Host-induced gene silencing of \textit{BcTOR} in \textit{Botrytis cinerea} enhances plant resistance to grey mould

FANGJIE XIONG\textsuperscript{1,2,\#}, MEI LIU\textsuperscript{1,2,\#}, FENGPING ZHUO\textsuperscript{1,2,3,\#}, HUAN YIN\textsuperscript{1,2}, KEXUAN DENG\textsuperscript{1,2}, SHUN FENG\textsuperscript{1,2}, YUDONG LIU\textsuperscript{1,2}, XIUMEI LUO\textsuperscript{1,2,\#}, LI FENG\textsuperscript{1,2}, SHUMIN ZHANG\textsuperscript{1,2}, ZHENGGUO LI\textsuperscript{1,2} AND MAOZHI REN\textsuperscript{1,2,4,*}

\textsuperscript{1}School of Life Sciences, Chongqing University, Chongqing 401331, China
\textsuperscript{2}Key Laboratory of Plant Hormone and Developmental Regulation of Chongqing, Chongqing 401331, China
\textsuperscript{3}School of Chemistry and Chemical Engineering, Chongqing University of Science and Technology, Chongqing 401331, China
\textsuperscript{4}Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences/National Chengdu Agricultural Science and Technology Center, Chengdu 610000, China

\section*{SUMMARY}

\textit{Botrytis cinerea} is the causal agent of grey mould for more than 200 plant species, including economically important vegetables, fruits and crops, which leads to economic losses worldwide. Target of rapamycin (TOR) acts as a master regulator to control cell growth and proliferation by integrating nutrient, energy and growth factors in eukaryotic species, but little is known about whether TOR can function as a practicable target in the control of plant fungal pathogens. Here, we characterize TOR signalling of \textit{B. cinerea} in the regulation of growth and pathogenicity as well as its potential value in genetic engineering for crop protection by bioinformatics analysis, pharmacological assays, biochemistry and genetics approaches. The results showed that conserved TOR signalling occurs, and a functional FK506-binding protein 12 kD (FKBP12) mediates the interaction between rapamycin and \textit{B. cinerea} TOR (BcTOR). RNA sequencing (RNA-Seq) analysis revealed that BcTOR displayed conserved functions, particularly in controlling growth and metabolism. Furthermore, pathogenicity assay showed that BcTOR inhibition efficiently reduces the infection of \textit{B. cinerea} in plant leaves of \textit{Arabidopsis} and potato or tomato fruits. Additionally, transgenic plants expressing double-stranded RNA of \textit{BcTOR} through the host-induced gene silencing method could produce abundant small RNAs targeting \textit{BcTOR}, and significantly block the occurrence of grey mould in potato and tomato. Taken together, our results suggest that BcTOR is an efficient target for genetic engineering in control of grey mould, and also a potential and promising target applied in the biocontrol of plant fungal pathogens.

\textbf{Keywords:} \textit{Botrytis cinerea}, host-induced gene silencing, mycelial growth, pathogenicity, target of rapamycin.

\section*{INTRODUCTION}

\textit{Botrytis cinerea}, a widespread necrotrophic fungal plant pathogen that can infect more than 200 plant species, including economically important vegetables, fruits and crops, and lead to grey mould rot or botrytis blight, causes widespread losses every year worldwide (Choquer \textit{et al.}, 2007; Dean \textit{et al.}, 2012; Williamson \textit{et al.}, 2007). More than 40% of these losses occur in greenhouse-grown and field-grown crops if chemical control is not used (Pedras \textit{et al.}, 2011; Villa-Rojas \textit{et al.}, 2012). Thus, chemical pesticides are widely required for the control of \textit{B. cinerea}. However, the widespread use of chemical pesticides results in serious water, soil, food and environmental pollution (Malhat \textit{et al.}, 2015; Oliveira \textit{et al.}, 2015; Tomenson and Matthews, 2009). To overcome this issue, biofungicides with low toxicity and high efficiency against key targets of pathogens have been developed and applied to control plant fungal disease. In addition, genetic engineering methods have also been extensively employed to improve resistance to plant pathogens in crop protection. For example, host-induced gene silencing (HIGS), an RNA interference (RNAI)-based approach in which small RNAs (sRNAs) are produced by the host plant to target invader transcripts, has emerged as an effective strategy for improving plant resistance against pathogens (Cai \textit{et al.}, 2018; Wang, Thomas \textit{et al.}, 2017).

HIGS has been widely used for crop protection and a wide range of transgenic crops acquire durable resistance against diseases by expressing double-stranded RNA (dsRNA) that is subsequently processed into sRNAs targeting essential genes regulating growth or pathogenicity (Cheng \textit{et al.}, 2015; Ghag \textit{et al.}, 2014; Govindarajulu \textit{et al.}, 2015; Koch \textit{et al.}, 2013; Nowara \textit{et al.}, 2010; Nunes and Dean, 2012; Xu \textit{et al.}, 2018; Zhang \textit{et al.}, 2016; Zhu \textit{et al.}, 2017). For instance, engineered corn plants expressing a vacuolar ATPase (V-ATPase) dsRNA can be protected from western corn rootworm feeding damage; durable resistance to Fusarium head blight and seedling blight can be acquired by...
of TOR to form a ternary complex, resulting in abolishment of interacts with the FKBP12-rapamycin binding domain (FRB). FKBP12 forms a binary complex with rapamycin and further dicer-like genes of B. cinerea and V. dahliae exhibited enhanced resistance to both pathogens (Wang et al., 2016).

Target of rapamycin (TOR), a Ser/Thr protein kinase of large molecular weight with multiple functional domains, is a well-known essential gene in eukaryotic species, but little is known about whether or not TOR can be developed and established as a key target for biological control of plant fungal pathogens. TOR was first discovered in yeast (Saccharomyces cerevisiae) by a genetic screen of mutants insensitive to rapamycin (Heitman et al., 1991). Rapamycin, a well-known medicine produced by Streptomyces hygroscopicus, is able to efficiently repress TOR kinase activity. FK506 binding protein 12 kD (FKBP12), as a receptor of rapamycin, mediates inhibition of TOR by rapamycin and its defects confer resistance to rapamycin (Heitman et al., 1991). In the presence of rapamycin, FKBP12 forms a binary complex with rapamycin and further interacts with the FKBP12-rapamycin binding domain (FRB) of TOR to form a ternary complex, resulting in abolishment of TOR activity (Chiu et al., 1994; Heitman et al., 1991; Koltin et al., 1991; Sabatini et al., 1994; Vezina et al., 1995). Based on the rapamycin-FKBP12-TOR system, the TOR signalling pathway has been extensively studied and gradually elucidated in yeast and mammals (Benjamin et al., 2011). The TOR protein consists of five highly conserved domains: HEAT repeats (Huntingtin, elongation factor 3 (EF3), a subunit of protein phosphatase 2A (PP2A) and TOR1), a FAT (FRAP, ATM and TRRAP) domain, a FRB (FKBP12-rapamycin binding) domain, a kinase and a FATC (carboxy-terminal FAT) domain, which reside in the TOR protein between the N-terminal and the C-terminal (Baretic and Williams, 2014; Sauer et al., 2013; Yang et al., 2013). Most eukaryotic organisms have one copy of the TOR gene, but two and three TOR genes have also been identified in yeast and Leishmania major, respectively (Heitman et al., 1991; Madeira da Silva and Beverley, 2010). In some eukaryotes, based on diverse components recruited by TOR, the TOR protein can form two different types of multiprotein complex: TOR complex 1 (TORC1) and TOR complex 2 (TORC2). The TOR protein recruits a regulatory-associated protein of TOR (RAPTOR) and a lethal with SEC13 protein8 (LST8) to form TORC1, but combines LST8, stress-activated map kinase-interacting protein 1 (SIN1) and rapamycin-insensitive companion of TOR (RICTOR) to form TORC2 in yeast and animals (Loewith et al., 2002; Wullschleger et al., 2006). Due to this difference, TORC1 is sensitive to rapamycin, but TORC2 shows insensitivity to rapamycin (Loewith et al., 2002). In addition, rapamycin-sensitive TORC1 plays a major role in cell growth, development and proliferation in a temporal manner, while rapamycin-resistant TORC2 seems to particularly regulate the development of the cell cytoskeleton (Feldman et al., 2009; Loewith et al., 2002; Takahara and Maeda, 2013; Wang and Proud, 2009; Wullschleger et al., 2006).

Various studies have shown that TOR is a central coordinator of energy, nutrient and stress signalling networks from yeast to mammals and plants (Dobrenel et al., 2016; Henriques et al. , 2014; Loewith and Hall, 2011; Rexin et al., 2015; Schmelzle and Hall, 2000; Xiong and Sheen, 2015). However, little attention has been paid to TOR signalling study in phytopathogenic fungi are relatively less studied. Rapamycin was discovered in the early 1970s as an antifungal agent against the pathogenic yeast Candida albicans (Sehgal et al., 1975). Subsequent studies also found that rapamycin is effective against many phytopathogenic fungi, such as Botrytis cinerea, Mucor cirrinheloides, Fusarium fusikuroi, Fusarium oxysporum and Fusarium graminearum (Bastidas et al., 2012; Lopez-Berges et al., 2010; Melendez et al., 2009; Teichert et al., 2006; Yu et al., 2014), indicating that a conserved TOR pathway also exists in plant pathogenic fungi. In addition, the research on TOR signalling in F. graminearum elucidated the TOR components and conserved functions in growth and development as well as its role in virulence (Yu et al., 2014). In B. cinerea, it was reported that a functional FKBP12 homologous protein existed with conserved function in bridging rapamycin, and the BcFKBP12 deletion relieved growth inhibition of rapamycin and reduced virulence of the strain T4 while not affecting the pathogenic development of the strain B05.10 (Girot et al., 2006). Additionally, BcFKBP12 was reported to likely be involved in sulphur regulation and this regulation appears to be unrelated to TOR signalling (Melendez et al., 2009). To date, information on the TOR signalling pathway in B. cinerea is very limited, and TOR function remains to be determined.

In the present study, we functionally characterized B. cinerea TOR (BcTOR) in the regulation of vegetative growth and development as well as virulence of the B. cinerea strain B05.10 by bioinformatics analysis, pharmacological assays and chemical genetics approaches. We created transgenic potato and tomato plants expressing dsRNA specific to BcTOR based on the HIGS method, and found that transgenic plants could produce abundant sRNA molecules targeting BcTOR, significantly blocking the occurrence of grey mould caused by B. cinerea. Our results suggest that BcTOR can function as a potential and promising target in the control of grey mould disease.

RESULTS

Molecular components of the TOR complex in B. cinerea

To investigate TOR signalling in B. cinerea, a yeast TOR protein sequence was used to search for the homologous sequences in the B. cinerea genome (http://fungi.ensembl.org/Botrytis_cinerea/
The search results revealed a single TOR homologue gene (named BcTOR), located on the first chromosome of the B. cinerea genome (Fig. 1A). Further analysis showed that the full-length genomic DNA of BcTOR spans about 8.12 kb and contains five exons and four introns in the B. cinerea genome (Fig. 1A), including a 7296 bp full-length coding sequence encoding a protein of 2431 amino acid residues with a predicted molecular mass of 275 kDa. Alignment of BcTOR with TOR protein sequences from other species showed similar domain organization with significant identification and conservation of the FRB and kinase domains as well as the FATC domain at the C-terminus (Fig. 1B), which are conserved in the phosphatidylinositol 3-kinase related protein kinase family (Fruman and Rommel, 2014; Sauer et al., 2013; Schmelzle and Hall, 2000; Yang et al., 2013). Additionally, a conserved motif sequence named HEAT, reported to be involved in protein–protein interactions (Hara et al., 2002; Schmelzle and Hall, 2000), was found to be distributed throughout the N-terminal region of BcTOR (Fig. 1B).

Phylogenetic analysis revealed that BcTOR is evolutionarily conserved and depicts a closer evolutionary relationship with ScTOR1/2 in comparison with other TORs (Fig. 1C). In yeast or

Fig. 1 The information for the TOR homologue in Botrytis cinerea. (A) The gene locus and structure of the TOR homologue in B. cinerea. (B) Domain organization of BcTOR protein and comparison of the BcTOR amino acid sequence with those of TOR proteins from other organisms. (C) Phylogenetic analysis of BcTOR with that from other species. Bc, Botrytis cinerea; Sc, Saccharomyces cerevisiae; Hs, Homo sapiens; At, Arabidopsis thaliana; Dm, Drosophila melanogaster; Ce, Caenorhabditis elegans. Protein domain diagram shows number of HEAT (Huntingtin, elongation factor 3 (EF3), a subunit of protein phosphatase 2A (PP2A) and TOR1) repeats; FAT (FRAP, ATM and TRRAP) domain; and carboxy-terminal FAT (FATC) domain.
animals, TOR protein was reported to form two structurally and functionally distinct multiprotein complexes through recruiting shared and distinct TOR-interacting components (Helliwell et al., 1994; Wullschleger et al., 2006). RAPTOR and LST8, as the core components of TORC1, each depicted one homologous gene through homologous comparison in the B. cinerea genome (Table 1). Additionally, RICTOR, a specific component of TORC2, also was found in the B. cinerea genome (Table 1), illustrating the existence of TORC2. These results indicate the existence of conserved TOR signalling in B. cinerea.

**BcFKBP12 rescues Arabidopsis sensitivity to rapamycin**

Early studies demonstrated that the FKBP12 protein from yeast or humans could restore rapamycin sensitivity in Arabidopsis (Mahfouz et al., 2006; Ren et al., 2012; Sormani et al., 2007; Xiong et al., 2017). Our previous study also indicated that tomato FKBP12 could restore the inhibitory effect of rapamycin to TOR (Xiong et al., 2016). Likewise, in B. cinerea, FKBP12 homologous protein was found with conserved function in bridging rapamycin and FK506, and it was also reported that BcFKBP12 is involved in T4 strain virulence and sulphur regulation (Gioti et al., 2006; Melendez et al., 2009). Here, to further confirm BcFKBP12 function in TOR signalling, we cloned and expressed BcFKBP12 in Arabidopsis. As shown in Fig. 2A, there was no change in morphological phenotypes observed in these transgenic lines as compared with wild-type (WT) plants. However, as expected, the transgenic lines expressing BcFKBP12 showed sensitivity to rapamycin and displayed a retarded growth phenotype with small cotyledons, reduced biomass (fresh weight) and shorter primary roots than that of WT under rapamycin treatment (Fig. 2A–E). In addition, this acquired response was dose-independent (Fig. 2D,2). Similar results were observed in Arabidopsis expressing ScFKBP12, while no difference was found in WT under rapamycin treatment in comparison to DMSO treatment (Fig. 2D,E). These observations further confirm the conserved function of BcFKBP12 in mediating the inhibitory effect of rapamycin on TOR activity.

**BcTOR regulates mycelial growth and conidiation, but not conidial germination of B. cinerea**

Rapamycin is used to study the TOR signalling pathway in eukaryotic species. Previous reports indicated that rapamycin is able to suppress growth mediated by the FKBP12 orthologue in B. cinerea, but there is a lack of detailed description about TOR function in these studies. Here we set up a series of concentrations of rapamycin from 1 nM to 1 μM to observe the inhibition morphology of B. cinerea caused by rapamycin. The results show that radial growth of B. cinerea is severely inhibited on potato dextrose agar (PDA) amended with rapamycin even at a very low dose (1 nM) and nearly ceases growth with about 98% inhibitory rate at 100 nM (Fig. 3A,B,D). Microscopic observation of hyphal morphology showed that hyphae treated with rapamycin had more branches with increasing dose of rapamycin compared with the solvent control (Fig. 3C). Furthermore, twisted hyphal morphology was observed (Fig. 3C), similar to a previous study in F. graminearum (Yu et al., 2014). In accordance with retarded mycelial growth, there was a significant decline in biomass of B. cinerea when treated with rapamycin under liquid culture conditions (Fig. S1). These results suggest

**Table 1**  TORC1 and TORC2 homologues in Botrytis cinerea for various species

|            | Hs | Sc   | Bc    |
|------------|----|------|-------|
| **TORC1**  |    |      |       |
| mTOR       |    | TOR1/2| TOR (Bcin01g11360) |
| RAPTOR     |    | Kog1 | RAPTOR (Bcin06g02850) |
| LST8       |    | Lst8 | LST8 (Bcin04g05980) |
| PRAS40     |    | -    | -     |
| DEPTOR     |    | -    | -     |
| **TORC2**  |    |      |       |
| mTOR       |    | TOR2 | TOR   |
| SIN1       |    | Avo1 | SIN1 (Bcin16g01100) |
| Avo2       |    | -    | -     |
| RICTOR     |    | Avo3 | RICTOR (Bcin03g06820) |
| LST8       |    | Lst8 | LST8  |
| PRR5       |    | Bit61| -     |
| DEPTOR     |    | -    | -     |

Hs, Homo sapiens; Sc, Saccharomyces cerevisiae; Bc, Botrytis cinerea. TORC1, target of rapamycin complex 1; TORC2, target of rapamycin complex 2; mTOR, mammalian target of rapamycin; RAPTOR, regulatory-associated protein of mTOR; LST8, lethal with SEC13 protein8; SIN, stress-activated map kinase-interacting protein 1; RICTOR, rapamycin-insensitive companion of mTOR. (–) indicates that there are no shown/obvious homologues of the indicated proteins in the corresponding organisms.
that TOR plays an important role in regulating the vegetative growth of *B. cinerea*.

In addition, rapamycin also exhibited a strong inhibitory effect on sporulation. Microscopic observation showed twisted hyphae with few conidiophores on 10 nM rapamycin plates compared to the control plate (Fig. 4A). Furthermore, spore number statistics showed that there is no difference in spore production between treatment of 1 nM rapamycin and DMSO solvent, while conidium production was dramatically decreased under 10 nM rapamycin treatment (Fig. 4B). The spores were not observed on PDA plates with 100 nM rapamycin (Fig. 4B). These results suggest that TOR activity is required for sporulation of *B. cinerea*. Subsequently, we investigated the effects of rapamycin on conidial germination through dropping spore suspensions on PDA plates supplemented with rapamycin or DMSO. As shown in Fig. 4C, after 3 days of incubation we observed smaller colonies on the rapamycin plates than on the DMSO plates.
However, there was almost no difference in conidial germination at different doses of rapamycin compared with DMSO when observed at indicated time points (Fig. 4D), suggesting BcTOR is not involved in regulating conidial germination of *B. cinerea*.

**BcTOR inhibition reprograms gene expression to modulate growth and metabolism**

To further investigate the role of BcTOR in the growth of *B. cinerea*, we performed whole genome expression profiling analysis on rapamycin and DMSO treatments through RNA sequencing (RNA-Seq). RNA-Seq data indicated that there were 3257 differentially expressed genes (DEGs), consisting of 1448 up-regulated DEGs and 1809 down-regulated DEGs (Fig. 5A and Table S1). Gene ontology (GO) enrichment analysis showed that these DEGs were categorized into 238 significantly enriched GO terms (*q*-value < 0.01) (Table S2). In the top 30 of the most significantly enriched GO terms, most were associated with transcription, translation and ribosome biogenesis (Fig. 5B), which all belong to the conserved function of TOR signalling in regulation of growth (Loewith and Hall, 2011; Mahoney et al., 2009; Thoreen et al., 2012; Wullschleger et al., 2006). Furthermore, the DEGs classified in these top 30 terms displayed an almost unanimous down-regulated trend (Fig. 5B and Table S2), suggesting that rapamycin represses BcTOR function in regulation of growth of *B. cinerea*. In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that many DEGs were mainly enriched on 169 KEGG pathways, including 91 down-regulated pathways and 78 up-regulated pathways (Tables S3 and S4). In the top 20 most enriched pathways, the majority were involved in anabolism in down-regulated groups, such as ‘ribosome’ and ‘ribosome biogenesis’, which were the two most significantly enriched pathways (Fig. 5C and Table S3). Conversely, in the up-regulated top 20 enriched pathways, most participated in catabolism, such as ‘valine, leucine and isoleucine degradation’ and ‘fatty acid degradation’ (Fig. 5D and Table S4). These results show consistency with the conserved function of TOR in modulating metabolism (Robaglia et al., 2012; Wullschleger et al., 2006). Taken together, the above data indicate there is a conserved TOR function in the regulation of growth and metabolism in *B. cinerea*.

**BcTOR inhibition reduces infection by *B. cinerea***

To investigate whether TOR affects infection by *B. cinerea*, detached leaves of *Arabidopsis* and potato plants, as well as mature red tomato fruits from the supermarket, were drop-inoculated with *B. cinerea* spore suspension mixed with indicated doses of rapamycin. After 3 days of incubation, larger water-soaked infection lesions and more pathogen colonization were observed on leaves of both *Arabidopsis* and potato when infected with *B. cinerea* under DMSO treatment compared with that of rapamycin treatment (Fig. 6A–C). In particular, infected spots were
hardly observed in the case of treatment with 1 μM of rapamycin (Fig. 6A). Similarly, tomato fruits showed smaller lesions and less pathogen biomass when infected with *B. cinerea* under rapamycin conditions at 7 days after incubation (Fig. 6A-C). Furthermore, after 15 days of incubation, tomato fruits exhibited severe decay covered by mycelium under the solvent conditions, while rapamycin efficiently reduced infection by *B. cinerea* and even blocked the infection at high concentrations (Fig. S2). These findings suggest that BcTOR is required for pathogenicity of *B. cinerea*.

**BcTOR silencing by plant-mediated RNAi enhances resistance to *B. cinerea* in plants**

HIGS has become a promising strategy in genetic engineering methods for crop protection. Transgenic plants and crops expressing dsRNAs that target essential growth and virulence genes of eukaryotic pathogens and pests are less prone to disease (Cai *et al.*, 2018; Nunes and Dean, 2012; Wang, Thomas *et al.*, 2017). Our previous results on BcTOR function showed that BcTOR could be a potential target in HIGS for crop protection. In order to test its potential, three fragments of *BcTOR* (BcRI1, BcRI2 and BcRI3) were selected to construct the RNA interference vector, and transgenic potato was first generated due to the ease of genetic transformation (Fig. 7A). A total of 25 independent transgenic potato lines were generated, including six BcRI1 lines (BcRI1-1 to BcRI1-9), nine BcRI2 lines (BcRI2-1 to BcRI2-6) and ten BcRI3 lines (BcRI3-1 to BcRI3-10), in which several lines were selected for pathogen infection experiment (Fig. S3). As shown in Fig. 7B, the selected transgenic potato lines all showed enhanced resistance to *B. cinerea* to different extents, manifested in smaller lesions and less pathogen biomass compared with WT or green fluorescent protein (GFP) dsRNA-expressing plants (Fig. 7B-D). In particular, BcRI3-3 and BcRI3-6 lines displayed near-complete resistance to *B. cinerea* so water-soaked lesions were hardly observed (Fig. 7A). In order to confirm that the enhanced resistance of transgenic potato is related to BcTOR transcript level, we examined the silencing efficiency of *BcTOR* by qRT-PCR in these infected leaves. In accordance with the above observation, the transcript level of *BcTOR* was significantly decreased in the infected *BcTOR*-silenced transgenic lines in comparison with WT or GFP dsRNA-expressing lines.
**Fig. 5** RNA-Seq analysis of TOR function on growth of *Botrytis cinerea*. RNA sequencing was performed between rapamycin and DMSO treatment. Each treatment contained three biological replicates. (A) The number of DEGs. (B) Top 30 most significantly enriched GO terms. (C) Top 20 most enriched KEGG pathways for down-regulated DEGs. (D) Top 20 most enriched KEGG pathways for up-regulated DEGs.
plants (Fig. 7E). These results suggest that plants expressing dsRNA targeting BcTOR could efficiently reduce susceptibility to B. cinerea by interfering with the expression of BcTOR.

The same constructs were used to generate transgenic tomato and a total of 27 independent transgenic lines were obtained (Fig. S4). Southern blotting results showed that among the selected lines, the BcRI1 and BcRI3 transgenic tomato lines contained two or more T-DNA insertions, while both BcRI2 transgenic lines had a single copy (Fig. S5A–C). To demonstrate the specificity of the BcTOR sRNA molecules generated in these transgenic lines, we carried out sRNA-Seq. The data showed that a large number of BcTOR-specific sRNA molecules were generated in these transgenic lines (Fig. 8A–C and Tables S5–S7). Corresponding to copy number, BcRI1 and BcRI3 lines had a higher abundance of sRNA molecules than BcRI2 lines. In addition, BcRI1 and BcRI3 lines also contained more species of BcTOR-specific sRNAs compared with BcRI2 lines or WT plants (Fig. 8A–C and Tables S5–S7). These results further confirm the presence of BcTOR-specific sRNAs compared with BcRI2 lines or WT plants (Fig. 8A–C and Tables S5–S7). These results further confirm the presence of sRNA molecules derived from BcTOR RNAi constructs in transgenic tomato seedlings. Furthermore, pathogen infection experiments showed that, similar to the observation from the T1 generation, the T2 generation plants also displayed enhanced resistance against B. cinerea, suggesting that the trait is stable among generations (Figs 9A–E and S6A–D). Specifically, under normal growth conditions there was no difference between transgenic tomato plants expressing dsRNA targeting BcTOR and WT tomato plants; however, dsRNA-expressing plants showed a distinct growth advantage after infection by B. cinerea compared with WT plants, where leaves showed serious decay (Fig. 9A). Additionally, consistent with BcTOR-specific sRNA levels, tomato fruits from BcRI1 and BcRI3 lines had smaller lesions and less pathogen biomass than BcRI2 lines or WT fruits, as well as a decline in transcript levels of BcTOR during infection (Fig. 9B–E). Taken together, these results further demonstrate that BcTOR dsRNA-expressing plants could constitutively produce BcTOR-specific sRNAs and effectively inhibit the expression of BcTOR to resist B. cinerea, suggesting that BcTOR is an effective target for genetic engineering in the control of grey mould.

**DISCUSSION**

To control grey mould disease, the traditional strategy is to apply fungicides, but fungicide residues derived from the widespread use of chemical pesticides have caused serious environmental pollution issues (Malhat et al., 2015; Oliveira et al., 2015; Tomenson and Matthews, 2009). Biofungicides for key targets of pathogens are a promising application for controlling diseases. On the other hand, genetic engineering methods also are extensively applied to improve resistance to plant pathogens for crop protection. For instance, HIGS has been proved to be an efficient tool to unravel
gene function as well as a promising approach to improve resistance to plant fungal pathogens by targeting key genes associated with growth and virulence (Cai et al., 2018; Wang, Thomas et al., 2017). However, selection of efficient targets is the key for HIGS.

TOR, a functionally and structurally conserved protein kinase, is a master regulator that controls cell growth by integrating nutrient, energy and growth factors in all eukaryotic species (van Dam et al., 2011; Takahara and Maeda, 2013; Wullschleger et al., 2006). TOR is extensively used as an excellent target in the control or therapy of diseases like Alzheimer’s disease, Huntington’s chorea and some cancers by using rapamycin or other active-site TOR inhibitors (Blenis, 2017; Ciuffreda et al.,...
Fig. 8  The abundance of sRNAs targeting BcTOR in transgenic tomato lines expressing BcTOR dsRNA. Two-week-old transgenic and wild-type (WT) seedlings were harvested and used for small RNA (sRNA) sequencing. (A) sRNAs derived from BcRI1 construct lines. (B) sRNAs derived from BcRI2 construct lines. (C) sRNAs derived from BcRI3 construct lines.
HIGS of BcTOR enhances resistance to grey mould

2010; Easton and Houghton, 2006; Jung et al., 2018; Santos et al., 2011; Wang, Valera et al., 2017), implying that TOR could also be a potential target in pathogens for disease control. To date, TOR signalling in phytopathogenic fungi such as B. cinerea, M. circinelloides and Fusarium spp. has been reported. TOR signalling was particularly well characterized in F. graminearum (Bastidas et al., 2012; Lopez-Berges et al., 2010; Melendez et al., 2009; Teichert et al., 2006; Yu et al., 2014), suggesting that a conserved TOR pathway also exists in plant pathogenic fungi.

Genome scanning revealed the presence of conserved TOR signalling including conserved TOR protein and TOR components such as RAPTOR, LST8 of TORC1 and RICTOR of TORC2 in B. cinerea (Fig. 1A–C and Table 1), as well as the FKBP12 homologue, suggesting there is conserved TOR signalling in B. cinerea. Previous studies showed that the BcFKBP12 deletion relieved the growth inhibition of rapamycin, suggesting the role of BcFKBP12 in associating the conserved inhibitory effect of rapamycin to TOR in B. cinerea (Gioti et al., 2006). Based on the characteristics of this dependency, functional FKBP12 is usually used in studying TOR function in some rapamycin-insensitive species, especially in the model plant Arabidopsis (Mahfouz et al., 2006; Ren et al., 2012; Sormani et al., 2007; Xiong et al., 2017). For example, Arabidopsis plants depicted susceptibility to rapamycin by expressing FKBP12 from yeast or human (Mahfouz et al., 2006; Ren et al., 2012; Sormani et al., 2007; Xiong et al., 2017). Consistent with these observations, expression of BcTOR dsRNA showed enhanced resistance to Botrytis cinerea. (A) Phenotype of BcTOR dsRNA-expressing tomato plants and wild-type (WT) before and after infection by B. cinerea. Scale bar represents 1 cm. (B) Infection phenotype of B. cinerea on fruits from transgenic tomato and WT plants. Photographs were taken at 3 days post-inoculation. Scale bar represents 1 cm. (C, D) Infection lesion size and relative biomass of B. cinerea. Error bars indicate the SEM (n ≥ 10). The experiments were repeated three times. (E) Relative expression level of BcTOR in B. cinerea during infection of transgenic and WT tomato fruits. Data are represented as means ± SD (n = 3). *P < 0.05, **P < 0.01 (Student’s t-test).
with the previous studies, BcFKBP12 also restored the sensitivity of Arabidopsis plants to rapamycin (Fig. 2A–E). This result further confirmed the conserved function of BcFKBP12 in bridging the inhibitory effect of rapamycin to TOR.

The TOR signalling pathway plays critical roles in controlling cell growth and proliferation, and in modulating downstream transcription, translation, autophagy and metabolism processes in a variety of eukaryotes (Dobrenel et al., 2016; Henriques et al., 2014; Mahoney et al., 2009; Rexin et al., 2015; Thoren et al., 2012; Xiong and Sheen, 2015). Previous reports indicated that rapamycin suppresses growth of B. cinerea, but there is no detailed description of TOR function in these studies. Previously it has been reported that inhibition of TOR by rapamycin caused serious growth retardation, displaying twisted hyphal morphology, more branches and increased septation in F. graminearum (Yu et al., 2014). In this study, similar results were observed in B. cinerea when treated with rapamycin (Figs 3A–C and S1), suggesting that TOR plays a crucial role in regulating vegetative growth in B. cinerea. For mycelial growth, the retarded growth caused by rapamycin was supported by RNA-Seq data that showed that many DEGs associated with ribosome, transcription and translation were down-regulated under TOR inhibition (Fig. S5–D and Tables S2–S4). These data also reflect conserved functions of TOR signalling for ribosome biogenesis and assembly, transcription and translation (Takahara and Maeda, 2013; Wullschleger et al., 2006). In addition, similar to previous reports in F. graminearum, TOR inhibition also led to severe suppression of conidiation of B. cinerea when concentrations of more than 10 nM rapamycin were used (Fig. 4A,B). This implies that restricting the spread of B. cinerea by inhibiting TOR could be a promising approach. TOR inhibition reduces pathogenicity in F. graminearum associated with regulating mycelial growth and virulence (Yu et al., 2014), which offers an impressive example of TOR function on the virulence of plant fungal pathogens. Similarly, we observed reduction and even loss of infectivity in B. cinerea under TOR inhibition during infection (Fig. 6A–C), implying that BcTOR could be a potential target used to control B. cinerea.

HIGS is an effective strategy for crop protection and has also been proven to be effective in controlling necrotrophic fungal pathogens. For instance, Arabidopsis plants expressing dsRNA targeting BcDCL1 and BcDCL2 are more resistant to B. cinerea (Zhang et al., 2016). Here, we generated transgenic potato and tomato plants expressing dsRNA targeting BcTOR based on the HIGS method. In general, the transgenic potato and tomato plants displayed enhanced, or near-complete resistance to B. cinerea, although there were some differences in resistance between transgenic lines (Figs 7B–E and 9A–E). sRNA-Seq showed that a large number of BcTOR-specific sRNA molecules were generated in these transgenic lines, and BcRI1 and BcRI3 lines had a higher abundance of sRNA molecules than BcRI2 lines (Fig. 8A–C and Tables S5–S7). The data suggest that BcTOR dsRNA could be constitutively and efficiently processed into sRNA molecules (Fig. 8A–C and Tables S5–S7). These data also explain the difference in resistance between transgenic tomato lines (Figs 8A–C and 9A–E, and Tables S5–S7). In addition, the efficiency of dsRNA being processed into sRNAs may be responsible for differences among lines from different constructs. BcRI2 lines produced significantly fewer types of sRNA than BcRI1 and BcRI3 lines (Fig. 8A–C); an off-target effect may also be the cause of the differences among these constructs. Through aligning BcTOR RNAi fragments with the cDNA database of tomato or B. cinerea, we found no possible off-target genes in tomato plants, but in B. cinerea there were only two genes with more than 19 bases matched for each fragment (Table S9). Compared to BcRI2 lines, BcRI1 and BcRI3 contained more sRNA reads matching to non-target genes, but these multiple target sRNA reads only account for very few of the total sRNA reads in BcRI1 and BcRI3 lines (Tables S8 and S9). In summary, our results demonstrate that BcTOR dsRNA-expressing plants were able to effectively produce BcTOR-specific sRNAs against B. cinerea, reducing infection. This study suggests that TOR is a potential and promising target in the biocontrol of plant fungal pathogens.

**EXPERIMENTAL PROCEDURES**

**Fungal strains and culture conditions**

Botrytis cinerea strain B05.10 was incubated on PDA plates at 25 °C unless indicated otherwise. For the growth inhibition assay, 0.4 mm diameter agar plugs with fungal mycelia were placed on PDA plates with different concentrations of rapamycin (1, 10, 100, 1000 nM) or dimethylsulphoxide (DMSO, solvent control). The diameter of the colony was measured every day, and the hyphal tip growth and branching patterns were observed after 2 days’ growth. For measurement of conidia production, spores were collected and counted after 10 days’ cultivation. For the spore germination experiment, a spore suspension (1 × 10⁶ spores/mL) was spread on PDA plates supplemented with rapamycin or DMSO for incubation, and spore germination was observed at different time points (2, 4 and 6 h).

In order to quantify the biomass of mycelia, potato dextrose broth (PDB) was used for liquid culture of mycelia. Five agar plugs with fungal mycelia were placed in a 100 mL triangular bottle with 50 mL PDB supplemented with the indicated concentrations of rapamycin or DMSO (solvent control), then the bottles were placed in an incubator at 25 °C. After 4 days’ incubation, the mycelia were collected and freeze-dried to constant weight before measuring the biomass of mycelia. To detect the expression level of related genes, mycelia cultivated in PDB for 4 days were treated by adding the indicated concentrations of rapamycin or DMSO.
(solvent control) for 12 h, and then the mycelia were collected for RNA extraction.

**Plant material and growth conditions**

The plant materials used in this work included *A. thaliana* ecotype Columbia (Col-0), *Solanum lycopersicum* 'Micro-Tom' and *Solanum tuberosum* ‘Desirée’. All plants were grown in soil in an artificial climate culture room at 25/18 °C of day/night (except *Arabidopsis* plants were grown at 22 °C), 80% humidity and under a long-day photoperiod consisting of a 16 h light regime. In addition, cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) fruits purchased from the supermarket were also used in infection experiments.

**Vector construction and plant transformation**

Vector construction was based on a Gateway system according to previous reports (Earley et al., 2006; Xiong et al., 2017). Vector p35S-IN4-8GWN was used as the entry vector, and the plant binary vector KANA303, modified pEarleyGate303, was the destination vector (Xiong et al., 2017). For RNAi vector construction, three different segments of 350-bp DNA sequences of *BcTOR* were selected, and the sense and antisense orientation fragments were then amplified with the primers (BcRI-L-Fu-F/BcRI-L-Fu-R) and linked into linearized p35S-IN4-8GWN (linearized by NotI/SbfI) to generate p35S-BcRI(L)-IN4-8GWN by seamless cloning using a In-Fusion HD Cloning Kit (Clontech, Palo Alto, CA, USA) following the user’s manual. The sense fragments were amplified with primers (BcRI-R-Fu-F/BcRI-R-Fu-R) and linked into linearized p35S-BcRI(L)-IN4-8GWN (digested by BbvCI/Ascl) to obtain p35S-BcRI(L)-IN4-BcRI(R)-8GWN by the seamless cloning method. Finally, RNAi fragments were cloned into the destination vector KANA303 using Gateway LR clonase (Invitrogen, Carlsbad, CA, USA).

The *BcTOR-RNAi* binary plasmid was transferred into *Agrobacterium* strain GV3101. Transgenic tomato was generated by *Agrobacterium*-mediated transformation following the protocols described by Fillatti et al. (1987), and transgenic potato created by *Agrobacterium*-mediated transformation according to the protocol described in Millam (2007). Transgenic plants were identified by PCR with primers of neomycin phosphotransferase II (*NPTII*) and *BcTOR* fragments (Table S10a). The positive transgenic plants were selected and used for subsequent experiments.

To create *BcFKBP12*-expressing *Arabidopsis*, the coding sequence of *BcFKBP12* (Bcin12g01360.1) was cloned in the plant binary vector KANA303 according to the experimental protocols described in previous reports (Xiong et al., 2016). The resulting destination vectors were transferred into *Agrobacterium* strain GV3101 for plant transformation. The floral dipping method was employed for generating transgenic *Arabidopsis* (Zhang et al., 2006). The transformation and screening of primary transformants were performed according to Zhang et al. (2006), and T3 generation transgenic plants were used for subsequent experiments.

**Botrytis cinerea infection**

For inoculation of plants, conidia were harvested from sporulating colonies in sterile water with 0.1% Tween-20 and filtered with glass wool as described by Stefanato et al. (2009). For droplet inoculations, the concentration of spores was adjusted to 1 × 10⁵ spores/mL and 5 μL of spore suspension with the indicated dose of rapamycin or DMSO was dropped onto the surface of single detached rosette leaves of 5-week-old *Arabidopsis* plants or 45-day-old potato plants starting from tissue culture plantlets or mature red cherry tomato fruits from supermarket and mature red tomatoes (cv. Micro-Tom) harvested at 14 days after breaker stage from transgenic plants. Before inoculations, all detached leaves or fruits were surface cleaned or sterilized with 75% ethanol. The inoculated leaves or fruits were kept under a transparent plastic film cover to maintain high humidity and kept at 25 °C in the dark. Infection symptoms were observed and photographed at 3 days post-incubation (dpi). For the incubation of intact tomato plants, the same density of spore suspension was applied for spray inoculations of 45-day-old intact tomato plants. Plants were kept prior to and during infection under sealed hoods at high humidity, and infection symptoms were observed and photographed at 15 dpi. The lesion area was measured using ImageJ software (http://imagej.nih.gov/ij/) from digitally computed images of leaves or tomato fruits.

**DNA extraction and DNA quantification**

To quantify *B. cinerea* growth by real-time quantitative PCR (qPCR), 1 cm² of leaf tissue or 1 cm³ of fruit tissue around the infected lesion area was collected at 3 dpi, and then ground into powder in liquid nitrogen. Total DNA was isolated using a plant genomic DNA extraction kit (Biotekie, Beijing, China) following the manufacturer’s user manual. To estimate the amount of fungal DNA in inoculated samples, purified DNAs were used for qPCR as described by Zhang et al. (2013). The primers listed in Table S10b were used.

**Expression profiling sequencing and analysis**

RNA-Seq was carried out by Novogene Bioinformatics Technology Co. Ltd (Beijing, China). Hyphae of *B. cinerea* were grown for 4 days in PDB medium at 25 °C and then treated by adding 100 nM rapamycin or DMSO (as solvent control). For each treatment, three independent biological replicates were performed. After incubation for 12 h, the mycelia were collected and total RNA of *B. cinerea* mycelium was isolated using a RNAprep Pure Plant Kit (Tiangen, Beijing, China). RNA integrity was assessed using the RNA Nano 6000...
performed using GO sequencing software (Young et al., 2010). The
were annotated in the B. cinerea genome database website (http://
by DESeq were assigned as differentially expressed. All the DEGs
ontology enrichment (corrected P-value < 0.05) of the DEGs was
fungi.ensembl.org/Botrytis_cinerea/Info/Index?db=core). Gene
was obtained using KOBAS software (Kanehisa et al., 2008; Mao
et al., 2012, 2013), and genes with an adjusted P-value < 0.05 found
in KEGG pathways (corrected P-value < 0.05) was obtained using KOBAS software (Kanehisa et al., 2008; Mao et al., 2005). RNA-Seq data was validated by real-time qRT-PCR for
the reference B. cinerea genome using TopHat2 software (Kim et al.,
2013). Transcript abundance was also normalized by transforming
the data to reads per kilobase of exon model per million mapped
reads (FPKM) method. Cufflinks and Cuffdiff were used to assem-
ble the mapped reads and identify DEGs, respectively (Trapnell et al.,
database. The resulting samples were reverse-transcribed using Superscript II
reverse transcriptase. Amplification was executed for the PCR
products. All steps were performed according to the manufac-
turer’s protocols. sRNA libraries were analysed for quality control
and the average size of inserts was approximately 140–150 bp.
The sequencing library was then sequenced on a Hiseq platform
(Illumina) by Shanghai Personal Biotechnology Cp. Ltd (Shanghai,
China). The quality information of raw data in FASTQ format was
calculated and the raw data were filtered using the Personalbio self-
developed script. Clean data were obtained by removing adapter
and low-quality sequence. Filter Clean Reads from 18 to 36 nt
in length and perform deduplication to obtain Unique Reads for
subsequent analysis. The unique reads were aligned using BLAST
with the corresponding target fragment of BcTOR. The reads count
value of the sRNA was counted based on the number of sequences
aligned to the corresponding target fragment of BcTOR.

**Statistics**
All experiments were repeated and yielded reproducible results. The
most representative data are shown in this paper. Data are presented
as means ± standard error of the mean, unless stated otherwise.
Paired or unpaired, two-tailed Student’s t-tests were used to com-
pare group differences. P-values < 0.05 were considered significant.

**ACKNOWLEDGEMENTS**
We thank Guozheng Qin (Chinese Academy of Sciences, Beijing,
China) for kindly providing B. cinerea strain B05.10. This work was
supported by the following grants: the National Natural Science
Foundation of China (nos. 31801913, 31972469, 31672206 and
31801271), the China Postdoctoral Science Foundation (nos.
2017M622958 and 2018M633320), the Project of Chongqing
Science and Technology Commission (nos. cstc2016jcymjA0822,
cstc2019jcyjX0127 and cstcjkjcxljrc15), Chengdu Agricultural
Science and Technology Center local financial special fund pro-
ject (NASC2019TI13), the Fundamental Research Funds for
the Central Institutes and the Chinese Academy of Agricultural
Sciences (19-001-09).

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

**REFERENCES**
Barettic, D. and Williams, R.L. (2014) The structural basis for mTOR function.
Semin. Cell Dev. Biol. 36, 91–101.
Bastidas, R.J., Shertz, C.A., Lee, S.C., Heitman, J. and Cardenas, M.E.
(2012) Rapamycin exerts antifungal activity in vitro and in vivo against
Mucor circinelloides via FKBP12-dependent inhibition of Tor. Eukaryot Cell,
11, 270–281.
Baum, J.A., Bogaert, T., Clinton, W., Heck, G.R., Feldmann, P., Ilagan, O., Johnson, S., Plaatnick, G., Muniyikwa, T., Pleau, M., Vaughan, T. and Roberts, J. (2007) Control of coleopteran insect pests through RNA interference. Nat. Biotechnol. 25, 1322–1326.

Benjamin, D., Colombi, M., Moroni, C. and Hall, M.N. (2011) Rapamycin passes the torch: a new generation of mTOR inhibitors. Nat. Rev. Drug Discov. 10, 868–880.

Bennis, J. (2017) TOR, the gateway to cellular metabolism, cell growth, and disease. Cell, 171, 10–13.

Cai, Q., He, B., Kogel, K.H. and Jin, H. (2018) Cross-kingdom RNA trafficking and environmental RNAi-nature’s blueprint for modern crop protection strategies. Curr. Opin. Microbiol. 46, 58–64.

Cheng, W., Song, X.S., Li, H.P., Cao, L.H., Sun, K., Qiu, X.L., Xu, Y.B., Yang, P., Huang, T., Zhang, J.B., Qu, B. and Liao, Y.C. (2015) Host-induced gene silencing of an essential chitin synthase gene confers durable resistance to Fusarium head blight and seedling blight in wheat. Plant Biotechnol. J. 13, 1335–1345.

Chiu, M.I., Katz, H. and Berlin, V. (1994) RAPT1, a mammalian homolog of the immunosuppressant rapamycin in yeast. Science, 253, 905–909.

Hellinwell, S.B., Wagner, P., Kunz, J., Deuter-Reinhard, M., Henriquez, R. and Hall, M.N. (1994) TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. Mol. Cell. Biol. 5, 105–118.

Henriques, R., Borges, L., Horvath, B. and Magyar, Z. (2014) Balancing act: matching growth with environment by the TOR signalling pathway. J. Exp. Bot. 65, 2691–2701.

Jung, S., Gamez-Diaz, L., Proietti, M. and Grimmer, B. (2018) “Immunic TOR-opathies,” a novel disease entity in clinical immunology. Front. Immunol. 9, 966.

Kanekia, M., Araki, M., Goto, S., Hattori, M., Hirakawa, M., Itoh, M., Katayama, T., Kawashima, S., Okuda, S., Tokimatsu, T. and Yamanishi, Y. (2008) KEGG for linking genomes to life and the environment. Nucleic Acids Res. 36, D480–484.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R. and Salzberg, S.L. (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 14, R36.

Koch, A., Kumar, N., Weber, L., Keller, H., Imani, J. and Kogel, K.H. (2013) Host-induced gene silencing of cytochrome P450 lanosterol C14α-demethylase-encoding genes confers strong resistance to Fusarium species. Proc. Natl. Acad. Sci. USA, 110, 19324–19329.

Koltin, Y., Faucette, L., Bergsma, D.J., Levy, M.A., Cafferkey, R., Koser, P.L., Riki, K.R. and Livi, G.P. (1991) Rapamycin sensitivity in Saccharomyces cerevisiae is mediated by a peptide-tyr cis-trans isomerase related to human FK506-binding protein. Mol. Cell. Biol. 11, 1718–1723.

Loewith, R. and Hall, M.N. (2011) Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics, 189, 1177–1201.

Loewith, R., Jacinto, E., Wullschleger, S., Lorberg, A., Cafferey, R., Koser, P.L., Oppfiger, W., Jenne, P. and Hall, M.N. (2002) Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. Mol. Cell, 10, 457–468.

Lopez-Berges, M.S., Rispail, N., Prados-Rosales, R.C. and Di Pietro, A. (2010) A nitrogen response pathway regulates virulence functions in Fusarium oxysporum via the protein kinase TOR and the bZIP protein MeaB. Plant Cell, 22, 2459–2475.

Madeira da Silva, L. and Beverley, S.M. (2010) Expansion of the target of rapamycin (TOR) kinase family and function in Leishmania shows that TOR3 is required for acidicolysosome biogenesis and animal infectivity. Proc. Natl. Acad. Sci. USA, 107, 11956–11970.

Mahfouz, M.M., Kim, S., Delaunay, A.J. and Verma, D.P. (2006) Arabidopsis TARGET OF RAPAMYCIN interacts with RAPTOR, which regulates the activity of S6 kinase in response to osmotic stress signals. Plant Cell, 18, 477–490.

Mahoney, S.J., Dempsey, J.M. and Blenis, J. (2009) Cell signaling in protein synthesis ribosome biogenesis and translation initiation and elongation. Prog. Mol. Biol. Transl. Sci., 90, 53–107.

Malhat, F.M., Hagga, M.N., Loutfy, N.M., Osman, M.A. and Ahmed, M.T. (2015) Residues of organochlorine and synthetic pyrethroid pesticides in honey, an indicator of ambient environment, a pilot study. Chemosphere, 120, 457–461.

Mao, X., Cai, T., Olyarchuk, J.G. and Wei, L. (2005) Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. Bioinformatics, 21, 3787–3793.

Melendez, H.G., Billon-Grand, G., Fevre, M. and Mey, G. (2009) Role of the Botrytis cinerea FKBP12 ortholog in pathogenic development and in sulfur regulation. Fungal Genet. Biol. 46, 308–320.

Millam, S. (2007) Potato (Solanum tuberosum L.). In Agrobacterium Protocols (K. Wang ed.), pp. 25–35. Totowa, NJ: Humana Press.

Nowara, D., Gay, A., Lacomme, C., Shaw, J., Ridout, C., Douchkov, D., Hensel, G., Kuzleman, J. and Schweizer, P. (2010) HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen Blumeria graminis. Plant Cell, 22, 3130–3141.
**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of this article at the publisher’s web site:

**Fig. S1** Biomass of *B. cinerea* under rapamycin and DMSO treatment in a liquid culture condition.

**Fig. S2** Phenotype of tomato fruits infected by *B. cinerea* under TOR inhibition at 15 dpi.

**Fig. S3** Identification of transgenic potato by PCR with primers of *neomycin phosphotransferase II (NPTII)* and *BcTOR* fragments.

**Fig. S4** Identification of transgenic tomato by PCR with primers of *neomycin phosphotransferase II (NPTII)* and *BcTOR* fragments.

**Fig. S5** DNA gel blotting analysis of tomato transgenic lines.

**Fig. S6** Phenotype of *BcTOR* dsRNA expressing tomato in T1 generation.

**Fig. S7** Validation of RNA-seq data by real-time PCR.

**Table S1** DEGs between rapamycin and DMSO treatment

**Table S2** Enriched GO terms

**Table S3** Enriched KEGG pathway (down)

**Table S4** Enriched KEGG pathway (up)

**Table S5** Abundance statistics of sRNA target to *BcTOR*

**Table S6** sRNA molecule sequence information

**Table S7** sRNA blast information

**Table S8** Potential off-target genes in *B. cinerea*

**Table S9** sRNAs matching to non-targeting genes

**Table S10** Primers used in this study