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RESEARCH ARTICLE

The brassinosteroid-responsive protein OCTOPUS is a novel regulator of *Arabidopsis thaliana* immune signaling

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Abstract

Phloem is a critical tissue for transport of photosynthates and extracellular signals in vascular plants. However, it also represents an ideal environment for pathogens seeking access to valuable host nutrients. Although many vascular pathogens induce economically relevant crop damage, there is still little known about the mechanisms by which immune signaling operates through the phloem. An existing phosphoproteomic dataset was mined to identify proteins that were both phosphorylated in response to the defense-elicitor flagellin (flg22) and expressed in vascular cells. A single candidate, OCTOPUS (OPS), is polarly associated with the plasma membrane of sieve element cells and has been characterized as an inhibitor of brassinosteroid insensitive-2 in promotion of brassinosteroid-related phytohormone signaling. The observation that OPS is differentially phosphorylated in response to flg22 led us to the examine whether OPS may also regulate flg22-induced immune signaling. Two independent alleles of *ops* exhibited enhanced immunity outputs across multiple signaling branches of PAMP-triggered immunity (PTI), constitutively and in response to flg22 treatment. Together with our observation that interactions between OPS and brassinosteroid insensitive-2 were disrupted by induction of salicylic acid and depletion of brassinosteriod, these data support a model whereby OPS modulates brassinolide and immune signaling to control downstream responses. We present OPS as a novel addition to the list of proteins with documented roles in PAMP-PTI signaling. These results further indicate that immune signaling in the phloem may be a significant and unique component of the host detection and response to pathogens in vascular plants.

KEYWORDS
Arabidopsis, BIN2, FLS2, gene expression, phloem, plant immunity

Kaitlyn N. Greenwood, Courtney L. King, and Isabella Melena contributed equally to this work.

1 | INTRODUCTION

The phloem is a critical tissue in vascular plants that transports photosynthetic sugars from source tissues (leaves) to sink tissues (roots, flowers, and fruit), but this carbon-rich nutrient content also makes the phloem an ideal tissue for pathogenic microbes to colonize (Jiang et al., 2019). There are many examples of phloem-limited bacterial pathogens, including the devastating citrus pathogen *Candidatus* Liberibacter asiaticus (CLas), the causal agent of citrus-greening disease (or "Huanglongbing"). CLas gains access to the phloem of citrus trees after being deposited by the brown planthopper or related insect vectors (Ammar et al., 2020; Ghanim et al., 2017). The phloem is such a rich source of nutrients that some pathogens that do not colonize the phloem directly will stimulate the aberrant development of phloem cells in other tissues to tap its resources (Walerowski et al., 2018). Two distinct cell types comprise the phloem transport system in plants: sieve elements and companion cells. Sieve elements are elongated cells whose primary function is to transport sap contents (photosynthetic sugars, RNA, peptides, and other small organic molecules) from source to sink. Adjacent to sieve elements are the companion cells, which are responsible for loading sap contents into sieve elements (Breia et al., 2021).

Current models of immune signaling in response to a pathogen generally assume that all host cells must individually recognize extracellular pathogen-associated molecular patterns (PAMPs). PAMPs are detected by plasma membrane (PM)-localized pattern recognition receptor proteins that, after PAMP binding, can initiate intracellular signaling and activate robust defenses (Couto & Zipfel, 2016; Nicaise et al., 2009). The classic example of a pattern recognition receptor is FLAGELLIN SENSING 2 (FLS2), a member of the leucine-rich repeat receptor-like kinase family of receptors. FLS2 binds to the bacterial motor protein flagellin or to a conserved 22-amino acid peptide, flg22, derived from bacterial flagellin, to initiate a cascade of intracellular signaling events that contribute to the host immune response (Gómez-Gómez & Boller, 2000). FLS2-associated signaling events have been categorized into three branches, each involving the activation of distinct sets of marker genes (Korasick et al., 2010; Smith et al., 2014). For example, the calcium-dependent branch of FLS2 signaling induces expression of *PHOSPHATE-INDUCIBLE 1* (*PHI1*), the mitogen-activated protein kinase pathway activates *FLG22-INDUCED RECEPTOR KINASE* (*FRK1*), and activation of the salicylic acid (SA) pathway causes increased expression of *PATHOGENESIS-RELATED1* (*PR1*) (DeFalco & Zipfel, 2021; Monaghan & Zipfel, 2012; Nicaise et al., 2009). In addition to changes in gene expression, FLS2 also initiates the production of extracellular reactive oxygen species and the deposition of callose at the cell wall. Combined, these independent signaling events promote the restriction of pathogen growth and promote host immunity (DeFalco & Zipfel, 2021).

Largescale phosphoproteomic screens have been used to detect proteins that are differentially phosphorylated in response to flg22, potentially identifying proteins involved in the regulation of flg22-induced signaling (Kalde et al., 2007; Nühse et al., 2007; Smith et al., 2014). To find potential regulators of flg22-induced signaling specifically occurring in the phloem, we mined existing flg22 phosphoproteomic datasets and identified a single candidate protein, OCTOPUS (OPS), which exhibits differential phosphorylation in *Arabidopsis* cell culture (Benschop et al., 2007). Previous data indicate that OPS is exclusively expressed in procambial and phloem tissue and has a role in phloem development but is completely uncharacterized in the context of immune signaling (Breda et al., 2017; Kang et al., 2017; Roschzttardtz et al., 2014; Ruiz Sola et al., 2017; Truernit et al., 2012). *ops* knockout lines display decreased phloem pattern complexity and contain undifferentiated sieve element cells, resulting in gaps in phloem strands of the root (Breda et al., 2017; Gujas et al., 2020; Ruiz Sola et al., 2017; Truernit et al., 2012). Further investigations indicated that OPS may regulate phloem developmental processes by promoting signaling events after perception of brassinolide (BL), a member of the brassinosteriod (BR) class of phytohormones. BL is an endogenous phytosteroid detected by the brassinosteroid insensitive-1 (BRI1) receptor kinase. When BRI1 binds BL, a series of downstream signaling events occurs, leading to cell elongation and growth. OPS interacts with a member of the GLYGOGEN SYNTHASE KINASE3 (GSK3) family, brassinosteroid insensitive-2 (BIN2), at the PM (Anne et al., 2015). The retention of BIN2 at the PM is known to prevent BIN2 from inhibiting transcriptional changes needed to induce cell growth, a phenomenon also associated with increased immune signaling. As a relatively uncharacterized regulatory protein, OPS is an ideal candidate for studying flg22-signaling in the phloem.

Despite the devastating effects of phloem-dwelling pathogens on crops, it is not yet known in detail how the cells of the phloem detect the presence of bacterial pathogens or how they respond to them (Jiang et al., 2019). Identifying the contributions of the phloem to pathogen detection and response will increase our knowledge of the tissue-specific mechanisms immune signaling. Using loss-of-function T-DNA mutants and gene expression analysis, we identify a new role for the sieve element protein OCTOPUS (OPS) in the regulation of flg22-induced signaling events in *Arabidopsis* seedlings and demonstrate that its interactions with BIN2 are influenced by BL and SA. To our knowledge, this is the first example of a protein localized in sieve elements with such a role in flg22 signaling.

2 | RESULTS

2.1 | flg22-induced immune signaling is enhanced in *ops* mutants

To explore a potential role for OPS in flg22-elicited immune signaling, we obtained T-DNA insertion mutant lines *ops-3* and *ops-4* (Roschzttardtz et al., 2014). Consistent with previous studies using different alleles (Truernit et al., 2012), seedlings from both lines displayed shorter roots with an increased amount of branching (Figure 1a,b).

We hypothesized that because OPS was identified as a protein differentially phosphorylated after flg22 treatment in a phosphoproteomic screen (Benschop et al., 2007), OPS might have a role in the regulation of flg22-induced signaling events in *Arabidopsis*. In WT plants, OPS

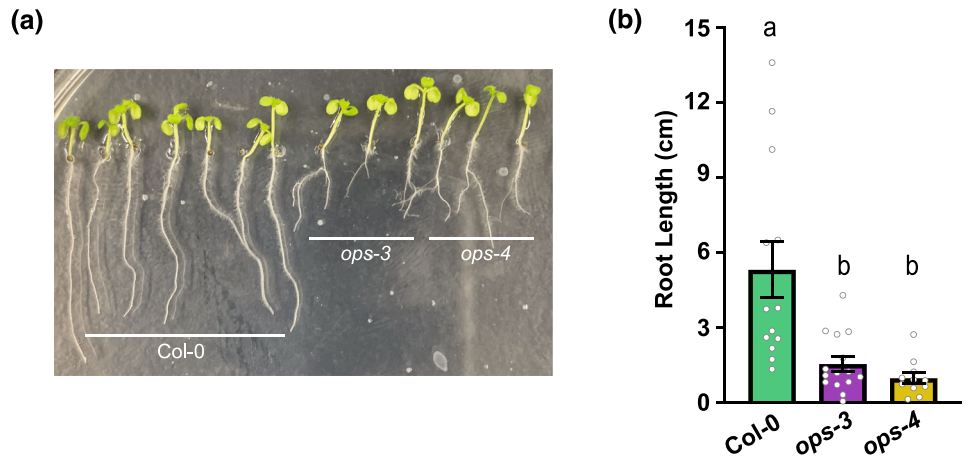

FIGURE 1 Phenotypic characterization of *ops-3* and *ops-4*. (a) Comparison of root length between 10-day old Col-0, *ops-3*, and *ops-4* seedlings grown vertically for 7 days. Representative image is shown. (b) Analysis of root length in Col-0, *ops-3*, and *ops-4* using ImageJ, $n = 15$. Statistical significance determined by ANOVA with Tukey MCS, $p < .05$. Error bars are 1 S.E.

expression is induced in response to flg22 treatment (Figure 2a). We sought to determine how plants lacking OPS would respond to flg22 using a standard seedling growth inhibition assay (Anderson et al., 2011; Gómez-Gómez & Boller, 2000). Col-0 and both mutants were grown in liquid media supplemented with mock or 1 μM flg22 for 2 weeks before measuring their fresh weight. Interestingly, both *ops* mutants showed significantly increased flg22-dependent growth inhibition relative to Col-0 (Figure 2b). These data suggest that OPS may function as an inducible repressor to attenuate FLS2 signaling.

In *Arabidopsis*, when FLS2 binds flg22, a network of multiple signaling pathway branches are initiated, with each independent branch resulting in the expression of one or more pathway-specific marker genes (Korasick et al., 2010; Smith et al., 2014). To test if OPS has a role in one or more of these specific pathways, we measured expression levels of these marker genes in the *ops-3* and *ops-4* mutants. In Col-0 seedlings treated with 100 nM flg22, *PHI1* expression remains low prior to flg22 exposure (0 min), peaks within 30 min, and then falls to near base levels for 1–3 h (Figure 3a). When *ops-3* and *ops-4* seedlings were treated with 100 nM flg22 for the same time course, the expression pattern of *PHI1* was observably different. Both *ops-3* and *ops-4* mutants showed constitutively enhanced *PHI1* expression relative to Col-0 and while *PHI1* peaked in both mutants 30 min after treatment, expression levels were still significantly higher than Col-0 (Figure 3a). Constitutive induction of *FRK1* expression was also observed in both *ops-3* and *ops-4* seedlings (Figure 3b,c). Finally, we evaluated SA-dependent FLS2 signaling by measuring *PR1* expression. Consistent with our previous marker genes tested, *PR1* was constitutively induced in both *ops-3* and *ops-4* (Figure 3d). Together, these data suggest that OPS represses a broad suite of flg22-induced immune responses.

2.2 | OPS expression and behavior is phytohormone-responsive

Previous investigations of *OPS* overexpression lines in *Arabidopsis* showed that OPS functions alongside the GSK3-like kinase BRASSINOSTEROID-INSENSITIVE 2 (BIN2) at the PM, likely preventing BIN2 from inhibiting transcriptional changes needed to induce cell growth (Anne et al., 2015). These data indicate that OPS plays a role in promoting BL-induced cellular responses which may explain some of thephloem developmental defects observed in *ops* mutants (Rodriguez-Villalon et al., 2014, 2015; Truernit et al., 2012).

To further describe a role for OPS in BL signaling using the *ops* knockout lines, we treated Col-0 seedlings with 1 μM epibrassinolide (24-epiBL) to establish *OPS* expression patterns. In contrast to our flg22 treatment data, but in alignment with established relationship between SA-mediated and BL-mediated signaling, *OPS* expression was attenuated by 24-epiBL treatment (Figure 4a). This response is consistent with other critical BL pathway components in the presence of exogenous BL (Kim et al., 2006). To connect this expression pattern to developmental phenotype, Col-0 and *ops* mutants were treated with 1 μM 24-epiBL and hypocotyl elongation was measured as a part of a standard BL sensitivity assay. Elongation in both *ops-3* and *op-4* mutants was reduced relative to Col-0 (Figure 4b), a finding that is consistent with previous data from OPS overexpression lines (Anne et al., 2015). Dark-grown *Arabidopsis* seedlings exhibit elongated hypocotyls in a BL-dependent manner (Fujioka et al., 1997). To support our previous findings, we measured the hypocotyls of Col-0, *ops-3* and *ops-4* seedlings grown in light and dark conditions. Col-0 hypocotyls elongate significantly in the dark, and while the length of hypocotyls of both *ops-3* and *ops-4* seedlings do elongate, they are significantly shorter than those of Col-0 (Figure 4b). These results provide additional evidence that OPS is BL-responsive and a necessary signaling component for BL-related hypocotyl elongation.

The association of OPS with BIN2 at the plasma membrane presents an attractive model by which the disruption of the protein complex allows for activation of downstream signaling responses in an elicitor-dependent manner. To test this idea, we cloned the coding regions of *BIN2* and *OPS* into split-luciferase expression constructs and expressed these transgenes in *N. enthamiana* leaf epidermal cells. Interaction was observed between OPS-cLUC and BIN2-nLUC relative to a non-interacting GUS-nLUC control, in agreement with previously reported bimolecular fluorescence complementation data (Anne et al., 2015). However, protein interaction was significantly attenuated by elicitation of the leaf tissue with either SA or the BL biosynthesis

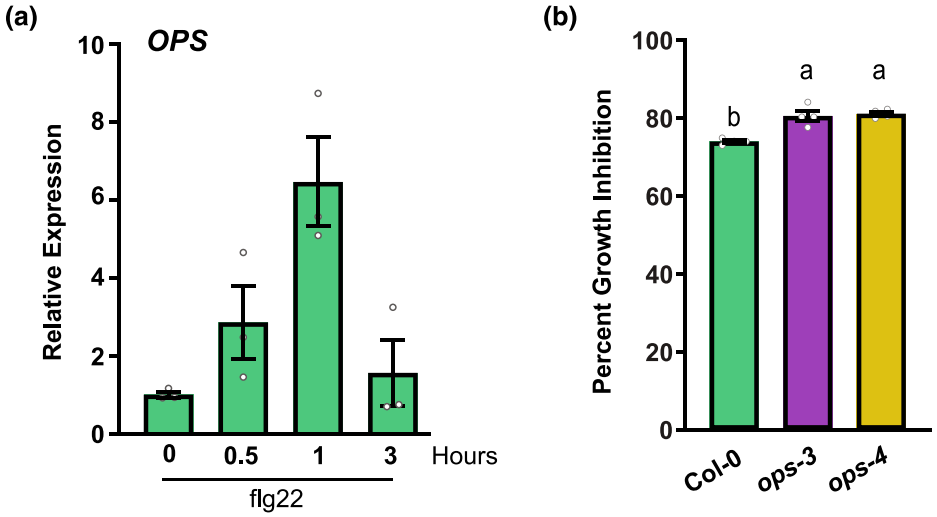

FIGURE 2 *ops* mutants exhibit enhanced flg22-induced growth inhibtion. (a) Flg22-induced expression of *OPS* mRNA in 10-day old Col-0 seedlings with treated with 1 μM flg22. Transcript level was measured using qPCR normalized to the housekeeping gene *ACT2*, $n = 3$. (b) Comparison of flg22-induced root inhibition between Col-0, *ops-3*, and *ops-4* seedlings. Four-day old seedlings were transfered to liquid MS media and grown for 2 weeks under mock or 1 μM flg22 conditions. Shown are percent change measurements pooled from 4 independent experiments, $n = 40–60$ in each replicate. Statistical significance determined by ANOVA with Tukey MCS, $p < .05$. Error bars are 1 S.E.

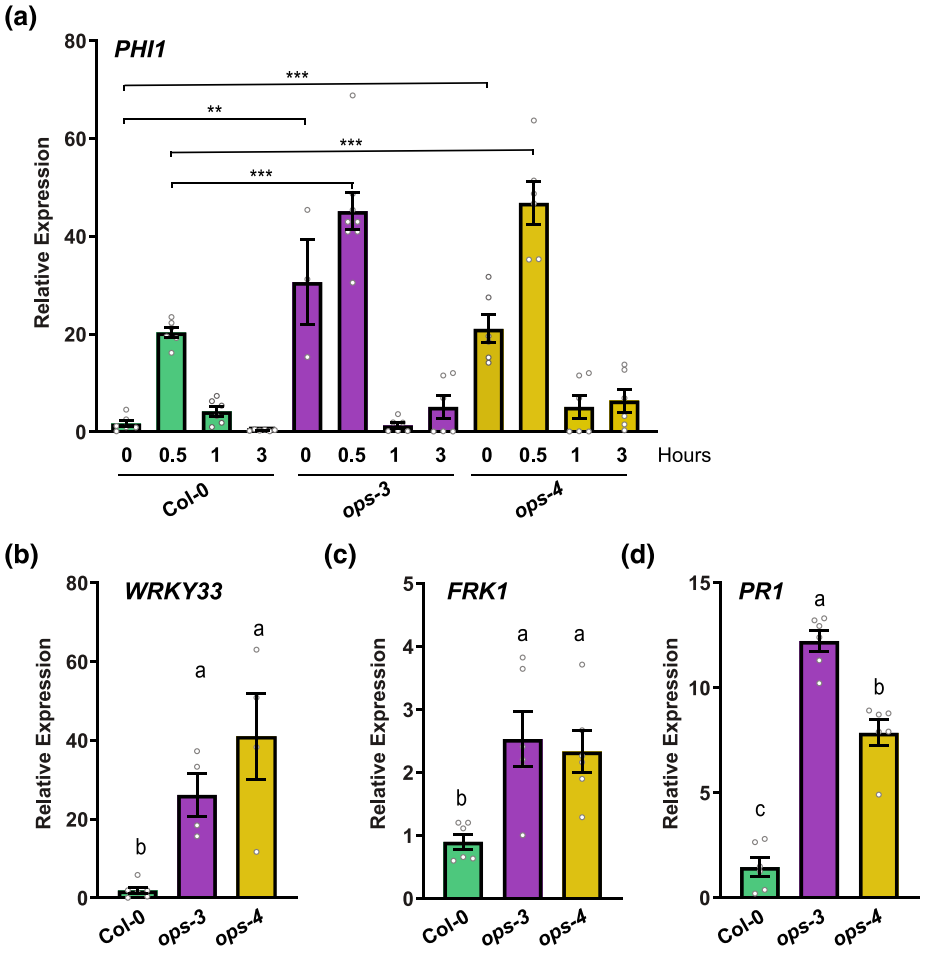

FIGURE 3 Multiple flg22 immune signaling pathways are constitutively enhanced in *ops* mutants. (a) Ten-day old Col-0, *ops-3*, and *ops-4* seedlings treated with 100 nM flg22 and mRNA was sampled across a 3-h timecourse. qPCR was performed for the marker gene *PHI1* normalized to the housekeeping gene *ACT2*. Shown are the means of pooled seedling samples, $n = 4$. Significance determined by Student's *t*-test, $^{**}p < .01$, $^{***}p < .0001$. (b–d) qPCR analysis of untreated 10-day old Col-0, *ops-3*, and *ops-4* seedlings. Expression of the marker genes *WRKY33* (b), *FRK1* (c), and *PR1* (d) was examined relative to *ACT2*. Significance determined by ANOVA with Tukey MCS, $p < .01$, $n = 6$. Error bars are 1 S.E.

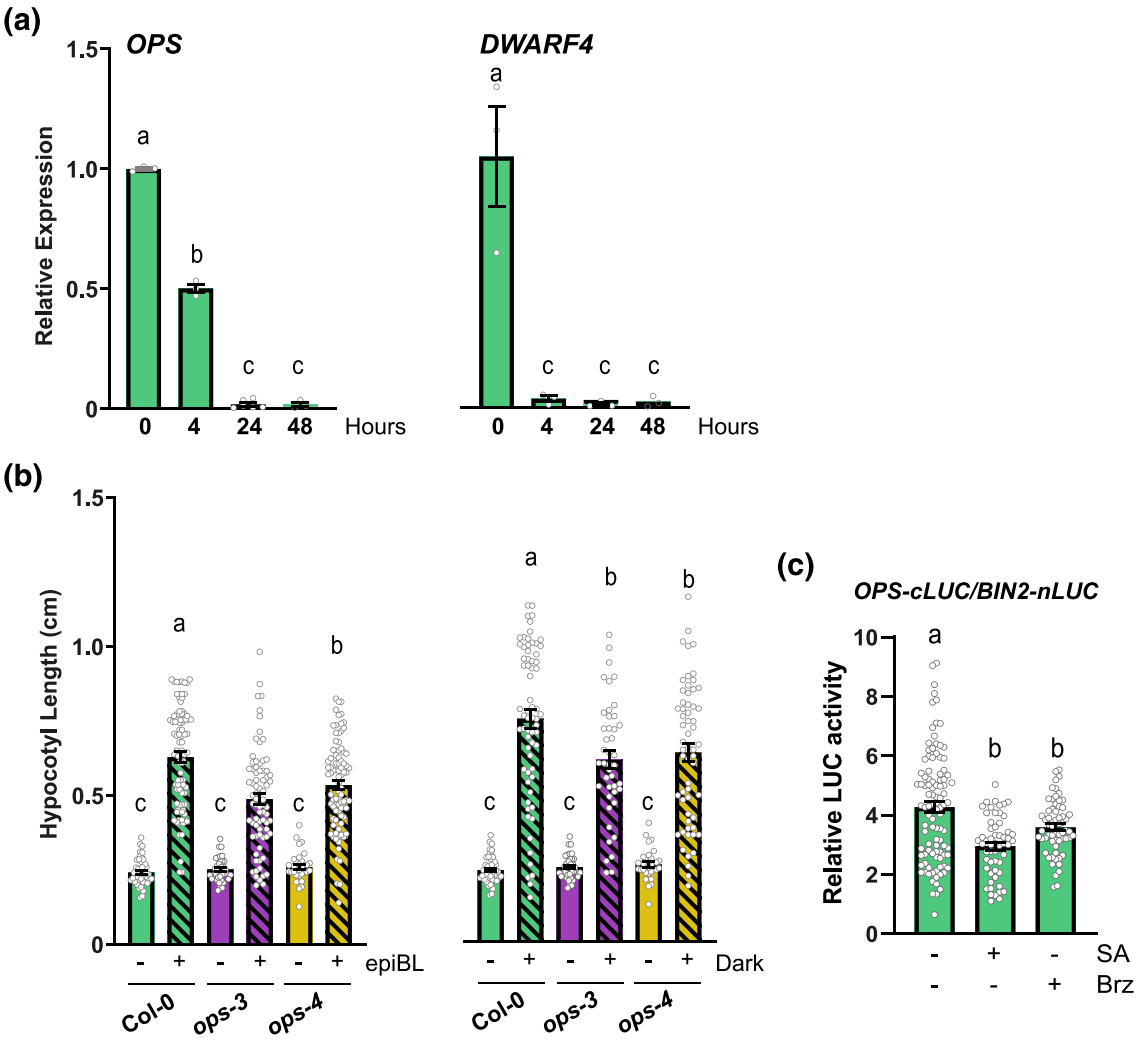

FIGURE 4 OPS expression and behavior is phyothormone-responsive. (a) Ten-day old Col-0 seedlings were treated with 1 μM 24-epibrassinolide and expression of *OPS* and marker gene *DWARF4* were measured by qPCR normalized to the housekeeping gene *ACT2*, $n = 3–6$. Error bars are 1 S.E. (b) Hypocotyl length measurements were taken of 10-day old Col-0, *ops-3*, and *ops-4* seedlings treated with mock (−) or 1 μM 24-epibrassinolide (+) using ImageJ. Values shown are pooled from three independent experiments, $n = 60–90$. Measurements were also taken of 10-day old seedlings grown under light (−) or dark (+) conditions. Values shown are pooled from three independent experiments, $n = 30–60$. (c) Interactions between OPS and BIN2 were evaluated by split-luciferase assay in *N. benthamiana* leaf epidermal cells. Tissue was sprayed with mock (−) or 1 μM solutions of either SA or Brz (+) 24 h prior to sampling. Total RLUs were normalized to the non-interacting control (GUS-nLUC) level as relative luciferace activity. Data were pooled from three independent experiments, $n = 60–100$. Significance in (a)–(c) determined by ANOVA with Tukey MCS, $p < .05$. Error bars are 1 S.E.

inhibitor brassinazole (Brz) 24 h prior to sampling (Figure 4c). These data align with our earlier findings with *ops* mutants exhibiting enhanced SA signaling, but repressed BL signaling. This positions OPS as a regulator of both BL and SA-related signaling, and BIN2 as a putative component of this regulatory mechanism.

3 | DISCUSSION

Phloem-limited pathogens cause devastating crop losses, but our understanding of how vasculature tissue responds to pathogen invasion is incomplete, making it imperative to study the contributions of tissue-specific immune signaling to overall plant defenses. A recent study demonstrated for the first time that when *Citrus sinensis* (Valencia) trees are infected with the phloem-limited pathogen CLas they exhibit increased reactive oxygen species production and callose deposition indicative of a PAMP-triggered immune response (Ma et al., 2022). Here, we present new findings on an *Arabidopsis* protein exclusively expressed in sieve elements of the phloem that describe a novel role in SA-related flg22-induced immune responses to complement its involvement in BL signaling. Notably, there is a conserved homolog to the protein in *C. sinensis* (Figure S1) that could potentially be playing a similar role in those species. These recent advances highlight the likelihood that phloem cells are involved in the detection of bacterial pathogens and activity in response signaling.

We demonstrate that two independent *Arabidopsis* lines harboring T-DNA insertions in the *OPS* gene lack *OPS* expression and display constitutive and flg22-induced expression of several immunity-related marker genes. These data indicate that OPS functions as a negative regulator of these flg22-induced responses and to exhibit a suppressive effect on this pathway (Figure 5). While the specific mechanisms of OPS function in flg22 signaling remains unclear, OPS may suppress activation of *PHI1*, *FRK1*, *WRKY33*, and *PR1* expression by directly inhibiting a signaling event in the flg22 pathway. BIN2 was recently shown to be activated in the presence of SA treatment, leading to induction of immune signaling (Kim et al., 2022); in agreement with our model, this could be tied to reduced suppression by OPS under these conditions via other complex proteins. Another possibility is OPS may be directly targeting another member of the GSK3 protein family that is instead known to regulate flg22-induced signaling, such as *Arabidopsis* Protein Kinase α (ASK α) (Stampfl et al., 2016). Because genes from three independent branches of FLS2-flg22 signaling show the same increased expression in the *ops* mutant backgrounds, it is likely that if OPS is acting directly to regulate these signals, it must function early in flg22 signaling. A third explanation remains that OPS has an indirect role in flg22 signaling. OPS is expressed in the sieve elements of the phloem in *Arabidopsis* and previous studies identified that *ops* mutants display in incomplete sieve element differentiation which results in a discontinuous protophloem and metaphloem cell file in the roots (Rodriguez-Villalon et al., 2014; Ruiz Sola et al., 2017; Truernit et al., 2012). Therefore, our observation that *ops* mutants exhibit increased flg22 marker gene expression could be the result of incomplete phloem transport, resulting in the loss of transport of a yet unknown phloem-mobile regulator of flg22 responses.

Our results are consistent with reports that FLS2 expression is detected in the vasculature of *Arabidopsis* cotyledons in roots (Beck et al., 2014; Wyrsch et al., 2015); however, detection of FLS2 could only be confirmed in the stele, which comprises both sieve elements and companion cells. FLS2 expression in the stele suggests that some cells of the vasculature are capable of detecting and responding to flg22, and while it has yet to be determined whether FLS2 expressed in the vasculature induces flg22 signaling events, our results indicate that some element of flg22 signaling does occur in the phloem.

In addition to our data demonstrating a role for OPS in flg22 signaling, we found that *OPS* expression is repressed by BL treatment in the Col-0 background and *Arabidopsis* loss-of-function *ops* mutants are impaired in BL and dark-responsive hypocotyl elongation. This is consistent with other work showing that OPS overexpression lines display increased BR responses (Anne et al., 2015), and confirms that OPS promotes BR-induced signaling for hypocotyl elongation. Finding that OPS functions in both the BRI1 and FLS2 signaling pathways is particularly interesting because these signaling pathways intersect downstream of initial receptor-ligand perception. Initiation of the BRI1 signaling pathway induces expression of several *WRKY* family transcription factors that inhibit the activation of gene expression of some flg22-induced genes (none that were tested in this work) resulting in a suppression of defense responses (Belkhadir et al., 2014; Lozano-Duran et al., 2013). That OPS functions as a positive regulator of BL signaling may explain why in *ops* mutants, we observe increased flg22-induced immune responses. While there is much left to understand about the function of OPS, it remains one of the few phloem-localized proteins identified with a role in immunity-related signaling events. Because it remains unknown whether sieve elements or companion cells can directly test and respond to PAMPs, future studies could use OPS as a target regulatory component to begin answering these questions.

4 | METHODS

4.1 | Plant material and growth conditions

Arabidopsis thaliana T-DNA insertion lines *ops-3* SALK_089722 and *ops-4* SALK_042563 were obtained from the Arabidopsis Biological Resource Center at The Ohio State University (https://abrc.osu.edu/). Surface-sterilized seeds were sown on .5× Murashige and Skoog

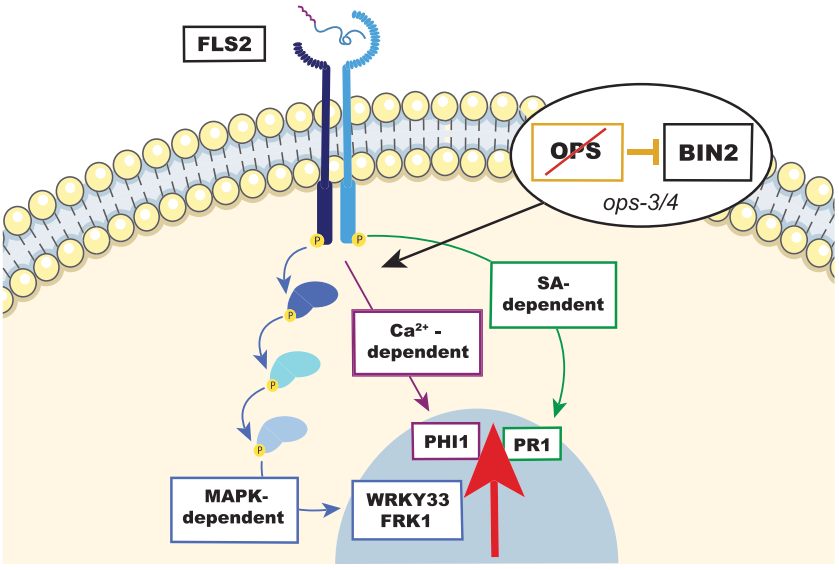

FIGURE 5 Proposed summary model of *ops* signaling phenotypes. Loss of the protein OPS in *ops-3* and *ops-4* results in constitutively increased expression of marker genes from three independent branches of the FLS2 signaling network (red arrow). Loss of OPS or elicitation of immune signaling in WT plants may de-repress BIN2 to promote SA-mediated immune signaling.

medium +1% (w/v) sucrose solidified with .6% (w/v) agar as described. After 2 days stratification at 4°C, seedlings were germinated and grown at 22°C with a 10-h light/14-h dark photoperiod. Unless otherwise noted, seedlings were grown under these conditions for 10 days before sample analysis.

4.2 | Peptides and hormones

The peptide flg22 (QRLSTGSRINSAKDDAAGLQIA) was purchased from Genscript and used for elicitation at the indicated concentrations and for the indicated times as described. 24-Epibrassinolide (BL) was purchased from MilliporeSigma and used for the indicated times and concentrations described.

4.3 | Root and hypocotyl measurements

Root length measurements were performed as described (Ekanayake et al., 2021). Ten-day old seedlings grown under the conditions described, were traced using Fiji Free-hand tool. For hypocotyl measurements, 4-day old seedlings were treated with the indicated concentration of BL in liquid media and placed at 22°C for an additional 7 days. Seedlings were removed and hypocotyls were traced using the Fiji Free-hand tool.

4.4 | Flg22 seedling growth inhibition

Seedling growth inhibition was measured as previously described (Anderson et al., 2011). Briefly, 4-day old seedlings were aseptically transferred from MS agar to wells of a 12-well microtiter plate (three seedlings per well) containing 1 ml of liquid MS medium with or without 1 μM flg22. After 14 days, seedling fresh weights were recorded.

4.5 | RNA isolation and RT-qPCR

RNA isolation, cDNA synthesis, and RT-qPCR were performed as previously described (Collins et al., 2020). Unless stated otherwise, for each sample, three to five seedlings were elicited with the indicated concentration of flg22 peptide and placed at 22°C for the time indicated. Tissue was flash frozen in liquid nitrogen at the indicated time points. Total RNA was isolated from tissue using Trizol reagent (Sigma-Aldrich) according to the manufacturer's protocol. RT-qPCR was performed on cDNA using a Rotor-Q Real-Time PCR Cycler from Qiagen using gene-specific primers and normalized to the *ACT2* housekeeping gene, verified in our system to be a suitable housekeeping gene for flg22 responses (Figure S2). Primers used are listed in Table S1.

4.6 | Molecular cloning

Full-length cDNAs of *OPS* and *BIN2* without stop codons were amplified from cDNA template synthesized from Col-0 gDNA and cloned into the Gateway-compatible donor vector pDONR221 (Invitrogen). Coding regions were moved by LR reaction into split luciferase vector pCAMBIA-NLuc, and pCAMBIA-CLuc (Chen et al., 2008). Primers used are listed in Table S1.

4.7 | Split luciferase assays

N. benthamiana split luciferase assays were performed as previously described (Spears et al., 2022). Briefly, GUS/OPS-nLUC and BIN2-cLUC constructs were transformed into *A. tumefaciens* strain C58C1 and co-inoculated at OD_{600} .2 into Benthi leaves by syringe infiltration. Twenty-four hours prior to sampling, leaves were sprayed with 1 μM SA, 1 μM Brz, or mock solution with .01% Tween-20. After 72-h incubation, leaf discs were taken by a sharp .5 cm diameter (#2) bore and floated abaxial side down on 100 μl of infiltration solution (50 mM MES pH 5.6, 10 mM $MgCl_2$, .5% DMSO) in a white 96-well plate. Infiltration solution was removed and replaced with 100 μl reaction solution (1× infiltration solution, 1 mM luciferin). Luminescence was quantified in 10 min increments over a 2-h period.

AUTHOR CONTRIBUTIONS

Carina A. Collins and Benjamin J. Spears supervised the experiments. Carina A. Collins, Kaitlyn N. Greenwood, Courtney L. King, Isabella Melena, Katherine A. Stegemann, Maura Donnelly, and Anna Childers designed experiments and analyzed the data. Carina A. Collins, Kaitlyn N. Greenwood, Courtney L. King, Isabella Melena, Katherine A. Stegemann, Raegan Mozal, and Benjamin J. Spears wrote the article. Benjamin J. Spears agrees to serve as the author responsible for contact and ensures communication.

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CONFLICT OF INTEREST STATEMENT

Carina A. Collins is currently an employee of Eli Lilly and Company.

PEER REVIEW

The peer review history for this article is available in the Supporting Information of this article.

DATA AVAILABILITY STATEMENT

Data and biological resources described in this manuscript are available upon request to the author.

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