

## 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.