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The Oomycete Microbe-Associated Molecular Pattern Pep-13 triggers SERK3/BAK1-independent plant immunity

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Key message **Oomycetes MAMP Pep-13 can trigger SERK3/BAK1-independent PTI. Silencing of SERK3/BAK1 in solanaceous plants resulted in reduced expression of brassinosteroid marker genes and enhanced PTI transcriptional responses to Pep-13 treatment.**

Abstract To prevent disease, pattern recognition receptors (PRRs) are responsible for detecting microbe-associated molecular patterns (MAMPs) to switch on plant innate immunity. SOMATIC EMBROYOGENESIS KINASE 3 (SERK3) / BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 (BAK1) is a well-characterized receptor-like kinase (RLK) that serves as a pivotal co-receptor with PRRs to activate immunity

following recognition of MAMPs including flg22, EF-Tu, INF1 and XEG1. However, the requirement for SERK3/BAK1 in many Pattern-triggered immune (PTI) signaling pathways is not yet known. Pep-13 is an oomycete MAMP that consists of a highly conserved motif (an oligopeptide of 13 amino acids) shared in *Phytophthora* transglutaminases. Quantitative RT-PCR analysis reveals that the transcripts of three PTI marker genes (*WRKY7*, *WRKY8* and *ACRE31*) rapidly accumulate in response to three different MAMPs: flg22, chitin and Pep-13. Whereas silencing of *SERK3/BAK1* in *Nicotiana benthamiana* or potato compromised transcript accumulation in response to flg22, it did not attenuate *WRKY7*, *WRKY8* and *ACRE31* up-regulation in response to chitin or Pep-13. This indicates that Pep-13 triggers immunity in a SERK3/BAK1-independent manner, similar to chitin. Surprisingly, silencing of *SERK3/BAK1* led to significantly increased accumulation of PTI marker gene transcripts following Pep-13 or chitin treatment, compared to controls. This was accompanied by reduced expression of brassinosteroid (BR) marker genes *StSTDH*, *StEXP8* and *StCAB50* and *StCHL1,* which is a negative regulator of PTI, supporting previous reports that SERK3/BAK1-dependent BR signaling attenuates plant immunity. We provide Pep-13 as an alternative to chitin as a trigger of SERK3/BAK1-independent immunity.

Key words: MAMP; disease resistance; transcriptome; flagellin; late blight

***Abbreviations*-** PRRs, pattern recognition receptors; MAMPs, microbe-associated molecular patterns; BAK1, BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1; PTI, Pattern-triggered immune; ETI, effector-triggered immunity; RLK, receptor-like kinase.

Introduction

The interactions of microbes with plants can lead to a range of outcomes, including

disease or disease resistance. To escape the challenges that pathogens present, plants utilize a wide range of membrane-localized pattern recognition receptors (PRRs) to perceive a multitude of conserved microbe-associated molecular patterns (MAMPs) secreted by potential invaders (Couto and Zipfel, 2016). This initiates Pattern-Triggered Immunity (PTI) and provide broad-spectrum disease resistance (Jones and Dangl, 2006). Subsequently, many pathogens can deliver “effectors” into plant cells or into the intercellular space to manipulate plant immunity or other host processes to weaken plant defense. Pathogen effectors can be recognized by plant intracellular nucleotide-binding leucine-rich repeat domain-like receptors (NLRs) in a direct or indirect way, and result in race-specific resistance known as effector-triggered immunity (ETI) (Caplan et al. 2008; Jones et al. 2016).

Receptor-like kinases (RLKs) and receptor-like proteins (RLPs) are two major types of plant membrane proteins linked to complex networks of interacting signal transduction pathways that allow plants to respond to diverse extracellular signals, including MAMPs. SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3 / BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 (SERK3/BAK1) is a well-characterized RLK that was originally identified in association with the Brassinosteroid (BR) Insensitive 1 (BRI1) receptor mediating BR signaling (Li et al. 2002; Nam and Li, 2002). SERK3/BAK1 serves as a pivotal co-receptor participating in plant responses resulting from the recognition of many MAMPs, including flagellin or a conserved 22-amino acid peptide (flg22) and elongation factor (EF-Tu) or an 18-amino acid peptide (elf18) from bacteria, and INF1 (a secreted elicitin) and XEG1 (Xyloglucan-specific Endo Glucanase) from *Phytophthora* spp., in *Arabidopsis* or *Nicotiana benthamiana* (Chinchilla et al. 2007; Heese et al. 2007; Du et al. 2015; Ma et al. 2015). The MAMP flagellin contains a highly conserved N-terminal 22-amino-acid peptide (flg22) that is sensed by the flagellin receptor (FLAGELLIN-SENSING 2; FLS2) (Gómez-Gómez and Boller, 2000). Flg22 binding induces the interaction of FLS2 with SERK3/BAK1 (Chinchilla et al. 2007; Heese et al. 2007; Sun et al. 2013). In *Arabidopsis*, chitin is recognized by CHITIN ELICITOR RECEPTOR KINASE 1

(CERK1) (Miya et al. 2007, Liu et al. 2012; Cao et al. 2014). Chitin is present as a structural component in the cell walls of fungi, the exoskeleton of crustaceans and insects (Gopalakannan et al. 2006; Lee et al. 2008), and the eggshells of nematodes (Brodaczewska et al. 2015). In contrast to flg22, the perception of chitin and signaling through CERK1 does not depend on SERK3/BAK1 (Shan et al. 2008; Kemmerling et al. 2011; Ranf et al. 2011). Therefore, chitin and flg22 detection are regarded as two contrasting MAMP perception models.

The flg22 peptide has been widely used in plant research to trigger and study PTI. Its perception by FLS2 and co-receptor SERK3/BAK1 has provided a model to study immune signal transduction processes, the contribution of cellular trafficking processes to PTI (Ben Khaled et al. 2015) and how this crosstalks with growth and development (Belkhadir et al. 2014), or with symbiotic interactions (Antolin-Llovera et al. 2014). Chitin has also been used in many studies, revealing structural insights into MAMP perception, and how MTI crosstalks with symbiosis (Desaki et al. 2018). Mostly studies of chitin have used its derivative chitosan from shellfish, which has poor solubility at neutral pH (Jung et al. 2014). Other sources of chitin such as fungal chitin remain unexplored (Elieh Ali Komi et al. 2018). However, chitin originating from different sources may have diverse structures, be associated with glucans and glycosylated proteins, and be structurally linked to cell wall components, so purification is a challenging process (Elieh Ali Komi et al. 2017).

MAMPs originating from oomycetes include conserved 10-kDa elicitins, beta-glucans, cellulose-binding elicitor lectin (CBEL), a small cysteine-rich (SCR) necrosis-inducing *Phytophthora* protein 1 (NPP1), necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs), secreted xyloglucanase XEG1, and the conserved component of transglutaminase Pep-13 (Hein et al. 2009; Fellbrich et al. 2002; Bohm et al. 2014; Ma et al. 2015; Brunner et al. 2002). Pep-13, a specific 13-amino-acid peptide, is from the first proteinaceous oomycete PAMP transglutaminase (TGase) and has been shown to be necessary and sufficient to stimulate PTI responses in parsley cells (Brunner et

al. 2002; Nürnberger et al. 1994). The dynamic regulation of MAPK cascade proteins involved in the Pep-13 mediated activation of 'early' defense gene expression has been elucidated (Kroj et al. 2003; Lee et al. 2004). Furthermore, the defense responses induced by Pep-13 require both SA and JA in potato (Halim et al. 2009). The involvement of SERK3/BAK1 in Pep-13 perception is unknown.

In this work, qRT-PCR approaches were taken to determine whether SERK3/BAK1 is required for plant innate immunity triggered by Pep-13, with flg22 and chitin used as positive and negative controls respectively. qRT-PCR data shows that all three early PTI marker genes (*WRKY7*, *WRKY8* and *ACRE31*) respond to each of the three PAMPs strongly in both potato and *N. benthamiana*. Furthermore, following flg22 treatment, *WRKY7*, *WRKY8* and *ACRE31* transcript expression levels were found to significantly drop in *SERK3/BAK1* RNAi potatoes and *N. benthamiana* VIGS plants compared with their respective controls. However, with Pep-13 treatment all three genes were significantly up-regulated in *SERK3/BAK1* RNAi and VIGS plants over and above the levels induced in control plants. This increase in PTI marker gene activation was also observed in *SERK3/BAK1* RNAi plants following chitin treatment. To explain this increase in PTI marker gene expression the basal levels of four downstream BR signal pathway genes, *CHL1*, *EXP8*, *STSTDH* and *CAB50* (Turnbull et al. 2017) were examined and found to be significantly reduced in *SERK3/BAK1*-silenced plants. A reasonable hypothesis is that the overstimulation of PTI may be due to the decrease of negative regulation mediated by BR signaling which is down regulated in *SERK3/BAK1* silenced plants.

Materials and Methods

Plant Materials and Growth Conditions

Transgenic potato plantlets were propagated on MS medium supplemented with 4% sucrose and 0.7% agar and raised in a climate room under controlled conditions (16/8 h light/dark cycle at 20°C). Four week old transgenic potato lines were transferred and

grown in individual pots. For *N. benthamiana*, two-week-old seedlings were transplanted in individual pots. Potato and *N. benthamiana* plants were grown in general purpose compost under glasshouse conditions of 16/8 h light /dark cycle at 22°C and 70% humidity.

Generation of StSERK3/BAK1 transgenic potatoes

LBA4404 Agrobacterium culture (OD_{600} 0.5) containing a fragment (344 bp-640 bp) of StBAK1 in the pHellsgate8 vector was spun at 4°C, 6 000 g, 6 min. and then resuspended in Murashige & Skoog Medium (MS) liquid medium. 1-2 mm slices of two-month-old *in vitro* tubers were co-cultivated with the Agro/MS medium for 10 min. The microtuber slices were blot dried and transferred to solid A1 (MS, 3% saccharose, 0.2 mg l^{-1} IAA, 2 mg l^{-1} GA3, 0.5 mg l^{-1} BA+2 mg l^{-1} ZT) medium and kept in the dark for 2 days at 24°C, then subsequently transferred to solid A1 medium containing 100 mg l^{-1} kanamycin and 400 mg l^{-1} cefotaxime for a further 6 weeks to develop callus at 24°C and 16-h light. Explants with well-developed callus were transferred to A3 medium (MS, 3% saccharose, 75 mg l^{-1} kanamycin, 200 mg l^{-1} cefotaxime, pH5.8) until shoots developed. Transgenic shoots were propagated on MS medium for mass tissue culture plantlet propagation.

TRV-based VIGS in *N. benthamiana*

Virus-induced gene silencing (VIGS) was performed by using a tobacco rattle virus vector. A TRV construct expressing GFP was used as a control. The TRV-SERK3/BAK1 construct was described previously (Chaparro-Garcia et al. 2011). *A. tumefaciens* strains containing a mixture of RNA1 and each VIGS construct at OD_{600}=0.5 were infiltrated into upper leaves of 4-leaf-stage *N. benthamiana* plants. Systemic leaves were detached analyzed by qRT-PCR, and used for PAMP treatment assays 2 to 3 weeks later. Primers for qRT-PCR are in Supplemental Table S1.

PTI Elicitor Treatment

Peptide flg22 was dissolved at 40 µM in sterile distilled water (SDW) before it was used

to infiltrate leaves of transgenic potato and VIGS plants. Pep13 peptide was solubilized at 100 µM in SDW. Chitin (C9752, SIGMA) was dissolved in 48 µM dilute hydrochloric acid to make 1 mg ml^{-1} solution for inoculation.

Gene Expression Analysis

RNA was isolated from plant tissue with the EASY spin plant RNA extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing, China) according to the manufacturers' instructions, including the on-column DNase treatment. RNA was quantified using a Nanodrop 1000 (Thermo Scientific) and cDNA synthesized using Hiscript Reverse Transcriptase (Vazyme Biotech Co., Ltd, Nanjin China) and oligo dT primers (Vazyme). qRT-PCR was performed using Bio-Rad SYBR green supermix, and reactions were incubated 40 cycles of 95°C for 5 min, 95°C for 10 s, 58°C 10 s and 72°C 30 s. Tubulin gene or *EF1α* was used as the internal control to normalize expression levels in potato or *N. benthamiana*. All primers for qRT-PCR were listed in Supplemental Table S1. Gene expression levels were calculated by a comparative Ct method as described by Cikos et al. (2007).

***P. infestans* colonization test**

P. infestans strain 3928A was grown on rye agar at 18-20°C, 14-day-old plates were flooded with sterile distilled water and the suspension was spun down at 2,750 rpm for 10 min at 4°C. *P. infestans* was inoculated at a concentration of 1×10^5 sporangia ml^{-1} for potato inoculation. Then 10 µl droplets were pipetted onto the surface of detached leaves and were kept in darkness for the first 24 h. Lesions were measured at the widest point 5 days post infiltration (dpi) for potato transgenic lines.

Results and Discussion

Conserved early transcriptional responses to flg22, chitin and pep13

In order to study early PTI transcriptional responses to diverse MAMPs in *Solanaceae*, we selected previously described marker genes. It has been reported that the marker

genes *WRKY7*, *WRKY8* and *ACRE31* are rapidly upregulated following treatment with flg22 at 1 h in potato (Turnbull et al. 2017) and 3 h in *N. benthamiana* (He et al. 2015; McLellan et al. 2013). Here, we confirmed this observation (Fig. 1A, 1B; Suppl Fig. S1) and extended the analysis to two additional MAMPs: fungal chitin and a conserved peptide in oomycete transglutaminases, Pep-13. In both potato and *N. benthamiana*, *WRKY7, WRKY8* and *ACRE31* were maximally induced at 3 h after chitin treatment (Fig. 1C, 1D; Suppl Fig. S2). The peak of transcript accumulation in response to Pep-13 was, for all three marker genes, at 1 h after treatment in potato and 0.5 h in *N. benthamiana* (Fig. 1E, 1F; Suppl Fig. S3). We thus conclude that, whilst the dynamics of transcriptional response vary between the two plants, and between the treatments, all three marker genes are induced early in response to all three MAMPs, and can thus be used to investigate the role of SERK3/BAK1 in response to each.

StSERK3/BAK1 RNAi lines are more susceptible to *Phytophthora infestan*.

The involvement of SERK3/BAK1 in perception of the oomycete MAMP Pep-13 is unknown. To address the involvement of SERK3/BAK1 in perception of the oomycete MAMP Pep-13, StSERK3/BAK1 RNAi potatoes were generated. As was the case for *N. benthamiana* (Chaparro-Garcia et al. 2011), two copies of *StSERK3/BAK1* were evident in the potato genome, called *StSERK3A* and *StSERK3B* (Suppl Fig. S4). To silence both copies, a 296 bp fragment was cloned for RNAi in potato (Suppl Fig. S5A).

Three potato RNAi lines were selected for detailed analysis (Ri-9, Ri-11 and Ri-34), each of which revealed an approximately 90 % reduction in *StSERK3/BAK1* transcript accumulation compared to the untransformed control, potato cultivar E3 (Suppl Fig. S5B). To investigate whether the three *StSERK3/BAK1* RNAi lines have an immune phenotype, a concentrated spore suspension of *P. infestans* was inoculated on detached leaves of E3 control plants and *StSERK3/BAK1* RNAi lines. As anticipated, infected leaf lesion diameter and the number of *P. infestans* sporangia per ml were significantly increased in *StSERK3/BAK1* RNAi lines compared to E3 (Fig. 2A and 2B). The extent of pathogen colonization was strikingly evident using UV light (Fig. 2C).

Consistent with previous work in *N. benthamiana* (Chaparro-Garcia et al. 2011), this demonstrates a role for StSERK3/BAK1 in potato immune responses to the late blight pathogen *P. infestans.*

The early transcriptional PTI response to Pep-13 is SERK3/BAK1-independent

Silencing of *NbSERK3/BAK1* by virus-induced gene silencing (VIGS) in *N. benthamiana* significantly reduces early up-regulation of PTI marker genes after flg22 treatment (Heese et al. 2007). We therefore investigated whether this was also the case for *StSERK3/BAK1* potato RNAi lines. At 1 h after flg22 treatment, a significant reduction in *StWRKY7*, *StWRKY8*, and *StACRE31* transcript abundance was observed in *StBAK1*-Ri-9, *StBAK1*-Ri-11 and *StBAK1*-Ri-34 plants, compared with the potato E3 control (Fig. 3A, 3B and 3C). Similarly, as anticipated, VIGS of *NbSERK3/BAK1* in *N. benthamiana* (Suppl Fig. S6), also attenuated transcript abundance of *NbWRKY7, NbWRKY8* and *NbACRE31* at 3 h after treatment with flg22 (Fig. 4A, 4B and 4C).

Early transcriptional PTI responses to chitin perception have been shown to be *SERK3/BAK1*-independent (Schwessinger et al. 2011; Kemmerling et al. 2011; Ranf et al. 2011). As expected, RNAi of *StSERK3/BAK1* in potato (Fig. 3D, 3E and 3F) or silencing of *NbSERK3/BAK1* by VIGS in *N. benthamiana* (Fig. 4 D, 4E and 4F), did not result in reduced transcript abundance in response to chitin at 3 h after treatment, supporting observations that chitin perception is SERK3/BAK1-independent. Interestingly, transcript abundance in response to Pep-13, at 1 h after treatment in potato and 0.5 h in *N. benthamiana*, was also not attenuated in either potato RNAi lines (Fig 3G, 3H and 3I) or *N. benthamiana* VIGS plants (Fig. 4G, 4H and 4I). This suggests that, similar to chitin perception, and in contrast to flg22 perception, Pep-13 recognition is SERK3/BAK1-independent.

Surprisingly, transcript abundance of *WRKY7* and *ACRE31* in response to chitin or Pep-13 was significantly enhanced by silencing *SERK3/BAK1* in both plants. Moreover, significantly enhanced transcript abundance of *WRKY8* was also observed in both plants in response to Pep-13 and in potato in response to chitin. This unexpected

observation suggests that SERK3/BAK1 contributes to negative regulation of immunity, at least in response to chitin or Pep-13.

Brassinosteroid-responsive transcript abundance is decreased in *StSERK3/BAK1* RNAi lines

Previously, it was shown that treatment of *Arabidopsis* with the brassinosteroid (BR) epiBl (epi-brassinolide) attenuated MTI in response to the SERK3/BAK1-dependent PRR FLS2, and also to the SERK3/BAK1-independent chitin receptor CERK1 (Albrecht et al. 2012). It was subsequently shown that the transcriptional regulator BZR1, activated by the BR pathway, induces a number of transcription factors that are negative regulators of immunity (Lozano-Duran et al. 2013). Similarly, in potato we have observed that activation of the BR pathway results in increased transcript abundance of BR-responsive genes, including the basic helix-loop-helix transcriptional regulator StCHL1, which suppresses immunity (Turnbull et al. 2017). We thus hypothesized that silencing of *StSERK3/BAK1* in the potato RNAi lines resulted in reduced transcript abundance of BR-responsive marker genes, including the negative regulator of immunity, *StCHL1*. Indeed, we observed a significant decrease in resting-state transcript levels for the BR-responsive genes *StCHL1, StSTDH, StCAB50* and *StEXP8* (Fig. 5). These genes were recently shown to be constitutively up-regulated in Avr2-expressing transgenic potato plants which are BR-overactive and more susceptible to *P. infestans* (Turnbull et al. 2017). A possible explanation for increased MTI marker gene transcript abundance in *StSERK3/BAK1* RNAi lines in response to either chitin or pep13 may due to reduced levels of immune suppressors such as StCHL1. It is also worth noting that silencing of BAK1 results in a heightened PTI response to Pep-13 and chitin in potato and *N. benthamiana*, but increased susceptibility to *P. infestans*. BAK1 is a very important coreceptor of many PRRs in plant immunity (Tang et al., 2017). Silencing BAK1 also may impair the PTI signalling initiated by perception of as-yet unknown *P. infestans* MAMPs that signal through BAK1.

Conclusion

NbSERK3/BAK1 is required for multiple MAMP-mediated responses in *N. benthamiana* (Heese et al. 2007). Here we confirmed StSERK3/BAK1 also shows similar functions during MTI signaling in potato. In addition, we found that, like chitin, perception of Pep-13 MAMP is also SERK3/BAK1-independent. Flg22, a SERK3/BAK1-dependent MAMP, is widely used in research to dissect plant immune signaling. Similar to flg22, Pep-13 is a small peptide and can be easily synthesized. Thus Pep-13 provides a new tool to study BAK1-independent PTI signaling in plants instead of chitin which has poor solubility and is difficult to source in a pure form. In addition, we provide further evidence of the crosstalk between BR and PTI signaling. This work suggests that SERK3/BAK1 plays a role in PTI suppression *via* BR-BRI1 signaling. Upon down regulation of *SERK3/BAK1*, BR signaling is reduced, allowing the release of negative regulation of PTI mediated by downstream immune suppressing BR-responsive transcription factors such as StCHL1. But silencing of SERK3/BAK1 may also disturb the perceptions of unknown MAMPs that signal through BAK1. In conclusion, our work reveals that, like fungi, oomycetes present MAMPs that can trigger SERK3/BAK1-independent PTI.

Author contributions

Z.D.T. and P.R.J.B designed the study. H.W., H.H., and Y.Q. performed the experiments. Z.T performed the potato transformations. H.W. analyzed data and drafted the manuscript. H. M., Z.D.T. and P.R.J.B revised the manuscript. Authors have no conflict of interest.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Supporting Information

Fig. S1 Expression time courses of flg22 induced transcripts in potato and *Nicotiana benthamiana*.

Fig. S2 Expression time course of chitin induced transcripts in potato and *N. benthamiana*.

Fig. S3 Expression time courses of Pep13 induced transcripts in potato and *N. benthamiana*.

Fig. S4 Alignment of *Solanum tuberosum* (potato) and *Nicotiana benthamiana* SERK3/BAK1 full-length nucleotide sequences.

Fig. S5 The silencing of BAK1 in *Solanum tuberosum*.

Fig. S6 Virus-induced gene silencing (VIGS) of BAK1 in *Nicotiana benthamiana*.

Table S1. Primer sequences used in this study.

Figure legends

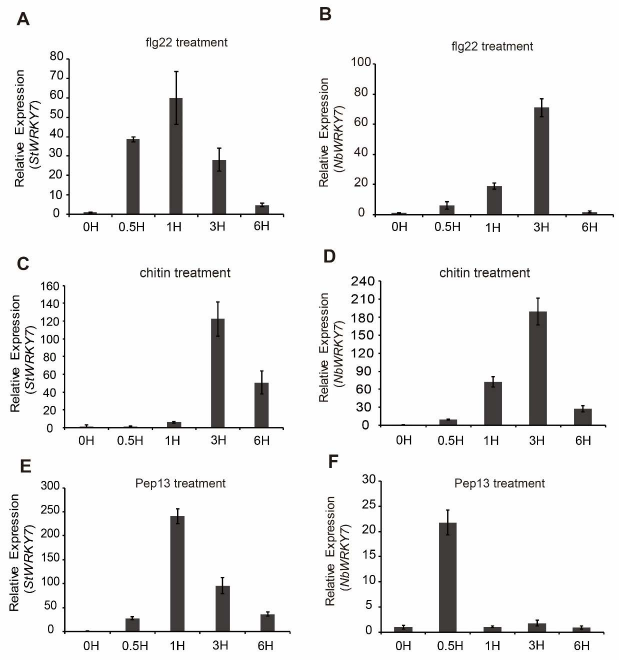
Fig. 1. Time courses of transcriptional responses following three MAMP (flg22, chitin and Pep-13) treatments in potato and *Nicotiana benthamiana*. (A), (C) and (E) *StWRKY7* transcript accumulation in potato. (B), (D) and (F) *NbWRKY7* transcript accumulation in *N. benthamiana.* Three biological replicates.

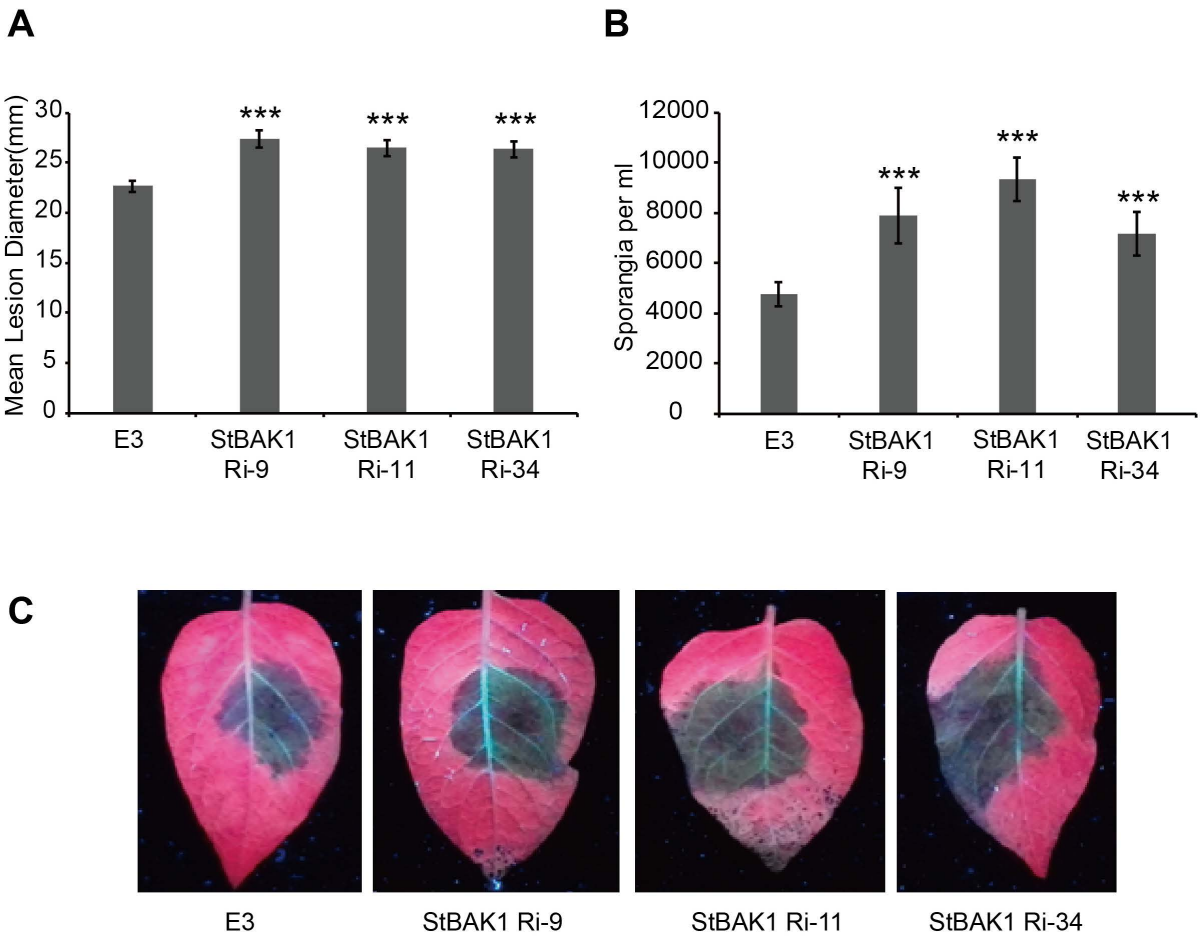
Fig. 2. StBAK1 RNAi potatoes are more susceptible to *Phytophthora infestans*. (A) Graph showing mean lesion diameter at 5 dpi with *P. infestans* on the control (E3), StBAK1-Ri9, StBAK1-Ri11 and StBAK1-Ri34 potato plants. These experiments were repeated at least three times. Statistical analysis was carried out using ANOVA with pairwise comparisons performed with a Holm-Sidak test. Asterisks denote the p value as follows *** p≤0.001; error bars show standard error. (B) Graph shows the number of sporangia recovered per ml at 4 dpi from *P. infestans* infected leaves silenced for StBAK1 compared to the E3 control. (C) Representative images showing disease lesions on the leaves of StBAK1 RNAi and control plants. Photo taken under UV light.

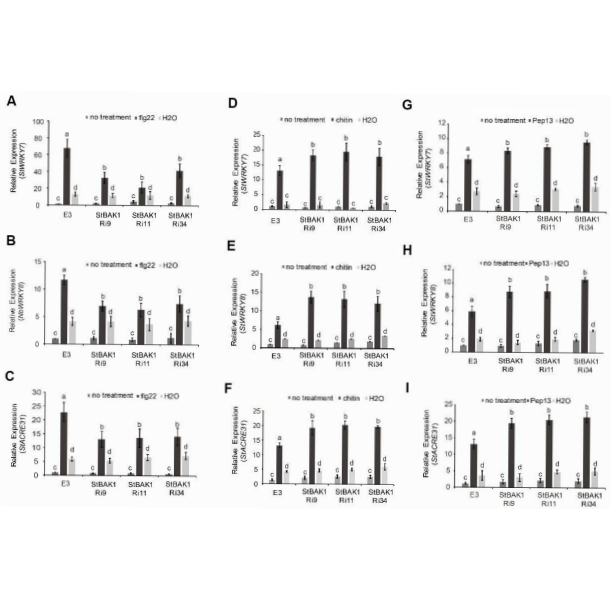
Fig. 3. PTI marker gene transcript responses on *StBAK1* silenced potatoes treated with each PAMP. (A-C) Expression levels of *StWRKY7*, *StWRKY8* and *StACRE31* on wild-type E3 and transgenic potatoes carrying the StBAK1 RNAi construct were analyzed after treatment with 40 µM flg22 using quantitative RT-PCR (D-F) with 1 µg ml^{-1} chitin and (H-I) with 40 µM Pep-13. Data are the means ± SD calculated using three biological replicates. Statistical analysis was carried out using ANOVA with pairwise comparisons performed with a Holm-Sidak test. Lower case letters a, b, c and d denote statistically significant changes.

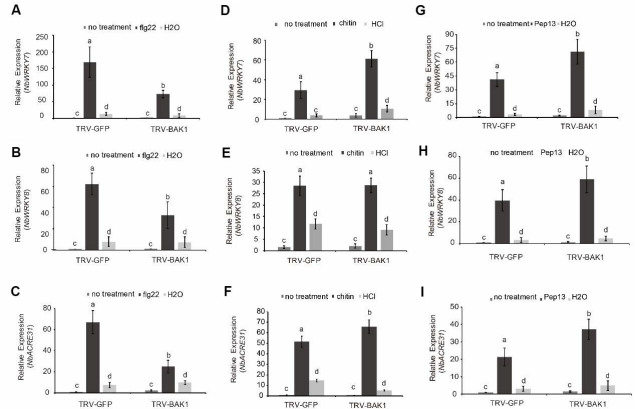
Fig. 4. PTI marker gene transcript responses during virus-mediated gene silencing of *NbBAK1* in *N. benthamiana* treated with three PAMPs. **(**A)-(C) Expression levels of *NbWRKY7*, *NbWRKY8* and *NbACRE31* in TRV: GFP controls and in Nb: SERK3/BAK1 silenced plants analyzed using quantitative RT-PCR after treatment with 40 µM flg22. (D-F) with 1 µg ml^{-1} chitin and (G)-(I) with 40 µM Pep-13. Data are the means ± SD calculated using three biological replicates. Statistical analysis was carried out using ANOVA with pairwise comparisons performed with a Holm-Sidak test. Lower case letters a, b, c and d denote statistically significant changes.

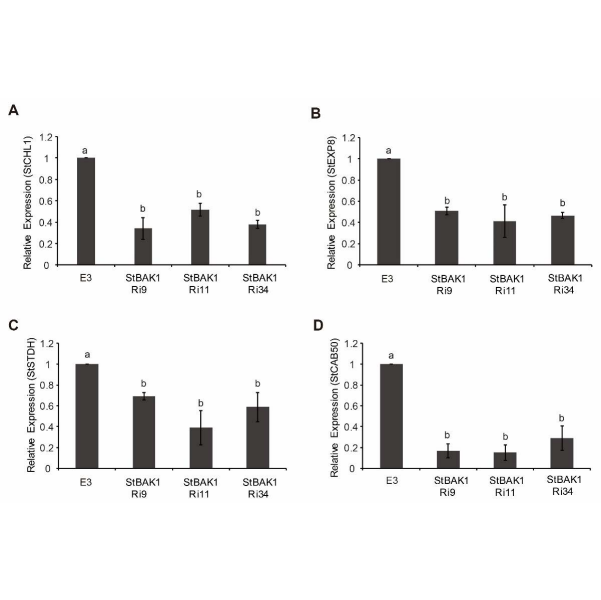
Fig. 5. BR related gene transcript responses on *StBAK1* silenced potatoes. (A) - (D) Expression levels of *StCHL1*, *StEXP8*, *StSTDH* and *StCAB50*. Data are the means ± SD calculated using three biological replicates. Statistical analysis was carried out using ANOVA with pairwise comparisons performed with a Holm-Sidak test. Lower case letters a and b denote statistically significant changes.


A
flg22 treatment
Relative Expression (StWRKY7)
80
70
60
50
40
30
20
10
0
0H
0.5H
1H
3H
6H
B
flg22 treatment
Relative Expression (NbWRKY7)
100
80
60
40
20
0
0H
0.5H
1H
3H
6H
C
chitin treatment
Relative Expression (StWRKY7)
160
140
120
100
80
60
40
20
0
0H
0.5H
1H
3H
6H
D
chitin treatment
Relative Expression (NbWRKY7)
240
210
180
150
120
90
60
30
0
0H
0.5H
1H
3H
6H
E
Pep13 treatment
Relative Expression (StWRKY7)
300
250
200
150
100
50
0
0H
0.5H
1H
3H
6H
F
Pep13 treatment
Relative Expression (NbWRKY7)
30
25
20
15
10
5
0
0H
0.5H
1H
3H
6H


A
Mean Lesion Diameter(mm)
30
25
20
15
10
5
0
***
***
***
E3
StBAK1 Ri-9
StBAK1 Ri-11
StBAK1 Ri-34
B
Sporangia per ml
12000
10000
8000
6000
4000
2000
0
***
***
***
E3
StBAK1 Ri-9
StBAK1 Ri-11
StBAK1 Ri-34
C
E3
StBAK1 Ri-9
StBAK1 Ri-11
StBAK1 Ri-34


A
B
C
D
E
F
G
H
I
no treatment
flg22
chitin
Pep13
H2O
Relative Expression (StWRKY1)
Relative Expression (StWRKY6)
Relative Expression (StACRE31)
E3
StBAK1 Ri9
StBAK1 Ri11
StBAK1 Ri34


A
no treatment
flg22
H2O
Relative Expression (NbWRKY7)
TRV-GFP
TRV-BAK1
B
Relative Expression (NbWRKY8)
C
Relative Expression (NbACRE31)
D
chitin
HCl
Relative Expression (NbWRKY7)
E
Relative Expression (NbWRKY8)
F
Relative Expression (NbACRE31)
G
Pep13
Relative Expression (NbWRKY7)
H
Relative Expression (NbWRKY8)
I
Relative Expression (NbACRE31)


A
Relative Expression (StCHL1)
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
E3
StBAK1 Ri9
StBAK1 Ri11
StBAK1 Ri34
B
Relative Expression (StEXP8)
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
E3
StBAK1 Ri9
StBAK1 Ri11
StBAK1 Ri34
C
Relative Expression (StSTDH)
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
E3
StBAK1 Ri9
StBAK1 Ri11
StBAK1 Ri34
D
Relative Expression (StCAB50)
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
b
E3
StBAK1 Ri9
StBAK1 Ri11
StBAK1 Ri34

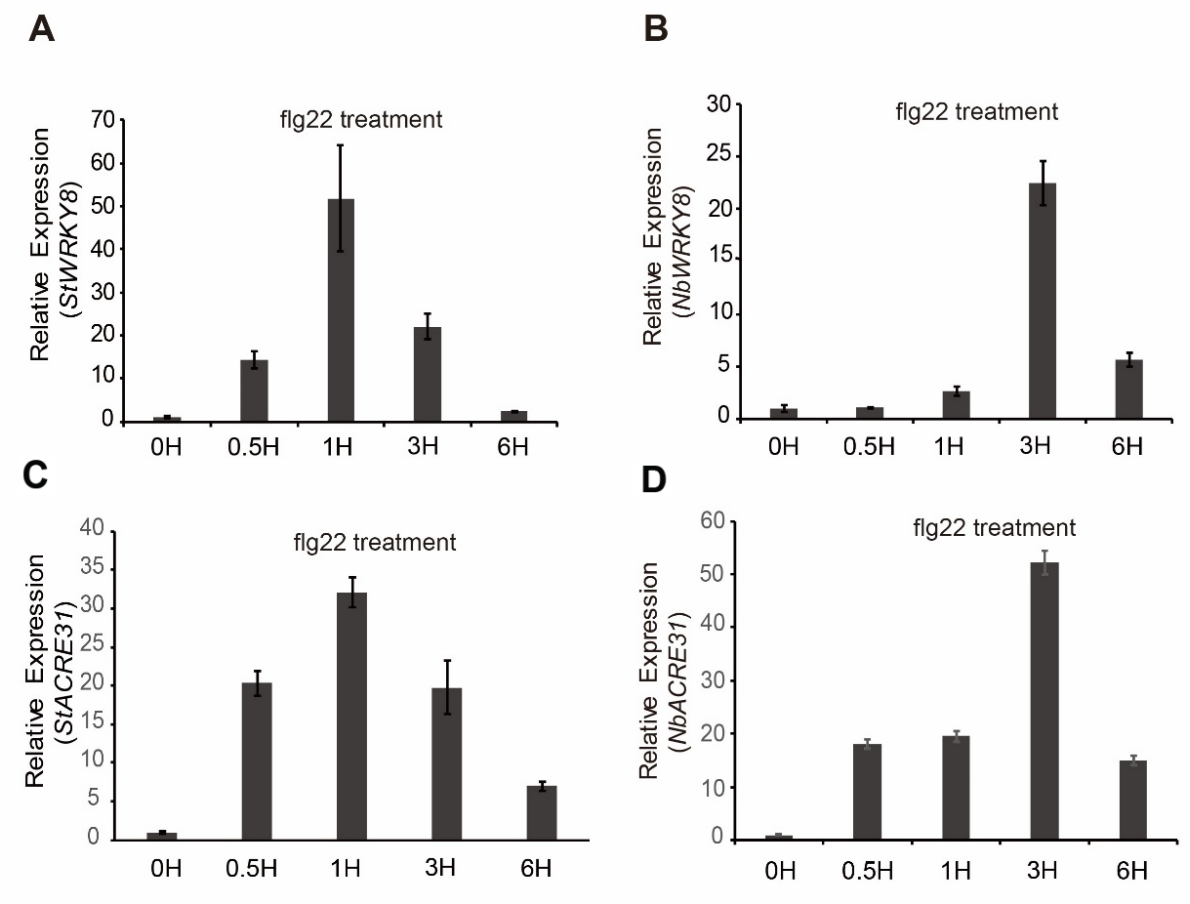


Fig. S1 Expression time courses of flg22 induced transcripts in potato and *Nicotiana benthamiana*. (A) and (C) *StWRKY8* and *StACRE31* transcript accumulation at 1 h post-inoculation (hpi) of flg22 in potato. (B) and (D) *NbWRKY8* and *NbACRE31* transcript accumulation at 3 h post-inoculation (hpi) in *N. benthamiana.*

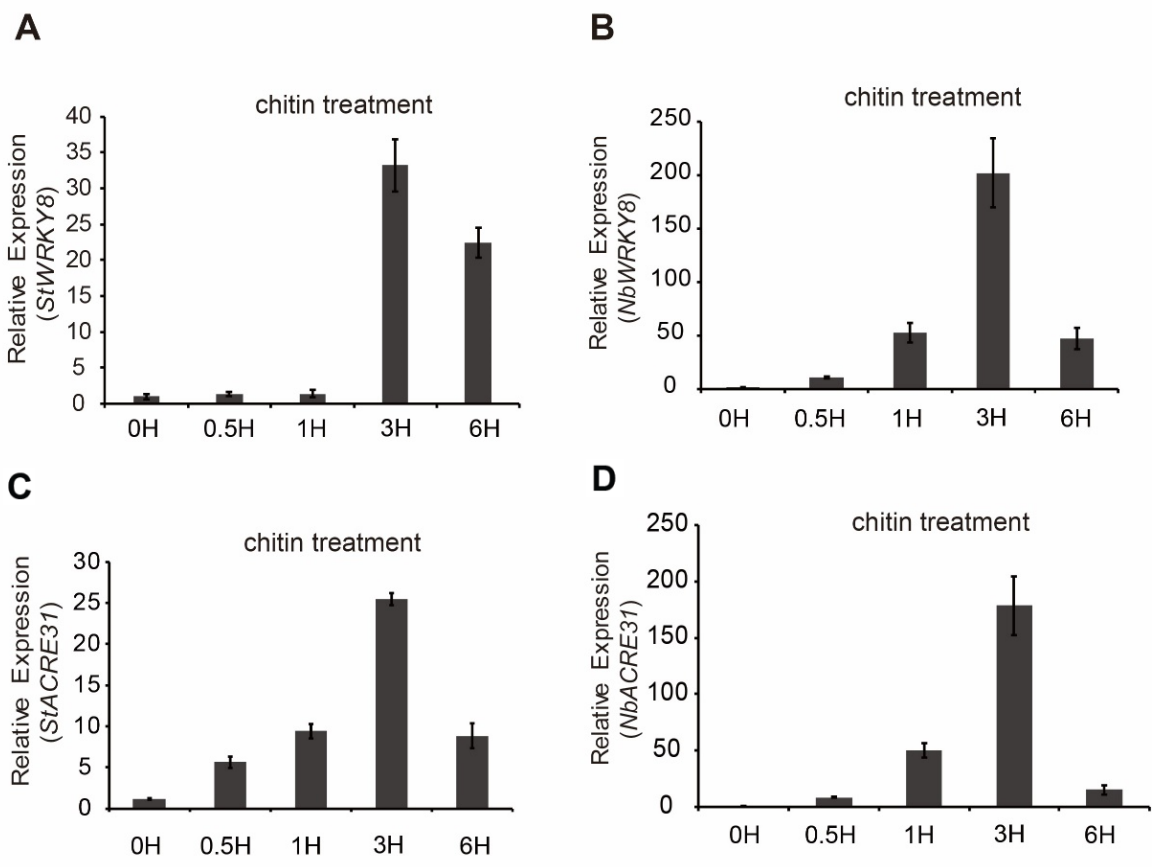


Fig. S2 Expression time course of chitin induced transcripts in potato and *N. benthamiana*. (A) and (C) *StWRKY8* and *StACRE31* transcript accumulation at 3 h post-inoculation (hpi) of chitin in potato. (B) and (D) *NbWRKY8* and *NbACRE31* transcript accumulation at 3 h post-inoculation (hpi) in *N. benthamiana.*

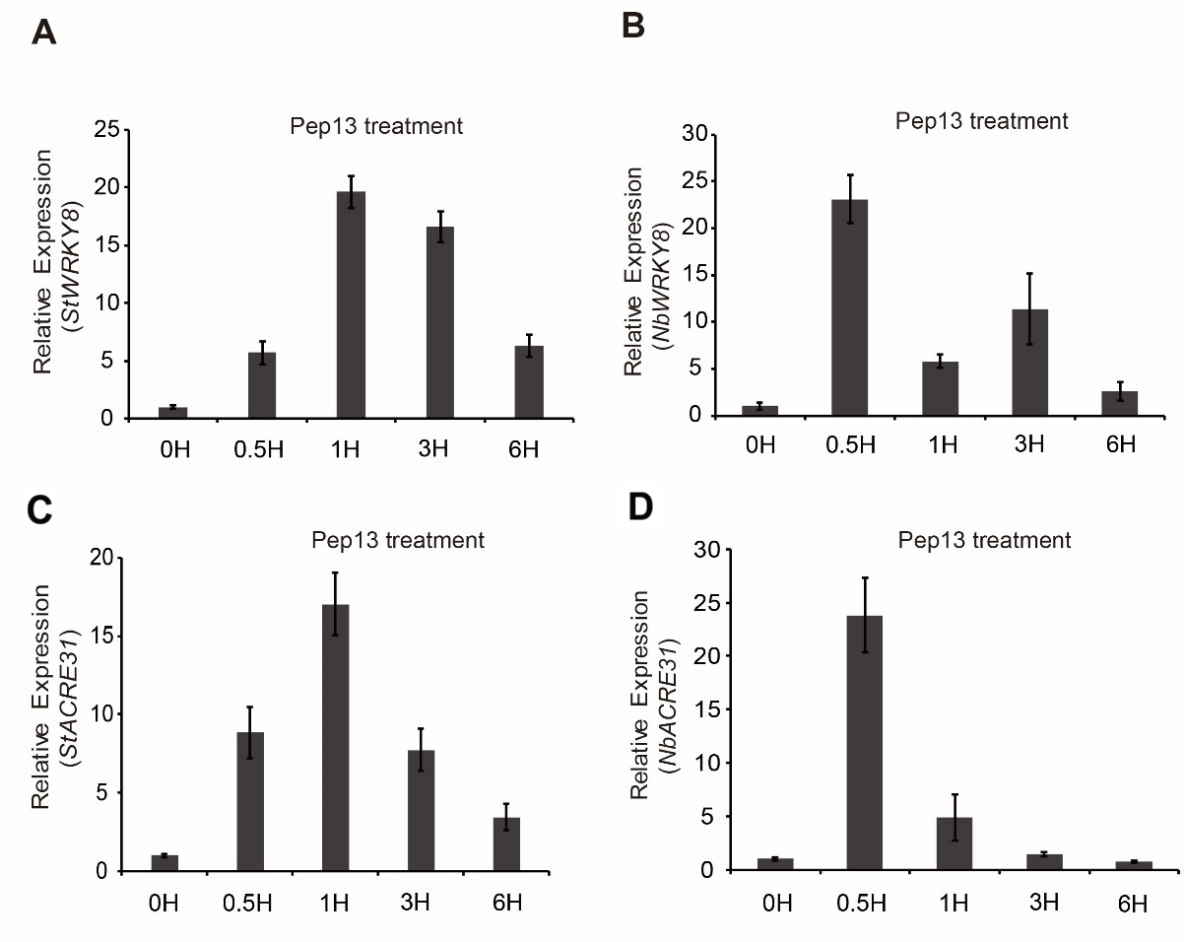


Fig. S3 Expression time courses of Pep13 induced transcripts in potato and *N. benthamiana*. (A) and (C) *StWRKY8* and *StACRE31* transcript accumulation at 1 h post-inoculation (hpi) of Pep13 in potato. (B) and (D) *NbWRKY8* and *NbACRE31* transcript accumulation at 0.5 h post-inoculation (hpi) in *N. benthamiana.*

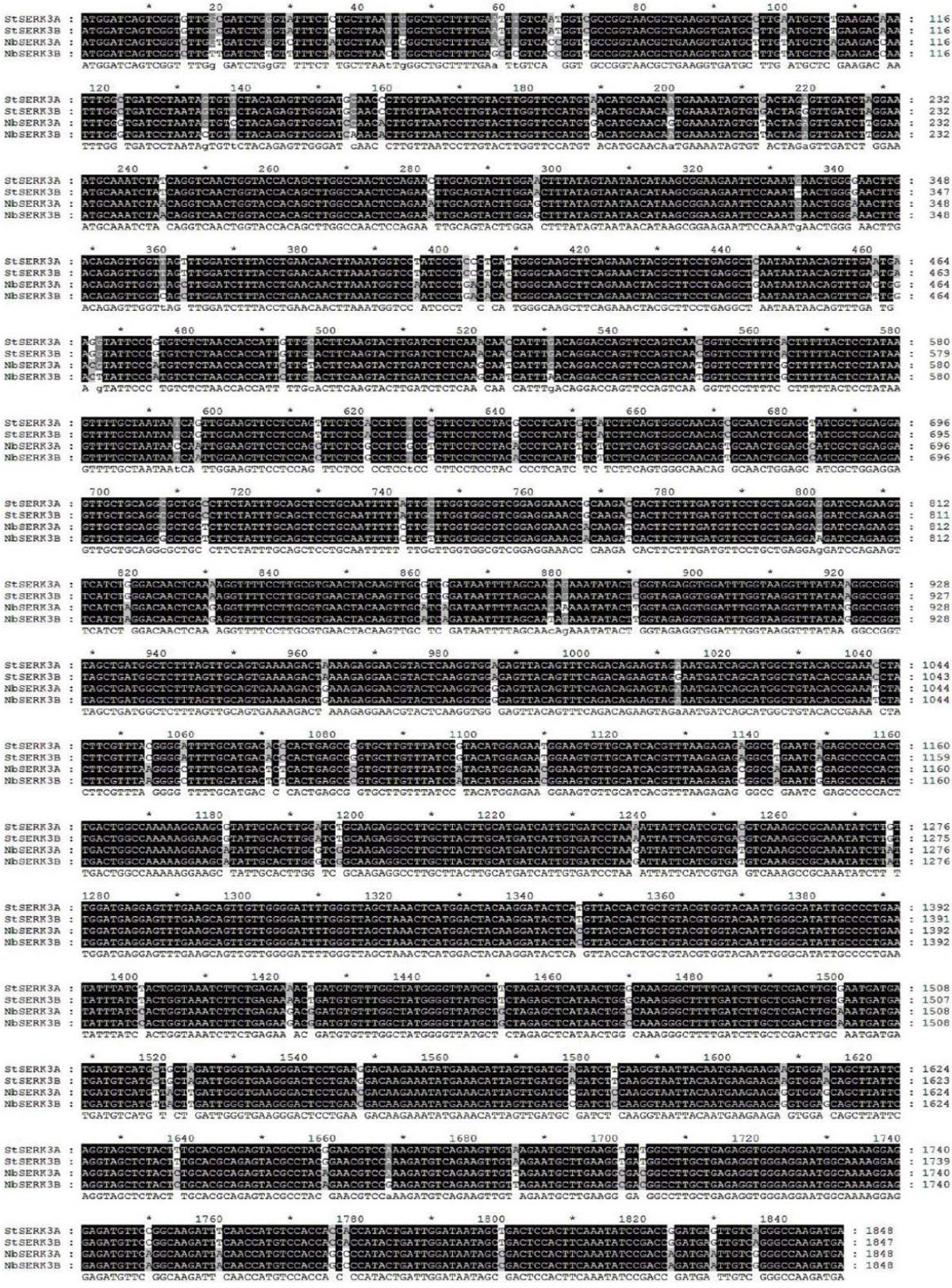


Fig. S4 Alignment of *Solanum tuberosum* (potato) and *Nicotiana benthamiana* SERK3/BAK1 full-length nucleotide sequences. Sequences were aligned using ClustalX2 and the graphic view generated in GeneDoc.

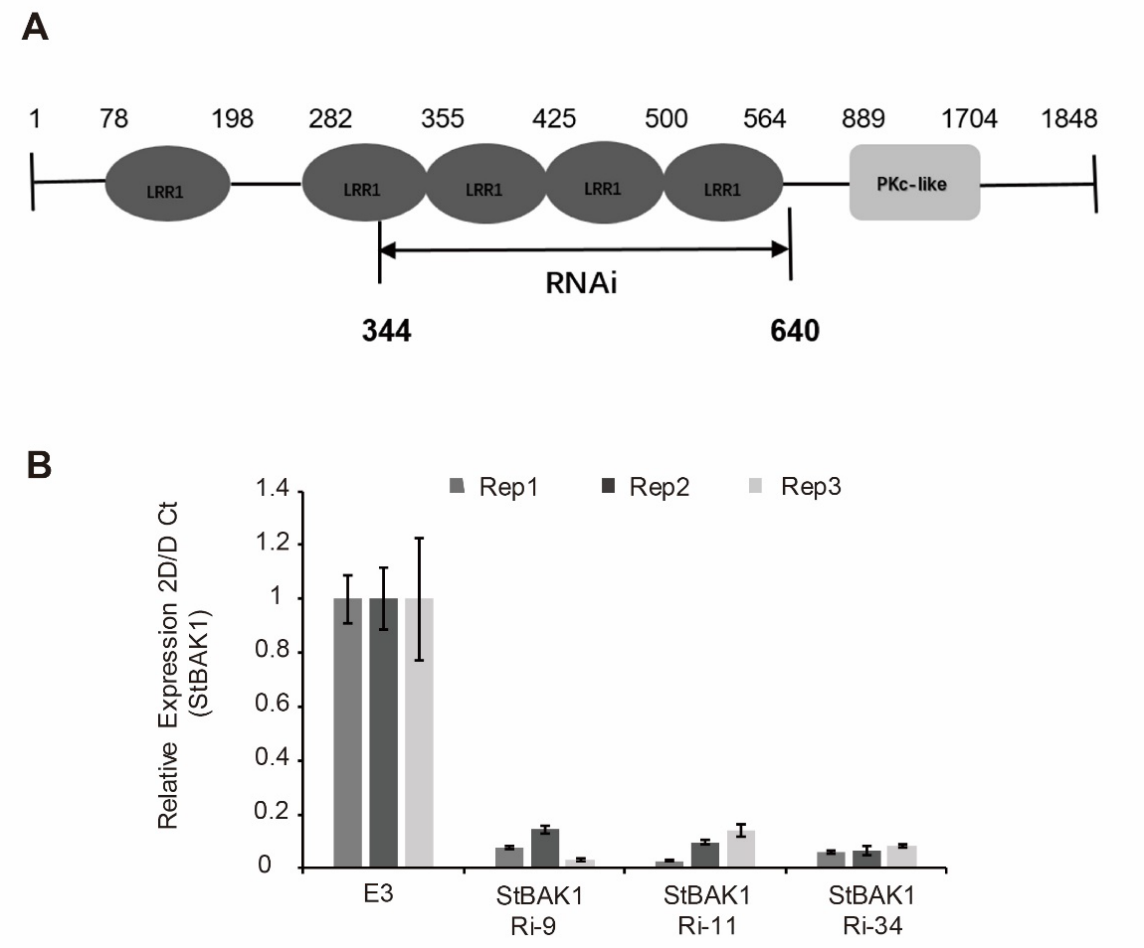


Fig. S5 Silencing efficiency of *BAK1* in *Solanum tuberosum*. (A) Schematic representation of the location of the *StBAK1* fragment that was cloned into the pHellsgate8 silencing construct (Arrows). (B) Graph showing the expression reduction of *StBAK1* in the three RNAi plants compare to the E3 control plants (three independent replicates).

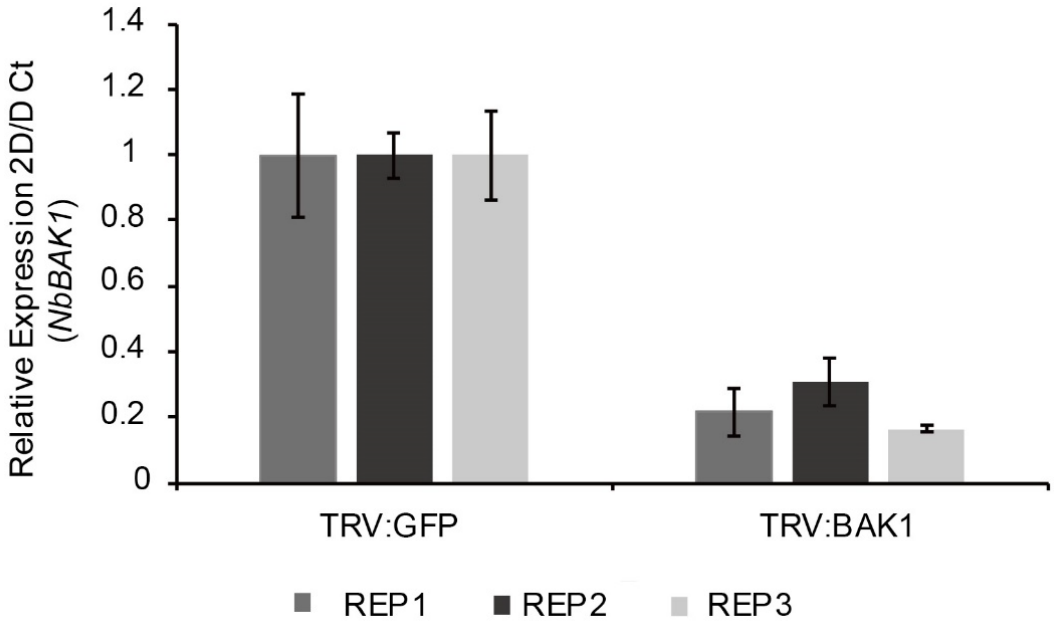


Fig. S6 Virus-induced gene silencing (VIGS) of *BAK1* in *Nicotiana benthamiana*. Graph shows that VIGS of *NbBAK1* resulted in a reduction in expression of *NbBAK1* in TRV: BAK1 plants compared to the TRV: GFP control plants (three independent replicates).

Table S1. Primer sequences used in this study.

Gene name	Primer Seqence 5' - 3'
attB 1_StSERK3/BAK1F	A AAAAGCAGGCT ACTTGACAGAGTTGGTTAGTT
attB2_StSERK3/BAK1R	AGAAAGCTGGGT TAGGAGGAAGGGGAGGAGGT
qRT_StSERK3/BAK1F	CACCTCCTCCCCTTCCTCCTA
qRT_StSERK3/BAK1R	CCGACGCCACCAAGCAATAA
qRT_StACRE31F:	CAGGATGAATCGGATCTGAAA
qRT_StACRE31R:	CGGCAATCCCAATTTCTCTA
qRT_StWRKY7F:	CCAACTGGAAGCAACAACAA
qRT_StWRKY7R:	CCTGATTAGAATGATTAGCCAACA
qRT_StWRKY8F:	CCTACTGTGACATCTCATCAATCC
qRT_StWRKY8R:	GGGTGCTCCCATTTCAGAC
qRT_StTUBF	CAAATGTGGGATGCCAAGAA
qRT_StTUBR	AGCTGTCAGGTAACGTCGTCCATGA
qRT_Nb- ef1aF	TGGACACAGGGACTTCATCA
qRT_Nb- ef1aR	CAAGGGTGAAAGCAAGCAAT
qRT_NbAcre31F	AATTCGGCCATCGTGATCTTGGTC
qRT_NbAcre31R	GAGAAACTGGGATTGCCTGAAGGA
qRT_NbWRKY7F	CACAAGGGTACAAACAACACAG
qRT_NbWRKY7R	GGTTGCATTTGGTTCATGTAAG
qRT_NbWRKY8F	AACAATGGTGCCAATAATGC
qRT_NbWRKY8R	TGCATATCCTGAGAAACCATT
qRT_StEXP8F	TGTTGGAGGTGCTGGTGATA
qRT_StEXP8R	AATTTTGGCCCCAATTTCTT
qRT_StCAB50F	GCCGATCCAGAAACTTTTGC
qRT_StCAB50R	CAGCTTCACCGAACTTGACA
qRT_StSTDHF	TGCAACATGCCCACATTATC
qRT_StSTDHR	TGCTCCTTCCCATCAAGTAAA