Different requirement of immunity pathway components by oomycete effectors-induced cell death

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Abstract
Plant pathogenic oomycete species pose a worldwide threat to crop production and ecosystems. During infection, oomycete pathogens secrete a series of effectors to manipulate plant immunity. Many of these effectors, which are indicated as avirulence gene candidates, will use components of immunity pathway to induce cell death in plants. This response given by plants is known as effector-triggered immunity (ETI). The identification of avirulence genes from pathogenic oomycete species opens a way to investigating their virulence function and uncovering related R gene repertoires in resistant plants. In this study, we screened eight cell death-inducing effectors from oomycete species in N. benthamiana and tested the requirements of ETI signaling components to induce cell death. SGT1 was required for PsAvh163- and PcRXLR25-mediated cell death, while silencing NbHSP90 abolished PcRXLR25-, PsAvh163-, PsAvh241- and PsCRN63-triggered cell death. The cell death induced by the tested effectors does not depend on EDS1, NDR1, NRG1 and ADR1. PcRXLR25- and PsAvh163-induced cell death was found to require NRC2/3/4, indicating that these two effectors are avirulence protein candidates. Finally, we found that auto-activated NRC2/3/4 also required SGT1 and HSP90 to induce hypersensitive response.

Keywords: Phytophthora, Effector-triggered immunity, RXLR, CRN, R gene

Background
Oomycete pathogens continue to hamper crop production and damage ecosystems on a global scale (Pais et al. 2013). A notorious group of pathogens are found within the Phytophthora genus and Pythium genus, among which members such as Phytophthora infestans and Phytophthora sojae cause severe yield losses in potato, tomato and soybean crops, while others, such as Pythium ultimum is a rapidly emerging pathogen with a broad host range (Kamoun et al. 2015). Oomycetes belong to heterokont/chromist clade (Riisberg et al. 2009), within the ‘Straminipila-Alveolata-Rhizaria’ superkingdom (Burki et al. 2008). The common management method for controlling pathogenic fungi may fail to prevent infections of oomycete pathogens due to the great diversity between these two types of pathogens. Therefore, there is an urgent need to understand the mechanisms underpinning the parasitism of this important group of eukaryotes.

For successful colonization in host plants, oomycete pathogens deliver diverse groups of effectors into plant cells to subvert host immunity (Dou and Zhou 2012). Oomycete pathogens mostly secrete two classes of effectors: apoplastic effectors such as necrosis and ethylene-inducing peptide-like proteins (NLPs), and cytoplasmic effectors such as RXLRs (Arg-X-Leu-Arg, where X is any amino acid) and CRNs (crinkling and necrosis proteins) (Dou and Zhou 2012). Specifically, hundreds of effectors are encoded by oomycete pathogens and many of them trigger hypersensitive response (HR)-like phenotype in...
Plants use their sophisticated immune system to antagonize the invasion of external pathogens. Plants employ two main regulatory strategies to defend themselves against pathogens: pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl 2006). PTI requires membrane-localized pattern recognition receptors to recognize pathogen- or microbe-associated molecular patterns (Zipfel 2008). However, virulent pathogens can secrete effectors to escape from the recognition by pattern-recognition receptors and therefore reach the aim of manipulating host immunity (Tsuda and Katagiri 2010). To address this problem, ETI, which is mediated by disease resistance (R) proteins, can directly or indirectly recognize the presence of effectors secreted by pathogens (Tsuda and Katagiri 2010). Compared with PTI, ETI induces a stronger and faster defense response against pathogens and is often accompanied by local cell death, a characteristic feature of the hypersensitive response (HR) (Dodds and Rathjen 2010).

Upon pathogen recognition, conformational changes and/or translocation of the sensors would activate the downstream immunity signaling pathways. Thence, misfolded or used sensors become a threat to host cells and must be inactivated and discarded immediately to avoid inappropriate activation of downstream pathways. Fortunately, several critical components of the ETI pathway have been identified to correct these misfolded and overused sensors, such as SGT1 and HSP90 (Shirasu 2009). SGT1-HSP90 pair, a structurally and functionally conserved chaperone complex in eukaryotes, is required for the maintenance of nucleotide-binding leucine-rich repeat (NLR)-type sensors in a correct state (Shirasu 2009). Another ETI regulator, ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1), is a conserved lipase-like protein that can transduce signals from pathogen-activated intracellular NLR receptors to transcriptional defense response and host cell death (Dongus and Parker 2021). In addition, NONRACE-SPECIFIC DISEASE RESISTANCE (NDR1), a positive plant immunity regulator, is required for both PTI and ETI (Knepper et al. 2011b; McNeece et al. 2017). NDR1 is also important for mediating electrolyte leakage because of its plasma membrane-localization (Knepper et al. 2011a).

NLR-mediated immune responses often require the presence and activity of so called ‘helper’ NLRs (hNLRs) (Wu et al. 2017; Qi et al. 2018). There are three described hNLR families, all encoding coiled-coil (CC)-NLRs (CNLs): the ACTIVATED DISEASE RESISTANCE 1 (ADR1) family (Bonardi et al. 2011), the N REQUIRED GENE 1 (NRG1) family (Peart et al. 2005) and HR-associated cell death (NRC) family (NB-LRR protein) (Gabriels et al. 2007). It seems that hNLRs serve as downstream signaling hubs for a diverse array of senser NLRs (Jubic et al. 2019).

In this study, we tested the requirement of ETI components for eight oomycete effectors to induce cell death in N. benthamiana. ETI-related genes were silenced by virus-induced gene silencing (VIGS) and effectors were then expressed in silenced leaves. SGT1 was found to be essential for INF1, VdEIX3, PcRXLR25 and PsAvh163 to induce cell death in N. benthamiana, while silencing NbHSP90 abolished PcRXLR25-, PsAvh163-, PsAvh241- and PsCRN63-triggered cell death. HR induced by PcRXLR25 and PsAvh163 required NRC2/3/4. No effectors induced cell death through EDS1, NDR1, NRG1 and ADR1. PcRXLR25 and PsAvh163 depended on both SGT1/HSP90 and NRC2/3/4 to induce HR, and this inspired us to investigate the relationship of NRC2/3/4 with SGT1 and HSP90. Moreover, we found auto-activated NRC2/3/4 required SGT1 and HSP90 to induce HR. Interestingly, however, there was no interaction between NRC2/3/4 and SGT1 or HSP90 by conducting a luciferase complementation assay.

Results
Eight oomycete effectors induce HR-like phenotype in N. benthamiana

Our laboratory has reported that many effectors from oomycete species induce cell death in N. benthamiana (Li et al. 2019; Ai et al. 2020). To figure out whether the oomycete effector-induced cell death requires ETI components, a transient expression screening was conducted. Eight effectors listed as INF1, PoNLP5, PoNLP7, PcRxLR25, PsAvh105, PsAvh163, PsAvh241 and PsCRN63 were chosen in this assay (Additional file 1: Table S1). VdEIX3, a fungal effector that induces pattern-triggered immunity (PTI), was selected as a control (Yin et al. 2021). VdEIX3 was found to be essential for INF1, VdEIX3, PcRXLR25 and PsAvh163 to induce cell death in N. benthamiana. GFP and VdEIX3 were used as negative and positive control, respectively. All the eight effectors and VdEIX3 induced obvious cell death in infiltration sites (Fig. 1a), while leaves expressing GFP did not. Confocal images and Western blot assay indicated that all the proteins were expressed correctly (Fig. 1b and Additional file 2: Figure S1).
VdEIX3, INF1, PcrXLR25 and PsAvh163 induce SGT-dependent HR, while INF1, PcrXLR25, PsAvh163, PsAvh241 and PsCRN63 cause HSP90-dependent HR

SGT1 and HSP90 jointly regulate the stability and accumulation of NLR protein in plants. Many NLR proteins require the complex of ubiquitin ligase-related protein SGT1 and the heat shock protein HSP90 to activate cell death (Bos et al. 2006; Li et al. 2015). To test whether the cell death triggered by the selected effectors depends on NbSGT1 or NbHSP90, we individually silenced the two genes by VIGS. Compared with TRV-GUS control leaves, the expression levels of NbSGT1 and NbHSP90 were reduced ~90% and ~70% in indicated silenced leaves, respectively (Additional file 2: Figure S2). NbSGT1- and NbHSP90-silenced plants showed impaired growth phenotype (Additional file 2: Figure S3). Furthermore, INF1 induced cell death was abolished in SGT1- and HSP90-silenced plants (Fig. 2a). These results are similar to the previous report on SGT1- and HSP90-silenced N. benthamiana (Bos et al. 2006), indicating that NbSGT1 and NbHSP90 were silenced successfully.
Silencing *NbSGT1* abolished VdEIX3-, INF1-, PoNL25-, and PsAvh163-triggered cell death in *N. benthamiana*, while silencing *NbHSP90* abolished INF1-, PoNL25-, PcRXLR25-, PsAvh163-, PsAvh241- and PsCRN63-triggered cell death (Fig. 2a). It was reported that protein accumulation might be affected in SGT1-VIGS plants (Yu et al. 2019). In our western blot assay, all proteins did accumulate correctly in GUS-, SGT1- and HSP90-silenced lines (Fig. 2b). Based on these results, we speculated that these effectors-induced cell deaths might be related to the ETI pathway.

The cell death caused by eight effectors in *N. benthamiana* does not depend on *NbNDR1* and *NbEDS1*

NDR1 and EDS1 are vital components of the ETI pathway (Aarts et al. 1998; McDowell et al. 2000; Day et al. 2006). We next tested whether the selected effector-triggered cell death is NDR1- or EDS1-dependent. As shown in Fig. 3a, silencing *NDR1* or *EDS1* led to no effect on the cell death induced by all eight effectors. NDR1 and EDS1 expression levels were reduced ~90% in the indicated gene-silenced leaves (Fig. 3a). *Agrobacterium* infiltration with any one of the eight effectors on EDS1- and NDR1-silenced leaves led
to the same cell death phenotype as those on GUS-silenced leaves (Fig. 3b), indicating that these effectors triggered EDS1- and NDR1-independent cell death.

**The cell death caused by eight effectors in** _N. benthamiana_ **does not depend on** RPW8-type helper NLR _NbADR1_ **and** _NbNRG1_ **N**

NRG1 and ADR1 are RPW8-containing helper NLRs that are required for cell death triggered by recognition between AVR proteins and sensor NLRs (Castel et al. 2019; Wu et al. 2019). It was reported that NRG1 is an EDS1 downstream factor to regulate TIR-type sensor NLRs (TNLs)-mediated immunity in _N. benthamiana_ (Qi et al. 2018). Besides, ADR1 is required for normal function of many TNLs (Wu et al. 2019). _NRG1_ - and _ADR1_-silenced lines were obtained and the eight effectors were individually expressed in the leaves (Fig. 3a). The expression levels of _ADR1_ and _NRG1_ were both down-regulated between AVR proteins and sensor NLRs (Castel et al. 2019; Wu et al. 2019). It was reported that NRG1 is a EDS1 downstream factor to regulate TIR-type sensor NLRs (TNLs)-mediated immunity in _N. benthamiana_ (Qi et al. 2018). Besides, ADR1 is required for normal function of many TNLs (Wu et al. 2019). _NRG1_ - and _ADR1_-silenced lines were obtained and the eight effectors were individually expressed in the leaves (Fig. 3a). The expression levels of _ADR1_ and _NRG1_ were both down-regulated
by more than 60%, indicating that the silencing efficiency is acceptable (Fig. 3a). The eight effectors still induced cell death in infiltration sites (Fig. 3b), which indicated that the cell death induced by the eight effectors did not require NRG1 or ADR1.

There is function redundancy between ADR1 and NRG1 (Saile et al. 2020). We thus silenced ADR1 and NRG1 simultaneously and found knock-down of both ADR1 and NRG1 did not abolish the cell death phenotype triggered by these effectors (Fig. 3a, b).

**PcRXLR25- and PsAvh163-triggered cell death in N. benthamiana depends on NRC helpers**

Except for RPW8-type helper NLRs, *N. benthamiana* has another significant type of helper NLRs, NRCs (NRC2, NRC3 and NRC4), which were reported to participate in defense responses in oomycetes, bacteria, viruses and nematodes (Wu et al. 2016; Derevnina et al. 2021). NbNRC4 is indispensable for Rpi-blb2 to recognize the *P. infestans* effector AVRblb2 in *N. benthamiana* (Wu et al. 2017). NRC2 and NRC3 are required for Pto-induced HR (Wu et al. 2016), while Bs2 and Sw5b trigger NRC2/3/4-dependent cell death in *N. benthamiana* (Wu et al. 2017). To figure out whether the NRC helpers have a role in the cell death caused by these eight effectors in *N. benthamiana*, we silenced NRC2, NRC3 and NRC4 separately and then expressed the eight effectors individually (Fig. 4a). The silencing efficiency of the three genes was confirmed and the eight effectors still triggered cell death in the silenced leaves (Fig. 4a, b).

However, when all the three NRCs were knocked down, the cell death induced by PcRXLR25 from *Phytophthora capsici* and PsAvh163 from *P. sojae* was totally inhibited (Fig. 5a, b). Western blot assay indicated that all the proteins accumulated normally in the silenced leaves (Fig. 5c). These results showed that PcRXLR25 and PsAvh163 triggered an NRCs-dependent cell death, indicating that PcRxLR25- and PsAvh163-triggered cell death in *N. benthamiana* was related to the ETI pathway.
Cell death triggered by auto-activated NbNRC2/3/4 is dependent on SGT1 and HSP90

According to the above experimental results, we concluded that HR induced by PcRXLR25 and PsAvh163 relied on SGT1/HSP90 and NbNRC2/3/4. It is interesting to figure out whether the correct functions of NbNRC2/3/4 require SGT1 and HSP90. The HSP90-SGT1 chaperone complex interacts with NLRs and plays a critical role in maintaining the stability of NLR proteins (such as Rx, RPS2, RPS5) and NLR-mediated resistance (Takahashi et al. 2003; Holt et al. 2005; Botër et al. 2007). Meanwhile, this chaperone complex contributes to the hybrid incompatibility in Nicotiana (Katsuyama et al. 2021). To investigate the functional relationship between NRCs and SGT1 or HSP90, NRCs auto-activated mutant vector (NbNRC2^{H480R}, NbNRC3^{D480V}, NbNRC4^{D478V}) was constructed (Derevnina et al. 2021). As shown in Fig. 6a, NbNRC2^{H480R}, NbNRC3^{D480V} and NbNRC4^{D478V} induced cell death in control leaves, which confirmed that NbNRC2^{H480R}, NbNRC3^{D480V} and NbNRC4^{D478V} are auto-activated mutants. However, all the auto-activated mutants failed to induce cell death in SGT1- and HSP90-silenced plants (Fig. 6a), indicating that the correct function of NbNRC2/3/4 required both SGT1 and HSP90.

We next tested the interaction of NbNRC2/3/4 with SGT1 and HSP90 using a luciferase complementation assay. NbNRC2/3/4 were fused with nLUC in their
C terminal and co-expressed with SGT1 or HSP90 fused with cLUC in N terminal in leaves. The combination of FLS2 (flagellin-sensitive2) and Gβ (G protein β-subunit1) from Arabidopsis thaliana, a well-known associated proteins (Liang et al. 2016), were used as positive control. No chemiluminescence signal could be detected in the infiltration sites where the combination of NRCs and HSP90/SGT1 was co-expressed, while positive control (FLS2-nLUC and cLUC-Gβ) displayed strong signals (Additional file 2: Figure S4). This indicated that NRC2/3/4 and SGT1/HSP90 may not interact with each other in our system.

Discussion

Many cytoplasmic effectors secreted by oomycete pathogens could induce cell death in plants. According to the results from two large-scale screenings of RXLR effectors, nearly 6–7% RXLR effectors from P. sojae and P. capsici could trigger cell death in N. benthamiana (Li et al. 2019; Wang et al. 2011). Likewise, RXLR effectors from Pythium species are able to cause cell death in plants (Ai et al. 2020). Same situation can be applied to CRN effectors (Stam et al. 2013). These cell death-induced effectors may be toxic effectors capable of killing cells directly. Alternatively, some of them may be AVR...
proteins recognized by R proteins and trigger HR. In this study, we identified two effectors, both of which are RXLR effectors and have potential to be AVR candidates.

Ectopic expression of some CRN effectors from oomycetes and fungi leads to severe cell death in plant, insect and animal cells (Schornack et al. 2010; Stam et al. 2013; Ramirez-Garcés et al. 2016; Shen et al. 2019). In this study, no CRN effector was selected as an AVR protein candidate. The screening scale of this study is not broad enough, so whether there is a chance for CRN effectors to be AVR proteins is still unknown. In many circumstances, CRN effector-induced cell death requires nuclear localization of CRN effectors (Schornack et al. 2010; Stam et al. 2013). Similarly, some NLR proteins need nucleus localization for correct functions (Shen et al. 2007; Xu et al. 2014). It is needed to figure out whether there is an R gene that can recognize CRN effectors.

Apoplastic effectors such as NLP effectors are toxic effectors that bind to plant-specific glycosylinositol phosphorylceramide (GIPC) sphingolipids to kill plant cells (Lenarcic et al. 2017). As expected, PoNLP5- and PoNLP7-triggered cell death did not require any of the ETI components tested in this study. Interestingly, VdEIX3, an effector to induce PTI, also relied on SGT1 to produce cell death. However, it did not depend on other ETI components like EDS1 or helper NLRs. Such phenomenon may be explained by the recently uncovered tight association between ETI and PTI (Ngou et al. 2021). Similarly, another PTI-related apoplastic effector, INF1, also required SGT1 and HSP90 to induce cell death.

HR mediated by PcRXLR25 and PsAvh163 was dependent on Solanaceae-specific helper NLR, NRC2/3/4, but not EDS1 or other types of helper NLR, which indicated that the sensor NLR of PcRXLR25 and PsAvh163 might be CNL-type R genes. In addition, PcRXLR25- and PsAvh163-induced HR depended on NRC2/3/4, SGT1 and HSP90 simultaneously. This inspired us to test the association of NRC2/3/4 with SGT1 and HSP90, and the luciferase complementation assay showed that no interaction was found between them. However, since the interaction between NLR and other proteins is a complicated issue, we can only conclude that no interaction was observed in our system. Surprisingly, we detected that the auto-activated NRC2/3/4 required SGT1 and HSP90 to induce HR. The SGT1 and HSP90 complex is crucial in stabilizing NLR-type sensors (Shirasu 2009). As a result of our study, SGT1 and HSP90 complex may also help to stabilize helper NLRs.

According to the iceberg model, the vast majority of AVR/R pairs are hidden (Thordal-Christensen 2020). It is a promising way to reveal the hidden AVR proteins via investigating the requirement of cell death-induced effector for ETI signaling components. By screening effectors in gene-silenced tobacco leaves, we have successfully identified two putative AVR proteins, PcRXLR25 and PsAvh163. In future studies, the virulent function of these two effectors should be investigated and their cognate R proteins should be identified. Furthermore, we have found that SGT1 and HSP90 are required for normal function of NRC2/3/4. Next, we need to determine how SGT1 and HSP90 regulate NRC2/3/4.

Conclusions
In this study, we screened eight cell death-induced effectors from oomycete pathogens in N. benthamiana to figure out which one is associated with ETI (Fig. 6b). The cell death phenotypes caused by these effectors were tested in the absence of vital ETI signaling components. As a result, we found that PsAvh163, VdEIX3 and PcRXLR25 require SGT1 to induce cell death, while silencing NbHSP90 abolishes PcRXLR25-, PsAvh163-, PsAvh241- and PsCRN63-triggered cell death. None of the eight effectors induce cell death through EDS1, NDR1, NRG1 and ADR1. HR induced by PcRXLR25 and PsAvh163 requires NRC2/3/4, suggesting that these two effectors are related to ETI. Hence, they are probably AVR protein candidates. Finally, we found that auto-activated NRC2/3/4 also relies on both SGT1 and HSP90 to generate HR.

Methods
Plasmid construct
The effectors used in the study are all existing vectors in the laboratory. Briefly, GFP (as a control), INF1 (as a control), VdEIX3, PoNLP5, PoNLP7 were cloned into pBin3xHA. PcRXLR25, PsAvh105, PsAvh163, PsAvh241 and PsCRN63 (without signal peptide) were cloned into pBinGFP2. NRC2, NRC3, NRC4, SGT1 and HSP90 were amplified from N. benthamiana. For luciferase complementation assay, NRC2, NRC3 and NRC4 were cloned into pCAMBIA1300-35S-HA-Nluc-RBS, SGT1 and HSP90 were cloned into pCAMBIA1300-35S-Cluc-RBS. To overexpress auto-activated NbNRC2/3/4 in N. benthamiana, NRC2H480R, NRC3D480V and NRC4D478V were constructed in pBin3xHA. In order to silence SGT1, HSP90, EDS1, NDR1, ADR1, NRG1, NRC2, NRC3 or NRC4 in N. benthamiana, the reported gene fragment was inserted into pTRV2 vector individually (Burch-Smith et al. 2004). Primers are listed in the Additional file 1: Table S2.

Plant growth conditions
N. benthamiana were grown in greenhouse at 25 °C with 60% relative humidity and a 16-h light/8-h dark photoperiod. VIGS-treated N. benthamiana plants used in this study were grown in the greenhouse at a temperature of...
22 °C under a 16-h light/8-h dark photoperiod and 58% relative humidity.

**Transient expression and VIGS in N. benthamiana**
The indicated recombinant constructs were transformed into *Agrobacterium tumefaciens* strain GV3101. For infiltration, *Agrobacterium* strains were cultured at 28 °C and 220 rpm for 48 h, and the cells were collected after centrifugation at 4000 rpm for 4 min. The cells were washed and then re-suspended in infiltration medium [10 mM MgCl₂, 10 mM MES (pH 5.7) and 200 μM acetylsyringone] to an appropriate optical density (OD) at 600 nm (0.4 – 0.6). Five-week-old *N. benthamiana* leaves were infiltrated for transient expression.

For *Agrobacterium*-mediated VIGS, TRV vectors pTRV-RNA1 and pTRV-RNA2, namely pTRV-SGT1, pTRV-HSP90, pTRV-EDS1, pTRV-NDR1, pTRV-ADR1, pTRV-NRG1, pTRV-NRC2, pTRV-NRC3, pTRV-NRC4, pTRV-ADR1/NRG1, pTRV-NRC2/3/4, pTRV-GUS (negative control), and pTRV-PDS (positive control), were introduced into *A. tumefaciens* strain GV3101 by electroporation. *Agrobacterium* suspensions containing pTRV-RNA1 and pTRV-RNA2 derivatives were mixed at an equal ratio and inoculated into the true leaves of 20-day-old soil-grown *N. benthamiana*. Treated *N. benthamiana* plants were maintained at 22 °C under a 16-h light/8-h dark photoperiod for 25 days before *Agrobacterium* transient expression.

**RT-qPCR analysis and bioinformatics analysis**
For RT-qPCR analysis, total RNA was extracted from *N. benthamiana* leaves with an RNA-simple Total RNA Kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the operating instructions. *N. benthamiana* cDNA was synthesized with the HiScript II Q RT SuperMix for qPCR (Vazyme Biotech Co., Ltd., Nanjing, China). Real-time PCR was performed by using a SYBR Premix Ex Taq Kit (Takara Bio Inc., Shiga, Japan) on an ABI Prism 7500 Fast Real-Time PCR system following the instructions. Gene expression levels were normalized to the expression of *NbEF1a*, a stably expressed reference gene in *N. benthamiana*. The primers used are listed in Additional file 1: Table S2. All the RT-qPCR results shown in this study were calculated from three independent biological replicates.

**Western blotting**
To extract proteins from *N. benthamiana*, leaves were frozen in liquid nitrogen and polished to a fine powder. For normal western blot assay, extraction buffer (50 mM HEPES, 150 mM KCl, 1 mM EDTA, and 0.1% Triton X-100; pH 7.5), supplemented with 1 mM DTT and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), was used for protein extraction from plant materials. Anti-HA (1:5, 000; #M20013; Abmart Inc., Shanghai, China), anti-GFP (1:5, 000; #M20004; Abmart), antibodies were used to bind the protein with the corresponding tag.

**Luciferase complementation assay**
The coding sequence of indicated genes was cloned into pCAMBIA1300-35S-HA-Nluc-RBS or pCAMBIA1300-35S-Cluc-RBS and then was transferred into *A. tumefaciens* strain GV3101. *Agrobacterium* strains carrying the indicated Cluc and Nluc constructs were infiltrated into *N. benthamiana* leaves. Leaves were sprayed with 1 mM luciferin (Biovision) and luminescence was detected with a microlight reader (BioTek, Beijing, China) after 15 min.

**Confocal microscopy**
The GFP-fused constructs were expressed in *N. benthamiana* leaves by *Agrobacterium*-mediated transient expression. The signal of fluorescence was imagined using a confocal microscope under a 488 nm excitation wavelength.

**Accession number**
The primary accession codes for INF1, VdEIX3, PoNLP5, PoNLP7, RXLR25, PsAvh105, PsAvh163, PsAvh241 and PsCRN63 that support the finding of this study were shown as AY830090.1 (INF1), VDAG_06165 (VdEIX3), PYOLI_00013111-RA (PoNLP5), PYOLI_00013113-RA (PoNLP7), PHYCAscaffold_81:176234-177661 (RXLR25), Ps138565 (PsAvh105), Ps141933 (PsAvh163), Ps133912 (PsAvh241) and HQ231783.1 (PsCRN63), respectively.

**Abbreviations**
ADR1: Activated disease resistance 1; CRN: Crinkling- and necrosis-inducing protein; EDS1: Enhanced disease susceptibility 1; ETI: Effector-triggered immunity; HR: Hypersensitive response; NDR1: Nonrace-specific disease resistance 1; NLR: Nucleotide-binding leucine-rich repeat; NRG: NRG1: N requirement gene 1; PTI: Pattern-triggered immunity.

**Supplementary Information**
The online version contains supplementary material available at https://doi.org/10.1186/s42483-022-00109-1.

**Additional file 1: Table S1.** Eight effectors from oomycetes that trigger cell death in *N. benthamiana*. Table S2. Primers used in this study.

**Additional file 2: Figure S1.** Observation of GFP-tagged effectors using confocal microscope. Confocal microscopy images were taken at 36 hpi. Scale bars = 50 μm. Figure S2. The silencing efficiency of *NbSGT1* and *NbHSP90*. Transcript levels of *SGT1* and *HSP90* were analyzed by RT-qPCR. The *EF1a* gene was used as an internal reference. Bars represent standard errors from three independent biological replicates (mean ± SD; n = 3; **, P < 0.01 compared with the GUS-silenced lines; Student’s t-test). Figure
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Authors’ contributions
DD, XD and GA conceived and designed the project, jointly performed data analysis and wrote the manuscript. XD, CX and WP performed the experiments. XD and GA analysed data. DD, XD, GA and ZY wrote and modified the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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