doi:10.1093/plphys/kiab334 PLANT PHYSIOLOGY 2021: 187: 1679–1689

Plant Physiology®

Research Article

The co-chaperone HOP3 participates in jasmonic acid signaling by regulating CORONATINE-INSENSITIVE 1 activity

Alfonso Muñoz,[1,2] M. Estrella Santamaria ,[1] Nuria Fernández-Bautista,[1] Silvina Mangano,[1,3] René Toribio,[1] Manuel Martínez,[1,4] Marta Berrocal-Lobo ,[1,5] Isabel Diaz[1,4] and M. Mar Castellano [1,*,†]

1 Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM) – Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Campus Montegancedo UPM, 28223-Pozuelo de Alarcón (Madrid), Spain
2 Departamento de Botánica, Ecología y Fisiología Vegetal, Campus de Rabanales, Edificio Severo Ochoa, Universidad de Córdoba, Córdoba 14071, Spain
3 Fundación Instituto Leloir and Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBA, CONICET), Av. Patricias Argentinas 435, Buenos Aires, C1405BWE, Argentina
4 Departamento de Biotecnología-Biología Vegetal, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, UPM 28040, Madrid, Spain
5 Departamento de Sistemas y Recursos Naturales, E.T.S.I. Montes, Forestal y del Medio Natural, Ciudad Universitaria s/n, 28040, Madrid, Spain

*Author for communication: castellano.mar@inia.es
These authors have contributed equally (N.F.-B., S.M., R.T.).
†Senior author.

A.M. designed and performed the co-immunoprecipitation experiments, carried out the different crosses with the *hop3-1* mutant and performed COI1 activity. M.E.S. co-designed and performed the analyses of susceptibility to *T. urticae*. M.M. and I.D participated in the design of these experiments and supervised them. M.B.-L. designed and performed the analyses of susceptibility to *Botrytis cinerea* with the collaboration of N.F.B. S.M. performed the expression analysis in response to MeJA and along with N.F.B. carried out the root growth assays. R.T. carried out expression analyses and prepared the figures. M.M.C. participated in the design, supervised the experiments, and wrote the article with contributions of all the authors.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plphys/pages/general-instructions) is: M. Mar Castellano (castellano.mar@inia.es).

Abstract

HOPs (HSP70–HSP90 organizing proteins) are a highly conserved family of HSP70 and HSP90 co-chaperones whose role in assisting the folding of various hormonal receptors has been extensively studied in mammals. In plants, HOPs are mainly associated with stress response, but their potential involvement in hormonal networks remains completely unexplored. In this article we describe that a member of the HOP family, HOP3, is involved in the jasmonic acid (JA) pathway and is linked to plant defense responses not only to pathogens, but also to a generalist herbivore. The JA pathway regulates responses to *Botrytis cinerea* infection and to *Tetranychus urticae* feeding; our data demonstrate that the Arabidopsis (*Arabidopsis thaliana*) *hop3-1* mutant shows an increased susceptibility to both. The *hop3-1* mutant exhibits reduced sensitivity to JA derivatives in root growth assays and downregulation of different JA-responsive genes in response to methyl jasmonate, further revealing the relevance of HOP3 in the JA pathway. Interestingly, yeast two-hybrid assays and *in planta* co-immunoprecipitation assays found that HOP3 interacts with COI1, suggesting that COI1 is a target of HOP3. Consistent with this observation, COI1 activity is reduced in the *hop3-1* mutant. All these data strongly suggest that, specifically

Received November 30, 2020. Accepted June 21, 2021. Advance access publication July 22, 2021

among HOPs, HOP3 plays a relevant role in the JA pathway by regulating COI1 activity in response to JA and, consequently, participating in defense signaling to biotic stresses.

Introduction

Jasmonic acid (JA) is a signaling molecule that is involved in different developmental processes such as fertility, root elongation, and senescence (Huang et al., 2017). Furthermore, JA also plays an essential role in plant defense, especially against chewing-biting herbivores (such as *Tetranychus urticae*, an acarus also known as two spotted spider mite) and necrotrophic pathogens (such as *Botrytis cinerea)* (Gimenez-Ibanez et al., 2016). Upon *T. urticae* and *B. cinerea* attack, the microbe- and damage-associated molecular patterns and, in the case of the herbivore, the herbivore-associated molecular patterns are perceived by the specific pattern recognition receptors of the plant. This recognition triggers a transduction cascade that leads, among other processes, to the biosynthesis of different hormones including JA, which plays a critical role in the induction of defensive molecules and in plant defense against the spider mite and the necrotrophic fungus (Zhurov et al., 2014; Martel et al., 2015; AbuQamar et al., 2017; Santamaria et al., 2021).

After biosynthesis, the JA bioactive form (+) -7-iso-JA-Ile (JA-Ile) binds intracellularly to the CORONATINE INSENSITIVE 1 (COI1) protein, which acts as a JA co-receptor (Yan et al., 2007; Fonseca et al., 2009). In addition, COI1 forms part of the Skp, Cullin, F-box containing complex (SCF^{COI1} complex), which, in the presence of JA-Ile, binds to the transcriptional repressors JASMONATE-ZIM DOMAIN PROTEIN (JAZs) and prompts JAZ degradation by the proteasome (Chini et al., 2007; Thines et al., 2007). Since JAZ proteins interact with and repress JA-related transcription factors, including the transcription factor MYC2 (Boter et al., 2004; Lorenzo et al., 2004), JAZ degradation upon JA signaling releases the transcriptional repression, triggering the expression of JA-regulated genes and the establishment of the JA response (Chico et al., 2008; Chini et al., 2009; Fonseca et al., 2009; Huang et al., 2017; Wasternack and Song, 2017).

Due to the central role of COI1 in JA perception and signaling, COI1 activity has been proven essential for the proper establishment of the JA-dependent processes. Indeed, COI1 was identified in a mutant screen for Arabidopsis plants insensitive to growth inhibition by the JA analog coronatine (Feys et al., 1994) and, subsequently, *coi1* mutants were widely reported to be compromised in multiple JA responses, including inhibition of plant growth and defense against biotic stresses (Xie et al., 1998; Stintzi et al., 2001; Yan et al., 2007; Zhang and Turner, 2008). Remarkably, it has been specifically reported that *coi1* mutants are highly susceptible to *B. cinerea* infection (Ferrari et al., 2007; Rowe et al., 2010), and that the mite *T. urticae* prefers to feed and lays more eggs on the tomato (*Lycopersicon esculentum*) *coi1* mutant over wild-type plants (Li et al., 2004). These observations reinforce the relevant role of COI1 in plant defense against this specific fungus and herbivore, respectively.

The relevance of COI1 in the JA response has also been highlighted by the use of HSP90 and HSP70 inhibitors. In the presence of these inhibitors, COI1 turns unstable, which leads to the downregulation of JA-responsive genes and a reduced sensitivity to JA in root growth assays (Zhang et al., 2015). These data suggest that the HSP70-HSP90 complex plays a major role promoting COI1 stability maintenance, affecting COI1 activity and the establishment of the JA response.

HOPs (HSP70–HSP90 organizing proteins) are a highly conserved family of HSP70 and HSP90 co-chaperones, whose role in assisting the folding of signaling proteins, such as transcription factors, kinases, and nuclear receptors, has been extensively studied in nonplant eukaryotes (Schopf et al., 2017). Specifically in plants, Arabidopsis HOP proteins interact in vivo with HSP70 and HSP90, and their role has been mainly associated with plant responses to stress (Fernandez-Bautista et al., 2017, 2018; Toribio et al., 2020). Indeed, the HOP family modulates Arabidopsis capacity to acclimate to high temperatures for long periods by regulating the heat-induced transcriptional response and the maintenance of protein quality control (QC; Fernandez-Bautista et al., 2018). In addition, it was demonstrated that one of the members of the HOP family in Arabidopsis, HOP3, plays an essential role in the alleviation of the ER stress associated with the high accumulation of misfolded proteins during specific developmental programs and in response to adverse environmental cues (Fernandez-Bautista et al., 2017). Probably related to its role in this cellular stress, the rice homolog of AtHOP3 seems to be required, along with HSP90, for the efficient maturation of the CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) and for its transport to the plasma membrane, where it participates in chitin recognition and the activation of the innate response to *Magnaporthe grisea* (Chen et al., 2010). In addition, HOP has been recently identified as a cellular determinant of Potato virus Y (PVY) symptom development in tobacco (*Nicotiana tabacum*; Lamm et al., 2017). In this case, although the virus is able to replicate, antiviral host defense response and symptom appearance are suppressed in the HOP-RNAi transgenic lines, indicating that HOP also facilitates PVY perception in tobacco plants.

Despite these data strongly suggesting that HOPs play a main role in multiple stress responses, including specific aspects of plant defense, it is largely unknown whether plant HOPs could be also involved in the regulation of the hormonal networks, which is the case with the chaperones

HSP70 and HSP90 and with HOPs' mammalian counterpart. In this article, we describe that HOP3 is involved in different JA-associated processes and plays a major role in JA pathway through the regulation of COI1 activity in Arabidopsis (*Arabidopsis thaliana*).

Results

In a previous study, we described that the triple *hop1 hop2 hop3* mutant showed an altered gene expression in response to heat (Fernandez-Bautista et al., 2018). During that study, we observed that, among the missexpressed genes, some belonged to the GO category of "JA responsive". Therefore, in order to analyze if this altered expression could uncover a role of HOP in JA pathway, we decided to assess the performance of *hop* mutants during JA-related processes. For this, we initially analyzed the defense response to the spider mite *T. urticae* and to the necrotrophic fungus *B. cinerea*.

hop mutants show an increased susceptibility to *T. urticae* feeding

As cited above, it is well established that JA pathway plays a major role in plants during *T. urticae* infestation (Zhurov et al., 2014; Martel et al., 2015; Santamaria et al., 2021); therefore, to evaluate the possible implication of HOP proteins in this JA-associated process, we analyzed the susceptibility of the *hop1 hop2 hop3* triple mutant *(hop-tm)* to this sucking herbivore in terms of leaf damage . It was previously observed that, among the members of the HOP family, *HOP3* is the only member of the family highly induced in response to spider mite feeding (Supplemental Figure S1A), and based on this, in these experiments, along with the Col-0 wild-type plants and the *hop1 hop2 hop3* triple mutant, we also included the *hop3-1* single mutant and a *hop3-1* complemented line described in Fernandez-Bautista et al. (2017). As shown in Figure 1A, the *hop1 hop2 hop3* triple mutants showed higher damage (~47%) than wild-type plants at 4 d after mite feeding. A comparable increase of damage was also observed when, instead of the triple mutant, the single *hop3-1* mutant was assayed. This damage was reduced to wild-type levels in the *hop3-1* complemented lines. These results suggest that *hop* mutants show an increased susceptibility to *T. urticae* feeding and that HOP3 is the main player in this response. Based on this, we focused for the rest of the study on the analysis of the role of HOP3 in JA-associated processes.

To further evaluate plant defense in this mutant, we also analyzed ion leakage and mite fecundity rate after infestation. In accordance with the quantification of damage, *hop3-1* mutant showed an increased rate of ion leakage upon mite feeding (Figure 1B). The higher susceptibility to the mite was also highlighted by the higher rate of accumulation of mite eggs on the leaf surface of the *hop3-1* mutant compared to wild-type plants (Figure 1C). Both parameters were reduced to wild-type levels in the *hop3-1* complemented line, further suggesting that *hop3-1* shows an increased susceptibility to spider mite feeding.

hop3-1 mutant displays a higher susceptibility to *B. cinerea* infection

It is well demonstrated that JA also plays a main role in the immune response against *B. cinerea* (AbuQamar et al., 2017). Since among the members of the *AtHOP* family, *HOP3* expression is specifically induced during *B. cinerea* infection (Supplemental Figure S1B) and a preliminary analysis suggested that HOP3 could have an important role in this response (Supplemental Figure S2A), we decided to further characterize the involvement of HOP3 in the plant defense against this necrotrophic fungus. For this, leaves from wild-type plants (Col-0), the *hop3-1* mutant and the *hop3-1* complemented line were inoculated with *B. cinerea* spores and symptom development was analyzed at 1 and 4 d post-inoculation (dpi). As shown in Figure 2, A and B, leaves of the *hop3-1* mutant exhibited bigger lesions and displayed more intense areas of trypan blue staining than those from Col-0 or the complemented line. These data also parallel with the increase in ion leakage in the *hop3-1* mutant (1.95-fold) compared to the other two genotypes in response to the infection (Figure 2C), while no significant difference was observed in plants in the absence of stress (Supplemental Figure S2B). Finally, to provide molecular evidence for the higher susceptibility of *hop3-1* mutant to this necrotrophic fungus, we also analyzed the level of expression of *PLANT DEFENSIN 1.2* (*PDF1.2*), a gene highly induced in response to *B. cinerea* that has been used as a marker of defense in this plant-pathogen system (Dombrecht et al., 2007). As shown in Figure 2D, compared to the wild-type genotype, *PDF1.2* induction level during the infection was significantly reduced (4.7-fold) in the *hop3-1* mutant, while the induction of *PDF1.2* upon *B. cinerea* inoculation was similar in wild-type plants and in the *hop3-1* complemented genotype. Together, all these data strongly suggest that *hop3-1* mutant shows an impaired establishment of the defense response and a higher susceptibility to this necrotrophic fungus.

hop3-1 mutant is partially insensitive to methyl jasmonate

The provided susceptibility data indicated that HOP3 is involved in plant defense against piercing–sucking herbivores and necrotrophic fungi, two processes highly regulated by the JA pathway. This opened the possibility that HOP3 may somehow participate in the response to this phytohormone. This hypothesis was also in agreement with the reduced expression of *PDF1.2* in response to *B. cinerea* since, apart from being a marker for defense, *PDF1.2* is a well-known JA-responsive gene (Dombrecht et al., 2007). Despite these data suggesting a role of HOP3 in the JA pathway, it has to be considered that defense responses are especially complex and that their output could be affected by intricate hormonal and regulatory pathways. Therefore, to provide evidence of the possible involvement of HOP3 in the JA pathway, we directly analyzed the sensitivity of the *hop3-1* mutant to methyl jasmonate (MeJA) in root growth assays.

As expected from previous studies, the exogenous application of JA derivatives (in this case MeJA) had an inhibitory

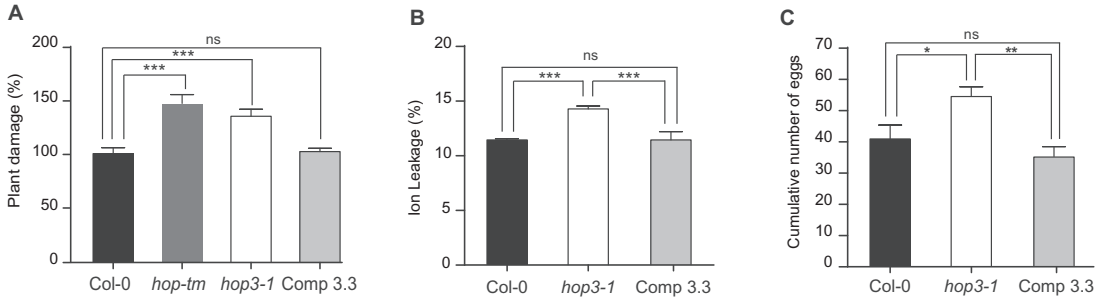

Figure 1 HOP mutants show an increased susceptibility to *T. urticae* infestation. A, Damage quantification on leaves from Col-0, *hop1 hop2 hop3* triple mutant (*hop_tm*), *hop3-1* mutant and *hop3-1* mutant complemented line 3.3 (Comp 3.3) after *T. urticae* feeding for 4 d. Data, normalized to the value of Col-0 that was assigned value 100%, are shown as means ± SE from 18 replicates. Statistically significant differences, using Kruskal–Wallis followed by Dunn's multiple comparison test, are highlighted by asterisks ***($P < 0.001$); ns: nonsignificant differences. B, Electrolyte leakage measurements upon *T. urticae* infestation. Data are shown as means ± SE from three replicates, each replicate involving five leaf disks. Ion leakage analyses in the absence of stress are shown in Supplementary Figure S2, where no significant changes were observed among genotypes. C, Quantification of the number of mite eggs on leaves from Col-0 and the *hop* mutant genotypes after *T. urticae* feeding for 36 h. Data are means ± SE from eight replicates. For B and C, statistically significant differences using one-way ANOVA with post hoc Tukey honestly significant difference test are highlighted by asterisks *($P < 0.05$), **($P < 0.01$), and ***($P < 0.001$); ns, non significant differences.

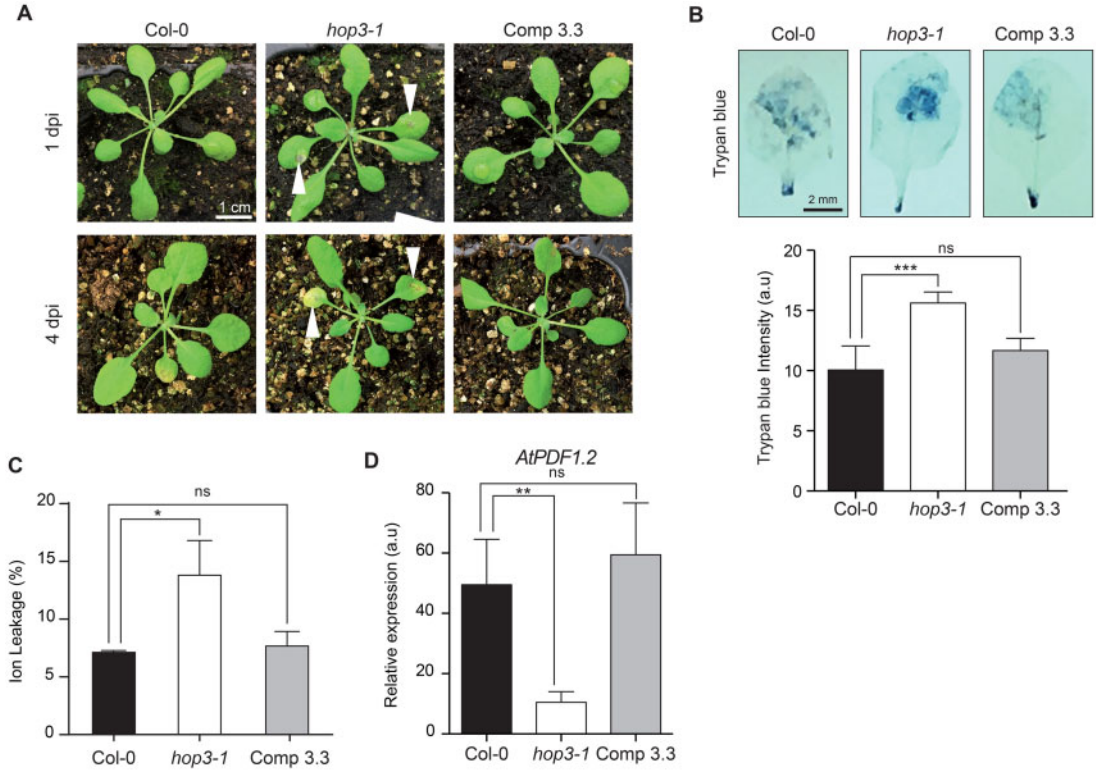

Figure 2 HOP3 participates in plant defense response to *B. cinerea* infection. A, B, Representative pictures of (A) the visual lesions (marked with white arrows) observed in the leaves from WT (Col-0), *hop3-1* mutant and the *hop3-1* complemented line 3.3 (Comp 3.3) inoculated with *B. cinerea* spores at 1 and 4 dpi or (B) after trypan blue staining at 2 dpi (B, upper); quantification of the trypan blue intensity on leaves from each genotype after *B. cinerea* infection (B, lower). Histograms show the mean ± SD of $n = 4$ leaves. C, Ion leakage analyses upon *B. cinerea* inoculation at 2 dpi. D, RT-qPCR analysis of *PLANT DEFENSIN 1.2 (AtPDF1.2)* expression at 1 dpi. The values represent changes in transcript abundance (fold change related to mock-inoculated plants). C, D, Histograms show the mean ± SE of $n = 3$ independent experiments. In all cases, statistically significant differences, using one-way ANOVA with post hoc Tukey honestly significant difference test, are highlighted by asterisks *($P < 0.05$), **($P < 0.01$), and ***($P < 0.001$); ns, nonsignificant differences. Scale bars in (A) and (B) correspond to 1 cm and 0.25 cm, respectively.

effect on primary root growth in Arabidopsis wild-type Col-0 plants (Xie et al., 1998; Chen et al., 2011; Fernandez-Calvo et al., 2011). As shown in Figure 3, A and B, MeJA inhibited root elongation and this inhibition increased at higher concentrations of MeJA. However, this inhibition was only partial in the *hop3-1* mutant, since the roots of this mutant

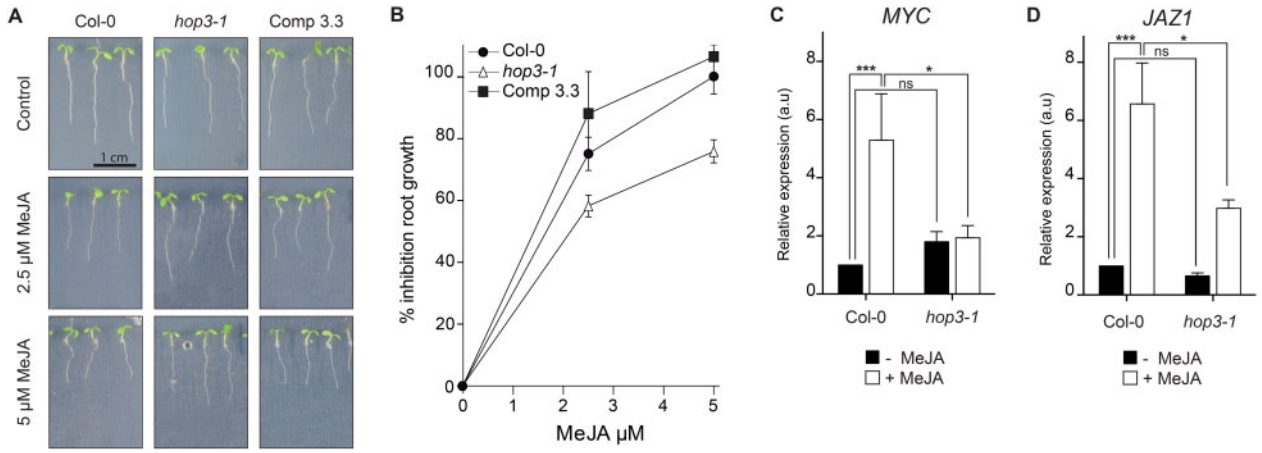

Figure 3 *hop3-1* mutant shows impaired response to MeJA. A, Representative pictures of 7-d-old seedlings from Col-0, *hop3-1*, and the *hop3-1* complemented line 3.3 (Comp 3.3) grown under control conditions (0 µM MeJA) or in the presence of 2.5 and 5 µM MeJA. Scale bar = 1 cm. B, Percentage of root elongation inhibition of Col-0, *hop3-1* mutant and *hop3-1* complemented line 3.3 (Comp 3.3) by increasing concentrations of MeJA. Data represent mean and SE of $n = 3$ independent experiments (each containing 28 seedlings for each genotype and condition). Statistically significant differences ($P < 0.01$), calculated using two-way ANOVA and Bonferroni multiple tests, were obtained for comparison between Col-0 and *hop3-1* at both MeJA concentrations and for the comparison between *hop3-1* and line 3.3 at 5 µM MeJA; no significant differences were observed for the rest of the multiple comparisons. C, D, RT-qPCR expression analysis of the JA-responsive genes *MYC2* (C) and *JAZ1* (D) in roots from 7-d-old seedlings from Col-0 and from the *hop3-1* mutant. Expression values (fold change in relation to the expression of each gene in Col-0 under control conditions, which was arbitrarily assigned value 1 after normalization with the calibrator gene *ACT7* (At5g09810)) are shown as mean ± SEM from $n = 3$ independent samples with three technical replicates. Statistically significant differences were calculated using a GLM analysis through Wald Chi-square followed by Bonferroni multiple comparison test and are highlighted by asterisks *($P < 0.05$) and ***($P < 0.001$); ns, nonsignificant differences.

responded but their growth was inhibited to a lesser extent (16.6% and 24.29%) than those of wild-type plants in the presence of 2.5 and 5 µM MeJA, respectively. In contrast, *hop3-1* complemented plants showed a restored sensitivity to the chemical, displaying a reduction in root length similar to that in wild-type plants. Remarkably, when assayed in parallel, the *hop3-1* mutant and the *hop1 hop2 hop3* triple mutant showed a similar sensitivity to MeJA (Supplemental Figure S3A). In addition to MeJA, a partial inhibition of root growth in the *hop3-1* (comparable to the response of the *hop1 hop2 hop3* triple mutant) was also observed in response to coronatine (Supplemental Figure S3B). All these data indicated that HOP3 plays a main role in JA-mediated root growth inhibition.

It is well established that JA signaling pathway induces in the presence of JA the expression of JA-responsive genes. Therefore, in order to gain insight into whether this reduced sensitivity to MeJA impinges on the transcriptional output of the JA pathway, we selected two well-known JA responsive genes (*MYC2* and *JAZ1*), and evaluated the expression of these genes in the wild-type genotype and *hop3-1* mutant under control conditions and after MeJA treatment (Pauwels et al., 2008). As shown in Figure 3, C and D, as expected, both genes were induced in the wild-type plants in response to MeJA. Despite the fact that both genes showed a similar level of expression under control conditions, their induction in the presence of MeJA was significantly reduced in the *hop3-1* mutant compared to Col-0. Since this mutant seemed unable to fully accomplish the JA transcriptional response when triggered with MeJA, these results confirm, as was also suggested by the reduced response to the assayed JA derivatives in root length assays, that this mutant is partially insensitive to JA. In addition, all these results strongly suggest that HOP3 is involved in JA perception or signaling.

HOP3 interacts with COI1 and modulates JAZ degradation levels

It was previously reported that HSP70 and HSP90 complexes are involved in the stabilization of COI1 (Zhang et al., 2015). Since HOP3 interacts with HSP70 and HSP90 in Arabidopsis (Fernandez-Bautista et al., 2017), and, according to our results, HOP3 is involved in JA perception and signaling, we reasoned that HOP3 could be part of the complexes that modulate COI1 activity. To test this hypothesis, we firstly analyzed the possible interaction between COI1 and HOP3 by directed yeast two-hybrid assays. As shown in Figure 4A, yeast cells co-transformed with plasmids expressing COI1 (fused to the Gal4-BD) and HOP3 (fused to the Gal4-AD) were able to grow on selective media, while independent co-transformations of the same constructs with vectors expressing the bare Gal4-AD and Gal4-BD, respectively, showed impaired growth in the same conditions. These data indicate that HOP3 interacts with COI1 in the two-hybrid assay. To validate this interaction in planta, we expressed different combinations of COI1-myc and GFP-HOP3 in *N. benthamiana* leaves and we carried out a HOP3

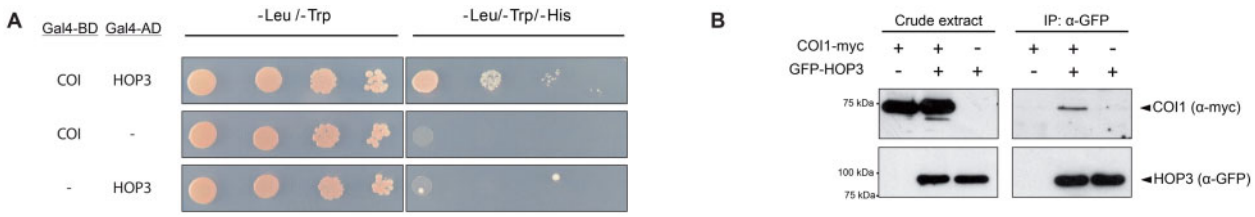

Figure 4 HOP3 interacts with COI1 in yeast two-hybrid assays and in vivo. HOP3 interaction with COI1 was tested using the yeast-two hybrid system (A) or by co-immunoprecipitation in *N. benthamiana* leaves (B). A, The proteins fused to the Gal4-BD and Gal4-AD that were co-expressed in the AH109 strain are shown on the left of the panel. The constructs expressing the bare Gal4-BD and Gal4-AD were used as controls (−). Independent co-transformants were tested for growth in nonselective medium (-Leu-Trp) or prototrophy-selective medium (-Leu, -Trp, -His). B, Protein extracts (crude extracts) from *N. benthamiana* leaves transiently expressing, under the control of the 35S promoter, different combinations of COI1-myc and GFP-HOP3 were subjected to immunoprecipitation using anti-GFP beads. The presence of the different proteins in the crude extracts and in the eluted fractions from HOP3 immunoprecipitations (IP:α-GFP) was analyzed by western-blot using anti-myc and anti-GFP antibodies.

immunoprecipitation analysis. As shown in Figure 4B, we observed a clear band corresponding to COI1 in the eluate from HOP3 immunoprecipitations (IP: anti-GFP), but not in the eluates lacking HOP3, demonstrating that HOP3 also interacts with COI1 in vivo. This in vivo interaction was also tested by similar co-immunoprecipitation analyses expressing a different set of HOP3 and COI1 fusion proteins (HA-HOP3 and COI1-IgG-myc or COI1-GFP) and swapping the immunoprecipitated protein (i.e. immunoprecipitating COI1). In these experiments (Supplemental Figure S4), as expected from the previous results, HOP3 was co-immunoprecipitated with COI1 but not with a nonrelated protein GFP (Supplemental Figure S4B), further verifying the specific interaction between HOP3 and COI1.

Once the role of HOP3 in the JA pathway and its interaction with COI1 was confirmed, we wondered whether HOP3's function in JA signaling could be exerted through the regulation of COI1 activity, which triggers the JA-dependent degradation of the JAZ repressors (Chini et al., 2007; Fonseca et al., 2009). In Arabidopsis, JAZ proteins are encoded by 12 genes designated *JAZ1* to *JAZ12*. Among these gene products, JAZ10 has been shown to interact with COI1 in the presence of coronatine in yeast two-hybrid and pull-down assays, and to be degraded in the presence of MeJA (Shyu et al., 2012; Moreno et al., 2013; Garrido-Bigotes et al., 2020). In addition, JAZ10 was shown to directly participate in JA-dependent defense response and in the inhibition of root elongation (Moreno et al., 2013; de Torres Zabala et al., 2016), which makes JAZ10 a good candidate to analyze COI1 activity in our assays. Therefore, to gain insight into the possible role of HOP3 in COI1 activity, we introgressed a *JAZ10* reporter line in the *hop3-1* mutant by crossing *hop3-1* with the previously reported line *p35S:JAZ10-GUS* in Col-0 (Chico et al., 2014). Homozygous plants expressing similar levels of the reporter gene in the *hop3-1* and Col-0 backgrounds were selected (Figure 5A). These lines, as expected by the expression levels, showed a similar level of JAZ10 (measured as GUS activity) under control conditions (i.e. in the absence of the JA derivative; Figure 5B). However, in the presence of MeJA, JAZ10 degradation was significantly reduced to 70% in the *hop3-1* mutant compared to the wild-type background (Figure 5C), demonstrating that COI1 activity is reduced in the *hop3-1* mutant and highlighting the role of HOP3 in COI1 regulation.

Discussion

HOP3 is involved in different JA-mediated processes

JA plays a main role in the regulation of many physiological processes including inhibition of root elongation and plant defense, especially against wounding herbivores and necrotrophic fungi (Gimenez-Ibanez et al., 2016). Our results show that the *hop3-1* mutant is more susceptible to *T. urticae* feeding and to infection by the necrotrophic fungus *B. cinerea*. In addition, the *hop3-1* mutant also shows a reduced inhibition of root elongation in response to MeJA and coronatine and an altered expression of JA-responsive genes in the presence of MeJA. All these data highlight that HOP3 is involved in different processes associated with the JA pathway. This involvement is reinforced by the in vivo interaction of HOP3 with COI1 and the reduced activity of COI1 in the *hop3-1* lines in response to MeJA. Since it has been previously described that COI1 activity is required for the proper establishment of the cited JA-associated processes (Li et al., 2004; Ferrari et al., 2007; Rowe et al., 2010; Moreno et al., 2013), the reduced activity of COI1 contributes to explaining the impaired defense and the root elongation inhibition phenotypes observed in the *hop3-1* mutant.

We have shown here that a HOP member is involved in the defense against leaf-sucking herbivores. Nevertheless, in rice, it has been previously reported that OsHOP participates in the defense against *Magnaporthe grisea* by promoting the maturation and transport of the chitin receptor CERK1 (Chen et al., 2010). This makes it possible to speculate that in the specific case of necrotrophic fungi, HOP could have a dual role in defense: on the one hand, by facilitating chitin perception through its role in CERK1 maturation and, on the other hand, by assuring the proper activation of the JA pathway through the regulation of COI1 activity. This may not be the case for the defense against

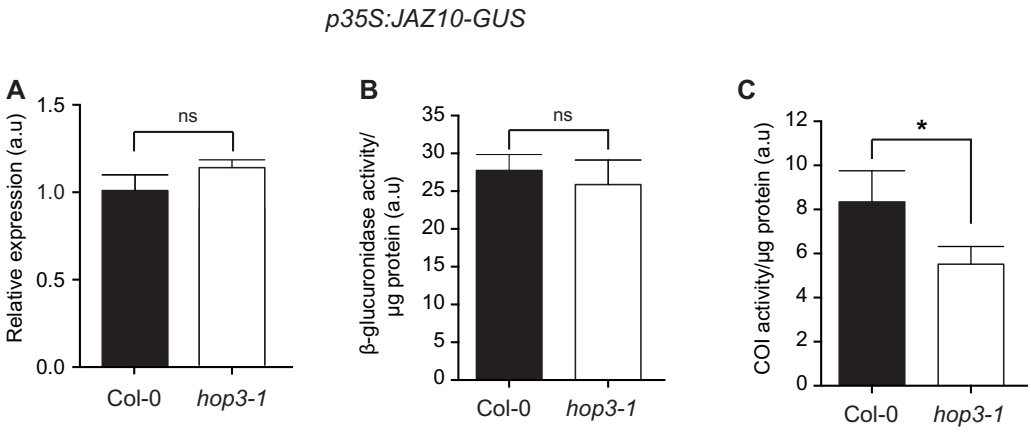

Figure 5 HOP3 modulates COI1 activity. A, RT-qPCR analysis of *GUS* expression in 7-d-old seedlings expressing the *p35S:JAZ10-GUS* reporter construct in the Col-0 or *hop3-1* mutant backgrounds. Values are means and SE, $n = 3$ independent experiments. B, Quantification of β-glucuronidase activity under control conditions in the cited lines. C, Quantification of COI1 activity in seedlings expressing the *p35S:JAZ10-GUS* reporter construct in the Col-0 and *hop3-1* mutant backgrounds upon treatment with 1μM MeJA for 1 h. For B and C, data represent mean and SE of $n = 5$ independent experiments (each containing 40 plants per condition and genotype). In all cases, statistically significant differences were analyzed using *t* test; *($P = 0.029$); ns, non significant differences.

spider mite, since in contrast to the important role of chitin perception in the response to *B. cinerea*, a relevant role of chitin signaling in the defense against *T. urticae* has not been reported (e.g. possible changes in susceptibility to *T. urticae* associated with the chitin receptor mutants has not been described). Based on this, it might be possible to speculate that the enhanced susceptibility of the *hop3-1* mutant to *T. urticae*, as is the case in the inhibition of root length, could mainly reflect the role of HOP3 in the JA pathway.

hop3-1 mutant is partially insensitive to JA derivatives

Compared to wild-type plants, *hop3-1* mutant shows a partial insensitivity to MeJA and coronatine in root length assays (i.e., inhibition of root elongation is not fully abolished in the *hop3-1* mutant but is partially blocked). This implies that this mutant is partially impaired in JA response. This observation is consistent with the reduced (but not absent) activity of COI1 in the *hop3-1* mutant and its reduced expression of JA-responsive genes in response to the hormone. In mammals, the function of HOP in hormone signaling has been deeply studied in the context of the folding of the glucocorticoid receptor (GR). In these eukaryotes, HOP is not strictly required for GR folding, but substantially increases the yield in the acquisition of GR's native conformation (Morishima et al., 2000). Based on our data, we speculate that this could also be the case in plants, where HOP3 could facilitate the acquisition of COI1 native conformation and stability.

HOP3 modulates COI1 activity

Protein activity depends on protein conformation and, in this sense, protein folding is an essential step to achieve high activity yields. Folding also affects protein stability, since, in general terms, misfolded proteins are usually recognized by the QC machinery and targeted for degradation (Diaz-Villanueva et al., 2015).

During our study, we tried to test whether HOP3, through the folding of COI1, directly modulates COI1 stability. For this reason, we introgressed the *p35S:COI1-FLAG* constructs into the *hop3-1* background. Unfortunately, the transgene *p35S:COI1-FLAG* got silenced in the mutant background. Furthermore, the lack of good commercial antibodies against COI1 also precluded the direct assessment of COI1 stability in the *hop3-1* lines. Despite these technical problems, our data clearly demonstrate that COI1 activity (i.e. JAZ degradation) is reduced in the *hop3-1* mutant. This observation is highlighted by the reduced expression of JA-responsive genes upon MeJA treatment in the mutant background. All these results demonstrate that HOP3, probably through the folding and stabilization of COI1, modulates COI1 activity.

HOP3 may participate with other co-chaperones in JA signaling

It is well known that the HSP70–HSP90 folding cycle is assisted by a cohort of different co-chaperones that act in cooperation to promote client proper folding and activity (Li et al., 2012; Prodromou, 2012; Schopf et al., 2017; Bohush et al., 2019). These co-chaperones modulate different aspects of chaperone function such as substrate selection, ATPase activity or their capacity to form multiprotein complexes. In addition, it is well established that some co-chaperones indirectly regulate the binding of other co-chaperones to the HSP70–HSP90 complex. This is the case, for example, for the FK506-BINDING PROTEINS, the CYCLOPHILIN 40 (CyP40) or the H(+)-ATPASE 1 (AHA1), whose interactions with HSP90 are inhibited in the presence of HOP (Owens-Grillo et al., 1996; Harst et al., 2005; Ebong et al., 2016). All these observations suggest that different sets of co-chaperones could mediate quality control (including the folding and degradation) of specific substrates, which makes the

identification of the precise set of co-chaperones that regulate important signaling proteins, such as, COI1 extremely interesting.

In this sense, it is worth mentioning that, although AtHOP3 belongs to a highly conserved family of proteins in Arabidopsis that is composed of two other constitutively expressed members (AtHOP1 and AtHOP2), the single *hop3-1* mutant displays, presumably in the presence of HOP1 and HOP2, an increased susceptibility to *T. urticae* feeding and to *B. cinerea* infection and a decreased sensitivity to JA derivates and COI1 activity. All these data suggest that HOP3 plays a prevalent role that does not fully overlap with HOP1 and HOP2 in these JA-associated processes. This prevalent role is also reinforced by the comparable responses of *hop3-1* and *hop1 hop2 hop3* mutants in the processes we analyzed. Although our data do not fully discard a role for HOP1 and HOP2, these data highlight the possible specificity of the different members of the HOP family in plants (Toribio et al., 2020).

In plants, another HSP70-HSP90 co-chaperone from the Suppressor of G2 allele SKP1 (SGT1) family, SGT1b, has been involved in disease resistance R protein accumulation and in JA pathway through the binding and stabilization of COI1 (Zhang et al., 2015). Since HOP3 and SGT1b associate with the HSP70 and HSP90 chaperones and share the same client protein (COI1), it is tempting to speculate that HOP3 could participate, along with SGT1b, in the folding of COI1. In this case, a partial folding of COI1 may be achieved by SGT1b in the absence of HOP3. This partial folding may lead to a reduced but not fully abolished COI1 activity, as observed in the *hop3-1* mutant. This hypothesis is also consistent with the partial reduction of the levels of COI1 and the moderate alteration (compared to the *coi1* mutants) of the expression of different JA-responsive genes in the *sgt1b* mutants (Zhang et al., 2015). SGT1b was also involved in the regulation of TIR1 stability (Gray et al., 2003; Zhang et al., 2015; Wang et al., 2016). Furthermore, COI1 and TIR1 share similar structures, where both are E3 ligases that contain an F-box and different leucine-rich repeats motifs. These parallelisms open the possibility that HOP3 could also regulate TIR1 stability. Nevertheless, whether HOP proteins (or HOP3) show an intrinsic specificity for proteins with special structures or motifs is an aspect that should be further investigated.

Taken together, this study uncovers the specific role of HOP3 as a regulator of COI1 activity and points out its relevant role in the JA signaling pathway and in plant defense. Furthermore, our results demonstrate that HOP3 forms part of the specific set of HSP70 and HSP90 co-chaperones that regulate the activation of the JA pathway.

Materials and methods

Plant material and growth conditions

Arabidopsis T-DNA insertion *hop3-1* mutant (SALK_00794) was acquired from the Arabidopsis Biological Resource Center. The *hop3-1* mutant and the *hop3-1* line complemented with the construct *pHOP3:HOP3-HA* (line 3.3) were previously characterized (Fernandez-Bautista et al., 2017). The *hop1 hop2 hop3* triple mutant was described in Fernandez-Bautista et al. (2018). *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) was used as wild-type genetic background control. Unless otherwise stated, all seeds were surface-sterilized, stratified at 4°C for 48 h and grown at 22°C using a 16-h-light photoperiod. For growth on plates, Murashige and Skoog (MS) medium supplemented with 1% (w/v) sucrose was used in all cases, unless otherwise stated.

Phytopathogen strains and growth conditions

Tetranychus urticae, London strain (Acari: Tetranychidae) was provided by Dr Miodrag Grbic (University of Western Ontario, Canada). Spider mite maintenance was previously reported in Santamaria et al. (2019). *Botrytis cinerea* strain was kindly provided by Plant Response Biotech S. L. This fungal pathogen was grown on potato dextrose agar medium at 28°C for 8 d as previously described in Berrocal-Lobo et al. (2002) and Windram et al. (2012). Spores were collected in sterile water, filtered, quantified with a Neubauer chamber and stored in 20% (v/v) glycerol at −80°C until use.

Plant damage, ion leakage, and fecundity assays upon *T. urticae* feeding

Quantification of plant damage after arthropod feeding was done as described in Santamaria et al. (2019). Briefly, 3-week-old plants were infested with 20 *T. urticae* adults, which were carefully transferred with a brush to the leaf surface. After 4 d of feeding, leaf damage was assessed by scanning the entire rosette using a scanner (HP Scanjet 5590 Digital Flatbed Scanner series) according to Cazaux et al. (2014). Six rosettes from each genotype were included for each of the three assayed experiments. Leaf damage was calculated in mm^2 using Adobe Photoshop CS software.

For ion leakage analyses, leaf disks (1-cm diameter) were infested with 10 mites for 24 h. In each case, ion leakage was determined as described in Santamaria et al. (2017). Three replicates were assayed, each of them including five disks per genotype.

Fecundity assays were performed on detached leaves from 3-week-old plants. The newest emerged leaf (about 1 cm long) from each plant was fit in special dishes and infested with 12 adult synchronized females. After 36 h of infestation, the number of eggs was counted. Eight replicates were analyzed per plant genotype.

Botrytis cinerea inoculation and phenotypic assessment

Either 2- or 3-week-old Arabidopsis plants were used for pathogen inoculation experiments. Plant leaf surfaces were inoculated with 5 µL of *B. cinerea* fungal inoculum (2×10^5 spores·mL^{-1}) in potato dextrose broth medium. Inoculated plants were placed in a growth chamber, allowing fungal growth until harvesting. Infection progress, necrosis, and cell death development in infected leaves were monitored for 1 week. Ion leakage analysis, conductivity measurements and

determination of cell death by trypan blue staining were performed as described in Fernández-Bautista et al. (2016). Four independent replicates were assayed, each of them including five leaves per genotype. Intensities of trypan blue staining (obtained from intensity profile plots) were estimated using ImageJ software. Four independent biological replicates were used for quantification.

Gene expression analysis by RT-qPCR

For the analysis of gene expression during *B. cinerea* infection, four leaves per genotype from four different mock- or *B. cinerea*-inoculated plants were harvested at 1 dpi. RNA isolation was carried out using the TRIzol reagent (GIBCO-Invitrogen-LifeTechnologies). Reverse transcription polymerase chain reaction (RT-qPCR) analysis was performed as described in Berrocal-Lobo et al. (2010), using 1 μg of RNA for cDNA synthesis and β-*ACTIN* (At3g18780) for normalization. Three biological replicates, each of them including three technical replicates, were analyzed. Primer sequences for the RT-qPCR experiments are shown in Supplemental Table S1.

For the analysis of gene expression upon MeJA treatment, 7-d-old seedlings were grown in a vertical position in MS medium in the absence or in the presence of 5 μM MeJA. For each treatment, the roots of 25 seedlings were collected. RNA isolation and RT-qPCR analyses were performed as described in Fernandez-Bautista et al. (2017) using *ACT7* (At5g09810) for normalization.

For quantification of *JAZ10-GUS* expression, approximately 20 seedlings from the *p35S:JAZ10-GUS* lines in Col-0 or *hop3-1* backgrounds were grown for 7 d on MS in a vertical position. RT-qPCR were performed as described in Echevarria-Zomeno et al. (2015), using *PP2AA3* (At1g13320) for normalization. Each experiment was conducted in three technical replicates with three biological replicates.

Root growth inhibition assays

Arabidopsis seedlings from Col-0, *hop3-1* mutant or *hop3-1* mutant complemented line 3.3 seedlings were grown in a vertical position side-by-side on MS in the absence or in the presence of 2.5 and 5 μM MeJA. After 7 d of growth, pictures were taken and root length was calculated with ImageJ as described in Reveglia et al. (2018). From each experiment, at least 20 seedlings were included. Each experiment was repeated three times, obtaining similar results.

Interaction assays

The yeast two-hybrid constructs *pGBK:COI1* and *pGADT7:HOP3* were previously reported in Chini et al. (2007) and Fernandez-Bautista et al. (2017), respectively. Yeast transformation into AH109 and growth in selective media were carried out as described in Castellano and Sablowski (2008). Co-immunoprecipitation analyses in *N. benthamiana* leaves were carried out as described in Fernandez-Bautista et al. (2017) and Munoz and Castellano (2018). The assay was repeated three times obtaining similar results.

JAZ degradation assays

The *p35S:JAZ10-GUS* line in the wild-type background was previously described (Chico et al., 2014). This marker line was introgressed into the *hop3-1* background by crossing, and a double homozygous line was used for further analyses. For quantification of JAZ10-GUS protein degradation, approximately 40 seedlings from the *p35S:JAZ-GUS* lines in Col-0 or *hop3-1* backgrounds were grown for 7 d on MS in a vertical position and subsequently treated for 1 h with 1 μM MeJA (Reveglia et al., 2018). After the treatment, roots were collected and frozen in liquid nitrogen. GUS and total protein quantifications from the root extracts were carried out as described in Chini et al. (2018) with minor modifications. COI1 activity was calculated as the difference between JAZ10 initial quantity and final quantity per time unit and per microgram of total protein. Statistically significant differences of $n = 5$ independent experiments were analyzed by *t* test.

Statistical analyses

Statistical analyses were performed using GraphPadPrismv6.01. In all cases, data sets were subjected to normality and homoscedasticity tests in order to select the proper statistical analyses. The statistical analyses used for each set of experiments were the following: in the case of calculations of electrolyte leakage, *T. urtiace* egg laying, trypan blue intensity and *PDF1.2* expression, one-way ANOVA with post hoc Tukey honestly significant difference test; for the analyses of leaf damage, Kruskal–Wallis followed by Dunn's multiple comparisons tests; for root growth inhibition assays, two-way ANOVA and Bonferroni multiple tests; for gene expression upon MeJA treatment, GLM analysis through Wald Chi-square followed by Bonferroni multiple comparison test; finally, for the analysis of β-glucuronidase activity in the Col-0 and *hop3-1* mutant backgrounds, *t* test.

Accession numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: *HOP1* (AT1G12270), *HOP2* (AT1G62740), *HOP3* (AT4G12400), *COI1* (AT2G39940) and *JAZ10* (AT5G13220).

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. Expression analyses of the different members of the *AtHOP* family in response to *T. urticae* infestation or to *B. cinerea* infection.

Supplemental Figure S2. Preliminary analysis of *hop* mutants' susceptibility to *B. cinerea* infection and ion leakage analyses in the absence of stress (controls for Figure 2C).

Supplemental Figure S3. Percentage of root elongation inhibition of different *hop* mutants by increasing concentrations of MeJA or coronatine (COR).

Supplemental Figure S4. HOP3 specifically interacts with COI1 by co-immunoprecipitation analyses in *N. benthamiana* leaves.

Supplemental Table S1. List of primers used in the study.

Acknowledgments

We thank Andrea Chini and Roberto Solano for sharing with us the *JAZ*-reporter and *COI1* two-hybrid constructs and for their valuable technical and scientific advice. We also thank Ana Arnaiz and Alicia Tapias for their valuable technical help with different aspects of the manuscript.

Funding

This work is supported by the projects RTI2018-095946-B-I00, BIO2017-83472-R from MICIU and by "Severo Ochoa Programme for Centres of Excellence in R&D" from the Agencia Estatal de Investigación of Spain (grant SEV-2016-0672 [2017–2021] to the CBGP). In the frame of this latter program R. T. and S. M. were supported with postdoctoral contracts.

Conflict of interest statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

AbuQamar S, Moustafa K, Tran LS (2017) Mechanisms and strategies of plant defense against Botrytis cinerea. Crit Rev Biotechnol **37**: 262–274

Berrocal-Lobo M, Molina A, Solano R (2002) Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. Plant J **29**: 23–32

Berrocal-Lobo M, Stone S, Yang X, Antico J, Callis J, Ramonell KM, Somerville S (2010) ATL9, a RING zinc finger protein with E3 ubiquitin ligase activity implicated in chitin- and NADPH oxidase-mediated defense responses. PLoS One **5**: e14426

Bohush A, Bieganowski P, Filipek A (2019) Hsp90 and its co-chaperones in neurodegenerative diseases. Int J Mol Sci **20**

Boter M, Ruiz-Rivero O, Abdeen A, Prat S (2004) Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and Arabidopsis. Genes Dev **18**: 1577–1591

Castellano MM, Sablowski R. 2008. Phosducin-Like Protein 3 is required for microtubule-dependent steps of cell division but not for meristem growth in Arabidopsis. Plant Cell **20**: 969–981

Cazaux M, Navarro M, Bruinsma KA, Zhurov V, Negrave T, Van Leeuwen T, Grbic V, Grbic M (2014) Application of two-spotted spider mite Tetranychus urticae for plant-pest interaction studies. J Vis Exp (89): 51738

Chen L, Hamada S, Fujiwara M, Zhu T, Thao NP, Wong HL, Krishna P, Ueda T, Kaku H, Shibuya N, et al. (2010) The Hop/Sti1-Hsp90 chaperone complex facilitates the maturation and transport of a PAMP receptor in rice innate immunity. Cell Host Microbe **7**: 185–196

Chen Q, Sun J, Zhai Q, Zhou W, Qi L, Xu L, Wang B, Chen R, Jiang H, Qi J, et al. (2011) The basic helix-loop-helix transcription factor MYC2 directly represses PLETHORA expression during jasmonate-mediated modulation of the root stem cell niche in Arabidopsis. Plant Cell **23**: 3335–3352

Chico JM, Chini A, Fonseca S, Solano R (2008) JAZ repressors set the rhythm in jasmonate signaling. Curr Opin Plant Biol **11**: 486–494

Chico JM, Fernandez-Barbero G, Chini A, Fernandez-Calvo P, Diez-Diaz M, Solano R (2014) Repression of jasmonate-dependent defenses by shade involves differential regulation of protein stability of MYC transcription factors and their JAZ repressors in Arabidopsis. Plant Cell **26**: 1967–1980

Chini A, Boter M, Solano R (2009) Plant oxylipins: COI1/JAZs/MYC2 as the core jasmonic acid-signalling module. FEBS J **276**: 4682–4692

Chini A, Cimmino A, Masi M, Reveglia P, Nocera P, Solano R, Evidente A (2018) The fungal phytotoxin lasiojasmonate A activates the plant jasmonic acid pathway. J Exp Bot **69**: 3095–3102

Chini A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia-Casado G, Lopez-Vidriero I, Lozano FM, Ponce MR, et al. (2007) The JAZ family of repressors is the missing link in jasmonate signalling. Nature **448**: 666–671

de Torres Zabala M, Zhai B, Jayaraman S, Eleftheriadou G, Winsbury R, Yang R, Truman W, Tang S, Smirnoff N, Grant M (2016) Novel JAZ co-operativity and unexpected JA dynamics underpin Arabidopsis defence responses to Pseudomonas syringae infection. New Phytol **209**: 1120–1134

Diaz-Villanueva JF, Diaz-Molina R, Garcia-Gonzalez V (2015) Protein folding and mechanisms of proteostasis. Int J Mol Sci **16**: 17193–17230

Dombrecht B, Xue GP, Sprague SJ, Kirkegaard JA, Ross JJ, Reid JB, Fitt GP, Sewelam N, Schenk PM, Manners JM, Kazan K (2007) MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. Plant Cell **19**: 2225–2245

Ebong IO, Beilsten-Edmands V, Patel NA, Morgner N, Robinson CV (2016) The interchange of immunophilins leads to parallel pathways and different intermediates in the assembly of Hsp90 glucocorticoid receptor complexes. Cell Discov **2**: 16002

Echevarria-Zomeno S, Fernandez-Calvino L, Castro-Sanz AB, Lopez JA, Vazquez J, Castellano MM (2015) Dissecting the proteome dynamics of the early heat stress response leading to plant survival or death in Arabidopsis. Plant Cell Environ **39**: 1264–1278

Fernández-Bautista N, Domínguez-Núñez JA, Moreno MMC, Berrocal-Lobo M (2016) Plant tissue trypan blue staining during phytopathogen infection. Bio-protocol **6**: e2078

Fernandez-Bautista N, Fernandez-Calvino L, Munoz A, Castellano MM (2017) HOP3, a member of the HOP family in Arabidopsis, interacts with BiP and plays a major role in the ER stress response. Plant Cell Environ **40**: 1341–1355

Fernandez-Bautista N, Fernandez-Calvino L, Munoz A, Toribio R, Mock HP, Castellano MM (2018) HOP family plays a major role in long-term acquired thermotolerance in Arabidopsis. Plant Cell Environ **41**: 1852–1869

Fernandez-Calvo P, Chini A, Fernandez-Barbero G, Chico JM, Gimenez-Ibanez S, Geerinck J, Eeckhout D, Schweizer F, Godoy M, Franco-Zorrilla JM, et al. (2011) The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant Cell **23**: 701–715

Ferrari S, Galletti R, Denoux C, De Lorenzo G, Ausubel FM, Dewdney J (2007) Resistance to Botrytis cinerea induced in Arabidopsis by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. Plant Physiol **144**: 367–379

Feys B, Benedetti CE, Penfold CN, Turner JG (1994) Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell **6**: 751–759

Fonseca S, Chico JM, Solano R (2009) The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr Opin Plant Biol **12**: 539–547

Garrido-Bigotes A, Valenzuela-Riffo F, Torrejon M, Solano R, Morales-Quintana L, Figueroa CR (2020) A new functional JAZ degron sequence in strawberry JAZ1 revealed by structural and interaction studies on the COI1-JA-Ile/COR-JAZs complexes. Sci Rep **10**: 11310

Gimenez-Ibanez S, Chini A, Solano R (2016) How microbes twist jasmonate signaling around their little fingers. Plants (Basel) 5

Gray WM, Muskett PR, Chuang HW, Parker JE (2003) Arabidopsis SGT1b is required for SCF(TIR1)-mediated auxin response. Plant Cell **15**: 1310–1319

Harst A, Lin H, Obermann WM (2005) Aha1 competes with Hop, p50 and p23 for binding to the molecular chaperone Hsp90 and

contributes to kinase and hormone receptor activation. Biochem J **387**: 789–796

Huang H, Liu B, Liu L, Song S (2017) Jasmonate action in plant growth and development. J Exp Bot **68**: 1349–1359

Lamm CE, Kraner ME, Hofmann J, Bornke F, Mock HP, Sonnewald U (2017) Hop/Sti1 - a two-faced cochaperone involved in pattern recognition receptor maturation and viral infection. Front Plant Sci **8**: 1754

Li J, Soroka J, Buchner J (2012) The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones. Biochim Biophys Acta **1823**: 624–635

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. Plant Cell **16**: 126–143

Lorenzo O, Chico JM, Sanchez-Serrano JJ, Solano R (2004) JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. Plant Cell **16**: 1938–1950

Martel C, Zhurov V, Navarro M, Martinez M, Cazaux M, Auger P, Migeon A, Santamaria ME, Wybouw N, Diaz I, et al. (2015) Tomato whole genome transcriptional response to Tetranychus urticae identifies divergence of spider mite-induced responses between tomato and Arabidopsis. Mol Plant Microbe Interact **28**: 343–361

Moreno JE, Shyu C, Campos ML, Patel LC, Chung HS, Yao J, He SY, Howe GA (2013) Negative feedback control of jasmonate signaling by an alternative splice variant of JAZ10. Plant Physiol **162**: 1006–1017

Morishima Y, Kanelakis KC, Silverstein AM, Dittmar KD, Estrada L, Pratt WB (2000) The Hsp organizer protein hop enhances the rate of but is not essential for glucocorticoid receptor folding by the multiprotein Hsp90-based chaperone system. J Biol Chem **275**: 6894–6900

Munoz A, Castellano MM (2018) Coimmunoprecipitation of interacting proteins in plants. Methods Mol Biol **1794**: 279–287

Owens-Grillo JK, Czar MJ, Hutchison KA, Hoffmann K, Perdew GH, Pratt WB (1996) A model of protein targeting mediated by immunophilins and other proteins that bind to hsp90 via tetratricopeptide repeat domains. J Biol Chem **271**: 13468–13475

Pauwels L, Morreel K, De Witte E, Lammertyn F, Van Montagu M, Boerjan W, Inze D, Goossens A (2008) Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured Arabidopsis cells. Proc Natl Acad Sci USA **105**: 1380–1385

Prodromou C (2012) The 'active life' of Hsp90 complexes. Biochim Biophys Acta **1823**: 614–623

Reveglia P, Chini A, Mandoli A, Masi M, Cimmino A, Pescitelli G, Evidente A (2018) Synthesis and mode of action studies of N-[(-)-jasmonyl]-S-tyrosin and ester seiridin jasmonate. Phytochemistry **147**: 132–139

Rowe HC, Walley JW, Corwin J, Chan EK, Dehesh K, Kliebenstein DJ (2010) Deficiencies in jasmonate-mediated plant defense reveal quantitative variation in Botrytis cinerea pathogenesis. PLoS Pathog **6**: e1000861

Santamaria ME, Garcia A, Arnaiz A, Rosa-Diaz I, Romero-Hernandez G, Diaz I, Martinez M (2021) Comparative transcriptomics reveals hidden issues in the plant response to arthropod herbivores. J Integr Plant Biol **63**: 14

Santamaria ME, Martinez M, Arnaiz A, Ortego F, Grbic V, Diaz I (2017) MATI, a novel protein involved in the regulation of herbivore-associated signaling pathways. Front Plant Sci **8**: 975

Santamaria ME, Martinez M, Arnaiz A, Rioja C, Burow M, Grbic V, Diaz I (2019) An Arabidopsis TIR-lectin two-domain protein confers defense properties against Tetranychus urticae. Plant Physiol **179**: 1298–1314

Schopf FH, Biebl MM, Buchner J (2017) The HSP90 chaperone machinery. Nat Rev Mol Cell Biol **18**: 345–360

Shyu C, Figueroa P, Depew CL, Cooke TF, Sheard LB, Moreno JE, Katsir L, Zheng N, Browse J, Howe GA (2012) JAZ8 lacks a canonical degron and has an EAR motif that mediates transcriptional repression of jasmonate responses in Arabidopsis. Plant Cell **24**: 536–550

Stintzi A, Weber H, Reymond P, Browse J, Farmer EE (2001) Plant defense in the absence of jasmonic acid: the role of cyclopentenones. Proc Natl Acad Sci U S A **98**: 12837–12842

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature **448**: 661–665

Toribio R, Mangano S, Fernández-Bautista N, Muñoz A, Castellano MM (2020) HOP, a co-chaperone involved in response to stress in plants. Front Plant Sci **11**

Wang R, Zhang Y, Kieffer M, Yu H, Kepinski S, Estelle M (2016) HSP90 regulates temperature-dependent seedling growth in Arabidopsis by stabilizing the auxin co-receptor F-box protein TIR1. Nat Commun **7**: 10269

Wasternack C, Song S (2017) Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. J Exp Bot **68**: 1303–1321

Windram O, Madhou P, McHattie S, Hill C, Hickman R, Cooke E, Jenkins DJ, Penfold CA, Baxter L, Breeze E, et al. (2012) Arabidopsis defense against Botrytis cinerea: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. Plant Cell **24**: 3530–3557

Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG (1998) COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science **280**: 1091–1094

Yan Y, Stolz S, Chetelat A, Reymond P, Pagni M, Dubugnon L, Farmer EE (2007) A downstream mediator in the growth repression limb of the jasmonate pathway. Plant Cell **19**: 2470–2483

Zhang XC, Millet YA, Cheng Z, Bush J, Ausubel FM (2015) Jasmonate signalling in Arabidopsis involves SGT1b-HSP70-HSP90 chaperone complexes. Nat Plants **1**: 15049

Zhang Y, Turner JG (2008) Wound-induced endogenous jasmonates stunt plant growth by inhibiting mitosis. PLoS One **3**: e3699

Zhurov V, Navarro M, Bruinsma KA, Arbona V, Santamaria ME, Cazaux M, Wybouw N, Osborne EJ, Ens C, Rioja C, et al. (2014) Reciprocal responses in the interaction between Arabidopsis and the cell-content-feeding chelicerate herbivore spider mite. Plant Physiol **164**: 384–399