ORIGINAL PAPER

CrossMark

Light enhanced the biosynthesis of terpenoid indole alkaloids to meet the opening of cotyledons in process of photomorphogenesis of *Catharanthus roseus*

Bofan Yu[1] · Yang Liu[1] · Yajie Pan[1] · Jia Liu[1] · Hongzheng Wang[1] · Zhonghua Tang[1]

Abstract

Light-induced photomorphogenesis is vital for plant growth and development. It was reported that secondary metabolites played an effective role during plant growth and tightly regulated by ambient light condition. However, the dynamic changes and possible functions of these compounds during photomorphogenesis were rarely reported. As one major class of secondary metabolites, terpenoid indole alkaloids (TIAs) dominate defense function and medicinal value in *Catharanthus roseus*. Here, the TIAs accumulations and their biosynthetic pathway gene expressions in light- and dark-grown seedlings were compared. Our results showed that the presence of light successfully induced seedling photomorphogenesis, including opened apical hook, inhibited hypocotyl elongation, opened and expanded cotyledons, and well developed roots. Generally, the contents of photosynthetic pigments and the ratio of chlorophyll a to b in cotyledons were continuously enhanced during this phase. Furthermore, light quickly activated the transcriptional expressions of the TIAs pathway enzymes and TIAs accumulations, namely tabersonine, catharanthine, vindoline, vinblastine and vincristine. Interestingly, these TIAs simultaneously peaked in the 3rd day after the cotyledons emerged out of growth substrate (DACE), exactly meeting the morphology of cotyledons opening. We propose that TIAs might play important roles for the cotyledons acclimatization to environmental illumination. Through scanning the absorption spectrums, TIAs showed strong absorptions in ultraviolet light. So we supposed that enhanced TIAs biosynthesis during photomorphogenesis might act as protective compounds to help the tender seedlings survive excessive light radiation.

Keywords *Catharanthus roseus* · Photomorphogenesis · Terpenoid indole alkaloids biosynthesis · Transcriptional expression

Abbreviations

CVs	Coefficients of variations
DACE	Days after the cotyledons emerged
DAT	Deacetylvindoline 4-*O*-acetyltransferase
DMRT	Duncan's Multiple Range test
D4H	Desacetoxyvindoline 4-hydroxylase
PRX1	Peroxidase 1
ROS	Reactive oxygen species
SGD	Strictosidine β-glucosidase
SLS	Secologanin synthase
STR	Strictosidine synthase
TDC	Tryptophan decarboxylase
TIAs	Terpenoid indole alkaloids
UV-B	Ultraviolet-B.

✉ Hongzheng Wang
  hzwang@nefu.edu.cn

✉ Zhonghua Tang
  tangzh@nefu.edu.cn

[1] Key Laboratory of Plant Ecology, Northeast Forestry University, Harbin 150040, China

Introduction

Undoubtedly, light is one of the most essential environment factors for seed germination and plants growth (Li et al. 2015), actively regulating photomorphogenesis (Jiao et al. 2007). After germination, the terrestrial plants undergo two distinct developmental programs depending on the availability of light, termed skoto- and photomorphogenesis (Pfeiffer et al. 2016). Skotomorphogenesis exhibits elongated

hypocotyl, apical hook topped by tightly-closed, underdeveloped cotyledons, a limited root system and the plastid sluggishly developing into etioplast (Chen et al. 2004). By contrast, photomorphogenesis is characterized by inhibition of hypocotyl elongation, expanded and green cotyledons, in favor of seedlings for photosynthesis and autotrophic growth (Nemhauser and Chory 2002; Xu et al. 2015). In this process, the light stimuli contributes to regulate development pattern depending on several receptors (Chen et al. 2012; Casal 2013; Kanegae and Kimura 2015; Ma et al. 2016). However, light signal exceeding the acceptable range of the activated response center will create light damage, and then lead to photoinhibition and reduce the photochemical and carbon assimilation rates (Alboresi et al. 2011; Nishiyama and Murata 2014; Zhang et al. 2017). Therefore, scavenging for harmful reactive intermediates operates the function of photoprotection, which is essential for plant growth and photosynthesis (Aluru et al. 2009; Zhang et al. 2017).

Numerous studies have stated that a series of secondary metabolites are synthesized to cope with various sorts of external potential threats, playing roles for defense (Ferreres et al. 2011). Intensive studies reported that synthesis of phenolic compounds requires light or would be elevated by light, and production of flavonoids absolutely depended on light intensity and density (Hemm et al. 2004). And it was believed that anthocyanins and flavonoids protected the tender seedlings from more damage of light in de-etiolation (Bowler et al. 2013; Li et al. 2017). C6C3C6-type phenolic compounds are considered to provide photo-protective function as filters against ultraviolet irradiation (Liu et al. 2016a, b). In addition, previous reports suggested that enhancement of flavonoid, ferulic acid and cinnamic acid in plants maybe closely related with the effective protection of photosynthetic apparatus (Hura et al. 2010).

The medicinal plant *Catharanthus roseus* produces more than 150 terpenoid indole alkaloids (TIAs) in different organs (Dutta et al. 2005; Pan et al. 2010; Liu et al. 2016a, b). Extensive progresses have been made for the TIAs biosynthetic pathway over the past decades (Fig. S1). Overall, the pathway can be recognized for three stages, including the formation of strictosidine, monomeric alkaloids and bisindole alkaloids (Rischer et al. 2006; Liu et al. 2007; Suttipanta et al. 2011; Zhu et al. 2014; Pan et al. 2015). TIAs are widely studied in virtue of displaying a large variety of pharmaceutical activities (De Luca and Laflamme 2001). Extensive evidence indicated that light signal was involved in regulating TIAs biosynthesis in *C. roseus*. Many reports agreed that productions of various indole alkaloids were elevated in *C. roseus* induced by light treatment, particularly by ultraviolet-B (UV-B) (He et al. 2011). UV-B light treatment for 5 min also enhanced the productions of catharanthine and vindoline by threefold and 12-fold, respectively (Ramani and Jayabaskaran 2008). In intact plant of *C. roseus*, artificial

UV-B and natural sunlight induced accumulation of TIAs and the expression of the TDC gene (Hirata et al. 1993; Ouwerkerk et al. 1999). In addition, phytochrome was probably assumed in the activation of D4H and light was not necessary for emerging of the structural cells where D4H and TDC exclusively localized (Vazquez-Flota and De Luca 1998; Vazquez-Flota et al. 2000).

Compared with other developmental periods, light has a more critical significance for plants during photomorphogenesis. Therefore, it is important to reveal how light regulates secondary metabolism in this process (Vazquez-Flota and De Luca 1998). In the present study, the light- and dark-grown seed germination was compared to reveal their morphological and metabolic difference during photomorphogenesis or skotomorphogenesis process. Our results provide more insight into the implication of light in the regulation of TIA metabolism and possible role in the process of photomorphogenesis in *C. roseus*.

Materials and methods

Plant material and treatment

Seeds of *C. roseus* were disinfected in sequence with 75% ethanol and 6% sodium hypochlorite for 0.5 and 10 min, and then washed with flowing water for 5 min. After an imbibition for 2 days, the seeds were sowed in the growth pots using pearlite as the culture substrate and they were placed in trays. Two treatments were carried out in our study, the dark group was in continuous dark environment and separated from the outside light via blackout fabric, in contrast, the light group was set to periodic dark (12 h)/light (12 h) and achieved by artificial plants cultured lamps (MXF9a-Y14., China) with an illumination flux of 100 µmol m^{-2} s^{-1} as well as a spectral range from 260 to 700 nm. Both the treatments were conducted in greenhouse with temperature of 25 °C and maintained relative humidity of 60%. Each treatment was conducted three biological replications and every replication included not < 20 individuals.

Determination of morphological parameters

The length of root and hypocotyl was determined by a ruler with precision of 1 mm. The cotyledons angle was determined by a protractor. Specifically, place the clean seedlings on white paper, and the cotyledon angle can be obtained by depicting the contours of the pair of cotyledons for measurement. The cotyledon area was measured following the method of Chadhary et al. (2012). Briefly, the cotyledon and a known area object were taken a photo together by a digital camera and the image was transformed to CIELAB color spaces. Using invert and region filling technique, a

full shape of the cotyledon was obtained, and then the area of cotyledon was calculated using the known object's area.

Determination of photosynthetic pigment contents

The photosynthetic pigment contents were determined following the method of Gitelson et al. (2003) with small modifications. Fresh cotyledons were exactly weighted and cut into pieces with size of about 0.5 cm, and then fast grinded into homogenate in a pre-cooled mortar, with 2 ml 100% acetone and a little quartz sand. The homogenate was centrifuged with $50,000 \times g$ for 5 min and the absorption values at 470, 663 and 645 nm of the supernatant were immediately assayed by a spectrophotometer (LKB Biochron Pharmacia 4060 Ultrospec, USA). The coefficients of Chlorophyll a, b, total Chlorophyll and carotenoids reported by Lichtenthaler were used for the calculation of contents of Chlorophyll a, b, total Chlorophyll and carotenoids.

Determination of TIA contents

Contents of TIAs were measured according to the method of Liu et al. (2016a, b). Accurately weighted 1.00 g fresh tissue was grinded with a high-throughput grinder (Xinyi-24, Ningbo Xinyi ultrasound device Lt., China). TIAs were extracted with 20 ml methanol for 1 h in a low-frequency ultrasonic machine (KQ-500DB., China) and the extract was centrifuged at $8000 \times g$ for 10 min and then repeated as above. All the supernatant was merged and filtered through 0.22 µm filter membrane for the following determination.

RNA extraction and qRT-PCR analysis

We analyzed the transcriptional level of genes through quantitative real-time reverse transcription PCR according to Pan et al. (Pan et al. 2015; Wang et al. 2016). The gene-specific primers were listed in Table S1. An internal control (RPS9) was served to evaluate the expression of the whole C. roseus

plants and employing the $2^{-\Delta\Delta Ct}$ method to obtain the relative expression value.

Scan of the adsorption spectrums of TIAs

Standard substances of tabersonine, vindoline, catharanthine, vinblastine and vincristine were dissolved in methanol and the final concentration was 0.02 mg ml^{-1}. The adsorption spectrums of the solutions were scanned with an UV/vis spectrophotometer (UV-5500, Shanghai Metash, China) from wavelength of 200–800 nm, using methanol as the blank.

Statistical analysis

All the results were conducted with three replicates. Statistical analysis was performed using two-way analysis of variance followed by Duncan's Multiple Range test (DMRT) (SPSS 17.0, SPSS Inc., USA) (Pan et al. 2015). The values are mean $\pm$ SE for three samples in each group. In the same way, data herein containing pathway gene expression level and alkaloids accumulation were analyzed by Pearson correlation via SPSS 17.0. P value ≤ 0.05 was considered as significant.

Results

The morphological characteristics during skoto- and photomorphogenesis

Light treatment induced opening of the hooks and the cotyledons in the 2nd and 3rd days after the cotyledons emerged (DACE) (Fig. 1; Table 1). Compared with the unopened cotyledons in skotomorphogenesis, the pair of cotyledons opened with an angle of 86° in photomorphogenesis in the 3rd DACE and fully extended in the 5th DACE. Additionally, the light promoted the expanding of the cotyledons. The

Fig. 1 Typical characters of skoto- and photomorphogenesis. *C. roseus* seedlings under the continuous dark condition and periodic supplying of the artificial light in DACE for 5 days (*DACE* day after the cotyledons emerged)
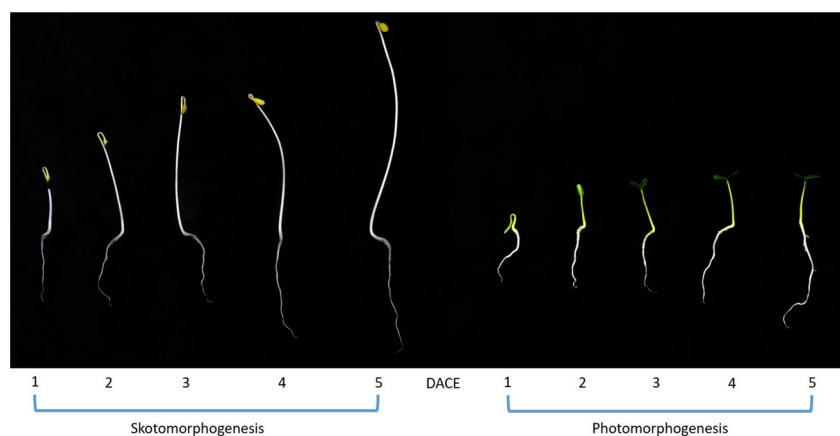
Table 1 Morphological parameters of *C. roseus* seedlings proceeding skoto- and photomorphogenesis. Different letters indicate significant difference (p<0.05 by ANOVA)

Treatment	DACE	Stem length (cm)	Root length (cm)	Cotyledons angle (°)	Cotyledons size (cm^2)	Lateral root numbers
Skoto	1st	2.033 ± 0.088e	1.713 ± 0.112c	0 ± 0a	0.019 ± 0.0021d	0b
	2nd	2.373 ± 0.241d	2.567 ± 0.033b	0 ± 0a	0.031 ± 0.0012c	0b
	3rd	3.86 ± 0.031c	2.733 ± 0.088b	0 ± 0a	0.031 ± 0.0015c	0b
	4th	4.5 ± 0.058b	2.687 ± 0.070b	0 ± 0a	0.044 ± 0.0012b	0b
	5th	5.117 ± 0.017a	3.467 ± 0.088a	0 ± 0a	0.060 ± 0.0009a	1a
Photo	1st	0.477 ± 0.012c	1.693 ± 0.093d	0 ± 0d	0.031 ± 0.0005e	0b
	2nd	1.103 ± 0.058b	2.133 ± 0.088c	0 ± 0d	0.061 ± 0.0009d	0b
	3rd	1.26 ± 0.070b	2.15 ± 0.076c	86 ± 2.082c	0.117 ± 0.0145c	0b
	4th	1.75 ± 0.076a	3.117 ± 0.017b	136.7 ± 1.667b	0.153 ± 0.0067b	0b
	5th	1.907 ± 0.070a	4.25 ± 0.104a	180.3 ± 0.882a	0.21 ± 0.0058a	3 ± 0.578a

area of cotyledons in photomorphogenesis was more than threefold of that in skotomorphogenesis (Table 1). The elongation of epicotyl was severely inhibited by the light, and its length in photomorphogenesis was only 37% of that in skotomorphogenesis in the 5th DACE. On the contrary, the root development was significantly improved by the light. In the 5th DACE, the root length and amount of lateral roots were 1.2-fold and threefold of those in skotomorphogenesis, respectively (Table 1).

Photosynthetic pigment contents in cotyledon during skoto- and photomorphogenesis

Biosynthesis of the photosynthetic pigments is crucial for seedling photomorphogenesis, we determined the contents of photosynthetic pigments (Fig. S2). Accumulations of all these pigments were gradually enhanced, with an exception of the decline of carotenoids content in the 5th DACE (Fig. S2a–d). However, in skotomorphogenesis, enhancements of these pigments were greatly reduced. In the 5th DACE, the contents of Chlorophyll a, Chlorophyll b, total Chlorophyll and carotenoids in cotyledons of seedlings when proceeding photomorphogenesis were 7.5, 2.8, 5.9 and 1.7-fold of that proceeding skotomorphogenesis (Fig. S2a–d). Furthermore, light treatment induced continuous increase of the ratio of Chlorophyll a/b and reached its peak in the 4th DACE. By contrast, no obvious increase of the ratio was found in the whole process of skotomorphogenesis (Fig. S2e).

TIAs accumulations during skoto- and photomorphogenesis

To study the effects of morphogenesis on TIAs accumulation in seedlings, the contents of tabersonine, catharanthine, vindoline, vinblastine and vincristine were also monitored (Fig. 2a–e). In skotomorphogenesis, three trends of the

contents were found: a slight increase followed by a decline in contents of tabersonine and catharanthine (Fig. 2a, b), a continuously increase in content of vindoline (Fig. 2c) and a continuously decline in contents of vinblastine and vincristine (Fig. 2d, e). However, only one change trend was found in the process of photomorphogenesis. Contents of the five kinds of TIAs gradually increased in the 1st–3rd DACE, followed by simultaneous decline in 4th–5th (Fig. 2a–e). In the 3rd DACE, these compounds in light-grown seedlings were 3.59, 4.28, 4.14, 1.61 and 4.15-fold of those in dark-grown ones, respectively (Fig. 2a–e). Furthermore, the content of loganin, an important precursor of TIAs, also reached its peak in the 3rd DACE in light-grown seedlings. On the contrary, only a little decrease was found in the 4th DACE under dark condition (Fig. 2f).

To compare the influences of light treatment and the intrinsic developmental rhythmicity on TIA accumulations, we also calculated the coefficients of variations (CVs) of the contents of loganin and the TIAs (Fig. S3). The results showed that the average of CVs in photomorphogenesis was higher than that in skotomorphogenesis, suggesting that light treatment exerted lager influence on the changes of TIAs accumulations than the intrinsic rhythmicity.

Gene transcripts during skoto- and photomorphogenesis

Subsequently, we investigated the gene expressions of TIAs (Fig. 3). For the enzymes in the first stage of TIAs biosynthesis pathway, gene expressions of SLS, STR TDC undergone fluctuations during this process. Anyhow, in light-grown seedlings, their expressions synchronously reached the peaks in 3rd DACE, followed by declines in the next day (Fig. 3a–c). D4H and DAT transcripts were higher in light-grown seedlings than in dark-grown ones in 1–5 DACE, and reached their peak in 3rd DACE following continuous declines in the next (Fig. 3d, e). SGD gene expressed slightly

Fig. 2 Accumulation of detected TIAs and loganin in *C. roseus* seedlings proceeding skoto- and photomorphogenesis. These seedlings were conducted under continuous dark and artificial light conditions in DACE for 5 days (mg g^{-1} FW). **a** Content of tabersonine, **b** catharanthine, **c** vindoline, **d** vinblastine, **e** vincristine and **f** loganin. The average of three measurements, with *error bars* representing standard deviation. Different *letters* indicate significant difference (p < 0.05 by ANOVA)
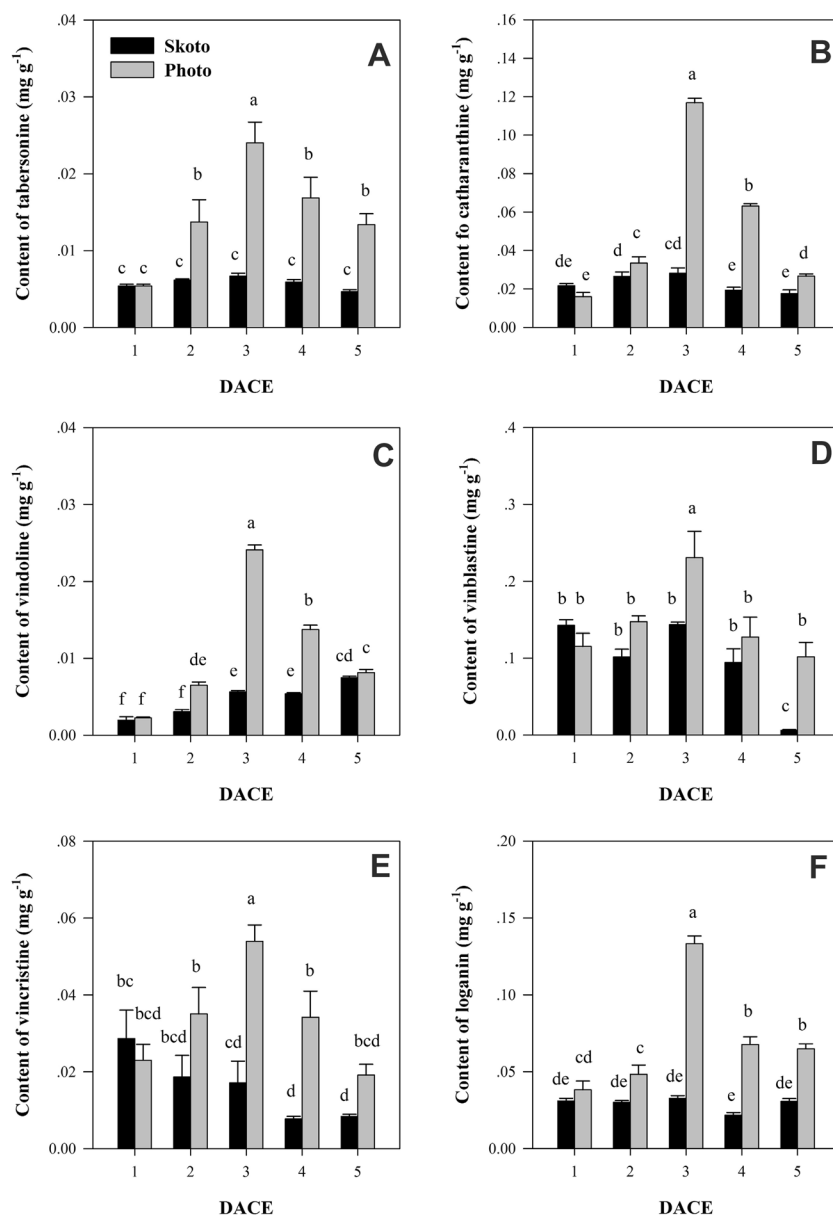

higher in photomorphogenesis than that in skotomorphogenesis in 3rd–4th DACE (Fig. 3f). The transcripts of PRX1, functioning for the bisindole alkaloid formation, were lower in light-grown seedlings than that in dark ones (Fig. 3g). The further correlation coefficients between gene expressions of the seven synthases with TIAs accumulations were conducted (Table 2). On the whole, there is lack of tight relation found in dark-grown seedlings. In contrast, in light-grown ones, gene expressions strongly correlated with the contents of TIAs in a positive way, except for vinblastine.

The potential UV light absorption capacity of TIAs

Since the peak of TIAs biosynthesis and opening of the cotyledons happened in the same time, we supposed that the TIAs accumulation likely protected the just opened cotyledons from the excessive light. To evaluate the protective role, we determined the absorption spectrums of the five TIAs (Fig. 4). Tabersonine efficiently adsorbed the light with wave length ranging from 290 to 380 nm, including all UV-B and most UV-A. Vindoline, vinblastine and vincristine showed strong adsorption capacity of all UV-B and a little part of UV-A. Catharanthine also had a weakest UV light adsorption ability and could only adsorb UV-B light. Evidently, accumulations of TIAs effectively reduced the UV radiation entering to inner cells of cotyledons which opened and received the direct strong light at that moment.

Fig. 3 Relative expression of the TIAs pathways genes in *C. roseus* seedlings proceeding skoto- and photomorphogenesis. These seedlings were conducted under continuous dark and artificial light conditions in DACE for 5 days. The relative gene expression was determined by qRT-PCR, using comparative Ct method and the *RPS* gene was applied as the internal control. **a** Relative gene expression of *SLS*, **b** *STR*, **c** *TDC*, **d** *D4H*, **e** *DAT*, **f** *SGD* and **g** *PRX1*. The average of three measurements of alkaloids, with *error bars* representing standard deviation. Different *letters* indicate significant difference (p < 0.05 by ANOVA)
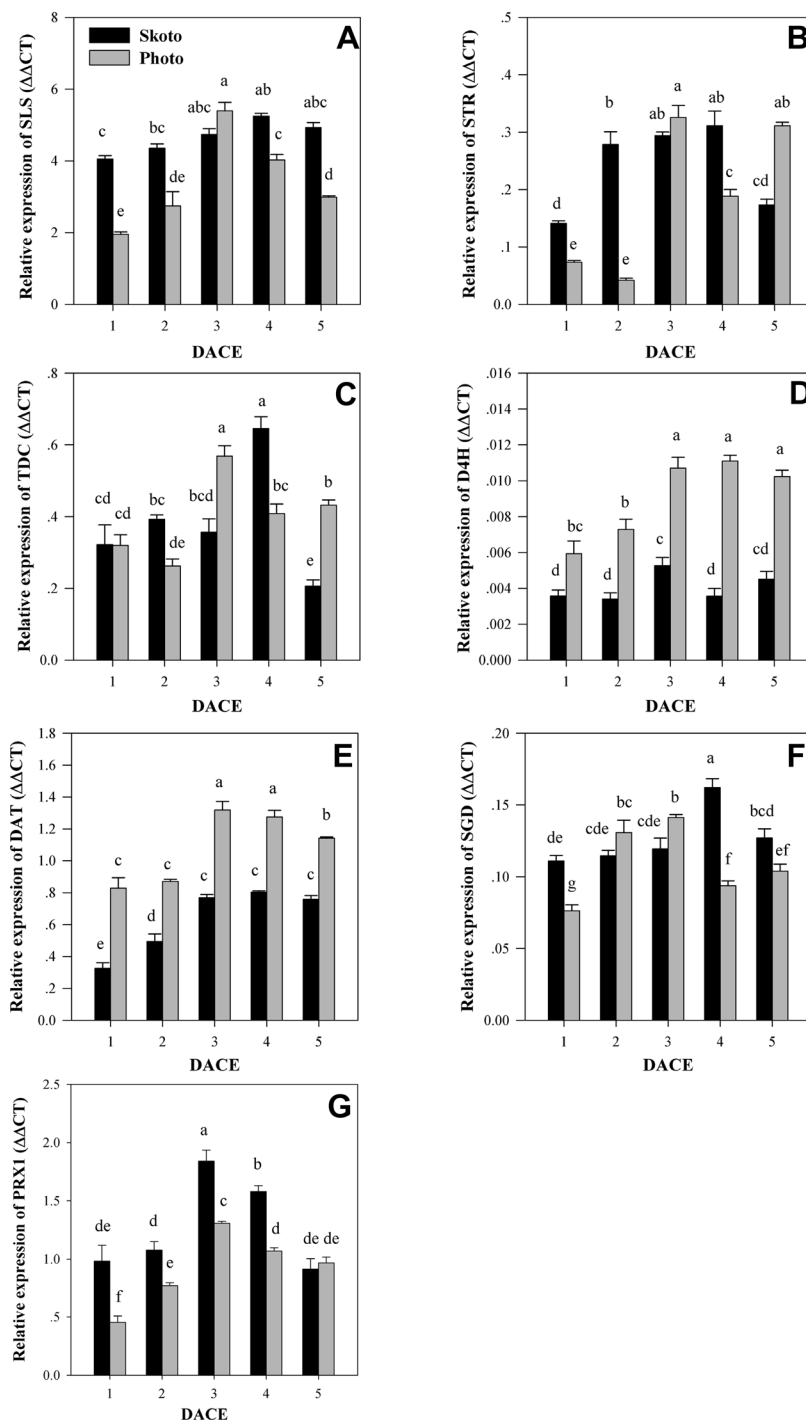
Discussions

Seedling photomorphogenesis is dramatically and highly affected by variations of light (Whitelam et al. 1998). When exposure to light, it can initiate seedling de-etiolation process for turning into photomorphogenesis, which basically employ limited hypocotyl growth, extended cotyledon development (Arsovski et al. 2012). In our study, completely

consistent with previous researches, *C. roseus* seedlings under continuous dark condition exhibited skotomorphogenesis features containing rapid elongation of hypocotyls, undeveloped and etiolated cotyledons. On the contrary, light stimulation induced opening of hooks and cotyledons in the 2nd and 3rd DACE, respectively, and the greening cotyledons developed continuously in photomorphogenesis (Fig. 1). To cope with external stimulus, the synthesis of

Table 2 Pearson correlation coefficients of relative expression of seven genes for synthases and contents of five TIAs proceeding skoto- and photomorphogenesis. The asterisk indicates significant effects of the levels of TIAs and genes. (*p<0.05; **p<0.01)

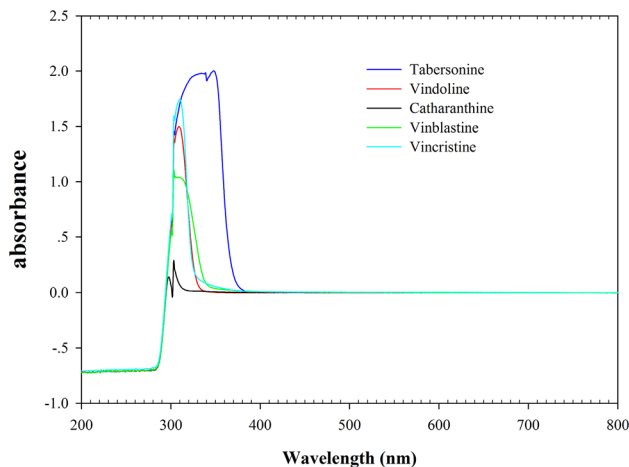|	Tabersonine	Vindoline	Catharanthine	Vinblastine	Vincristine	Total
Photo						
SLS	0.837**	0.884**	0.894**	0.586*	0.708**	0.790**
TDC	0.680**	0.830**	0.781**	0.521*	0.528*	0.685**
STR	0.556*	0.698**	0.588*	0.313	0.243	0.463
SGD	0.684**	0.613*	0.638*	0.608*	0.704**	0.689**
D4H	0.594*	0.714**	0.619*	0.304	0.329	0.483
DAT	0.637*	0.833**	0.750**	0.423	0.380	0.603*
PRX	0.861**	0.917**	0.841**	0.547*	0.569*	0.739**
Skoto						
SLS	− 0.072	0.703**	− 0.114	− 0.385	− 0.475	− 0.400
TDC	0.393	− 0.127	− 0.013	0.286	− 0.205	0.211
STR	0.643**	0.191	0.398	0.189	− 0.353	0.150
SGD	0.036	0.434	− 0.238	− 0.255	− 0.611*	− 0.329
D4H	− 0.019	0.496	0.186	0.051	0.004	0.076
DAT	0.108	0.837**	− 0.216	− 0.384	− 0.621*	− 0.426
PRX	0.700**	0.217	0.362	0.427	− 0.216	0.378

Fig. 4 Absorption spectrums of these five TIAs. For instance, tabersonine, vindoline, catharanthine, vinblastine and vincristine

chlorophylls and development of chloroplasts can assist seedlings to resist light damage in this conversion for de-etiolation (Para et al. 2016; Warpeha and Montgomery 2016). Most of photosynthetic pigments exhibited gradually elevation when proceeding photomorphogenesis except carotenoids in 5th DACE. Skotomorphogenesis, by contrary, demonstrated the extremely slight enhancement of pigments contents (Fig. S2). In plants, light induces chlorophyll production for the assembling of functional photosynthetic apparatus functioning as protective roles against light stress environment (Reinbothe et al. 1996; Mochizuki et al. 2010; Cheminant et al. 2011).

In addition, for higher plants, light also represents a crucial environmental signal for the regulation of biosynthesis and accumulation of multiple secondary metabolites (Guo et al. 2014; Lu et al. 2014). TIAs, as a typical and significant class of secondary metabolites in *C. roseus*, are highly regulated by plant developmental and environmental situations, such as ethylene (Pan et al. 2015; Wang et al. 2016). Our work revealed that TIAs metabolism was tightly regulated by photomorphogenesis, compared with skotomorphogenesis. The cotyledon opening accompany with increased levels of TIAs and and their common precursor loganin tested in this study (Figs. 1, 2). The multi-step TIA biosynthetic pathway in *C. roseus* is quite complex and is under strict molecular regulation (Vazquez-Flota et al. 1997; De Luca and Laflamme 2001; Memelink et al. 2001; Dutta et al. 2005). Accumulation of TIAs legitimately originated from the expression level of pathway enzymes genes, including SLS, STR, D4H and DAT, which exhibited peak when cotyledons opening (Fig. 3a, b, d, e). In addition, the presence of pathway genes involved in alkaloids biosynthesis were identified more obviously positive relation to TIAs than in skotomorphogenesis (Table 2).

It was reported that vindoline biosynthesis is dependent on light presence and regulated by D4H and DAT, which require the involvement of phytochrome (Vazquez-Flota and De Luca 1998; Zhou et al. 2009). In the light-grown seedlings, we also found more accumulations of tabersonine and vindoline (Fig. 2a, c). Furthermore, etiolated seedlings expressed D4H and DAT in an inactive form and light exposure appeared to increase their expression activity and positively correlated with related alkaloids (Fig. 3d, e) (Table 2). Vindoline and catharanthine are the unique two specific reaction substrates for the biosynthesis of bisindole alkaloids vinblastine (Verma et al. 2012), which was basically exceeded over other TIAs by light exposure in this

study (Fig. 2d, e). Additionally, CVs of the three alkaloids tabersonine, vindoline, catharanthine and their common precursor loganin in photomorphogenesis were correspondingly greater than those in skotomorphogenesis (Fig. S3). Functioning as the exclusive enzyme for the formation of bisindole alkaloids, PRX1 expressed lower in photomorphogenesis while with a promoted mode in 3rd DACE (Fig. 3g), similarly corresponding to the morphology of cotyledons opening (Fig. 1). Addition of secologanin to the cultures would increase accumulation of strictosidine, and the addition of upstream products is likely to promote the contents of downstream products TIAs (Moreno et al. 1993). Our results confirmed that SLS was triggered by light stimulation to catalyze the formation of secologanin (Figs. 2f, 3a).

Upon light, a young seedling expands its cotyledons to increase the light capturing surface and uses petioles to position blades towards the light source (Arsovski et al. 2012). Light modulates the chloroplast development and biosynthesis of many precursors for TIAs in the developed chloroplasts (Liu et al. 2011). The previous findings suggested that productions of TIAs and several related pathway gene expression were improved by light stress, mainly upon UV-B treatment (Ramani and Jayabaskaran 2008; He et al. 2011). Our results showed that the increase of photosynthetic pigments and TIAs accompanied with cotyledons opening (Figs. 1, 2). In this phase, seedlings are sensitive to light and often employ various compounds to absorb excess light spectrum. For example, flavonoids are considered to be crucial antioxidant to cope with the excessive sunlight radiation in plants (Bernal et al. 2013). The increased TIAs were supposed to contribute to absorb the harmful light irradiation and protect plant cell from damage (Ouwerkerk et al. 1999). We assumed that TIAs accumulation during photomorphogenesis also play protective roles. Our analysis of UV light absorption capacity of these five TIAs revealed that accumulation of TIAs effectively reduced the UV radiation for the inner cells of cotyledons which opened and faced to strong light stress directly (Fig. 4).

Conclusions

In the present study, the light- and dark-grown *C. roseus* seed germination was investigated to reveal their morphological and metabolic difference during photomorphogenesis or skotomorphogenesis process. Under light condition, we observed that seeds can successfully complete photomorphogenesis, including opened apical hook, inhibited hypocotyl elongation, opened and expanded cotyledons. The de-etiolated seedlings displayed enhancement of photosynthetic pigments in cotyledons. Furthermore, light quickly activated the transcripts of the key TIAs pathway enzymes and TIAs accumulations, namely tabersonine, catharanthine,

vindoline, vinblastine and vincristine. It was noted that TIAs peaked in the 3rd day when the cotyledons emerged out of growth substrate (DACE), exactly meeting the morphology of cotyledons opening. We propose that TIAs might play important roles for the cotyledons acclimatization to environmental illumination. Through scanning the absorption spectrums, TIAs showed strong absorptions in ultraviolet light. Our results provide more insight into the implication of light in the regulation of TIA metabolism and possible role in the process of photomorphogenesis in *C. roseus*.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Alboresi A, Dall'Osto L, Aprile A, Carillo P, Roncaglia E, Cattivelli L, Bassi R (2011) Reactive oxygen species and transcript analysis upon excess light treatment in wild-type Arabidopsis thaliana vs a photosensitive mutant lacking zeaxanthin and lutein. BMC Plant Biol 11:22

Aluru MR, Zola J, Foudree A, Rodermel SR (2009) Chloroplast photooxidation-induced transcriptome reprogramming in Arabidopsis immutans white leaf sectors. Plant Physiol 150(2):904–923

Arsovski AA, Galstyan A, Guseman JM, Nemhauser JL (2012) Photomorphogenesis. The Arabidopsis book. Am Soc Plant Biol 10:e0147

Bernal M, Llorens L, Julkunen-Tiitto R, Badosa J, Verdaguer D (2013) Altitudinal and seasonal changes of phenolic compounds in *Buxus sempervirens* leaves and cuticles. Plant Physiol Biochem 70:471–482

Bowler C, Botto J, Deng X-W (2013) Photomorphogenesis, B-Box transcription factors, and the legacy of Magnus Holm. Plant Cell 25(4):1192–1195

Casal JJ (2013) Photoreceptor signaling networks in plant responses to shade. Ann Rev Plant Biol 64:403–427

Chaudhary P, Godara S, Cheeran AN, Chaudhari KA (2012) Fast and accurate method for leaf area measurement. Int J Comput Appl 49(9):22–25

Cheminant S, Wild M, Bouvier F, Pelletier S, Renou JP, Erhardt M, Hayes S, Terry MJ, Genschik P, Achard P (2011) DELLAs regulate chlorophyll and carotenoid biosynthesis to prevent photooxidative damage during seedling deetiolation in Arabidopsis. Plant Cell 23(5):1849–1860

Chen M, Chory J, Fankhauser C (2004) Light signal transduction in higher plants. Ann Rev Genet 38:87–117

Chen F, Shi X, Chen L, Dai M, Zhou Z, Shen Y, Li J, Li G, Wei N, Deng XW (2012) Phosphorylation of FAR-RED ELONGATED HYPOCOTYL1 is a key mechanism defining signaling dynamics of phytochrome A under red and far-red light in Arabidopsis. Plant Cell 24(5):1907–1920

De Luca V, Laflamme P (2001) The expanding universe of alkaloid biosynthesis. Curr Opin Plant Biol 4(3):225–233

Dutta A, Batra J, Pandey-Rai S, Singh D, Kumar S, Sen J (2005) Expression of terpenoid indole alkaloid biosynthetic pathway genes corresponds to accumulation of related alkaloids in *Catharanthus roseus* (L.) G. Don. Planta 220(3):376–383

Ferreres F, Figueiredo R, Bettencourt S, Carqueijeiro I, Oliveira J, Gil-Izquierdo A, Pereira DM, Valentao P, Andrade PB, Duarte P, Barcelo AR, Sottomayor M (2011) Identification of phenolic compounds in isolated vacuoles of the medicinal plant *Catharanthus roseus* and their interaction with vacuolar class III peroxidase: an H_2O_2 affair? J Exp Bot 62(8):2841–2854

Gitelson AA, Gritz Y, Merzlyak MN (2003) Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. J Plant Physiol 160(3):271–282

Guo XR, Chang BW, Zu YG, Tang ZH (2014) The impacts of increased nitrate supply on *Catharanthus roseus* growth and alkaloid accumulations under ultraviolet-B stress. J Plant Interact 9(1):640–646

He LH, Yang L, Tan RH, Zhao SJ, Hu ZB (2011) Enhancement of vindoline production in suspension culture of the *Catharanthus roseus* cell line C20hi by light and methyl jasmonate elicitation. Anal Sci 27(12):1243–1248

Hemm MR, Rider SD, Ogas J, Murry DJ, Chapple C (2004) Light induces phenylpropanoid metabolism in Arabidopsis roots. Plant J 38(5):765–778

Hirata K, Asada M, Yatani E, Miyamoto K, Miura Y (1993) Effects of near-ultraviolet light on alkaloid production in *Catharanthus roseus* plants. Planta Med 59(1):46–50

Hura T, Hura K, Grzesiak M (2010) Early stage de-etiolation increases the ferulic acid content in winter triticale seedlings under full sunlight conditions. J Photochem Photobiol B 101(3):279–285

Jiao Y, Lau OS, Deng XW (2007) Light-regulated transcriptional networks in higher plants. Nat Rev Genet 8(3):217–230

Kanegae T, Kimura I (2015) A phytochrome/phototropin chimeric photoreceptor of fern functions as a blue/far-red light-dependent photoreceptor for phototropism in Arabidopsis. Plant J 83(3):480–488

Li W, Khan MA, Yamaguchi S, Liu X (2015) Hormonal and environmental regulation of seed germination in salt cress (*Thellungiella halophila*). Plant Growth Regul 76(1):41–49

Li J, Lv X, Wang L, Qiu Z, Song X, Lin J, Chen W (2017) Transcriptome analysis reveals the accumulation mechanism of anthocyanins in 'zijuan' tea (camellia sinensis, var. asssamica, (masters) kitamura) leaves. Plant Growth Regul 81(1):1–11

Liu DH, Jin HB, Chen YH, Cui LJ, Ren WW, Gong YF, Tang KX (2007) Terpenoid indole alkaloids biosynthesis and metabolic engineering in *Catharanthus roseus*. J Integr Plant Biol 49(7):961–974

Liu Y, Zhao DM, Zu YG, Tang ZH, Zhang ZH, Jiang Y, Shi DY (2011) Effects of low light on terpenoid indole alkaloid accumulation and related biosynthetic pathway gene expression in leaves of *Catharanthus roseus* seedlings. Bot Stud 52(2):191–196

Liu J, Liu Y, Wang Y, Zhang ZH, Zu YG, Efferth T, Tang ZH (2016a) The combined effects of ethylene and MeJA on metabolic profiling of phenolic compounds in *Catharanthus roseus* revealed by metabolomics analysis. Front Physiol 7:11

Liu J, Liu Y, Pan YJ, Zu YG, Tang ZH (2016b) Determination of alkaloids in *Catharanthus roseus* and vinca minor by high-performance liquid chromatography-tandem mass spectrometry. Anal Lett 49(8):1143–1153

Lu Z, Liu Y, Zhao L, Jiang X, Li M, Wang Y, Xu Y, Gao L, Xia T (2014) Effect of low-intensity white light mediated de-etiolation on the biosynthesis of polyphenols in tea seedlings. Plant Physiol Biochem 80:328–336

Ma DB, Li X, Guo YX, Chu JF, Fang S, Yan CY, Noel JP, Liu HT (2016) Cryptochrome 1 interacts with PIF4 to regulate high temperature-mediated hypocotyl elongation in response to blue light. Proc Natl Acad Sci USA 113(1):224–229

Memelink J, Verpoorte R, Kijne JW (2001) ORCAnization of jasmonate-responsive gene expression in alkaloid metabolism. Trends Plant Sci 6(5):212–219

Mochizuki N, Tanaka R, Grimm B, Masuda T, Moulin M, Smith AG, Tanaka A, Terry MJ (2010) The cell biology of tetrapyrroles: a life and death struggle. Trends Plant Sci 15(9):488–498

Moreno PR, van der Heijden R, Verpoorte R (1993) Effect of terpenoid precursor feeding and elicitation on formation of indole alkaloids in cell suspension cultures of *Catharanthus roseus*. Plant Cell Rep 12(12):702–705

Nemhauser J, Chory J (2002) Photomorphogenesis. The Arabidopsis book. Am Soc Plant Biol 1:e0054

Nishiyama Y, Murata N (2014) Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. Appl Microbiol Biotechnol 98(21):8777–8796

Ouwerkerk PB, Hallard D, Verpoorte R, Memelink J (1999) Identification of UV-B light-responsive regions in the promoter of the tryptophan decarboxylase gene from *Catharanthus roseus*. Plant Mol Biol 41(4):491–503

Pan QF, Chen Y, Wang Q, Yuan F, Xing SH, Tian YS, Zhao JY, Sun XF, Tang KX (2010) Effect of plant growth regulators on the biosynthesis of vinblastine, vindoline and catharanthine in *Catharanthus roseus*. Plant Growth Regul 60(2):133–141

Pan YJ, Liu J, Guo XR, Zu YG, Tang ZH (2015) Gene transcript profiles of the TIA biosynthetic pathway in response to ethylene and copper reveal their interactive role in modulating TIA biosynthesis in *Catharanthus roseus*. Protoplasma 252(3):813–824

Para A, Muhammad D, Orozco-Nunnelly DA, Memishi R, Alvarez S, Naldrett MJ, Warpeha KM (2016) The dehydratase ADT3 affects ROS homeostasis and cotyledon development. Plant Physiol 172(2):1045–1060

Pfeiffer A, Janocha D, Dong YH, Medzihradszky A, Schone S, Daum G, Suzaki T, Forner J, Longenecker T, Rempel E et al (2016) Integration of light and metabolic signals for stem cell activation at the shoot apical meristem. eLife 5:21

Ramani S, Jayabaskaran C (2008) Enhanced catharanthine and vindoline production in suspension cultures of *Catharanthus roseus* by ultraviolet-B light. J Mol Signal 3:9

Reinbothe S, Reinbothe C, Lebedev N, Apel K (1996) PORA and PORB, two light-dependent protochlorophyllide-reducing enzymes of angiosperm chlorophyll biosynthesis. Plant Cell 8(5):763–769

Rischer H, Oresic M, Seppanen-Laakso T, Katajamaa M, Lammertyn F, Ardiles-Diaz W, Van Montagu MC, Inze D, Oksman-Caldentey KM, Goossens A (2006) Gene-to-metabolite networks for terpenoid indole alkaloid biosynthesis in *Catharanthus roseus* cells. Proc Natl Acad Sci USA 103(14):5614–5619

Suttipanta N, Pattanaik S, Kulshrestha M, Patra B, Singh SK, Yuan L (2011) The transcription factor CrWRKY1 positively regulates the terpenoid indole alkaloid biosynthesis in *Catharanthus roseus*. Plant Physiol 157(4):2081–2093

Vazquez-Flota FA, De Luca V (1998) Developmental and light regulation of desacetoxyvindoline 4-hydroxylase in *Catharanthus roseus* (L.) G. Don. Evidence Of a multilevel regulatory mechanism. Plant Physiol 117(4):1351–1361

Vazquez-Flota F, De Carolis E, Alarco AM, De Luca V (1997) Molecular cloning and characterization of desacetoxyvindoline-4-hydroxylase, a 2-oxoglutarate dependent-dioxygenase involved in the biosynthesis of vindoline in *Catharanthus roseus* (L.) G. Don. Plant Mol Biol 34(6):935–948

Vazquez-Flota FA, St-Pierre B, De Luca V (2000) Light activation of vindoline biosynthesis does not require cytomorphogenesis in *Catharanthus roseus* seedlings. Phytochemistry 55(6):531–536

Verma P, Mathur AK, Srivastava A, Mathur A (2012) Emerging trends in research on spatial and temporal organization of terpenoid

indole alkaloid pathway in *Catharanthus roseus*: a literature update. Protoplasma 249(2):255–268

Wang X, Pan YJ, Chang BW, Hu YB, Guo XR, Tang ZH (2016) Ethylene-induced vinblastine accumulation is related to activated expression of downstream TIA pathway genes in *Catharanthus roseus*. Biomed Res Int 2016(9):8

Warpeha KM, Montgomery BL (2016) Light and hormone interactions in the seed-to-seedling transition. Environ Exp Bot 121:56–65

Whitelam GC, Patel S, Devlin PF (1998) Phytochromes and photomorphogenesis in Arabidopsis. Philos Trans R Soc Lond B Biol Sci 353(1374):1445–1453

Xu XS, Paik I, Zhu L, Huq E (2015) Illuminating progress in phytochrome-mediated signaling pathways. Trends Plant Sci 20(10):641–650

Zhang Q, Liu M, Ruan J (2017) Metabolomics analysis reveals the metabolic and functional roles of flavonoids in light-sensitive tea leaves. BMC Plant Biol 17(1):64

Zhou ML, Shao JR, Tang YX (2009) Production and metabolic engineering of terpenoid indole alkaloids in cell cultures of the medicinal plant *Catharanthus roseus* (L.) G. Don (*Madagascar periwinkle*). Biotechnol Appl Biochem 52(Pt 4):313–323

Zhu X, Zeng X, Sun C, Chen S (2014) Biosynthetic pathway of terpenoid indole alkaloids in *Catharanthus roseus*. Front Med 8(3):285–293