Host target modification as a strategy to counter pathogen hijacking of the jasmonate hormone receptor

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In the past decade, characterization of the host targets of pathogen virulence factors took a center stage in the study of pathogenesis and disease susceptibility in plants and humans. However, the impressive knowledge of host targets has not been broadly exploited to inhibit pathogen infection. Here, we show that host target modification could be a promising new approach to "protect" the disease-vulnerable components of plants. In particular, recent studies have identified the plant hormone jasmonate (JA) receptor as one of the common targets of virulence factors from highly evolved biotrophic/hemibiotrophic pathogens. Strains of the bacterial pathogen *Pseudomonas syringae*, for example, produce proteinaceous effectors, as well as a JA-mimicking toxin, coronatine (COR), to activate JA signaling as a mechanism to promote disease susceptibility. Guided by the crystal structure of the JA receptor and evolutionary clues, we succeeded in modifying the JA receptor to allow for sufficient endogenous JA signaling but greatly reduced sensitivity to COR. Transgenic *Arabidopsis* expressing this modified receptor not only are fertile and maintain a high level of insect defense, but also gain the ability to resist COR-producing pathogens *Pseudomonas syringae* pv. *tomato* and *P. syringae* pv. *maculicola*. Our results provide a proof-of-concept demonstration that host target modification can be a promising new approach to prevent the virulence action of highly evolved pathogens.

plant hormone | plant immunity | bacterial virulence | coronatine | jasmonate

Studies during the past two decades have revealed that plants possess a sophisticated, multilayered immune signaling network that is regulated by several stress hormones (1). Most prominently, jasmonate (JA) plays a central role in regulating plant defense against a variety of chewing insects and necrotrophic pathogens, whereas salicylic acid (SA) is critical for plant defense against biotrophic or hemibiotrophic pathogens (1–3). During host–pathogen coevolution, however, many successful plant pathogens developed mechanisms to attack or hijack components of the plant immune signaling network as part of their pathogenesis strategies (4–6). As a result, the plant immune system, although powerful, is often fallible in the face of highly evolved pathogens.

The JA signaling cascade has been a subject of intense study, and many important players in this hormone signal transduction system have been identified. Higher plants synthesize different forms of JA, including the most bioactive form jasmonoyl-L-isoleucine (JA-Ile) (7–11). Perception of JA-Ile occurs through a coreceptor, composed of CORONATINE INSENSITIVE1 (COI1), the F-box subunit of a Skp/Cullin/F-box–type ubiquitin ligase complex, and JASMONATE ZIM DOMAIN (JAZ) proteins, which are transcriptional repressors (12–16). In the absence of hormone signal, JAZ repressors bind to and repress the transcription factors (e.g., MYC2) both directly and through the recruitment of the NOVEL INTERACTOR OF JAZ (NINJA) adapter and TOPLESS (TPL) corepressor proteins (10, 14, 17–20). In response to developmental or environmental cues, JA-Ile concentration rises, which promotes the interaction between COI1 and JAZs and subsequent degradation of JAZ repressors through the 26S proteasome (10, 21). Activation of MYC and other JAZ-interacting transcription factors leads to transcriptional reprograming and results in a plethora of JA-mediated physiological responses (22–24).

Although activation of the JA signal transduction pathway is essential for plant resistance to chewing insects and necrotrophic pathogens, it also leads to inhibition of SA signaling through hardwired molecular cross-talk between the two pathways (1, 22, 25–27). Because the SA signaling pathway is critical for plant defense against biotrophic and hemibiotrophic pathogens, activation of JA signaling makes plants vulnerable to biotrophic and hemibiotrophic pathogens. In fact, some strains of the hemibiotrophic bacterial pathogen *Pseudomonas syringae* have evolved an ability to produce a potent JA-mimicking phytotoxin, coronatine (COR), to activate JA signaling as an effective means of inhibiting SA defense and promote plant susceptibility (4, 26, 28, 29). Furthermore, COR-like compounds are produced by pathogens of other taxa (30, 31) and proteinaceous effectors from both bacterial and fungal pathogens have been shown to target the COI1–JAZ coreceptor (32–34). These recent findings suggest that the COI1–JAZ coreceptor is a common target of manipulation by diverse plant pathogens and represents a prominent vulnerable point of the plant immune network.

COR structurally mimics JA-Ile and directly binds to the COI1–JAZ coreceptor to activate the JA signaling pathway (7, 13, 15). The molecular mimicry of COR is remarkable, as illustrated by its high binding affinity (equal to or higher than JA-Ile) to the COI1–JAZ

Significance

Pathogen infections can cause significant crop losses worldwide and major disturbances in natural ecosystems. Understanding the molecular basis of plant disease susceptibility is important for the development of new strategies to prevent disease outbreaks. Recent studies have identified the plant jasmonate (JA) hormone receptor as one of the common targets of pathogen virulence factors. In this study, we modified the JA receptor and showed that transgenic *Arabidopsis* plants with the modified JA receptor gained resistance to bacterial pathogens that secrete a potent JA-mimicking toxin to promote infection. Our results suggest that host target modification may be developed as a new strategy to protect the disease-vulnerable components of the susceptible plant against highly evolved pathogens.

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coreceptor, and by the fact that all previously reported COI1 mutations that affect the action of JA-Ile also affect the action of COR (7, 10, 13, 15, 35, 36). Interestingly, coronatine-*O*-methyloxime (COR-MO), a potent and highly specific JA-Ile antagonist, was found to inhibit both JA signaling and COR action in *Arabidopsis* and *Nicotiana benthamiana* (37). To date, no COI1 mutations have been shown to differentially affect the action of JA-Ile vs. COR, illustrating the difficulty in uncoupling the molecular actions of these ligands. Nevertheless, a systematic mutagenesis of the COI1–JAZ coreceptor has not been reported.

Guided by the crystal structure of the COI1–JAZ coreceptor and evolutionary clues, we report here the successful generation of a modified JA receptor with a single amino acid substitution in the JA-Ile-binding pocket of the COI1 protein, which allows for sufficient signal transduction of endogenous JA hormone, fertility, and plant defense against insects, but confers resistance against COR-producing pathogens, *P. syringae* pv. *tomato* (*Pst*) DC3000 and *P. syringae* pv. *maculicola* (*Psm*) ES4326. Our results provide a proof-of-concept demonstration that host target modification could be a promising new approach to prevent hijacking of host targets by highly evolved pathogens.

Results

A Large-Scale, Targeted Alanine Substitution Mutagenesis of the COI1 Protein. We began our study by conducting an expanded mutagenesis of the COI1 protein to identify amino acid residues that might differentially affect the actions of JA-Ile vs. COR. We selected a total of 42 amino acids in or near the COI1 ligand binding pocket for alanine substitution mutagenesis (a detailed description of the rationale and results can be found in *Supplemental Description of Results of Alanine Scanning Mutagenesis of the COI1 Protein*). With yeast two-hybrid (Y2H) assay, we were able to identify 21 alanine substitutions that disrupted COR-dependent COI1–JAZ9 interaction, including eight COI1 residues that make direct contact with JA-Ile (36) (Fig. S1 and Table S1). However, none disrupted only COR-dependent interaction but still maintained JA-Ile–dependent COI1–JAZ9 interaction (Fig. S2 *A* and *B*). Our results therefore strengthen the notion that COR is a remarkable mimic of JA-Ile and that most, if not all, COI1 residues that are important for the action of JA-Ile are also important for COR action.

Structure-Guided Modeling of JA-Ile/COR Binding Sites in COI1. Our initial mutagenesis was based on alanine substitution, which resulted in a reduction of the side-chain size for all of the amino acid residues targeted for mutagenesis, except for G357A. Next, we considered increasing the side-chain sizes of residues that are in contact with JA-Ile/COR. We noted that although COR and JA-Ile are highly similar in structure, the flexibilities of COR and JA-Ile in the binding pocket are different. For example, the cyclohexene ring and the ethyl-cyclopropane group of COR appear more rigid than the equivalent parts (the pentenyl side-chain and the isoleucine side-chain, respectively) of JA-Ile (36). We hypothesized that the higher rigidity of the cyclohexene ring and the ethyl-cyclopropane group of COR may be more prone than the equivalent parts of JA-Ile to physical hindrance from an increased size of the amino acid side-chain with which COR/JA-Ile are in direct contact.

Based on the above hypothesis, residues A86 and A384 attracted our attention for two reasons. First, in silico analysis of the putative JA-Ile binding pockets in diverse plant species for which the COI1 protein sequences are available revealed that, although most residues in the JA binding site are highly conserved across taxa, residues at positions 86 and 384 exhibit a higher degree of polymorphism (Fig. S3*A*). In the moss species *Physcomitrella patens*, for example, isoleucine or valine occupy the corresponding position of A384 (Fig. S3*A*). Positions of A86 and A384 in *Selaginella moellendorffii* are replaced by isoleucine/valine and serine, respectively (Fig. S3*A*). Previous studies have shown that, although core JA signaling genes are found in *P. patens* (38), neither JA nor JA-Ile could be detected in *P. patens* (39). On the other hand, (9*S*,13*S*)-12-oxophytodienoic acid [*cis*-(+)-OPDA], the precursor of JA biosynthesis, is synthesized in *P. patens*, suggesting that *P. patens* may produce an alternative, OPDA-related ligand (39). We speculated that, during plant evolution, the polymorphism at positions 86 and 384 in the putative COI1 binding pocket may provide a basis for accommodating related ligands of distinct structural features. If so, mutations at these amino acid positions may have a higher chance of producing differential effects on different ligands compared with more highly conserved residues, which are expected to affect different ligands similarly.

Second, we noted that, in the JA-Ile/COR-binding pocket, A86 and A384 make direct contacts with the ligand (Fig. S3*A*) and are situated close to the cyclohexene ring and the ethyl-cyclopropane group of COR or the equivalent parts of JA-Ile, the pentenyl side-chain, and the isoleucine side-chain, respectively (Fig. 1 *A* and *B* and Fig. S3 *B* and *C*). The Cβ atom of A86 is 3.6 Å from the nearest C-atom in the pentenyl side-chain of JA-Ile and 3.7 Å from the ethyl group attached to the cyclohexene ring of COR in their respective crystal structures. The Cβ atom of A384 is 4.0 Å from the nearest C-atom of the isoleucine side chain of JA-Ile and 3.6 Å from the ethyl-cyclopropane group of COR. In silico mutagenesis followed by energy minimization revealed that the A384V substitution, in particular, would create steric clash with the isoleucine side-chain of JA-Ile or the ethyl group attached to the cyclopropane moiety of COR (Fig. 1 *C* and *D*). However, the flexibility of the isoleucine side-chain of JA-Ile would likely allow for its readjustment to fit the mutated

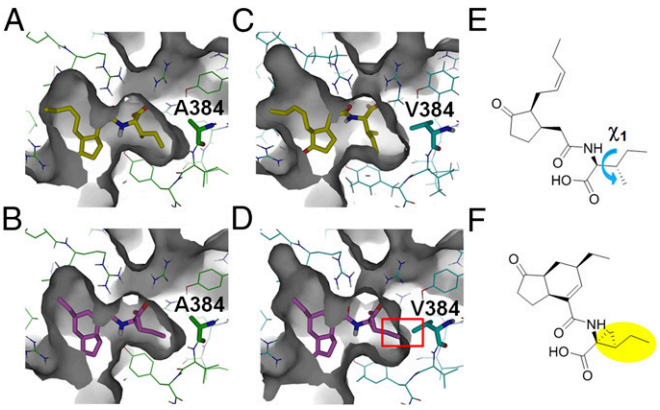

Fig. 1. Computer modeling of JA-Ile or COR in the ligand-binding site of COI1 or COI1^{A384V}. (*A* and *B*) Binding pose of JA-Ile (*A*) and COR (*B*) in the ligand-binding site of COI1 in the crystal structures of the COI1–JAZ1 complex (PDB ID codes 3OGL and 3OGK, respectively). Amino acid contacts in the ligand pocket were described by Sheard et al. (36). (*C* and *D*) Computer modeling of the A384V substitution showing expected steric clash with the isoleucine side-chain of JA-Ile or the ethyl group attached to the cyclopropane moiety of COR. However, the isoleucine side-chain of JA-Ile can be adjusted in the mutant ligand binding site by rotation of the side-chain dihedral angle, χ1 of isoleucine (*C*). In contrast, the steric clash (highlighted in red box) impairs COR binding in the ligand-binding site because the rotatable bond at the equivalent position is absent in COR (*D*). The ligand-binding site in COI1 is shown in gray-colored surface representation. Ligands and A384/V384 residues are shown in stick representation, whereas all other atoms in the protein are shown in line representation. C-atoms in the wild-type and mutant COI1 proteins are shown in green and cyan, respectively; those in JA-Ile and COR are shown in yellow and magenta, respectively. In protein and ligand molecules N-, O-, and H-atoms are colored in blue, red, and gray, respectively, and, for clarity, nonpolar H-atoms are not shown. (*E*) Molecular structure of JA-Ile with χ1 torsion angle shown in cyan arrow. (*F*) Molecular structure of COR in which the cyclopropane moiety restricts the rotational freedom of the terminal ethyl group. The cyclopropane moiety along with the ethyl substitution is highlighted in yellow.

ligand-binding pocket, whereas the rigidity of the ethyl-cyclopropane group of COR would not (Fig. 1 *E* and *F*). Taken together, our in silico and structural modeling analyses suggest the possibility that mutating alanine to valine at position 384 may result in a ligand-binding pocket that is more unfavorable to the chemical structure of COR than that of JA-Ile.

Effects of Amino Acid Substitutions at Positions 86 and 384 on JA-Ile/COR–Dependent Formation of the COI1-JAZ9 Coreceptor. To test the hypothesis that mutating A384 or A86 may create a ligand-binding pocket that is more unfavorable to COR than to that of JA-Ile, we first substituted these two alanine residues with the corresponding residues found in lower plant species *P. patens* and *S. moellendorffii* (Fig. S3*A*). Specifically, the following COI1 mutants were generated: COI1^{A86I}, COI1^{A86V}, COI1^{A384I}, COI1^{A384S}, and COI1^{A384V}. Quantitative liquid Y2H assays revealed that both COI1^{A86I} and COI1^{A86V} abolished JA-Ile–dependent COI1–JAZ9 interaction, and reduced COR-dependent COI1–JAZ9 interaction (Fig. 2*A*). This indicated that A86 is critical for the action of both JA-Ile and COR, albeit more critical for JA-Ile than COR.

Substitutions at position 384 exhibited more diverse effects than those at position 86 on JA-Ile/COR–dependent COI1–JAZ9 interaction (Fig. 2*A*). COI1^{A384I} disrupted both JA-Ile– and COR-dependent interaction, whereas COI1^{A384S} only reduced JA-Ile–dependent interaction. Most interestingly, COI1^{A384V} greatly reduced COR-dependent interaction, but had less effect on JA-Ile–dependent COI1–JAZ9 interaction (Fig. 2*A*). We also found that 10 μM JA-Ile, which contains a mixture of active and inactive isomers of JA-Ile, was equivalent to 0.1 μM pure COR in promoting the COI1–JAZ9 interaction in yeast (Fig. 2*B*).

We made seven additional substitutions at A384 to determine whether these substitutions would have an effect similar to that of COI1^{A384V}. Of these seven substitutions (representing different types of side-chains), A384C reduced, and A384D, A384G, A384L, A384N, A384P, and A384T completely disrupted JA-Ile– and COR-dependent interaction (Fig. S2*C*). In all, no additional substitutions affected COR-dependent COI1–JAZ9 interaction more than JA-Ile–dependent COI1–JAZ9 interaction. Therefore, through extensive mutagenesis efforts we succeeded in identifying a specific amino acid substitution, A384V, in the JA-Ile binding pocket that preferably affects COR-dependent COI1–JAZ9 interaction, compared with JA-Ile–dependent COI1–JAZ9 interaction in yeast.

Transgenic *Arabidopsis* Plants Expressing COI1^{A384V} Are Fertile but Exhibit Differential Sensitivities to Methyl Jasmonate and COR in Vivo. To determine the physiological relevance of the results from Y2H assays, we produced transgenic *Arabidopsis* plants (in *coi1-30*–null mutant background) that express COI1^{A384V} from the *COI1* native promoter (*pCOI1:COI1^{A384V}-4xc-Myc*; COI1^{A384V} hereafter). As controls, we also generated transgenic lines that express wild-type COI1 in the *coi1-30* background (*pCOI1:COI1^{WT}-4xc-Myc*; COI1^{WT} hereafter). First, we determined whether COI1^{A384V} complements the male sterile phenotype in *coi1-30*. JA is essential for male fertility and *coi1* mutants are male sterile (40). Consistent with Y2H results showing that COI1^{A384V} maintained substantial JA-Ile interaction, 83% of COI1^{A384V} lines (10 of 12 lines analyzed) were fertile (Fig. 3*A*). Four fertile COI1^{A384V} lines were randomly chosen for protein expression analysis and all were found to produce the c-Myc–tagged COI1^{A384V} protein (Fig. 3*B*). No fertility penalty was detected in COI1^{A384V} plants, as judged by the number of developed siliques and the number of seeds per silique, which are similar to wild-type plants (Table S2).

Next, we performed COI1–JAZ9 pull-down experiments to compare the responsiveness of plant-expressed COI1^{WT} and COI1^{A384V} proteins to serial concentrations of JA-Ile and COR using *Escherichia coli*-expressed JAZ9 protein, following the procedure reported previously (13). These experiments confirmed

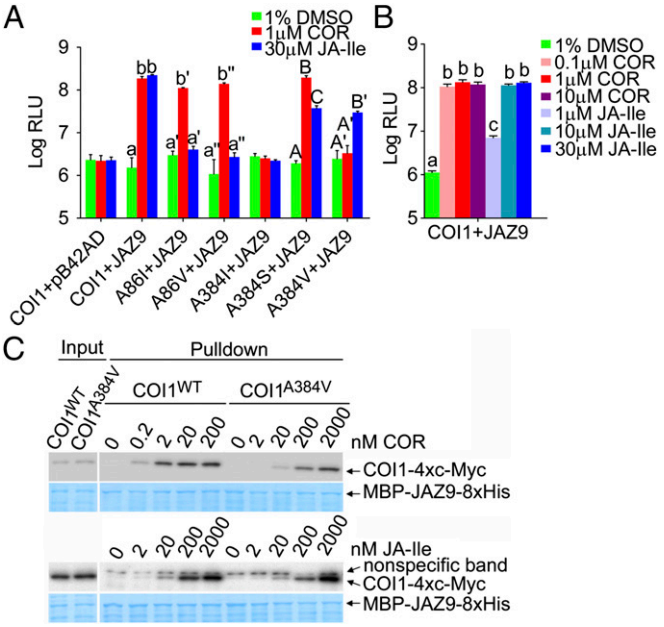

Fig. 2. Y2H and pull-down assays for physical interactions between COI1 and JAZ9 proteins. (*A*) Liquid Y2H results of JAZ9 and mutant COI1 proteins containing amino acid substitutions at position 86 or 384 in the presence of 1 μM COR or 30 μM JA-Ile. (*B*) Liquid Y2H results of COI1–JAZ9 interaction in the presence of different concentrations of JA-Ile and COR. Relative light units (RLU) indicated the degree of interaction between COI1 mutants and JAZ9. One-percent DMSO treatment was used as mock treatment. Different letters of the same type above columns indicated significant differences ($P < 0.05$) between different treatments (i.e., DMSO, JA-Ile, or COR) for the same set of interacting proteins ($n = 3$, error bars, SEM). For those interacting proteins that do not have letter labels above columns, no significant differences were detected between treatments. Two-way ANOVA with Bonferroni posttest was used for *A*. One-way ANOVA with Tukey's multiple comparison test was used for *B*. (*C*) Results of coreceptor pull-down assays. Pull-down assays were performed with protein extracts from *pCOI1:COI1$^{WT/A384V}$-4xc-Myc* plants and recombinant *E. coli*-expressed MBP-JAZ9-8xHis in the presence of COR or JA-Ile at indicated concentrations. Proteins bound to MBP-JAZ9-8xHis were analyzed by immunoblotting. Anti–c-Myc antibody was used for detection of COI1$^{WT/A384V}$-4xc-Myc protein. The Coomassie blue-stained gel shows the amounts of MBP-JAZ9-8xHis pulled down by the Ni affinity resin.

that a much higher (~100-fold) concentration of COR was required for robust formation of the COI1^{A384V}–JAZ9 coreceptor than for the COI1^{WT}–JAZ9 coreceptor, whereas similar concentrations of JA-Ile were needed to promote the formation of the COI1^{A384V}–JAZ9 and COI1^{WT}–JAZ9 coreceptors (Fig. 2*C*).

Finally, we conducted further analyses with two representative COI1^{A384V} lines, L1 and L2, to determine their responses to JA- or COR-induced root growth inhibition. Dose–response experiments showed that the effect of 10 μM methyl jasmonate (MeJA), which is converted to the active form JA-Ile *in planta*, was equivalent to that of 0.2 μM COR in wild-type Col-0 plants (Fig. 3*C* and Fig. S4). Unlike wild-type Col-0 plants, the root growth inhibition of COI1^{A384V} plants was significantly less sensitive to 0.2 μM COR than to 10 μM MeJA (Fig. 3*C* and Fig. S4). The potency of 0.2 μM COR in inhibiting root growth in COI1^{A384V} plants was comparable to 0.1 μM MeJA, indicating ~100-fold less effectiveness of 0.2 μM COR in COI1^{A384V} than in Col-0 and COI1^{WT} (Fig. S4). These results were consistent with the differential effects of the A384V substitution on JA-Ile– vs. COR-dependent formation of the COI1–JAZ9 coreceptor observed in both Y2H and COI1–JAZ9 coreceptor pull-down assays, and confirmed that COI1^{A384V} transgenic plants are differentially sensitive to MeJA vs. COR in vivo.

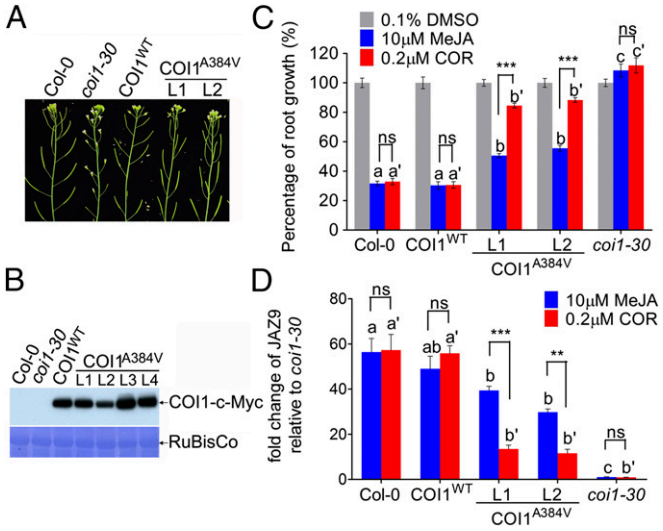

Fig. 3. Phenotypes of transgenic COI1^{WT} and COI1^{A384V} plants. (*A*) A picture showing restoration of male fertility in transgenic *coi1/COI1*^{WT} and *coi1/COI1*^{A384V} plants. (*B*) COI1 protein levels in *pCOI1:COI1*^{WT}*-4xc-Myc* and *pCOI1:COI1*^{A384V}*-4xc-Myc* transgenic plants. Coomassie blue-stained ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) protein was used as loading control. (*C*) Quantification of root growth assay with 10 μM MeJA or 0.2 μM COR application. Relative root length was compared with mock treatment (0.1% DMSO). Different letters of the same type above columns indicated significant differences ($P < 0.05$) between different plant genotypes with the same treatment (MeJA or COR) ($n = 15$, error bars, SEM, except for *coi1-30*, $n = 7$), as determined by two-way ANOVA with Bonferroni posttest. ***$P < 0.001$ indicated significant differences between two ligand treatments of the same plant genotype (ns: not significant). (*D*) Fold-changes of *JAZ9* gene expression in Col-0, transgenic COI1^{WT}, COI1^{A384V}, and *coi1-30* plants after 10 μM MeJA or 0.2 μM COR induction, relative to those in *coi1-30* plants with 10 μM MeJA. Internal control: the PROTEIN PHOSPHATASE 2A SUBUNIT A3 gene (*PP2AA3, AT1G13320*). Different letters of the same type above columns indicated significant differences ($P < 0.05$) of gene expression between different plant genotypes with the same ligand treatment (MeJA or COR) ($n = 4$, error bars, SEM), by two-way ANOVA with Bonferroni posttest. **$P < 0.01$ and ***$P < 0.001$ indicate significant differences between two different ligand treatments of the same plant genotype (ns, not significant).

Transgenic *Arabidopsis* Plants Expressing COI1^{A384V} Exhibit Differential Expression of JA Response Marker Genes in Response to MeJA vs. COR. We next examined JA response gene expression in COI1^{A384V} transgenic plants. For this purpose, the expression of the JA-responsive marker gene *JAZ9* was measured by quantitative PCR (qPCR). As expected, *JAZ9* gene expression was induced in Col-0 and COI1^{WT} plants after MeJA or COR application (Fig. 3*D*). In COI1^{A384V} lines, however, *JAZ9* gene expression in response to COR treatment was significantly reduced compared with Col-0 or COI1^{WT} plants, whereas *JAZ9* expressions in response to MeJA treatment was less affected in this same comparison (Fig. 3*D*). For example, in COI1^{A384V} L1, MeJA treatment induced the expression of *JAZ9* by 38-fold compared with that in *coi1-30* plants. However, induction of *JAZ9* gene expression in COI1^{A384V} L1 was only eightfold higher than that in *coi1-30* plants after COR treatment. These results are consistent with the conclusion that the A384V substitution greatly affects the action of COR, while maintaining JA signaling required for substantial JA response gene expression. We also examined the expression of SA-responsive genes *PATHOGENESIS-RELATED GENE 1* (*PR1*) and *SALICYLIC ACID INDUCTION DEFICIENT 2* (*SID2*) in COI1^{A384V} plants and found that *PR1* and *SID2* gene expression were similarly low in Col-0, COI1^{WT} and COI1^{A384V} plants (Fig. S5), indicating that the SA signaling pathway remained quiescent in COI1^{A384V} plants, as in Col-0 and COI1^{WT} plants.

COI1^{A384V} Transgenic Plants Gained Resistance to *Pst* DC3000 and *Psm* ES4326, While Maintaining High-Level Defense Against Chewing Insects. Our analyses so far suggested that we might have succeeded in engineering a modified JA receptor that substantially uncouples endogenous hormone signaling from pathogen hijacking via COR. If so, we expected that the COI1^{A384V} transgenic plants would gain resistance to COR-producing bacterial pathogens, while retaining substantial defense against chewing insects. To test this possibility, we conducted bioassays using *Pst* DC3000 and *Psm* ES4326, two well-known COR-producing hemibiotrophic pathogens that infect *Arabidopsis* (41, 42), and *Spodoptera exigua*, a generalist chewing insect that is susceptible to COI1-dependent defenses in *Arabidopsis* (43). As expected, Col-0 and COI1^{WT} plants were highly susceptible to *Pst* DC3000 (Fig. 4 *A* and *B*). COI1^{A384V} plants, however, exhibited significantly increased resistance to *Pst* DC3000, as evidenced by greatly reduced bacterial growth and disease symptoms (Fig. 4 *A* and *B*). Quantitatively, *Pst DC3000* populations in COI1^{A384V} lines were 254- to 42-fold lower than those in Col-0 plants and 189- to 31-fold lower than those in COI1^{WT} transgenic plants (Fig. 4*B*). Similarly, COI1^{A384V} plants exhibited significantly increased resistance to *Psm* ES4326 compared with wild-type Col-0 or COI1^{WT} plants (Fig. 4 *C* and *D*). Control experiments showed that *coi1-30* plants were highly resistant to both pathogens in these assays (Fig. 4 *B* and *D*).

Next, we conducted disease assays using *Pst* DC3118 and DB29, which are mutants of *Pst* DC3000 defective in COR production (44, 45). Similar levels of bacterial growth were observed in Col-0, COI1^{WT}, and COI1^{A384V} plants, suggesting that the gained resistance in COI1^{A384V} plants to *Pst DC3000* was largely COR-dependent (Fig. S6).

Finally, we performed insect feeding assays using *S. exigua* neonate larvae. As expected, *S. exigua* grew much more slowly on Col-0 plants than on *coi1-30* mutant plants (Fig. 5), consistent with previous reports (46, 47). The average weight of larvae feeding on *coi1-30* plants was sixfold higher than larvae reared on COI1^{WT} plants and fivefold higher than those grown on COI1^{A384V} plants

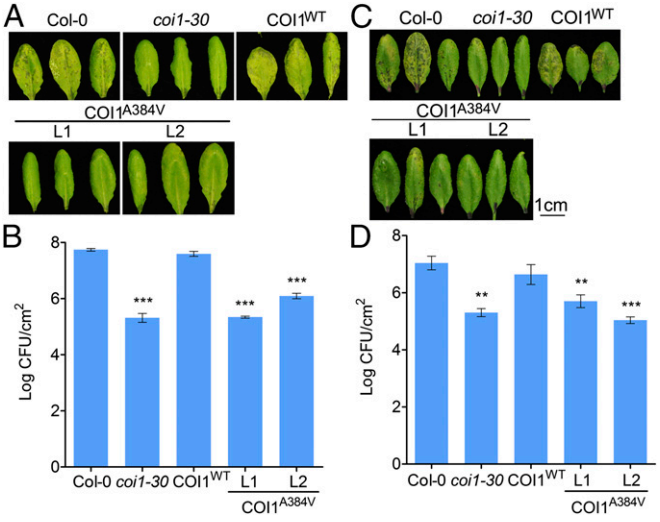

Fig. 4. Results of bacterial infection assays with *Pst* DC3000, *Psm* ES4326, and *Pst* DC3118 and *Pst* DB29 (two COR-deficient mutants of *Pst* DC3000). (*A* and *B*) Disease symptoms (*A*) and bacterial populations (*B*) 3 d after dip-inoculation with 1×10^8 cfu/mL *Pst* DC3000. ***$P < 0.001$ indicates significant difference between mutant lines and wild-type Col-0 by one-way ANOVA with Dunnett test ($n = 4$, error bars, SEM). (*C* and *D*) Disease symptoms (*C*) and bacterial populations (*D*) 3 d after dip-inoculation with 1×10^8 cfu/mL *Psm* ES4326. **$P <$ 0.01 and ***$P < 0.001$ indicate significant difference between mutant lines and Col-0 wild-type by One way ANOVA with Dunnett test ($n = 4$, error bars, SEM).

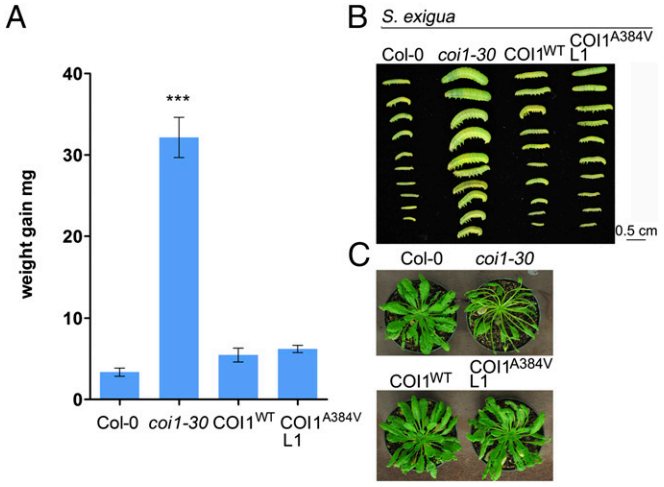

Fig. 5. Results of insect feeding assays on COI1^{WT} and COI1^{A384V}. (*A*) Average weights of 12-d-old *S. exigua* larvae fed on Col-0, *coi1-30*, COI1^{WT} or COI1^{A384V} plants. ***$P < 0.001$ indicates a significant difference in comparisons to Col-0 using One-way ANOVA with Dunnett test ($n = 10$, error bars, SEM). No significant difference was detected in the weight of larvae reared on Col-0, COI1^{WT} and COI1^{A384V} L1 plants. (*B*) A picture of representative larvae 12 d after feeding. (*C*) Pictures of *Arabidopsis* plants after insect challenge.

(Fig. 5*A*). Thus, COI1^{A384V} plants maintained an almost wild-type level of defense against *S. exigua*.

Discussion

In the past decade, numerous host targets of bacterial, fungal, oomycete, and nematode virulence factors have been identified, representing major advances in our understanding of plant–microbe interactions. However, this fundamental knowledge has largely not yet been exploited to inhibit disease development. COR was one of the first bacterial virulence factors of which the host target was clearly identified (7, 13, 15) and its molecular action on the host target (the JA receptor) was elucidated at the crystal structural level (36). In this study, guided by the crystal structure of the JA receptor, we identified a single amino acid substitution (A384V) in the JA-binding pocket of the COI1 protein that greatly reduces *Arabidopsis* sensitivity to COR and confers substantial resistance of *Arabidopsis* to COR-producing *Pst* DC3000 and *Psm* ES4326. Our study provides a proof-of-concept demonstration for the feasibility of making a simple modification to a host target as a promising new approach to counter pathogen virulence, thus expanding the range of pathogens that a plant can defend against.

The COR toxin is produced not only by *P. syringae* pv. *tomato* and *P. syringae* pv. *maculicola*, but also *P. syringae* pvs. *atropurpurea*, *glycinea*, *morsprunorum*, and *porri*, which collectively infect a wide range of plants, including ryegrass, soybean, crucifers, cherry, plum, leeks, and tomato (29, 30). Furthermore, production of COR/COR-like compounds has been reported beyond the *P. syringae* species, including *Pseudomonas cannabina* pv. *alisalensis*, *Streptomyces scabies*, and *Xanthomonas campestris* pv. *phormiicola* (29–31). Finally, gene clusters for COR biosynthesis have been found in *Pseudomonas savastanoi* pv. *glycinea* and *Pectobacterium atrosepticum* (syn. *Erwinia carotovora* subsp. *atroseptica*) (48, 49). Importantly, transposon insertion mutants of coronafacic acid-like polyketide phytotoxin gene clusters in *P. atrosepticum* were shown to have reduced pathogen virulence (49). However, it remains to be determined whether these COR-like toxins target the COI1–JAZ coreceptor for their virulence activity. If so, modification of COI1 at A384 or other residues in the JA binding pocket could represent a broadly applicable approach to improve plant resistance to diverse pathogens. In addition, because of the simplicity of constructing amino acid substitutions, generation of COI1^{A384V} plants seems particularly amenable through CRISPR-mediated genome editing.

Although our study is focused on uncoupling JA signaling from COR toxin action, recent studies have shown that the JA receptor is also a host target of proteinaceous effectors delivered into the host cell by bacterial pathogens and fungal symbionts (32–34). For example, *P. syringae* pv. *syringae*, which is not known to produce COR or COR-like toxins, delivers the effector protein HopZ1a to acetylate and induce JAZ protein degradation, thereby activating JA signaling (33). *P. syringae* pv. *tabaci*, which also does not produce COR or COR-like toxins, delivers the effector protein HopX1 into the host cell to interact with and degrade JAZ via its cysteine protease activity (34). The *Laccaria bicolor* fungal effector protein MiSSP7 (mycorrhiza-induced small secreted protein 7) interacts with the host *Populus* PtJAZ6 protein and inhibits JA-induced degradation of PtJAZ6 to promote symbiosis (32). Hence, the COI1–JAZ coreceptor has emerged as a common host target for diverse effector proteins of pathogens and symbionts. Further study to elucidate how these effector proteins modify JAZ proteins could guide future efforts to develop JAZ-based modifications to counter pathogen virulence and enhance beneficial symbiosis. For example, innovative methods may be developed to disrupt the interaction between JAZs and HopZ1a/HopX1 or to block proteolytic degradation of JAZ proteins by HopZ1a/HopX1 as means of protecting plants from pathogen hijacking of the JA receptor.

Together with a recent demonstration of ABA receptor engineering against abiotic stress (50), our study illustrates that fundamental insights into the plant hormone receptors could indeed lead to innovative methods to manipulate plant hormone receptor signaling with the ultimate goal of improving plant growth and tolerance to abiotic and biotic stresses.

Materials and Methods

All experiments reported in this work were performed three or more times with similar results. For computer modeling, coordinates for JA-Ile or COR were obtained from the crystal structures of COI1–JA-Ile/COR–JAZ degron peptide complex (PDB ID codes 3OGL and 3OGK, respectively). In Y2H and *in planta* assays, we standardize the relative potencies of different ligands used (COR, MeJA, and JA-Ile) before a new set of experiments. Because of the limited amounts of JA-Ile available for this study, we used other forms of JA if the use of JA-Ile was not absolutely needed. For example, MeJA can be converted to JA-Ile *in planta* and is commonly used in the study of JA signaling (11). Therefore, we used MeJA, instead of JA-Ile, for *in planta* assays. However, for Y2H experiments we used JA-Ile, because JA or MeJA are not active in yeast (12, 15). Detailed procedures for gene cloning, site-directed mutagenesis, protein and RNA analyses, production of transgenic *Arabidopsis*, and assays for root inhibition, protein–protein interaction, disease susceptibility, and insect resistance can be found in *SI Materials and Methods*. See Table S3 for gene identifiers of the *COI1* genes in seven plant species.

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