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***Rpi-blb2*-Mediated Hypersensitive Cell Death Caused by *Phytophthora infestans* AVRblb2 Requires *SGT1*, but not *EDS1*, *NDR1*, Salicylic Acid-, Jasmonic Acid-, or Ethylene-Mediated Signaling**

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Potato *Rpi-blb2* encodes a protein with a coiled-coil-nucleotide binding site and leucine-rich repeat (CC-NBS-LRR) motif that recognizes the *Phytophthora infestans* AVRblb2 effector and triggers hypersensitive cell death (HCD). To better understand the components required for *Rpi-blb2*-mediated HCD in plants, we used virus-induced gene silencing to repress candidate genes in *Rpi-blb2*-transgenic *Nicotiana benthamiana* plants and assayed the plants for AVRblb2 effector. *Rpi-blb2* triggers HCD through *NbSGT1*-mediated pathways, but not *NbEDS1*- or *NbNDR1*-mediated pathways. In addition, the role of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) in *Rpi-blb2*-mediated HCD were analyzed by monitoring of the responses of *NbICS1*-, *NbCOI1*-, or *NbEIN2*-silenced or *Rpi-blb2::NahG*-transgenic plants. *Rpi-blb2*-mediated HCD in response to AVRblb2 was not associated with SA accumulation. Thus, SA affects *Rpi-blb2*-mediated resistance against *P. infestans*, but not *Rpi-blb2*-mediated HCD in response to AVRblb2. Additionally, JA and ET signaling were not required for *Rpi-blb2*-mediated HCD in *N. benthamiana*. Taken together, these findings suggest that *NbSGT1* is a unique positive regulator of *Rpi-blb2*-mediated HCD in response to AVRblb2, but *EDS1*, *NDR1*, SA, JA, and ET are not required.

Keywords : AVRblb2 effector, EDS1, hypersensitive cell death, NDR1, *Rpi-blb2*, SGT1

Many late blight resistance (*R*) genes were introgressed into potato cultivars from the wild species (*Solanum* sp.) through breeding strategies (Vleeshouwers et al., 2011). Among the potato *R* genes, the *Rpi-blb1* and *Rpi-blb2* genes were cloned from the diploid species (*Solanum bulbocastanum*). These genes could confer durable resistance to *Phytophthora infestans* (van der Vossen et al., 2003; 2005). Especially, a potato *Rpi-blb2*, an *Mi-1* homolog conferring resistance to nematodes in tomato, was cloned from *S. bulbocastanum* (van der Vossen et al., 2005). *Rpi-blb2* has a coiled-coil (CC)-domain in its N-terminus with nucleotide-binding and leucine-rich-repeats (NB-LRRs), and recognizes *P. infestans* AVRblb2, activating effector-triggered immunity (ETI; Oh et al., 2009). AVRblb2 can function in Avr activity that directly activates hypersensitive cell death (HCD) and associated disease-resistance responses mediated by *Rpi-blb2* (Oh et al., 2014). HCD is a sign of induced defense responses and resembles programmed cell death in animals (Jones and Dangl, 2006). Little is known, however, about the AVRblb2 and *Rpi-blb2* recognition mechanism or defense responses in plants (Bozkurt et al., 2011; Oh et al., 2009; 2014).

Mutational and knock-down analyses in plants led to the identification of proteins downstream of the *R* proteins (Botër et al., 2007; Kadota et al., 2010). For example, *SGT1* (suppressor of the G2 allele of *skp1*), *RAR1* (Required for *Mla12* resistance), and *HSP90* (Heat Shock Protein 90) interact with *R* proteins or are required for *R* functions (Kadota et al., 2010). Specifically, the ubiquitin ligase-associated protein *SGT1* is required for the function of *R* genes (Austin et al., 2002; Azevedo et al., 2006). *SGT1* homologues in *Nicotiana benthamiana* are indispensable for *Rx*-dependent HCD in response to Potato virus *X* (PVX) and *R3a*-dependent HCD in response to *AVR3a*^{KI} (Azevedo et al., 2006; Bos et al., 2006; Peart et al., 2005). *Rpi-blb2*-mediated late blight resistance or HCD required *SGT1* in *N. benthamiana*

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indicating that the knockdown of *SGT1* in plants results in reduced *R* gene function (Oh et al., 2014).

Moreover, other resistance signaling components are enhanced disease susceptibility-1 (*EDS1*) and non-race-specific disease resistance (*NDR1*) proteins (Bhattacharjee et al., 2011; Century et al., 1997). *EDS1* functions as a regulator of basal resistance and ETI mediated by Toll-interleukin-1 (TIR)-NB-LRR proteins, including *RPP1* and *RPP4* genes in response to oomycete pathogens or *N* gene in response to *Tobacco mosaic virus* (Aarts et al., 1998; Bhattacharjee et al., 2011; Peart et al., 2002). In contrast, *NDR1* is required for disease resistance to bacterial pathogens expressing *avr* genes and several oomycete pathogens (Century et al., 1997; Tornero et al., 2002). In addition, *NDR1* is required for R-proteins that contain the CC-NB-LRR domain, such as *RPS2*, *RPM1*, and *RPP5* (Aarts et al., 1998; Century et al., 1997; Tornero et al., 2002). However, there is no information available about *Rpi-blb2*-mediated HCD- or resistance-signaling pathways.

Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play major functions in the regulation of plant defense responses to various pathogens (Glazebrook, 2005). Moreover, these molecules are involved in the regulation of different kinds of plant cell death responses (Mur et al., 2008; Rao et al., 2000). Specifically, SA acts synergistically with reactive oxygen species (ROS) accumulation to drive HCD during ETI in plants (Mur et al., 2008). Likewise, in *R*-gene triggered responses, SA and SA-pathway amplifications are required for HCD and defense-related gene functions, including HCD in response to many *R* genes (Glazebrook, 2005; Oh et al., 2014). Therefore, to elucidate the role of SA, JA, and ET signaling components in *Rpi-blb2* gene-mediated HCD, we used virus-induced gene silencing (VIGS) technology with *ICS1* (*Isochorismate synthase 1*, enzyme for SA biosynthesis), *COI1* (*Coronatine insensitive 1*, JA-insensitive), and *EIN2* (*Ethylene insensitive 2*) genes in *Rpi-blb2*-transgenic plants.

To understand the signaling pathway of *Rpi-blb2*-mediated HCD caused by *AVRblb2*, we examined the molecular functions of *Rpi-blb2* in *N. benthamiana* using *Agrobacterium*-mediated gene expression or VIGS methods. Through gain-of-function and loss-of-function studies, we demonstrate the necessity of *SGT1*, but not *EDS1*, *NDR1*, or the SA- JA-, and ethylene-mediated signaling pathways, for eliciting *Rpi-blb2*-mediated HCD in response to the *P. infestans* effector *AVRblb2*.

Materials and Methods

Bacteria, plants, and culture conditions. *Escherichia*

coli DH5 α and *Agrobacterium tumefaciens* GV3101 were grown in LB media with appropriate antibiotics at 37°C and 28°C, respectively. *P. infestans* 88069 was cultured on rye agar medium supplemented with 2% sucrose at 20°C. Homozygous transgenic *N. benthamiana* expressing *Rpi-blb2* under the control of native promoters were obtained using pBINplus based plasmids described by van der Vossen et al (2005). All *N. benthamiana* seedlings were grown in a growth chamber and maintained at 22–25°C under a 16/8 hr light-dark photoperiod.

RT-PCR analysis. Total RNA was extracted from *N. benthamiana* using TRI reagent, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). RT-PCR was performed with equal amounts of total RNA using the ReverTraAce RT-PCR kit (TOYOBO Co.Osaka, Japan). Time courses of *P. infestans* infection on detached *N. benthamiana* leaves were performed using agar plugs as described by elsewhere (van der Vossen et al., 2005). Total RNA isolated from infected leaves of *N. benthamiana*, 6 days after inoculation (dai), from non-infected leaves (0 dai), and genomic DNA isolated from *P. infestans* mycelium grown in synthetic medium (My) was amplified with primer sets from the two genes. The oligonucleotides used to amplify *P. infestans* elongation factor 2 alpha [Table S1, (Oh et al., 2009)]. The expression of *PiEF2 α* gene was controlled with primer pair, which is specific for the constitutively expressed *NbActin* gene (Table S1).

In planta HR cell death assays. For agroinfiltration assays, recombinant *A. tumefaciens* was grown as described elsewhere (van der Hoorn et al., 2000). *A. tumefaciens* carrying the respective constructs were mixed in a 2:1 ratio in inducing media (10 mM MgCl₂, 10 mM MES, pH 5.6 and 150 μ M acetosyringone), and then incubated at room temperature for 3 hr before infiltration. *Agrobacterium* solutions were infiltrated at an adjusted OD₆₀₀ of 0.4 (Oh et al., 2009). Transient co-expression of *Rpi-blb2* and *AVRblb2* was performed by mixing the appropriate *Agrobacterium* in induction buffer at a ratio of 2:1 (final OD₆₀₀ of 0.6:0.3). For HR assays, *Agrobacterium* expressing the pGR106-*AVRblb2* or controls (pGR106-dGFP) were infiltrated with a final OD₆₀₀ of 0.3 into transgenic *N. benthamiana* plants, respectively. Full length- or deleted-*Rpi-blb2* clones were co-infiltrated with *AVRblb2* in 20 inoculation sites per five plants.

Virus-induced gene silencing in *N. benthamiana*. For the VIGS assay, cultures containing *Agrobacterium* with a TRV derivative vector (TRV2-dGFP, -*NbSGT1*, *NbEDS1*,

NbNDR1, *NbICS1*, *NbCOII*, and *NbEIN2* constructs) and *Agrobacterium* GV3101 (TRV1) were transferred into LB media with antibiotics (50 mg/L kanamycin, 25 mg/L rifampicin) and grown at 28°C to an A_{600} of 0.8. The culture containing *Agrobacterium* (with TRV2-*dGFP*, -*NbSGT1* constructs) were mixed in a 1:1 ratio ($OD_{600}=0.3$) with GV3101 (TRV1) and infiltrated into the expanded leaves of 4-week-old wild type and all transgenic *N. benthamiana* (Liu et al., 2002). On day 21 after inoculation, transient expression of pGR106 or pGR106-AVRblb2 was performed by infiltration *A. tumefaciens* carrying each individual gene in an induction buffer at a ratio of 1:1 (final OD_{600} of 0.6). For RT-PCR analysis, total RNA was extracted from control (*dGFP*) and *NbSGT1*, *NbEDS1*, *NbNDR1*, *NbICS1*, *NbCOII*, and *NbEIN2*-silenced *N. benthamiana* leaves using the TRIZOL solution (Invitrogen, Carlsbad, CA). Primers used to amplify *NbSGT1*, *NbEDS1*, *NbNDR1*, *NbICS1*, *NbCOII*, and *NbEIN2* annealed outside the VIGS target region (Table S1).

Results and Discussion

ETI strengthens basal defenses and elicits *R* proteins through the recognition of pathogen-derived effectors in plants (Jones and Dangl, 2006). To understand the function of *Rpi-blb2*-mediated HCD in response to the *P. infestans* effector AVRblb2, we developed gain-of-function *Rpi-blb2*-transgenic and loss-of-function *Rpi-blb2*-silenced *N. benthamiana* plants. The *Rpi-blb2*-transgenic plants were resistant to *P. infestans*, which is in agreement with studies in tomato and potato plants (van der Vossen et al., 2005). Therefore, we further characterized *Rpi-blb2*-mediated HCD using *Rpi-blb2*-transgenic and *Rpi-blb2*-silenced *N. benthamiana* plants.

Full-length *Rpi-blb2* is required for AVRblb2-specific HCD induction and confers late blight resistance. Previously, we reported that AVRblb2 is an AVR effector that triggers *Rpi-blb2*-dependent HCD or resistance responses in *N. benthamiana*. Additionally, a 34-amino acid region in the C-terminal region of AVRblb2 is sufficient for triggering *Rpi-blb2* hypersensitivity (Oh et al., 2009; 2014). However, little is known about the regions of *Rpi-blb2* that govern this response. We used a *N. benthamiana* transient expression assay to define the regions of the *Rpi-blb2* gene that are necessary for induction of cell death. We constructed full-length (CC-NBS-LRR and *Rpi-blb2*), N-terminal (CC domain), and C-terminal (LRR domain) deletion constructs in a 35S promoter *Agrobacterium* binary vector and as-

sayed them in *N. benthamiana* (Fig. 1A).

Agrobacterium co-expression of full-length *Rpi-blb2* with AVRblb2 induced HCD 3 to 4 days after infection, whereas co-expression with the control vector or the *Rpi-blb2* N- and C-terminal deletions did not trigger HCD in *N. benthamiana* (Fig. 1A). Thus, both the CC and LRR domains, including the functional NB domain of *Rpi-blb2*, are necessary to induce HCD. Additionally, a full-length *Rpi-blb2* genomic DNA construct was fully effective in plants and triggered *Rpi-blb2*-mediated HCD (Fig. 1A). RT-PCR analysis of AVRblb2 and *Rpi-blb2*, *Rpi-blb2* N-, or *Rpi-blb2* C-terminal deletion transcripts confirmed that the transcript levels of *Rpi-blb2*, *Rpi-blb2* N-, or *Rpi-blb2* C-terminal deletion were upregulated following co-expression in *N. benthamiana* (Fig. 1B). Although the exact mechanism of the interaction between the AVRblb2 effector and the *Rpi-blb2* protein remains unclear, *in planta* interactions resulted in HCD in *N. benthamiana* (Oh et al., 2009; 2014). These results indicate that all regions of *Rpi-blb2* are required for AVRblb2 recognition and induction of cell death.

Rpi-blb2-overexpressing tomato, potato, and *N. benthamiana* plants have heightened resistance responses against *P. infestans* infections (Oh et al., 2014; van der Vossen et al., 2005). To confirm the effect of *Rpi-blb2* on the disease resistance response *in planta*, *Rpi-blb2*-transgenic *N. benthamiana* leaves were inoculated with *P. infestans*. Disease symptoms were visible in the control plants within 4 to 6 dai. However, the area of infection was significantly smaller in *Rpi-blb2*-transgenic *N. benthamiana* plants than in control plants, which correlates with reduced oomycete colonization (Fig. 1C). At 4–6 dai, visible disease symptoms were observed in 10–32.5% of the total leaf area in control plants, which was significantly larger than the 3.3–3.5% observed in the transgenic plants (Fig. 1D). Quantitative RT-PCR analyses revealed that *PiEF2a* transcript levels were significantly lower in *Rpi-blb2* transgenic *N. benthamiana* plants than in control plants (Fig. 1E). The *N. benthamiana* *Actin* gene (*NbActin*) was used as a loading control and as an internal control for RNA quality. Additionally, *Rpi-blb2* transcript expression was confirmed in *Rpi-blb2*-transgenic *N. benthamiana* plants (Fig. 1E). Reduced biomass may have resulted from a loss of viable tissue during disease progression. Thus, it was found that *Rpi-blb2*-transgenic *N. benthamiana* plants are resistant to *P. infestans*, which is in concordance with tomato and potato (van der Vossen et al., 2005). Hence, we were used for further characterization of *Rpi-blb2*-mediated HCD using VIGS techniques.

Fig. 1. The Rpi-blb2 are required to initiate HR and confer resistance to late blight disease. Deletion mutants of *Rpi-blb2* were coexpressed with *AVRblb2* by agro-infiltration in *N. benthamiana* to determine the *Rpi-blb2* domains required for induction of *AVRblb2*-derived HR. (A) Schematic of the different mutant and deletion constructs is shown on the left. HR index with plus and minus signs indicate the presence and absence of *AVRblb2*-*Rpi-blb2* function, respectively. Accumulation of auto fluorescent phenolic compounds associated with cell death induced by the *Rpi-blb2* and *AVRblb2* combination (Green) and the *Rpi-blb2* and *AVRblb2* combination were visualized under ultraviolet (UV) light. (B) Full-length *Rpi-blb2* genomic DNA, and CC-NBS-LRR, N- (CC domain) and C-terminal (LRR domain) deletion constructs in a 35S promoter *Agrobacterium* binary vector. Amplification of *Rpi-blb2*-gDNA from pBINplus-*gRpi-blb2* plasmid (Left) and RT-PCR analysis of the expression levels of pBINplus-*gRpi-blb2*-expressing (cDNA) *N. benthamiana* (Right). The *NbActin* gene was used as a control. (C) Typical disease symptoms after inoculation of *N. benthamiana* leaves with *P. infestans*. (D) Quantitative RT-PCR of *P. infestans* colonization levels in *Rpi-blb2*-expressing and WT plants. The expression of *PiEF2α* was controlled with primer *NbActin*, which is specific for the constitutively expressed *NbActin* gene. (E) Leaf area covered with disease lesion in *Rpi-blb2*-expressing and WT after inoculation with *P. infestans*. Bars indicate standard deviation (SD). All assays were repeated at least three times with similar results. CC, Coiled-coil; NBS, Nucleotide-Binding Sites; LRR, Leucine Rich Repeats; My, *P. infestans* mycelium.

SGT1, but not EDS1 or NDR1, functions in Rpi-blb2-mediated HCD in response to P. infestans AVRblb2.

Knockdown analyses in *N. benthamiana* led to the identification of signaling components downstream of R proteins (Lu et al., 2003). EDS1, NDR1, and SGT1 are key components of the signal-transduction pathway leading to disease resistance in control plants and induce HCD in response to specific effectors (Aarts et al., 1998; Bhattacharjee et al., 2011; Kadota et al., 2010). Specifically, EDS1 functions as

a regulator of basal resistance and ETI mediated by TIR-NB-LRR proteins (Bhattacharjee et al., 2011; Falk et al., 1999). CC-NB-LRR protein function requires NDR1 (Aarts et al., 1998; Shapiro et al., 2001; Tornero et al., 2002) or SGT1 (Kadota et al., 2010; Oh et al., 2014).

To examine whether HCD is induced by co-expression of *AVRblb2* and *Rpi-blb2*, we silenced *EDS1*, *NDR1*, and *SGT1* using VIGS by agro-infiltration in *Rpi-blb2*-transgenic *N. benthamiana* plants (Fig. 2A). *Rpi-blb2*-transgenic

plants were infiltrated at the four- to five-leaf stage with mixtures of *Agrobacterium* carrying combinations of TRV1, TRV2-dGFP (control vector), TRV2-*NbSGT1*, -*NbEDS1*, or -*NbNDR1*. Twenty-one dai, plants with silenced TRV2-*NbSGT1* or -*NbEDS1* had abnormal phenotypes indicative of successful silencing of *SGT1* and *EDS1*, respectively. We also confirmed *EDS1*, *NDR1*, and *SGT1* silencing by RT-PCR. Expression of all specific transcripts was reduced in the silenced plants compared with control vector-containing plants (TRV2-dGFP; Fig. 2B). The silenced *N. benthamiana* plants were infiltrated with *Agrobacterium* containing *AVRblb2*. HCD induced by *AVRblb2* was completely suppressed in *SGT1*-silenced *Rpi-blb2*-transgenic plants, but was not suppressed in plants infected with

TRV2-dGFP or in *EDS1*- and *NDR1*-silenced plants. Cell death was also quantified by ion leakage from leaves after *AVRblb2* infiltration (Fig. 2C).

In *EDS1*- and *NDR1*-silenced *Rpi-blb2*-transgenic plants, *AVRblb2* caused visible HCD at 3–4 dai in approximately 95% of the infiltration sites in three independent experiments (Fig. 2D). However, in *SGT1*-silenced plants, HCD was observed in only approximately 5% of the infiltration sites (Fig. 2D). In TRV2-dGFP (vector control)-treated plants, the *Rpi-blb2* and *AVRblb2* combination resulted in visible HCD at 4 dai in approximately 95% of the infiltration sites (Fig. 2D). Our findings indicate that *Rpi-blb2* elicits HCD through *SGT1*-mediated signaling in *N. benthamiana*. Likewise, silencing *RAR1* or *HSP90* in *N. benthamiana* plants did not abolish *Rpi-blb2*-mediated HCD in response to *P. infestans* AVRblb2 (Oh et al., 2014). Moreover, *EDS1* and *NDR1* are not required for HCD in response to *P. infestans* AVRblb2 in *Rpi-blb2*-transgenic *N. benthamiana*. Although we did not address the question of how *SGT1* regulates *Rpi-blb2* function here, we hypothesize that *NbSGT1* affects other processes, including the translation of *Rpi-blb2* and/or the maintenance of pre-activated *Rpi-blb2* protein stability. We also assumed that *SGT1*-dependent HCD could be due to the *Rpi-blb2* signaling complex in *N. benthamiana* in response to specific AVRblb2 effector proteins.

SA, JA, and ET are not required for HCD elicited by the *Rpi-blb2*-*AVRblb2* interaction. Plant hormones such as SA, JA, and ET activate defense responses to pathogen attacks and cross talk with other signaling pathways (Glazebrook, 2005; Robert-Seilantantz et al., 2011). In addition, the occurrence of HCD leads to activation of SA, JA, and ET signaling throughout the plant (Glazebrook, 2005; Overmyer et al., 2003). To functionally validate the involvement of SA, JA, and/or ET in *Rpi-blb2* to *Rpi-blb2*-mediated cell death, we performed a TRV2-based VIGS assay. *ICS1* (an enzyme for SA biosynthesis; Wildermuth et al., 2001), *COII* (a JA signaling component; Xu et al., 2002), or *EIN2* (ethylene insensitive; Alonso et al., 1999) genes were independently silenced in *Rpi-blb2*-transgenic *N. benthamiana* and *AVRblb2* was transiently infiltrated in the silenced plants (Fig. 3A). RT-PCR analysis of *NbICS1*, *NbCOII*, and *NbEIN2* transcripts confirmed that their levels were downregulated by VIGS (Fig. 3B). HCD was induced in *NbICS1*, *NbCOII*, and *NbEIN2*-silenced plants, but was not induced in TRV2-dGFP control plants (Fig. 3C). Therefore, these results indicate that SA, JA, and ET are not essential for *Rpi-blb2*-mediated HCD in response to *P. infestans* AVRblb2.

Fig. 3. *Rpi-blb2*-mediated HCD in response to AVRblb2 did not require SA, JA, or ethylene signaling. (A) Evidence that each signaling component was silenced by VIGS. (B) RT-PCR was performed with 30 cycles for *NbICS1* and 35 cycles for *NbEIN2* and *NbCOI1*. Signaling components that did not affect *Rpi-blb2*-mediated HR in response to AVRblb2. *ICS1*, *EIN2* or *COI1* was silenced and AVRblb2 was transiently overexpressed by agro-infiltration in *Rpi-blb2*-transgenic *N. benthamiana* of leaves. (C) Percentages of infiltrated leaves with *Rpi-blb2*-mediated HR in response to AVRblb2. Bars indicate SD. Similar results were obtained at least twice from independent replications. Leaves of wild type, *Rpi-blb2*, *NahG*, and *Rpi-blb2::NahG* -transgenic *N. benthamiana* plants were agro-infiltrated with the pGR106-*AVRblb2* vector. *Rpi-blb2*-transgenic plants showed HR symptoms activated by AVRblb2 at 3–4 dai. (D) Percentages of infiltrated leaves with *Rpi-blb2*-mediated HR. All values represent means and SD from at least three independent experiments with three replications.

Studies have demonstrated that SA is required for the full function of a number of *R* genes. SA signaling-associated mutants in *Arabidopsis* have been used to characterize the role of *R* gene-mediated HCD and pathogen resistance (Glazebrook, 2005; Hammond-Kosack and Parker, 2003). We also tested the effects of *NahG*, which encodes a salicylate hydroxylase (Delaney et al., 1994), on the HCD response to AVRblb2. We generated *Rpi-blb2::NahG*-transgenic plants from crosses between *Rpi-blb2*-transgenic- and *NahG*-transgenic plants (Oh et al., 2014). After infiltration

by *P. infestans* AVRblb2, we observed that HCD lesions in wild-type (WT), *Rpi-blb2*-, *NahG*-, and *Rpi-blb2::NahG*-transgenic plants were similar (Fig. 3D). Silencing *SGT1* on *Rpi-blb2*- or *NahG*-transgenic backgrounds completely suppressed *Rpi-blb2*-mediated HR in response to *P. infestans* AVRblb2 (Fig. 3E). These results confirmed that *NbSGT1* is associated with *Rpi-blb2*-mediated HCD in response to AVRblb2, but *NbSGT1* is not associated with the coordinated SA accumulation. Moreover, SA itself does not activate HCD, but elicits a HCD response following H_2O_2 accumulation (Oh et al., 2014).

Here, we demonstrate that SGT1 is required for HCD response in *Rpi-blb2*-transgenic *N. benthamiana* in response to *P. infestans* AVRblb2, but not EDS1 or NDR1-mediated signaling. In addition, even though SA affects *Rpi-blb2*-mediated resistance against *P. infestans* but *Rpi-blb2*-mediated HCD in response to AVRblb2 did not affect. We also suggest that JA and ET signaling were not required for *Rpi-blb2*-mediated HCD in *N. benthamiana* against AVRblb2. Hence, further analysis of other processes and their corresponding products will help to unravel the complexity of *Rpi-blb2*-mediated HCD responses to and resistance pathways against *P. infestans* AVRblb2 in plants.

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