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ACCUMULATION OF TERPENOID INDOLE ALKALOIDS IN JASMONIC ACID ELICITED *CATHARANTHUS ROSEUS* PLANTS BEFORE AND DURING FLOWERING

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Abstract

Jasmonates analogues including jasmonic acid (JA) and methyl-jasmonate (MeJA) are plant-signaling molecules involved in defense against insects and pathogens. In *Catharanthus roseus*, jasmonates play a key role in regulating the biosynthesis of pharmaceutically important terpenoid indole alkaloids (TIAs). In the present study, *C. roseus* plants were elicited with JA before and during flowering to investigate the induction of TIA biosynthesis in different organs during the two developmental stages concerning via HPLC and qRT-PCR methods. The results showed that JA stimulates the TIA accumulation before flowering but had less effect during flowering. TIA accumulations in different organs (flower, leaf, and root) also showed a different response to JA elicitation. Moreover, transcriptional analysis showed that JA elicitation had a greater effect on the expression levels of key TIA biosynthetic genes (such as STR, SGD, DAT and PRX1) in *C. roseus* before flowering than during flowering. In *C. roseus* control (non-treated) plants the level of jasmonic acid was higher before flowering than during flowering, and in JA-treated plants JA was accumulated more before flowering than during flowering. The study provided an insight into the effect of flowering on JA-induced TIA biosynthesis in *C. roseus* plants.

Key words: *Catharanthus roseus*, Jasmonic acid, Terpenoid indole alkaloids, Flowering.

Introduction

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 & Devoto, 2008). Induction of secondary metabolites production is an important defense response, which might be regulated by jasmonates as the regulatory signals (Memelink *et al*., 2001).

Jasmonic acid (JA), as a member of the jasmonate class of plant hormones, has been reported to regulate the biosynthesis of secondary metabolites, e.g., terpenoid indole alkaloids (TIAs) in *Catharanthus roseus* (Van der Fits & Memelink, 2000). 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 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 biosynthetic 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 (Peebles *et al*., 2009; Shukla *et al*., 2010; El-Sayed & Verpoorte, 2004). 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 & Verpoorte, 2004), but had little effect on the TIA accumulation when it was applied on the flowering *C. roseus* plants (Pan *et al*., 2010). There are also less reports about JA or MeJA induction on vindoline biosynthetic enzyme deacetylvindoline 4-O-acetyltransferase (DAT) and anhydrovinblastine biosynthetic enzyme PRX1. 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.

Previous researches showed that different organs of *C. roseus* (root, stem, leaf and flower) had very different metabolic and transcriptional profiles (El-Domyati *et al*., 2017; Pan *et al*., 2014). Leaves and flowers accumulate higher level of vindoline, catharanthine and anhydrovinblastine while roots have higher level of ajmalicine, vindolinine and serpentine (Pan *et al*., 2016). Moreover, the levels of monoindole alkaloids decreased while bisindole alkaloids increased with leaf aging (Pan *et al*., 2016). In this study, the effect of JA on the TIA biosynthesis in different organs (flower, leaf, stem and root) was investigated in *C. roseus* plants before and during flowering at both transcriptional and metabolic levels, aiming for the better understanding of the regulation of TIA biosynthesis.

Materials and Methods

Plant materials and cultivation methods: Seeds of *C. roseus* (cv. Pacific Cherry Red) were purchased from Pan American 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 & Skoog, 1962) basal medium. Cultures were grown under 16 h light and 8 h dark photoperiod at 25 ± 2°C. After germination for 2 weeks, seedlings were transplanted into soil and grown in the greenhouse at 25±3°C.

JA treatments: Jasmonates (JA) was purchased from Sigma-Aldrich Chemie (Steinheim, Germany). The stock solutions were prepared by dissolving JA 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 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. Nine plantlets were put in one tray and totally 4 trays plantlets were used during the experiment. Each tray were sprayed with a volume of 250 ml work solution to make them all wet at 9:00 am on the first experiment day. Two trays were sprayed with 0.1 mM JA and the other two trays with water as control (CK). The flowers, upper leaves (3 layers leaves from the top), lower layer leaves (3 layers leaves from the bottom) and roots were collected at 9:00 am in the following three days including those of the control plants for the following transcriptional and alkaloid analysis. Samples at 0 h were collected before spraying on the first experiment day. The experiments were conducted in randomized block design (RBD) with three replicate samples for each time point of the time course.

Relative gene expression analysis by qRT-PCR: For qRT-PCR, both JA-treated and non-treated upper leave samples before and during flowering were used. Total RNA of each collected sample was isolated. DNA contamination was removed using DNase I following the protocol provided by the manufacturer (TaKaRa, Japan). The cDNAs were synthesized from the RNA samples using Prime ScriptTM Reverse Transcriptase Reagent according to the manufacturer's instructions, using oligo (dT) as the primer. The qRT-PCR analysis was performed in a Peltier Thermal Cycler PTC200 (Bio-Rad), using the cDNAs as a template and gene-specific primers for analysis. The primers for these genes (STR, SGD, TDC, DAT, PRX1 and RSP9) are listed as the reference (Pan *et al*., 2012). Ribosomal protein subunit 9 (Rsp9) was used as an internal control to evaluate all *C. roseus* plants. SYBR Green (SYBR Premix Ex Taq; TaKaRa) was used in the PCR reactions to quantify the amount of dsDNA. The relative Ct (threshold cycle value) method (User Bulletin 2, ABIPRISM700 Sequence Detection System, update 2001; PerkinElmer/Applied Biosystems) was used to estimate the initial amount of template present in the reactions.

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 used was described in the previous study (Pan *et al*., 2016). 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 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°C. 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 trime thylsilyldiazomethane 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°C, increase from 80°C to 200°C at 10°C /min, and subsequently increase to 300°C at 20°C /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.

Data statistical analysis: 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.

Results and Discussion

Comparison of TIA contents in upper leaves, lower leaves and roots of *Catharanthus roseus* before and during flowering: TIA contents (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) for better understanding the regulation of JA signaling on TIA biosynthesis. 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 were accumulated at higher levels before flowering while anhydrovinblastine was 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.

Figures 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 an increase of 30% at 72 h over the controls (Fig. 1). 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 Figs. 1, 2) and had the highest level before flowering. After JA treatment before flowering, compared to the controls, catharanthine levels showed an increase of 28% 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). 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).

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). JA elicitation seems to slightly, though not statistically significant, 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 showed that the ajmalicine levels in the roots and upper leaves were higher before flowering than during flowering (Table 1, Figs. 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.

Table 1. TIA contents in upper leaf, lower leaf and root of *Catharanthus roseus* before and during flowering.

Compounds	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

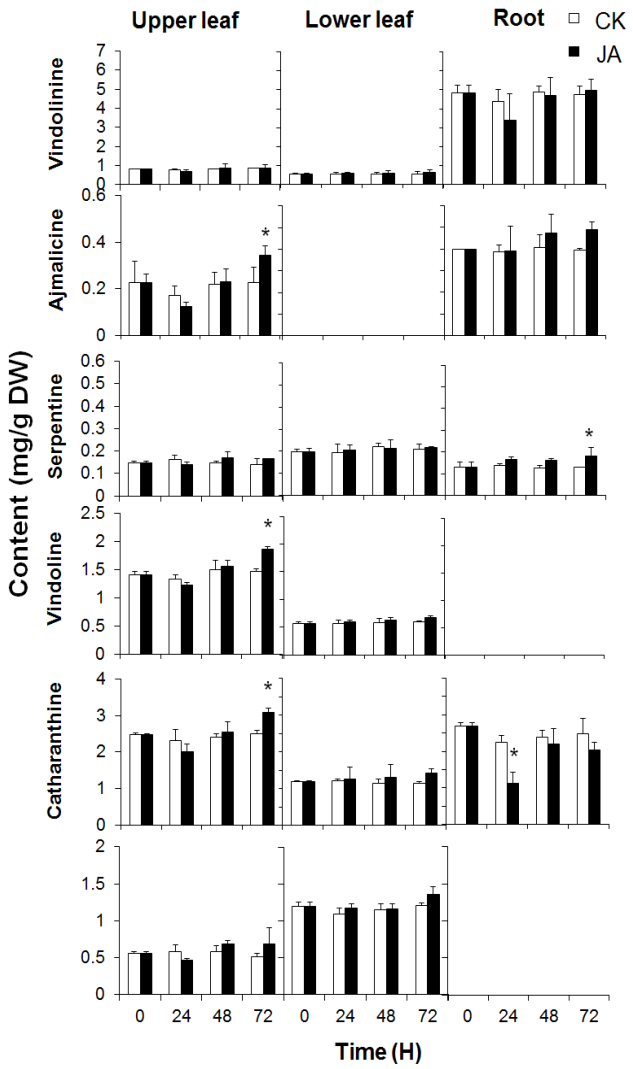

Fig. 1. The contents of vindolinine, ajmalicine, serpentine, vindoline, catharanthine and anhydrovinblastine in the upper leaves, lower leaves and roots of *Catharanthus roseus* under JA treatment before flowering. CK: the control plants; JA: plants treated with JA. DW: dry weight. *: significant difference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

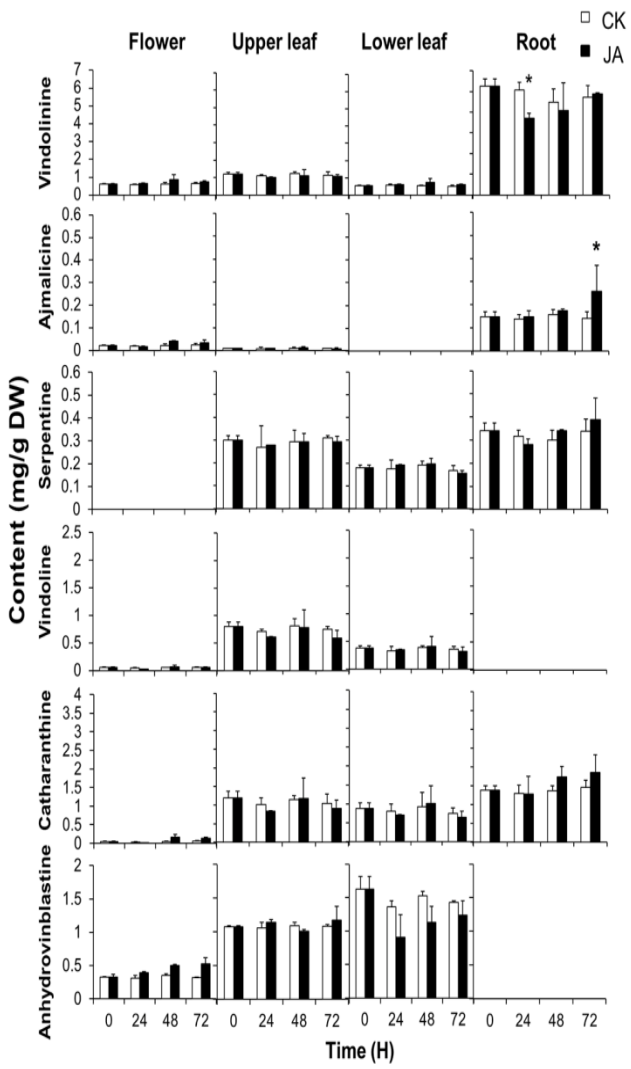

Fig. 2. The contents of vindolinine, ajmalicine, serpentine, vindoline, catharanthine and anhydrovinblastine in the flowers, upper leaves, lower leaves and roots of *Catharanthus roseus* under JA treatment during flowering. CK: the control plants; JA: plants treated with JA. DW: dry weight. *: significant 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 & 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. 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 was increased under JA treatment. It has been reported that jasmonates can induce ajmalicine production in hairy roots (Ruiz-May *et al*., 2008).

Transcriptional analysis of the structural genes in TIA pathway: The upper leaves of *C. roseus* have a more complete alkaloid profile than other organs. Transcripts of the key enzyme genes in the TIA pathway (TDC, STR, SGD, DAT and PRX1) in the upper leaves of *C. roseus* plants were analyzed by qRT-PCR under JA treatment before and during flowering (Fig. 3). The results showed that these genes had different patterns of their response to JA induction before and during flowering. Before flowering, expression levels of STR, SGD and DAT displayed similar trends, which all increased with the maximum response occurring at 24 h and followed by a decline to the control levels by 72 h after JA treatment. DAT transcript was increased 2.58 fold, STR was increased 2.15 fold and SGD was increased 1.69 fold compared to the control. PRX1 transcripts showed a slight increase of 39% at 48 h while TDC transcripts had a decrease of 92% at 48 h after JA elicitation compared to the controls. During flowering, except that PRX1 transcripts showed an increase of 53% at 48 h compared to the control, transcripts of TDC, STR, SGD and DAT had no great change and little response to JA induction.

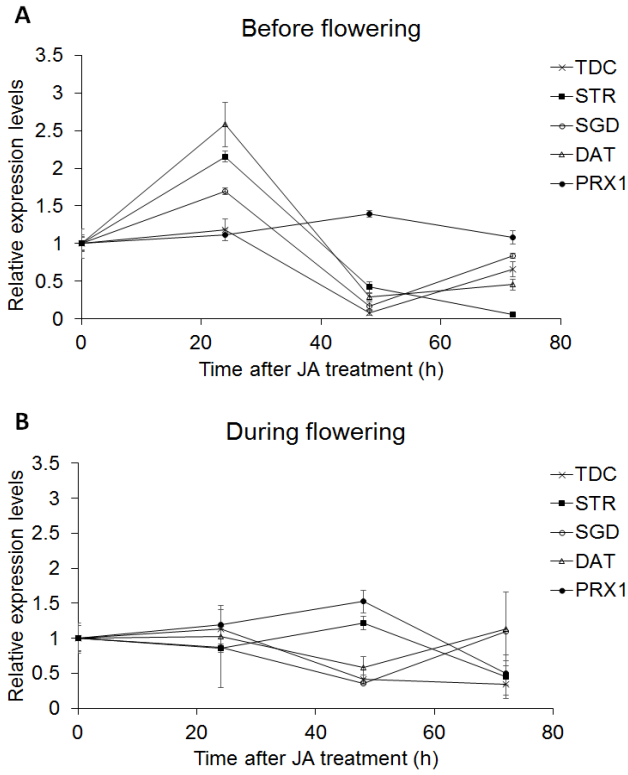

Fig. 3. Relative expression levels of TDC, STR, SGD, DAT and PRX1 in *Catharanthus roseus* upper leaves under JA treatment before flowering (A) and during flowering (B). Results are the mean of 3 replicates ± standard deviation.

Previous studies have reported that JA or MeJA treatment enhanced the expression levels of TDC, STR and SGD, which led to the increase of alkaloids (vindoline, catharanthine, serpentine and so on) contents in *C. roseus* cells, hairy roots and seedlings (Peebles *et al*., 2009; Zhou *et al*., 2010). Our transcriptional results further convinced that JA elicitation increased not only the expression levels of STR and SGD in the upper-stream TIA pathway but also DAT transcript in the down-stream TIA pathway, which gave a good explanation of increased vindoline accumulation in the upper leaves of *C. roseus* before flowering under JA treatment. Transcriptional levels of these key genes were correlated with the alkaloid contents. However, it seems that JA has slight effect on the expression level of PRX1, which is the last step enzyme of anhydrovinblastine and vinblastine biosynthesis.

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 whereas DHJA (226.14 m/z), which is used as the internal standard, appeared at 13.88 min. According to normalized areas under the curve of JA to the internal standard (DHJA), JA levels were quantified as relative amounts.

The results showed that JA was at a 6-fold higher level before flowering than during flowering in leaves without JA treatment (Fig. 4). 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 was 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.

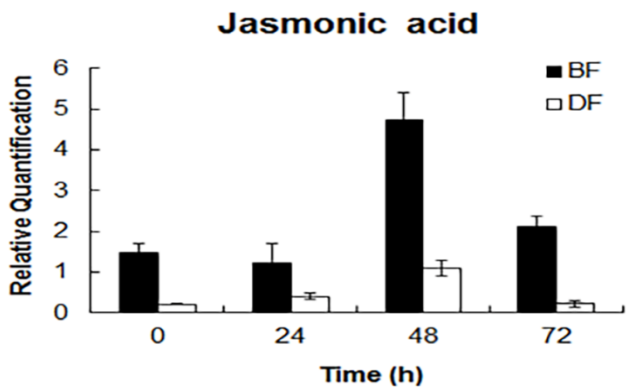

Fig. 4. Relative quantification of jasmonic acid in *Catharanthus roseus* leaves under JA treatment before flowering (BF) and during flowering (DF). Results are the mean of 3 replicates ± standard deviation.

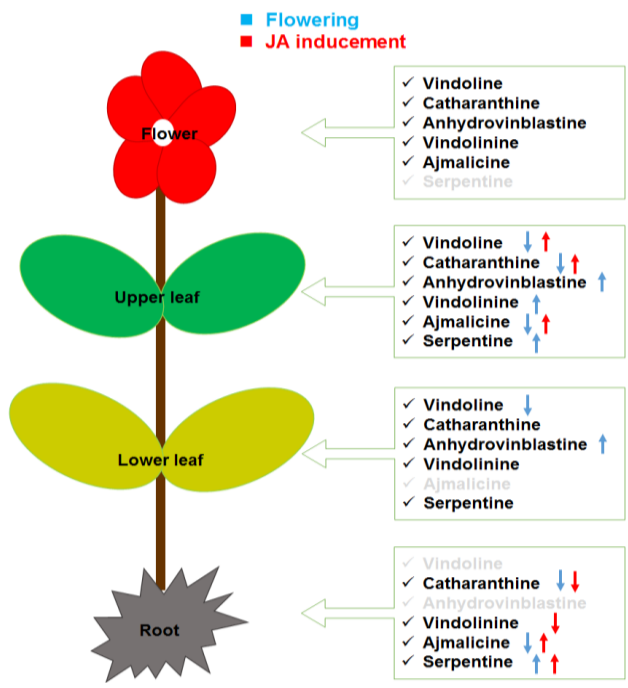

Fig. 5. A schematic diagram of the accumulation of six TIAs (vindolinine, ajmalicine, serpentine, vindoline, catharanthine and anhydrovinblastine) in different organs under JA inducement at different developmental stages (before and during flowering). TIAs in grey represented not detectable in the organs. ↑, means that TIAs contents increased significantly. ↓, means that TIAs contents decreased significantly. Arrows in blue mean that TIA contents changed with flowering. Arrows in red mean that TIA contents changed under JA inducement.

Conclusions

This study provides information on the TIA accumulation upon JA elicitation analyzed in different organs at different developmental stages (Fig. 5). 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 leaves was much higher before flowering than during flowering.

In *C. roseus* plants, JA stimulates the transcripts of key enzyme genes in the TIA pathway, which resulted in TIAs accumulation before flowering. But JA elicitation had less effect on TIA biosynthesis at both transcriptional and metabolic levels during flowering. 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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