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Short communication

Plum pox virus capsid protein suppresses plant pathogen-associated molecular pattern (PAMP)-triggered immunity

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SUMMARY

The perception of pathogen-associated molecular patterns (PAMPs) by immune receptors launches defence mechanisms referred to as PAMP-triggered immunity (PTI). Successful pathogens must suppress PTI pathways via the action of effectors to efficiently colonize their hosts. So far, plant PTI has been reported to be active against most classes of pathogens, except viruses, although this defence layer has been hypothesized recently as an active part of antiviral immunity which needs to be suppressed by viruses for infection success. Here, we report that Arabidopsis PTI genes are regulated upon infection by viruses and contribute to plant resistance to *Plum pox virus* (PPV). Our experiments further show that PPV suppresses two early PTI responses, the oxidative burst and marker gene expression, during Arabidopsis infection. *In planta* expression of PPV capsid protein (CP) was found to strongly impair these responses in *Nicotiana benthamiana* and Arabidopsis, revealing its PTI suppressor activity. In summary, we provide the first clear evidence that plant viruses acquired the ability to suppress PTI mechanisms via the action of effectors, highlighting a novel strategy employed by viruses to escape plant defences.

Keywords: *Arabidopsis thaliana*, capsid protein, effector, flg22 signalling, PAMP-triggered immunity, plant antiviral defences, *Plum pox virus*.

INTRODUCTION

Animal and plants possess an elaborate immune system, whose first layer enables the identification of pathogens by pattern recognition receptors (PRRs) that perceive pathogen-associated molecular patterns (PAMPs) (Kumar *et al.*, 2011; Zipfel, 2014). Once activated, PRRs trigger signalling cascades that launch transcriptional and physiological changes within host cells, ultimately hampering pathogen growth and establishing PAMP-triggered immunity (PTI). To counteract this defence strategy, successful pathogens deploy a range of effectors, the primary function of which is to evade/interfere with PTI (Jones and Dangl, 2006).

The best-studied PTI pathway in plants relies on the Arabidopsis receptor kinase FLAGELLIN-SENSING 2 (FLS2), which perceives bacterial flagellin (or its active epitope flg22) (Gómez-Gómez *et al.*, 2000; Zipfel *et al.*, 2004). FLS2 activation requires the association with the co-receptor BRI1-ASSOCIATED KINASE 1 (BAK1), or its closest paralog BAK1-LIKE 1 (BKK1), within plasma membrane (PM)-localized PRR complexes (Chinchilla *et al.*, 2007; Heese *et al.*, 2007; Roux *et al.*, 2011; Sun *et al.*, 2013). Activated FLS2 dissociates with the receptor-like cytoplasmic kinases (RLCKs) BOTRYTIS-INDUCED KINASE 1 (BIK1) and AVRPPHB SUSCEPTIBLE 1-LIKE 1 (PBL1) (Kadota *et al.*, 2014; Li *et al.*, 2014; Lu *et al.*, 2010; Zhang *et al.*, 2010). The following downstream cascade comprises a reactive oxygen species (ROS) burst, activation of mitogen-activated protein kinases (MAPKs) and transcriptional reprogramming (Bigeard *et al.*, 2015).

Over the last 20 years, it has become clear that PTI mechanisms allowing both plants and animals to resist pathogen attacks follow conserved signalling strategies (Arpaia and Barton, 2011; Lester and Li, 2014; Schwessinger and Ronald, 2012; Thompson *et al.*, 2011; Zipfel and Felix, 2005). Antiviral PRR pathways have been studied extensively in mammals, and the mechanisms whereby viral effectors manipulate PTI defences have been well characterized (Harris and Coyne, 2013; Hiscott *et al.*, 2006; Kumar *et al.*, 2011; Schröder and Bowie, 2007; Yokota *et al.*, 2010). In contrast, hardly anything is known in plants, although indications concerning the existence of PTI mechanisms targeting plant viruses have emerged recently (Kørner *et al.*, 2013; Nicaise, 2014; Zvereva and Pooggin, 2012).

Plant antiviral defences rely mainly on RNA interference, in which the cellular machinery targets virus-derived nucleic acids, and resistance (R) proteins which recognize virus avirulence factors and trigger an array of physiological and biochemical defence processes broadly targeting pathogens (Nicaise, 2014). Interestingly, a recent model hypothesizes: (i) the action of PTI mechanisms within plant immunity against viruses in parallel with RNA interference and R proteins; and (ii) the existence of specialized effectors encoded by successful plant viruses to bypass PTI, in

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parallel with the well-characterized viral silencing suppressors (Nicaise, 2014; Zvereva and Pooggin, 2012).

Potyviruses constitute one of the largest and most successful genera of plant viruses (Revers and García, 2015). Their single-stranded RNA genome is packed into filamentous particles and encodes 11 highly multifunctional proteins (Charon *et al.*, 2016), including the capsid protein (CP), which is primarily characterized by its structural role in forming the protective shell around the viral genome. In addition to being the causal agent of Sharka, the most damaging viral disease affecting stone fruit trees, *Plum pox virus* (PPV) is a representative model of RNA viruses, a dual feature that has led to its classification among the Top 10 plant viruses of scientific and economic importance (Decroocq *et al.*, 2006; García *et al.*, 2014; Rimbaud *et al.*, 2015; Scholthof *et al.*, 2011).

We address here the question of the existence of virus-encoded effectors suppressing PTI mechanisms, using the *Arabidopsis thaliana*–PPV pathosystem. In this report, we show that: (i) PTI genes contribute to Arabidopsis immunity to PPV; (ii) PPV suppresses early PTI responses during plant infection; and (iii) PPV CP acts as an effector suppressing PTI mechanisms, underlining a novel strategy employed by a plant virus to counteract host defences.

RESULTS AND DISCUSSION

Plant infection by viruses is associated with cellular perturbations, including a massive reprogramming of host gene expression (Hanley-Bowdoin *et al.*, 2013; Lindbo *et al.*, 2001; Pallas and García, 2011; Whitham *et al.*, 2006). The analysis of previously published transcriptomic data derived from Arabidopsis tissues infected with viruses (Ascencio-Ibáñez *et al.*, 2008; Babu *et al.*, 2008; Espinoza *et al.*, 2007; Fernandez-Calvino *et al.*, 2014; Ishihara *et al.*, 2004; Marathe *et al.*, 2002; Pierce and Rey, 2013; Rodrigo *et al.*, 2012; Yang *et al.*, 2007) has indicated that plant colonization is associated with the transcriptional regulation of genes encoding key factors from PTI pathways, such as PRRs themselves, co-receptors, regulators, MAPKs and transcription factors (Table S1, see Supporting Information). This suggests that cellular components belonging to the PTI machinery may play a role in antiviral defences in plants.

In order to clarify the contribution of the PTI machinery in plant–virus interactions, we investigated Arabidopsis susceptibility to PPV in different genotypes altered in PTI signalling. For the sake of inoculation efficiency/reproducibility, whilst causing minimal injury to leaf tissues, Arabidopsis leaves were inoculated by agroinfiltration with a PPV infectious construct on a small area at the tip of each leaf, and the virus loads were quantified in the rest of the leaf by semi-quantitative double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) at 11 days post-inoculation (dpi). Wild-type (WT) ecotypes (Columbia-0 and Landsberg erecta) were used as susceptible controls (Fig. 1). The role of the plant PRRs FLS2, EF-Tu receptor (EFR) and CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) is well characterized in antibacterial and antifungal immunity (Zipfel, 2014). Arabidopsis null mutants for the corresponding genes were as susceptible to PPV as WT plants (Fig. 1A), suggesting that these PRRs do not participate in anti-PPV immunity. This is in accordance with the observation that plant PRRs are specialized in the recognition of specific classes of pathogen (Zipfel, 2014). BAK1 and BKK1 are involved in a wide array of pathways related to development and defence (Schwessinger and Rathjen, 2015). Notably, they are both key PTI activators against various non-viral pathogens (Chaparro-Garcia *et al.*, 2011; Chinchilla *et al.*, 2007; Heese *et al.*, 2007; Kim *et al.*, 2013; Peng and Kaloshian, 2014; Prince *et al.*, 2014; Roux *et al.*, 2011) and their contribution to plant resistance against viruses has been reported (Kørner *et al.*, 2013; Yang *et al.*, 2010). In our experiments, the single mutants *bak1-4*, *bak1-5* and *bkk1* were not significantly affected in PPV susceptibility (Fig. 1B), unlike the results observed previously with tobamoviruses and carmoviruses (Kørner *et al.*, 2013; Yang *et al.*, 2010). However, the double mutant *bak1-5 bkk1* displayed a strong increase in viral accumulation (Fig. 1B), indicating that both BAK1 and BKK1 contribute to immunity against PPV, probably in a redundant manner. In various PTI pathways, PRR downstream signalling is positively regulated by BIK1 and PBL1 (Kadota *et al.*, 2014; Li *et al.*, 2014; Lu *et al.*, 2010; Zhang *et al.*, 2010). The mutant *bik1* was more susceptible to PPV (Fig. 1C), indicating that this kinase positively contributes to Arabidopsis basal resistance against PPV, whereas the loss of PBL1 failed to increase significantly the *bik1* phenotype. PTI signalling is mediated by MAPK cascades comprising MPK3 and MPK6, which activate PTI responses, whereas MPK4 acts as a negative regulator of immune pathways (Rasmussen *et al.*, 2012). Here, we observed that, although statistical analyses do not validate the role of MPK3 in our conditions, the mutants *mpk6* and *mpk4* are more susceptible and more resistant to PPV respectively (Fig. 1D), respectively, indicating that these two MAPKs seem to be actively involved in plant–virus interactions. Taken together, these results show that a range of host proteins previously described as key PTI factors contribute to Arabidopsis immunity to PPV.

Successful cellular pathogens from both animals and plants must suppress PTI pathways to efficiently colonize their hosts. In the case of acellular microorganisms, only animal viruses have so far been described as interfering with PTI pathways (Yokota *et al.*, 2010). To determine whether plant viruses employ such a strategy during host infection, we evaluated the impact of PPV infection on Arabidopsis early PTI responses. For this purpose, Arabidopsis plants were first agroinoculated with the PPV infectious construct and systemic tissues were sampled at 4 and 11 dpi. The ability to activate PTI was then evaluated by measuring the responsiveness

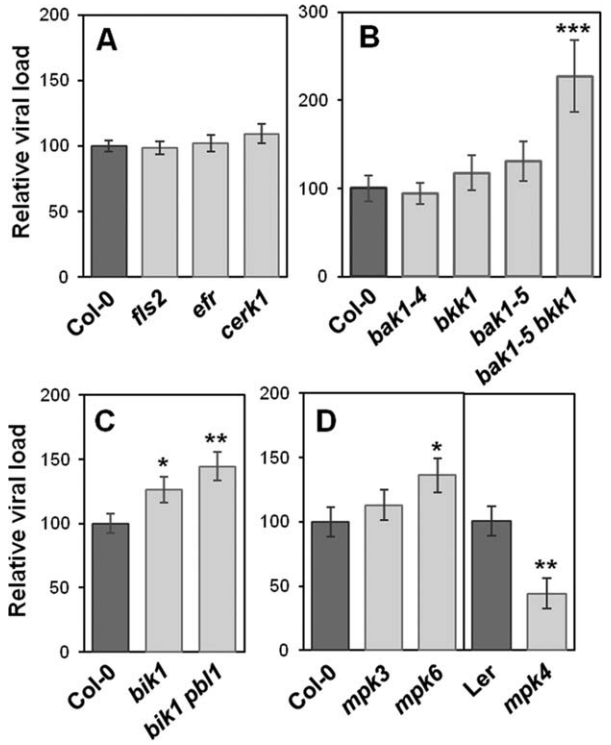

Fig. 1 Pathogen-associated molecular pattern-triggered immunity (PTI) machinery contributes to Arabidopsis resistance to *Plum pox virus* (PPV). Arabidopsis susceptibility to PPV was evaluated at 11 days post-inoculation (dpi) by measuring the viral loads by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) in inoculated leaves from mutants affected in the expression of PTI components, such as pattern recognition receptors (PRRs) (A), co-receptors (B), positive regulators (C) and mitogen-activated protein kinases (MAPKs) (D). In each panel, values were normalized relative to wild-type (WT) samples (Col-0 for all lines, except for *mpk4*, where the WT ecotype used is Ler). Values presented are the average of 18–24 samples from at least three experiments ± standard error. Values labelled with asterisks are statistically significantly different from WT samples: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. Col-0, Columbia-0; Ler, Landsberg erecta.

of infected tissues to the heterologous bacterial PAMP flg22, an efficient elicitor of PTI responses in Arabidopsis (Zipfel *et al.*, 2004). Mock controls consisting of leaves infiltrated with WT agrobacteria were always analysed in comparison. Care was taken to collect leaf discs outside the agroinfiltrated area to avoid any impact of the presence of agrobacteria and/or agroinfiltration-associated wound lesions on PTI assays (Fig. S1A, see Supporting Information). At an early infection stage (4 dpi), virus accumulation outside the inoculated area was highly limited and only detectable by reverse transcription-polymerase chain reaction (RT-PCR), whereas the 11-dpi stage displayed full-blown PPV infection (Fig. S1B, C). At 4 dpi, infected tissues displayed a PPV-specific oxidative burst, whereas ROS production returned to the basal level at 11 dpi (Fig. 2A). Interestingly, PPV infection had a strong impact on the PTI-related oxidative burst: at 4 dpi, infected tissues treated with flg22 produced a greater amount of ROS than flg22-treated mock samples (Fig. 2A), suggesting that there is an additive effect between the PPV- and flg22-induced oxidative burst. In contrast, the flg22 responsiveness of PPV-inoculated tissues was reduced at 11 dpi compared with flg22-treated mock samples (Fig. 2A). In consequence, our results suggest that, at a very early PPV infection stage (when only a few viral particles are present), infection triggers ROS production, confirming the previous reports on ROS release during viral infections in plants (Allan *et al.*, 2001; Díaz-Vivancos *et al.*, 2008; Love *et al.*, 2005; Manacorda *et al.*, 2013; Nováková *et al.*, 2015). However, at a late PPV infection stage (with full-blown virus accumulation), PPV impairs both the PPV- and PTI-related oxidative burst. With the intention of confirming a possible negative effect of PPV infection on the early PTI response, the expression of PTI-related genes was evaluated during Arabidopsis infection by PPV at 11 dpi. Classically used as flg22-induced marker genes (Boudsocq *et al.*, 2010), *AtFRK1* and *AtNHL10* were observed to be induced upon PAMP treatment in mock samples, as expected (Fig. 2B). Interestingly, the infected tissues displayed a decrease in transcript accumulation without PAMP treatment, compared with the basal levels measured in mock samples (Fig. 2B). Moreover, PPV accumulation strongly impaired gene induction triggered by flg22 treatment (Fig. 2B), revealing that plant infection by PPV suppresses the expression of PTI-related marker genes. These results indicate that PPV negatively regulates early PTI responses during plant infection.

We hypothesized that one (or several) virus genome-encoded protein(s) may act as PTI-suppressing effectors. Overlapping immune signalling induced by different classes of pathogen enables the successful identification of effectors suppressing PTI responses triggered by heterologous PAMPs (Bos *et al.*, 2010; Chen *et al.*, 2013; Jaouannet *et al.*, 2013; Park *et al.*, 2012; Pel *et al.*, 2014; Zheng *et al.*, 2014). Thus, we sought to determine the effects of the expression of PPV proteins on early PTI responses triggered by flg22. For this purpose, agrobacteria expressing green fluorescent protein (GFP)-tagged versions of PPV-encoded proteins were infiltrated into *Nicotiana benthamiana* leaves. At 2 days post-agroinfiltration (dpa), leaf discs were collected inside the agroinfiltrated area (Fig. S2A, see Supporting Information) and flg22 responsiveness was evaluated. We found that *in planta*-expressed CP-GFP (detected in Fig. S2C, E) strongly reduced the flg22-triggered oxidative burst relative to GUS-GFP (Fig. 3A). The genes *NbACRE31* and *NbACRE132* are rapidly up-regulated upon flg22 treatment in *N. benthamiana* (Heese *et al.*, 2007; Segonzac *et al.*, 2011). Although flg22 responsiveness was not affected in leaves overexpressing GUS-GFP and those infiltrated with WT agrobacteria, the induction of the flg22 marker genes was suppressed in CP-expressing samples (Fig. 3B). Transient expression experiments performed in Arabidopsis seedlings (Fig. S2B, D) confirmed these results, as the induction of flg22

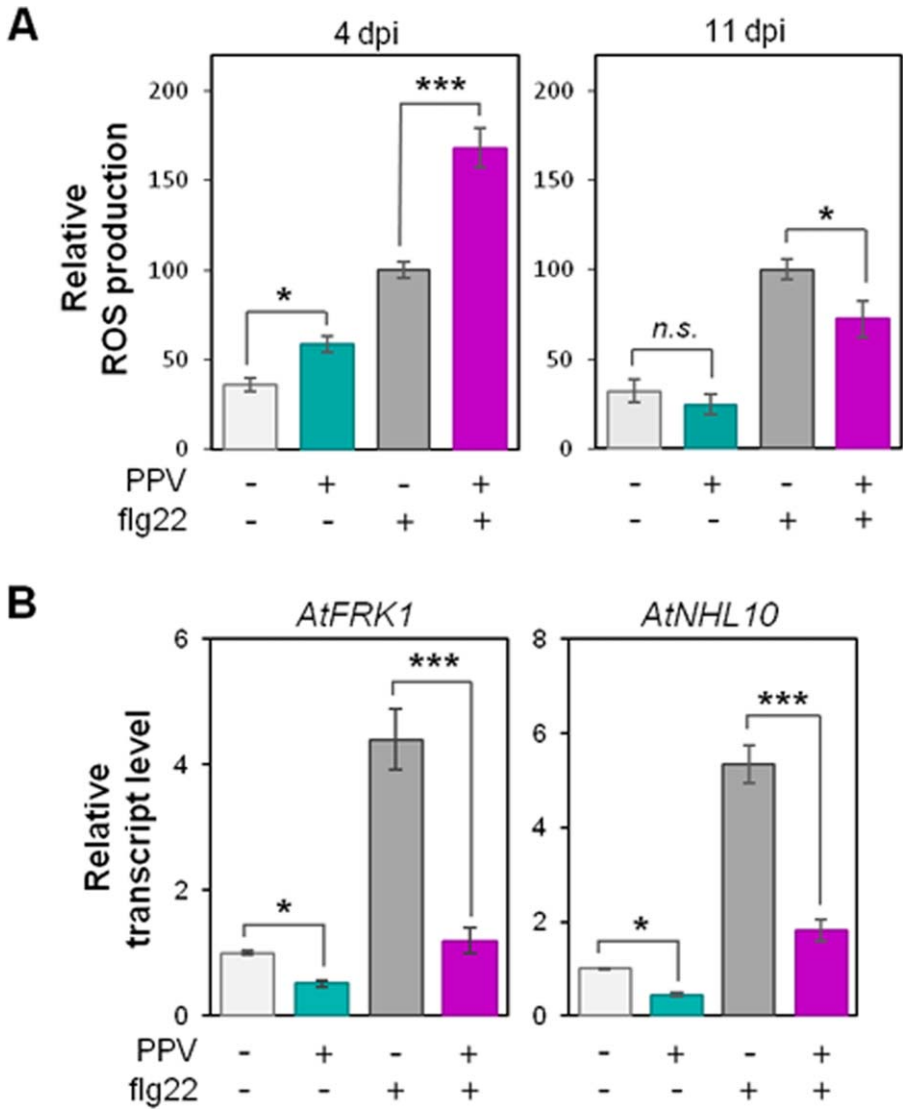

Fig. 2 *Plum pox virus* (PPV) suppresses early pathogen-associated molecular pattern-triggered immunity (PTI) responses during Arabidopsis infection. (A) The PTI-related oxidative burst is affected upon PPV infection. Reactive oxygen species (ROS) production was measured in PPV-inoculated (+) or mock-inoculated (–) leaves at 4 days post-inoculation (dpi)/11 dpi in response to treatment with 200 nM flg22. The results are presented as the total photon count during 40 min of treatment, normalized in comparison with mock-inoculated leaves treated with flg22. The values presented are the average of 24–30 samples from at least three experiments ± standard error. Connecting lines with asterisks indicate two statistically significantly different values: $*P < 0.05$ and $***P < 0.001$; *n.s.*, not significant. (B) PTI marker gene expression is suppressed upon PPV infection. The transcript accumulation of Arabidopsis PTI marker genes *AtFRK1* and *AtNHL10* was assessed by quantitative reverse transcription-polymerase chain reaction (RT-PCR) in PPV-inoculated (+) or mock-inoculated (–) leaves at 11 dpi, 30 min after treatment with 1 μM flg22. Values are the average of 12 samples from three experiments ± standard error presented as fold induction compared with untreated mock-inoculated samples. Connecting lines with asterisks indicate two statistically significantly different values: $*P < 0.05$ and $***P < 0.001$.

marker genes, *AtFRK1* and *AtNHL10*, was also inhibited upon PPV CP expression, compared with the negative controls (Fig. 3C). Therefore, our results show that PPV CP suppresses early PTI responses, revealing, for the first time, the existence of a plant virus PTI-suppressing effector.

PTI governs a fast and powerful defence line that has been reported to be active in many eukaryotic organisms. In plants, pathogens from various lifestyle classes have been shown to be controlled by PTI, most models excluding viruses (Boller and Felix, 2009; Dangl *et al.*, 2013; Schwessinger and Ronald, 2012). The contribution of key PTI components in Arabidopsis resistance against PPV suggests that plants, similar to animals, defend themselves against viruses using PTI machinery. Since the submission of this work, viral double-stranded RNAs (dsRNAs) have been

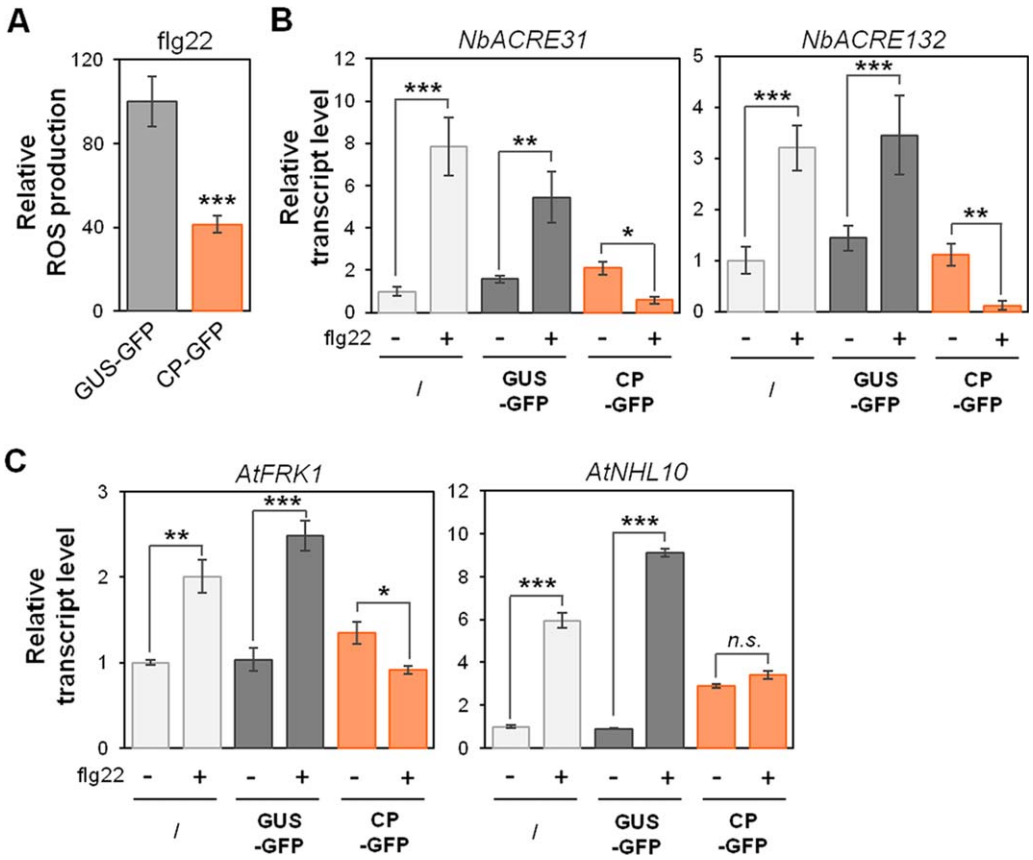

Fig. 3 *Plum pox virus* (PPV) capsid protein (CP) suppresses early pathogen-associated molecular pattern-triggered immunity (PTI) responses in *Nicotiana benthamiana* and Arabidopsis. (A) *In planta*-expressed PPV CP impairs the PTI-associated oxidative burst in *Nicotiana benthamiana*. Reactive oxygen species (ROS) production in response to treatment with 200 nM flg22 was measured on leaves transiently overexpressing CP-green fluorescent protein (CP-GFP) or β-glucuronidase-GFP (GUS-GFP) at 2 days post-agroinfiltration (dpa). ROS production is presented as the total photon count during 40 min of treatment, normalized relative to GUS-GFP-expressing leaves. Values presented are the average of 24 samples from three biological experiments ± standard error. Values labelled with asterisks are statistically significantly different: $***P < 0.001$. (B) *In planta*-expressed PPV CP suppresses PTI-associated gene expression in *Nicotiana benthamiana*. Transcript accumulation of PTI marker genes *NbACRE31* and *NbACRE132* was assessed by quantitative reverse transcription-polymerase chain reaction (RT-PCR) on leaf tissues overexpressing CP-GFP and GUS-GFP or infiltrated with wild-type agrobacteria (control samples represented by '/') at 2 dpa, 1 h after treatment with 1 μM flg22. Values are the average of 12 samples from three biological experiments ± standard error presented as fold induction compared with mock-treated control samples. Connecting lines with asterisks indicate two statistically significantly different values: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. (C) *In planta*-expressed PPV CP suppresses flg22-induced gene expression in Arabidopsis seedlings. Transcript accumulation of PTI marker genes *AtFRK1* and *AtNHL10* was assessed at 2 dpa by quantitative RT-PCR on seedlings overexpressing CP-GFP or GUS-GFP or on control seedlings treated with wild-type agrobacteria (represented by '/'), 30 min after treatment with 1 μM flg22. Values are the average of eight samples from two biological experiments ± standard error presented as fold induction compared with mock-treated control samples. Connecting lines with asterisks indicate two statistically significantly different values: $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$; *n.s.*, not significant.

reported to act as PAMPs that trigger PTI responses and protection against viral infection in Arabidopsis (Niehl *et al.*, 2016), confirming the existence of antiviral PTI in plants. In this context, the discovery of the first PTI-targeting effector encoded by a plant virus illustrates further the biological significance of PTI for antiviral defences in plants.

Most animal PRRs involved in virus perception are intracellular (Kumar *et al.*, 2011). Whether plant PRRs specialized in virus perception fulfil similar canonical structures and subcellular localizations has yet to be determined. Notwithstanding, the role of PM-localized PTI actors (such as BAK1 and BKK1) in plant–virus interactions raises the question of the way in which intracellular pathogens could be perceived extracellularly. Although this point remains unknown, a similar situation occurring in animal cells indicates that extracellular treatment with viral PAMPs could activate immune pathways via a clathrin-dependent endocytic pathway and/or the activation of PRRs located at the cell surface, in addition to their classical inner membrane-associated localization (Itoh *et al.*, 2008; Pohar *et al.*, 2013).

It is reasonable to consider that antiviral PTI pathways may display specificity relative to the mechanisms identified in other plant pathosystems. However, the fact that key PTI components involved in defence against non-viral pathogens contribute to defence against viruses confirms that immune pathways against various classes of pathogen share certain signalling components. Nonetheless, it is worth mentioning that BAK1, BKK1 and BIK1, in addition to their role in PTI, are involved in signalling pathways associated with BRI1 (Brassinosteroid-insensitive 1), a steroid phytohormone receptor involved in plant development (He *et al.*, 2007; Lin *et al.*, 2013), which could suggest that brassinosteroid (BR) signalling has an impact on plant defence against PPV, as reported for other plant viruses (Ali *et al.*, 2014; Baebler *et al.*, 2009; Deng *et al.*, 2016; Nakashita *et al.*, 2003; Zhang *et al.*, 2015). Although the use of the mutant *bak1*-5 [carrying a point mutation impairing PTI responses, but not BR signalling (Schwessinger *et al.*, 2011)] favours the role of PTI on PPV infection, the possible cross-talk between PTI and BR pathways in plant–virus interactions has yet to be clarified. In addition, future investigations will need to focus on possible interconnections between immune pathways triggered by viral PAMPs and host DAMPs (danger-associated molecular patterns) released by cells during the infection process.

In this work, we hypothesize that PPV infection triggers PTI mechanisms, which are afterwards suppressed by the viral CP to enable plant colonization (hypothesis developed in Fig. S3, see Supporting Information). PPV is a representative model of potyviruses and more broadly of RNA viruses. Hence, it is likely that other plant viruses have evolved strategies to suppress PTI mechanisms. Remarkably, the fact that the expression of PTI genes seems to be mostly down-regulated during plant infection (Table S1) could suggest that DNA and RNA viruses from different families share a common feature that involves the targeting of plant PTI pathways, notably at the gene expression level.

In accordance with the current concept of plant innate immunity, effector proteins can act as both a virulence factor (suppressing PTI) and avirulence factor [triggering effector-triggered immunity (ETI)]. Although many proteins from all cellular pathogens meet this definition (Dangl *et al.*, 2013), only the avirulence factor side has so far been reported for viruses, including many CPs recognized by plant *R* genes (De Ronde *et al.*, 2014). The fact that PPV CP displays PTI suppressor activity emphasizes, for the first time, that plant viruses integrate the host–pathogen conceptual arms race illustrated by the zig–zag model (Jones and Dangl, 2006).

Although CPs from animal and plant viruses were initially characterized for their role as structural proteins in forming protective shells around viral genomes, they possess numerous non-encapsidation activities, including the regulation of host immune defences (Ni and Cheng Kao, 2013; Weber and Bujarski, 2015). Here, our findings provide evidence that PPV CP possesses PTI suppressor activity. Remarkably, potyviral CPs are intrinsically disordered (Baratova *et al.*, 2001; Charon *et al.*, 2016; Ksenofontov *et al.*, 2013; Rantalainen *et al.*, 2008), a structural feature shared by many pathogen effectors to efficiently bypass the immune system (Marín *et al.*, 2013).

Plant viruses have been known for some time to successfully evade/manipulate host defences via specific proteins, the most widely known being the virus-encoded silencing suppressors (Csorba *et al.*, 2015; Nakahara and Masuta, 2014; Pumplin and Voinnet, 2013). The present work reports the existence of a plant virus-encoded PTI suppressor, and therefore a novel strategy employed by a plant virus to escape host defences. By providing evidence that viruses belong to the list of plant pathogens that suppress PTI, our findings raise considerable questions about the tight molecular dialogue underlying plant–virus interactions.

EXPERIMENTAL PROCEDURES

All primers used in this work are described in Methods S1 (see Supporting Information).

Plant material

All plants were grown in a glasshouse at 20–22 °C with a 16-h light/8-h dark photoperiod. Arabidopsis genotypes were in the Columbia-0 background, except for the *mpk4-1* mutant, which was in the Landsberg erecta background. Arabidopsis mutants have been published previously: *fls2* (=*fls2c*; Zipfel *et al.*, 2004); *efr* (=*efr-1*; Zipfel *et al.*, 2006); *cerk1* (=*cerk1-2*; Gimenez-Ibanez *et al.*, 2009); *bak1-4* (Chinchilla *et al.*, 2007); *bkk1* (He *et al.*, 2007); *bak1-5* and *bak1-5 bkk1* (Schwessinger *et al.*, 2011); *bik1* and *bik1 pbl1* (Zhang *et al.*, 2010); *mpk3* (=*mpk3-1*; Bartels *et al.*, 2009); *mpk4* (=*mpk4-1*; Petersen *et al.*, 2000); *mpk6* (*mpk6-2*; Bartels *et al.*, 2009).

Virus inoculation and detection

The PPV isolate used in this work was PPV-R3-GFP (D strain). Rosette leaves from 6–7-week-old plants were inoculated on a small area at the tip of each leaf (Fig. S1) by infiltration with *Agrobacterium tumefaciens* C58C1 cells [optical density at 600 nm (OD_{600}) = 0.15; ~80 μL per leaf tip] carrying the infectious clone pBIN-PPV-NK-GFP construct, as described previously (Jiménez *et al.*, 2006; Nicaise *et al.*, 2007). DAS-ELISA experiments were performed using anti-PPV polyclonal antibodies on an EPOCH microplate spectrophotometer (Biotek, Colmar, France). RT-PCRs were performed on cDNAs synthesized using Superscript® II Reverse Transcriptase (Invitrogen, Paisley, UK) from total RNAs isolated using the TRI-Reagent® method (Sigma-Aldrich, Saint Louis, USA).

ROS measurement and PTI marker gene expression

ROS production was measured using an Infinite® 200 PRO photon counting reader (TECAN, Mannedorf, Switzerland), as described previously (Zipfel *et al.*, 2004), in the presence of 17 mM L-012 (Wako Chemical Inc., Richmond, USA), 1 μM horseradish peroxidase (Sigma-Aldrich) and 200 nM flg22 (Peptron Inc., Deajeon, South Korea).

Total RNAs were isolated using the TRI-Reagent® method (Sigma-Aldrich) and treated with Turbo DNA-free DNase (Ambion, Austin, USA). First-strand cDNA was synthesized using Superscript® II Reverse Transcriptase (Invitrogen) and an oligo(dT) primer, according to the manufacturer's instructions. cDNAs were amplified in triplicate or quadruplicate by quantitative PCR using SYBR Green JumpStart Taq ReadyMix (Sigma-Aldrich) and the LightCycler® 480 System (Roche, Meylan, France). *AtU-box* and *NbEF1α* genes were used as internal controls (in Arabidopsis and *N. benthamiana* experiments, respectively). The expression in control samples was used to normalize with the expression level set to unity. Relative expression was determined using the comparative *Ct* method ($2^{-\Delta\Delta Ct}$).

DNA constructs

The PPV capsid coding sequence was cloned into the pENTR/D-TOPO vector (Invitrogen). A control pENTR/D-TOPO-GUS was also obtained in parallel. Entry constructs were recombined by LR reaction into the Gateway-compatible pK7FWG2.0 (Ghent University, Belgium).

In planta transient expression

Transient expression in *N. benthamiana* was performed as described previously (Bos *et al.*, 2010) on leaves from 5-week-old plants. Transient expression in Arabidopsis *efr* seedlings was performed as described previously (Wu *et al.*, 2014).

Protein extraction, purification and western blotting

In planta-expressed tagged proteins were extracted and immunoprecipitated with GFP-Trap® agarose beads (Chromotek, Planegg-Martinsried, Germany), as described previously (Nicaise *et al.*, 2013). Proteins were fractionated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto poly(vinylidene difluoride) (PVDF) membrane and detected using anti-GFP antibodies (Ambion, Abingdon, UK) and peroxidase-conjugated anti-rabbit antibodies (Sigma-Aldrich). Immunodetection was performed using the reagent SuperSignal™ West-Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, USA).

Statistical analyses

Statistical significance based on one-way analysis of variance (ANOVA) was determined with InStat 3.10 software (GraphPad, La Jolla, CA, USA).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1 *Plum pox virus* (PPV) accumulation in inoculated leaves during Arabidopsis infection.

Fig. S2 *In planta* transient over-expression of capsid protein-green fluorescent protein (CP-GFP) and β-glucuronidase-GFP (GUS-GFP) in *Nicotiana benthamiana* leaves and Arabidopsis seedlings.

Fig. S3 Schematic model depicting capsid protein (CP)-mediated pathogen-associated molecular pattern-triggered immunity (PTI) suppression during *Plum pox virus* (PPV) infection. ETS, effector-triggered susceptibility.

Table S1 Pathogen-associated molecular pattern-triggered immunity (PTI) machinery-related genes are regulated upon virus infection in Arabidopsis. The table represents the PTI-related genes up-regulated (in red) or down-regulated (in green) by RNA and DNA viruses during Arabidopsis infection. The absence of colour indicates that the corresponding genes are not up- or down-regulated. Data were extracted from transcriptomic analyses published previously (Ascencio-Ibáñez *et al.,* 2008; Babu *et al.,* 2008; Espinoza *et al.,* 2007; Fernandez-Calvino *et al.,* 2014; Ishihara *et al.,* 2004; Marathe *et al.,* 2002; Pierce and Rey, 2013; Rodrigo *et al.,* 2012; Yang *et al.,* 2007). ER-QC, endoplasmic reticulum-quality control; CDPK, calcium-dependent protein kinase; MAPK, mitogen-activated protein kinase; PRR, pattern recognition receptor; RBP, RNA-binding protein; SERK, somatic embryogenesis receptor kinase (protein family containing BAK1/SERK3 and BKK1/SERK4). DNA viruses: CaLCuV, *Cabbage leaf curl begomovirus*; SACMV, *South African cassava mosaic begomovirus*. RNA viruses: CMV, *Cucumber mosaic bromovirus*; PPV, *Plum pox potyvirus*; TCV, *Turnip crinkle carmovirus*; TEV, *Tobacco etch potyvirus*; TMV, *Tobacco mosaic tobamovirus*; TRV, *Tobacco rattle tobravirus*; TuMV, *Turnip mosaic potyvirus*.

Methods S1 Primers used in this study.