Ciencia Nicolaita 94:27-38; Agosto de 2025 https://doi.org/10.35830/cn.vi94.850

Jasmonic acid induces trichome formation in *Arabidopsis* leaves via the receptor CORONATINE INSENSITIVE 1, and crosstalk with specific auxin signaling components and cell patterning genes

Javier Raya González[1], Aarón Munguía Rodríguez[2], José López Bucio[2]

[1] Facultad de Químico Farmacobiología, Universidad Michoacana de San Nicolás de Hidalgo.
[2] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo. Edificio B3, Ciudad Universitaria; 58030, Morelia, Michoacán, México.

Dates
Received: 7 February 2025
Accepted: 30 May 2025
Published: August 2025

***Corresponding author**
Javier Raya González
javier.raya@umich.mx
ORCID: 0000-0002-6685-5090

Abstract

Trichomes are leaf epidermal cells with important adaptive functions and represent useful systems for studying cell fate determination. Jasmonic acid (JA) and auxin (indole-3-acetic acid; IAA) share signaling elements that influence growth and development, but their roles in epidermal cell differentiation in leaves have been scarcely investigated. In this work, we show that trichome formation was stimulated in a JA concentration-dependent manner in leaves of *Arabidopsis* seedlings, but not in JA resistant *coi1-1* mutants defective on the JA receptor CORONATINE INSENSITIVE 1, or in auxin signaling double mutant *arf7/arf19* or cell specification mutants *gl2* and *rhd6*. Noteworthy, jasmonic acid-related mutants *jar1* and *axr1-3* as well as the triple mutant *tir1*/*afb2*/*afb3* defective in auxin receptors and *slr1*, displayed normal responses to trichome formation induced by jasmonic acid. Our results indicate that the jasmonic acid receptor CORONATINE INSENSITIVE 1 mediates leaf epidermal cell differentiation likely acting in concert with ARF7 and ARF19, GL2 and RHD6 transcription factors.

Keywords: *Arabidopsis thaliana*, differentiation, trichomes, jasmonic acid, auxin.

Introduction

Trichomes are differentiated epidermal cells from the aerial part of plants such as leaves, stems, and flowers (Wagner *et al.,* 2004; Kabir *et al.,* 2024). Trichomes can be single-celled or multicellular, branched or unbranched, and glandular secretory or non-glandular; characteristics often used for species identification (Hauser, 2014). Trichomes assist seed dispersal and function as barriers to protect plants against herbivores and insect attack, fungal infection, parasitic plants, UV radiation, and modulate transpiration (Wagner *et al.,* 2004; Ishida *et al.*, 2008; Runyon *et al.,* 2010).

In *Arabidopsis*, trichomes are unicellular structures that can be either unbranched or have two to five branches (Mathur and Chua, 2000; Schnittger and Hülskamp, 2002). The genetic mechanisms for trichome formation involve the R2R3 MYB/basic helix-loop-helix (bHLH)/WD40 (MBW) complex that includes GLABRA1 (GL1), MYB23, MYB5 and WEREWOLF (WER), required for differentiation of shoot or root epidermal cells (Pesch *et al.,* 2015). The MBW complex transcriptionally activates, among other downstream genes, the homeodomain transcription factor *GLABRA2* (*GL2*) for trichome and root hair differentiation (Rerie *et al.,* 1994; Di Cristina *et al.,* 1996; Masucci *et al.,* 1996; Hung *et al.,* 1998). GL1 acts specifically in trichome patterning, whereas WER modulates root hair patterning, MYB23 works redundantly in both trichome and root hair patterning, and MYB25 regulates mucilage synthesis, seed coat development and trichome morphogenesis (Lee and Schiefelbein, 1999; Kirik *et al.,* 2001; 2005; Li *et al.,* 2009). However, additional genetic components have been involved in root hair formation and development, including the transcription factors ROOT HAIR DEFECTIVE 6 (RHD6) and CAPRICE (CPC) (Wada *et al.,* 1997; Menand *et al.,* 2007). CPC moves into trichoblast cells to establish cell identity, through a positive regulation of RHD6, which is critical for root hair emergence (Menand *et al.,* 2007). *Arabidopsis* mutants affected in CPC or RHD6 are defective in root hair number or emergence, respectively, indicating their essential role in epidermal cell patterning.

Jasmonic acid (JA) and indole-3-acetic acid (IAA) are two important phytohormones that interact in various

developmental processes such as seed germination, root growth, senescence, lateral root formation, and epidermal cell differentiation (Raya-González *et al.,* 2012; Wasternack and Hause, 2013; Pérez-Alonso *et al.,* 2021). Several proteins involved in jasmonate biosynthesis or signaling have been isolated and characterized including the CORONATINE INSENSITIVE1 (COI1), JASMONIC ACID RESISTANT1 (JAR1), and AUXIN RESISTANT1 (AXR1) (Wasternack and Hause, 2013; Browse, 2009; Mittal *et al.,* 2024). Among these, JAR1 conjugates JA to isoleucine forming the bioactive (+)-7-iso-JA-Ile that is perceived by COI1, acting as a jasmonate receptor (Yan *et al.,* 2009; Mittal *et al.,* 2024). The function of AXR1 in both JA and IAA signaling indicates that these regulators interact in modulating developmental processes (Tiryaki and Staswick, 2002), but its possible commonalities orchestrating trichome formation remain to be investigated.

In the present work, we tested the effects of JA on trichome formation on leaves of *Arabidopsis* seedlings. We show that JA increases trichome density in a dose-dependent manner and analyzed this process in a variety of *Arabidopsis* mutants defective in JA and auxin signaling, and epidermal cell patterning. Our results show that COI1, but not JAR1 or AXR1, is involved in jasmonate-induced trichome formation. Indeed, genetic analysis demonstrated that the ARF7 and ARF19 transcription factors, previously involved in the formation of lateral roots in response to auxin, directly or indirectly crosstalk with GL2 and RHD6 to orchestrate trichome development in *Arabidopsis* leaves.

Materials and methods

Biological material and growth conditions

For the different experimental designs, *Arabidopsis thaliana* seeds from Columbia (Col-0) and Wassilewskija (Ws) ecotypes were used, as well as mutants resistant to JA, *coi1-1* (Feys *et al.,* 1994), *jar1-1* (Staswick and Tiryaki, 2004) and *axr1-3 (*Lincoln *et al.,* 1990), mutants defective in auxin signaling, *tir1/afb2/afb3* (Parry *et al.,* 2009), *arf7/arf19* (Wilmoth *et al.,* 2005), *slr1-1* (Fukaki *et al.,* 2002), mutants affected in epidermal cell differentiation *cpc* (Wada *et al.,* 1997), *rhd6* (Masucci and Schiefelbein, 1994), *gl2* (Rerie *et al.,* 1994) and its respective reporter construct *GL2:uidA* (Masucci *et al.,* 1996). Seeds were surface sterilized by placing them in Eppendorf tubes, where 95% (v/v) ethanol was added and shaking applied for 5 min. Ethanol was then removed and 20% (v/v) bleach was added for 7 min. Subsequently, bleach was removed and 5 washes were performed with sterile distilled water, seeds were incubated in darkness at 4 °C for 48 h.

The disinfected seeds were germinated and grown under sterile conditions on agar plates containing 0.2x MS salts (Murashige and Skoog, 1962) supplemented with sucrose 0.6% (w/v), phytagar 1% (w/v) and pH adjusted to 7. Plates were placed vertically at an angle of 65° in a growth chamber (Percival AR-95L) under controlled photoperiod of 16 h of light and 8 h of darkness, light intensity of 100 µmol m^{-2} s^{-1}, temperature of 22 °C and 80% humidity. JA was dissolved in ethanol and added to the media at the required concentrations. In control treatments, the solvent was supplied to the medium in a volume equivalent to the highest concentration of JA tested in the bioassay. MS basal salts mixture and JA were acquired from Sigma-Aldrich, while Phytagar, which was used to solidify the media, was purchased from PhytoTechnology.

For *coi1-1* mutant selection, seeds from a *coi1-1*/*COI1* segregating population were screened for normal primary root growth in agar solidified 0.2x MS medium supplemented with 4 µM JA. Putative JA resistant mutants with long primary roots were selected and transferred to plates with different treatments to analyze trichome development.

Trichome measurements

Arabidopsis trichomes were analyzed using a stereoscopic microscope (Leica, MZ6/L2), at 2X objective. The images were captured using a SAMSUNG SCC 131-A digital camera adapted to the microscope. To determine trichome density in wild-type and mutant seedlings under control conditions and in response to JA, Image J software was used to indicate an area of 1 mm^2 on the leaf surface, and the number of trichomes within the area was quantified. Trichome quantification was performed on the second true leaf of *Arabidopsis* 12 days after germination. For all experiments with wild-type and mutant lines, data were statistically analyzed using the STATISTICA 10 program (Dell StatSoft, Austin, Texas, USA). Univariate and multivariate analysis with a Tukey's post hoc test were performed. Different letters were used to indicate means with significant difference ($P < 0.05$).

Histochemical analysis

For β-glucuronidase activity analysis, transgenic *Arabidopsis* seedlings expressing the *GL2:uidA* marker (Szymanski *et al.,* 1998) were incubated overnight at 37 °C in a micro-plate with 0.1% X-Gluc (5-bromo-4-chloro-

3-indol β-D-glucuronide) dissolved in a phosphate buffer (NaH_2PO_4 and Na_2HPO, 0.1 M, pH 7) with the addition of 2 mM potassium ferrocyanide and 2 mM potassium ferricyanide. For tissue clearing, X-Gluc solution was removed and seedlings were incubated 60 min at 62 °C with 0.24 N HCl in 20% methanol (v/v). The solution was substituted by 7% NaOH (w/v) in 60% ethanol (v/v) for 20 min at room temperature. Seedlings were dehydrated with ethanol treatments at 40%, 20% and 10% (v/v) for a 20 min period each, and fixed in 50% glycerol (v/v). A representative seedling was chosen for each treatment and photographed using the Leica CME microscope. For each treatment at least ten seedlings were analyzed.

Results

Jasmonic acid induces trichome formation in *Arabidopsis*

Previous reports indicate that JA regulates trichome formation (Traw and Bergelson, 2003). However, to the best of our knowledge, detailed information about the connection between JA and IAA in this developmental response is still lacking. The effects of JA on leaf trichome patterning were analyzed in *Arabidopsis* (Col-0) seedlings, which were germinated and grown on agar-solidified Petri plates supplied with 0.2x MS medium in the presence of the solvent (control) or increasing concentrations of JA. **Figure 1** shows that JA increases trichome density in a concentration-dependent manner, stimulating up to twice its numbers in JA concentrations of 16 and 32 µM (**Figure 1a**). Besides increasing trichome number, JA slightly induced anthocyanin production on leaves, particularly at the highest concentrations tested (**Figure 1b**). These results indicate that JA modulates cell differentiation in leaf epidermal cells.

The *coi1-1* mutant is insensitive to jasmonic acid effects in trichome formation

The *CORONATINE INSENSITIVE1* (*COI1*) gene encodes for a jasmonate receptor (Katsir *et al.,* 2008). To understand the involvement of COI1 receptor on

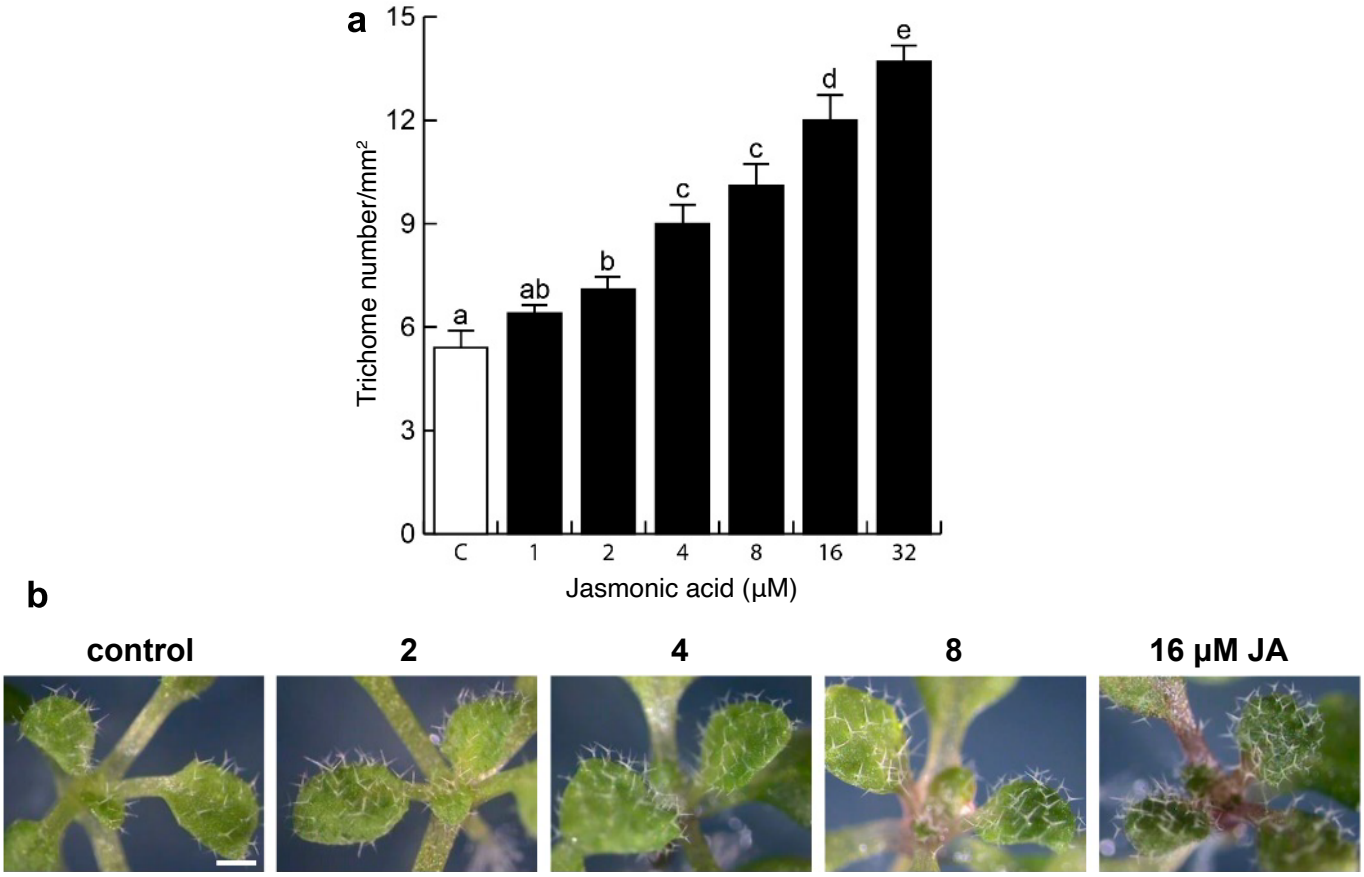

Figure 1. Jasmonic acid promotes trichome formation in *Arabidopsis thaliana*. (**a**) Trichome density in leaves from 12 day-old *Arabidopsis thaliana* seedlings treated with the solvent or increasing jasmonic acid concentrations. Trichome density was determined by counting the number of trichomes per mm^2 of leaf surface (n = 10). Bars indicate the means and standard errors. (**b**) Representative images of leaves from seedlings grown in the different treatments. Scale bar = 1 mm. The experiment was repeated three times with comparable results.

trichome formation, the effect of JA in wild-type and *coi1-1* mutant seedlings was compared. Because *coi1-1* mutant is infertile, homozygous mutant seeds are not available, we then performed a previous selection supplying 8 µM JA and analyzing primary root growth in seedlings from a *coi1-1*/*COI1* segregating population, taking as main indicator the insensitivity of homozygous *coi1* to JA on root growth (Feys *et al.,* 1994). Wild-type and *coi1* seedlings were transferred to agar-solidified MS 0.2x medium supplied with the solvent, or increasing JA concentrations. When compared to the WT, *coi1-1* mutants developed fewer trichomes in leaves in medium without JA. Indeed, the mutants were clearly resistant to the application of JA on trichome formation and anthocyanin production (**Figure 2a, b**). These results indicate that JA requires COI1 to induce trichome formation in leaves.

Jasmonic acid induces trichome formation independently of JAR1 and AXR1

JA signaling involves the genetic components JAR1, which encodes an enzyme that conjugates JA to isoleucine forming JA-Ile (Staswick and Tiryaki, 2004) and AXR1, an *Arabidopsis* auxin-resistance gene, which encodes a protein related to ubiquitin-activating enzyme E1 (Leyser *et al.,* 1993). Initially, both genes were iden-

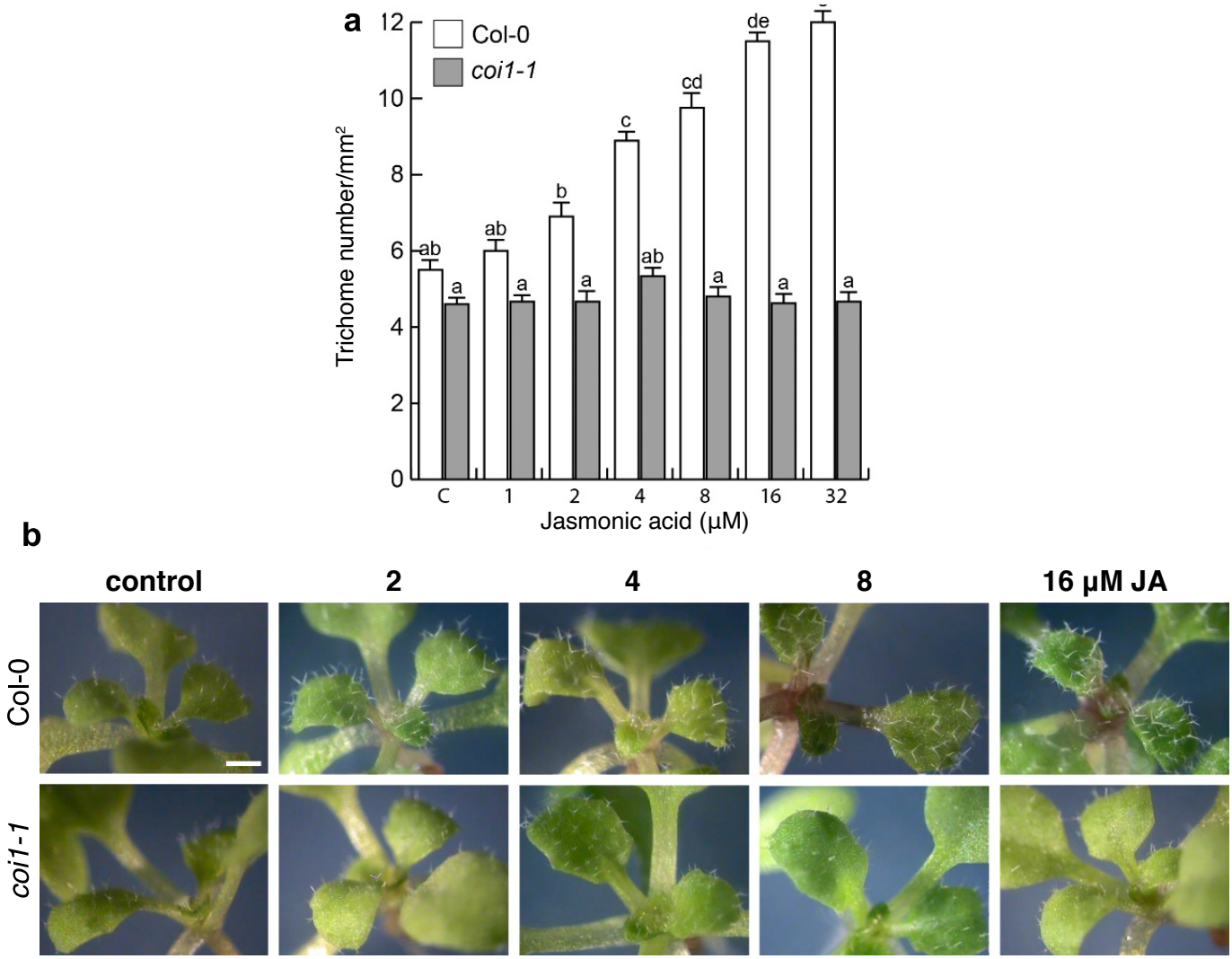

Figure 2. The jasmonic acid receptor *coronatine insensitive 1* (*COI1*) mediates trichome response to jasmonic acid. Wild-type seedlings (Col-0) were germinated and grown during 4 days in MS 0.2X medium and *coi1-1* homozygous seedlings were selected from the segregating population *coi1-1*/*COI1* in medium supplemented with 4 µM JA. Four day-old seedlings were transferred and grown side by side in MS 0.2X medium supplied with the solvent or different concentrations of jasmonic acid. 8 days after transfer, trichome density (**a**) was determined. Scale bar = 1 mm. (**b**) Representative images of leaves from wild-type and *coi1-1* seedlings under different jasmonic acid treatments. The bars in (A) represent the means ± standard error. The experiment was repeated three times with comparable results.

tified from mutants that are resistant to JA on primary root growth. However, there is poor knowledge about their participation in trichome formation in response to JA. Therefore, the responses of WT and JA signaling mutants *axr1-3* and *jar1-1* to the effect of JA on trichome formation were evaluated in leaves of plants 12 days after germination. It was found that both mutant lines presented a similar response to the WT under control conditions and when supplied with increasing JA concentrations (**Figure 3a, b**). These results indicate that trichome formation in response to JA is specifically altered in *coi1* but not in other JA-related *Arabidopsis* mutants.

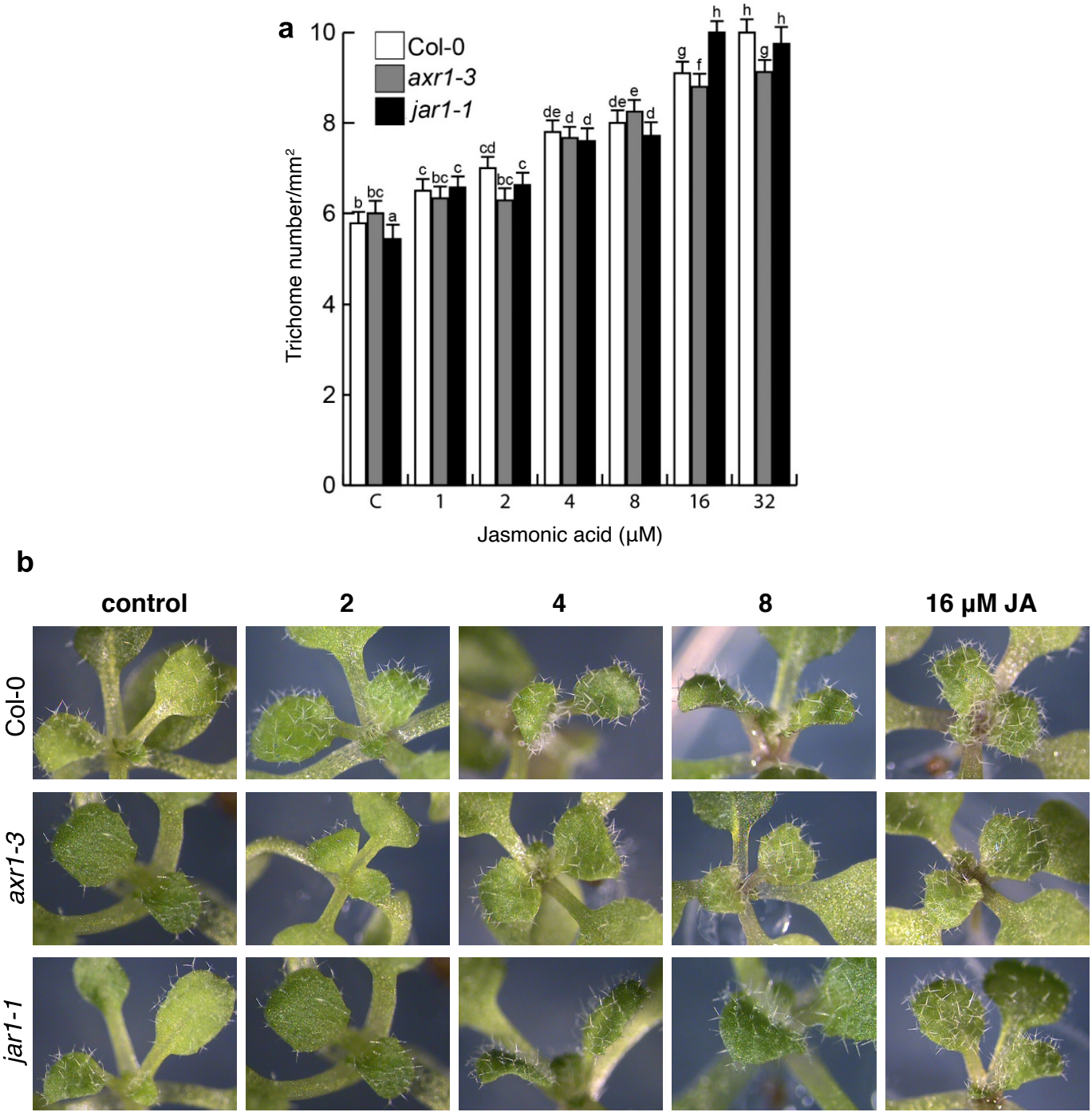

Figure 3. Induction of trichomes by jasmonic acid occurs independently of the signaling elements JAR1 and AXR1. (**a**) Trichome density per mm² on leaves of *Arabidopsis thaliana* seedlings. (**b**) Representative images of leaves from plants grown under varied jasmonic acid supplementation. Scale bar = 1 mm. The experiment was repeated twice with comparable results.

AUXIN RESPONSE FACTORS 7 and 19 mediate jasmonic acid induction of trichomes

JA and IAA interact to orchestrate several developmental programs (Pérez-Alonso *et al.,* 2021). A possible interaction between JA signaling and IAA in trichome formation was determined at the genetic level by analyzing the responses of the WT and a battery of mutants defective in auxin perception and signaling including *tir1*/*afb2*/*afb3*, *arf7*/*arf19* and *slr1* in response to increasing JA supplementation. It was found that the *arf7/arf19* double mutant is resistant to JA promoting effect on trichome formation (**Figure 4**), whereas *tir1/afb2/afb3* and *slr1* undergo a comparable response to wild-type seedlings (**Figure 4**). These results imply ARF7 and ARF19 in JA-induced trichome formation and define a novel JA-IAA genetic interaction at the level of epidermal cell specification.

Role of epidermal specification genes *GL2*, *RHD6* and *CPC* in trichome formation in response to jasmonic acid

The GL2 protein selectively regulates epidermal cell differentiation in *Arabidopsis*, acting as a positive regulator of trichome formation (Masucci *et al.,* 1994). To determine the possible participation of GL2 in JA-induced trichome formation, we evaluated the effect of JA on leaves of wild-type, transgenic *Arabidopsis* seedlings expressing the *GL2:uidA* marker, and *gl2* mutant seedlings. Trichome density was increased in a JA concentration-dependent manner in the WT (**Figure 5a**), which correlated with increased expression of *GL2:uidA* on these structures at the leaf epidermis as the JA concentration increased. On the other hand, under control conditions, *gl2* mutant seedlings lacked trichomes (**Figure 5b**), a phenotype not restored with JA application at low concentrations (1 and 4 μM). Moreover, at a high concentration of JA (16 μM), the formation of aberrant, unbranched trichomes was observed at leaf margins (**Figure 5b**). Thus, JA stimulates trichome formation and differentiation via GL2.

The *RHD6* and *CPC* play critical roles in root hair initiation and epidermal cell differentiation, however, their possible participation on trichome formation remains unknown (Shibata *et al.* 2019). We next decided to study the effect of JA on mutants affected in these genes. As in the WT (Ws ecotype), JA increased trichome density in *Arabidopsis cpc* mutants, being even more sensitive than the WT, since JA increased trichome density by about 20% in *cpc* compared to Ws seedlings (**Figure 6**). Interestingly, *rhd6* forms less trichomes than the WT with or without JA supplementation (**Figure 6**). These results indicate that RHD6 and CPC play opposite roles in JA-induced trichome formation.

Discussion

Trichomes are specialized leaf epidermal cells with relevant protective functions to biotic and abiotic stress. Here, we tested the possible crosstalk between auxin and jasmonic acid signaling in trichome formation in *Arabidopsis* leaves at the genetic level. JA supplementation to the growth medium promoted trichome formation in a dose-dependent manner, an effect coincident to that reported for foliar treatment with JA, or during the leaf wounding response, which increases trichome formation (Traw and Bergelson, 2003).

To determine whether trichome formation in response to JA is orchestrated via canonical components of the JA or IAA signaling pathways, we evaluated the effect of JA on WT *Arabidopsis* seedlings and selected mutants. The F-box protein COI1 acts as a JA receptor, which directly binds JA-Ile (Xie *et al.,* 1998; Yan *et al.,* 2009), while JAR1 is responsible for JA conjugation to isoleucine (Staswick and Tiryaki, 2004) and AXR1 is a subunit of the RUB1-activating enzyme that is necessary for protein degradation required for responses to either JA or IAA (Lincoln *et al.,* 1990). Quantification of trichome numbers in leaves from WT, *coi1-1*, *jar1-1* and *axr1-3*, showed that only *coi1-1* showed resistance to JA-induced trichome formation, which indicates that the formation of these epidermal structures requires the JA receptor COI1, but not the signaling elements JAR1 and AXR1, opening the possibility that other JA conjugates with lower activity may be responsible for trichome formation in those mutants since JAR1 mutation does not dismiss all JA-amino acid conjugates (Staswick and Tiryaki, 2004). These results are consistent with previous reports, which indicate that COI1 is a key component, whereas JAR1 and the JA-signaling transcription factor MYC2, are not involved in trichome formation in response to JA (Traw and Bergelson, 2003; Yoshida *et al.,* 2009). Interestingly, although AXR1 is not implicated in trichome formation, it has been considered a key player in trichome morphogenesis. Recently, Liu *et al.* 2023, isolated an *Arabidopsis* mutant named *aberrantly branched trichome3-1* (*abt3-1*), which showed reduced trichome branching. Genetic mapping indicated that *ABT3* is a new allele of *AXR1* (Liu *et al.*, 2023). Genetic and biochemical analysis showed that AXR1/ABT3 physically interacts with ROP2, a member of Rho GTPase of plants (ROP) family. Transgenic

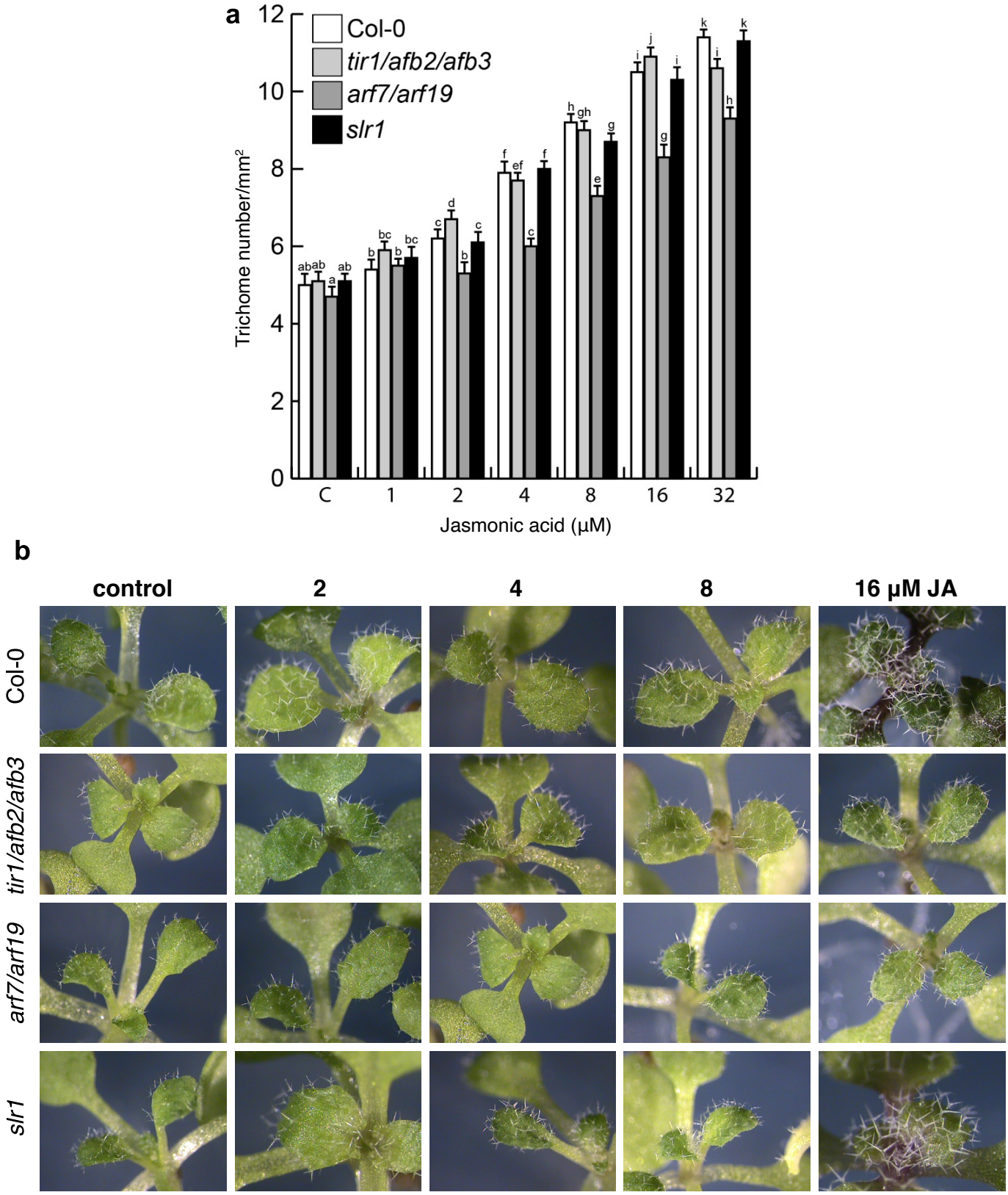

Figure 4. The induction of trichomes by jasmonic acid occurs independently of canonical auxin signaling elements. (**a**) Effect of JA on trichome formation in wild-type and auxin related *tir1*/*afb2*/*afb3*, *arf7*/*arf19* and *slr1* mutant seedlings germinated and grown in medium with the solvent (control) or increasing concentrations of JA. (**b**) Representative images of leaves from plants grown under varied jasmonic acid supplementation. Graph bars represent the means ± standard error. The experiment was repeated twice with comparable results. Scale bar= 1 mm.

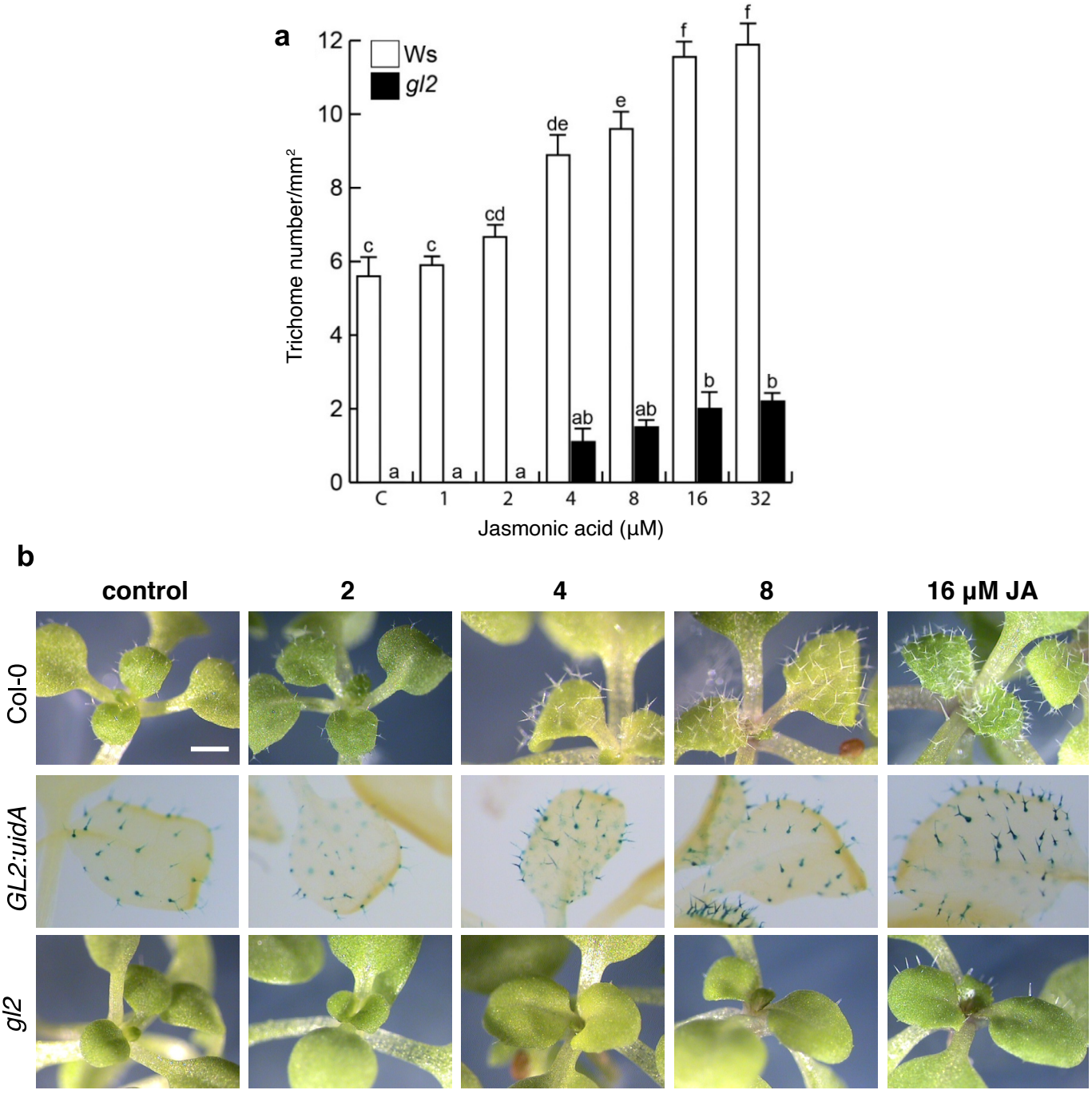

Figure 5. Jasmonic acid induces trichome formation and patterning via GL2. Wild-type (Col-0) *Arabidopsis* seedlings and *gl2* mutants were germinated and grown during 12 days in MS 0.2X medium supplemented with solvent (control) and increasing JA concentrations. (**a**) Trichome number per mm^2 in leaves was determined. The bars represent standard error. (**b**) Representative images of leaves of Col-0, *GL2:uidA* expressing line and *gl2* mutant seedlings grown at increasing JA supplementation. The experiment was repeated three times with similar results. Scale bar = 1 mm.

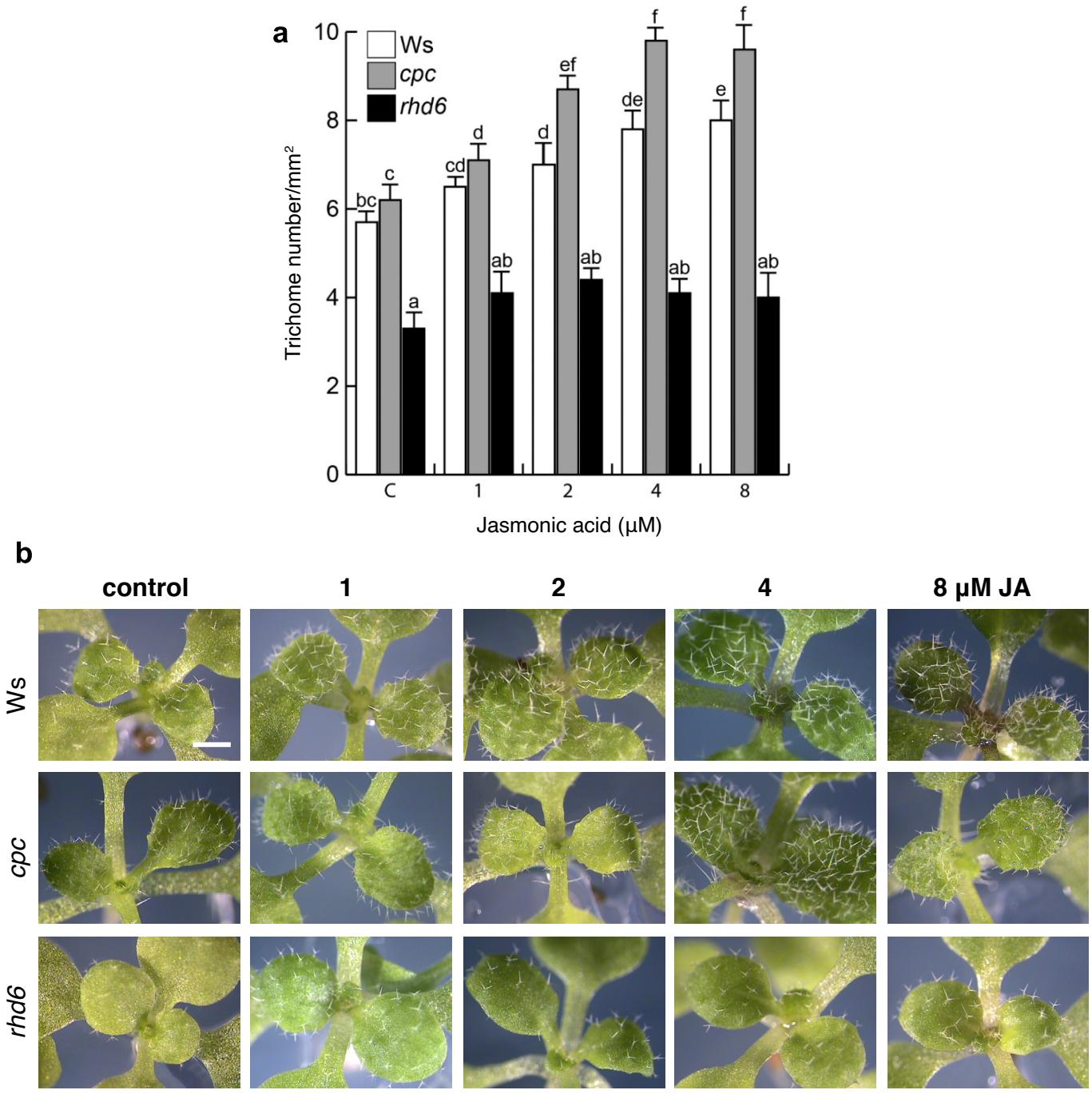

Figure 6. Roles of root hair patterning genes CPC and RHD6 in jasmonic acid induced trichome formation. Wild-type (Ws) seedlings, *cpc* and *rhd6* mutants were germinated and grown for 12 days in MS 0.2X medium supplemented with the solvent (control), 1, 2, 4, and 8 **µ**M JA. (**a**) Number of trichomes per mm^2 was determined by microscopical analysis. Bars represent standard error. (**b**) Representative images of leaves of WT (Wassilewskija, Ws ecotype) besides *cpc* and *rhd6* mutant seedlings grown under different JA treatments. Scale bar = 1 mm. The experiment was repeated three times with comparable results.

plants expressing an active form of ROP2 (CA-ROP2) reduced trichome branching, whereas elevated AXR1 expression in CA-ROP2 transgenic plants, repressed ROP2 expression and rescued the defects of trichome branching in CA-ROP2, indicating that AXR1 negatively regulates ROP2 via ubiquitin-proteasome pathway (Liu *et al.,* 2023).

Examples of crosstalk between IAA and JA signaling involve the auxin-related mutant *axr1*, which is resistant to exogenous supplementation of JA in primary root growth inhibition assays (Tiryaki and Staswick, 2002). Moreover, JA promotes auxin biosynthesis by up-regulating YUCCA8 and YUCCA9 (Hentrich *et al.,* 2013) and homeostasis of IAA and JA can simultaneously be regulated by amidohydrolases (Zhang *et al.,* 2016). However, to the best of our knowledge, whether JA affects trichome development via auxin or auxin-related genetic elements remained to be investigated. The receptors from the TIR1/AFB family and the auxin-signaling repressors Aux/IAAs have well defined functions in growth and epidermal cell specification. For instance, the *tir1* mutant, along with its paralogs *afb1*, *afb2*, and *afb3*, had decreased root hair growth (Dharmasiri *et al.,* 2005). We found that the *tir1/afb2/afb3* and *slr1-1* mutants were equally sensitive to the WT seedlings in trichome formation, whereas the *arf7/arf19* was somewhat resistant to this effect. These data suggest that JA epidermal cell response requires specific components of the auxin perception cascade.

In the *Arabidopsis* root epidermis, hair cell types are specified in a distinct position-dependent pattern determined by transcriptional feedback loops in which CAPRICE (CPC), and GLABRA2 (GL2) genes play a critical role. In addition, the ROOT HAIR DEFECTIVE6 (RHD6) is required for root hair initiation acting in an auxin signaling pathway (Masucci *et al.,* 2004). To further understand the roles of these genes in leaf epidermal patterning, we examined the phenotypes of WT, *gl2*, *rhd6* and *cpc* mutants. At low JA concentrations, no formation of trichomes occurred in leaves of *gl2* mutants, however at the highest JA concentration tested, the formation of aberrant trichomes on leaf margins could be observed, suggesting that a GL2 independent mechanism is employed by JA to promote trichome initiation, but it requires GL2 for a normal cell differentiation program underlying trichome morphology.

Transgenic plants carrying the *GL2:uidA* reporter gene show GUS activity at all developmental stages of trichomes, and in epidermal cells that surrounds the site of trichome initiation (Ohashi *et al.,* 2002). Additionally, we found that the induction of trichome formation

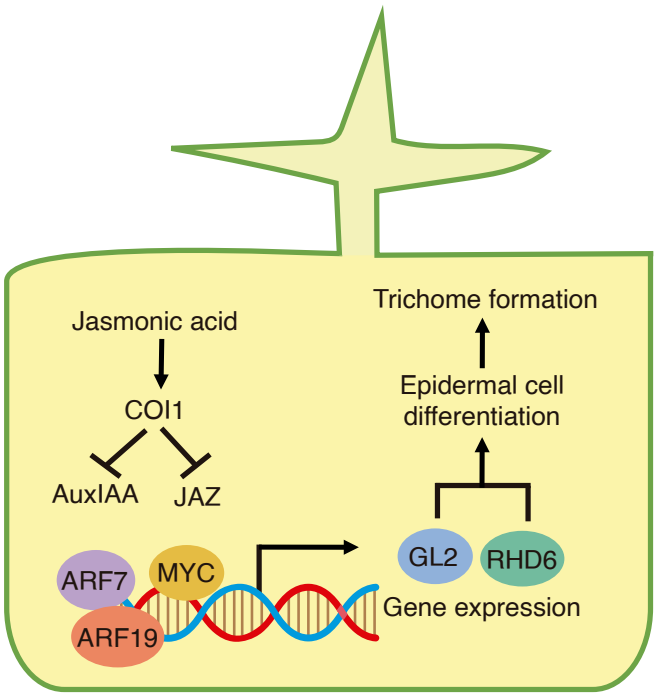

Figure 7. Proposed model for the role of JA in trichome formation. After activation of defense responses (e.g. herbivory), JA is produced and perceived by its receptor COI1, which regulates the activity of the transcription factors ARF7, ARF19, and MYC to control gene expression and epidermal cell differentiation, which leads to trichome formation.

in response to JA was accompanied by *GL2:uidA* expression in leaves indicating that GL2 plays positive roles in mediating the JA-regulated trichome formation. It was observed that seedlings of *rhd6* mutants develop fewer trichomes on leaves, phenotype that could not be rescued by JA. On the other hand, we observed more trichomes in *cpc* mutants compared with the WT irrespective of JA supplementation, thus indicating that CPC acts as a negative regulator for the JA-induced trichome formation in opposition to the proposed role of GL2. A schematic model that summarizes the role of JA on trichome formation is presented in **Figure 7**, in which JA is recognized by its receptor COI1, which triggers the degradation of the JA-signaling repressors of the JAZ family, and also auxin-signaling repressors Aux/IAA. Then, the transcription factors of JA and auxin-responsive genes, including MYC, ARF7, and ARF17, regulate GL2 and RHD6 gene expression, two essential elements for epidermal cell differentiation and trichome formation.

Taken together, our results indicate that JA alters the leaf epidermal cell differentiation program via COI1, and by crosstalk with specific auxin signaling components and cell patterning genes. The possibility that JA could influence this same group of genes for specification

of other kinds of epidermal cells, such as root hairs or stomata, is an open question.

Acknowledgments

Drs. Claire Grierson, Luis Herrera-Estrella, and Mark Estelle are thanked for providing us with *Arabidopsis* mutant seedlings. This work was supported by the Consejo de la Investigación Científica (UMSNH, México).

References

Browse J (2009). The power of mutants for investigating jasmonate biosynthesis and signaling. *Phytochemistry* 70:1539-1546.

Dharmasiri N, Dharmasiri S, Weijers D, Lechner E, Yamada M, Hobbie L, Ehrismann JS, Jurgens G, Estelle M (2005). Plant development is regulated by a family of auxin receptor F box proteins. *Dev. Cell.* 9:109-119.

Di Cristina M, Sessa G, Dolan L, Linstead P, Baima S, Ruberti I, Morelli G (1996). The Arabidopsis Athb-10 (GLABRA2) is an HD-Zip protein required for regulation of root hair development. *Plant J* 10:393-402.

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.

Fukaki H, Tameda S, Masuda H, Tasaka M (2002). Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of *Arabidopsis*. *Plant J* 29:153-168.

Hauser MT (2014). Molecular basis of natural variation and environmental control of trichome patterning. *Front Plant Sci* 5:320.

Hentrich M, Sánchez-Parra B, Pérez Alonso MM, Carrasco Loba V, Carrillo L, Vicente-Carbajosa J, Medina J, Pollmann S (2013). YUCCA8 and YUCCA9 overexpression reveals a link between auxin signaling and lignification through the induction of ethylene biosynthesis. *Plant Signal Behav.* 8(11):e26363.

Hung CY, Lin Y, Zhang M, Pollock S, Marks MD, Schiefelbein J (1998). A common position-dependent mechanism controls cell-type patterning and GLABRA2 regulation in the root and hypocotyl epidermis of *Arabidopsis. Plant Physiol.* 117:73-84.

Ishida, T, Kurata, T, Okada K, Wada, T (2008). A genetic regulatory network in the development of trichomes and root hairs. *Annu. Rev. Plant Biol.* 59:365-386.

Kabir N, Wahid S, Rehman S, Qanmber G (2024). The intricate world of trichome development: From signaling pathways to transcriptional regulation. *Environ. Exper. Bot.* 217,105549.

Katsir L, Schilmiller AL, Staswick PE, He SY, Howe GA (2008). COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proc. Natl. Acad. Sci. USA.* 105:7100-7105.

Kirik V, Lee MM, Wester K, Herrmann U, Zheng Z, Oppenheimer D, Schiefelbein J, Hulskamp M (2005). Functional diversification of MYB23 and GL1 genes in trichome morphogenesis and initiation. *Development*. 132(7):1477-85.

Kirik V, Schnittger A, Radchuk V, Adler K, Hülskamp M, Bäumlein H (2001). Ectopic expression of the Arabidopsis AtMYB23 gene induces differentiation of trichome cells. *Dev Biol*. 235(2):366-77.

Lee MM, Schiefelbein J (1999). WEREWOLF, a MYB-related protein in Arabidopsis, is a position-dependent regulator of epidermal cell patterning. *Cell*. 99(5):473-83.

Leyser HM, Lincoln CA, Timpte C, Lammer D, Turner J, Estelle M (1993). *Arabidopsis* auxin-resistance gene AXR1 encodes a protein related to ubiquitin-activating enzyme E1. *Nature* 364:161-164.

Lincoln C, Britton JH, Estelle M (1990). Growth and development of the *axr1* mutants of *Arabidopsis*. *Plant Cell* 2:1071-1080.

Liu L, Niu L, Ji K, Wang Y, Zhang C, Pan M, Wang W, Schiefelbein J, Yu F, An L (2023). AXR1 modulates trichome morphogenesis through mediating ROP2 stability in Arabidopsis. *Plant J.* 116(3):756-772.

Masucci JD, Schiefelbein JW (1994). The *rhd6* mutation of *Arabidopsis thaliana* alters root-hair initiation through an auxin- and ethylene-associated process. *Plant Physiol*. 106:1335-1346.

Masucci JD, Rerie WG, Foreman DR, Zhang M, Galway ME, Marks MD, Schiefelbein JW (1996). The homeobox gene GLABRA2 is required for position-dependent cell differentiation in the root epidermis of *Arabidopsis thaliana. Development* 122:1253-1260.

Masucci JD, Schiefelbein JW (1994). The *rhd6* mutation of *Arabidopsis thaliana* alters root-hair initiation through an auxin- and ethylene-associated process. *Plant Physiol*. 106(4):1335-1346.

Mathur J, Chua NH (2000). Microtubule stabilization leads to growth reorientation in *Arabidopsis* trichomes. *Plant Cell* 12:465-477.

Menand B, Yi K, Jouannic S, Hoffmann L, Ryan E, Linstead P, Schaefer DG, Dolan L (2007). An ancient mechanism controls the development of cells with a rooting function in land plants. *Science*. 316(5830):1477-80.

Mittal D, Gautam JK, Varma M, Laie A, Mishra S, Behera S, Vadassery J (2024). External jasmonic acid isoleucine mediates amplification of plant elicitor peptide receptor (PEPR) and jasmonate-based immune signalling. *Plant Cell Environ*. 47(4):1397-1415.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Ohashi Y, Oka A, Ruberti I, Morelli G, Aoyama T (2002). Ectopically additive expression of GLABRA2 alters the frequency and spacing of trichome initiation. *Plant J*. 29:359-369.

Parry G, Calderon-Villalobos LI, Prigge M, Peret B, Dharmasiri S, Itoh H, Lechner E, Gray WM, Bennett M, Estelle M (2009). Complex regulation of the TIR1/AFB family of auxin receptors. *Proc. Natl. Acad. Sci*. 106:22540-22545.

Pérez-Alonso MM, Sánchez-Parra B, Ortiz-García P, Santamaría ME, Díaz I, Pollmann S (2021). Jasmonic acid-dependent MYC

transcription factors bind to a tandem G-Box motif in the *YUCCA8* and *YUCCA9* promoters to regulate biotic stress responses. *Int. J. Mol. Sci*. 22(18):9768.

Pesch M, Schultheiss I, Klopffleisch K, Uhrig JF, Koegl M, Clemen CS, Simon R, Weidtkamp-Peters S, Hulskamp M (2015). TRANSPARENT TESTA GLABRA1 and GLABRA1 compete for binding to GLABRA3 in *Arabidopsis*. *Plant Physiol.* 168:584-597.

Raya-González J, Pelagio-Flores R, López-Bucio J (2012). The jasmonate receptor COI1 plays a role in jasmonate-induced lateral root formation and lateral root positioning in *Arabidopsis thaliana*. *J. Plant Physiol.* 169:1348-1358.

Rerie WG, Feldmann, KA, Marks MD (1994). The GLABRA2 gene encodes a homeo domain protein required for normal trichome development in *Arabidopsis. Genes Dev.* 8:1388-1399.

Runyon JB, Mescher MC, De Moraes CM (2010). Plant defenses against parasitic plants show similarities to those induced by herbivores and pathogens. *Plant Signal. Behav*. 5:929-931.

Schnittger A, Hülskamp M (2002). Trichome morphogenesis: a cell-cycle perspective. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 357:823-826.

Shibata M, Sugimoto K (2019). A gene regulatory network for root hair development. *J Plant Res.* 132(3):301-309.

Staswick PE, Tiryaki I (2004). The oxylipin signal jasmonic acid Is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis. Plant Cell* 16:2117-2127.

Szymanski DB, Jilk RA, Pollock SM, Marks MD (1998). Control of *GL2* expression in *Arabidopsis* leaves and trichomes. *Development* 125:1161-1171.

Tiryaki I, Staswick PE (2002). An Arabidopsis mutant defective in jasmonate response is allelic to the auxin-signaling mutant *axr1*. *Plant Physiol.* 130:887-894.

Traw MB, Bergelson J (2003). Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. *Plant Physiol*. 133:1367-1375.

Wada T, Tachibana T, Shimura Y, Okada K (1997). Epidermal cell differentiation in *Arabidopsis* determined by a Myb homolog, CPC. *Science* 277:1113-1116.

Wagner GJ, Wang E, Shepherd RW (2004). New approaches for studying and exploiting an old protuberance, the plant trichome. *Ann. Bot*. 93:3-11.

Wasternack C, Hause B (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Ann. Bot.* 111:1021-1058.

Wilmoth JC, Wang S, Tiwari SB, Joshi AD, Hagen G, Guilfoyle TJ, Alonso JM, Ecker JR, Reed JW (2005). NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *The Plant J.* 43:118-130.

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

Yoshida Y, Sano R, Wada T, Takabayashi J, Okada K (2009). Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in Arabidopsis. *Development*. 136(6):1039-48.

Zhang T, Poudel AN, Jewell JB, Kitaoka N, Staswick P, Matsuura H, Koo AJ (2016). Hormone crosstalk in wound stress response: wound-inducible amidohydrolases can simultaneously regulate jasmonate and auxin homeostasis in *Arabidopsis thaliana. J. Exp. Bot*. 67:2107-2120.