Control of Western flower thrips through jasmonate-triggered plant immunity

Chen, G.

Chapter 5

Site-dependent induction of jasmonic acid-associated chemical defenses against Western flower thrips in chrysanthemum

Gang Chen, Hye Kyong Kim, Peter G. L. Klinkhamer, Rocío Escobar-Bravo

Plants have evolved numerous inducible defense traits to resist or tolerate herbivory, which can be activated locally at the site of the damage, or systemically through the whole plant. Here we investigated how activation of local and systemic chemical responses upon exogenous application of the phytohormone jasmonic acid (JA) varies along the plant canopy in chrysanthemum, and how these responses correlate with resistance to Western flower thrips (WFT). Our results showed that JA application reduced WFT damage per plant when applied to all the plant leaves or when locally applied to apical leaves, but not when only basal leaves were locally treated. Local application of JA to apical leaves resulted in a strong reduction in WFT damage in new leaves developed after the JA application. Yet, activation of a JA-associated defensive protein marker, polyphenol oxidase, was only locally induced. Untargeted metabolomics analysis further showed that JA increased the concentrations of sugars, phenylpropanoids, flavonoids and some amino acids in locally induced basal and apical leaves. However, local application of JA to basal leaves barely affected the metabolomics profiles of systemic non-treated apical leaves, and vice versa. Our results suggest that JA-mediated activation of systemic chemical defense responses is spatially variable and depends on the site of the application of the hormone in chrysanthemum.

Keywords: chrysanthemum, *Frankliniella occidentalis*, jasmonic acid, local and systemic induced defenses, metabolomics

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1 Introduction

Plants defend themselves against herbivory by employing a plethora of physical and chemical arsenals. Chemical defenses can exert repellent, anti-nutritive, and/or toxic effects on herbivores, or attract their natural enemies (Howe & Jander, 2008). Physical defenses, such as leaf toughness and trichomes, can also increase plant fitness by negatively affecting herbivore performance and preference. Furthermore, these plant defenses can be classified according to their differential regulation as constitutive or inducible defenses (Agrawal & Karban, 1999). Constitutive defenses are defined as morphological or chemical-based defensive traits that are always expressed in the plant, irrespective of herbivore attack (Agrawal, 2007). Induced plant defenses, however, can be physical- or chemical-related traits that are initiated or elevated upon herbivory (Agrawal, 2007). Plant inducible defense responses to herbivory are mainly modulated by the phytohormones jasmonic acid (JA), salicylic acid (SA) and ethylene (Bari & Jones, 2009; Smith *et al.*, 2009). In general, chewing-biting and cell-content herbivores, certain phloem feeders and necrotrophic pathogens activate the JA signaling pathway (Walling, 2000; Glazebrook, 2005; Lazebnik *et al.*, 2014), while the SA pathway is generally activated by biotrophic pathogens and phloem feeders (Walling, 2000; De Vos *et al.*, 2005; Glazebrook, 2005).

Induction of plant defenses by herbivory can occur locally at the site of attack and systemically in undamaged parts of the plant located at a substantial distance from the challenged area (Pieterse *et al.*, 2014). Although these defense responses have been reported to occur within minutes in both local and systemic tissues, they often vary in their magnitude, space and time within and among plant species. This variation can be explained by the genetic background, the development plasticity, transmission of long-distance signals, and the vascular architecture of the plant (Van Dam *et al.*, 2001; Arnold & Schultz, 2002; Arimura *et al.*, 2004; Orians, 2005; Howe & Jander, 2008). For example, glucosinolates, which are important chemical defenses against biotic stresses, are reported to be locally and systemically induced by herbivore feeding in tap and lateral roots of several *Brassica* species, but not in fine roots (Tsunoda *et al.*, 2018). This has been explained by the capacity of plants to increase the protection of tissues that contribute most to plant fitness, such as primary roots. Importantly, these variations can affect herbivore distribution along the plant canopy, and modulate plant-mediated interactions among different herbivore species (Lee *et al.*, 2017).

Knowledge about variation in induced defenses against insect herbivores is important to develop strategies for plant protection in agri- and horticulture. In both Western flower thrips (WFT) [*Frankliniella occidentalis,* (Pergande)] is one of the most important insect pests (Steenbergen *et al.*, 2018). WFT feeding damage on flowers, fruits and plant leaves can reduce growth and yield, and affect product appearance and quality (De Jager, C *et al.*, 1995; de Jager, KM *et al.*, 1995). Western flower WFT infestation activates the JA signaling pathway in Arabidopsis (*Arabidopsis thaliana*) (Abe *et al.*, 2008; Abe *et al.*, 2011), turnip (*Brassica rapa*) (Abe *et al.*, 2009), and tomato (*Solanum lycopersicum*) (Li *et al.*, 2002; Escobar-Bravo *et al.*, 2017). Activation of JA-associated defenses play a prominent role in plant resistance against this pest (Steenbergen *et al.*, 2018). Previous experiments carried out in our laboratory have shown that exogenous application of JA enhances resistance against WFT in chrysanthemum [*Chrysanthemum* × *morifolium* Ramat. (Asteraceae)] as well (see Chapter 4 in this thesis). However, when this phytohormone was locally applied on basal chrysanthemum leaves it did not seem to have a significant effect on WFT resistance (Chen *et al*. unpublished data), suggesting possible constraints in the induction of systemic defenses against this pest.

Here we have investigated whether local and systemic chemical defense responses to the exogenous application of JA vary along the plant canopy in chrysanthemum. In addition, we have determined whether a differential JA-mediated induction of local and systemic chemical responses correlates with WFT susceptibility. For this, we have conducted insect bioassays to determine the effects of local and systemic JA application on WFT-associated feeding damage along the plant canopy. In addition, we have determined the activation of JA signaling upon local or systemic application of JA by analyzing the induction levels of a JA-responsive defensive protein marker, polyphenol oxidase (Thaler *et al.*, 1999). Finally, we have performed a comprehensive non-targeted metabolomic analysis to determine how JA application affects chrysanthemum chemical defenses upon local or systemic induction. Our study offers a comprehensive analysis of induced chemical defenses in chrysanthemum, one of the most important cultivated ornamental species for which WFT represent one of the most damaging insect pests affecting their production worldwide.

2 Materials and Methods

2.1 Plant material and insects

Chrysanthemum [*Chrysanthemum* × *morifolium* Ramat. (Asteraceae)] cuttings (cv. Baltica) were provided by Deliflor Chrysanten (Maasdijk, The Netherlands). The cuttings were individually planted in small plastic trays (2 cm × 2 cm) filled with potting soil and placed in a climate room provided with 20°C, 70% RH, 113.6 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) and L16:D8 photoperiod. At 10 days after planting, plants were transplanted to plastic pots (9 cm × 9 cm × 10 cm) containing the same potting soil.

The Western flower thrips (WFT) (*Frankliniella occidentalis*) [Pergande] were maintained on chrysanthemum flowers (cultivar Euro Sunny) in a climate room at 23°C, 60% RH and L12:D12 photoperiod.

2.2 Experimental design

To determine the effect of jasmonic acid (JA) on the induction of local and systemic chemical defenses against WFT we carried out the following induction treatments (Fig. 1): (1) application of JA or mock solution to all the plant leaves, (2) local application of JA or mock solution to leaves 4 and 5 from the bottom (basal leaves), or (3) local application of JA or mock solution to leaves 9 and 10 from the bottom (apical leaves). Leaves were sprayed with 3 mM of JA (Cayman, Ann Arbor, Michigan, USA) in 0.8% aqueous ethanol solution as described in Redman *et al*. (2001). Control plants were sprayed with 0.8% aqueous ethanol (mock) solution. Mock- and JA-treated plants were placed in separate climate rooms for 45 min after the treatment. Thereafter, both control and JA-treated plants were randomly placed in a climate room at 20°C, 70% RH, 113.6 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) and L16:D8 photoperiod. At 7 days after JA or mock solutions application, basal (4-5) and apical (9-10) leaves of 5 plants per treatment were sampled for metabolomics analyses by NMR, and leaves 5, 6, 8, 9, 13 and 14 of 5 plants of each treatment were sampled for polyphenol oxidase (PPO) activity. The remaining plants were subjected to non-choice whole-plant thrips bioassays (see below).

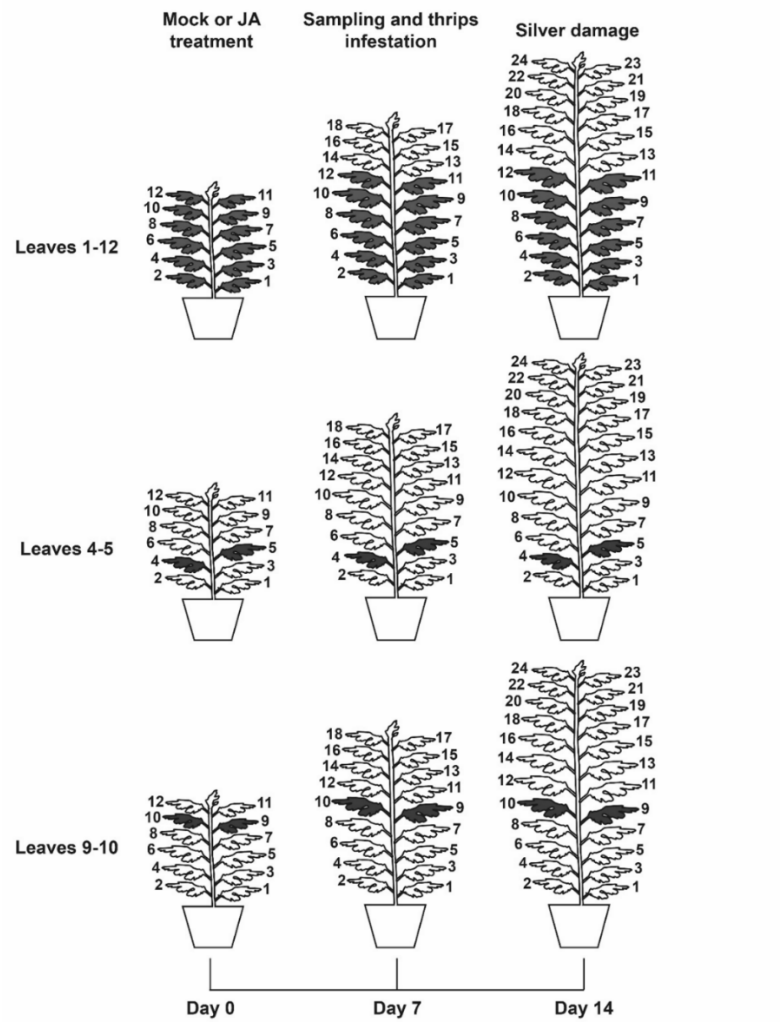

Fig. 1 Schematic representation of the experimental design. Jasmonic acid (JA) or mock solutions were applied to (1) all leaves, (2) basal leaves (4-5) or (3) apical leaves (9-10) of chrysanthemum plants at day 0. Seven days after the hormone treatments, JA- and mock-treated plants (n = 5) were sampled for determination of polyphenol oxidase (PPO) activity on leaves 5, 6, 8, 9, 13 and 14 from the bottom. Another set of plants were sampled for NMR analysis on leaves 4-5 and 9-10 from the bottom (n = 5). The remaining plants (n = 10 per treatment) were infested with Western flower thrips (WFT). Evaluation of WFT feeding damage ('silver damage') was carried out at 7 days after WFT infestation (day 14). The leaves filled with black were treated with JA or mock solutions on day 0.

2.3 Non-choice whole plant thrips bioassay

Plants were individually placed into WFT-proof cages as described in Leiss *et al*. (2009a) (n = 10 for each treatment). Ten adult WFT (8 females and 2 males) were added to each plant. All cages were randomly placed in a climate room provided with 113.6 µmol photons m^{-2} s^{-1} of PAR, 16L:8D of photoperiod, 25°C and 70% RH. Seven days after WFT infestation, WFT-associated feeding damage (hereafter referred as 'silver damage') was evaluated in all the leaves of the plant and expressed as the damaged area in mm^2 per plant or the silver damage

caused by WFT in four groups of leaves: i.e. leaf 1-6, leaf 7-12, leaf 13-18 and leaf 19-24 from the bottom.

2.4 Determination of polyphenol oxidase activity

Polyphenol oxidase (PPO) activity was determined following the methodology described in Stout *et al*. (1998). Briefly, 0.150 g of leaf tissue was flash-frozen in liquid nitrogen, ground in a tissue lyser (Qiagen, Hilden, Germany), and homogenized in a 2 ml tube with 1.25 ml ice-cold 0.1 M pH 7.0 phosphate buffer containing 7% polyvinyl-polypyrrolidone and 0.4 ml of 10% Triton X-100. The homogenate was vortexed for 2 min and centrifuged for 10 min at 11,000 g at 4ºC. Five microliters of the extract were added to 1 ml of 2.92 mM chlorogenic acid solution in pH 8.0 potassium phosphate buffer. The optical density (OD) at 470 nm was recorded in a spectrophotometer (UV-1800, Shimadzu) every 10 sec for one minute. PPO activity was calculated as the increment of OD values per min per gram of fresh weight.

2.5 Nuclear Magnetic Resonance (NMR) analysis

NMR analysis was performed on basal (leaves 4 and 5) and apical (leaves 9 and 10) leaves at 7 days after the hormone or mock treatments (n = 5). Leaves 4 and 5, and 9 and 10, were pooled prior to analysis. Plant material was freeze-dried and ground using a tissue lyser (Qiagen, Hilden, Germany). Twenty milligrams of fine powder were extracted with 1.5 ml of 80% methanol-*d4* in KH_2PO_4 buffer (90 mM, pH = 6.0) containing 0.02% (w/v) trimethyl silyl-3-propionic acid sodium salt-*d4* (TMSP). Plant extracts were vortexed for 1 min, ultra-sonicated for 15 min and centrifuged at 13,000 rpm for 15 min at room temperature. Eight hundred microliters of the supernatant were transferred to the NMR tubes for analysis. The ^{1}H NMR spectra were acquired using a 600 MHz Bruker AV-600 spectrometer equipped with cryo-probe operating at a proton NMR frequency of 600 MHz at 25°C, as described in López-Gresa *et al* (2012). Deuterated methanol served as internal lock. ^{1}H NMR spectrum consisted of 128 scans requiring 10 min acquisition time with a digital resolution of 0.25 Hz/point, a pulse angle of 30° (10.8 μs), and a recycle delay of 1.5 s per scan. A pre-saturation sequence was used to suppress the residual water signal with low power selective irradiation at the H_2O frequency during the recycle delay. Spectra were Fourier transformed with a 0.3 Hz line broadening and zero-filled to 32 K points. Phase and baseline correction of the resulting spectra were done manually, followed by a calibration to TMSP at 0.00 ppm using Topspin (version 2.1, Bruker). ^{1}H NMR spectra was then converted and saved as ASCII files using AMIX (v. 3.7, Bruker Biospin). Spectral intensities were scaled to the intensity of the internal standard TMSP and reduced to integrated regions, referred to as buckets, of equal width (0.04 ppm) corresponding to the region of δ 10.0-0.2. The Regions in the range of δ 5.0-4.7 and δ 3.34-3.28, corresponding to water and methanol, respectively, were removed prior to statistical analyses.

2.6 Statistical analysis

Statistical analyses were performed using the SPSS software package (version 23; SPSS Inc., Chicago, IL, USA). Normality and homogeneity of the residuals were first checked using Kolmogorov-Smirnov and Levene's tests, respectively. Differences in silver damage symptoms per plant between JA- and mock-treated plants were analyzed by student-t tests. Effects of the main factors JA and groups of leaves based on their position in the plant (1-6, 7-12, 13-18 and 19-23 from the bottom) and their interaction on silver damage symptoms were analyzed by Generalized Linear Models (GLMs) using linear distribution and identity link function. Differences in PPO activity among leaves 5, 6, 8, 9, 13 and 14 detected in JA and mock-treated plants were analyzed by GLMs using linear distribution and identity link

function. Data on silver damage and PPO activity determined in plants receiving local application or systemic JA application were Log-transformed prior to analysis. Effects of JA, leaf position (4-5 and 9-10) and their interaction on levels of metabolites identified in the NMR analysis were analyzed by GLMs using linear distribution and identity link function. Differences among groups were tested by Fisher's least significant difference (LSD) post-hoc test. Patterns of chemical shifts detected by NMR in leaves 4-5 and 9-10 of mock- and JA-treated plants were analyzed by Partial Least Squares Discriminant analysis (PLS-DA) using the SIMCA-P 15 software package (Umetrics, Sweden). This analysis determines the variation in X variables (chemical shifts) modeled by the Y explanatory variable, i.e. mock and JA solution application on basal (leaves 4 and 5), apical (leaves 9 and 10) or on all leaves. The final model was selected according to the minimum number of latent variables showing the highest predicted variation in Y (Q^2). The chemical shifts with a variable importance in projection (VIP) > 1 were selected as the important X variables. Detailed statistical results are shown in Supplementary Table S1, S2 and S3.

3 Results

3.1 Systemic or local application of JA to apical leaves, but not local application to basal leaves, reduces silver damage per plant

Application of JA to all the leaves of chrysanthemum plants significantly reduced silver damage symptoms per plant (**Fig. 2A**, Table S1; student *t*-test, $P < 0.05$). This reduction was statistically significant for leaves 1-6, 7-12 and 13-18 (**Fig. 2B**). Local application of JA to basal leaves (4-5) did not significantly reduce the silver damage per plant (**Fig. 2C**; student *t*-test, $P = 0.592$), although there was a significant reduction in leaves 13-18 compared to their controls (**Fig. 2D**). Local application of JA on apical leaves (9-10) significantly reduced the silver damage symptoms per plant (**Fig. 2E**; student *t*-test, $P = 0.038$). This reduction was significant for leaves 13-18 and, although not significant, also evident for leaves 7-12. (**Fig. 2F**). Overall, silver damage symptoms were higher in leaves 7-12 and 13-18 compared to leaves 1-6 and 19-24 (**Fig. 2B, D,** and **F**).

3.2 JA induces polyphenol oxidase activity in local but not in systemic leaves

When JA was applied to all the leaves of chrysanthemum plants, PPO activity was significantly induced in leaves 5, 6, 8, 9 and 13, but not in leaf 14, at 7 days after the JA treatment (**Fig. 3A**, Table S1). Application of JA to basal leaves (4-5) significantly increased PPO activity in leaf 5, while in the other leaves there was a very small and non-significant increase (**Fig. 3B**). Likewise, application of JA to the apical leaves (9-10) induced PPO locally, i.e. on leaf 9, but not in non-treated leaves (**Fig. 3C**). Notably, PPO activity levels were higher in the youngest leaves (13 and 14) in both mock- and JA-treated plants.

3.3 JA effects on the leaf metabolome are locally but not systemic

All leaves treated with JA. A total of 246 signals were detected in the ^{1}H NMR analysis of leaves corresponding to mock- and JA-treated chrysanthemum plants. PLS-DA analysis of the metabolomics profiles of basal (4-5) and apical (9-10) leaves of plants from which all the leaves were treated with JA or mock solutions resulted in a model with five latent variables (LVs). This model explained 75.5% of the total metabolomics variation and 95.5% of the treatment variation, with a 77.3% total model predictability (model statistics: $R^2X = 0.755$, $R^2Y = 0.951$ and $Q^2 = 0.773$; CV-ANOVA, $P < 0.001$) (Fig. **4A**). The first LV separated JA-treated basal leaves (4-5) from JA-treated apical leaves (9-10) and mock-treated basal and apical leaves, explaining 40.1% of the metabolomic variation. The second LV explained 15.8% and separated basal leaves (4-5) from apical leaves (9-10) of both treatments. Differences

among treatments were mainly explained by 101 signals with variable importance for projection (VIP) scores higher than 1 (Fig. **4B** and Fig. S1). Among these, fourteen signals were identified corresponding to sugars (fructose, glucose and sucrose), amino acids (valine, threonine, alanine, arginine, glutamine, asparagine, adenine), organic acids (citric acid), phenylpropanoids (chlorogenic acid, 3,5-dicaffeoylquinic acid) and flavonoids (luteolin 7-*O*-glucoside). JA application significantly increased the levels of sucrose, glucose, threonine, asparagine, phenypropanoids (chlorogenic acid and 3,5-dicaffeoylquinic acid), the flavonoid luteolin 7-*O*-glucoside and camphor, and it reduced the levels of citric acid in basal leaves (4-5) at 7 days after the hormone treatment (**Fig. 4C**, Table S2 and S3). Overall JA application affected the metabolomics profile of apical (9-10) leaves less strongly than those of basal leaves, but a significant reduction in the leaf content of some amino acids (alanine and glutamine) and a significant induction of adenine, and phenylpropanoids (chlorogenic acid and 3,5-dicaffeoylquinic acid) were observed (**Fig. 4C**, Table S2 and S3). Apical leaves (9-10) showed lower levels of sugars (fructose and sucrose) and phenylpropanoids (chlorogenic acid and 3,5-dicaffeoylquinic acid), and higher levels of amino acids (valine, threonine, alanine, arginine, and adenine) than basal (4-5) leaves, independently of the treatment.

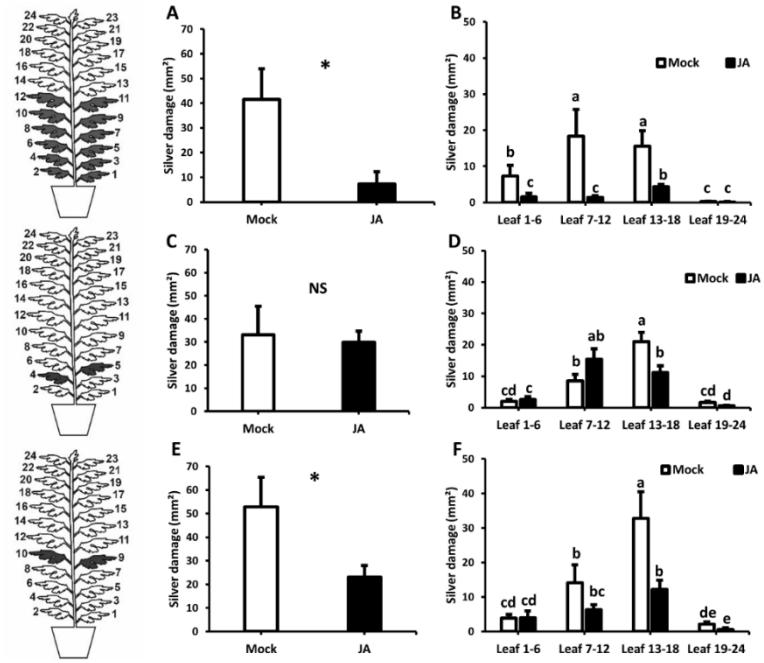

Fig. 2 Effect of systemic and local JA treatment on chrysanthemum resistance to Western flower thrips. Silver damage symptoms (mean ± SEM, n = 10) were determined for the whole plant or separately in four groups of leaves along the plant canopy in mock- and jasmonic acid (JA)-treated plants at 7 days after Western flower thrips infestation. Mock or JA solutions were applied to all the plant leaves (**A** and **B**), basal leaves (4-5 from the bottom; **C** and **D**) or to apical leaves (9-10 from the bottom; **E** and **F**). Asterisks denote significant differences determined by unpaired t-test at $P \leq 0.05$. Different letters indicate significant differences among groups compared by Fisher's LSD test at $P \leq 0.05$. n.s. = not significant.

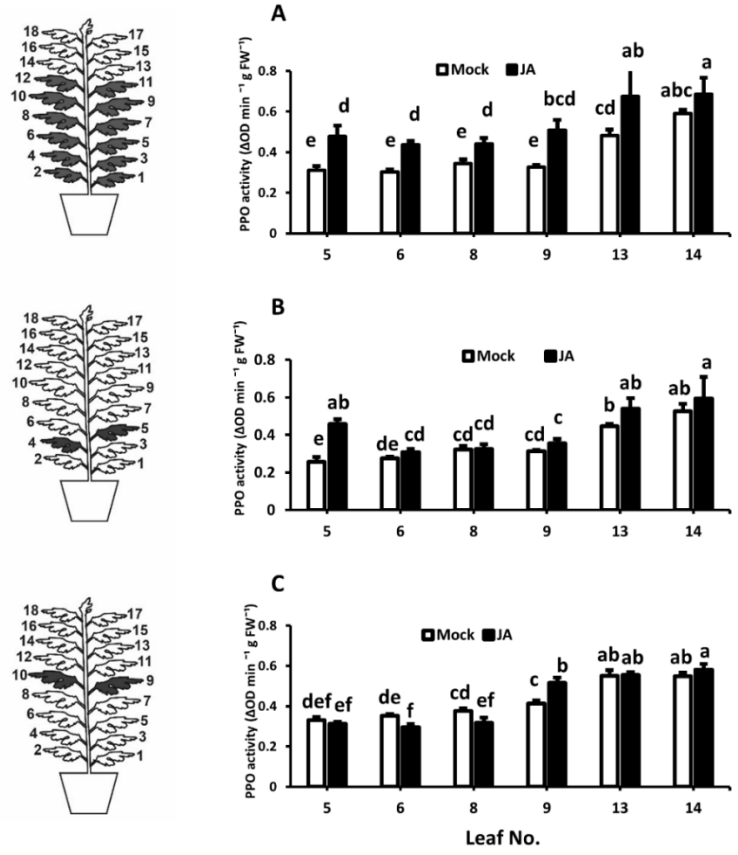

Fig. 3 Effect of systemic and local JA induction on polyphenol oxidase activity in chrysanthemum. Polyphenol oxidase (PPO) activity (mean ± SEM, $n = 5$) was determined on leaf 5, 6, 8, 9, 13 and 14 from the bottom of mock- and jasmonic acid (JA)-treated plants. Mock or JA solutions were applied to all plant leaves (**A**), basal leaves (**B**) or apical leaves (**C**). Plants were sampled at 7 days after the hormone treatments. Different letters indicate significant differences among groups compared by Fisher's LSD test at $P \leq 0.05$.

Basal leaves treated with JA. When plants were treated locally with JA on basal leaves (4-5), the metabolomic responses to the hormone treatment were only evident in those local leaves, while barely altering the chemistry of systemic apical leaves (9-10) (**Fig. 5A**). The PLS-DA analysis resulted in a model with three LVs explaining 63.1% of the total metabolomic variation and 80.9% of the treatment response, with a 49.4% total model predictability ($R^2X = 0.631$, $R^2Y = 0.809$ and $Q^2 = 0.494$; CV-ANOVA, $P = 0.025$). The first LV explained 42.7% of the variance and separated JA-treated basal leaves (4-5) from apical leaves (9-10) of mock- and JA-treated plants. The second LV explained 14.8% of the variance and separated mock-treated basal leaves (4-5) from the other leaves. These differences were mainly explained by 125 signals with VIP scores higher than 1 (**Fig. 5B** and Fig. S2). JA application to basal leaves (4-5) reduced the levels of sugars (fructose, glucose and sucrose) and the amino acid glutamine, while increasing the levels of the amino acid arginine, phenolic acids and flavonoids in these leaves (**Fig. 5C**, Table S2 and S3). No significant differences

in the levels of these compounds were observed for the apical leaves, except for a slight but significant reduction in citric acid and alanine levels (Table S2 and S3).

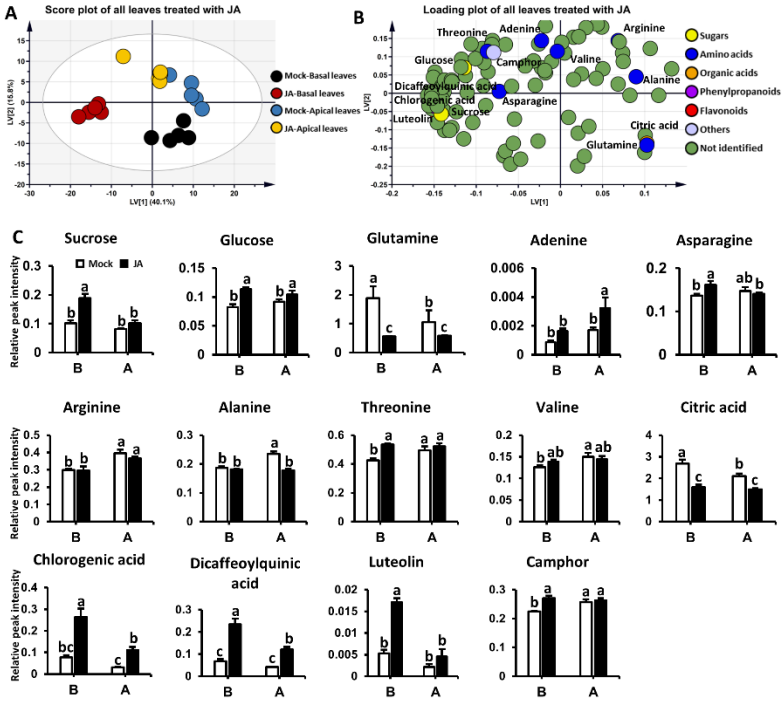

Fig. 4 Metabolic responses of basal and apical chrysanthemum leaves to systemic application of JA to all the plant leaves. Leaf metabolites were analyzed by NMR in basal (leaf 4-5) and apical (leaf 9-10) leaves of chrysanthemum plants at 7 days after the application of mock or jasmonic acid (JA) solutions to all plant leaves. Partial least square-discriminant analysis (PLS-DA) was performed on the obtained ^{1}H NMR spectra (n = 5). (**A**) Score plot showing the first two latent variables (LVs). The ellipse represents the Hotelling T2 with 95% confidence. (**B**) Loading plot showing important chemical shifts that contribute most to the model (variable importance in projection, VIP > 1). The identified compounds are shown in the plot. (**C**) Relative peak intensity (mean ± SEM, n = 5) of the identified compounds in basal (B) and apical (A) leaves of mock- and JA-treated plants are shown. Different letters indicate significant differences among groups compared by Fisher's LSD test at $P \leq 0.05$.

Apical leaves treated with JA. Finally, plants treated locally with JA on apical (9-10) leaves also displayed local metabolomic responses at 7 days after the hormone induction (**Fig. 6A**). The PLS-DA analysis resulted in a model with four LVs explaining 61.0% of the total metabolomic variation and 91.3% of the treatment response, with a 68.2% total model predictability (R^2X = 0.610, R^2Y = 0.913 and Q^2 = 0.682; CV-ANOVA, P = 0.018). The first LV explained 25.5% of the variance and separated basal leaves (4-5) from apical (9-10) leaves regardless of the hormone treatment. The second LV explained 23.0% of the variance and separated the JA-treated apical leaves from their controls. No clear metabolic separation was observed between basal leaves of mock- and JA-treated plants. Differences among treatments were explained by 100 signals with VIP scores higher than 1 (**Fig. 6B** and Fig. S3). JA significantly reduced the levels of fructose, glutamine and citric acid, and it increased the levels of glucose, adenine, phenylpropanoids (chlorogenic acid and 3,5-dicaffeoylquinic acid) and the flavonoid luteolin-7-*O*-glucoside in apical leaves (9-10). Notably, although the

local application of JA to apical leaves barely affected the overall metabolomic profiles of basal leaves, significant lower levels of sugars (fructose and sucrose), some amino acids (valine, alanine, and glutamine), camphor and *myo*-inositol were observed (**Fig. 6C**, Table S2 and S3). Levels of sugars (fructose, glucose and sucrose), phenylpropanoids (chlorogenic acid 3,5-dicaffeoylquinic acid), the flavonoid luteolin-7-*O*-glucoside and some amino acids (glutamine) were significantly lower in mock-treated apical leaves when compared to mock-treated basal leaves, while levels of some amino acids (threonine, alanine and arginine) were significantly higher (Table S2 and S3).

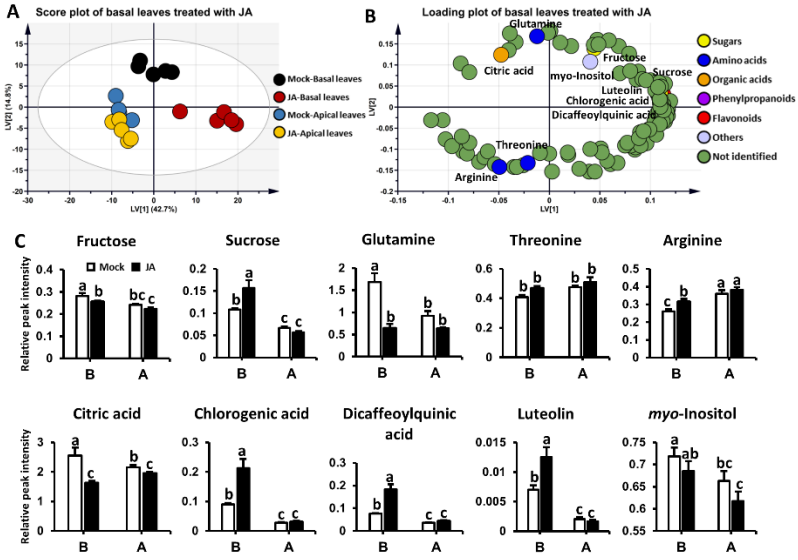

Fig. 5 Metabolomic responses of basal and apical chrysanthemum leaves to local application of JA to basal leaves. Leaf metabolites were analyzed by NMR in basal (leaf 4-5) and apical (leaf 9-10) leaves of chrysanthemum plants at 7 days after the local application of mock or jasmonic acid (JA) solutions to basal leaves (4-5 from the bottom). Partial least square-discriminant analysis (PLS-DA) was performed on the obtained ^{1}H NMR spectra (n = 5). (**A**) Score plot showing the first two latent variables (LVs). The ellipse represents the Hotelling T2 with 95% confidence. (**B**) Loading plot showing important chemical shifts that contribute most to the model (variable importance in projection, VIP > 1). The identified compounds are shown in the plot. (**C**) Relative peak intensity (Mean ± SEM, n = 5) of the identified compounds in basal (B) or apical (A) leaves of mock- and JA-treated plants are shown. Different letters indicate significant differences among groups compared by Fisher's LSD test at $P \leq 0.05$.

4 Discussion

In this study we have demonstrated that JA-mediated induction of local and systemic chemical defense responses varies along the plant canopy in chrysanthemum. We showed that either systemic or local application of JA to apical, but not to basal leaves, increased systemic plant resistance against the Western flower thrips (WFT) *Frankliniella occidentalis*. Variation in the systemic induction of chemical defense was not explained by the vertical alignment of local and systemic leaves, and thus their direct vascular connections, nor differences in the responses to the hormone treatment between apical and basal leaves. On the contrary, levels of constitutive and inducible chemical defenses were higher in basal leaves.

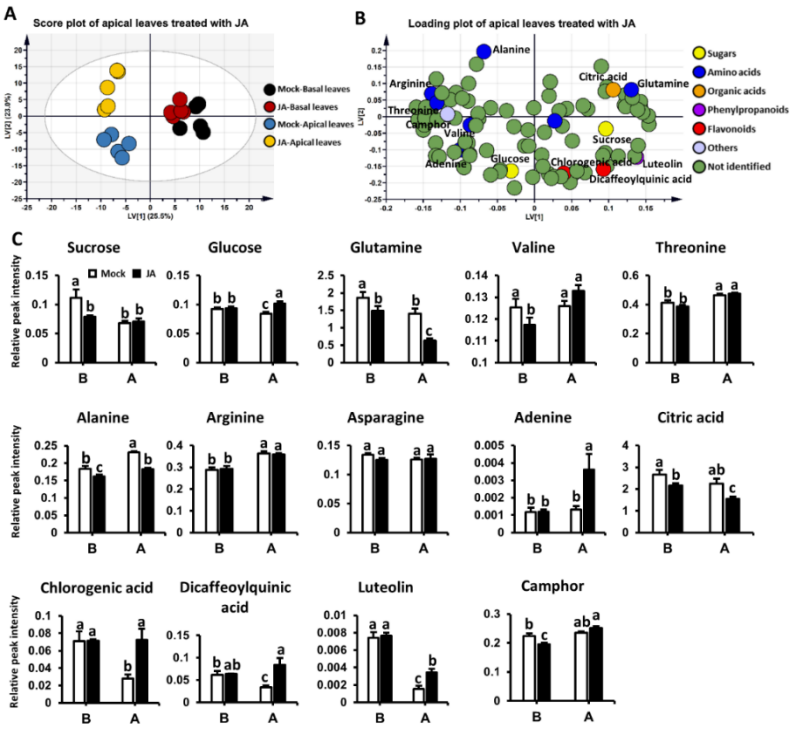

Fig. 6 Metabolomic responses of basal and apical chrysanthemum leaves to local application of JA to apical leaves. Leaf metabolites were analyzed by NMR in basal (leaf 4-5) and apical (leaf 9-10) leaves of chrysanthemum plants at 7 days after the local application of mock or jasmonic acid (JA) solutions to basal leaves (9-10 from the bottom). Partial least square-discriminant analysis (PLS-DA) was performed on the obtained ^{1}H NMR spectra (n = 5). (**A**) Score plot showing the first two latent variables (LVs). The ellipse represents the Hotelling T2 with 95% confidence. (**B**) Loading plot showing important chemical shifts that contribute most to the model (variable importance in projection, VIP > 1). The identified compounds are shown in the plot. (**C**) Relative peak intensity (Mean ± SEM, n = 5) of the identified compounds in basal (B) or apical (A) leaves of mock- and JA-treated plants are shown. Different letters indicate significant differences among groups compared by Fisher's LSD test at $P \leq 0.05$.

Our results first showed that WFT-associated feeding damage differed along the plant canopy in chrysanthemum, being basal leaves (leaves 1-6 from the bottom) more resistant than medium-apical leaves (leaves 7-12 and 13-18) (Fig. 2B, D and F). Likewise, van Haperen *et al*. (2019) described a higher susceptibility in young/apical leaves of a WFT-susceptible accession of sweet pepper (*Capsicum* spp.). These results, however, differ from previous studies reporting higher WFT susceptibility in basal/old leaves of *Rhododendron simsii* (Scott-Brown *et al.*, 2016), tomato (*S. lycopersicum*) (Chen *et al.*, 2018), *Senecio* (Leiss *et al.*, 2009a) and in a resistant accession of sweet pepper (van Haperen *et al.*, 2019). This might be explained by differences in nutrients and defense-related metabolites and their distribution within the plant, as these components are important determinants of herbivore performance (Behmer *et al.*, 2002; Köhler *et al.*, 2015).

Exogenous application of JA has been previously reported to confer plant resistance against WFT in Arabidopsis (Abe *et al.*, 2008), cabbage (Abe *et al.*, 2009), tomato (Escobar-Bravo *et al.*, 2017) and chrysanthemum (see chapter 4 in this thesis). Here we showed that

silver damage symptoms were significantly reduced in the chrysanthemum plants when all the leaves were treated with JA. It should be noted that leaves that were developing during WFT infestation (leaf 19-24) were barely damaged independently of the treatment (Fig. 2B), probably because their small size, a shorter period exposed to WFT and/or they were better defended. We further showed that local JA application to basal leaves (4-5) did not affect WFT resistance in systemic apical leaves (7-12), and it slightly reduced silver damage in leaves 13-18 only. Conversely, local JA induction of apical leaves (9-10) strongly reduced WFT damage in these and the adjacent leaves developed after the induction (13-18), which contributed most to the overall reduction in silver damage per plant (Fig. 2E, F). Systemic induction of defenses against pathogens and herbivores has been amply studied in different plant species (Hilleary & Gilroy, 2018). For instance, Cohen *et al* (1993) showed that local application of JA to basal tomato leaves triggered systemic resistance to a fungal pathogen. We thus hypothesized that diminished systemic induction of resistance against WFT when JA is locally applied to basal chrysanthemum leaves might be explained by a lower capacity of these leaves to respond to the hormone treatment and, therefore, activate JA signaling.

In a first attempt to investigate whether basal and apical leaves differ in their responses to JA, we determined the induction of the JA-associated marker enzyme polyphenol oxidase (PPO) along the chrysanthemum canopy. First, our results showed that PPO activity levels were higher in apical leaves (13-14) than in basal leaves (5-9) of mock-treated plants. Augmented PPO activity levels have been reported to confer enhanced plant resistance to arthropod herbivores (Wang & Constabel, 2004; Mahanil *et al.*, 2008). As young apical chrysanthemum leaves are more susceptible to WFT, our results suggest that differences in constitutive levels of PPO within the plant canopy might not explain the degree of susceptibility to WFT. Application of JA to all the plant leaves increased PPO levels in basal and apical leaves (5-9) (Fig. 3A), suggesting that both groups are responsive to the hormone treatment. Interestingly, application of JA to basal or apical leaves induced PPO levels only locally. This is in strong contrast with previous studies in tomato, where the activity of this defense-related protein has been reported to be induced in systemic leaves after local wounding, JA application or herbivory (Stout *et al.*, 1994; Stout *et al.*, 1996).

Despite the lack of systemic induction of PPO after local application of JA, we did observe an increased resistance to WFT in systemic leaves (Fig. 2C, D). Thus, we further investigated whether JA affected other local and systemic chemical defenses against WFT by analyzing changes in the leaf metabolome. First, our results showed that constitutive chemical defenses of basal and apical leaves significantly differed (Fig. 4). Basal (4-5) leaves of mock-treated plants contained higher levels of phenolics (i.e. chlorogenic acid, dicaffeoylquinic acid and flavonoid luteolin-7-*O*-glucoside), organic acids and sugars (sucrose and glucose), while levels of amino acids were overall reduced, when compared to apical (9-10) leaves. Essential amino acids like threonine and arginine are important nitrogen sources for herbivore growth and development (Chen *et al.*, 2005). Reduced concentrations of these primary metabolites might explain why basal leaves were less preferred by WFT. Furthermore, enhanced levels of phenolics and sugars might have contributed to the higher levels of WFT resistance observed in basal leaves. Both chlorogenic acid and caffeoylquinic acid have been reported to contribute to WFT resistance in chrysanthemum (Leiss *et al.*, 2009b). Similarly, enhanced levels of the flavonoid luteolin have been associated to WFT resistance in carrot (*Daucus carota* L.) (Leiss *et al.*, 2013). Sugars, including glucose, fructose and sucrose, are reported to be involved in plant development and defenses as well (Sheen *et al.*, 1999; Smeekens *et al.*, 2010; Trouvelot *et al.*, 2014), as they can act as signaling molecules and/or provide resources for the constitutive and inducible production of C-based compounds, such as phenolics (Arnold *et al.*, 2004; Guo *et al.*, 2013). Thus, a higher

concentration of sugars in basal leaves might have increased constitutive and hormone-mediated induced defenses. These findings, however, contradict the optimal defense theory (ODT) (McKey, 1974; Ohnmeiss & Baldwin, 2000). ODT predicts that within-plant allocation of defense-associated metabolites positively correlate with the fitness value of specific tissues. Younger leaves are generally of a greater relative fitness value than older/mature leaves (Iwasa *et al.*, 1996; Ohnmeiss & Baldwin, 2000) and they are reported to display higher levels of chemical and/or physical defenses (van Dam *et al.*, 1994; Zangerl & Rutledge, 1996; Scott-Brown *et al.*, 2016; Eisenring *et al.*, 2017; Chen *et al.*, 2018). Further comprehensive work is thus needed to evaluate the influence of these induction strategies on plant fitness in chrysanthemum.

Notably, when JA was applied to all the leaves of chrysanthemum plants, the metabolomic responses of basal (4-5) leaves were stronger than those of apical (9-10) ones (Fig. 4). JA increased the concentrations of sugars, phenylpropanoids, flavonoids and the amino acid asparagine, and reduced the levels of glutamine and citric acid in both basal and apical leaves, but these differences were slightly larger in basal leaves. Induction of phenolic acids (dicaffeoylquinic and chlorogenic acid) and luteolin by the volatile form of JA, methyl jasmonate (MeJA), has been previously reported in wild tobacco (*N. attenuata*), carrot (*Daucus carota* L.) and rice (*Oryza sativa* L.) (Keinänen *et al.*, 2001; Kong *et al.*, 2004; Heredia & Cisneros-Zevallos, 2009). Also, MeJA application has been reported to reduce the amino acid glutamine in *Arabidopsis* (Hendrawati *et al.*, 2006), which is a predominant amino acid constituent of the insect gut (Yoshinaga *et al.*, 2003). Taken together, induction of JA-associated chemical defenses (i.e. phenolics and sugars) and reduction in the nitrogen content of the hormone-treated leaves might have contributed to the observed enhanced resistance to WFT (Fig. 2A, B).

Our results also showed that local application of JA to basal leaves barely affected the metabolomic profiles of systemic apical leaves, and vice versa, at 7 days after the hormone application. This might explain why the local induction of basal leaves (4-5) did not alter WFT susceptibility in apical leaves (9-10) (Fig. 2D). Systemic defense responses are often found to be highly variable in space and time, and many studies have reported differences in local and systemic defense responses to herbivory or exogenous hormone application (Babst *et al.*, 2009; Moreira *et al.*, 2009; Lee *et al.*, 2017; Kundu *et al.*, 2018). Systemically induced resistance can be achieved by the systemic transport, through the plant vascular system, of defensive metabolites and/or signals from the induced tissues that activate *de novo* expression of resistance-associated traits (Heil & Ton, 2008). Distribution of defenses within plants is then often controlled by their vascular architecture, and the translocation of leaf compounds occurs mainly among leaves that are in an approximate vertical row (orthostichy) in many plant species (Orians, 2005). For instance, in Eastern Cottonwood (*Populus deltoides*) (Jones *et al.*, 1993), tobacco (*Nicotiana attenuata*) (Schittko & Baldwin, 2003), tomato (*Lycopersicon esculentum* Mill. `Moneymaker') (Rhodes *et al.*, 1999), cotton (*Gossypium* sp.) (Eisenring *et al.*, 2017) and Arabidopsis (Ferrieri *et al.*, 2015) leaves with direct vascular connections to the damaged leaf are reported to display stronger chemical defense inductions than leaves without these vascular connections. Our results showed that local application of JA to both basal or apical leaves increased plant resistance to WFT in leaves developed after the induction treatment (13-18) (Fig. 2C, D), albeit at different magnitudes. This suggests that they might share direct vascular connections. Alternatively, a stronger sink strength in these new developed leaves (13-18) might have attenuated the systemic responses in mature leaves (9-10). For instance, Arnold and Schultz (2002) showed that JA treatment enhanced sink strength in the developing leaves of hybrid poplar saplings, which resulted in a higher import of carbohydrates and production of

condensed tannins in those leaves. Additional analyses are needed to explore if the induced systemic resistance to WFT in leaves 13-18 correlates with increases in imported resources and chemical defenses from the adjacent leaves (9-10).

In conclusion, we showed that local and systemic induction of JA-mediated chemical defenses in chrysanthemum is spatially variable and dependent on the site of the induction. Furthermore, we showed that higher levels of constitutive and inducible defenses in basal leaves might explain the distribution of WFT-associated feeding within the chrysanthemum plant canopy. Yet, our data also demonstrate that apical leaves, which were preferred by WFT, induced a stronger systemic protection against WFT in leaves that were developed after the hormone induction, contributing most to the enhanced resistance to this insect. Our study has important implications for agriculture systems, as it highlights the variability in within-plant induction of chemical defenses in one of the most important cultivated ornamental species worldwide.

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Supplementary Materials

Table S1 Detailed statistical analysis performed for data displayed in Fig. 2 and 3.
Table S2 Relative intensity of the identified compounds detected by NMR in basal (leaf 4-5 from the bottom) and apical (leaf 9-10 from the bottom) leaves of chrysanthemum plants at 7 days after the application of mock or jasmonic acid (JA) solutions to all the plant leaves, basal or apical leaves.
Table S3 Statistical analysis of identified chemical compounds.
Fig. S1 Heatmap of important NMR signals detected in chrysanthemum basal and apical leaves of plants treated with mock or jasmonic acid (JA) in all the leaves.
Fig. S2 Heatmap of important NMR signals detected in chrysanthemum basal and apical leaves of plants treated with mock or jasmonic acid (JA) in basal leaves.
Fig. S3 Heatmap of important NMR signals detected in chrysanthemum basal and apical leaves of plants treated with mock or jasmonic acid (JA) in apical leaves.

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Supplementary Materials

Table S1 Detailed statistical analysis performed for data displayed in Fig. 2 and 3.

Figure	Panel	Statistical test	Factor and statistic value	df	*P*
Fig. 2	A	Student t-test	JA vs Mock; $t = 2.408$	18	$P = 0.027$
	B	GLM	Age group; *Wald* $\chi^2 = 68.352$	3	$P < 0.001$
			JA vs Mock; *Wald* $\chi^2 = 30.761$	1	$P < 0.001$
			Interaction; *Wald* $\chi^2 = 10.378$	3	$P = 0.016$
	C	Student t-test	JA vs Mock; $t = 0.545$	18	$P = 0.592$
	D	GLM	Age group; *Wald* $\chi^2 = 121.898$	3	$P < 0.001$
			JA vs Mock; *Wald* $\chi^2 = 0.798$	1	$P = 0.373$
			Interaction; *Wald* $\chi^2 = 9.285$	3	$P = 0.026$
	E	Student t-test	JA vs Mock; $t = 2.236$	18	$P = 0.038$
	F	GLM	Age group; *Wald* $\chi^2 = 81.662$	3	$P < 0.001$
			JA vs Mock; *Wald* $\chi^2 = 8.332$	1	$P = 0.004$
			Interaction; *Wald* $\chi^2 = 1.842$	3	$P < 0.606$
Fig. 3	A	GLM	Leaf age; *Wald* $\chi^2 = 83.272$	5	$P < 0.001$
			JA vs Mock; *Wald* $\chi^2 = 45.994$	1	$P < 0.001$
			Interaction; *Wald* $\chi^2 = 5.713$	5	$P = 0.335$
	B	GLM	Leaf age; *Wald* $\chi^2 = 113.699$	5	$P < 0.001$
			JA vs Mock; *Wald* $\chi^2 = 17.369$	1	$P < 0.001$
			Interaction; *Wald* $\chi^2 = 20.997$	5	$P = 0.001$
	C	GLM	Leaf age; *Wald* $\chi^2 = 406.573$	5	$P < 0.001$
			JA vs Mock; *Wald* $\chi^2 = 0.005$	1	$P = 0.942$
			Interaction; *Wald* $\chi^2 = 29.185$	5	$P < 0.001$

Table S2 Relative intensity of the identified compounds detected by NMR in basal (leaf 4-5 from the bottom) and apical (leaf 9-10 from the bottom) leaves of chrysanthemum plants at 7 days after the application of mock or jasmonic acid (JA) solutions to all the plant leaves, basal or apical leaves.

Categories	Compounds	Chemical shift	Hormone application											
			All leaves				Basal leaves				Apical leaves			
			Leaf 4-5		Leaf 9-10		Leaf 4-5		Leaf 9-10		Leaf 4-5		Leaf 9-10	
			Mock	JA	Mock	JA	Mock	JA	Mock	JA	Mock	JA	Mock	JA
Sugars	Fructose	4.08	0.2677± 0.0166 a	0.2882± 0.0078 a	0.2317± 0.0089 b	0.2370± 0.0107 b	0.2823± 0.0128 a	0.2561± 0.0046 b	0.2412± 0.0062 bc	0.2234± 0.0073 c	0.3193± 0.0214 a	0.2533± 0.0072 bc	0.2951± 0.0422 ab	0.2278± 0.0026 c
	Glucose	5.20	0.0825± 0.0048 b	0.1133± 0.0036 a	0.0916± 0.0045 b	0.1045± 0.0068 a	0.0819± 0.0069 b	0.1056± 0.0039 a	0.0798± 0.0037 b	0.0891± 0.0055 b	0.0924± 0.0026 b	0.0938± 0.0030 b	0.0845± 0.0030 c	0.1017± 0.0038 a
	Sucrose	5.40	0.1015± 0.0099 b	0.1882± 0.0149 a	0.0818± 0.0029 b	0.1015± 0.0097 b	0.1079± 0.0040 b	0.1567± 0.0179 a	0.0666± 0.0037 c	0.0567± 0.0023 c	0.1116± 0.0142 a	0.0789± 0.0024 b	0.0683± 0.0038 b	0.0709± 0.0056 b
Amino acids	Valine	1.04	0.1262± 0.0038 b	0.1401± 0.0036 ab	0.1497± 0.0095 a	0.1456± 0.0060 ab	0.1211± 0.0054 c	0.1269± 0.0029 bc	0.1387± 0.0076 ab	0.1433± 0.0086 a	0.1253± 0.0040 a	0.1173± 0.0034 b	0.1260± 0.0023 a	0.1329± 0.0027 a
	Threonine	1.32	0.4261± 0.0105 b	0.5343± 0.0068 a	0.4961± 0.0249 a	0.5226± 0.0220 a	0.4078± 0.0162 b	0.4707± 0.0123 a	0.4746± 0.0102 a	0.5114± 0.0312 a	0.4121± 0.0182 b	0.3872± 0.0093 b	0.4640± 0.0104 a	0.4744± 0.0070 a
	Alanine	1.48	0.1861± 0.0053 b	0.1813± 0.0019 b	0.2354± 0.0086 a	0.1773± 0.0054 b	0.1762± 0.0081 b	0.1705± 0.0036 b	0.2225± 0.0088 a	0.1824± 0.0078 b	0.1831± 0.0083 b	0.1623± 0.0043 c	0.2314± 0.0031 a	0.1821± 0.0041 b
	Arginine	1.72	0.2991± 0.0079 b	0.2974± 0.0213 b	0.3948± 0.0211a	0.3659± 0.0105 a	0.2597± 0.0137 c	0.3165± 0.0140 b	0.3603± 0.0208 a	0.3819± 0.0147 a	0.2871± 0.0122 b	0.2928± 0.0133 b	0.3634± 0.0099 a	0.3586± 0.0065 a
	Glutamine	2.44	1.8832± 0.1427 a	0.5525± 0.0451 c	1.0538± 0.1888 b	0.5830± 0.0442 c	1.6859± 0.2011 a	0.6535± 0.0844 b	0.9240± 0.1056 b	0.6381± 0.0299 b	1.8569± 0.1702 a	1.4902± 0.1378 b	1.4044± 0.1468 b	0.6249± 0.0706 c
	Asparagine	2.84	0.1363± 0.0046 b	0.1612± 0.0091 a	0.1474± 0.0087 ab	0.1405± 0.0035 b	0.1347± 0.0040 a	0.1409± 0.0056 a	0.1397± 0.0024 a	0.1361± 0.0053 a	0.1339± 0.0034 a	0.1250± 0.0032 a	0.1261± 0.0030 a	0.1273± 0.0070 a
	Adenine	8.20	0.0009± 0.0001 b	0.0016± 0.0002 b	0.0017± 0.0002 b	0.0032± 0.0007 a	0.0012± 0.0003 ab	0.0010± 0.0002 b	0.0016± 0.0002 a	0.0015± 0.0003 ab	0.0012± 0.0002 b	0.0012± 0.0001 b	0.0013± 0.0002 b	0.0036± 0.0009 a
Small organic acid	Citric acid	2.72	2.6841± 0.1854 a	1.5876± 0.1175 c	2.1100± 0.1058 b	1.4910± 0.0483 c	2.5476± 0.2692 a	1.6300± 0.0677 c	2.1578± 0.0686 b	1.9504± 0.0500 c	2.6584± 0.2234 a	2.1682± 0.0970 b	2.2466± 0.2354 ab	1.5565± 0.1000 c
Phenylpropanoids	Chlorogenic acid	6.36	0.0777± 0.0100 bc	0.2642± 0.0378 a	0.0320± 0.0013 c	0.1108± 0.0141 b	0.0903± 0.0041 b	0.2122± 0.0313 a	0.0271± 0.0034 c	0.0311± 0.0029 c	0.0713± 0.0109 a	0.0714± 0.0017 a	0.0279± 0.0047 b	0.0725± 0.0128 a
	3,5-Dicaffeoylquinic acid	6.48	0.0673± 0.0025 c	0.2339± 0.0269 a	0.0412± 0.0025 c	0.1205± 0.0125 b	0.0757± 0.0028 b	0.1835± 0.0220 a	0.0354± 0.0030 c	0.0443± 0.0034 c	0.0615± 0.0088 b	0.0635± 0.0015 ab	0.0339± 0.0041 c	0.0844± 0.0152 a
Flavonoids	Luteolin-7-*O*-glucoside	7.44	0.0053± 0.0008 b	0.0171± 0.0010 a	0.0022± 0.0007 b	0.0046± 0.0017 b	0.0070± 0.0007 b	0.0126± 0.0017 a	0.0020± 0.0004 c	0.0017± 0.0002 c	0.0074± 0.0007 a	0.0077± 0.0004 a	0.0016± 0.0003 c	0.0035± 0.0004 b
Others	Camphor	0.96	0.2241± 0.0036 b	0.2703± 0.0088 a	0.2573± 0.0102 a	0.2632± 0.0076 a	0.2162± 0.0069 a	0.2416± 0.0131 ab	0.2543± 0.0191 a	0.2409± 0.0155 ab	0.2236± 0.0094 b	0.1957± 0.0048 c	0.2357± 0.0046 ab	0.2511± 0.0063 a
	myo-Inositol	3.64	0.7137± 0.0367 ab	0.7632± 0.0233 a	0.6919± 0.0255 ab	0.6761± 0.0346 b	0.7185± 0.0197 a	0.6852± 0.0220 ab	0.6631± 0.0219 bc	0.6172± 0.0218 c	0.8158± 0.0407 a	0.7101± 0.0084 b	0.6813± 0.0107 bc	0.6426± 0.0202 c

The mean and SEM for each compound is shown. Different letters denote significant differences among groups compared by LSD test at $P \leq 0.05$.

Table S3 Statistical analysis of identified chemical compounds.

Categories	Compounds	Chemical shift	Hormone application								
			All leaves			Basal leaves			Apical leaves		
			JA treatment	Leaf position	Interaction	JA treatment	Leaf position	Interaction	JA treatment	Leaf position	Interaction
Sugars	**Fructose**	**4.08**	$P = 0.211$	$P < 0.001$	$P = 0.463$	$P = 0.003$	$P < 0.001$	$P = 0.571$	$P = 0.002$	$P = 0.247$	$P = 0.974$
	Glucose	**5.20**	$P < 0.001$	$P = 0.973$	$P = 0.049$	$P < 0.001$	$P = 0.044$	$P = 0.121$	$P = 0.001$	$P = 0.988$	$P = 0.004$
	Sucrose	**5.40**	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.021$	$P < 0.001$	$P < 0.01$	$P = 0.034$	$P < 0.001$	$P = 0.013$
Amino acids	**Valine**	**1.04**	$P = 0.384$	$P = 0.010$	$P = 0.107$	$P = 0.371$	$P = 0.003$	$P = 0.923$	$P = 0.840$	$P = 0.004$	$P = 0.008$
	Threonine	**1.32**	$P < 0.001$	$P = 0.067$	$P = 0.010$	$P = 0.004$	$P = 0.002$	$P = 0.451$	$P = 0.500$	$P = 0.001$	$P = 0.101$
	Alanine	**1.48**	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.009$	$P < 0.001$	$P < 0.001$	$P = 0.003$
	Arginine	**1.72**	$P = 0.296$	$P < 0.001$	$P = 0.352$	$P = 0.006$	$P < 0.001$	$P = 0.221$	$P = 0.961$	$P < 0.001$	$P = 0.586$
	Glutamine	**2.44**	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.091$
	Asparagine	**2.84**	$P = 0.148$	$P = 0.441$	$P = 0.010$	$P = 0.750$	$P = 0.978$	$P = 0.221$	$P = 0.330$	$P = 0.489$	$P = 0.205$
	Adenine	**8.20**	$P = 0.001$	$P = 0.001$	$P = 0.281$	$P = 0.395$	$P = 0.031$	$P = 0.822$	$P = 0.007$	$P = 0.003$	$P = 0.008$
Small organic acid	**Citric acid**	**2.72**	$P < 0.001$	$P = 0.003$	$P = 0.032$	$P < 0.001$	$P = 0.789$	$P = 0.006$	$P < 0.001$	$P = 0.001$	$P = 0.527$
Phenylpropanoids	**Chlorogenic acid**	**6.36**	$P < 0.001$	$P < 0.001$	$P = 0.004$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.004$	$P = 0.007$	$P = 0.005$
	3,5-Dicaffeolquinic acid	**6.48**	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P = 0.677$	$P = 0.003$
Flavonoids	**Luteolin-7-*O*-glucoside**	**7.44**	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.002$	$P < 0.001$	$P < 0.001$	$P = 0.011$	$P < 0.001$	$P = 0.051$
Others	**Camphor**	**0.96**	$P < 0.001$	$P = 0.067$	$P = 0.005$	$P = 0.640$	$P = 0.146$	$P = 0.131$	$P = 0.285$	$P < 0.001$	$P < 0.001$
	***myo*-Inositol**	**3.64**	$P = 0.538$	$P = 0.046$	$P = 0.233$	$P = 0.039$	$P = 0.001$	$P = 0.742$	$P = 0.001$	$P < 0.001$	$P = 0.114$

The effects of the hormone treatment, leaf position and their interaction on the relative intensity of the identified compounds were tested by Generalized linear models.

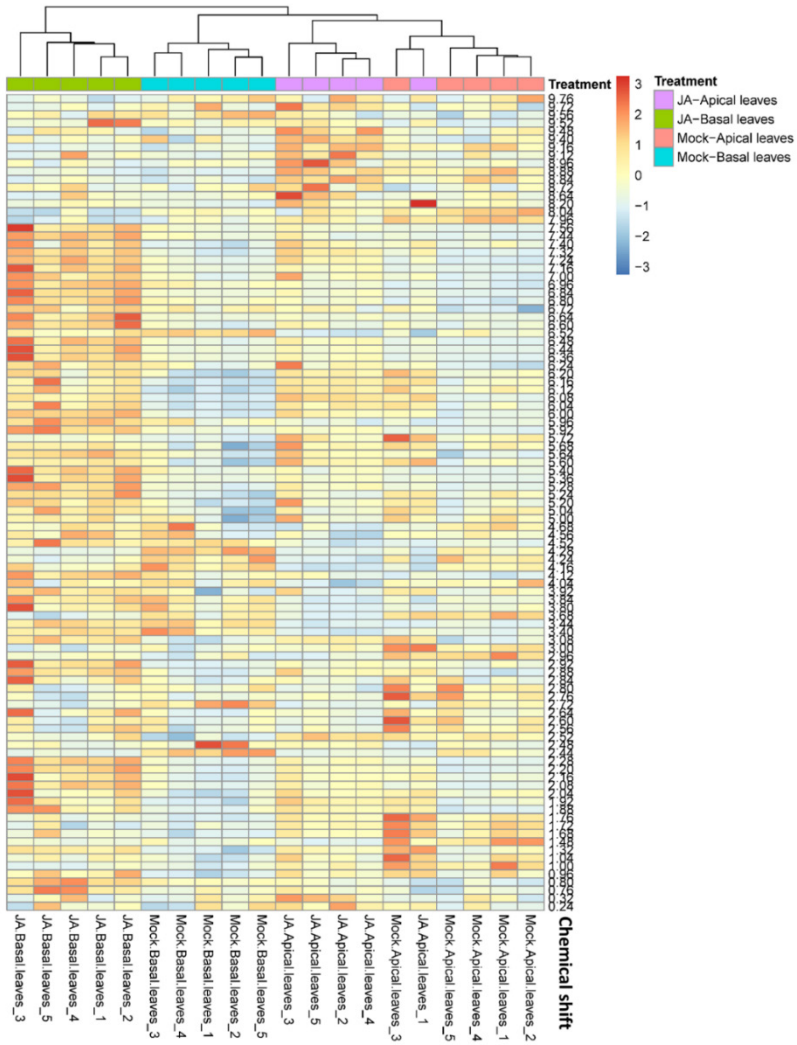

Fig. S1 Heatmap of important NMR signals detected in chrysanthemum basal and apical leaves of plants treated with mock or jasmonic acid (JA) in all the leaves. The NMR signals were selected according to their variable importance in projection (VIP > 1) obtained based on the partial least square-discriminant analysis (PLS-DA). The heatmap shows the standard relative peak intensity of the important chemical shifts of basal (leaf 4-5) or apical (leaf 9-10) leaves of chrysanthemum plants at 7 days after the hormone treatment. Hierarchical classifications with attributes (HCAs) were performed to group the chemical profiles in each treatment according to their Euclidean distance.

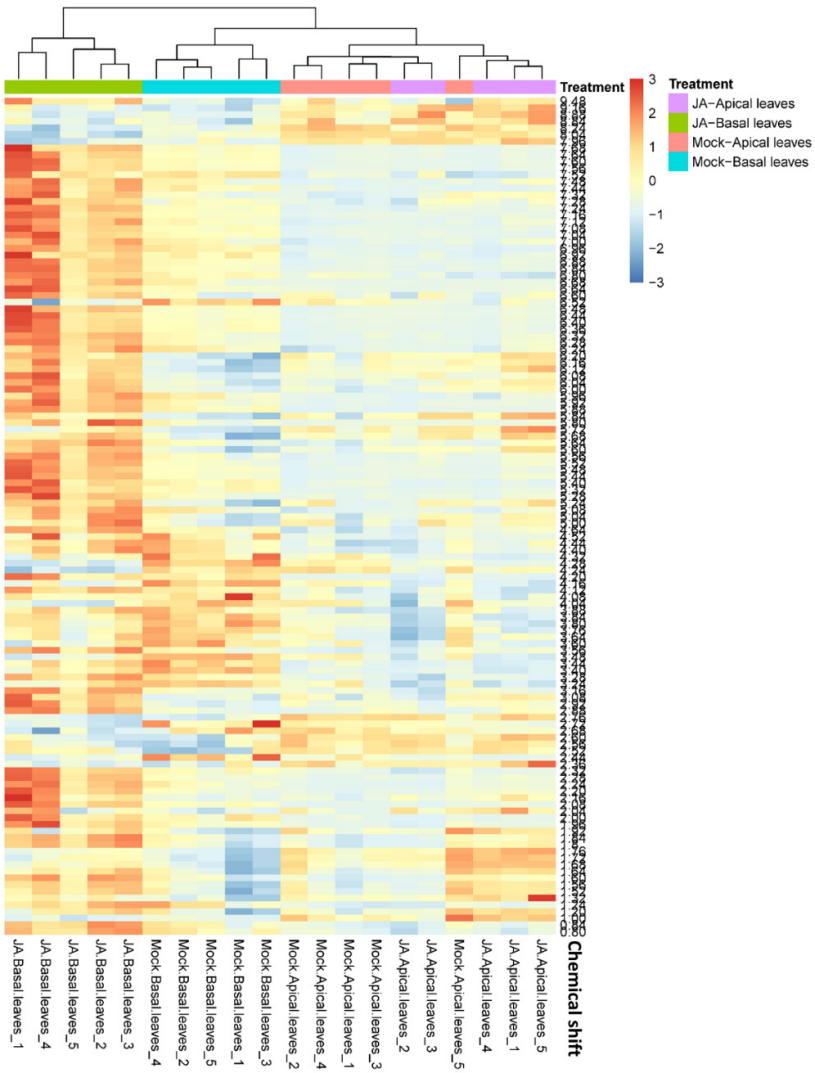

Fig. S2 Heatmap of important NMR signals detected in chrysanthemum basal and apical leaves of plants treated with mock or jasmonic acid (JA) in basal leaves. The NMR signals were selected according to their variable importance in projection (VIP > 1) obtained based on the partial least square-discriminant analysis (PLS-DA). The heatmap shows the standard relative peak intensity of the important chemical shifts of basal (leaf 4-5) or apical (leaf 9-10) leaves of chrysanthemum plants at 7 days after the hormone treatment. Hierarchical classifications with attributes (HCAs) were performed to group the chemical profiles in each treatment according to their Euclidean distance.

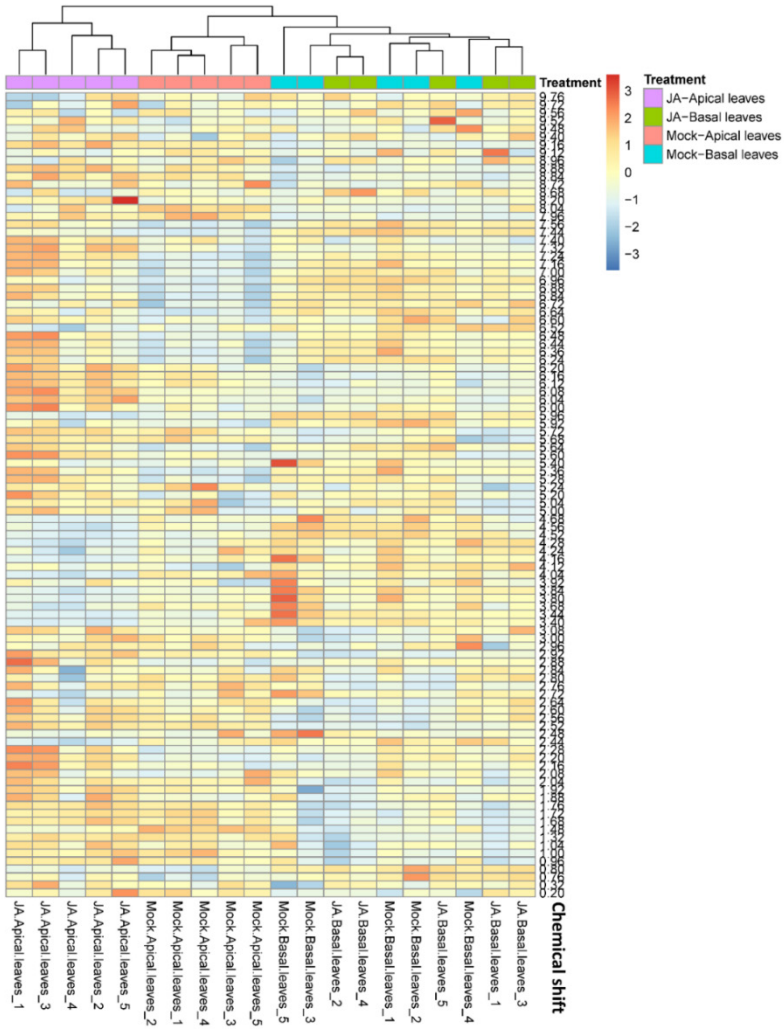

Fig. S3 Heatmap of important NMR signals detected in chrysanthemum basal and apical leaves of plants treated with mock or jasmonic acid (JA) in apical leaves. The NMR signals were selected according to their variable importance in projection (VIP > 1) obtained based on the partial least square-discriminant analysis (PLS-DA). The heatmap shows the standard relative peak intensity of the important chemical shifts of basal (leaf 4-5) or apical (leaf 9-10) leaves of chrysanthemum plants at 7 days after the hormone treatment. Hierarchical classifications with attributes (HCAs) were performed to group the chemical profiles in each treatment according to their Euclidean distance.