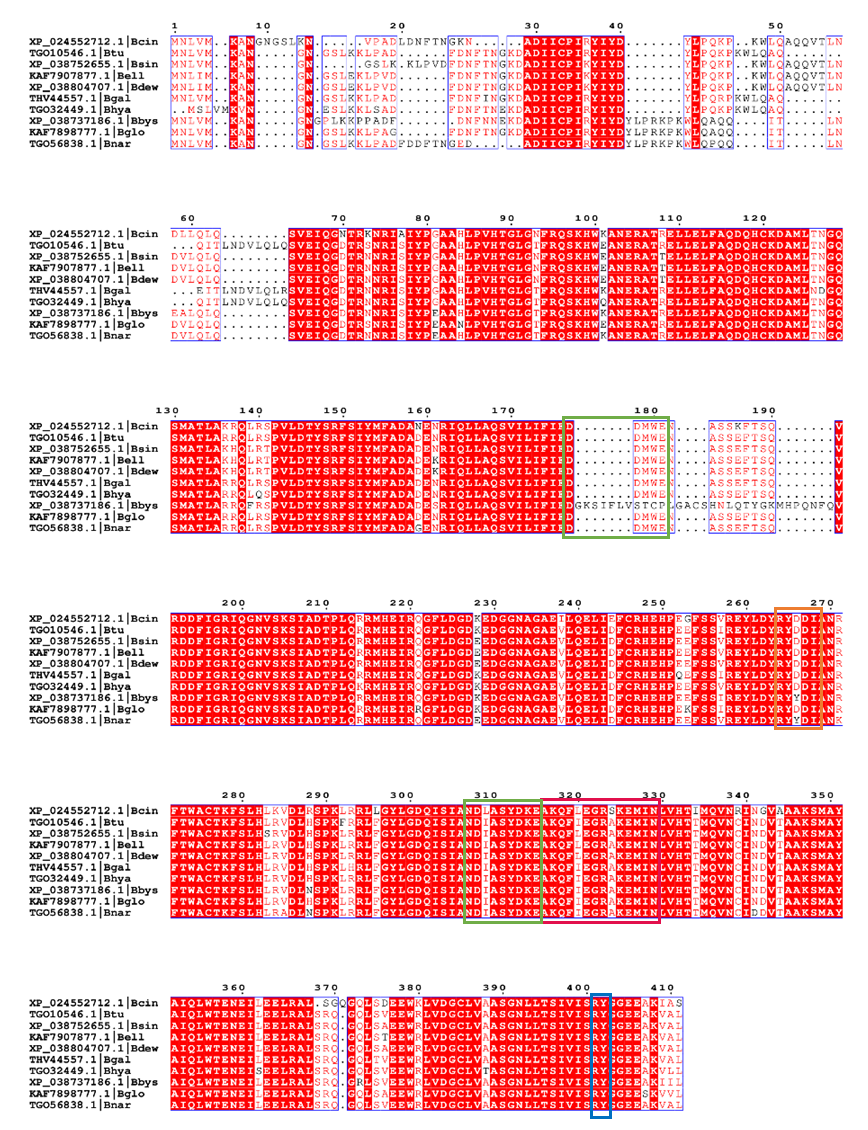
**Figure S1. Alignment of BcStc3 and homologous protein sequences of other *Botrytis* species.** Protein sequences were selected after running similarity search by BlastP using BcStc3 as query sequence. Multiple alignments of protein sequences were carried out with Clustal-Omega and were visualized using ESPript 3.0. Green boxes indicate the two conserved magnesium binding motifs. Orange box denotes conserved effector triad. Blue box indicates conserved R and Y residues involved in substrate binding. Red box indicates H1α-loop ending at the conserved asparagine (N329) residue. Bcin, *Botrytis cinerea;* Btu, *Botrytis tulipae*; Bsin, *Botrytis sinoallii*; Bell, *Botrytis elliptica*; Bdew, *Botrytis deweyae*; Bgal, *Botrytis galanthina*; Bhya, *Botrytis hyacinthi*; Bbys, *Botrytis byssoidea*; Bglo, *Botryotinia globosa*; Bnar, *Botryotinia narcissicola.* NCBI accession number of each sequence is shown.



**Figure S2**. **Gene expression of the *Bstc* gene family in the *B. cinerea* B05.10 strain. (A)** Relative expression levels of the *Bcstc* genes in ungerminated conidia. mRNA levels were quantified by qRT-PCR and normalized to *actA* and *B-tub* expression. Data are plotted as relative expression, compared to the *Bcstc1* expression level, set as 1. **(B)** Relative expression levels of the *Bcstc* genes in axenic culture and *in planta*. mRNA levels were quantified by qRT-PCR in mycelium grown in YGG medium for 96 hours (axenic culture) and in infected tissue of *Nicotiana tabaccum* leaves 96 hours post-inoculation with a conidial suspension (*in planta*). Values were normalized to *actA* and *B-tu*b genes expression and data are plotted as relative expression, compared to the expression level of each gene in ungerminated conidia. Error bars show standard deviation of three biological replicates (n = 3). Different letters above the bars in A mean that there are significant differences between genes with a *p*-value<0.05. \* in B means the existence of significant differences between axenic and *in planta* culture for each gene with a *p*-value<0.05.

Gráfico

Descripción generada automáticamente

Interfaz de usuario gráfica, Aplicación

Descripción generada automáticamente**Figure S3. Virulence assays.** Different hosts were inoculated with 5 µL droplets containing 2500 conidia of the indicated strain in TGGK solution, incubated in the dark at 20°C under conditions of high humidity and the degree of damage was estimated. **(A)** The damage on inoculated tomato fruits was estimated using a qualitative scale ranging from 1 (very good appearance) to 4 (bad appearance) and results are presented as the percentage of fruits in each disease grade to the total number of infected fruits at 2, 3 and 4 days post-inoculation. **(B)** Aqualitative scale ranging from 1 (very good) to 4 (bad appearance) was used to estimate the damage on inoculated grapefruits and results are presented as the percentage of fruits in each disease grade to the total number of infected fruits at 2-, 3- and 4-days post-inoculation. **(C)** Representative images of the lesions produced by each fungal strain on gerbera and **(D)** detached tobacco leaves 4 days after inoculation.

**Table S1.** Oligonucleotides used in this work.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primer** | **Sequence (5’ ⭢ 3’)** | **Features** | **Used for** | **References** |
| ***Hph*-F** | GTCGGAGACAGAAGATGATATTGAAGGAGC | P*trpC* | Cloning – amplification of P*trpC*::*hph* for Δ*bcstc3* construct | [1] |
| ***Hph*-R** | GTTGGAGATTTCAGTAACGTTAAGTGGAT | *Hph* | Cloning – amplification of P*trpC*::*hph* for Δ*bcstc3* construct | [1] |
| ***TrpC*-P2** | CCTCCACTAGCTCCAGCCAAGCCC | P*trpC* | Diagnostic PCR – homologous integration at *bcstc3-*5' | [1] |
| ***TrpC*-T** | GGAATAGAGTAGATGCCGACCGG | T*trpC* | Diagnostic PCR – homologous integration at *bcstc3-*3' | [1] |
| ***Bcstc3-*5F** | GTAACGCCAGGGTTTTCCCAGTCACGACGTTTGTCTGCCCTTTGACGGA | pRS426-5F – *bcstc3*-5’ | Cloning KO construct– amplification of 5' flank for Δ*bcstc3* construct | This study |
| ***Bcstc3-*5R** | ATCCACTTAACGTTACTGAAATCTCCAA CCCAAGCCGTTAATGATTCG | *Hph – bcstc3*-5’ | Cloning – amplification of 5' flank for Δ*bcstc3* construct | This study |
| ***Bcstc3-*3R** | GCGGATAACAATTTCACACAGGAAACAGCATCTGGCGCAATAAGGAGTG | pRS426-3R – *bcstc3*-3’ | Cloning – amplification of 3' flank for Δ*bcstc3* construct | This study |
| ***Bcstc3-*3F** | CTCCTTCAATATCATCTTCTGTCTCCGACCTACTGAAATAGCTTCCTTGCAC | P*trpC – bcstc3*-3’ | Cloning – amplification of 3' flank for Δ*bcstc3* construct | This study |
| ***Bcstc3-*hi5F** | GGAGGACGCATATAGATCCA | *Bcstc3-*5’ | Diagnostic PCR – homologous integration at *bcstc3-*5' | This study |
| ***Bcstc3-*hi3R** | GTGGAGGAATCTGGACTTGA | *Bcstc3*-3’ | Diagnostic PCR – homologous integration at *bcstc3-*3' | This study |
| ***Bcstc3*-WT-F** | CTTGCCAAACGACAGCTCCG | *Bcstc3* ORF | Diagnostic PCR – detection of *bcstc3* alleles | This study |
| ***Bcstc3*-WT-R** | CCCGTACAGATGAAAATCCC | *Bcstc3* ORF | Diagnostic PCR – detection of *bcstc3* alleles | This study |
| **PactA-amp-F** | GGGGTTGATAAATTAAGACGTTAAGTCCTATTACCGTATTCATTTGG | PactA – bcstc3-5’ | Cloning – amplification of PactA for overexpression construct and Diagnostic PCR – homologous integration at bcniaD-3' | This study |
| **PactA-PtrpC-R** | GCCCAAAAAATGCTCCTTCAATGTCACTAGTTGTGCGTCCTCTTCTGCCTACCCA | PtrpC – PactA | Cloning – amplification of PactA for overexpression construct | [1] |
| **TtrpC-Bcstc3-F** | TGACATGGAGCTATTAAATCACTAAGCGGCCGCTGGTACTTCTTTAAGCTTAGT | TtrpC – bcstc3 | Cloning – amplification of bcstc3 locus for overexpression construct | This study |
| **PactA-*Bcstc3*-R** | GACTTAACGTCTTAATTTATCAACCCCGAACTTTCCAAATGAATACGGTAATAG | PactA – bcstc3 | Cloning – amplification of bcstc3 locus for overexpression construct and Diagnostic PCR – homologous integration at bcniaD-5' | This study |
| ***BcniaD*-5F** | GTGAATGGGATTCATTGTTTATTTC | *BcniaD-5’* | Cloning – amplification of overexpression fragment | [1] |
| ***BcniaD*-3R** | GCATTGGATTAATAATTGTTGCTAAGC | *BcniaD*-3’ | Cloning – amplification of overexpression fragment | [1] |
| ***BcniaD-*hi5F** | CGCATATCAGCATATCGAGATGTCC | *BcniaD* locus | Diagnostic PCR – homologous integration at *bcniaD*-5' | [1] |
| ***BcniaD-*hi3R** | GAGTACCCATCCGATGGAGTTGTTG | *BcniaD* locus | Diagnostic PCR – homologous integration at *bcniaD*-3' | [1] |
| ***BcniaD-WT-F*** | GCCACAGACTCCGCCAGATTCTAATG | BcniaD locus | Diagnostic PCR – detection of BcniaD alleles | [1] |
| ***BcniaD-WT-R*** | CAACCATTTCACGCTGCGACCACC | BcniaD locus | Diagnostic PCR – detection of BcniaD alleles | [1] |
| **Bcstc1fw** | TATGAGCAGCAACAGTACGG | Bcstc1-5’ | mRNA level quantification of the gene *Bcstc1* | [2,3] |
| **Bcstc1rv** | GCTGCTTCAATTCCTGGGTG | Bcstc1-3’ | mRNA level quantification of the gene *Bcstc1* | [2,3] |
| **Bcstc2fw** | CATCATCGATGATTGCGTCC | Bcstc2-5’ | mRNA level quantification of the gene *Bcstc2* | [2,3] |
| **Bcstc2rv** | GAACCTTCATCCAACCATCC | Bcstc2-3’ | mRNA level quantification of the gene *Bcstc2* | [2,3] |
| **Bcstc3fw** | GGAAAGCAAATGAGAGGGCG | Bcstc3-5’ | mRNA level quantification of the gene *Bcstc3* | [2,3] |
| **Bcstc3rv** | TGTCCATTGGTTAGCATGGC | Bcstc3-5’ | mRNA level quantification of the gene *Bcstc3* | [2,3] |
| **Bcstc4fw** | ATCCAGTACCAGAGGTACCC | Bcstc4-5’ | mRNA level quantification of the gene *Bcstc4* | [2,3] |
| **Bcstc4rv** | CACGCGTACGCTATTTACGC | Bcstc4-3’ | mRNA level quantification of the gene *Bcstc4* | [2,3] |
| **Bcstc5fw** | GCACGAGTTGTTAAGAAGG | Bcstc5-5’ | mRNA level quantification of the gene *Bcstc5* | [2,3] |
| **Bcstc5rv** | CCATCTGAATTGCCTTCTGC | Bcstc5-3’ | mRNA level quantification of the gene *Bcstc5* | [2,3] |
| **Bcstc7fw** | TGTATGAGGTGCTGCACTGG | Bcstc7-5’ | mRNA level quantification of the gene *Bcstc7* | [2,3] |
| **Bcstc7rv** | GACTTCATTCTGATGACGCG | Bcstc7-3’ | mRNA level quantification of the gene *Bcstc7* | [2,3] |
| **BcactAfw** | TTTGAGACCTTCAACGCCCC | BcactA-5’ | mRNA level quantification of the gene *BcactA* | [2,3] |
| **BcactArv** | ACGTGAGTAACTCCGTCACC | BcactA-3’ | mRNA level quantification of the gene *BcactA* | [2,3] |
| **Bctubfw** | TCCTTTCGGTCAACTCTTCCG | Bctub-5’ | mRNA level quantification of the gene *Bctub* | [2,3] |
| **Bctubrv** | CACCCTCAGTGTTAATGACCC | Bcstub-3’ | mRNA level quantification of the gene *Bctub* | [2,3] |

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