Metabolomic characteristics of Catharanthus roseus plants in time and space

Qifang, P.

Chapter 5

Accumulation of terpenoid indole alkaloids in jasmonates-elicited *Catharanthus roseus* plants before and during flowering

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Abstract

Jasmonic acid (JA) analogues including methyl-jasmonate (MeJA) are plant-signaling molecules that play key roles in defense against insects and pathogens. Interestingly, in *Catharanthus roseus* jasmonates regulate the biosynthesis of pharmaceutically important terpenoid indole alkaloids (TIAs). Exogenous JA/MeJA application stimulates TIA metabolism in cell cultures, hairy roots and seedlings of *C. roseus.* In the present study, *C. roseus* plants were elicited with JA or MeJA before and during flowering to investigate the induction of TIA biosynthesis during different plant developmental stages. The results showed that there was no significant difference between JA and MeJA induction of TIA biosynthesis. The level of jasmonic acid was higher before flowering than during flowering in *C. roseus* control (non-treated) plants. In *C. roseus* plants, JA slightly stimulates the TIA accumulation before flowering but had less effect during flowering. TIA biosynthesis in different organs (flower, leaf, and root) showed a different response to JA elicitation.

Introduction

Methyl jasmonate (MeJA) is a volatile compound formed from jasmonic acid (JA) which is catalyzed by *S*-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (Seo *et al.*, 2001). Jasmonates play a central role in inter- and intra-plant signaling and function as important cellular regulators mediating diverse developmental processes such as senescence, root growth, pollen production, wounding responses, and plant resistance to insects and pathogens (Turner *et al.*, 2002; Balbi and Devoto, 2008). Induction of secondary metabolites production is an important defense response, which might be regulated by jasmonates as the regulatory signals (Gundlach *et al.*, 1992; Memelink *et al.*, 2001).

Jasmonates (JAs) have been also reported to regulate the biosynthesis of secondary metabolites, e.g. terpenoid indole alkaloids (TIAs) in *Catharanthus roseus*. More than 130 TIAs have been identified in this species, among which some are of pharmaceutically important metabolites such as vinblastine and vincristine, which have antitumor activity (van der Heijden *et al.*, 2004). In *C. roseus*, JA/MeJA is first converted to the bioactive jasmonate JA-Ile and its perception by CrCOI1 results in the degradation of CrJAZ proteins which repress the activity of CrMYC2. CrMYC2 then activates the transcription of genes encoding the ERF transcription factors ORCA2 and ORCA3, which in turn activate the expression of TIA biosynthesis genes (Zhang *et al.*, 2011).

Elicitation strategies using exogenous JAs have been implemented in *C. roseus* cell cultures, hairy roots, and plantlets aiming at increasing TIA production. The presence of jasmonates results in transcriptional activation of tryptophan decarboxylase (TDC) and strictosidine glucosidase (SGD), enhancing some steps of the TIA biosynthetic network, however, some pathways like the one leading to vindoline are not induced in *C. roseus* cell cultures (Shukla *et al.*, 2010). In *C. roseus* hairy roots, JA or MeJA treatment increases the transcripts level of TIA pathway genes (e.g. *ORCAs*, *ASa*, *TDC*, *DXS*, *DXR*, *G10H*, *CPR*, *SLS*, *STR*, *SGD*, *ZCTs*) and the concentrations of ajmalicine, catharanthine, serpentine, and tabersonine (Ruiz-May *et al.*, 2008; Peebles *et al.*, 2009; Zhou *et al.*, 2010). Similarly, MeJA elicitation increased the accumulation of vindoline and catharanthine in *C. roseus* seedlings (Aerts *et*

al., 1996; El-Sayed *et al.*, 2004), but had little effect on the TIA accumulation when it was applied on the flowering *C. roseus* plants (Pan *et al.*, 2010). Apparently besides the jasmonate signaling also the cellular differentiation and development stages of the plant are involved in the regulation of TIA biosynthesis. Therefore, it is essential to study the effect of jasmonates on the TIA accumulation through the developmental stages of *C. roseus* plants such as before and during flowering.

In this study, the effect of JA or MeJA on the TIA accumulation in different organs (flower, leaf, stem and root) and endogenous JA content were investigated through the developmental stages of *C. roseus* plants by HPLC and GC-MS, aiming at better understanding the regulation of TIA biosynthesis.

Materials and methods

Plant materials and cultivation methods

Seeds of *C. roseus* (cv. Pacific Cherry Red) were purchased from PanAmerican Seed Company (West Chicago, IL, U.S.A.). The seeds were surface sterilized in 75% (v/v) ethanol for 2 min and 5% (v/v) $NaClO_2$ for another 5 min. Subsequently, seeds were washed 5 times with sterile distilled water and germinated on Petri dishes containing MS (Murashige and Skoog, 1962) basal medium. Cultures were grown under 16 h light and 8 h dark photoperiod at 25 ± 2 ℃. After germination for 2 weeks, seedlings were transplanted into soil and grown in the greenhouse at 25±3 ℃.

JA and MeJA treatments

Jasmonates (JA and MeJA) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). The stock solutions were prepared by dissolving JA and MeJA in ethanol to achieve a concentration of 1 M and subsequently diluted by water to provide 0.1 mM of the working solutions. For the experiment, the *C. roseus* plantlets were divided into two groups. The plantlets which had 6 to 7 layers of leaves at 45 days (before flowering) and 9 to 10 layers of leaves at 80 days (during flowering) were sprayed with 0.1 mM JA or MeJA, respectively. The flowers, upper leaves (3 layers leaves from the top), lower layer leaves (3

layers leaves from the bottom) and roots were collected in the following three days including those of the control plants (without JA/MeJA treatment). The mixed upper and lower leaves were used to analyse the difference between JA and MeJA treatments. The experiments were conducted in randomized block design (RBD) with three replicate samples for each time point of the time course.

Statistical analysis was performing using ANOVA by SPSS (version 20.0, Chicago, IL, USA). Homogeneity of variance was tested. The values are mean ± standard deviation for three samples in each group. Level of significance set at 0.05 (Alpha <0.05) was considered as significant.

TIA analysis using HPLC-DAD

Fresh samples were collected and ground in liquid nitrogen. Subsequently, the samples were lyophilized for 72 hours. The dried sample (30 mg) was extracted with 1 mL methanol and sonicated for 30 min in an Ultrasonic bath (DL-60D) (RADIOLINIJA UAB, Vilnius, Lithuania). Subsequently, the samples were centrifuged at 12,000 rpm for 10 min at room temperature and the supernatant was filtered through 0.45 μm PTFE membrane filter prior to HPLC analysis.

The chromatography was carried out using a Zorbax Eclipse XDB-C18 column (250 mm x 4.6 mm, 5μ) (Agilent Techologies, Santa Clara, CA, USA). The chromatographic system was an Agilent Technologies 1200 series consisting of a G1322A Vacuum Degasser，a G1310A Iso Pump, a G1329A AutoSampler，a G1316A Thermostated Column Compartment, and a G1315D Diode Array Detector.

The chromatographic method was optimized from Tikhomiroff and Jolicoeur (2002) for the qualitative and quantitative analysis of 10 TIAs, and 5 TIA precursors. The mobile phase consisted of a mixture of 5 mM Na_2HPO_4 (pH adjusted to 6 with HCl) (solvent A) and methanol (solvent B) at a flow rate of 1.5 mL per min. The eluent profile (volume of solvent A / volume of solvent B) was: 0-2 min, linear gradient from 86:14 to 14:86; 26-30 min, isocratic elution with 14:86 (v/v); 30-35 min, linear gradient from 14:86 to 86:14; 35-37 min, isocratic elution with 86:14(v/v). The sample injection volume was 30 μL. Peak identification was based on a comparison of the retention time and UV

spectra of the standard compounds. Samples were applied in triplicate and quantified using the calibration curves of the standard compounds.

The standard compounds for vindoline was bought from PhytoLab (Vestenbergsgreuth, Germany); vindolinine and ajmalicine were purchased from Sigma-Aldrich (St. Louis, MO, USA); serpentine was purchased from Roth (Karlsruhe, Germany); catharanthine and anhydrovinblastine were kind gifts from Pierre Fabre (Gaillac, France).

JA analysis using GC-MS

Fifty mg of freeze-dried sample was put into a glass tube. One hundred ng of dihydrojasmonic acid (DHJA) was added as the internal standard. The sample was mixed and vortexed with 1 mL of 2-propanol/water/36% of HCl (2:1:0.002 v/v/v) for 1 min. After sonicating for 30 min, 1 mL of methylene chloride was added and vortexed for another minute, and subsequently the sample was centrifuged at 3500 rpm for 15 min at 5 ℃. The bottom methylene chloride/2-propanol layer was collected with a syringe into a new glass tube and derivatized with 2 µl of 2 M trimethylsilyldiazomethane in *n*-hexane at room temperature for 30 min. Subsequently 2 µl of 2 M acetic acid in hexane was added to stop the reaction. The derivatized sample was concentrated under nitrogen gas flow and the residue was redissolved in 300 µl of methylene chloride for GC-MS analysis.

The chromatography system consisted of an Agilent 7890A gas chromatograph (GC) coupled to an Agilent 5975C mass-selective detector (Agilent Technologies). The detector was operated in electron impact (EI) ionization mode (electron energy 70 eV). The GC was fitted with a DB-5MS column (30 m x 0.25 mm x 0.25 µm) (J&W Scientific, Folsom, CA, USA). The flow rate of the gas carrier (helium) was 1 mL/min. GC conditions were isothermal for 2 min at 80 ℃, increase from 80 ℃ to 200 ℃ at 10 ℃ /min, and subsequently increase to 300 ℃ at 20 ℃ /min for 15 min. Quantification of JA was based on the integrated area under the curve of JA by comparison with the internal standard. Each treatment was measured in triplicate.

Results and discussion

Comparison of TIA contents in upper leaves, lower leaves and roots of Catharanthus roseus before and during flowering

The contents of vindolinine, ajmalicine, serpentine, vindoline, catharanthine and anhydrovinblastine in upper leaves, lower leaves and roots were compared and analyzed before and during the blooming period of *C. roseus* plants (Table 1).

Table 1 TIA contents in upper leaf, lower leaf and root of *Catharanthus roseus* before and during flowering. *-: not detected

	Upper leaf		Lower leaf		Root	
mg/g DW	**BF**	**DF**	**BF**	**DF**	**BF**	**DF**
Vindolinine	0.81 ± 0.01^{a}	1.21 ± 0.08^{b}	0.56 ± 0.01^{a}	0.54 ± 0.04^{a}	4.83 ± 0.44^{a}	5.25 ± 0.36^{a}
Ajmalicine	0.23 ± 0.09^{a}	0.01 ± 0.001^{b}	-	-	0.4 ± 0.003^{a}	0.15 ± 0.02^{b}
Serpentine	0.15 ± 0.01^{a}	0.3 ± 0.02^{b}	0.2 ± 0.01^{a}	0.18 ± 0.01^{a}	0.13 ± 0.02^{a}	0.34 ± 0.03^{b}
Vindoline	1.47 ± 0.07^{a}	0.81 ± 0.08^{b}	0.57 ± 0.03^{a}	0.38 ± 0.04^{b}	-	-
Catharanthine	2.51 ± 0.05^{a}	1.19 ± 0.19^{b}	1.18 ± 0.02^{a}	0.88 ± 0.14^{a}	2.69 ± 0.10^{a}	1.38 ± 0.12^{b}
Anhydrovinblastine	0.56 ± 0.03^{a}	1.07 ± 0.01^{b}	1.2 ± 0.06^{a}	1.63 ± 0.19^{b}	-	-

$^{a, b}$:significant difference between BF and DF in one column (Alpha < 0.05 by ANOVA)
BF: before flowering
DF: during flowering
DW: dry weight
Results are the mean of 3 replicates ± standard deviation.

Vindolinine in upper leaves showed a significantly higher level before flowering than during flowering, but in lower leaves and roots there was no difference in its accumulation between the two stages. In both upper leaves and roots ajmalicine contents were higher before flowering than during flowering. On the contrary, serpentine level was significantly lower in upper leaves and roots before flowering if compared to their levels during flowering. In both upper and lower leaves, vindoline and catharanthine accumulated at higher

levels before flowering while anhydrovinblastine accumulated more during flowering. The decrease in levels of the two precursors (vindoline and catharanthine) for the dimeric alkaloids during flowering coincides with an increase of the dimer anhydrovinblastine. Similarly the increase of serpentine during flowering compensates for the decrease in the level of its precursor ajmalicine.

Effect of JA elicitation on TIA biosynthesis in different organs of Catharanthus roseus before and during flowering

Flowers, upper-and lower leaves, and roots of *C. roseus* were collected for the study on the effect of JA treatment on the accumulation of TIA before and during flowering.

Figure 1 and 2 show that vindoline, one of the precursors for bisindole alkaloids, was present in the leaves and flowers, but it not in the roots. This is in accordance with the finding that vindoline biosynthesis requires chloroplasts only occurring in cells in the leaves of *C. roseus* (St-Pierre *et al.* 1999; Murata and De Luca 2005). The vindoline level in the upper leaves before flowering showed a small increase of 29% at 72 h over the controls (Fig. 1). A similar trend was observed in the experiment in which both JA and MeJA elicitation were compared (Fig. 6). During flowering, however, vindoline level in the upper layer leaves was not significantly changed after JA elicitation (Fig. 2). Regardless the developmental stages, the lower leaves contained a lower level of vindoline than the upper ones. No change in vindoline level was observed in the lower leaves after JA elicitation in both developmental stages.

In contrast to vindoline, the other precursor of the bisindole alkaloids, catharanthine, was present in all organs (flowers, leaves and roots in Fig. 1, 2) and had the highest level before flowering. After JA treatment before flowering, compared to the controls, catharanthine levels showed a small increase of 27% in upper leaves at 72 h and a significant decrease of 54% in roots at 24 h while no significant change of its levels was observed in lower leaves (Fig. 1). In Fig. 6 similar observations can be made for another experiment using either JA or MeJA as elicitor. Catharanthine accumulation in roots was much higher before flowering than during flowering. After JA treatment a transient reduction in its

level was observed in plants before flowering, followed by a small increase if compared to control. During flowering, JA treatment caused no change of catharanthine level in upper and lower leaves (Fig. 2, 6).

Catharanthine and vindoline are the precursors for the bisindole alkaloids. One of the bisindole alkaloids detected in this study was anhydrovinblastine. Anhydrovinblastine accumulates in flowers, leaves and roots with the growth of plants. During flowering, anhydrovinblastine content was two times higher in the upper leaves than before flowering. Before flowering, anhydrovinblastine was 2-fold higher in the lower leaves than the upper leaves (Fig. 1). This compensates the lower levels of its precursors, vindoline and catharanthine if compared to their levels in the upper leaves. After JA elicitation there seems a trend of a small increase of anhydrovinblastine in upper leaves before flowering whereas there is no effect during flowering. In the lower leaves in the blooming plant, after JA treatment the level of anhydrovinblastine decreased if compared with control (Fig. 2). The same trend is observed in Fig. 6. JA elicitation seems to slightly, though not statistically significantly, increase the level of anhydrovinblastine in flowers (Fig. 2).

Ajmalicine is a mono-TIA, which particularly accumulates in the roots of *C. roseus*. The results show that the ajmalicine levels in the roots and upper leaves were higher before flowering than during flowering (Table 1, Fig. 1 and 2). After JA elicitation, ajmalicine accumulation in upper leaves was increased 64% at 72 h in plants before flowering and 26% at 48 h in plants during flowering. JA elicitation resulted in 24% and 77% increase of ajmalicine in roots at 72 h in plants before and during flowering, respectively. The lower leaves did not accumulate ajmalicine. Up to 0.042 mg/g DW ajmalicine was detected in the flowers after 48 h of JA elicitation, 90% higher than the controls.

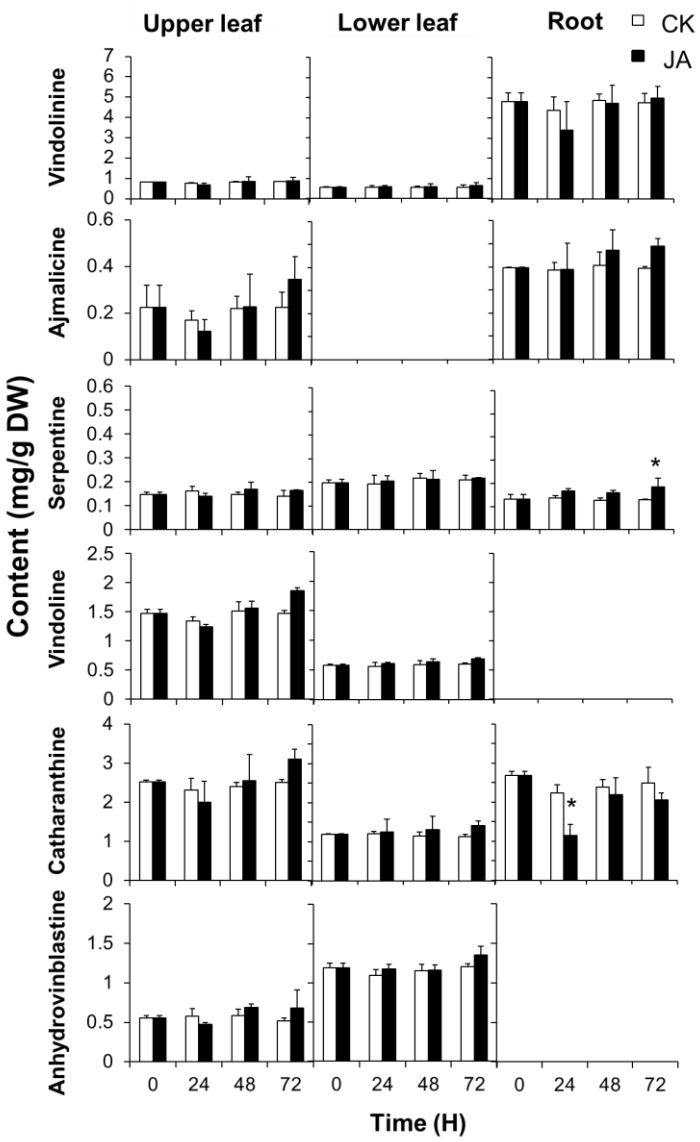


Fig. 1 The contents of vindolinine, ajmalicine, serpentine, vindoline, catharanthine and anhydrovinblastine in the upper leaves, lower leaves and roots of *Catharanthus roseus* under JA treatment before flowering. CK: the control plants; JA: plants treated with JA. DW: dry weight. *: significant difference (Alpha < 0.05 by ANOVA). Results are the mean of 3 replicates ± standard deviation. Empty panel means that the compound was not detected in the sample.

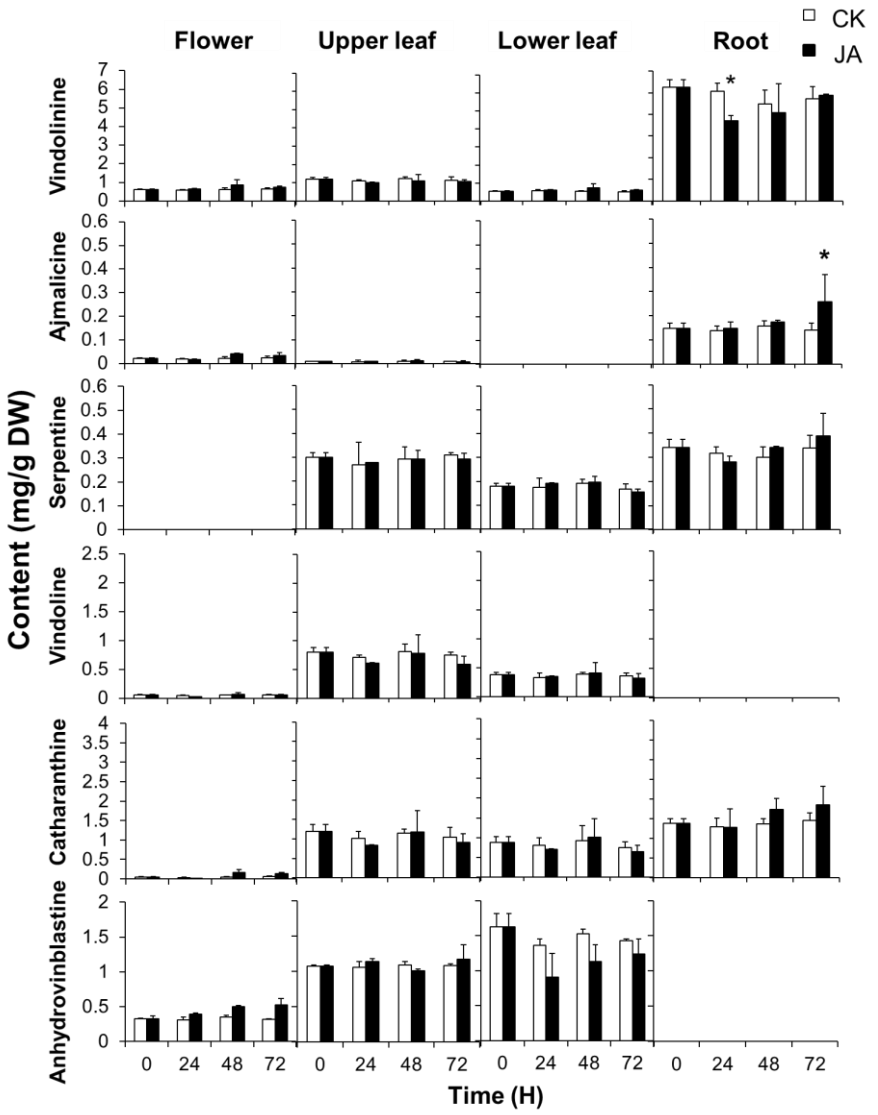


Fig. 2 The contents of vindolinine, ajmalicine, serpentine, vindoline, catharanthine and anhydrovinblastine in the flowers, upper leaves, lower leaves and roots of *Catharanthus roseus* under JA treatment during flowering. CK: the control plants; JA: plants treated with JA. DW: dry weight. *: significant difference (Alpha < 0.05 by ANOVA). Results are the mean of 3 replicates ± standard deviation. Empty panel means that the compound was not detected in the sample.

Serpentine is formed from ajmalicine by oxidation catalyzed by peroxidases. Serpentine accumulated in leaves and roots but was not detected in flowers (Fig. 2). In the flowering plants the level of serpentine in upper leaves and roots is higher than in plants before flowering, this is connected with a similar decrease in ajmalicine level in the upper leaves and roots of the flowering plants. Before flowering, JA treatment had no significant effect on serpentine accumulation in upper and lower leaves but a significantly increased production was observed in roots (40%) 72 hrs after treatment (Fig. 1). Changes in the accumulation of alkaloids such as ajmalicine and serpentine were also observed in *C. roseus* hairy roots after elicitation with MeJA (Ruiz-May *et al.*, 2008; Goklany *et al.*, 2009).

Vindolinine was detected in all organs of *C. roseus* plants (Fig. 2). Before flowering, JA treatment showed no effect on vindolinine accumulation in leaves and a small reduction after 24 hrs in roots (Fig.1). During flowering, vindolinine production was not much affected either by the elicitation. Only the roots showed a trend of an small transient reduction at 24 hrs for the non-flowering plants and the flowering plants.

The floral transition is a major metabolic change in a plant's life. One of the functions of JA signaling in *C. roseus* is, among others, to regulate the alkaloid biosynthesis in a narrow time interval of the development of leaves and roots (El-Sayed and Verpoorte 2004; Aerts *et al.*, 1994). MeJA hardly affects alkaloid contents when it was applied at later developmental stages of the seedlings (Aerts *et al.*, 1996). MeJA treatment increased the contents of vindoline and catharanthine but failed to increase vinblastine accumulation in seedlings (Aerts *et al.*, 1996). When applied to flowering plants, MeJA had little effect on the contents of vindoline, catharanthine and vinblastine (Pan *et al.*, 2010). Our present results are consistent with these previous studies. Although statistical analysis by ANOVA showed that in most cases of the individual experiments the results cannot be concluded to be significant, in a separate experiment similar small fluctuations in TIA contents were observed after JA and MeJA elicitation (compare results shown in Fig. 1 and Fig. 2 with Fig. 6). JA treatment stimulated the accumulation of vindoline and catharanthine in upper leaves, and serpentine and ajmalicine in roots before flowering, whereas during flowering JA induced the accumulation of catharanthine and ajmalicine

mainly in roots and flowers. Similar to protease inhibitors accumulation that is limited to the early stages of plant development, wound- and jasmonate-induced whole plant nicotine accumulation in *Nicotiana sylvestris* decreased during the plant's ontogeny (Van Dam *et al.*, 2001). In the case of *C. roseus*, JA induction of TIA biosynthesis was limited to the early stages of plant development as JA treatment of the flowering plants did not significantly increase TIA levels in leaves and roots.

The initiation of flowering is associated with changes in the relative defense requirements of different plant organs. Though the changes are relatively small, the results show that the response of TIA accumulation after JA induction does show some variation between different organs. Flowering slightly attenuated the JA effect on the accumulation of vindoline, catharanthine, ajmalicine, serpentine, and anhydrovinblastine in leaves and roots, but in flowers TIAs accumulated and responded to JA induction. These results indicate that TIA accumulation in flowers is more sensitive to JA elicitation than in leaves during flowering. As the reproductive tissues, flowers are more important than vegetative parts and plants seem to prioritize chemical protection to seed production over other functions (Diezel *et al.*, 2011). In terms of industrial production, the alkaloid content in the flowers is quite low if compared to the other plants parts, and thus not of interest for large scale extraction. In roots, the accumulation of catharanthine, ajmalicine and serpentine increased under JA treatment. It has been reported that jasmonates can induce ajmalicine production in hairy roots (Ruiz-May *et al.*, 2008).

Jasmonic acid levels in Catharanthus roseus leaves

The level of JA in *C. roseus* leaves after JA treatment before and during flowering was determined by GC-MS. Identification of JA was based on mass spectral data and retention time. JA (224 *m*/*z*) appeared at 13.82 min (Fig. 3) whereas DHJA (226.14 *m*/*z*), which is used as the internal standard, appeared at 13.88 min (Fig. 4). According to normalized areas under the curve of JA to the internal standard (DHJA), JA levels were quantified as relative amounts.

The results show that JA was at a 6-fold higher level before flowering than during flowering in leaves without JA treatment (Fig. 5). Before flowering, jasmonates level showed a 2-fold increase after 48 h and returned to a basal

level after 72 h of JA treatment. During flowering, jasmonates level increased 4-fold at 48 h and returned to a low level at 72 h after JA treatment. JA level was always observed to be higher before flowering than during flowering after JA treatment.

Flowering plants have an apparent control over herbivore-elicited phytohormones. Insect oral secretions (OS)-inducible JA bursts decline with the initiation of flowering in *N. attenuata* plants (Diezel *et al.*, 2011). Wound-elicited JA showed a decrease in *N. sylvestris* during its ontogeny (Ohnmeiss and Baldwin, 2000). The lower JA level in flowering plants and the reduced response to jasmonates treatment in these plants leaves may result from metabolic limitations because flowering plants prioritize the allocation of resources to seed production over other functions that are not directly involved in fitness output. The lower level of jasmonates may cause an attenuation of the TIA accumulation during flowering.

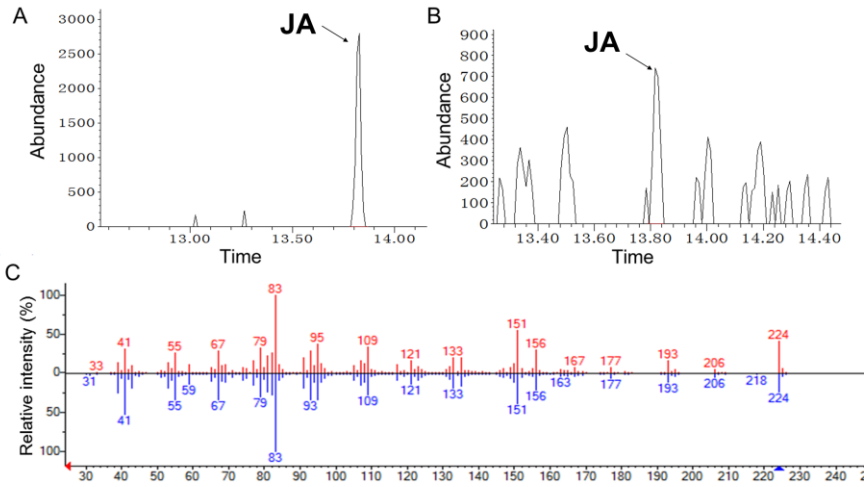


Fig. 3 Detection of jasmonic acid (JA) in *Catharanthus roseus* leaves by GC-MS. A: GC-ION extraction spectra of authentic JA; B: GC-ION extraction spectra of JA in samples; C: mass spectra of JA (In red was JA in samples and in blue was authentic JA).

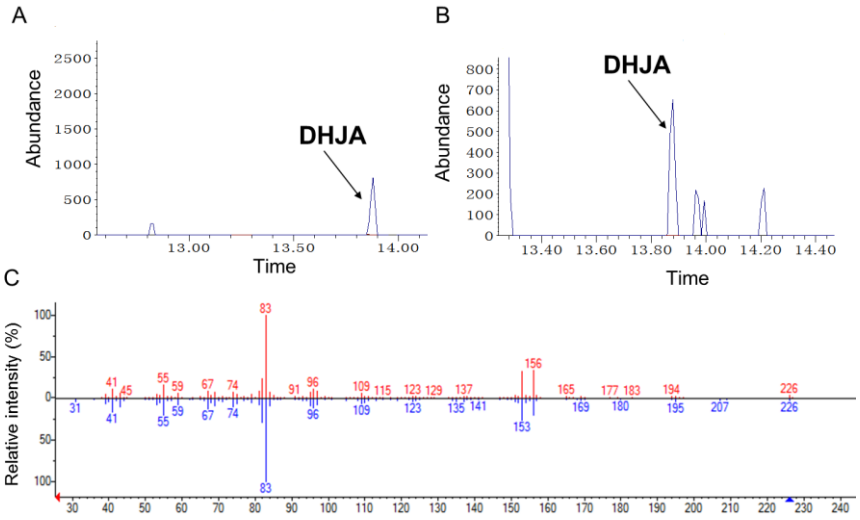
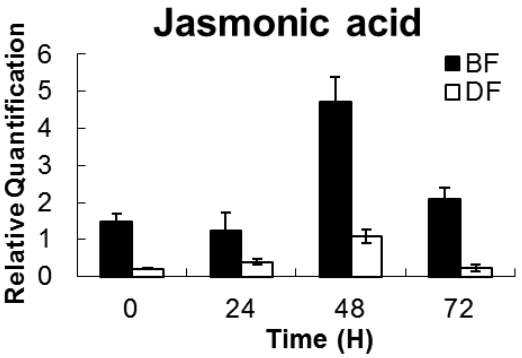


Fig. 4 Detection of internel standard DHJA in *Catharanthus roseus* leaves by GC-MS. A: GC-ION extraction spectra of authentic DHJA; B: GC-ION extraction spectra of authentic DHJA in samples; C: mass spectra of DHJA (In red was internal standard DHJA in samples and in blue was authentic DHJA).

Fig. 5 Relative quantification of JA in *Catharanthus roseus* leaves under JA treatment before flowering (BF) and during flowering (DF). Results are the mean of 3 replicates ± standard deviation.



Comparison between JA- and MeJA-induction on TIA biosynthesis in Catharanthus roseus leaves

In this set of experiments, the precursors of bisindole alkaloids, i.e.

vindoline and catharanthine were measured, as well as anhydrovinblastine, to investigate the total accumulation of those compounds in all the leaves (thus including both upper and lower leaves) of JA- or MeJA-elicited *C. roseus* plants before and during flowering.

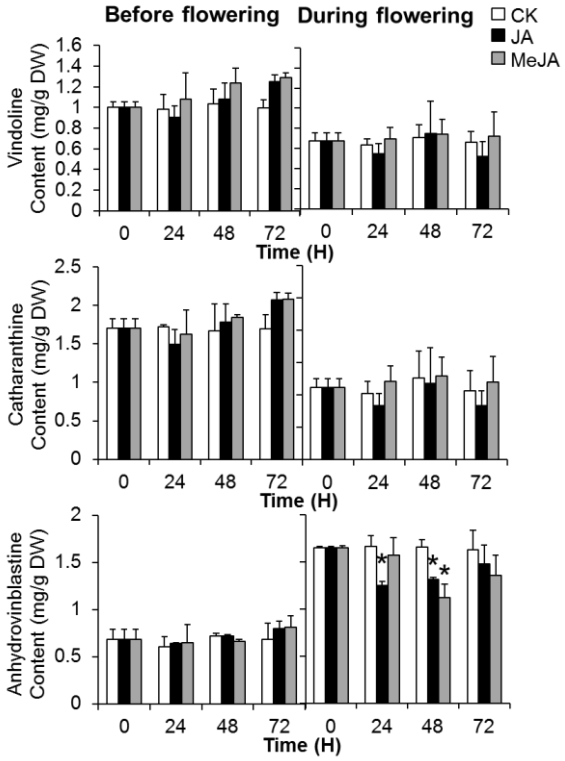


Fig. 6 Vindoline, catharanthine and anhydrovinblastine accumulation in *Catharanthus roseus* leaves under JA or MeJA treatment before and during flowering. MeJA: plants treated with MeJA; JA: plants treated with JA; CK: the control plants without MeJA or JA treatment. DW: dry weight. *: significant difference (Alpha < 0.05 by ANOVA). Results are the mean of 3 replicates ± standard deviation.

Figure 6 shows that before flowering, the contents of vindoline, catharanthine and anhydrovinblastine increased 25%, 22% and 16% respectively after 72 h compared to the controls. Similarly MeJA treatment

caused an increase of the accumulation of vindoline, catharanthine and anhydrovinblastine with 19%, 22% and 19%, respectively after 72 h if compared to the controls. Though statistical analysis showed that the increases were not significant, the trends observed were similar as in the experiments shown in figure 1 and 2. During flowering, both JA and MeJA treatment showed no differences on the accumulation of vindoline and catharanthine as compared to the non-treated leaves. However, it caused a significant decrease in anhydrovinblastine accumulation in the leaves of blooming plants. Anhydrovinblastine level had also significantly decreased 32% at 48 h after MeJA elicitation. The results indicate that JA or MeJA elicitation had a similar effect on TIA biosynthesis. Before flowering, both JA and MeJA, were able to modify the TIA accumulation while the effect declined during flowering. A previous study showed that most herbivore-resistant responses of JA-signaling in the wild-type *N. attenuata* plants, was when the plants were treated with exogenously applied MeJA. In *N. attenuata* plants, MeJA was first hydrolyzed to JA, which then induced a set of defense-related genes (Wu *et al.*, 2008).

Conclusions

This study provides information on the TIA accumulation upon JA elicitation analyzed in different organs at different developmental stages. The bisindole precursors i.e. vindoline and catharanthine were accumulated in the leaves at higher levels before flowering than during flowering. The JA level of was much higher before flowering than during flowering. No differences were found between JA and MeJA treatments, which indicates that the response to these different forms of jasmonate was similar in the plants.

In *C. roseus* plants, JA stimulates the TIA accumulation before flowering but had less effect during flowering. Though some of the trends observed are not statistically significant, the results are supported by similar trends in the first (Fig. 1 and 2) and the second set of experiments (Fig. 4). TIA biosynthesis in different organs (flower, leaf, and root) showed a different response to JA elicitation. The results may be of interest for optimizing commercial alkaloid production in the field, as young leaves before flowering have the highest level of the precursors for the synthesis of the dimers.

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