Cell surface-localized pattern-recognition receptors (PRRs) and intracellular nucleotide-binding and leucine-rich repeat domain-containing receptors (NLRs) represent two tiers of the plant surveillance system against invading organisms (Jones & Dangl, 2006). Both types of receptors perceive, directly or indirectly, invasion patterns from pathogens (Cook et al., 2015). PRRs mediate the perception of apoplastic elicitors (Ranf, 2017), often termed pathogen-associated molecular patterns (PAMPs), and their activation leads to the development of pattern (or PAMP)-triggered immunity (PTI) (Boller & Felix, 2009). Many well-studied PRRs have extracellular leucine-rich repeats (LRRs) and an intracellular kinase domain, and are therefore called LRR receptor-like kinases (LRR-RLKs); these PRRs mostly perceive proteinaceous PAMPs, while other extracellular domains mediate the perception of chemically diverse elicitors (Ranf, 2017). Other PRRs, named receptor-like proteins, lack intracellular signalling domains (Ranf, 2017). NLRs perceive effector proteins delivered inside plant cells, and their activation leads to effector-triggered immunity (ETI) (Jones & Dangl, 2006; Lolle et al., 2020). Immunity mediated by PRRs and NLRs confers disease resistance, although the duration and amplitude of the response triggered by different specific immune receptors varies. The activation of both PRRs and NLRs leads to convergent responses, such as ion fluxes, activation of mitogen-activated protein kinase (MAPK) cascades, production of reactive oxygen species (ROS), as well as transcriptional reprogramming, and therefore relies on similar or shared signalling components (Peng et al., 2018). Moreover, it has been reported that PTI and ETI require each other to confer robust disease resistance (Ngou et al., 2020; Yuan et al., 2020). The suppressor of the G2 allele of skp1 (SGT1), as a core immune regulator, is a protein required for the activation of NLR-mediated immunity. In this work, we examined the requirement of SGT1 for immune responses mediated by several LRR-RLKs in both Nicotiana benthamiana and Arabidopsis. Using complementary genetic approaches, we found that SGT1 is not limiting for early PRR-dependent responses or antibacterial immunity. We therefore conclude that SGT1 does not play a significant role in bacterial PAMP-triggered immunity.

**KEYWORDS**

LRR-RLK, PAMP-triggered immunity, ROS burst, SGT1, virus-induced gene silencing
MAPK activation or antibacterial immunity were not altered on SGT1 mutation or gene silencing. We therefore conclude that SGT1 does not play a significant role in bacterial PAMP-triggered immunity.

We recently found that SGT1s from different plant species are targeted by an effector protein, named RipAC, injected into plant cells by the bacterial pathogen *Ralstonia solanacearum* (Yu et al., 2020). RipAC targets SGT1s from different plant species and suppresses PAMP-triggered immunity.

**FIGURE 1** RipAC inhibits pathogen-associated molecular patterns (PAMPs)-triggered reactive oxygen species (ROS) in *Nicotiana benthamiana* leaves, which does not require NbSGT1. (a)–(d) RipAC suppresses ROS burst triggered by flg22Pto (a, b) and csp22Rsol (c, d). RipAC-GFP or green fluorescent protein (GFP, as control) was transiently expressed in 5-week-old (for 50 nM flg22 Pto) or 6-week-old (for 50 nM csp22Rsol) *N. benthamiana* leaves and ROS burst was analysed after treatment with the respective elicitors using a luminol-based assay (mean ± SEM, n = 24, t test, *p < 0.01). (e) and (f) Silencing NbSGT1 does not affect PAMP-triggered ROS burst in *N. benthamiana*. PAMP (50 nM flg22Pto or 50 nM csp22Rsol)-triggered ROS burst assay was performed 2 weeks after NbSGT1 virus-induced gene silencing (VIGS) using a luminol-based assay (mean ± SEM, n = 16, t test, n.s. indicates no statistical significance). Empty vector (pTRV2:EV) was used as control. (h) and (i) Silencing NbSGT1 does not affect PAMP-triggered MAPK activation in *N. benthamiana*. PAMP (100 nM flg22Pto or 1 uM csp22Rsol)-triggered MAPK activation was analysed 2 weeks after NbSGT1 VIGS using an anti-pMAPK antibody. Empty vector (pTRV2:EV) was used as control. (j)–(q) Silencing NbSGT1 does not affect PAMP-triggered ROS production suppression activity of RipAC in *N. benthamiana*. RipAC suppresses ROS burst triggered by 50 nM flg22Pto in either control plants (pTRV2:EV, j, k) or NbSGT1 VIGS plants (l, m). RipAC also suppresses ROS burst triggered by 50 nM csp22Rsol in either control plants (pTRV2:EV, n, o) or NbSGT1 VIGS plants (p, q). RipAC-GFP or GFP (as control) was transiently expressed in *N. benthamiana* leaves 2 weeks after VIGS and ROS burst was analysed after treatment with the respective elicitors using a luminol-based assay (mean ± SEM, n = 8, Student’s t test, **p < 0.01**). (r) Growth of *Pseudomonas syringae* pv. *tomato* (Pto) ΔhrCC in *N. benthamiana* undergoing VIGS. Control plants (pTRV2:EV) or NbSGT1 VIGS *N. benthamiana* plants were hand-infiltrated with the nonpathogenic Pto ΔhrCC mutant strain, and four inoculated leaf discs were taken as one sample at 3 days postinoculation (dpi) (mean ± SEM, n = 6). In (a), (c), (e), (f), (l), (m), (n), and (p), the graphs show ROS dynamics after PAMP treatment. In (b), (d), (g), (k), (m), (o), and (q), total relative luminescence units (RLU) was calculated within 60 min after PAMP treatment. In (h) and (i), the western blots were probed with the antibodies indicated in the figures. The accumulation of endogenous NbSGT1 was detected using an anti-SGT1 antibody. Coomassie brilliant blue (CBB) staining was used as loading control. Molecular weight (kDa) marker bands are indicated for reference. All these experiments were performed at least three times with similar results.
interferes with the MAPK-mediated phosphorylation of SGT1 to suppress ETI responses (Yu et al., 2020). In an independent approach to identify the additional activities of this effector, we found that RipAC is able to suppress the fast ROS burst triggered by bacterial PAMPs in N. benthamiana; RipAC expression almost completely blocked the early production of ROS upon treatment with the elicitors flg22Py (from Pseudomonas syringae pv. tomato [Pto]; Figure 1a,b) or csp22Red (from R. solanacearum; Figure 1c,d) (Wei et al., 2018), which are perceived by the LRR-RLKs FLS2 and CORE, respectively (Gómez-Gómez & Boller, 2000; Wang et al., 2016). Considering that RipAC suppresses ETI by targeting SGT1 and our new findings that RipAC also suppresses PAMP-triggered responses, we considered a potential role of SGT1 in the activation of PTI. Although SGT1 is transcriptionally induced by different stimuli, including PAMP perception (Azevedo et al., 2006; Noël et al., 2007), whether or not SGT1 is required for PTI has not been formally tested to date. To address this question, we first performed virus-induced gene silencing (VIGS) of NbSGT1 in N. benthamiana (Yu et al., 2019). Our NbSGT1 VIGS approach results in undetectable levels of NbSGT1 protein (Figure S1a) and abolishes SGT1-dependent ETI responses, such as the cell death triggered by the R. solanacearum effector RipE1 (Sang et al., 2020; Figure S1b). However, VIGS of NbSGT1 did not have a significant impact in the ROS burst (Figure 1e–g) or MAPK activation (Figure 1h,i) triggered by flg22Py or csp22Red, indicating that SGT1 is not required for early responses triggered by these bacterial PAMPs in N. benthamiana. As a control, we validated that we were able to detect a reduction in flg22-triggered ROS after performing VIGS of early PTI signalling components, such as the flg22 co-receptor BAK1 (NbBAK1; Figure S1c–e). Moreover, the ability of RipAC to suppress PAMP-triggered ROS was not affected in tissues that do not accumulate NbSGT1 (Figures 1j–q and S1f), confirming that RipAC suppresses PAMP-triggered ROS in an SGT1-independent manner. A previous report has shown that silencing NbSGT1 compromises the development of cell death during both compatible and incompatible plant-pathogen interactions, although its impact in bacterial growth varies depending on the inoculated strain (Wang et al., 2010). On inoculation with a non-pathogenic Pto ΔhrcC mutant, NbSGT1-silenced plants did not display enhanced bacterial growth (Figure 1r), suggesting that NbSGT1 is not required for the establishment of PTI against this strain.

RipAC associates with the two isoforms of Arabidopsis SGT1: AtSGT1a and AtSGT1b (Yu et al., 2020). In Arabidopsis, an AtsgtlAAtsgtlb double mutant is embryo lethal, suggesting that both genes have redundant functions in plant development besides their contribution to immunity (Azevedo et al., 2006). Alternatively, or in addition to their function in plant development, it is possible that SGT1 isoforms are guarded by NLRs, and their mutation leads to an autoimmune phenotype; this is in line with the observation that prolonged silencing of NbSGT1 leads to a weak activation of the expression of the immune marker gene NbPR1 (Yu et al., 2019). In short-day growth conditions, we observed that the knockout mutants sgt1a-1 (Ws-0 background) and sgt1b-3 (La-er background) showed different developmental phenotypes compared to their respective wild-type (WT) controls (Figure 2a); while sgt1a-1 mutant plants were larger than WT plants, sgt1b-3 were smaller than WT plants. To test PTI responses, because Ws-0 does not perceive flg22 (Gómez-Gómez et al., 1999) and the perception of csp22 is restricted to certain solanaceous plants (Wang et al., 2016), we treated plant tissues with the elicitor peptide elf18 (Kunze et al., 2004) from either P. syringae or R. solanacearum (Lacombe et al., 2010), which is perceived by the LRR-RLK EFR (Zipfel et al., 2006). Surprisingly, the sgt1a-1 mutant showed attenuated ROS burst, while the sgt1b-3 mutant showed enhanced ROS burst, compared to their respective WT controls (Figure 2b–i). It is noteworthy that, although AtSGT1a is transcriptionally induced after different biotic stress treatments, AtSGT1b transcripts stay almost unaltered on the same treatments (Azevedo et al., 2006; Noël et al., 2007). Moreover, considering that the PAMP-triggered ROS results were inversely proportional to the size of the plants (relative to their respective controls), it is possible that these opposite results are due to developmental effects caused by the respective mutations. Despite these differences, none of these mutants showed altered MAPK activation triggered by elf18Py (Figure 2j,k). Given the different effect of sgt1a and sgt1b mutations on PAMP-triggered ROS burst, we tested whether they are affected in resistance against a PTI-inducing strain. Figure 2l shows that none of the mutants presented altered resistance upon inoculation with a nonpathogenic Pto ΔhrcC mutant, suggesting that, although AtSGT1a and AtSGT1b may have a different contribution to early PTI signalling, or their mutation may cause developmental effects that have a physiological impact on PAMP-triggered ROS burst, they are not required for the establishment of PTI. These results are in line with previous studies that reported no differences in the susceptibility of Atsgtl single mutants to Pto DC3000 (Holt et al., 2005; Uppalapati et al., 2011).

It is noteworthy that, to date, the redundancy between AtSGT1a and AtSGT1b has hindered the detection of enhanced susceptibility against ETI-inducing P. syringae strains (Austin et al., 2002; Azevedo et al., 2006; Holt et al., 2005), despite the well-established role of SGT1 in NLR-mediated disease resistance. To overcome the observed partial redundancy of AtSGT1a and AtSGT1b, we performed VIGS of AtSGT1a in sgt1b-3 mutant plants (Figure 2n). AtSGT1a VIGS reduced the accumulation of AtSGT1a (Figure 2o) sufficiently to render silenced plants more susceptible to the ETI-inducing strain Pto AvrRpt2 (Figure 2p), suggesting that these plants have impaired SGT1 functions in the establishment of ETI. In contrast, the silenced plants were not more susceptible to a Pto ΔhrcC mutant or to Pto DC3000 (Figure 2q,r), suggesting that abolishing SGT1 function in Arabidopsis does not have a major impact on the establishment of PTI. Similarly, although Atsgtlb-3 (La-er) displays stronger ROS burst on elf18 treatment (Figure 2d,e,h,i), additional silencing of AtSGT1a in this background caused a minor reduction in ROS burst, which became similar to the levels observed in WT plants (Figure 2s,t). Altogether, our results indicate that the targeting of SGT1 does not account for the RipAC-mediated suppression of PTI, and SGT1 is not required for PTI against bacterial pathogens.

Plants use diverse immune receptors to perceive invasion signals, leading to immune responses of similar nature, but different magnitude and duration (Peng et al., 2018). Although activation of both PTI and ETI leads to convergent downstream molecular events, the
involvement and recruitment of different components before and after immune activation might be varied. In this work, we showed that although extensive research has proven the requirement of AtSGT1b for the establishment of ETI (Azevedo et al., 2006; Shirasu, 2009), SGT1 is not required for PTI responses in *N. benthamiana* or *Arabidopsis*. This is reminiscent of what has been described for the tomato NLR-required
for cell death (NRC) proteins, which are NLR helpers required for ETI responses mediated by numerous NLRs, but are not essential for immunity triggered by bacterial flagellin (Wu et al., 2020). Further studies are currently ongoing to identify and characterize the RipAC target(s) that underlie its ability to suppress PTI.

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AUTHOR CONTRIBUTIONS

G.Y. and A.P.M. designed experiments. G.Y., L.X., and H.Z. performed experiments. G.Y. and A.P.M. analysed the data and wrote the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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