# JAZ2 CONTROLS STOMATA DYNAMICS DURING BACTERIAL INVASION

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**ABSTRACT**

Bacterially produced coronatine (COR) facilitates bacterial entry into the plant apoplast by stimulating the opening of stomata, which are pores on the leaves surface controlling gas exchange. Despite the significance of bacterial penetration for pathogenesis, the exact signaling events controlling COR-induced virulence at stomata remain unclear. We found that the COR and JA-Ile co-receptor JAZ2 is constitutively expressed in stomata guard cells and modulate stomatal dynamics during bacterial invasion. *Arabidopsis jaz2* mutants are partially impaired in pathogen recognition-induced stomata closing and are more susceptible to *Pseudomonas syringae*. Conversely, gain-of-function mutations in JAZ2 fully prevent stomatal reopening by COR and are highly resistant to bacterial penetration. We also show that MYC2, MYC3 and MYC4, which are targets of JAZ2, regulate directly the expression of *ANAC19, ANAC55* and *ANAC72* to modulate stomata aperture. Therefore, our results demonstrate the existence of a COI1-JAZ2-MYC2,3,4-ANAC19,55,72 module responsible for the regulation of stomatal aperture that is hijacked by bacterial COR to promote infection. Due to the antagonistic interactions between the SA and JA defense pathways, efforts to increase resistance to biotrophic pathogens result in enhanced susceptibility to necrotrophs, and viceversa. Remarkably, due to its spatial expression the dominant *jaz2Δjas* mutant show unaltered levels of resistance against multiple necrotrophic pathogens. Our results provide a novel strategy for crop protection against bacterial infections that use stomata as entry ports by spatially uncoupling the SA-JA antagonism controlling resistance to both biotrophic and necrotrophic pathogens.

**INTRODUCTION**

Pathogen ingress into host tissue is a critical first step during infection. Phytopathogenic bacteria do not have the means to penetrate the leaf epidermis directly but rather rely on natural surface openings such as stomata, which represent major entry ports for foliar bacterial pathogens (Melotto et al., 2008). To defend themselves, plants have evolved sophisticated strategies to perceive their attacker during the infection process and to translate this perception into an effective immune response. Recognition of highly conserved microbe-associated molecular patterns (MAMPs) by host cell transmembrane pattern recognition receptors (PRRs) leads to MAMP-triggered immunity (MTI) that

restricts pathogen growth (Jones and Dangl, 2006). Upon MAMPs perception plants rapidly close stomata to inhibit the entry of pathogen and host tissue colonization (Melotto et al., 2006; Melotto et al., 2008; Zhang et al., 2008; Liu et al., 2009; Zeng and He, 2010; Zeng et al., 2011; Desclos-Theveniau et al., 2012; Kumar et al., 2012; Singh et al., 2012; Montillet and Hirt, 2013; Montillet et al., 2013; Du et al., 2014). Pathogen-induced stomatal closure requires microbial perception though immune PRRs such as FLAGELLIN-SENSING 2 (FLS2), which perceives the bacterial flagellum (Gomez-Gomez and Boller, 2000). It also requires the plant immune hormones salicylic acid (SA) and abscisic acid (ABA) (Melotto et al., 2006; Zhang et al., 2008; Zeng and He, 2010; Zeng et al., 2011; Du et al., 2014).

*Pseudomonas syringae* is a widespread bacterial pathogen that causes disease on a broad range of economically important plant species and a model pathogen for understanding plant-microbe interactions. In order to infect, *P. syringae* produces a number of phytotoxins and delivers effector proteins into eukaryotic cells (O'Brien et al., 2011; Xin and He, 2013). Many *P. syringae* strains have evolved a sophisticated strategy for manipulating the complex hormonal homeostasis in which plant immunity relies on, by producing coronatine (COR), a mimic of the bioactive jasmonate (JA) hormone, JA-isoleucine (JA-Ile) (Fonseca et al., 2009a). In general terms, SA signaling mediates resistance against biotrophic and hemi-biotrophic microbes such as *P. syringae*, whereas a combination of JA and ethylene (ET) pathways activates resistance against necrotrophs such as the fungal pathogen *Botrytis cinerea* (Robert-Seilaniantz et al., 2011). SA and JA/ET defense pathways generally antagonize each other and thus, elevated resistance against biotrophs is often correlated with increased susceptibility to necrotrophs, and vice versa (Glazebrook, 2005). COR contributes to disease symptomatology by inducing chlorotic lesions (Kloek et al., 2001; Brooks et al., 2004; Uppalapati et al., 2007) and facilitates bacterial entry into the plant host by stimulating the reopening of stomata after MAMP-triggered stomatal closure (Melotto et al., 2006; Melotto et al., 2008). COR also promotes bacterial growth in the apoplast by inhibiting SA-dependent defenses required for *P. syringae* resistance, because of its activation of the antagonistic JA pathway (Cui et al., 2005; Laurie-Berry et al., 2006) and enhances bacterial virulence systemically (Zheng et al., 2012).

COR, as the JA-Ile phytohormone, is perceived through a receptor complex formed by the F-box protein CORONATINE-INSENSITIVE 1 (COI1) and JASMONATE ZIM DOMAIN (JAZ) proteins (Xie et al., 1998; Chini et al., 2007; Thines et al., 2007; Katsir et al., 2008; Yan et al., 2009; Sheard et al., 2010). COI1 is the F-box component of a SCF-(Skip-cullin-F-box)-type E3 ubiquitin ligase required for all JA-dependent responses tested so far (Feys et al., 1994; Xie et al., 1998; Xu et al., 2002). COI1 controls the turnover of the JAZ co-receptors that negatively regulate the JA-signaling pathway by directly interacting with and repressing transcription factors (TFs), such as MYC2, that control JA-regulated genes (Chini et al., 2007; Thines et al., 2007; Sheard et al., 2010; Fernandez-Calvo et al., 2011; Pauwels and Goossens, 2011). MYC2 was the first identified direct target of JAZ repressors (Chini et al., 2007). Since then, many other JAZ-interacting TFs from different families have been described (Gimenez-Ibanez et al., 2015; Chini et al., in press). Repression of TFs by JAZ is executed by a double mechanism involving; i) the recruitment of the general co-repressor TOPLESS (TPL) by the adaptor protein NOVEL INTERACTOR OF JAZ (NINJA) (Pauwels et al., 2010) and ii) competition with the MEDIATOR25 (MED25) component of the general transcriptional activation machinery for interaction with MYCs (Zhang et al., 2015). Under stress conditions, COR or JA-Ile promotes the formation of JAZ-COI1 complexes, triggering JAZ ubiquitination and subsequent degradation via the 26S proteasome (Chini et al., 2007; Thines et al., 2007; Sheard et al., 2010). This leads to de-repression of the TFs that initiate the transcription of JA-dependent genes (Fonseca et al., 2009b).

During *Arabidopsis* infection, *P. syringae*-produced COR is perceived by COI1 and de-represses MYC2 activating the expression of *ANAC19, ANAC55* and *ANAC72* TFs, which are direct targets of MYC2. Subsequently, these ANACs inhibit SA biosynthesis, which facilitates reopening of the stomata and suppress SA-mediated plant immunity against the bacteria (Zheng et al., 2012). This NAC activity is conserved in other plants since the closely related NAC TFs, JA2 (JASMONIC ACID 2) and JA2L (JA2-LIKE) differentially regulate stomatal closure and reopening during *Pseudomonas* attack in tomato (Du et al., 2014). MYC2 can not be the only TF controlling COR-induced susceptibility because *myc2* loss-of-function mutants still induce *ANAC* expression in the presence of COR (Zheng et al., 2012). Indeed, it is currently unclear whether *Arabidopsis* plants lacking the *MYC2* gene are affected in COR-mediated stomatal

reopening during *Pseudomonas* pathogenesis as are the *anac19/anac55/anac72* triple mutants.

Despite our increasing understanding of JA signaling, the specific JAZ repressors and their TF targets that modulate COR-induced stomatal reopening during bacterial infection remain elusive. Here, we demonstrate that JAZ2 is the major JAZ co-receptor of COR in stomata guard cells that regulates stomatal aperture by a signaling cascade composed of the signaling module COI1-JAZ2-MYC2,3,4-ANAC19,55,72. This module is hijacked by bacterial-derived COR to promote infection by inducing JAZ2 degradation and the subsequent activation of MYC2, MYC3 and MYC4, which in turn activate ANAC19, ANAC55 and ANAC72. A dominant mutation in JAZ2, resistant to COR-induced degradation, fully prevents stomatal reopening by COR and provides resistance to bacterial infection. *Arabidopsis* plants lacking the *JAZ2* gene are partially impaired in stomata closure upon pathogen perception and are more susceptible to *Pseudomonas*. Remarkably, due to the fact that JAZ2 is expressed primarily at the stomata guard cells, these mutants show unaltered levels of resistance to necrotrophic pathogens because *jaz2* mutation does not significantly affect apoplastic defenses. Therefore, our results evidence novel cell type specific strategies for crop protection against bacterial infections that use stomata as entry ports, without compromising whole leaf resistance to necrotrophic pathogens.

## RESULTS

### *JAZ2* is expressed at stomata guard cells.

To identify *JAZ* genes specifically expressed at stomata, we produced transgenic *Arabidopsis* lines harboring a promoter construct covering around 2 Kb upstream of the ATG of each *JAZ* fused to the *β-glucuronidase* (*GUS*) reporter gene. We successfully obtained stable transgenic plants expressing the corresponding *JAZ* promoter:*GUS* fusions for seven out of the 13 *JAZ* genes (*JAZ1, JAZ2, JAZ3, JAZ5, JAZ6, JAZ9* and *JAZ12*). Detailed examination of *GUS* expression driven by *JAZ* regulatory sequences at the stomata revealed that *JAZ2* was the only one among these *JAZs* expressed in guard cells (Figure 1). In basal conditions, the *JAZ2* promoter was strongly and exclusively expressed in guard cells, indicating that JAZ2 has an important function at the stomata. JA treatment induced it in roots and mesophyll cells of young seedlings, which partially masked the guard cell signal. Histochemical analyses of *pJAZ2:GUS* transgenics into the *coi1-30* background revealed that basal expression of *JAZ2* at the stomata occurs in a COI1-independent manner (Figure 1). However, COI1 is required for JA-responsiveness of *JAZ2* in mesophyll and root cells.

### JAZ2 regulates stomata dynamics during bacterial pathogenesis.

The localization of *JAZ2* in guard cells suggested that regulates stomatal dynamics during bacterial invasion. In order to address this hypothesis, we first obtained an *Arabidopsis* transposon insertion mutant, designated *jaz2-3,* in the accession Nossen (No-0), and selected homozygous plants (Figure S1). Gene expression analyses supported that this mutant is a knock-out, or at least a severe knock-down, since it does not express the full-length *JAZ2* gene, and expresses very low levels of truncated mRNA 3' downstream the insertion (Figure S1).

We next analyzed the ability of *jaz2-3* to close stomata upon microbial perception and reopen it in the presence of COR. MAMPs in crude boiled *P. syringae* pv. *tomato* DC3000 (*Pto* DC3000) bacterial extracts induce stomata closing (Kunze et al., 2004; Gimenez-Ibanez et al., 2009a), whereas addition of COR to these extracts promote stomata reopening (Melotto et al., 2006; Melotto et al., 2008). Thus, we incubated whole leaves with crude *Pto* DC3000 bacterial extracts (MAMPs), MAMPs plus COR or a mock solution as a control. MAMPs induced stomatal closure in WT plants and *coi1-30* mutants. However, the single *jaz2-3* mutant was partially impaired in MAMP-

induced stomata closing (Figure 2A). Incubation of leaves with MAMPs and COR, simultaneously, triggered COR-induced stomata reopening in *jaz2-3* and WT plants, but not in *coi1-30* (Figure 2A). These results support previous observations indicating that COR-induced reopening of stomata is dependent on COR perception through the receptor COI1 (Melotto et al., 2006), and suggests that JAZ2 plays a role in modulating stomata closure after bacterial perception.

Pathogen-induced stomatal closure in *Arabidopsis* depends on SA synthesis and signaling pathways (Melotto et al., 2006; Zeng and He, 2010; Zeng et al., 2011; Arnaud and Hwang, 2015). Thus, we next evaluated the ability of WT and the single *jaz2-3* mutant to activate SA signaling upon MAMP perception by monitoring the expression of the SA marker genes *PR1, PR2* and *PR5*. MAMPs induced significantly *PR1, PR2* and *PR5* expression in WT plants after 5 hours of incubation (Figure 2B). In contrast, MAMP induced expression of these genes was strongly compromised in the single *jaz2-3* mutant compared to its control mock treatment. This suggests that the compromised ability of plants lacking *JAZ2* to trigger MAMP induced stomatal closure might be the consequence of a defective activation of SA signaling pathway upon pathogen perception.

In addition to induce stomatal reopening, COR is also essential for the bacteria to overcome the mesophyll cell-based defenses occurring in the apoplast (Cui et al., 2005; Laurie-Berry et al., 2006; Zeng et al., 2011; Zheng et al., 2012). To differentiate the contribution of JAZ2 to bacterial defense by either regulating stomata aperture and/or mesophyll cell-based defenses in the apoplast, we compared bacterial replication of *Pto* DC3000 on WT (No-0) and *jaz2-3 Arabidopsis* plants infected by spray inoculation or syringe infiltration. The spray inoculation technique mimics natural infection conditions and is one of the most sensitive techniques to assess plant susceptibility to bacterial pathogens (Zipfel et al., 2004). In contrast, the syringe infiltration bypasses the early stomatal level of regulation measuring mainly apoplastic cell-based defenses. Three days post-spray inoculation bacterial titers in *jaz2-3* plants were significantly higher than those in its respective WT control. However, the titers were more similar in *jaz2-3* and WT after infiltration (Figure 2C). These results indicate that JAZ2 positively regulates disease resistance to *Pseudomonas* bacteria and support a predominant role for

JAZ2 in early penetration stages of the bacteria through the stomata compared to the weak role of JAZ2 on apoplastic cell-based defenses.

**Dominant *jaz2Δjas* mutants are impaired in COR-induced stomata reopening and are resistance to *P. syringae* infections.**

Truncated JAZ forms lacking the C-terminal Jas domain (JAZΔJas) are resistant to COI1-dependent degradation and behave as constitutive active repressors, blocking the activity of TFs and conferring JA-insensitivity (Chini et al., 2007; Katsir et al., 2008; Sheard et al., 2010; Moreno et al., 2013). To further evaluate the effect of this dominant repressor in stomatal dynamics we searched for *Arabidopsis* T-DNA insertion mutants that would result in JAZ2ΔJas forms under the control of its natural genomic context. We identified a T-DNA line (GABI collection) that contained an insertion in the third exon (Figure S2), and therefore, translated *JAZ2* into an aberrant protein lacking the C-terminal Jas domain. This mutant was designated *jaz2Δjas.*

As a control for specificity, we also included in these analyses other *jazΔjas* mutants from *JAZ* genes not expressed at stomata. Thus, we used the previously described *jai3-1* dominant mutant (hereafter named *jaz3Δjas*) (Chini et al., 2007) and a newly identified *jaz1Δjas* in the *Arabidopsis* accession Col-7 (Figure S3), which also behaves as a dominant mutant as shown below. *JAZ3* was prevalently expressed in roots, both in basal and JA induced conditions (Figure S4). Similarly, *JAZ1* was also expressed in roots in basal conditions whereas JA treatment strongly induced *JAZ1* in roots and mesophyll cells (Figure S4). Therefore, *JAZ1, JAZ2* and *JAZ3* have different expression patterns, which suggest that they may have diverse functional specificities.

To assess the effect of these different mutations in stomatal and apoplastic immunity, we incubated whole leaves of *jaz1Δjas, jaz2Δjas* and *jaz3Δjas* mutants and their respective WT backgrounds with MAMPs, MAMPs plus COR or a mock control, as described above. Similar to WT and *coi1-30* plants, the dominant version of JAZ1, JAZ2 and JAZ3 closed stomata when incubated with MAMPs, indicating that these repressive truncated JAZs forms do not compromise stomata closing upon microbial perception (Figure 3A). When leaves were incubated with MAMPs and COR simultaneously, WT, *jaz1Δjas* and *jaz3Δjas Arabidopsis* plants induced COR-mediated stomatal reopening as expected (Figure 3A). However, similar to *coi1-30, jaz2Δjas*

mutants were fully impaired in COR-mediated stomatal reopening. This indicates that JAZ2 regulates stomata dynamics and that COR-induced stomata reopening require inhibition of JAZ2.

We next compared bacterial replication of *Pto* DC3000 on *Arabidopsis* WT (Col-0 and Col-7), *jaz1Δjas, jaz2Δjas, jaz3Δjas* and *coi1-30* plants infected by spray inoculation or syringe infiltration. Infections with sprayed *Pto* DC3000 bacteria showed similar symptom development on WT and *jaz3Δjas* plants (Figure 3B). In contrast, *jaz1Δjas* and *jaz2Δjas* were remarkably more resistant than WT and resembled *coi1-30* mutants, with very few chlorotic symptoms typical of *Pseudomonas* infections (Figure 3B). Plant symptomatology correlated well with bacterial titers. Similar to *coi1-30* plants, *jaz1Δjas* and *jaz2Δjas* leaves sprayed with *Pto* DC3000 contained remarkably lower bacterial titers than WT, whereas bacterial counts in *jaz3Δjas* plants were significantly higher and close to WT levels (Figure 3C). Still, bacterial levels in *jaz2Δjas* leaves were always slightly higher than those in *coi1-30* plants. We next performed *Pto* DC3000 infection assays by syringe infiltration of *Pto* DC3000 into the apoplast. *jaz3Δjas* leaves contained similar bacterial counts as those observed in WT (Col-0) plants, indicating that JAZ3 does not play a role in regulating COR-induced stomatal aperture nor apoplastic defense responses in aerial tissues (Figure 3C). In contrast, *jaz1Δjas* leaves contained still lower bacterial counts compared to WT plants when *Pto* DC3000 bacteria were infiltrated onto the leaf, suggesting that JAZ1 plays a major role in the regulation of apoplastic defense responses (Figure 3C). Remarkably, disease susceptibility could be mostly restored in the *jaz2Δjas* mutant when bypassing stomata regulation through syringe injection of bacteria (Figure 3C). Indeed, differences in bacterial growth observed when *Pto* DC3000 bacteria was sprayed were significantly diminished when the same bacteria were injected into the leaf, supporting the idea that JAZ2 functions during the early penetration process of *Pto* DC3000 bacteria by specifically regulating stomatal aperture. Still, *jaz2Δjas* leaves typically contained slightly less bacterial counts than WT plants which reflects a minor effect of JAZ2 on aploplastic defense.

To further evaluate whether COR contributes to promote bacterial pathogenicity by targeting JAZ2, we sprayed *Pto* DC3000 or the COR-deficient *Pto* DC3000 *cor*− bacteria in WT, *jaz2Δjas* and *coi1-30* plants. As we previously observed, three days

after spray with *Pto* DC3000, WT leaves contained higher bacterial titers than *jaz2Δjas* and *coi1-30* mutants (Figure 3D). In contrast, all WT, *jaz2Δjas* and *coi1-30* plants exhibited similar levels of bacterial growth when the COR-deficient *Pto* DC3000 *cor−* was sprayed onto the leaf (Figure 3D). These results indicate that the virulence effect of COR during the bacterial infective process requires elimination of JAZ2, and therefore, cannot be exerted in this constitutively active variant of JAZ2, which is resistant to degradation. Supporting this, further experiments to determine JAZ2 protein stability in the presence of COR at stomata indicated that concentrations as low as 50 μM of COR induced the rapid degradation of JAZ2 protein at guard cells in transgenic *Arabidopsis* plants ectopically expressing *JAZ2* fused to the *GUS* reported gene (Figure S5). Altogether, these data support a prominent role of JAZ2 in regulating stomatal dynamics during the infection process of phytopathogenic *Pseudomonas* bacteria.

**JAZ2 has a minor role in JA-responses outside the guard cell**

To further study other possible roles of JAZ2 outside guard cell regulation, we analyzed other typical JA-regulated responses, such as root-growth inhibition and anthocyanin accumulation. As shown in Supplemental Figure S6, root-growth inhibition in response to COR was unaffected in either *jaz2-3* or *jaz2Δjas* plants. In contrast, *jaz1Δjas* and *jaz3Δjas* were markedly insensitive to COR, which is consistent with their expression patterns. Regarding anthocyanin accumulation, *jaz1Δjas* plants were severely compromised in their ability to accumulate anthocyanin in response to COR, whereas *jaz2Δjas* and *jaz3Δjas* dominant JAZ versions showed an intermediate phenotype between WT and *coi1-30* plants. *jaz2-3* mutant plants showed completely normal anthocyanin accumulation in response to COR (Figure S6). These results are consistent with the expression patterns of JAZ1, JAZ2 and JAZ3, and indicate that JAZ2 has a primary role in the regulation of stomata aperture and only a minor role in other JA-regulated responses outside guard cells.

**MYC2, MYC3 and MYC4 regulate redundantly COR-mediated stomatal reopening**

COR-induced stomatal reopening and apoplastic defense is achieved through direct activation of *ANAC19, ANAC55* and *ANAC72* by the TF MYC2 (Zheng et al., 2012). However, COR-induced *NAC* induction is not completely abolished in a *myc2* mutant (Zheng et al., 2012), suggesting that additional TFs should play a redundant function

with MYC2 in regulating COR-induced stomatal reopening. Furthermore, it remains to be demonstrated whether *Arabidopsis myc2* mutants are affected in COR-mediated stomatal reopening during *Pseudomonas* pathogenesis as are the *anac19/anac55/anac72* triple mutants (Zheng et al., 2012). MYC3 and MYC4 are also targets of JAZ repressors, JAZ2 among others, and regulate redundantly with MYC2 some JA-Ile-dependent responses, including pathogen resistance (Cheng et al., 2011; Fernandez-Calvo et al., 2011; Niu et al., 2011; Moreno et al., 2013). To investigate whether MYC3 and/or MYC4 could play a redundant role with MYC2 in regulating COR-mediated stomata reopening, we firstly examined the tissue-specific expression patterns of these TFs. Gene expression data in public databases (http://bbc.botany.utoronto.ca/efp) indicated that MYC3 and MYC4 are expressed in mature guard cells, although the expression of MYC4 was much lower (Figure 4A). Promoter-GUS fusion assays of MYC3 and MYC4 confirmed these data (Figure 4B).

We next analyzed the ability of single *myc2*, double *myc2myc3* and triple *myc2myc3myc4* mutants to induce COR-mediated stomatal reopening. We included in these experiments *coi1-30* as a negative control. As expected, all mutants and WT plants closed stomata when incubated with MAMP extracts (Figure 4C). However, in the presence of MAMPs and COR, *myc2myc3myc4* triple mutants could not reopen stomata, similar to *coi1-30* (Figure 4C). *myc2myc3* mutants showed an intermediate phenotype, whereas the single *myc2* mutant induced COR-mediated stomatal reopening similar to WT plants. Altogether, these results indicate that depletion of these three MYCs is sufficient to render plants fully insensitive to COR in terms of stomata reopening and that MYC2, MYC3 and MYC4 play redundant roles in controlling COR-induced stomata reopening.

COI1 regulates both apoplastic defenses and stomatal reopening (Melotto et al., 2006; Zheng et al., 2012). Bacterial growth after infiltration or spray inoculation was very similar in *myc2myc3myc4* and *coi1-30*, further supporting that these MYCs also regulate both defensive processes (Figure 4D). Consistent with a redundant function, the single *myc2* and double *myc2myc3* mutants showed an intermediate phenotype between WT and the triple *myc2myc3myc4* when bacteria were sprayed inoculated onto the leaves or directly infiltrated into the apoplast (Figure 4D). These results are consistent with the effects of *myc2*, *myc3* and *myc4* mutants in stomatal aperture.

We further investigated to which extent COR contributes to promote bacterial pathogenicity through these MYCs. When COR-deficient *Pto* DC3000 *COR−* bacteria was sprayed onto WT and *myc2myc3myc4* plants, both exhibited rather similar levels of bacterial growth compared to the almost three log (cfu/cm$^2$) difference that we typically observed when we sprayed *Pto* DC3000 onto the same plants (Figure 4E), supporting the notion that the majority of the virulence effect of COR is mediated by these three MYCs.

Altogether, these results suggest that MYC2, MYC3 and MYC4 act redundantly controlling both COR-induced stomata reopening and apoplastic defenses.

**JAZ2, MYC2, MYC3 and MYC4 control COR-dependent expression of *ANAC19, ANAC55* and *ANAC72***

We have previously shown that MYC2, MYC3 and MYC4 interact *in vitro* and *in vivo* with JAZ2 (Fernandez-Calvo et al., 2011). We hypothesized that the inability of the dominant JAZ2ΔJas variant to induce COR-mediated stomatal reopening could be due to constitutive repression of these MYCs and, consequently, suppression of MYC-dependent COR-induced *ANAC19/55/72* genes expression. To test this hypothesis, we analyzed the expression of *ANAC19, ANAC55* and *ANAC72* in *jaz2Δjas*, *myc2, myc2myc3* and *myc2myc3myc4* mutants treated with COR or a mock solution for 5 and 20 hours. As shown in Figures 5 and S7, COR-induced expression of *ANAC19, ANAC55* and *ANAC72* was severely compromised in the *jaz2Δjas, myc2 and myc2myc3* mutants and almost completely abolished in the triple *myc2myc3mcy4* mutant. These results indicate that MYC3 and MYC4 are required for full COR-dependent induction of *ANAC19, ANAC55* and *ANAC72* and act additively with MYC2. Moreover, the results also support that JAZ2 is a repressor of *ANAC* gene expression mediated by MYCs in response to COR.

MYC2 can bind the promoter of *ANAC* genes and regulate their expression when overexpressed in transgenic plants (Zheng et al., 2012). Since overexpression is prone to artifacts, we tested whether MYCs, expressed under their natural genomic contexts, could bind directly to the promoter of *ANAC* genes. Thus, we performed chromatin

immuno-precipitation (ChIP) experiments using transgenic "recombineering" *Arabidopsis* plants expressing MYC2-GFP or MYC3-GFP under their native chromatin context, including native 5' and 3' regions (Ref). The results from ChIP showed that MYC2 and MYC3 bind efficiently to the promoters of *ANAC19, ANAC55* and *ANAC72* genes but not to control gene *ACT8* (Figure 6). These results indicate that both MYC2 and MYC3 activate the expression of the three *ANAC*s by direct interaction with their promoters under their natural genomic contexts.

**The *jaz2Δjas* mutant shows unaltered levels of resistance against necrotrophic pathogens**

JA and SA defense pathways generally antagonize each other and thus, strategies to enhance apoplastic defenses against necrotrophs often lead to increased susceptibility to biotrophs, and vice versa (Glazebrook, 2005; Grant and Lamb, 2006). It is well established that compromised JA defenses in mesophyll cells facilitate very aggressively the pathogenesis by necrotrophic fungi (Penninckx et al., 1998; Thomma et al., 1998; Diaz et al., 2002; Thaler et al., 2004; Stasevich and McNally, 2011). In contrast, compromised JA responses at stomata should not alter susceptibility to necrotrophs, which invade the plant tissue by active penetration through enzymes and/or appressoria-like structures that establishes a primary lesion through which can penetrate into the host surface (Mendgen et al., 1996). Thus, a main cell specific JAZ2 function at guard cells implies that *jaz2Δjas* mutation should not compromise apoplastic defenses to necrotrophs. To test this, we measured susceptibility of WT, *jaz1Δjas, jaz2Δjas, jaz3Δjas* and *coi1-30* plants to the necrotrophic fungus *B. cinerea.* Since JAZ1 is expressed in leaves and roots, JAZ3 is preferentially expressed in roots (Figure S4) and COI1 is widely expressed in most tissues, *jaz1Δjas, jaz3Δjas* and *coi1-30* mutants seemed appropriate controls. Consistent with published data, six days after *B. cinerea* infection *coi1-30 Arabidopsis* plants showed enhanced fungal symptoms and increased spore production compared to control plants (Figure 7A). Consistent with JAZ1 expression in leaves, *jaz1Δjas* also supported significantly higher number of spores compared to WT plants despite it was not comparable to the levels of susceptibility of *coi1-30* plants. In contrast, *jaz2Δjas* and *jaz3Δjas* behaved similar to WT in terms of fungal symptoms and spore production (Figure 7A), indicating that the *jaz2Δjas* mutation does not alter apoplastic susceptibility to the necrotrophic fungus *B. cinerea.*

Analyses of other necrotrophic fungi such as *Plectosphaerella cucumerina* (Figure 7B) *and Alternaria brassicicola* (Figure 7c) gave similar results, indicating that *jaz2Δjas* retains WT levels of resistance to a broad range of necrotrophs. Six days after *P. cucumerina* and *A. brassicicola* infection *coi1-30* plants showed massive fungal symptoms and increased spore production compared to WT plants. In contrast, *jaz2Δjas* resembled WT plants with similar symptoms and content of spores in leaves (Figure 7B and 7C). These results further support a key role of JAZ2 in regulating stomatal dynamics, but only a minor role in apoplastic defense responses. Moreover, these results highlight the potential of novel strategies for crop protection by manipulating JA/COR-dependent signaling events at the entry ports of specific microbes through the expression of JAZΔJas forms at guard cells. These new strategies will increase resistance to biotrophs without affecting susceptibility to other general pathogens due to the well-known antagonism between JA and SA.

Altogether, our data indicate that COR-COI1-JAZ2-MYC2/3/4-NAC19/55/72 forms a signaling module controlling stomatal responses during the invasive process of phytopathogenic *Pseudomonas syringae*, whereas other JAZs would likely form such modules to regulate apoplastic defenses.

**DISCUSSION**

Stomata have been classically assumed to merely be passive ports for pathogen entry. However, it is now clear that stomata are an integral part of the plant immune system, and regulation of its aperture prevents pathogen entry into leaves and subsequent colonization of host tissues (Melotto et al., 2006; Melotto et al., 2008; Zhang et al., 2008; Liu et al., 2009; Zeng and He, 2010; Zeng et al., 2011; Desclos-Theveniau et al., 2012; Singh et al., 2012; Montillet et al., 2013; Du et al., 2014). Pathogen-induced stomatal closure requires microbial perception though immune PRRs and the essential plant hormones SA and ABA (Melotto et al., 2006; Zhang et al., 2008; Zeng and He, 2010; Zheng et al., 2012; Du et al., 2014; Arnaud and Hwang, 2015). To evade the stomatal defense layer, *Pseudomonas* produces COR, a bacterial phytotoxin that suppresses stomatal immunity by activating the JA-Ile pathway. COR-induced stomatal reopening requires perception by COI1 and is achieved through activation of the three NAC TFs ANAC19, ANAC55 and ANAC72 that repress SA biosynthetic enzymes and SA accumulation (Zheng et al., 2012). Perception of COR by COI1 requires a JAZ co-

receptor (Sheard et al., 2010), which represses the activity of TFs and is degraded upon COR/JA-Ile-triggered ubiquitination by $SCF^{COI1}$. However, the identity of the specific JAZ repressor and its TFs targets suppressing stomatal immunity remained unknown.

In this work, we filled the signaling gap between the COI1 receptor and the NAC TFs by the identification of JAZ2 and its targets MYC2, MYC3 and MYC4. Thus, we showed that phytopathogenic *Pseudomonas* produces COR to hijack a COI1-JAZ2-MYC2/3/4-NAC19/55/72 signaling module controlling stomatal responses during the invasive process. Cell-specific expression of *JAZ2* at guard cells suggested that JAZ2 could regulate stomatal dynamics during bacterial invasion. Loss- and gain-of-function analyses using *jaz2-3* and *jaz2Δjas* mutants after infiltration or spray inoculation confirmed this hypothesis, and supported the idea that JAZ2 primarily functions at the guard cells. The gain-of-function *jaz2Δjas* allele blocked stomatal reopening and increased resistance to *Pto* DC3000*,* whereas the loss-of-function *jaz2-3* was partially impaired in stomatal closure and more susceptible to the bacteria. This partial phenotype of *jaz2-3* in stomatal closure could be due to redundancy among JAZ proteins. In fact, COR can still be perceived in the *jaz2-3* mutant at the stomatal guard cells, suggesting that in these cells other JAZ proteins should be co-receptors with COI1, in addition to JAZ2. We only succeed analyzing the expression patterns of 7 *JAZs*, and therefore, a role for the remaining JAZs (JAZ4, JAZ7, JAZ8, JAZ10, JAZ11 and JAZ13) at the stomata could be expected.

Similar to other *jaz* loss-of-function mutants (ie. *jaz10*; Yan et al., 2007), the lack of JAZ2 should relieve TFs from repression and, therefore, render the plant tissue hypersensitive to JA, thus antagonizing the SA-dependent closure (Melotto et al., 2006; Zeng and He, 2010; Khokon et al., 2011; Zeng et al., 2011). In line with this, we showed that MAMP-induced *PR* gene expression (SA markers), was strongly compromised in loss-of-function *jaz2-3* mutants compared to WT plants, indicating that the inability of this mutant to trigger stomata closing upon microbial perception is likely due to suppression of SA-dependent closure.

Several lines of evidence indicate that JAZ2 play a major role in controlling stomatal reopening during bacterial invasion and a minor (or redundant) role in apoplastic defenses. Fistly, *jaz2Δjas* plants were remarkably more resistant than WT when *Pto*

DC3000 was sprayed onto the leaves, but only very slightly more resistant when the stomata barrier was bypassed by leaf infiltration of the bacteria. Secondly, *jaz2Δjas* plants did not show altered resistance to necrotrophic fungi, which further supports that its repressive effect on JA signaling does not affect apoplastic defenses. Finally, further analysis of typical JA-mediated responses such as root-growth and anthocyanin accumulation are consistent with a prevalent role of JAZ2 in stomata, since only a minor effect in anthocyanin accumulation was observed in *jaz2Δjas* and none in *jaz2-3* mutants. Therefore, despite *JAZ2* expression is induced by JA in other plant tissues, its role outside guard cells seems to be rather limited. However, redundancy of JAZ2 with other JAZs in these tissues can not be excluded, since its expression is coincident with that of many other *JAZ* genes after JA induction (Boter & Solano, unpublished data) (Grunewald et al., 2009). An interesting conclusion of our results is that although JAZs are generally redundant, they show specificities that depend mainly on their tissue expression patterns. Therefore specific plant responses to JAs may be the consequence of the specific spatio-temporal expression patterns of each JAZ and its targets. This introduces a novel layer of control of JA signaling during abiotic and biotic interactions.

It was previously reported that COR induces *ANAC19*, *ANAC55* and *ANAC72* expression through MYC2, although defects in stomata dynamics have not been reported in *myc2* mutants (Zheng et al., 2012). We found that *MYC3* and *MYC4* are expressed in stomata guard cells and act redundantly with MYC2 in the regulation of stomata closure. Defects in COR-induced stomata reopening were obvious in plants lacking both *MYC2* and *MYC3* genes, and responses were fully blocked in a triple mutant lacking all three genes. Consistently, we found that MYC2, MYC3 and MYC4 additively control COR-dependent expression of *ANAC19, ANAC55* and *ANAC72*. MYC2 and MYC3, expressed under their own genomic contexts, directly bind the promoters and activate expression of *ANAC19, ANAC55* and *ANAC72*. This supports and complements previous data indicating that MYC2 binds to *ANAC* promoters when overexpressed in *Arabidopsis* (Zheng et al., 2012). The redundant role of MYC2, MYC3 and MYC4 in the regulation of stomatal opening is consistent with their identical DNA binding specificities towards the G-box element present in the *ANACs* promoters (Fernandez-Calvo et al., 2011; Zheng et al., 2012).

The function of ANAC19, ANAC55 and ANAC72 is not restricted to the guard cells, but also repress SA defenses in mesophyll cells (Zheng et al., 2012). Our results on susceptibility of *myc* single, double and triple mutants after infiltration or spray inoculation support that MYC2, MYC3 and MYC4 also regulate redundantly COR-induced susceptibility both at stomata and apoplast, similar to COI1 (Fernandez-Calvo et al., 2011; Zheng et al., 2012). In spite of this, *myc2myc3myc4* triple mutants still have higher levels of *ANAC* transcripts and are slightly more susceptible than *coi1-30* plants to *Pto* DC3000. This suggests that additional redundancy among MYC TFs may be expected. In the case of plant fertility, pollen development depends on four MYC TFs (MYC2, MYC3, MYC4 and MYC5; Qi et al., 2015), suggesting that MYC5 is a good candidate to regulate also stomata dynamics..

It is worth noting that our *ANAC* expression analyses were performed in whole seedlings and, therefore, we measured COR-induced *NAC* expression in stomata and apoplast. We were unable to perform these analyses in purified guard cells because stomata purification protocols are based on several enzymatic digestions to obtain protoplast, and protoplasting induces the expression of stress-associated genes including most JA signaling components such as JAZs and MYCs (Leonhardt et al., 2004; Koo et al., 2009; Koo and Howe, 2009; Wang et al., 2011; Obulareddy et al., 2013). Therefore, the lower reduction of *ANAC* gene induction by COR in *jaz2Δjas* compared with that in *myc2myc3myc4* and *coi1-30* likely reflects the induction of *ANACs* in mesophyll cells, not affected by JAZ2. The reduction in ANAC gene induction is higher in *myc2myc3myc4* and *coi1-30* because these genes regulate *ANAC* induction both at stomata and mesophyll cells. Altogether, our results indicate that JAZ2 functions during the early penetration process of *Pto* DC3000 bacteria and is specifically hijacked by COR to regulate stomatal aperture when bacteria is approaching to reach the more benign apoplastic spaces.

Genetic manipulation of defense pathway genes has succeeded enhancing resistance to specific kinds of pathogens. However, due to the antagonistic interactions between the SA and JA defense pathways, efforts to develop plants with broad-spectrum resistance by manipulation of signaling genes had limited success so far (Robert-Seilaniantz et al., 2011; Pieterse et al., 2012). In general, alteration of apoplastic defenses to increase resistance to biotrophic pathogens results in enhanced susceptibility to necrotrophs, and

vice versa. Solving this trade-off is a major challenge for a sustainable agriculture and requires uncoupling the antagonism between hormonal pathways. Remarkably, dominant *jaz2Δjas* mutants showed strong resistance to the hemi-biotroph *P. syringae*, without altering levels of resistance against a broad range of necrotrophic fungi such as *B. cinerea, P. cucumerina* and *A. brassicicola*, which do not use stomata to enter the host. Our results suggest that novel strategies for broad-spectrum crop protection could be possible by manipulating signaling events at the entry ports of specific microbes, which should not affect the JA-SA hormonal antagonism, and therefore, avoid the defense trade-off. In the particular case of JAZ2, the new genome editing technologies, such as TALEN or CRISPR/Cas (Boch et al., 2009; Christian et al., 2010; Belhaj et al., 2013), and the dominant nature of the *jaz2Δjas* mutation should facilitate its use in crops to improve disease resistance to biotrophic bacterial pathogens, without increasing susceptibility to necrotrophs. This strategy could potentially enable more durable and sustainable resistance in the field.

## MATERIAL AND METHODS

### Plant Materials and Growth Conditions

Transgenic *Arabidopsis* lines expressing *pJAZ:GUS* promoter fusions were developed as we previously reported (Fernandez-Calvo et al., 2011). *pJAZ2:GUS* in *coi1-30* background was generated by crossing the corresponding parental single homozygous lines. *Arabidopsis* lines expressing *pMYC3:GUS* and *pMYC4:GUS* promoter fusions, were previously reported (Fernandez-Calvo et al., 2011). The lines *jaz2-3* (RIKEN_ 13-5433-1) and *jaz2Δjas* (GABI_169B06) were obtained from the Nottingham *Arabidopsis* Stock Centre. The line *jaz1Δjas* was obtained from an *Arabidopsis* activation tagging library (Weigel et al., 2000). Knock out lines *myc2/jin1-2* (Lorenzo et al., 2004; Fernandez-Calvo et al., 2011), *myc2/myc3* (Fernandez-Calvo et al., 2011), *myc2/3/4* (Fernandez-Calvo et al., 2011), *jai3-1* (Chini et al., 2007) and *coi1-30* (Yang et al., 2012) were previously described. *coi1-1* was provided by J. Turner. Transgenic *Arabidopsis* plants expressing MYC2-GFP and MYC3-GFP under their native promoters to perform chromatin immuno-precipitation (ChIP) experiments were obatained from the laboratory of Joseph Ecker (Ref). Schweizer et al., 2013

For *in vitro* assays, seedlings of six to eleven days were growth in MS or Johnson's medium at 21ºC under a 16-h-light/8-h-dark cycle. For stomata analysis and bacterial growth assays, *A. thaliana* plants of four to six weeks were grown in controlled environment chambers at an average temperature of 22°C (range 16°C–24°C), with 45%–65% relative humidity under short day conditions (8 h light).

### Bacterial strains

*Pseudomonas* strains used in this study were *Pseudomonas syringae* pv. *tomato* (*Pto*) DC3000 and the coronatine-deficient *Pto* DC3000 strain (*Pto* DC3000 *COR*−) which is a *Pto* DC3000 AK87 mutant that carries mutations in cmaA (coronamic acid A) and cfa6 (coronafacic acid 6) [11].

### GUS staining

For *JAZ promoter:GUS* expression experiments, five to seven days-old seedlings were treated with a mock or 50 μM JA for 2 h and GUS stained as previously described (Fernandez-Calvo et al., 2011). For JAZ2 protein stability experiments, seven to nine

days-old seedlings were treated with a mock or 50 μM COR for 3 h. Then, samples were placed in staining solution containing 50 mM phosphate buffer, pH 7, 0.1% (v/v) Triton X-100 (Sigma-Aldrich), 2 mM 5-bromo-4-chloro-3-indolyl b-D glucuronic acid (X-Gluc, Glycosynth), 1 mM potassium-ferrocyanide (Sigma-Aldrich), and 1 mM potassium-ferricyanide (Sigma-Aldrich) and incubated at 37ºC overnight. After staining, the tissue was soaked several times in 75% ethanol and kept in 5% glycerol until being photographed with a Leica DMR UV/VIS microscope.

**Preparation of *Pto* DC3000 MAMP extracts**

Crude *Pto* DC3000 bacterial extracts (MAMPs) were prepared as previously described (Kunze et al., 2004; Gimenez-Ibanez et al., 2009a). Briefly, *Pto* DC3000 was grown in LB-medium at 28°C on a rotary shaker until $OD_{600}$~0.6-1.0. Bacteria were harvested by centrifugation, washed and resuspended in water (20 to 30% cells [fresh weight]/volume). Crude bacterial extracts were prepared by boiling the bacterial suspensions for 10 minutes and kept as cell containing *Pto* DC3000 MAMP crude extracts at -20 degrees. Typically, *Pto* DC3000 MAMPs were used at optical density at 600 nm $OD_{600}$=1,5 (7,5 $\times 10^8$ cfu/ml) for incubation with leaves (Stomatal Aperture Measurements) or *Arabidopsis* seedlings (Quantitative RT-PCR).

**Stomatal Aperture Measurements**

Whole leaves from 4- to 6-week-old *Arabidopsis* plants were collected and exposed to white light for 1.30 hours while floating in a solution containing 50 mM KCl, 10 μm $CaCl_2$, and 10 mm MES-KOH pH = 6.1 (opening stomata buffer) with moderate sacking (50 rpm) to induce homogenous stomatal aperture among all *Arabidopsis* lines to compare. To do the MAMP-induced closure assays, the previous solution was removed and whole leaves were immersed in opening stomata buffer containing *Pto* DC3000 MAMP extracts at $OD_{600}$=1,5 (7,5 $\times 10^8$ cfu/ml), 2 μM of coronatine or a mock solution. The samples were further incubated under the same previous conditions for 4-5 hours. Then, leaves were rapidly peeled and abaxial epidermal peels were placed on cover slips and observed with a microscope (Leica DMR). Stomatal aperture (width/length) was measured using ImageJ software. Values reported are the mean of twenty stomata. Error bars represent standard error of the mean (SEM). Stomatal experiments were repeated at least three independent times and representative results are shown.

**Bacterial Growth Curves**

Bacterial growth assays in *Arabidopsis* were performed as previously described (Gimenez-Ibanez et al., 2009b). For spraying infection assays, plants were sprayed with a bacterial suspension containing $10^8$ (cfu)/ml bacteria ($OD_{600}$ = 0.2) with 0.04% Silwet L-77. For bacterial growth assays by syringe infiltration, leaves were syringe infiltrated with a bacterial suspension containing $5x10^5$ (cfu)/ml bacteria ($OD_{600}$ = 0.001). Bacterial growth assays by spray inoculation and syringe infiltration were performed simultaneously and thus, results shown represent comparable experiments. Leaf discs were harvested after two or three days and ground in 10 mM $MgCl_2$. Serial dilutions of leaf extracts were plated on LB agar with appropriate antibiotics. Each data point represents the average of seven replicates, each containing two leaves discs from different plants. Error bars indicate standard error of the mean (SEM). These experiments were repeated at least three times with similar results, and representative results are shown.

**Statistical Methods**

Statistical significance based on Students´s *t* test analysis was developed by GraphPad Prism program (*p<0.05; **p<0.01; ***p<0.001).

**Quantitative RT-PCR**

Gene expression experiments were performed with RNA extracted from 7 to 10-day-old seedlings grown on liquid MS media that were treated with 1 µM of COR for 5 or 20 h or a mock solution. For experiments monitoring *PR* gene expression, seedlings were treated with DC3000 MAMP extracts at $OD_{600}$=1,5 (7,5 $\times 10^8$ cfu/ml), 2 µM of coronatine or a mock solution for 5 hours as stomata experiments were performed. Quantitative RT-PCR was performed as it was previously described (Fernandez-Calvo et al., 2011). Briefly, RNA extraction and cleanup was done using Trizol reagent (Invitrogen) followed by RNeasy mini kit (Qiagen) and DNase digestion to remove genomic DNA contamination. cDNA was synthesized from 0.5 to 1 µg of total RNA with the high-capacity cDNA reverse transcription kit (Applied Biosystems). Two microliters from one-tenth diluted cDNA was used to amplify selected genes. For quantitative PCR analysis, Power SYBR Green was used for gene amplification (Applied Biosystems). Quantitative PCR was performed in 96-well optical plates in a

7300 Real Time PCR system (Applied Biosystems). Data analysis shown was done using three technical replicates from one biological sample; similar results were obtained with at least three additional independent biological replicates. Primers for *ANAC19, ANAC55* and *ANAC72* genes used here were previously described (Zheng et al., 2012) and are as follows:

*AtANAC19*, 5′-GCATCTCGTCGCTCAG-3′ and 5′- CTCGACTTCCTCCTCCG-3′;

*AtANAC55*, 5′- GCGCTGCCTCATAGTC-3′ and 5′-CGAGGAATCCCCTCAGT-3′;

*AtANAC72*, 5′- TGGGTGTTGTGTCGAAT-3′ and 5′- ATCGTAACCACCGTAACT-3′; *AtACTIN8*, 5′-CCAGTGGTCGTACAACCGGTA-3′ and 5′-TAGTTCTTTTCGATGGAGGAGCTG-3′; *AtJAZ2* Full Length Forward (*AtJAZ2* FL_F), 5′-ATGCCAAGAGATTCATCTTAAAAAA-3′; *AtJAZ2* Full Length Reverse (*AtJAZ2* FL_R), 5′-GTGATCCTGTTAAAGGTTTACGAGTAG-3′; *Atjaz2-3* Forward (*Atjaz2-3*_F), 5′-TGGATTCTTCTGCTGGTCAA -3′; *Atjas2Δjas* Forward (*jas2Δjas*_F), 5′- TGCTTCTAGCCCAAATCCTG-3′; *Atjas2Δjas* Reverse (*jas2Δjas*_R), 5′- TCTTTGGTCCCAGAGGAAGA-3′; *AtPR1*, 5′-GTGGGTTAGCGAGAAGGCTA-3′ and 5′-ACTTTGGCACATCCGAGTCT-3′;

*AtPR2*, 5′-GTCTGAATCAAGGAGCTTAGCC-3′ and 5′-CCGTAGCATACTCCGATTTGT-3′; *AtPR5*, 5′-TCTCTTCCTCGTGTTCATCACA-3′ and 5′-ACGGTGGTAGGGCAATTGT-3′.

**ChIP-PCR**

For pMYC2:MYC2-GFP and pMYC3:MYC3-GFP ChIP, ten-days-old seedlings were treated with1 μM of COR for 4 h. ChIP was performed as previously described (Busch et al., 2010) and the samples were analyzed by qPCR. Input samples were first used to normalize the results. Fold difference was then calculated by taking ratios between normalized results from the probes (ChIP with antibody against GFP) and the corresponding control (ChIP with no antibody). Finally, the fold enrichment was calculated as the ratio between Col-0 plants and from pMYC2:MYC2-GFP or pMYC3:MYC3-GFP plants. Primers were designed to amplify positions of putative MYC2 and MYC3 binding sites in *ANAC019, ANAC055* and *ANAC072* promoters at -991, -625 and 776 base pairs from transcription start site were , respectively. The primers used for qPCR are as follows:

*AtANAC19* G-box (*At ANAC19* G-box F), 5′-AGCAGCTACTACGAGTTGTGT-3′;

*AtANAC19* G-box (*At ANAC19* G-box R), 5′-CGTGTCCACGTGTCTATCGT-3′;

*AtANAC55* G-box (*At ANAC55* G-box F), 5′-TGTGTCGGCTTGTGGTAGTT-3′;
*AtANAC55* G-box (*At ANAC55* G-box R), 5′-GGGATGAGTTCACTGGATGGT-3′;
*AtANAC72* G-box (*At ANAC72* G-box F), 5′-TGCAATCACTCAGCGGACTT-3′;
*AtANAC72* G-box (*At ANAC72* G-box R), 5′-GGCCGACCTTATCGATGTGT-3′;
and *AtACTIN8* ChIP, (*AtACTIN8* ChIP F) 5′-GACTCAGATCATGTTTGAGACCTTT-3′ and (*AtACTIN8* ChIP R) 5′-ACCGGTTGTACGACCACTGG-3′.

**Botrytis cinerea infection assays**

*B. cinerea* infection assays were performed as previously described (Monte et al., 2014). Briefly five-week-old *Arabidopsis* plants, grown directly in soil under short-day conditions and at 21 °C, were inoculated with 20 µl of a suspension of 5 $\times 10^6$ spores/ml PDB (Difco). At least 15 leaves (3 leaves per plant) were inoculated per treatment. Disease symptoms were scored 6 days after inoculation. Spores were quantified in a hemocytometer with a light microscope (Leica DMR UV/VIS). Five inoculated leaves of five different plants were pooled for each replicate. Three independent replicates were measured for each treatment. This experiment was repeated twice with similar results.

*P. cucumerina* and *A. brassicicola* strains were kindly provided by C. Castresana (CNB-CSIC). Plugs containing micelium were grown in PDA (OXOID). Spores were collected, resuspended in 15% glycerol and stored at −80 °C. *P. cucumerina* and *A. brassicicola* infections assays were performed as previously described for *B. cinerea* but inoculating each leaf with 20 µl of a suspension of 3 $\times 10^7$ (*P. cucumerina*) or 1 $\times 10^6$ (*A. brassicicola*) spores/ml PDB (Difco). Disease symptoms and spores were quantified as previously described for *B. cinerea.*

**Root growth and anthocyanin measurements**

Root growth, chlorophyll and anthocyanin measurements were performed as previously described (Fonseca et al., 2014). In both cases, 20 to 30 seedlings were measured seven to eleven days after germination in presence or absence of 1 µM of COR or a mock control. Values represent mean and standard deviation (SD). Results are representative of at least two independent experiments.

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## REFERENCES

**Arnaud, D., and Hwang, I.** (2015). A sophisticated network of signaling pathways regulates stomatal defenses to bacterial pathogens. Molecular plant **8,** 566-581.

**Belhaj, K., Chaparro-Garcia, A., Kamoun, S., and Nekrasov, V.** (2013). Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods **9,** 39.

**Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A., and Bonas, U.** (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. Science **326,** 1509-1512.

**Brooks, D.M., Hernandez-Guzman, G., Kloek, A.P., Alarcon-Chaidez, F., Sreedharan, A., Rangaswamy, V., Penaloza-Vazquez, A., Bender, C.L., and Kunkel, B.N.** (2004). Identification and characterization of a well-defined series of coronatine biosynthetic mutants of Pseudomonas syringae pv. tomato DC3000. Mol Plant Microbe Interact **17,** 162-174.

**Busch, W., Miotk, A., Ariel, F.D., Zhao, Z., Forner, J., Daum, G., Suzaki, T., Schuster, C., Schultheiss, S.J., Leibfried, A., Haubeiss, S., Ha, N., Chan, R.L., and Lohmann, J.U.** (2010). Transcriptional control of a plant stem cell niche. Dev Cell **18,** 849-861.

**Chen, Z., Kloek, A.P., Boch, J., Katagiri, F., and Kunkel, B.N.** (2000). The Pseudomonas syringae avrRpt2 gene product promotes pathogen virulence from inside plant cells. Mol Plant Microbe Interact **13,** 1312-1321.

**Cheng, Z., Sun, L., Qi, T., Zhang, B., Peng, W., Liu, Y., and Xie, D.** (2011). The bHLH transcription factor MYC3 interacts with the Jasmonate ZIM-domain proteins to mediate jasmonate response in Arabidopsis. Molecular plant **4,** 279-288.

**Chini, A., Gimenez-Ibanez, S., Goossens, A., and Solano, R.** (in press). Redundancy and specificity in jasmonate signalling Current Opinion in Plant Biology.

**Chini, A., Fonseca, S., Fernandez, G., Adie, B., Chico, J.M., Lorenzo, O., Garcia-Casado, G., Lopez-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., and**

**Solano, R.** (2007). The JAZ family of repressors is the missing link in jasmonate signalling. Nature **448,** 666-671.

**Christian, M., Cermak, T., Doyle, E.L., Schmidt, C., Zhang, F., Hummel, A., Bogdanove, A.J., and Voytas, D.F.** (2010). Targeting DNA double-strand breaks with TAL effector nucleases. Genetics **186,** 757-761.

**Cui, J., Bahrami, A.K., Pringle, E.G., Hernandez-Guzman, G., Bender, C.L., Pierce, N.E., and Ausubel, F.M.** (2005). Pseudomonas syringae manipulates systemic plant defenses against pathogens and herbivores. Proc Natl Acad Sci U S A **102,** 1791-1796.

**Desclos-Theveniau, M., Arnaud, D., Huang, T.Y., Lin, G.J., Chen, W.Y., Lin, Y.C., and Zimmerli, L.** (2012). The Arabidopsis lectin receptor kinase LecRK-V.5 represses stomatal immunity induced by Pseudomonas syringae pv. tomato DC3000. PLoS Pathog **8,** e1002513.

**Diaz, J., ten Have, A., and van Kan, J.A.** (2002). The role of ethylene and wound signaling in resistance of tomato to Botrytis cinerea. Plant Physiol **129,** 1341-1351.

**Doares, S.H., Syrovets, T., Weiler, E.W., and Ryan, C.A.** (1995). Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. Proc Natl Acad Sci U S A **92,** 4095-4098.

**Du, M., Zhai, Q., Deng, L., Li, S., Li, H., Yan, L., Huang, Z., Wang, B., Jiang, H., Huang, T., Li, C.B., Wei, J., Kang, L., Li, J., and Li, C.** (2014). Closely related NAC transcription factors of tomato differentially regulate stomatal closure and reopening during pathogen attack. Plant Cell **26,** 3167-3184.

**Fernandez-Calvo, P., Chini, A., Fernandez-Barbero, G., Chico, J.M., Gimenez-Ibanez, S., Geerinck, J., Eeckhout, D., Schweizer, F., Godoy, M., Franco-Zorrilla, J.M., Pauwels, L., Witters, E., Puga, M.I., Paz-Ares, J., Goossens, A., Reymond, P., De Jaeger, G., and Solano, R.** (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.

**Feys, B., Benedetti, C.E., Penfold, C.N., and Turner, J.G.** (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., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R., Miersch, O., Wasternack, C., and Solano, R.** (2009a). (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nat Chem Biol **5,** 344-350.

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

**Fonseca, S., Fernandez-Calvo, P., Fernandez, G.M., Diez-Diaz, M., Gimenez-Ibanez, S., Lopez-Vidriero, I., Godoy, M., Fernandez-Barbero, G., Van Leene, J., De Jaeger, G., Franco-Zorrilla, J.M., and Solano, R.** (2014). bHLH003, bHLH013 and bHLH017 are new targets of JAZ repressors negatively regulating JA responses. PloS one **9,** e86182.

**Gimenez-Ibanez, S., Ntoukakis, V., and Rathjen, J.P.** (2009a). The LysM receptor kinase CERK1 mediates bacterial perception in Arabidopsis. Plant Signal Behav **4,** 539-541.

**Gimenez-Ibanez, S., Boter, M., and Solano, R.** (2015). Novel players fine-tune plant trade-offs. Essays Biochem **58,** 83-100.

**Gimenez-Ibanez, S., Hann, D.R., Ntoukakis, V., Petutschnig, E., Lipka, V., and Rathjen, J.P.** (2009b). AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. Curr Biol **19,** 423-429.

**Glazebrook, J.** (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol **43,** 205-227.

**Gomez-Gomez, L., and Boller, T.** (2000). FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Molecular cell **5,** 1003-1011.

**Grant, M., and Lamb, C.** (2006). Systemic immunity. Curr Opin Plant Biol **9,** 414-420.

**Grunewald, W., Vanholme, B., Pauwels, L., Plovie, E., Inze, D., Gheysen, G., and Goossens, A.** (2009). Expression of the Arabidopsis jasmonate signalling repressor JAZ1/TIFY10A is stimulated by auxin. EMBO Rep **10,** 923-928.

**Jones, J.D., and Dangl, J.L.** (2006). The plant immune system. Nature **444,** 323-329.

**Katsir, L., Schilmiller, A.L., Staswick, P.E., He, S.Y., and Howe, G.A.** (2008). COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc Natl Acad Sci U S A **105,** 7100-7105.

**Khokon, A.R., Okuma, E., Hossain, M.A., Munemasa, S., Uraji, M., Nakamura, Y., Mori, I.C., and Murata, Y.** (2011). Involvement of extracellular oxidative

burst in salicylic acid-induced stomatal closure in Arabidopsis. Plant Cell Environ **34,** 434-443.

**Kloek, A.P., Verbsky, M.L., Sharma, S.B., Schoelz, J.E., Vogel, J., Klessig, D.F., and Kunkel, B.N.** (2001). Resistance to Pseudomonas syringae conferred by an Arabidopsis thaliana coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. Plant J **26,** 509-522.

**Koo, A.J., and Howe, G.A.** (2009). The wound hormone jasmonate. Phytochemistry **70,** 1571-1580.

**Koo, A.J., Gao, X., Jones, A.D., and Howe, G.A.** (2009). A rapid wound signal activates the systemic synthesis of bioactive jasmonates in Arabidopsis. Plant J **59,** 974-986.

**Kumar, A.S., Lakshmanan, V., Caplan, J.L., Powell, D., Czymmek, K.J., Levia, D.F., and Bais, H.P.** (2012). Rhizobacteria Bacillus subtilis restricts foliar pathogen entry through stomata. Plant J **72,** 694-706.

**Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., and Felix, G.** (2004). The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. Plant Cell **16,** 3496-3507.

**Laurie-Berry, N., Joardar, V., Street, I.H., and Kunkel, B.N.** (2006). The Arabidopsis thaliana JASMONATE INSENSITIVE 1 gene is required for suppression of salicylic acid-dependent defenses during infection by Pseudomonas syringae. Mol Plant Microbe Interact **19,** 789-800.

**Leonhardt, N., Kwak, J.M., Robert, N., Waner, D., Leonhardt, G., and Schroeder, J.I.** (2004). Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. Plant Cell **16,** 596-615.

**Liu, J., Elmore, J.M., Fuglsang, A.T., Palmgren, M.G., Staskawicz, B.J., and Coaker, G.** (2009). RIN4 functions with plasma membrane H+-ATPases to regulate stomatal apertures during pathogen attack. PLoS biology **7,** e1000139.

**Lorenzo, O., Chico, J.M., Sanchez-Serrano, J.J., and 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.

**Melotto, M., Underwood, W., and He, S.Y.** (2008). Role of stomata in plant innate immunity and foliar bacterial diseases. Annu Rev Phytopathol **46,** 101-122.

**Melotto, M., Underwood, W., Koczan, J., Nomura, K., and He, S.Y.** (2006). Plant stomata function in innate immunity against bacterial invasion. Cell **126,** 969-980.

**Mendgen, K., Hahn, M., and Deising, H.** (1996). Morphogenesis and mechanisms of penetration by plant pathogenic fungi. Annu Rev Phytopathol **34,** 367-386.

**Monte, I., Hamberg, M., Chini, A., Gimenez-Ibanez, S., Garcia-Casado, G., Porzel, A., Pazos, F., Boter, M., and Solano, R.** (2014). Rational design of a ligand-based antagonist of jasmonate perception. Nat Chem Biol **10,** 671-676.

**Montillet, J.L., and Hirt, H.** (2013). New checkpoints in stomatal defense. Trends Plant Sci **18,** 295-297.

**Montillet, J.L., Leonhardt, N., Mondy, S., Tranchimand, S., Rumeau, D., Boudsocq, M., Garcia, A.V., Douki, T., Bigeard, J., Lauriere, C., Chevalier, A., Castresana, C., and Hirt, H.** (2013). An abscisic acid-independent oxylipin pathway controls stomatal closure and immune defense in Arabidopsis. PLoS biology **11,** e1001513.

**Moreno, J.E., Shyu, C., Campos, M.L., Patel, L.C., Chung, H.S., Yao, J., He, S.Y., and Howe, G.A.** (2013). Negative feedback control of jasmonate signaling by an alternative splice variant of JAZ10. Plant Physiol **162,** 1006-1017.

**Moscatiello, R., Mariani, P., Sanders, D., and Maathuis, F.J.** (2006). Transcriptional analysis of calcium-dependent and calcium-independent signalling pathways induced by oligogalacturonides. Journal of experimental botany **57,** 2847-2865.

**Niu, Y., Figueroa, P., and Browse, J.** (2011). Characterization of JAZ-interacting bHLH transcription factors that regulate jasmonate responses in Arabidopsis. Journal of experimental botany **62,** 2143-2154.

**O'Brien, H.E., Thakur, S., and Guttman, D.S.** (2011). Evolution of plant pathogenesis in Pseudomonas syringae: a genomics perspective. Annu Rev Phytopathol **49,** 269-289.

**Obulareddy, N., Panchal, S., and Melotto, M.** (2013). Guard cell purification and RNA isolation suitable for high-throughput transcriptional analysis of cell-type responses to biotic stresses. Mol Plant Microbe Interact **26,** 844-849.

**Pauwels, L., and Goossens, A.** (2011). The JAZ proteins: a crucial interface in the jasmonate signaling cascade. Plant Cell **23,** 3089-3100.

**Pauwels, L., Barbero, G.F., Geerinck, J., Tilleman, S., Grunewald, W., Perez, A.C., Chico, J.M., Bossche, R.V., Sewell, J., Gil, E., Garcia-Casado, G., Witters,**

**E., Inze, D., Long, J.A., De Jaeger, G., Solano, R., and Goossens, A.** (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. Nature **464,** 788-791.

**Penninckx, I.A., Thomma, B.P., Buchala, A., Metraux, J.P., and Broekaert, W.F.** (1998). Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell **10,** 2103-2113.

**Pieterse, C.M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C.** (2012). Hormonal modulation of plant immunity. Annual review of cell and developmental biology **28,** 489-521.

**Qi, T., Huang, H., Song, S., and Xie, D.** (2015). Regulation of Jasmonate-Mediated Stamen Development and Seed Production by a bHLH-MYB Complex in Arabidopsis. Plant Cell.

**Robert-Seilaniantz, A., Grant, M., and Jones, J.D.** (2011). Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol **49,** 317-343.

**Sheard, L.B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T.R., Kobayashi, Y., Hsu, F.F., Sharon, M., Browse, J., He, S.Y., Rizo, J., Howe, G.A., and Zheng, N.** (2010). Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature **468,** 400-405.

**Singh, P., Kuo, Y.C., Mishra, S., Tsai, C.H., Chien, C.C., Chen, C.W., Desclos-Theveniau, M., Chu, P.W., Schulze, B., Chinchilla, D., Boller, T., and Zimmerli, L.** (2012). The lectin receptor kinase-VI.2 is required for priming and positively regulates Arabidopsis pattern-triggered immunity. Plant Cell **24,** 1256-1270.

**Stasevich, T.J., and McNally, J.G.** (2011). Assembly of the transcription machinery: ordered and stable, random and dynamic, or both? Chromosoma **120,** 533-545.

**Thaler, J.S., Owen, B., and Higgins, V.J.** (2004). The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. Plant Physiol **135,** 530-538.

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

**Thomma, B.P., Eggermont, K., Penninckx, I.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P., and Broekaert, W.F.** (1998). Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proc Natl Acad Sci U S A **95,** 15107-15111.

**Uppalapati, S.R., Ishiga, Y., Wangdi, T., Kunkel, B.N., Anand, A., Mysore, K.S., and Bender, C.L.** (2007). The phytotoxin coronatine contributes to pathogen fitness and is required for suppression of salicylic acid accumulation in tomato inoculated with Pseudomonas syringae pv. tomato DC3000. Mol Plant Microbe Interact **20,** 955-965.

**Wang, R.S., Pandey, S., Li, S., Gookin, T.E., Zhao, Z., Albert, R., and Assmann, S.M.** (2011). Common and unique elements of the ABA-regulated transcriptome of Arabidopsis guard cells. BMC Genomics **12,** 216.

**Weigel, D., Ahn, J.H., Blazquez, M.A., Borevitz, J.O., Christensen, S.K., Fankhauser, C., Ferrandiz, C., Kardailsky, I., Malancharuvil, E.J., Neff, M.M., Nguyen, J.T., Sato, S., Wang, Z.Y., Xia, Y., Dixon, R.A., Harrison, M.J., Lamb, C.J., Yanofsky, M.F., and Chory, J.** (2000). Activation tagging in Arabidopsis. Plant Physiol **122,** 1003-1013.

**Xie, D.X., Feys, B.F., James, S., Nieto-Rostro, M., and Turner, J.G.** (1998). COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science **280,** 1091-1094.

**Xin, X.F., and He, S.Y.** (2013). Pseudomonas syringae pv. tomato DC3000: A Model Pathogen for Probing Disease Susceptibility and Hormone Signaling in Plants. Annu Rev Phytopathol **51,** 473-498.

**Xu, L., Liu, F., Lechner, E., Genschik, P., Crosby, W.L., Ma, H., Peng, W., Huang, D., and Xie, D.** (2002). The SCF(COI1) ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. Plant Cell **14,** 1919-1935.

**Yan, J., Zhang, C., Gu, M., Bai, Z., Zhang, W., Qi, T., Cheng, Z., Peng, W., Luo, H., Nan, F., Wang, Z., and Xie, D.** (2009). The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. Plant Cell **21,** 2220-2236.

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

**Yang, D.L., Yao, J., Mei, C.S., Tong, X.H., Zeng, L.J., Li, Q., Xiao, L.T., Sun, T.P., Li, J., Deng, X.W., Lee, C.M., Thomashow, M.F., Yang, Y., He, Z., and He, S.Y.** (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc Natl Acad Sci U S A **109,** E1192-1200.

**Zeng, W., and He, S.Y.** (2010). A prominent role of the flagellin receptor FLAGELLIN-SENSING2 in mediating stomatal response to Pseudomonas syringae pv tomato DC3000 in Arabidopsis. Plant Physiol **153,** 1188-1198.

**Zeng, W., Brutus, A., Kremer, J.M., Withers, J.C., Gao, X., Jones, A.D., and He, S.Y.** (2011). A genetic screen reveals Arabidopsis stomatal and/or apoplastic defenses against Pseudomonas syringae pv. tomato DC3000. PLoS Pathog **7,** e1002291.

**Zhang, F., Yao, J., Ke, J., Zhang, L., Lam, V.Q., Xin, X.F., Zhou, X.E., Chen, J., Brunzelle, J., Griffin, P.R., Zhou, M., Xu, H.E., Melcher, K., and He, S.Y.** (2015). Structural basis of JAZ repression of MYC transcription factors in jasmonate signalling. Nature **525,** 269-273.

**Zhang, W., He, S.Y., and Assmann, S.M.** (2008). The plant innate immunity response in stomatal guard cells invokes G-protein-dependent ion channel regulation. Plant J **56,** 984-996.

**Zheng, X.Y., Spivey, N.W., Zeng, W., Liu, P.P., Fu, Z.Q., Klessig, D.F., He, S.Y., and Dong, X.** (2012). Coronatine promotes Pseudomonas syringae virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. Cell Host Microbe **11,** 587-596.

**Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E.J., Jones, J.D., Felix, G., and Boller, T.** (2004). Bacterial disease resistance in Arabidopsis through flagellin perception. Nature **428,** 764-767.

## FINANTIAL DISCLOSURE

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## COMPETING INTEREST

The authors declare that they have no competing interests.

## ABBREVIATIONS

MAMPs: Microbe-associated molecular patterns; COR: Coronatine; Cfu: Colony forming units; Dpi: Days post inoculation; EV: Empty vector; WT: wild type; Hpi: Hours post inoculation; GUS: β-glucuronidase; JA: Jasmonic acid; JA-Ile: Jasmonate-Isoleucine; SA: salycilic acid; *P. syringae : Pseudomonas syringae; Pto* DC3000: *P. syringae* pv. tomato stain DC3000; *B. cinerea*: *Botrytis cinerea.*

## FIGURE LEGENDS

**Figure 1. Tissue Expression Patterns of JAZ2.**

Histochemical GUS activity of 6 days old *Arabidopsis* transgenic seedlings expressing the GUS reporter gene under the control of the JAZ2 promoter (pJAZ2:GUS). Seedlings were treated with 50 μM of JA or mock for 2 hours . GUS activity was detected after overnight staining.

**Figure 2. JAZ2 regulates stomatal dynamics during *Pseudomonas* infection**

**(A)** Stomatal aperture in *Arabidopsis* No-0, No-0 *jaz2-3*, Col-0 and Col-0 *coi1-30* leaves measured after 5 hours of incubation with MAMPs ($OD_{600}$=1.5 of crude *Pto* DC3000 extracts), MAMPs plus 2 μM COR or a mock control. Error bars indicate standard error of the mean (SEM) ($n$ = 20). Different letters above columns indicate significant differences compared to mock-treated No-0 control sample (Student's $t$ test, $p<0.001$). The results are representative of four independent experiments.

**(B)** Quantitative RT-PCR analysis of *PR1, PR2* and *PR5* expression on *Arabidopsis* No-0 and *jaz2.3* seedlings induced for 5 hours with MAMPs ($OD_{600}$=1.5 of boiled *Pto* DC3000) or a mock control. The measurements (three technical replicates) represent the ratio of expression levels between each sample and mock treated No-0. All samples were normalized against the housekeeping gene *AtACT8*. Error bars represent standard deviation (SD). The results are representative of two independent experiments. Asterisks indicate statistically significant differences in the gene induction between MAMP and mock treatments in each *Arabidopsis* line (Student's $t$ test, *$p<0.05$; **$p<0.01$; ***$p<0.001$).

**(C)** Growth of *Pto* DC3000 on *Arabidopsis* No-0 and No-0 *jaz2-3* plants three days after spray inoculation with bacteria at $10^8$ colony-forming units $mL^{-1}$ (cfu/ml) or syringe infiltration with bacteria at $5x10^5$ cfu/ml. Error bars indicate SEM ($n$ = 7). Asterisks indicate statistically significant values in *jaz2-3* lines compared to its No-0 WT control (Student's $t$ test, *p<0.05; **p<0.01; ***p<0.001). The results are representative of three independent experiments.

**Figure 3. Dominant *jaz2Djas* mutants are impaired in COR-induced stomata reopening and resistance to *P. syringae* infections.**

**(A)** Stomatal aperture in *Arabidopsis* Col-7, *jaz1Δjas,* Col-0, *jaz2Δjas, jaz3Δjas* and *coi1-30* leaves measured after 5 hours of incubation with MAMPs ($OD_{600}$=1.5 of crude *Pto* DC3000 extracts), MAMPs plus 2 μM COR or a mock control. Error bars indicate SEM ($n$ = 20). Different letters above columns indicate significant differences compared to their respective mock-treated WT control sample (Col-7 or Col-0) (Student's $t$ test, p<0.001). The results are representative of three independent experiments.

**(B)** *Pto* DC3000 disease symptoms on Col-7, *jaz1Δjas,* Col-0, *jaz2Δjas, jaz3Δjas* and *coi1-30* plants after spray inoculation with *Pto* DC3000 bacteria at $10^8$ cfu/ml. Pictures were taken 4 days after inoculation and show typical chlorosis caused by *Pto* DC3000. Leaves show representative symptoms of three independent experiments.

**(C)** Growth of *Pto* DC3000 on *Arabidopsis* Col-7, *jaz1Δjas*, Col-0, *jaz2Δjas, jaz3Δjas* and *coi1-30* plants three days after spray inoculation with bacteria at $10^8$ cfu/ml or syringe infiltration with bacteria at $5x10^5$ cfu/ml. Error bars indicate SEM ($n$ = 7). Different letters above columns indicate significant differences compared to their respective WT (Col-7 or Col-0) control samples in each infection condition (Student's $t$ test, p<0.05). ns: no significant. The results are representative of three independent experiments.

**(D)** Growth of *Pto* DC3000 or a COR-deficient *Pto* DC3000 strain (*Pto* DC3000 *cor*-) on *Arabidopsis* Col-0, *jaz2Δjas* and *coi1-30* plants three days after spray inoculation with bacteria at $10^8$ cfu/ml. Error bars indicate SEM ($n$ = 7). Different letters above columns indicate significant differences compared to WT plants in each infection condition (Student's $t$ test, p<0.001). The results are representative of three independent experiments.

**Figure 4. MYC2, MYC3 and MYC4 are expressed at stomata and control redundantly COR-induced stomatal reopening**

**(A)** *MYC3* and *MYC4* expression patterns at stomata guard cells. Data from *Arabidopsis* eFP Browser, http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi

**(B)** Histochemical GUS staining of *MYC3* and *MYC4* expression at stomata guard cells.

**(C)** Stomatal aperture in *Arabidopsis* Col-0, *myc2, myc2myc3, myc2myc3myc4* and *coi1-30* leaves measured after 5 hours of incubation with MAMPs ($OD_{600}$=1.5 of crude *Pto* DC3000 extracts), MAMPs plus 2 μM COR or a mock control. Error bars indicate SEM (*n* = 20). Different letters above columns indicate significant differences compared to mock-treated Col-0 control sample (Student's *t* test, p<0.1). The results are representative of three independent experiments.

**(D)** Growth *Pto* DC3000 on *Arabidopsis* Col-0, *myc2, myc2myc3, myc2myc3myc4* and *coi1-30* plants three days after spray inoculation with bacteria at $10^8$ cfu/ml or syringe infiltration with bacteria at $5x10^5$ cfu/ml. Error bars indicate SEM (*n* = 7). Asterisks indicate statistically significant between the indicated *Arabidopsis* lines (Student's *t* test, *p<0.05; **p<0.01; ***p<0.001). ns: no significant. The results are representative of two independent experiments.

**(E)** Growth of *Pto* DC3000 or a COR-deficient *Pto* DC3000 strain (*Pto* DC3000 *cor*-) on *Arabidopsis* Col-0 and *myc2myc3myc4* plants three days after spray inoculation with bacteria at $10^8$ cfu/ml. Error bars indicate SEM (*n* = 7). Asterisks indicate statistically significant differences compared to WT plants in each infection condition at *p<0.05; **p<0.01; ***p<0.001). The results are representative of two independent experiments.

**Figure 5. MYC2, MYC3, MYC4 and JAZ2 are required for full COR-induction of *ANAC19, ANAC55* and *ANAC72* expression**

Quantitative RT-PCR analysis of *ANAC19, ANAC55* and *ANAC72* expression on *Arabidopsis* Col-0, *myc2, myc2myc3, myc2myc3myc4, jaz2Δjas* and *coi1-30* seedlings induced for 5 hours with 1 μM of COR or a mock solution. The measurements (three technical replicates) represent the ratio of expression levels between each sample and mock treated Col-0. All samples were normalized against the housekeeping gene *AtACT8*. Error bars represent standard deviation (SD). The results are representative of three independent experiments. Asterisks indicate statistically significant differences in the gene induction in each *Arabidopsis* line compared to the previous as indicated (Student's *t* test, *p<0.05; **p<0.01; ***p<0.001). ns: no significant

**Figure 6. MYC2 and MYC3 directly bind to the promoter of *ANAC19, ANAC55* and *ANAC72* genes.**

*MYC2-GFP* and *MYC3-GFP* transgenic plants under the control of their own genomic contexts were used for ChIP experiments. Graphic shows fold enrichment of Q-PCR data from ChIP assays with antibody against GFP using the *ACTIN8* gene as negative control. Error bars represent SD of two technical replicates. Each experiment was repeated twice with similar results. Asterisks indicate statistically significant differences compared to Col-0 for each gene (Student's *t* test, *p<0.05; **p<0.01; ***p<0.001).

**Figure 7. *jaz2Δjas* mutants retain WT resistance to necrotrophic fungi**

**(A)** *B. cinerea* disease symptoms (left) and determination of spore number (right) on Col-7, *jaz1Δjas,* Col-0, *jaz2Δjas, jaz3Δjas* and *coi1-30* plants 6 days after inoculation with $5 \times 10^6$ spores per ml.

**(B)** *P. cucumerina* disease symptoms and determination of spore number on Col-0, *jaz2Δjas* and *coi1-30* plants 6 days after inoculation with $3 \times 10^7$ spores per ml.

**(C)** *A. brassicicola* disease symptoms and determination of spore number on Col-0, *jaz2Δjas* and *coi1-30* plants 6 days after inoculation with $1 \times 10^6$ spores per ml.

Leaves in A, B and C show representative symptoms of at least two independent experiments. For quantification, three pools per treatment (containing five leaves from five independent plants per pool) were measured. Error bars in A, B and C represent standard deviation (SD). Asterisks indicate statistically significant differences with their respective WT controls (Col-0 or Col-7) (Student's *t* test, *p<0.05; **p<0.01; ***p<0.001).

**SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental Figure 1. Isolation of the *Arabidopsis jaz2-3* mutant.**

**(A)** *JAZ2* gene model showing *jaz2-3* transposon insertion sites. Primers used in B and C are shown.

**(B)** RT-PCR to detect full-length *JAZ2* transcript accumulation in WT and *jaz2-3* mutant after overnight treatment with 1 μM COR (+) or a mock solution (-).

**(C)** Quantitative RT-PCR to detect *JAZ2* transcript accumulation in WT and *jaz2-3* plants after treatment with 1 μM COR or a mock solution using primers that amplify an *AtJAZ2* C-terminal fragment located after the insertion.

**Supplemental Figure 2. Isolation of the *jaz2Δjas* mutant.**

**(A)** *JAZ2* gene model showing the T-DNA insertion site in *jaz2Δjas*. The Jas motif coding region is shown in red. Primers used in B are shown.

**(B)** RT-PCRs to detect *JAZ2* transcript accumulation in WT (Col-0) and *jaz2Δjas Arabidopsis* mutants after treatment with 50 μM JA (+) or a mock solution (-) for 2 hours. Three different regions of JAZ2 were amplified to characterize the *jaz2Δjas Arabidopsis* mutant: an AtJAZ2 full-length fragment, an AtJAZ2 N-terminal fragment located before the insertion and finally, an AtJAZ2 C-terminal fragment located after the insertion. Primers used are represented in A.

**Supplemental Figure 3. Isolation of the *jaz1Δjas* mutant.**

**(A)** *JAZ1* gene model showing the T-DNA insertion in *jaz1Δjas*. *jaz1Δjas* is a T-DNA insertion mutant in the accession Col-7 (in the Weigel collection; Weigel et al., 2000) that contains a deletion of 7 base pairs leading to a JAZ1 protein with a premature stop codon lacking the C-terminal Jas domain. The Jas motif coding region is shown in red.

Alternativa: (corregir Andrea)

We identified a T-DNA insertion mutant in *JAZ1* in the Weigel collection that we named *jaz1Δjas* (accession Col-7; Weigel et al., 2000). This mutant contained a deletion of 7 base pairs that led to a JAZ1 protein with a premature stop codon lacking the C-terminal Jas domain

**(B)** Amino acid sequence of full length JAZ1 and JAZ1ΔJas proteins. The Jas motif coding region is depicted in red. Common sequences between both proteins are shown in capital letters.

**Supplemental Figure 4. Tissue Expression Patterns of *JAZ1* and *JAZ3*.**

Histochemical GUS activity of 6 days old *Arabidopsis* transgenic seedlings expressing the GUS reporter gene under the control of the promoter of *JAZ1* (*pJAZ1*:GUS) or *JAZ3* (*pJAZ3*:GUS). For treatment with the hormone (JA panels), the seedlings were treated with 50 μM of JA for 2 hours. GUS activity was detected after overnight staining.

**Supplemental Figure 5. COR induced degradation of JAZ2-GUS at stomata.**

Histochemical GUS activity of 8 days old *Arabidopsis* transgenic seedlings expressing the GUS reporter gene or *JAZ2-GUS* under the control of the 35S promoter. Seedlings were treated with 50 μM of COR or a mock solution for 3 hours. GUS activity was detected after an overnight staining.

**Supplemental Figure 6. Effect of the *jaz2Δjas* mutation on root growth inhibition and anthocyanin accumulation by COR.**

**(A)** Root growth inhibition assays of 11 days old *Arabidopsis* seedlings from Col-7, *jaz1Δjas,* Col-0, *jaz2Δjas, jaz3Δjas,* No-0, No-0 *jaz2-3* and *coi1-30* plants grown in 1 μM COR or mock media. Results shown are the mean ± SD of measurements from 30 seedlings.

**(B)** Anthocyanin accumulation of 10 days old *Arabidopsis* seedlings from Col-7, *jaz1Δjas,* Col-0, *jaz2Δjas***,** *jaz3Δjas,* No-0, No-0 *jaz2-3* and *coi1-30* plants grown in 1 μM COR or mock media. Results shown are the mean ± SD of measurements from 30 seedlings.

**Supplemental Figure 7. MYC2, MYC3, MYC4 and JAZ2 are required for full COR-induction of *ANAC19, ANAC55* and *ANAC72* expression**

Quantitative RT-PCR analysis of *ANAC19, ANAC55* and *ANAC72* expression on *Arabidopsis* Col-0, *myc2, myc2myc3, myc2myc3myc4, jaz2Δjas* and *coi1-30* seedlings induced for 20 hours with 1 μM of COR or a mock solution. The measurements (three technical replicates) represent the ratio of expression levels between each sample and mock treated Col-0. All samples were normalized against the housekeeping gene *AtACT8*. Error bars represent standard deviation (SD). The results are representative of three biologically independent experiments. Asterisks indicate statistically significant differences in the gene induction in each *Arabidopsis* line compared to the previous as indicated (Student's *t* test, *p<0.05; **p<0.01; ***p<0.001). ns: no significant