PopP2 interacts with PAD4 in an acetyltransferase activity-dependent manner and affects plant immunity
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ABSTRACT
Plant pathogenic bacteria inject many of the effector proteins into host cell to manipulate host protein and promote pathogen development. Only a few effectors can be recognized by plant immune receptors called nucleotide-binding domain and leucine-rich repeat-containing proteins (NLRs). Enhanced disease susceptibility1 (EDS1) is an important regulator of plant basal and NLR receptor-triggered immunity. EDS1/PAD4 or EDS1/SAG101 heterodimers are recruited by Toll-interleukin1-receptor domain NLRs (TNLs) to transcriptionally mobilize resistance pathways. Type III effector PopP2 contributes to Ralstonia solanacearum virulence. PopP2 has an acetyltransferase activity and is recognized by Arabidopsis NLR pair RPS4/RRS1-1-R. On the other hand, PopP2 avirulence function is dependent on its enzymatic activity but target proteins in the host cell are still largely unknown. In this study, we found EDS1 and PAD4 are new host targets of PopP2 effector. Arabidopsis PAD4 lipase-like domain protein physically associates with enzymatic active PopP2 protein but not inactive PopP2C321A. PAD4-PopP2 interaction is disrupted by EDS1 immune regulator but not SAG101. We propose that acetyltransferase activity of PopP2 might confer specificity to PAD4 to manipulate plant immunity. As a counter strategy, EDS1 associates with PAD4 to form heterodimeric immune regulator complexes for activating basal resistance and interfering PopP2 physical interaction.

Keywords
lipase-like protein; PAD4; EDS1; ralstonia effector; PopP2; acetylation
not associations. yltransferase - protein Figure Figure 1b). Similarly, we performed co-IP with EDS1 and PopP2. EDS1 interacts with both PopP2 and PopP2C321A protein (Figure 2a) but PopP2/EDS1 interaction exhibited quite weak signals in coIP. EDS1 also shows no acetylation via acetyl-lysine specific antibody (Figure 2b). These data demonstrate that enzymatic active PopP2 specifically targets PAD4. EDS1 also associates with PopP2 independent of acetyltransferase enzymatic activity.

Next, we investigated whether EDS1 can disrupt PopP2/PAD4 associations. As shown in Figure 3a, PAD4 coIPs with PopP2 but not PopP2C321A protein. PAD4/PopP2 associations are disrupted by co-expression of EDS1 protein. To confirm this result, we used bimolecular fluorescence complementation (BiFC) assay. As expected, very weak BiFC signals between PopP2C321A-cCFP and nVenus-PAD4 were observed in the nucleus (Figure 3b). A strong BiFC signal between PopP2-cCFP and nVenus-PAD4 was observed in the nucleus but not co-expressed EDS1-Myc protein (Figure 3b), suggesting that formation of EDS1/PAD4 heterodimeric complexes might attenuate PopP2/PAD4 association.

We also tested the effect of PAD4 and SAG101 on PopP2 association using co-IP and BiFC assays in N. benthamiana leaves. In co-IP, PAD4 shows no association with SAG101 because of the absence of EDS1 (Figure 3a). PAD4/PopP2 interaction was not significantly altered by SAG101 co-expression both co-IP and BiFC (Figure 3a,b). These results indicated that EDS1 specifically inhibited PAD4/PopP2 association when transiently co-expressed in N. benthamiana.

To determine the effect of disruption of PopP2 interaction, we tested bacterial growth using P. syringae pv. tomato (Pto) DC3000-delivered AvrRps4N:PopP2149−488 system in Ws-2, rrs1-1, eds1-1, and 35S::EDS1 x 35S::PAD4 (rrs1-1 background) transgenic Arabidopsis plants. As expected,
Ws-2 Arabidopsis plants contained RRS1-R immune receptor exhibited strong effector triggered immunity but not rrs1-1 (Figure 3c). Mutant eds1-1 showed enhanced susceptibility to bacteria although RRS1-R still recognized PopP2. Overexpressing transgenic plants of PAD4/EDS1 in rrs1-1 showed strong basal defense (Figure 3c), suggesting that formation of EDS1/PAD4 dimeric complexes might have a role in inhibition of PopP2 interaction to enhanced plant immunity.

In conclusion, this study provides evidence that PopP2 effector targets both immune regulator EDS1 and PAD4 to suppress plant immunity. Basically, immune regulator EDS1/PAD4 heterodimeric complex can elevate plant basal defense signaling. Moreover, Phytophthora capsici effector PcAvh103 interacts with EDS1 to disrupt EDS1/PAD4 and inhibit plant defense signaling, suggesting that EDS1 and PAD4 might be general effector targets.21,23 On the other hand, EDS1 disrupts PAD4/PopP2 interaction but not SAG101. This result consistent with disruption of EDS1/AvrRps4 interaction by PAD4.21 Notably, we found PAD4 only associates with enzymatic active PopP2. It is possible that acetylation activity of PopP2 might modified PAD4 protein to enhance protein interaction affinity. We failed detection of acetylation in EDS1 and PAD4 using acetyl-lysine specific antibody and it might be required more specific method applications. Further studies are still needed to explore the PAD4 acetylation by PopP2 to understand host target modification. We therefore propose that PopP2 acetyltransferase activity may be required to specific interaction with host target proteins to suppress plant immunity. As a counter strategy, plant EDS1 makes heterodimeric immune regulator
complexes with PAD4 for activating basal resistance and interfering PopP2 physical interaction.

**Disclosure statement**

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