

# Insect Herbivory of Leaves Affects the Auxin Flux Along Root Apices in *Arabidopsis thaliana*

Suli Yan<sup>1,2</sup> · Chunyang Jiao<sup>1,2</sup> · Eric S. McLaamore<sup>3</sup> · Ningning Wang<sup>1,2</sup> · Hongjun Yao<sup>1</sup> · Yingbai Shen<sup>1,2</sup>

Received: 27 August 2016 / Accepted: 25 January 2017  
© Springer Science+Business Media New York 2017

**Abstract** Plants reduce their growth rate when confronted by insect attack, and in turn resources are redirected from growth to enhance resistance against herbivory. In this study, one possible signaling cascade was investigated for establishing the underlying mechanism for the slow growth rate that has been observed following insect herbivory. Our results showed that free jasmonate (JA) and auxin levels were elevated in both leaves and roots after *Plutella xylostella* L. attack which was accompanied by the transcriptional increase of the auxin biosynthetic *YUCCA3* and *YUCCA8* genes. Further examination of endogenous auxin flux using physiological micro-sensor profiling showed that near the surface of the root transition zone, the net auxin flux decreased after insect attack. Conversely, insect herbivory caused an increase in the net H<sup>+</sup> flux along the root surface with the most pronounced response occurring in the transition zone. Transcript levels of auxin transporter

genes *PIN1*, *PIN2*, *PIN3*, *PIN7*, and *AUX1* were also reduced after insect attack. Together, the auxin and H<sup>+</sup> flux results indicate that the reduced growth after insect attack was likely associated with a decrease of auxin flux and proton secretion along the root tip.

**Keywords** Insect herbivory · Jasmonic acid · Auxin flux · Root growth · *Arabidopsis thaliana*

## Introduction

Plants have evolved an arsenal of inducible defenses in response to insect herbivory (Glazebrook 2005). Herbivory-induced plant defense is associated with the relocation of photosynthates from growth and reproduction to the synthesis of defense compounds (Zebelo and others 2014). Therefore, under insect predation, plants inherently slow down the growth rate and invest energy into anti-herbivory defense mechanisms (Coley and others 1985). However, the chemical signals involved in the regulation of plant growth and defense during insect attack are complex, and detailed studies of phytohormone transport are needed to elucidate the underlying spatiotemporal mechanisms. Phytohormones such as auxin, abscisic acid, salicylic acid, and jasmonate are all involved in regulating growth and development (Chen and others 2005; Teale and others 2006; Wasternack and others 2007; Rivas-San Vicente and others 2011).

Auxin is an important growth regulator involved in almost every aspect of plant growth and development (Benková and others 2003; Friml 2003; Blilou and others 2005). The polar transport of auxin is responsible for distribution between root and shoot, and determines root elongation and morphogenesis (Grieneisen and others

Chunyang Jiao has contributed equally to this work.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00344-017-9688-4) contains supplementary material, which is available to authorized users.

✉ Suli Yan  
yansuli2005@126.com

✉ Yingbai Shen  
ybshen@bjfu.edu.cn

<sup>1</sup> College of Biological Sciences and Technology, Beijing Forestry University, Beijing 100 083, China

<sup>2</sup> National Engineering Laboratory for Tree Breeding, Beijing Forestry University, 35, Qinghua East Road, Haidian District, Beijing 100 083, China

<sup>3</sup> Agricultural and Biological Engineering Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, USA

2007). Absciscic acid (ABA) is known to interact with auxin in regulating the resistance of plant roots to water stress (Xu and others 2013). In the salicylic acid (SA)-mediated defense response, SA represses the expression of auxin receptor genes TIR1 and AFBs (Navarro and others 2006; Wang and others 2007). These studies and others suggest that the modulation of polar auxin transport and signaling by other phytohormones is one of the strategies for enhancing plant adaptability under stress.

Jasmonate (JA) also regulates a wide spectrum of plant signaling pathways, including normal development and growth processes as well as defense responses related to insect herbivory, environmental stressors, and pathogen infection. When plants are infected by the pathogen *Alternaria brassicicola*, the accumulation of JA up-regulates auxin biosynthesis and attenuates polar auxin transport in roots (Qi and others 2012). Exogenous methyl jasmonate (MeJA) is also known to modulate endocytosis and accumulation of PIN2 on the plasma membrane (Sun and others 2009). Zhang and Turner (2008) indicated that wounding reduces the growth of wild-type *Arabidopsis thaliana* plants, but not JA synthesis and perception in mutants (Zhang and Turner 2008), which suggests that JA could mediate the alteration of growth under insect herbivory. These studies shed light on the potential mechanism of root elongation inhibition during herbivory-related defense response. Thus, we hypothesize that the interactions between JA and auxin regulate the plant growth rate and root elongation under insect herbivory.

Auxin transport is accompanied by  $H^+$  transport (Taiz and Zeiger 2006). The  $H^+$  transport mediated by the plasma membrane (PM)  $H^+$ -ATPase not only keeps the cytosolic pH stable but also provides the driving force for other ion channels and transporters of secondary metabolites. PM  $H^+$ -ATPase-mediated proton secretion is downstream of the auxin signaling pathway and regulates cell elongation (Rober-Kleber and others 2003), primary root growth, and root hair development (Haruta and Sussman 2012). However, under insect herbivory, it is not known whether  $H^+$  transport and auxin coordinate to control root elongation.

In this study, the influence of insect herbivory on plant growth and root elongation was studied by monitoring free auxin and JA content in the leaves and roots of plants during insect attack. We show that auxin and  $H^+$  flux in *A. thaliana* roots were affected when the leaves were attacked by diamondback moth larva (*Plutella xylostella* L). Our results suggest that down-regulation of auxin transport is an important factor in the inhibition of plant growth and root elongation under insect herbivory.

## Methods

### Plant Material and Growth Conditions

Wild-type *A. thaliana* lines (WT, Columbia ecotype) were used for all experiments. The seeds were surface-sterilized for 10 min in 0.5% NaClO water solution, washed four times in distilled water, and plated on 1/2 MS medium with 7 g agar in 1 L 1/2 MS. Plants were stratified at 4 °C for 2 days in dark and then transferred to an incubator at  $22 \pm 1$  °C, with a white-light illumination of  $150 \mu\text{mol m}^{-2} \text{s}^{-1}$  in a 16/8 h diurnal cycle and a relative humidity of 70%.

### Root Length Measurement

Ten-day-old *Arabidopsis* seedlings were infested by placing first-instar larvae on one leaf ( $n \geq 20$ ). Larvae were removed after the leaf area lost about 30%. The seedlings were photographed every other day throughout a week. The root length was measured by Image-Pro Plus. Measurements were repeated on more than 20 plants. Least significance difference (LSD) (variance neat) or Dunnett's C (variance not neat) was used for significant differences. Error bars denote  $\pm$ SD. According to the Student's *t* test, characters in the figure represent statistically significant differences compared with the corresponding WT ( $a: P < 0.05$ ).

### Auxin and $H^+$ Measurement in Roots

Net flux of  $H^+$  was measured using the ~~non-invasive micro-test (NMT) technique~~ (NMT-100, Younger USA LLC, Amherst, MA) with ASET 2.0 (Science wares, Falmouth, MA) and iFluxes 1.0 Software (Younger USA, LLC.) (Xu and others 2006; Yan and others 2015a). Our measurements were performed as described in Shabala and others (2006) and Yan and others (2015a, b, 2016). An electrolyte solution ( $H^+$ : 40 mM  $\text{KH}_2\text{PO}_4$  and 15 mM NaCl, pH 7.0) was filled in silanized glass micropipettes (2–4  $\mu\text{m}$  aperture), and a selective liquid ion exchange (LIX) cocktail (Sigma) was front-filled to a LIX column length of approximately 25  $\mu\text{m}$ . After being filled, an Ag/AgCl electrode with a holder was inserted until the tip of the wire contacted the surface of the electrolyte solution, and DRI-REF-2 (World Precision Instruments, USA) was used as the reference electrode to form conductive path. The installed electrodes were calibrated with a test buffer (0.1 mM KCl, 0.1 mM  $\text{CaCl}_2$ , 0.1 mM  $\text{MgCl}_2$ , 0.5 mM NaCl, 0.3 mM MES, 0.2 mM  $\text{Na}_2\text{SO}_4$ , pH 5.5–6.5), and only electrodes with Nernstian slopes between +53 and +62 mV/[log- $H^+$ ] were used.

The primary root tips of 10-day-old seedlings with and without *P. xylostella* L. herbivory were fixed on the bottom

of a 35-mm dish incubated in the test buffer (0.1 mM KCl, 0.1 mM CaCl<sub>2</sub>, 0.1 mM MgCl<sub>2</sub>, 0.5 mM NaCl, 0.3 mM MES, 0.2 mM Na<sub>2</sub>SO<sub>4</sub>) at pH 6.0 for 20 min. The H<sup>+</sup> flux of individual roots was then measured by positioning the sensor 1–2 μm away from the root. Computer-controlled stepper motors were used to oscillate the electrode perpendicular to the tangent of the root surface at a distance of 20 μm. The proton concentration gradient (Δ[H<sup>+</sup>]) was continuously monitored while oscillating the electrode at a fixed distance (ΔX). Flux measurements were taken at the following distances from the root cap junction (linearized for root curvature): 0, 100, 200, 300, 400, 600, 700, 800, 900, 1000, 1100, and 1200 μm. The reported flux values were the calculated mean flux from six individual plants. The H<sup>+</sup> flux was calculated as reported by Porterfield and others (2009):

$$J = D \frac{\Delta[H^+] + B_t(LBC/K_a)\Delta[H^+]}{\Delta X},$$

where J is the free proton flux (pmol cm<sup>-2</sup> s<sup>-1</sup>), D is the proton molecular diffusion coefficient (9.22×10<sup>-5</sup> cm<sup>2</sup> s<sup>-1</sup>), [H<sup>+</sup>] is the measured proton concentration gradient (pmol ml<sup>-1</sup>), K<sub>a</sub> is the acid dissociation constant for the buffer (MES 0.000000794), and ΔX is the excursion distance for the microelectrode oscillation (20 μm).

Net auxin flux along the root surface was also measured using NMT. Sensor construction, surface modification, and calibration were performed using the methods described by McLamore and others (2010). Sample preparation and measurement were the same as described above in the H<sup>+</sup> flux measurement. The polarization voltage was +700 mV, and YG003-Y05 (Younger USA, LLC) was used as the reference electrode to complete the circuit. The IAA electrode was calibrated with 0, 2, 4, 6, and 8 (μM) IAA in PBS buffer. Only electrodes with a linear calibration slope ( $R^2 > 0.99$ ) were used in the test. The calibration slope of IAA electrode is shown in Fig. S1. Fick's first law of diffusion ( $J = -D \cdot \Delta C / \Delta X$ ) was used to calculate the auxin flux, where J is the free auxin flux (fmol cm<sup>-2</sup> s<sup>-1</sup>), D is the molecular diffusion coefficient (7×10<sup>-6</sup> cm<sup>2</sup> s<sup>-1</sup>) (Sussman and Goldsmith 1981), ΔC is the auxin concentration gradient, and ΔX is the excursion distance (20 μm).

### IAA and JA Measurement in Leaves and Roots

Eggs of the cruciferous plant-specific insect *P. xylostella* L. were hatched at 25–27°C, and the second-instar larvae were starved for 12 h before all feeding experiments. One second-instar larva was placed on each of the 10-day-old *Arabidopsis* seedlings. After 4–5 h, the larvae were removed and the degree of damage to the seedlings was

recorded. Then the leaves and roots were harvested for JA and IAA measurement.

The quantitative analysis of JA and IAA in plants was performed according to the method published by Pan and others (2010). In brief, (1) the plant tissue (leaves or roots) was ground into powder in liquid nitrogen; (2) a total of 100 mg powder was transferred to a 10-ml centrifuge tube and then mixed with 1 ml of working solution (2-propanol: H<sub>2</sub>O: HCl=2:1:0.002), with internal standard of 2 μl d<sub>5</sub>-IAA (2 ng μl<sup>-1</sup>, Aldrich) and 2 μl H<sub>2</sub>JA (2 ng μl<sup>-1</sup>, OlChemim); (3) the tubes were shaken at 100 rpm for 30 min at 4°C; (4) two milliliters of dichloromethane was added to each sample and shaken for another 30 min in a cold room (4°C); (5) the samples were centrifuged at 4°C and 13,000 g for 5 min, then a 2-ml aliquot of the solvent from the lower phase was transferred into a screw-cap vial and concentrated using a nitrogen evaporator with nitrogen flow, and the samples were re-dissolved in 0.4 ml methanol after complete drying; (6) finally, the re-dissolved extract was filtered through a 0.22-μm membrane filter and injected into a reverse-phase C18 (Agilent SB-C18, 50 mm×4.6 mm, 1.8 μm) Gemini HPLC column for HPLC–ESI–MS/MS analysis (AB QTRAP 5500). The detailed condition of LC–MS/MS is shown in Table S2.

### Quantitative RT-PCR

Leaves from non-treated and *Plutella xylostella* L.-attacked Col-0 seedlings (10-day-old) were used for all quantitative real-time PCR analyses. Total RNA was extracted using the extraction kit (Total RNA, Takara, Otsu, Japan). Followed by DNase treatment according to the manufacturer's instructions, 500 ng of total RNA was reverse transcribed using reverse transcriptase kit (Takara). The primer sequences are listed in Table S1 in the Supplemental Information. Quantitative real-time PCR was performed on a 7300 real-time PCR system (Applied Biosystems) according to the Power SYBR Green PCR Master Mix kit (Applied Biosystems, Foster, CA, USA) protocol and ΔCt method was used for transcript evaluation. The *ACTIN2* gene was used as a reference.

## Results

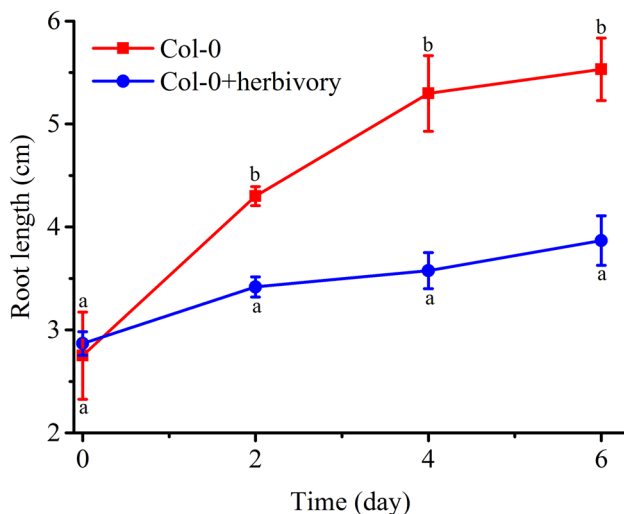
### *P. xylostella* Herbivory Reduces the Growth Rate of *A. thaliana* Seedlings

The effect of insect herbivory on primary root elongation was investigated using *A. thaliana* seedlings growing on 1/2 MS (with 7 g agar in 1 l 1/2 MS) with leaves under attack by *P. xylostella* L. In comparison to control 10-day-old seedlings without herbivory, the root growth rate

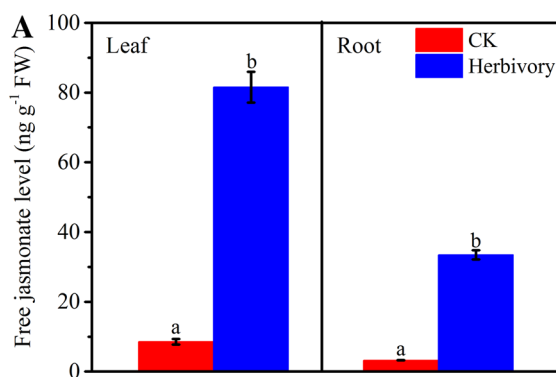
was significantly reduced after *P. xylostella* L. herbivory (Fig. 1).

### Herbivory Elevates Free JA and Auxin Levels in *A. thaliana* Seedlings

To further investigate the underlying mechanism of growth retardation induced by insect herbivory, we monitored the free JA and auxin content after *P. xylostella* L. attack. The free JA concentration in control leaves and roots was low ( $8.3 \pm 0.7$  and  $3.3 \pm 0.1$  ng g<sup>-1</sup> FW). After herbivory, the JA concentration in leaves and roots was nearly ten times higher ( $81.6 \pm 4.4$  and  $33.5 \pm 1.3$  ng g<sup>-1</sup> FW, respectively).



**Fig. 1** Effect of insect herbivory on the root growth of *A. thaliana* seedlings. 10-day-old seedlings were infested with second-instar larvae of *P. xylostella* L., and then the root length was measured every 2 days ( $n=20$ ; Mean  $\pm$  SD; statistical analysis was performed by ANOVA,  $a: P < 0.05$ ). The experiments are representative of at least three independent experiments



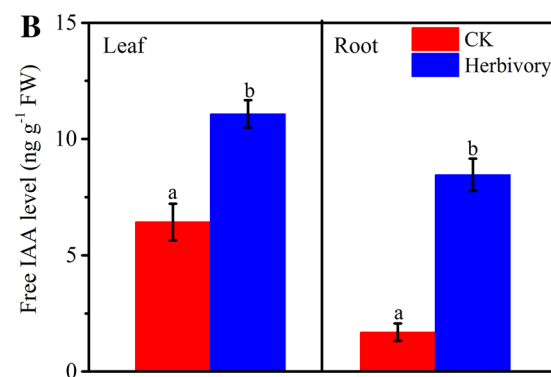
**Fig. 2** Free JA and IAA levels in the leaves and roots of *Arabidopsis* seedlings upon *P. xylostella* L. herbivory. Ten-day-old seedlings were attacked by *Plutella xylostella* L. After 5 h, the seedlings were collected for JA and IAA measurement. Data points represent aver-

In control plants, the auxin concentrations in leaves and roots were  $6.4 \pm 0.8$  and  $1.7 \pm 0.4$  ng g<sup>-1</sup> FW, respectively. The free auxin concentration was significantly elevated in leaves and roots after herbivory by *P. xylostella* L. (Fig. 2b). These results suggest that JA and IAA may contribute synergistically to regulate root growth retardation induced by *P. xylostella* L. herbivory.

### Herbivory Alters Auxin Flux and Promotes Proton Secretion in the Transition Zone of Seedling Roots

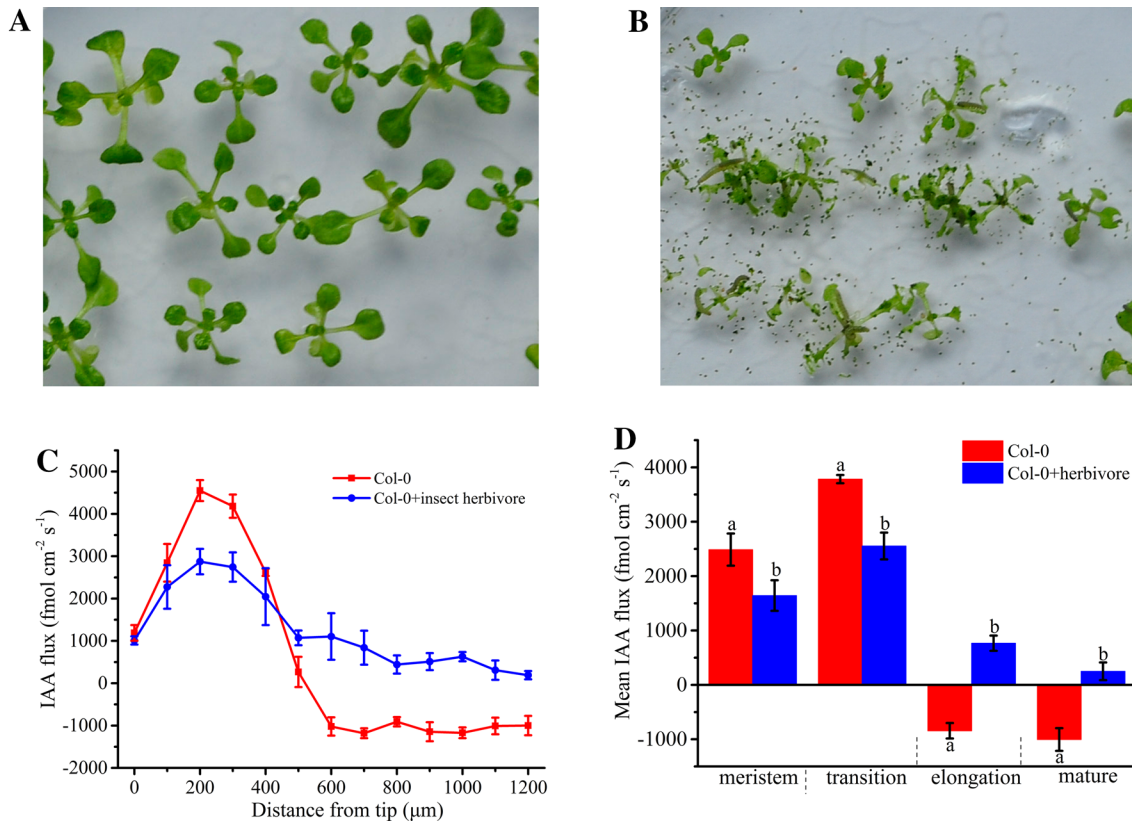
To investigate the physiological changes in root development, auxin flux along the root tip of 15-day-old seedlings was evaluated with an NMT technique after *P. xylostella* L. attack. Photographs of the samples are shown in Fig. 3a (before attack) and Fig. 3b (after attack). According to the research of Baluška, we divided the root tip into meristematic zone, transition zone, elongation zone, and mature zone (Baluška and others 2010). In the control seedling roots, the auxin flux rate was positive in the meristematic (0–100  $\mu$ m from the tip) and transition zone (100–400  $\mu$ m from the tip), suggesting strong auxin efflux (Fig. 3c). The highest auxin efflux was observed at 200  $\mu$ m from the root cap junction, whereas the elongation and mature zones showed negative values, indicating net auxin influx. After *P. xylostella* L. herbivory, the auxin efflux was significantly repressed in the meristematic and transition zones (0–400  $\mu$ m from the tip); however, in the elongation and transition zones, the herbivory did not induce auxin influx as shown in the control.

Previous studies indicate that auxin transport is accompanied by H<sup>+</sup> transport (Yang and others 2006). Therefore, H<sup>+</sup> flux in the apical region of roots after *P. xylostella* L. herbivory was also measured with NMT. In control seedlings, roots showed strong H<sup>+</sup> influx in the meristematic zone (0–100) and transition zone



ages  $\pm$  SD from three experiments. FW, fresh weight. Columns labeled with different letters are significantly different at  $p < 0.05$ . This experiment was conducted with two biological replicates



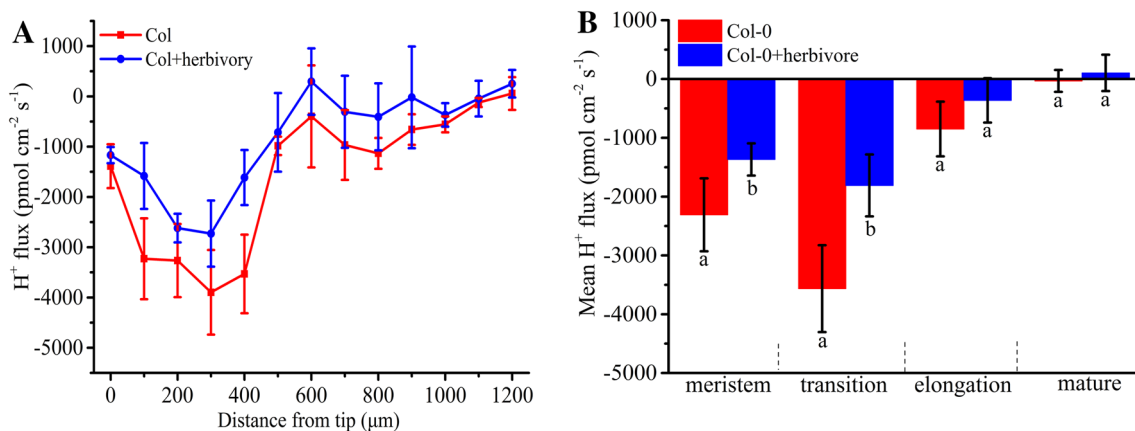


**Fig. 3** Effect of insect herbivory on auxin flux rate in *A. thaliana* root. **a** Pictures of 15-day-old seedlings of Col-0 control. **b** Picture of plants after *P. xylostella* L. attack for 12 h. **c** Auxin flux profile along the root apices of Col-0 (red) and Col-0 herbivoried by *P. xylostella*

L. (blue); ( $n=6$ ). Each value represented the mean of six individual measurements and bars represent the standard deviation. **d** Mean auxin flux in different zones of the roots. Statistical analysis was performed by ANOVA ( $n=6$ ; mean  $\pm$  SD,  $a: P < 0.05$ )

(100–400), with a maximum at 300  $\mu\text{m}$ . In the elongation and mature zones, the  $\text{H}^+$  flux rate showed a slightly negative value, indicating a net uptake of  $\text{H}^+$ . After insect herbivory, the net  $\text{H}^+$  influx decreased in both the

meristematic and transition zones (Fig. 4). However, in the elongation and mature zones, there was no significant change in the  $\text{H}^+$  flux compared to the controls.



**Fig. 4** Effect of insect herbivory on  $\text{H}^+$  flux in *A. thaliana* root. **a** Change of  $\text{H}^+$  flux rate along the roots of Col-0 induced by insect herbivory. Each value represented the mean of six individuals and

bars represent the standard deviation. **b** Mean  $\text{H}^+$  flux in different zones of the roots. Statistical analysis was performed by ANOVA ( $n=6$ ; mean  $\pm$  SD,  $a: P < 0.05$ )

## Herbivory Activates Auxin Biosynthesis and Down-regulates Auxin Transporter Genes

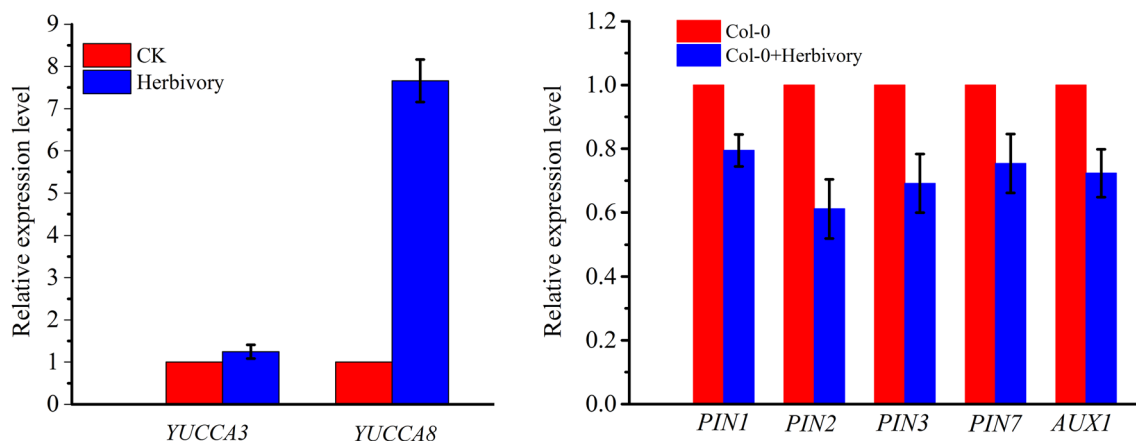
To investigate how insect herbivory alters auxin flux, the transcript levels of genes involved in auxin transportation and biosynthesis were further studied. Compared to the non-treated seedlings, the transcript levels of *YUCCA3* and *YUCCA8* were up-regulated after *P. xylostella* L. herbivory, and the expression levels of the auxin transporter genes *PIN1*, *PIN2*, *PIN3*, *PIN7*, and *AUX1* were down-regulated in the roots (Fig. 5a, b).

## Discussion

Plants have limited resources to manage both growth and defense. To survive herbivore attack or other unfavorable environmental conditions, plants reduce their growth rate and shuttle resources to defense mechanisms (Coley and others 1985). Schmidt recently suggested that plants reduce the carbon delivery to roots after insect herbivory, which is a possible reason for slow plant growth (Schmidt and others 2015). The interaction of phytohormones, especially JA and IAA, likely plays an important role in this defense response (Farmer and Ryan 1990; Duffey and Stout 1996; Bosch and others 2014). The transition zone located between the apical meristem and basal elongation region has a unique role in the determination of cell fate and root growth. This zone also integrates diverse inputs from endogenous (hormonal) and exogenous (sensorial) stimuli and translates them into signaling and motoric outputs as adaptive differentiation responses (Baluška and others 2001, 2004). In this study, we first show that the growth rate of *A. thaliana* seedlings decreases under *P. xylostella* L. herbivory. Insect herbivory

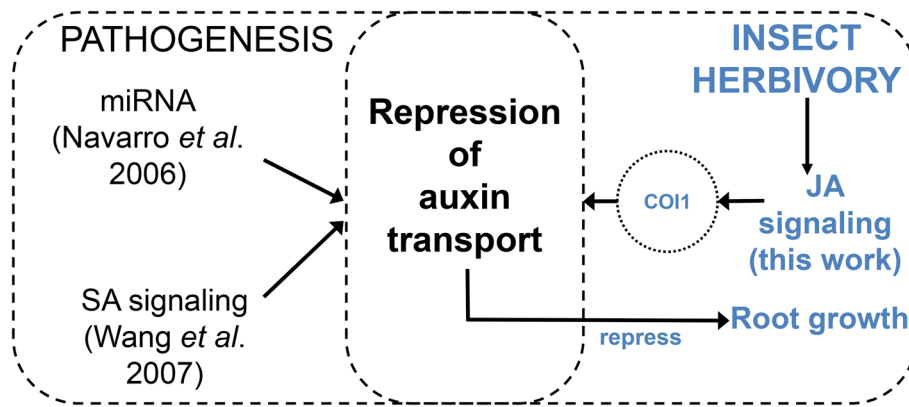
reduced primary root elongation and induced a defense response related to JA and IAA, redirecting energy used for growth to the defense response. These results are in agreement with previous studies showing that the overexpression of JA biosynthesis genes in plants alters development and inhibits primary root elongation (Xue and Zhang 2007).

Generally, the herbivory of chewing insects induces an increase of JA concentration in plant tissues (Turner and others 2002; Li and others 2002; Wasternack 2007; Furstenberg-Hagg and others 2013; Bosch and others 2014). Our results confirmed that when *P. xylostella* L. attack *Arabidopsis* leaves, the JA concentration was significantly elevated in both the roots and leaves, which suggests that JA can serve as a long-distance signal. These results are comparable to those of previous studies (Turner and others 2002; Li and others 2002; Wasternack 2007; Bosch and others 2014). It is known that JA can be transported throughout the whole plant as a result of insect herbivory (Furstenberg-Hagg and others 2013). Recent work demonstrated that MeJA or coronatine treatment promotes the production of auxin, including genes such as *CYP79B2*, *CYP79B3*, *ASA1*, *TAA1*, and *YUC* which are involved in auxin biosynthesis (Stepanova and others 2008; Sun and others 2009; Qi and others 2012; Hentrich and others 2013; Yang and others 2014). Our results show that *P. xylostella* L. herbivory also activates the expression of *YUCCA* genes in *A. thaliana* roots and leads to a high IAA concentration. Indeed, Qi and others (2012) showed that increased free IAA in roots was induced by *A. brassicicola* infection, but this was substantially impaired in the *coil-1* mutant. We previously observed that MeJA treatment did not alter the auxin flux rate and H<sup>+</sup> flux at the root tip of JA-insensitive mutant *coil-1* plants (Yan and others 2015 b). It is reasonable to assume that herbivory activated the JA signal



**Fig. 5** Insect herbivory regulates the expression of some auxin biosynthesis and transportation genes in Col-0 plants. RT-PCR assays showing JA-regulated expression of auxin synthesis genes *YUCCA3*,

*YUCCA8* and auxin transportation genes *PIN1*, *PIN2*, *PIN3*, *PIN7*, *AUX1*. The transcript levels of these genes were normalized according to the expression of *ACTIN-2* or *ACTIN-7*



**Fig. 6** Proposed model on the reduction of plant growth rate upon insect herbivory. Based on previous studies of the interaction of JA and auxin (Sun and others 2011; Qi and others 2012), the hypothetical signal sequences of insect herbivory reduction of the root growth

rate were as follows: firstly, insect herbivory elevated the JA concentration, then JA as a long-distance signal transported to the whole plant. Increased levels of JA reduced auxin transmembrane flux and also polar auxin transport, causing a reduction in root growth

pathway and the latter regulated the auxin flux rate at the root tip, and hereby the plant growth rate and root elongation were inhibited. Therefore, we conclude that the JA pathway is important for the elevation of free IAA concentrations in plant tissues after *P. xylostella* L. attack.

The interaction of JA and auxin in plant resistance to pathogen infection has been well studied. Qi and others (2012) showed that when *Arabidopsis* is resistant against *A. brassicicola* infection, the auxin polar transportation was reduced. Furthermore, Sun and others (2011) found that exogenous MeJA treatment reduced the endocytosis and the accumulation of the PIN2 protein on the plasma membrane. However, in these studies the auxin flux in roots was not studied. Therefore, we used NMT to monitor the auxin flux along the root tip of *Arabidopsis* after *P. xylostella* L. attack. Our results suggest that insect herbivory suppresses the auxin efflux in meristematic and transition zones, but promotes auxin influx in the elongation and mature zones. *P. xylostella* L. attack also down-regulates auxin transport genes, including *PIN1*, *PIN2*, *PIN3*, *PIN7*, and *AUX1*. This leads to reduced auxin transport in the meristematic and transition zones, which confirms the auxin flux results.

Based on the auxin flux and auxin transporter-related gene expression data herein, we hypothesize that auxin transport from leaves to roots is reduced after *P. xylostella* L. herbivory, and *PIN2*-, *PIN3*-, and *PIN7*-mediated auxin lateral redistribution is also attenuated. The down-regulation of auxin transport may lead to the accumulation of auxin in meristematic and transition zones, but the lack of auxin in the elongation and mature zones leads to reduced root elongation. Thus, the up-regulation of auxin biosynthesis after *P. xylostella* L. herbivory may be a compensation effect in response to the low level of auxin in roots.

There is evidence that polar auxin transport may lead to PM  $H^+$ -ATPase-mediated proton secretion in roots

(Pitts and others 1998; Staal and others 2011; Xu and others 2013) and subsequent root growth and development (Rober-Kleber and others 2003). We therefore examined the transmembrane  $H^+$  flux at the root tip after *P. xylostella* L. herbivory and found increased  $H^+$  flux in meristematic and transition zones, but not in the elongation and mature zones. According to the now generally accepted chemiosmotic model of polar auxin transport (Yang and others 2006; Taiz and Zeiger 2006), heightened  $H^+$  efflux could establish a proton motive force ( $\Delta E + \Delta pH$ ) that provides the driving force for auxin uptake, as the protonated auxin (IAAH) can enter cells by passive diffusion. The enhanced uptake of IAAH into cells may be the reason for the noted auxin efflux in the root tip after insect attack. In addition, the  $H^+$  efflux also provides a driving force for the  $2H^+$ -IAA- symporter, AUX1, to mediate the uptake of dissociated auxin ( $IAA^-$ ), which is the major form of auxin influx in cells. On the other hand, the large amount of  $H^+$  efflux decreased the concentration of  $H^+$  in the cytoplasm, which is also known to affect cell division and metabolism elongation (Morisawa and Steinhardt 1982), thus limiting root elongation.

In conclusion, based on our results and those from the previous studies, we characterize the potential mechanism of reduced growth after insect herbivory (Fig. 6). According to this model, *P. xylostella* herbivory leads to elevated JA concentration in leaves, from which JA is transported to other plant tissues, including roots, resulting in the activation of auxin biosynthesis in leaves and roots. Increased levels of JA reduced polar auxin transport, causing auxin accumulation in leaves. And reduced polar transport of auxin affected auxin distribution in the root, causing a reduction in root growth.

**Acknowledgements** We would like to thank Yue Xu from Xuyue (Beijing) Science and Technology Company for their technical support. This work was financially supported by the National Natural Science Foundation of China (31270655) and the National ‘863’ Plan Project (No. 2011AA10020102).

**Authors’ Contributions** Yingbai Shen and Suli Yan designed the project. Suli Yan and Chunyang Jiao performed the auxin flux measurement. Ningning Wang and Hongjun Yao performed the  $H^+$  flux measurement. Suli Yan and Chunyang Jiao analyzed the data and wrote the manuscript. Eric S. McLaMORE assisted with data analysis, NMT experiments, and manuscript preparation.

## References

- Baluška F, Volkmann D, Barlow PW (2001) A polarity crossroad in the transition growth zone of maize root apices: cytoskeletal and developmental implications. *J Plant Growth Regul* 20:170–181
- Baluška F, Mancuso S, Volkmann D, Barlow P (2004) Root apices as plant command centres: the ‘brain-like’ status of the root apex transition zone. *Biologia* 59:1–13
- Baluška F, Mancuso S, Volkmann D, Barlow PW (2010) Root apex transition zone: a signalling-response nexus in the root. *Trends Plant Sci* 15:402–408
- Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, Friml J (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115:591–602
- Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature* 433:39–44
- Bosch M, Berger S, Schaller A, Stintzi A (2014) Jasmonate-dependent induction of polyphenol oxidase activity in tomato foliage is important for defense against *Spodoptera exigua* but not against *Manduca sexta*. *BMC Plant Biol* 14:257–272
- Chen CW, Yang YW, Lur HS, Tsai YG, Chang MC (2005) A novel function of abscisic acid in the regulation of rice (*Oryza. Sativa* L.) root growth and development. *Plant Cell Physiol* 47(1):1–13
- Coley PD, Bryant JP, Chapin FS (1985) Resource availability and plant anti herbivore defense. *Science* 23:895–900
- Duffey SS, Stout MJ (1996) Antinutritive and toxic components of plant defense against insects. *Arch insect biochem* 32:3–37
- Farmer EE, Ryan CA (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proc Natl Acad Sci* 87:7713–7716
- Friml J (2003) Auxin transport-shaping the plant. *Curr opin plant biol* 6:7–12
- Fürstenberg-Hägg J, Zagrobelny M, Bak S (2013) Plant defense against insect herbivores. *Int J Mol Sci* 14:10242–10297
- Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu Rev Phytopathol* 43:205–227
- Grieneisen VA, Xu J, Marée AF, Hogeweg P, Scheres B (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* 449:1008–1013
- Haruta M, Sussman MR (2012) The effect of a genetically reduced plasma membrane protonmotive force on vegetative growth of Arabidopsis. *Plant physiol* 158:1158–1171
- Hentrich M, Böttcher C, Dücking P, Cheng Y, Zhao Y, Berkowitz O, Masle J, Medina J, Pollmann S (2013) The jasmonic acid signaling pathway is linked to auxin homeostasis through the modulation of *YUCCA8* and *YUCCA9* gene expression. *Plant J* 74:626–637
- Li L, Li C, Lee GI, Howe GA (2002) Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. *Proc Natl Acad Sci* 99:6416–6421
- McLaMORE ES, Porterfield DM (2011) Non-invasive tools for measuring metabolism and biophysical analyte transport: self-referencing physiological sensing. *Chem Soc Rev* 40:5308–5320
- McLaMORE ES, Diggs A, Calvo Marzal P, Shi J, Blakeslee JJ, Peer WA, Murphy AS, Porterfield DM (2010) Non-invasive quantification of endogenous root auxin transport using an integrated flux microsensor technique. *Plant J* 63:1004–1016
- Morisawa M, Steinhardt RA (1982) Changes in intracellular pH of *Physarum plasmodium* during the cell cycle and in response to starvation. *Exp Cell Res* 140:341–351
- Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JD (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science* 312:436–439
- Pan X, Welti R, Wang X (2010) Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography-mass spectrometry. *Nature Protoc* 5:986–992
- Pitts RJ, Cernac A, Estelle M (1998) Auxin and ethylene promote root hair elongation in Arabidopsis. *Plant J* 16:553–560
- Porterfield DM, McLaMORE ES, Banks MK (2009) Microsensor technology for measuring  $H^+$  flux in buffered media. *Sensor Actuat B* 136:383–387
- Qi L, Yan J, Li Y, Jiang H, Sun J, Chen Q, Li H, Chu J, Yan C, Sun X, Yu Y, Li C, Li C (2012) *Arabidopsis thaliana* plants differentially modulate auxin biosynthesis and transport during defense responses to the necrotrophic pathogen *Alternaria brassicicola*. *New Phytol* 195:872–882
- Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: its role in plant growth and development. *J Exp Bot* 62(10):3321–3338
- Rober-Kleber N, Albrechtová JT, Fleig S, Huck N, Michalke W, Wagner E, Speth V, Neuhaus G, Fischer-Iglesias C (2003) Plasma membrane  $H^+$ -ATPase is involved in auxin-mediated cell elongation during wheat embryo development. *Plant Physiol* 131:1302–1312
- Schmidt L, Hummel GM, Thiele B, Schurr U, Thorpe MR (2015) Leaf wounding or simulated herbivory in young *N. attenuata* plants reduces carbon delivery to roots and root tips. *Planta* 241:917–928
- Shabala S, Newman I, Whittington J, Juswono U (1998) Protoplast ion fluxes: their measurement and variation with time, position and osmoticum. *Planta* 204:146–152
- Shabala S, Shabala L, Gradmann D, Chen Z, Newman I, Mancuso S (2006) Oscillations in plant membranetransport: model predictions, experimental validation, and physiological implications. *J Exp Bot* 57:171–184
- Staal M, De Cnodder T, Simon D, Vandenbussche F, Van der Straeten D, Verbelen JP, Elzenga T, Vissenberg K (2011) Apoplastic alkalization is instrumental for the inhibition of cell elongation in the *Arabidopsis* root by the ethylene precursor 1-aminocyclopropane – 1-carboxylic acid. *Plant Physiol* 155:2049–2055
- Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie DY, Dolezal K, Schlereth A, Jürgens G, Alonso JM (2008) TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* 133:177–191
- Sun J, Xu Y, Ye S, Jiang H, Chen Q, Liu F, Zhou W, Chen R, Li X, Tietz O, Wu X, Cohen JD, Palme K, Li C (2009) *Arabidopsis ASA1* is important for jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. *Plant Cell* 21:1495–1511



- Sun J, Chen Q, Qi L, Jiang H, Li S, Xu Y, Liu F, Zhou W, Pan J, Li X, Palme K, Li C (2011) Jasmonate modulates endocytosis and plasma membrane accumulation of the *Arabidopsis* PIN2 protein. *New Phytol* 191:360–375
- Sussman MR, Goldsmith MH (1981) The action of specific inhibitors of auxin transport on uptake of auxin and binding of *N*-1-naphthylphthalamic acid to a membrane site in maize coleoptiles. *Planta* 152:13–18
- Taiz L, Zeiger E (2006) *Plant Physiology*, 4th Revised edn. Sinauer Associates, USA
- Teale WD, Paponov IA, Palme K (2006) Auxin in action: signalling, transport and the control of plant growth and development. *Nat Rev Mol Cell Biol* 7:847–859
- Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. *Plant Cell* 14:S153–S164
- Wang D, Pajeroska-Mukhtar K, Culler AH, Dong X (2007) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Curr Biol* 17:1784–1790
- Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann Bot* 100:681–697
- Xu Y, Sun T, Yin L (2006) Application of non-invasive microsensing system to simultaneously measure both  $H^+$  and  $O_2$  fluxes around the pollen tube. *J Integr Plant Biol* 48:823–831
- Xu W, Jia L, Shi W, Liang J, Zhou F, Li Q, Zhang J (2013) Abscissic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. *New Phytol* 197:139–150
- Xue R, Zhang B (2007) Increased endogenous methyl jasmonate altered leaf and root development in transgenic soybean plants. *J Genet and Genomics* 34:339–346
- Yan S, Luo S, Dong S, Zhang T, Sun J, Wang N, Yao H, Shen YB (2015a) Heterotrimeric G-proteins involved in the MeJA regulated ion flux and stomatal closure in *Arabidopsis thaliana*. *Funct Plant Biol* 42:126–135
- Yan S, McLamore ES, Dong S, Gao H, Taguchi M, Wang N, Zhang T, Su X, Shen Y (2015b) The role of plasmamembrane  $H^+$ -ATPase in jasmonate-induced ion fluxes and stomatal closure in *Arabidopsis thaliana*. *Plant J* 83:638–649
- Yan S, Zhang T, Dong S, McLamore Eric, Wang N, Shan X, Shen Y, Wan Y (2016) MeJA affects root growth by modulation of transmembrane auxin flux in the transition zone. *J Plant Growth Regul* 35:256–265
- Yang Y, Hammes UZ, Taylor CG, Schachtman DP, Nielsen E (2006) High-affinity auxin transport by the AUX1 influx carrier protein. *Curr Biol* 16:1123–1127
- Yang ZB, Geng X, He C, Zhang F, Wang R, Horst WJ, Ding Z (2014) TAA1-regulated local auxin biosynthesis in the root-apex transition zone mediates the aluminum-induced inhibition of root growth in *Arabidopsis*. *Plant Cell* 26:2889–2904
- Zebelo S, Piorkowski J, Disi J, Fadamiro H (2014) Secretions from the ventral eversible gland of *Spodoptera exigua* caterpillars activate defense-related genes and induce emission of volatile organic compounds in tomato, *Solanum lycopersicum*. *BMC Plant Biol* 14:140–151
- Zhang Y, Turner JG (2008) Wound-induced endogenous jasmonates stunt plant growth by inhibiting mitosis. *PLoS ONE* 3:e3699