTCGT | BAS2-GFP fusion expressing mutant |
| 27 | cBAS2-F2 | tctagaATGGTCCGCATCACTCT | Transient expression in rubber-tree protoplasts |
| 28 | cBAS2-R2 | gagctctGAAACCTTGCTTCTTGG | Transient expression in rubber-tree protoplasts |
