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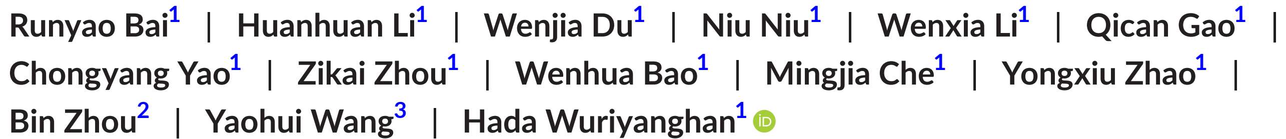
SHORT COMMUNICATION

Decoy engineering of the receptor-like cytoplasmic kinase StPBS1 to defend against virus infection in potato



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Abstract

Potato virus Y (PVY) is an important pathogen of potato (*Solanum tuberosum*). Although the PBS1–RPS5 immune system is well documented in *Arabidopsis thaliana*, it has not been reported in potato. In *Arabidopsis*, the bacterial effector AvrPphB cleaves AtPBS1 to trigger an immune response. Here, we show that the AvrPphB-triggered immune response is mediated by *StPBS1*, a close homologue of *AtPBS1* in potato. However, downstream signalling of StPBS1 was mediated by unknown resistance (R) proteins other than potato orthologues of AtRPS5 and HvPBR1, which is important for HvPBS1 signalling in barley. Immune signalling of StPBS1 is mediated by the AvrPphB C-terminal cleavage domain and an STKPQ motif, in contrast to AtPBS1-mediated immunity in which both AvrPphB cleavage fragments and an SEMPH motif are essential. The cleavage sequence of AvrPphB in StPBS1 was replaced with that of the PVY NIa-Pro protease to obtain StPBS1$^{\text{NIa}}$. *StPBS1*$^{\textit{NIa}}$ overexpression potato displayed stronger immunity to PVY infection than did the *StPBS1* transgenic lines. StPBS1$^{\text{NIa}}$ was cleaved at the expected target site by NIa-Pro protease from PVY. Thus, we characterized the function of StPBS1 in potato immunity and provide a biotechnology control method for PVY via transformation of decoy-engineered StPBS1$^{\text{NIa}}$.

KEYWORDS

AvrPphB, decoy engineering, immune signalling, potato virus Y, resistance protein, *StPBS1*, transgenic potato

Runyao Bai and Huanhuan Li contributed equally to the work.

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Plant immunity is divided into pathogen-associated molecular pattern-triggered immunity (PTI) and effector-triggered immunity. Resistance (R) proteins recognize pathogen avirulence (Avr) proteins and usually cause a hypersensitive response (HR) in plants (Yuan et al., 2021). The recognition spectrum of R proteins is limited; therefore, modification of R proteins for recognition of different pathogens is important for development of sustainable disease resistance in crops (Fuchs, 2017). Directed evolution aims to expand the recognition spectrum of a known R protein for new pathogens (De la Concepcion et al., 2019). Most effector proteins are recognized by indirect mechanisms, in which guard or decoy proteins synchronize with R protein-mediated defence responses (van der Hoorn & Kamoun, 2008; Sun et al., 2017b). AtRPS5 from *Arabidopsis thaliana* confers resistance to *Pseudomonas syringae* pv. *tomato* (Pst) carrying the effector protein AvrPphB (Warren et al., 1999). *AtPBS1* encodes a serine/threonine kinase (Swiderski & Innes, 2001). AvrPphB belongs to the cysteine protease subfamily and cleaves AtPBS1 to activate AtRPS5-mediated immune responses (DeYoung et al., 2012; Qi et al., 2014; Shao et al., 2003; Simonich & Innes, 1995). In parallel with AvrPphB–PBS1–RPS5, *Arabidopsis* possesses an AvrRpt2–RIN4–RPS2 immune pathway in which AvrRpt2 specifically cleaves RIN4 to trigger the RPS2 immune response. Based on this, Innes's group expanded the AtPBS1 recognition spectrum via substitution of the AvrPphB cleavage site with an AvrRpt2 cleavage site and verified that the modified $AtPBS1^{RCS2}$ could recognize Pst (AvrRpt2) to trigger AtRPS5 immune responses (Kim et al., 2016). The potyvirus protease NIa-Pro is indispensable for generation of functional viral proteins (Adams et al., 2005). Therefore, they further modified the AvrPphB cleavage site of AtPBS1 as the NIa-Pro cleavage site of two potyviruses, tobacco etch virus (TEV) and turnip mosaic virus (TuMV). The modified $AtPBS1^{TEV}$ and $AtPBS1^{TuMV}$ conferred resistance to TEV and TuMV, respectively, upon overexpression in *Arabidopsis* (Kim et al., 2016). This strategy is termed decoy engineering, as it is based on artificially modified bait proteins as decoys for new pathogens (Kourelis et al., 2016).

Potato virus Y (PVY) is a devastating pathogen of potato (*Solanum tuberosum*) and is regarded as one of the plant viruses of great economic significance in agriculture (Rybicki, 2015). In the present study, we characterized a PBS1 homologue from potato and engineered it for PVY resistance. First, we tested the existence of AvrPphB-triggered immunity in potato. Potato cultivar Shepody was inoculated with Pst DC3000 carrying either the AvrPphB effector or not (empty vector [EV]). Pst (AvrPphB) elicited strong HR symptoms, while Pst (EV) and control $MgCl_2$ did not induce a visible HR (Figure 1a). Reactive oxygen species (ROS) production in the Pst (AvrPphB)-infected area was confirmed by 3,3′-diaminobenzidine (DAB) staining (Figure 1b). In an ion leakage assay, Pst (EV) increased the electroconductivity 2.0-fold, while Pst (AvrPphB) increased it 3.6-fold as compared with control $MgCl_2$ (Figure 1c). The number of Pst colonies was significantly lower (44.3%) in Pst (AvrPphB)-infected leaves than in Pst (EV)-infected leaves (Figure 1d). The above results demonstrated that potato could recognize AvrPphB to trigger an immune response against Pst.

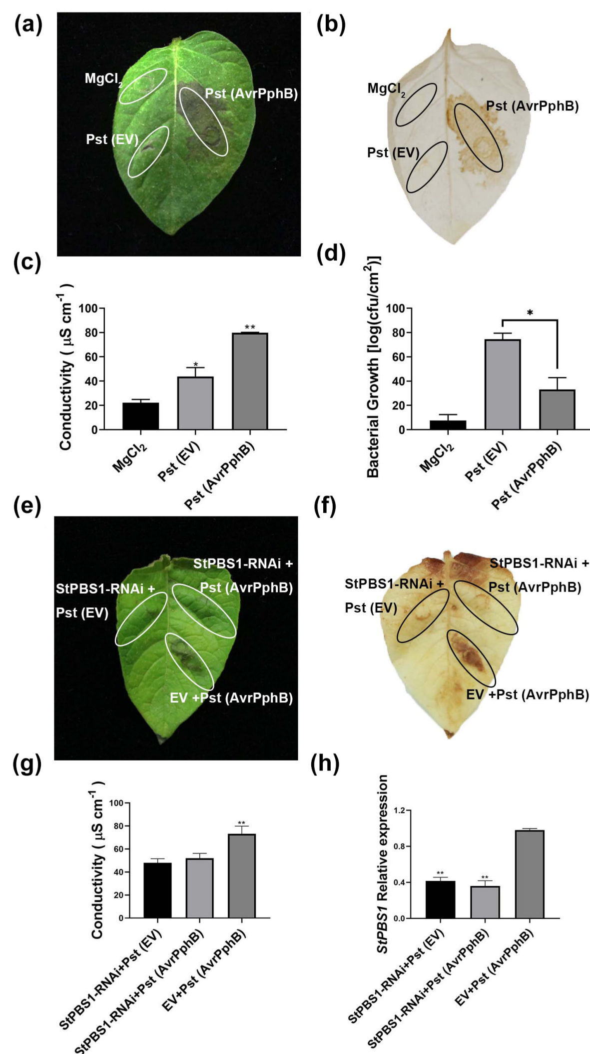

FIGURE 1 AvrPphB triggers immunity in potato via StPBS1. (a) Bacterial suspension (OD_{600} = 0.002) was inoculated onto 21-day-old potato seedlings. At 3 days postinoculation (dpi), hypersensitive response (HR) was visualized and photographed. Inoculated areas are circled with solid lines. (b) HR was visualized in the same leaves by 3,3′-diaminobenzidine (DAB) staining. (c) Cell conductivity assay. Electrolyte leakage was measured using a Horiba B-173 conductivity meter. The relative electrolyte leakage is expressed as the percentage of sample conductivity to total conductivity. (d) Bacterial numbers were determined for inoculated regions per area. (e), (f), and (g) Silencing of *StPBS1* compromised AvrPphB-triggered immunity. The experimental procedures were the same as in (a), (b), and (c), while *StPBS1* was silenced via inoculation of the StPBS1-RNAi vector at the indicated leaf areas. (h) Determination of the *StPBS1* silencing effect. Samples were taken from inoculated leaves and the *StPBS1* expression level was determined by reverse transcription-quantitative PCR. Potato *actin* was used as the reference gene. Three biological replicates were performed. The expression of the corresponding gene was calculated according to the $2^{-\Delta\Delta Ct}$ method, and statistical variance was analysed with SPSS v. 19.0 software. $^*p < 0.05$, $^{**}p < 0.01$

We next asked whether the AvrPphB-triggered immune response was mediated by a potato homologue of AtPBS1. We used the AtPBS1 amino acid sequence to query PBS1 homologues in a

potato database (http://potato.plantbiology.msu.edu/index.shtml). One potato protein, XM006359009.2, designated as StPBS1, showed the highest amino acid identity (73%) with AtPBS1 in full-length protein comparison among PBS1 homologues from several monocotyledonous and dicotyledonous plants and also AtPBL homologues from *Arabidopsis*. This suggests that the function of StPBS1 and AtPBS1 might be evolutionarily conserved (Figure S1a). AvrPphB cleavage is essential for AtPBS1 activation, and we found that the AvrPphB cleavage site (GDK/SHVS) is highly conserved in StPBS1 and other PBS1 homologues from several plant species, demonstrating that they might be cleavable by the effector AvrPphB, similar to AtPBS1 (Figure 2a). Silencing of *StPBS1* via agro-infiltration of the pCB2004B-*StPBS1* construct compromised the HR that was induced by Pst (AvrPphB) infection, indicating that AvrPphB-triggered immunity is dependent on *StPBS1* (Figure 1e–h).

While downstream signalling of AtPBS1 was mediated through AtRPS5, HvPBS1 from barley has been reported to signal through PBR1 in a PBS1–PBR1 immune pathway (Carter et al., 2019). In order to investigate whether StPBS1 signals through potato homologues of AtRPS5 or HvPBR1, we also retrieved their homologues (PGSC0003DMP400024285 and PGSC0003DMP400066527) from the potato genome database, and named them StRPS5 and StPBR1, respectively (Figure S1b).

We next investigated whether the StPBS1 immune signalling pathway was mediated by StRPS5 and StPBR1. *AtPBS1*, *AtRPS5*, *StPBS1*, *StRPS5*, *StPBR1*, *HvPBR1*, and *TaPBR1* were cloned from respective plant species and overexpression vectors were constructed in pCAMBIA1300. We used a *Nicotiana benthamiana* transient expression system to coexpress different combinations of the above constructs according to the AvrPphB–PBS1–RPS5 or the AvrPphB–PBS1–PBR1 immune pathway. Expression of the above genes in inoculated areas was confirmed by reverse transcription PCR (Figure S2). We did not observe any HR in the above combinations harbouring StPBS1, while the well-established AvrPphB–AtPBS1–AtRPS5 immune pathway caused a strong HR (Figure 2b). We speculate that the above results might be for two reasons. First, the StPBS1 immune pathway could only be activated in the native potato, because other cellular proteins are required that are absent from *N. benthamiana*. Second, StPBS1 might mediate immune responses through R proteins in potato other than StRPS5 and StPBR1. Therefore, we used a potato transient expression system to test our hypothesis. Although expression of *StPBS1* alone did not trigger visible HR, coexpression of *StPBS1* with Pst (AvrPphB) led to stronger HR compared with Pst (AvrPphB) alone (Figure 2c,d). Together with the fact that silencing of *StPBS1* reduced the HR induced by Pst (AvrPphB) infection (Figure 1e–h), the present result indicates that AvrPphB-induced immune responses in potato are mediated by StPBS1.

Insertion of five alanine residues in the AvrPphB cleavage site of AtPBS1, AtPBS1^{5A}, causes conformational changes in AtPBS1 and constitutively activates immune signalling when AvrPphB is absent (DeYoung et al., 2012). When the equivalent insertion was introduced between GDK and SHVS in the putative AvrPphB cleavage site of StPBS1 (Figure 3a), StPBS1^{5A} was self-activated and induced a robust HR in potato (Figure 2e,g). Both of two cleavage segments of AtPBS1 by AvrPphB are necessary for the activation of AtRPS5 (DeYoung et al., 2012). Therefore, we truncated StPBS1 at the predicted AvrPphB cleavage site to create expression constructs for N-terminal (StPBS1^{N}, amino acids 1–243) and C-terminal (StPBS1^{C}, amino acids 244–468) segments of StPBS1 (Figure 3a). Expression of StPBS1^{N} did not cause a visible HR, while expression of StPBS1^{C} alone triggered a strong HR, which was comparable to the HR in StPBS1^{5A} and was not affected by coexpression of StPBS1^{N} (Figure 2e,g). Expression of StRPS5 or StPBR1 did not cause an HR in the potato transient expression assay, demonstrating that they are not functional at least as regards the HR (Figure 2e,g). An HR by StPBS1^{5A} and StPBS1^{C} expression in potato was not detected in the *N. benthamiana* transient expression assay, confirming our hypothesis that additional proteins are involved in StPBS1 signalling in potato that are missing from *N. benthamiana* (Figure 2f). Furthermore, reverse transcription-quantitative PCR (RT-qPCR) analysis showed that *StPBS1* expression was induced by Pst (AvrPphB) infection, PVY infection, and treatment with the defence hormone salicylic acid (SA), indicating that StPBS1 is involved in the defence responses triggered by AvrPphB and PVY or mediated by SA in potato (Figure 2h). The above treatments did not alter *StRPS5* expression, while Pst (AvrPphB) infection or SA treatment slightly elevated or repressed *StPBR1* expression (Figure 2i,j). The above data showed that StPBS1 might affect immunity through potato proteins other than StRPS5 and StPBR1.

In the *Arabidopsis* AvrPphB–AtPBS1–AtRPS5 defence system, the C-terminal SEMPH motif of AtPBS1 is necessary for AtRPS5 recognition (DeYoung et al., 2012). The amino acid sequences corresponding to the SEMPH motif of AtPBS1 were divergent but the flanking sequences were conserved among different PBS1 proteins (Figure 2a). StPBS1 has more divergent sequences of STKPQ with three residues differing from STRPH of HvPBS1 and two residues differing from SEMPH of AtPBS1 (Figure 2a). We speculate that this sequence divergence might explain the different downstream R proteins of StPBS1; the two-residue difference of AtPBS1 and HvPBS1 led to the difference in downstream signalling by RPS5 and PBR1 (Carter et al., 2019; Sun et al., 2017a). To further demonstrate the importance of the STKPQ motif in StPBS1, we replaced the STKPQ motif of immune-competent StPBS1^{C} with SEMPH to obtain StPBS1$^{C\text{-}R}$ (Figure 3a). Expression of StPBS1$^{C\text{-}R}$ did not cause an HR in potato or *N. benthamiana*, indicating that immune signalling of StPBS1 is different from that of AtPBS1 and that the STKPQ motif is essential for StPBS1-triggered immune responses (Figure 2e).

Based on the above results, we aimed to develop decoy-engineered StPBS1 that could recognize PVY and initiate an immune response. The putative AvrPphB cleavage site (GDK/SHVS) of StPBS1 was replaced with the PVY NIa-Pro protease cleavage sequence (DVVVEQ/A) to obtain StPBS1^{NIa} (Figure 3a). As a comparison, we also introduced an STKPQ→SEMPH replacement at the C-terminus of StPBS1^{NIa} to obtain StPBS1^{NR} (Figure 3a). In *Arabidopsis*, localization at the plasma membrane by the N-terminal *S*-acylation

FIGURE 2 Determination of StPBS1 immune signalling. (a) Sequence alignment of three conserved regions of PBS1 homologues. Predicted functional motifs are underlined, and residue positions are shown above for StPBS1. At: *Arabidopsis thaliana*; Gm: *Glycine max*; Hv: *Hordeum vulgare*; St: *Solanum tuberosum*; Ta: *Triticum aestivum*. (b) and (f) Transient expression assay in *Nicotiana benthamiana*. The leaves were infiltrated with *Agrobacterium tumefaciens* GV3101 inoculum (OD_{600} = 1.0) carrying different recombinant vectors and visualized under natural light at 3 days postinoculation (dpi). (c) and (e) Transient expression assay in potato. The leaves were infiltrated with *A. tumefaciens* GV3101 inoculum (OD_{600} = 1.0) carrying different recombinant vectors and visualized under natural light at 3 dpi. (d) and (g) Electrolyte leakage was measured using a Horiba B-173 conductivity meter. The relative electrolyte leakage is expressed as the percentage of sample conductivity to total conductivity. (h), (i), and (j) Expression analysis of *StPBS1*, *StRPS5*, and *StPBR1*. Experimental procedures and data analysis were the same as in Figure 1h. All experiments included at least three biological repeats. Between-group differences were analysed by one-way analysis of variance. $StPBS1^{N}$: N-terminal (amino acids 1–243) segment of StPBS1; $StPBS1^{C}$: C-terminal (amino acids 244–468) segment of StPBS1; $StPBS1^{5A}$: StPBS1 with the insertion of five alanine residues at the AvrPphB cleavage site; $StPBS^{C\text{-}R}$: $StPBS1^{C}$ (C-terminal segment) harbouring replacement of the STKPQ motif with SEMPH of AtPBS1

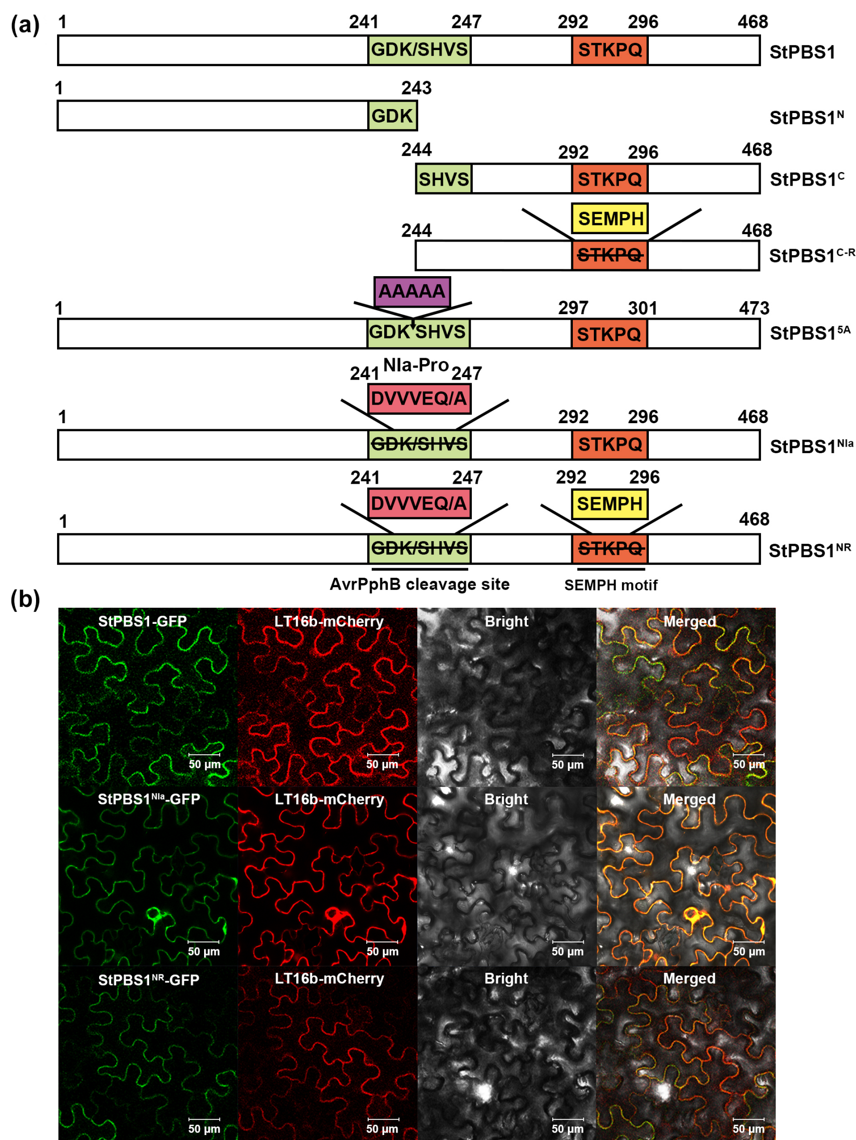

FIGURE 3 Modification of StPBS1 and subcellular localization. (a) Schematic diagram of StPBS1 modification and domain truncation. The substitution is shown above the respective motifs. The slashes indicate the exact cleavage site for AvrPphB or NIa-Pro. (b) Subcellular localization of StPBS1 and its derivatives. *Nicotiana benthamiana* leaves were infiltrated with *Agrobacterium tumefaciens* GV3101 containing the pEarleyGate103-SL-StPBS1 vector, and the subcellular localization of GFP-fused StPBS1 (StPBS1^{NIa}, StPBS1^{NR}) was observed by confocal microscopy at 36 h postinoculation. The plasma membrane marker protein LTI6b (Morsy et al., 2005) fused with mCherry is shown

site is essential for the AtPBS1-mediated immune response (Qi et al., 2014; Sun et al., 2017a). Sequence alignment showed that the *S*-acylation site was conserved among PBS1 homologues from different plant species and in particular it was the same between AtPBS1 and StPBS1, indicating that StPBS1 may also be localized at the plasma membrane (Figure 2a). To clarify the subcellular localization of StPBS1, we constructed the pEarleyGate103-SL-StPBS1 recombinant vector, transformed it into *Agrobacterium tumefaciens* GV3101, and coexpressed it with the membrane localization marker LTI6b-mCherry in tobacco leaves. StPBS1 was localized at the cell membrane (Figure 3b). TaPBS1 also localizes to the cell membrane, indicating the conservative subcellular localization of PBS1 from different plant species (Sun et al., 2017a). Similar to StPBS1, StPBS1^{NIa} and StPBS1^{NR} also localized at the cell membrane, suggesting that modified StPBS1^{NIa} and StPBS1^{NR} would not affect StPBS1 immune signalling by altered subcellular localization (Figure 3b). Inspired by the above results, we examined whether decoy-engineered StPBS1^{NIa} was cleaved by NIa-Pro protease. FLAG-tagged StPBS1^{NIa} and glutathione *S*-transferase (GST)-tagged NIa-Pro were expressed in *Escherichia coli* and purified. We showed that NIa-Pro cleaved StPBS1^{NIa} to generate the expected fragment sizes when the purified proteins were incubated in vitro, demonstrating the cleavage efficiency of NIa-Pro on StPBS1^{NIa} (Figure S3).

The recombinant vectors pCAMBIA1300-StPBS1, pCAMBIA1300-StPBS1^{NIa}, and pCAMBIA1300-StPBS1^{NR}, in which the HA epitope was fused with these genes, were transformed into the PVY-susceptible potato variety Shepody. The genomic DNA of transgenic lines was verified by PCR using a CaMV 35S promoter-specific forward primer and an *StPBS1*-specific reverse primer (Figure S4a). RT-qPCR analysis showed that *StPBS1* (*StPBS1^{NIa}*, *StPBS1^{NR}*) transcripts accumulated more abundantly in the transgenic lines compared with wild-type (WT) plants (Figure S4b). We verified the ectopic expression of 52-kDa StPBS1, StPBS1^{NIa}, and StPBS1^{NR} proteins in the respective transgenic lines via a western blot assay using an antibody against the HA epitope (Figure 4a). We first determined the effects of NIa-Pro expression on HR in these

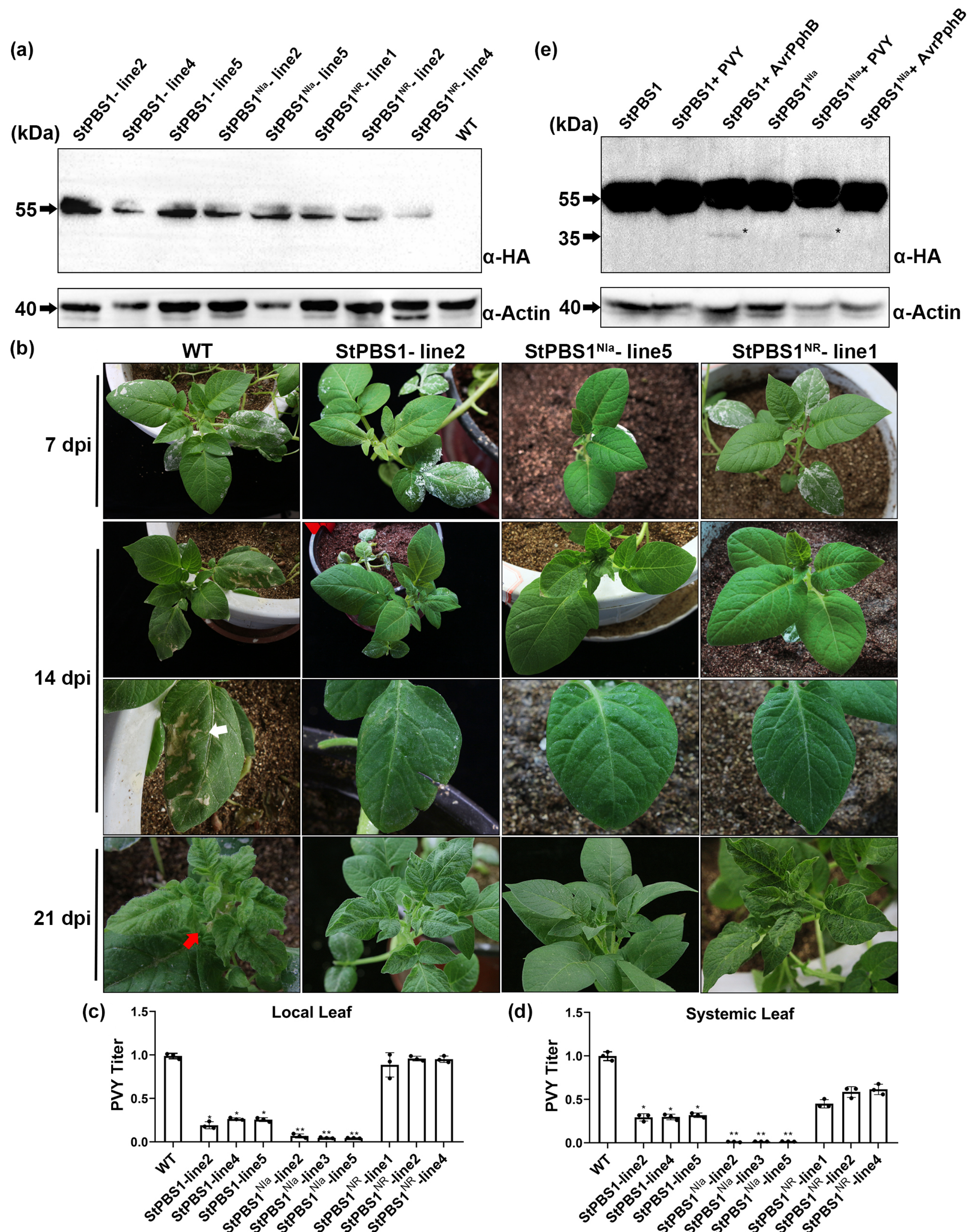

FIGURE 4 Analysis of StPBS1 transgenic potato. (a) Western blot analysis of protein expression. Leaf tissue was harvested for total protein isolation and recombinant StPBS1-HA was detected with anti-HA antibody. Potato actin (detected with anti-actin antibody) served as an internal control. (b) Virus inoculation and symptom detection in potato. Potato leaves were sap inoculated with PVY, and virus symptoms were recorded at different days postinoculation (dpi) as indicated. Systemic leaves at 21 dpi are shown. (c) and (d) Detection of PVY titres in inoculated leaves and systemic leaves at 14 dpi and 21 dpi, respectively. Viral titre denotes the abundance of viral RNA. Total RNA was extracted and cDNA was prepared. Reverse transcription-quantitative PCR was carried out using PVY coat protein gene-specific primers, and potato *actin* was used as internal control. Three biological replicates were performed, and the expression of the corresponding gene was calculated according to the $2^{-\Delta\Delta Ct}$ method. (e) Cleavage of StPBS1. Asterisks indicate the location of cleavage products by the respective proteases within the recognition sites

transgenic plants. To this aim, we expressed *NIa-Pro* in the leaves of StPBS1 and StPBS1^{NIa} transgenic plants. HR and ROS production could only be observed in StPBS1^{NIa} transgenic plants but not in StPBS1 transgenic plants upon transient expression of NIa-Pro protease, indicating the inducible immunity via coupling of NIa-Pro with StPBS1^{NIa} but not StPBS1 (Figure S5).

PVY was rub-inoculated on the leaves of 14-day-old seedlings, and virus symptoms were recorded over time. At 7 dpi, no visible symptoms were observed in the infected plants. At 14 dpi, WT plants showed obvious viral phenotypes such as leaf necrosis and curling while the transgenic plants showed no or much weaker symptoms (Figure 4b). In RT-qPCR analysis, the PVY titre was decreased in *StPBS1*, *StPBS1^{NIa}*, and *StPBS1^{NR}* transgenic plants by 74%–82%, 93%–95%, and 4%–10%, compared with that in WT plants, respectively (Figure 4c). The above data suggest that *StPBS1* has basal resistance to PVY, which is consistent with the previous results in which *StPBS1* expression was induced by PVY infection (Figure 2h). *StPBS1^{NIa}* overexpression further alleviated virus symptoms as compared with *StPBS1* overexpression, while STKPQ→SEMPH replacement in StPBS1^{NR} compromised this alleviation. Viral symptoms and virus titre were recorded in the newly emerging leaves at 21 dpi. WT plants showed symptoms of severe leaf mottling and wrinkling, while these symptoms were slightly weaker in the *StPBS1^{NR}* transgenic plants. The systemic leaves of *StPBS1* transgenic potato displayed slight chlorosis, while *StPBS1^{NIa}* transgenic lines showed no obvious phenotype (Figure 4b). The PVY titre was significantly decreased in the systemic leaves of *StPBS1*, *StPBS1^{NIa}*, and *StPBS1^{NR}* transgenic plants by 68%–71%, 98%–99%, and 38%–58% compared with that in WT plants, respectively. Therefore, viral infection severity was dramatically decreased in *StPBS1^{NIa}* transgenic lines compared with those in *StPBS1* transgenic lines, while it was increased in *StPBS1^{NR}* transgenic lines (Figure 4d). The above results demonstrate that engineered StPBS1^{NIa} confers resistance to PVY, possibly through recognition by the PVY-encoded NIa-Pro protease. Consistent with this, PVY infection caused the cleavage of StPBS1^{NIa} with the expected fragment size but did not cleave StPBS1. StPBS1 but not StPBS1^{NIa} could be cleaved by AvrPphB with the same fragment sizes, again proving their cleavages at the respective cleavage sites (Figure 4e).

PBS1 orthologues have been characterized in several crops such as wheat, barley, and soybean besides the well-studied AtPBS1 in *Arabidopsis*, and have all been shown to be cleaved by the bacterial effector AvrPphB (Carter et al., 2019; Helm et al., 2019; Sun et al., 2017a). Here, we showed that AvrPphB could cleave StPBS1 (Figure 4e) and coexpression of StPBS1 enhanced AvrPphB-triggered HR in potato. These results indicate that the recognition mechanism of AvrPphB via PBS1 is convergent in different plant species, which might be attributable to the conserved AvrPphB cleavage sites in these PBS1 orthologues. Despite this similarity, StPBS1 showed differences from other PBS1 orthologues in the following aspects. First, the STKPQ motif is essential for StPBS1 immune responses and StPBS1 signals through neither RPS5 nor PBR1 in potato, adding evidence for the existence of divergent R partners for PBS1 orthologues across different plant species. Previous studies have shown that the SEMPH motif in the C-terminal part of AtPBS1 is the key determinant for AtRPS5 recognition and the negatively charged glutamate residue is essential for this recognition (Sun et al., 2017a). However, the SEMPH motifs are divergent among PBS1 orthologues by two or three residue differences, which might underlie the differences in R proteins that directly interact with them, such as the HvPBR1 in barley for recognition of STRPH in HvPBS1 (Carter et al., 2019). Second, the C-terminal cleavage segment of StPBS1 is sufficient to trigger downstream signalling, which is different from AtPBS1, in which both cleavage fragments are necessary (DeYoung et al., 2012). This demonstrates the signal transduction difference between StPBS1 and other PBS1 orthologues. Immune competence of StPBS1^{C} instead of StPBS1^{N} again indicates that the signal relay part of StPBS1 is located in its C-terminal domain. Third, overexpression of native, unmodified *StPBS1* also conferred immunity to PVY. In consideration of the fact that *StPBS1* expression was induced by both PVY and Pst (AvrPphB) infection as well as SA treatment, *StPBS1* might work as a general defence gene and might be involved in the PTI pathway. The cytoplasmic receptor-like protein kinase VII subfamily contains many PBS1-like (PBL) proteins, among which Botrytis-Induced Kinase 1 (BIK1) is essential for PTI resistance to *P. syringae*, and other members such as PBL1 and PBL2 also contribute to PTI (Zhang et al., 2010). *P. syringae* secretes the effector protein AvrPphB, which can cleave many PBL proteins and target the components of the PTI signal transduction pathway to inhibit plant immunity (Block et al., 2008; Fu et al., 2007; Göhre et al., 2008). Therefore, we speculate that StPBS1 may also participate in the disease resistance response of PTI. Previously, Innes's group decoy engineered *GmPBS1* for soybean mosaic virus resistance and confirmed its effectiveness via protoplast assays and genetic transformation (Helm et al., 2019; Pottinger et al., 2020). Here, we extended this decoy strategy for StPBS1 engineering against PVY infection. Interestingly, overexpression of StPBS1^{NIa} strongly inhibited systemic spread of PVY. Furthermore, substitution of STKPQ with SEMPH significantly compromised the antiviral immunity, again supporting the importance of this motif. Identification of StPBS1-interacting R proteins for further elucidation of the StPBS1 signalling pathway is of great interest, similar to the case for identification of the signalling partner PBR1 for HvPBS1 (Carter et al., 2019). NIa-Pro is localized in the nucleus and also in the cytoplasm, where it might recognize StPBS1 at the cell membrane (Riechmann et al., 1992). Our study opens a way for pathogen control by engineering PBS1, as long as the pathogen secretes a protease. Several pathogens, including viruses, bacteria, oomycetes, fungi, and nematodes, express proteases during host infection, so this strategy may be very valuable and have great application prospects (Alfano & Collmer, 2004; Cheng et al., 2015; Hou et al., 2018).

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DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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