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ORIGINAL ARTICLE

Effects of light on production of camptothecin and expression of key enzyme genes in seedlings of *Camptotheca acuminate* Decne

Yuanyuan Hu[1,2] · Weiwu Yu[1,2] · Lili Song[1,2] · Xu Hua Du[3] · Xiaohua Ma[1,2] · Yang Liu[1,2] · Jiasheng Wu[1,2] · Yeqing Ying[1,2]

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Abstract *Camptotheca acuminata* (*C. acuminata*) is utilized in preparation of drugs and as constituent in functional foods of China due to high camptothecin (CPT) content in different plant parts. Light intensity is one of the most critical factors which affect plant growth and secondary metabolites. Pot experiment was conducted to study the effect of light intensity (i.e., 100 % irradiance (control), 75 % irradiance, 50 % irradiance and 25 % irradiance) on contents of CPT, activity of enzymes and genes expression related to CPT biosynthesis of *C. acuminata* seedlings. The study examined total leaf biomass, CPT content, activities of tryptophan synthase (TSB) and tryptophan decarboxylase (TDC), and relative expression of *TSB*, *TDC1*, and *TDC2* genes. Plants grown in 75 % irradiance possessed the greatest leaf biomass compared with 100 % light irradiance. Highest values of CPT contents were found after 60 days in plants grown in 50 % irradiance, followed by 25, 75 % and full sunlight. Furthermore, activities of TSB, TDC and relative expression of genes of *TSB*, *TDC1*, and *TDC2*, were significantly increased after 60 days of 50 % irradiance compared with full sunlight. Irradiance of 50 % up-regulated the expression of CPT biosynthesis-related genes and induced CPT biosynthesis. In addition to that lower or higher irradiance inhibited the expression of CPT biosynthesis-related genes and CPT biosynthesis. It is concluded that manipulating light intensity can be an effective means to achieve highest CPT yield in medicinal plants.

Keywords *Camptotheca acuminata* seedlings · Light intensity · Biomass production · Secondary metabolites gene expression

Abbreviations

CPT	Camptothecin
TIA	Terpenoid indole alkaloid
TSB	Tryptophan synthase
Trp	Tryptophan
TDC	Tryptophan decarboxylase

Introduction

Camptotheca acuminata (*C. acuminata*) Decne, a member of the Nyssaceae, is deciduous hardwood species native to China. It has been used for long time as functional food resource, such as medicinal liquor in China (Lin 2013). *C. acuminata* is essential due to high content of camptothecin (CPT) in plant parts. CPTs are natural secondary metabolites with properties of anti-tumor and immune deficiency disease resistance. It is consumed for control of gastric, colorectal, bladder and other cancers (Priel et al. 1991;

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Y. Hu and W. Yu contributed equally to this work.

✉ Jiasheng Wu
wujs@zafu.edu.cn

Yeqing Ying
yeqing@zafu.edu.cn

[1] The Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang A & F University, Hangzhou 311300, People's Republic of China

[2] School of Forestry and Biotechnology, Zhejiang A & F University, Lin'an, Hangzhou 311300, People's Republic of China

[3] Key Laboratory of High Efficient Processing of Bamboo of Zhejiang Province, China National Bamboo Research Center, Hangzhou 310012, People's Republic of China

Douillard et al. 2000; Li et al. 2002; Amna et al. 2006). CPT is mainly extracted from *C. acuminata* due to fact that artificially-synthesized CPT and its analogues have not been available until recently. Owing to multiple beneficial effects on human health and rapid growth of pharmaceutical market, the demand for CPT produced from *C. acuminata* species will increase in the future.

CPT is manufactured by modified terpenoid indole alkaloid (TIA) pathway (Fig. 1). Plants with alkaloid properties have identical upstream biosynthesis pathways for every TIA products which make use of strictosidine backbone and produced by condensation reaction among indole tryptamine and terpenoid secologanin catalyzed by enzyme strictosidine synthase (Sun et al. 2011). Strictosidine is altered to strictosamide through intermolecular cyclization. This compound is a precursor of CPT as apparent by incorporation of radiolabeled precursors (Lorence and Nessler 2004). During process of strictosidine biosynthesis in *C. acuminate,* different enzymes have been isolated and functionally identified. In synthesis of indole precursor tryptophan (Trp) and tryptaminee identified enzymes were Trp synthase (TSB) (Lu and McKnight 1999) and Trp decarboxylase (TDC) (López-Meyer and Nessler 1997). Trp biosynthesis begins with the conversion of chorismate to anthranilate by anthranilate synthase and TSB to form the final product. TSB were cloned and characterized from *C. acuminate* by Lu and McKnight (1999). In all inspected organs of *C. acuminata*, TSB mRNA and protein were noticed and their sufficient quantity were similar with CPT. When Trp is formed, TDC

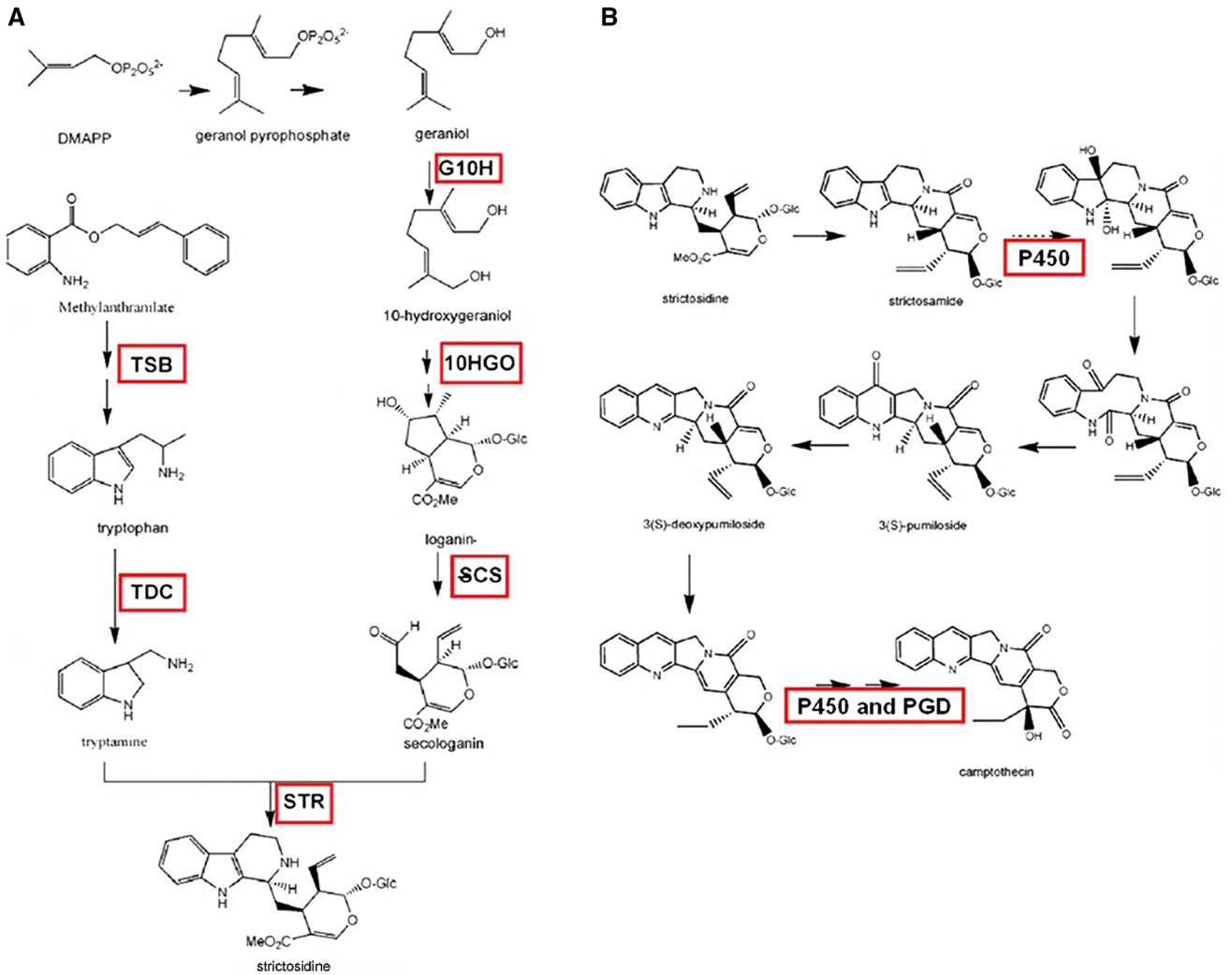

Fig. 1 Biosynthetic pathway of CPT from DMAPP to strictosidine and from strictosidine to CPT in *C. acuminata*. **A** The upstream pathway for the synthesis of the backbone of strictosidine. **B** The proposed branch pathway of CPT biosynthesis (steps after strictosidine synthesis). *TSB* b-subunit of tryptophan synthase; *TDC* tryptophan decarboxylase; *G10H* geraniol-10-hydroxylase; *SCS* secologanin synthase; *STR* strictosidine synthase; *10-HGO* 10-hydroxy geraniol oxidoreductase. *PGD* putative strictosidine b-D-glucosidase. The *arrow* with the *dotted line shaft* represents the step that was presumed in the study to be catalyzed by a CYP450 (quoted from Sun et al. 2011)

change it to tryptamine and perform an essential role in TIA biosynthesis through linking primary and secondary metabolism. Genes encoding for *TDCs* in *C. acuminata* include *TDC1* and *TDC2* was revealed by published research studies. *TDC1* may be component of increasing regulated chemical defence system in *C. acuminata*. However, *TDC2* act as part of defence system induced during pathogen challenge (Lorence and Nessler 2004). Previous studies have revealed that *C. acuminata* species tend to conduct CPT biosynthesis and accumulation when exposed to to both biotic and abiotic stresses such as light, water, temperature, soil type, nutritional status, cutting, plant growth regulators, and various developmental processes (Feng et al. 2002a, b; Li and Liu 2003; Sun and Yan 2008). CPT buildup in leaves of *C. acuminata* seedlings varied significantly in response to different nitrogen forms as reported by Sun and Yan (2008). However, effect of environment on molecular mechanisms regarding expressions of genes engaged in CPT biosynthesis are not yet fully understood.

Light is an important environmental factor which affects vegetative and reproductive growth of plants. *C. acuminata* is considered to be shade intolerant and CPT levels in *C. acuminata* seedlings generally tend to increase with reduction of light intensity, unless plant growth is inhibited (Dai et al. 2004). However, enough research studies have not been conducted to optimum light intensity for CPT production. Published research has not yet clarified how enzymatic activity and genes of the CPT biosynthesis pathway affect CPT concentration under different light conditions.

This study has investigated the effects of light intensity (namely 100 % irradiance, control, 1500 ± 30 μmol m^{-2} s^{-1}, 75 % irradiance, 50 % irradiance and 25 % irradiance) on CPT content, enzymatic activity and expression of genes engaged for biosynthesis of CPT in seedlings of *C. acuminate.* This study has examined that how enzymatic activity and genes of CPT biosynthesis pathway respond to different light qualities. Light intensity which optimizes CPT production has been determined. Information generated from this study is expected to be of great value in better understanding CPT biosynthesis and improving growth conditions for commercial production of high CPT-yielding plants.

Materials and methods

Plant material and treatments

The study was conducted using different shade cloths in controlled environment at Zhejiang A&F University (30°23′N, 119°72′E) during March 2013 in China. One-year-old healthy and uniform *C. acuminata* seedlings (mean basal diameter of 5.8 ± 5 mm and seedling height of 42.4 ± 2 cm) were shifted to plastic pots (16.5 cm inner diameter, 18 cm height, with holes in the bottom, one seedling per pot). The root media was substrate mixture of pine bark: peat: soil (4:4:2, v:v:v, 40 kg m^{-3} of organic manure). Homogenous sixty seedlings with age of 8 weeks were separated into four groups. Experiment was conducted in completely randomized design with five replications per treatment and three plants per replication. The seedlings per replication were moved to growth chamber under artificial light (six 400 W dysprosium lamps above 10-cm water layer serving as heat filter). Photosynthetically active radiation (PAR, 1500 ± 30 μmol m^{-2} s^{-1}) was provided by adjusting distance of lamps to plant canopies at 15 cm. Four irradiance levels were created by neutral shade cloths which have neutral effect on light quality (Yates 1989) over rigid frame, namely 100 % irradiance (non-shaded, control), 25 % irradiance (75 % shaded), 50 % irradiance (50 % shaded) and 75 % irradiance (25 % shaded). All treatments were kept at 30 ± 2 °C and 50–60 % relative humidity during day, 20 ± 2 °C at night and 50–60 % relative humidity with 8–10 h light: 14–16 h dark photoperiod. The light intensity was measured with Digital Lux Meter (TES-1339R, Taiwan). The plants were kept well-watered once a day until the end of experiment.

Third and fourth fresh leaves from top of plants were picked four times (i.e., 0, 20, 40, and 60 days after treatment) from five replication of same treatment. Surface contaimination from leaves were removed with tissue papers. Leaves were frozen in liquid nitrogen, and stored at −70 °C. The fresh weight of *C. acuminata* Decne leaves under different treatments was measured at the same time.

Plant growth and biomass

Leaves, stem, and roots were separated, weighed, and dried at 60 °C after 60 days of treatments. The growth of total dry mass was calculated by the dry biomass of leaves, stem and roots of each seedling before and after treatment. Similar procedure was followed for calculation of growth in leaves.

Extraction and analysis of CPT content

The third and fourth leaves from top were grounded finely with mortar and pestle. The sample of 1 g from 5 seedlings was extracted with 60 % methanol by sonication for 30 min at room temperature. Extract of 50 ml (methanolic) was filtered and evaporated at 40 °C in vacuum using rotavapor. The filtered and evaporated methanolic extract was redissolved in HPLC-grade methanol (1 ml). The HPLC system (Waters, Milford, MA, USA) consisted of

HPLC pump (1525 Binary HPLC Pump), a reversed phase column (Symmetry C18 4.6 × 250 mm) and a detector (2487 Dual λ Absorbance Detector) for CPT detection at 254 and 370 nm. The flow rate was 1 ml min^{-1}, and isocratic mobile phase consisted of water: acetonitrile (70/30, v: v). Based on retention time and absorbance spectra of CPT reference solutions (0.1, 0.05, 0.01, 0.05, and 0.001 mg ml^{-1}), identification and quantification of CPT was completed.(Sigma, St. Louis, MO, USA) (Valletta et al. 2010). CPT yield per plant = CPT content × leaves biomass per plant.

Analysis of TSB and TDC activity

Tryptophan Synthase (TSB) assays followed the method of Last et al. (1991). The frozen leaves samples were grounded finely in liquid nitrogen at 4 °C. Plant extracts were prepared by grinding 0.1 g of leaf tissues to a paste with prechilled mortar and pestle with 2 ml of 0.1 M potassium phosphate buffer (pH 8.2), 600 mg of 100-pm glass beads, and 600 mg of polyvinylpolypyrrolidone (PVPP) (Sigma). Homogenates were sonicated for 6 × 10 s and cleared by centrifugation at 12,000*g* for 15 min. The resultant supernatant fraction was used as enzyme extract. Sixty micromole of L-serine, 0.2 µmol of indole, 80 µmol of potassium phosphate (pH 8.2), and 10 µg of pyridoxal phosphate were incubated with 0.4 ml of plant extract with gentle agitation at 30 °C. The reaction was stopped with the addition of 0.5 ml of toluene after 90 min, and cleared by centrifugation at 7000*g* for 5 min. HPLC system with detector (2487 Dual λ Absorbance Detector) at 270 nm has measured indole content of the supernatant fraction. The flow rate was 1 ml min^{-1}, the isocratic mobile phase consisted of water: acetonitrile (50/50, v: v), and column temperature was 25 °C. Consuming indole as µmol h^{-1} g^{-1} has measured enzyme activity.

Crude protein extract was obtained from about 1 g frozen leaves using 9 ml sodium phosphate buffer (pH 7.8). Subsequently, Elisa kits were used to determine the enzymatic activity of TDC aided with Microplate spectrophotometer at 450 nm (Labsystems Multiskan MS 352, Finland). The activity of TDC was calculated based on standard curve of the Elisa kit standard.

Expression analysis of CPT biosynthesis-related genes

Total RNA was isolated from *C. acuminata* leaves (five samples for each treatment) following the method of Pang et al. (2005) and cDNAs were synthesized with a cDNA synthesis kit (Clontech, Palo Alto, California, USA) following manufacturer instructions. The expression levels of CPT biosynthesis-related genes (*TSB*, *TDC1*, and *TDC2*) along with an actin gene used as a reference were determined by quantitative real-time PCR (qRT-PCR) under an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) and a SYBR Green PCR Master Mix (Applied Biosystems) following manufacturer instructions (Xu et al. 2008) (Table 1 presents primer information for amplification of the analyzed genes).

Data analysis

Statistical analysis was conducted by one-way analysis of ari-ance (ANOVA) with statistical software package SPSS 11.5 for Windows, and Duncan's multiple range test was employed to detect differences between means at 0.05 level ($P \leq 0.05$).

Results

Variation in leaf biomass

Total leaf biomass of *C. acuminata* seedlings showed variable response to different light intensities. Table 2 revealed that total leaf biomass seedling^{-1} was enhanced by 45.1 % ($P < 0.05$) and 20.6 % ($P < 0.05$) at 75 and 50 % irradiance treatments and reduced by 16.5 % in 25 % irradiance treatments respectively, compared with 100 % light irradiance (control). Irradiance of 75 and 50 % has

Table 1 Sequences of primers used to amplify the actin gene (used as a reference), tryptophan synthase (*TSB*), tryptophan decarboxylase (*TDC1*) and tryptophan decarboxylase (*TDC2*) genes

Gene name	Sequence (5′ → 3′)	T_m (°C)
TSB	Up-5′CTGCACTATCGCCAGAGAGAT 3′	64
	Down-5′TTGGTCTCCAGATAGAGATCG 3′	62
TDC1	Up-5′ACTGAATCTCCGGCATCCGTT 3′	64
	Down-5′TCAGAATGCTCTCGAATGGCT 3′	62
TDC2	Up-5′AGCGGA ACTTGAGCTGGAGAT 3′	64
	Down-5′CTGCCACGTCAGCTTCTATCT 3′	64
Actin	Up-5′GTGACAATGGAACTGGAATGG3′	64
	Down-5′AGACGGAGGATAGCGTGAGG3′	62

Table 2 Response of the leaf biomass at 0, 20, 40 and 60 days in *C. acuminata* exposed to different levels of irradiance

Treatment (100 % level) (%)	Biomass of *C. acuminata* seedlings leaves (g)			
	0 days	20 days	40 days	60 days
100	17.25 ± 0.81^{a}	17.89 ± 0.86^{b}	18.02 ± 0.92^{b}	18.49 ± 0.85^{c}
75	17.25 ± 0.81^{a}	19.68 ± 0.77^{a}	21.46 ± 0.46^{a}	23.49 ± 0.44^{a}
50	17.25 ± 0.81^{a}	18.21 ± 0.95^{b}	19.36 ± 0.89^{b}	20.77 ± 0.49^{b}
25	17.25 ± 0.81^{a}	16.78 ± 0.67^{c}	15.46 ± 1.13^{c}	15.38 ± 1.14^{d}

The values presented are the mean ± SE

Different letters indicate significant differences between irradiance treatments ($P < 0.05$); $n = 5$. 100 % level = 1500 ± 30 μmol m^{-2} s^{-1}

Table 3 Response of CPT content at 0, 20, 40 and 60 days, in *C. acuminata* leaves exposed to different levels of irradiance

Treatment (100 % level) (%)	CPT content of *C. acuminata* seedlings leaves (mg g^{-1})			
	0 days	20 days	40 days	60 days
100	1.60 ± 0.01^{a}	1.57 ± 0.06^{d}	1.62 ± 0.02^{d}	1.68 ± 0.06^{c}
75	1.60 ± 0.01^{a}	1.86 ± 0.07^{c}	1.84 ± 0.06^{c}	1.82 ± 0.02^{d}
50	1.60 ± 0.01^{a}	2.26 ± 0.05^{b}	2.87 ± 0.09^{b}	3.56 ± 0.02^{a}
25	1.60 ± 0.01^{a}	2.78 ± 0.07^{a}	3.46 ± 0.13^{a}	3.20 ± 0.07^{b}

The values presented are the mean ± SE

Different letters indicate significant differences between irradiance treatments ($P < 0.05$); $n = 5$. 100 % level = 1500 ± 30 μmol m^{-2} s^{-1}

Table 4 Response of CPT yield at 0, 20, 40 and 60 days in *C. acuminata* leaves exposed to different levels of irradiance

Treatment (100 % level) (%)	CPT yield of *C. acuminata* seedlings leaves (mg $plant^{-1}$)			
	0 days	20 days	40 days	60 days
100	27.60 ± 1.31^{a}	28.08 ± 1.36^{d}	29.19 ± 1.32^{c}	31.10 ± 1.43d
75	27.60 ± 1.31^{a}	36.60 ± 1.07^{c}	39.48 ± 0.96^{b}	42.76 ± 0.81c
50	27.60 ± 1.31^{a}	41.15 ± 0.95^{b}	55.56 ± 1.89^{a}	73.94 ± 1.74a
25	27.60 ± 1.31^{a}	46.65 ± 1.67^{a}	53.84 ± 2.13^{a}	49.22 ± 3.66b

The values presented are the mean ± SE

Different letters indicate significant differences between irradiance treatments ($P < 0.05$); $n = 5$. 100 % level = 1500 ± 30 μmol m^{-2} s^{-1}

improved growth of *C. acuminata* seedlings compared with inhibited growth from 25 % irradiance.

Variation in CPT content and CPT yield per plant

Mean CPT content of *C. acuminata* seedlings significantly differed ($P < 0.05$) among different treatments and treatment periods (Table 3). CPT contents in plants growing in 100 % light conditions slightly increased over time. Compared to control, CPT content in 75, 50 and 20 % irradiance treatments increased with increasing time of treatment. Highest content was observed after 60 days of 50 % light irradiance treatments ($P < 0.05$), followed by 25 % ($P < 0.05$) and 75 % irradiance treatments ($P < 0.05$), respectively (Table 3). Interestingly, between days 20 and 40, CPT content in 25 % light irradiance treatments was higher than 100, 75 and 50 % irradiance treatments.

CPT yield of individual seedlings was estimated and the result was equal to CPT content multiplied by biomass of leaves (Table 4). The CPT yield was highest after 60 days of 50 % irradiance treatment, followed (in descending order) by the 25, 75, and 100 % light irradiance treatments. Highest value was equal to 2.4 ($P < 0.05$) times of lowest value (Table 4).

Variation in activity of key enzymes

The activity of key enzymes (TSB and TDC) in CPT biosynthesis pathway significantly differed ($P < 0.05$) among various treatments and treatment periods (Fig. 2). We observed slight increase in activities of TSB and TDC in plants grown in 100 % irradiance conditions after 60 days of treatment. Compared with control, TSB and TDC activity increased with reduction in light intensity. The highest levels of TSB and TDC activity in 25 %

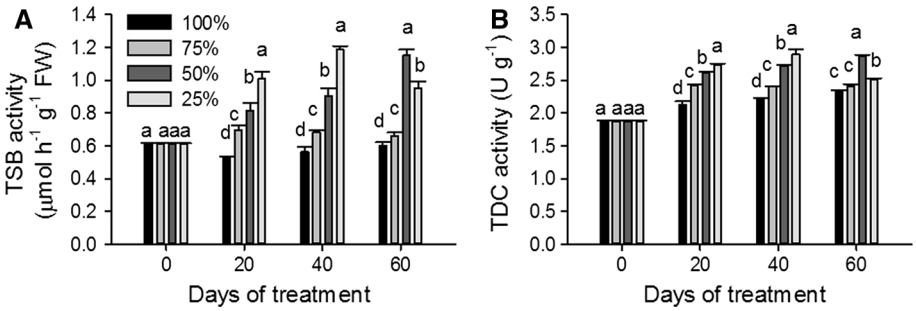

Fig. 2 Variation in activity of enzymes (TSB and TDC) in *C. acuminata* Decne leaves growing under different levels of irradiance. The values presented are the mean ± SE. *Different letters* indicate significant differences between irradiance treatments ($P < 0.05$); $n = 5$. 100 % level = 1500 ± 30 μmol m^{-2} s^{-1}

irradiance were observed between 20 and 40 days. Nevertheless, on day 60, the level of TSB and TDC activity in 50 % irradiance treatments was highest ($P < 0.05$) followed by descending order in 25 % ($P < 0.05$) and 75 % ($P < 0.05$) irradiance treatments respectively, while 100 % irradiance treatment ($P < 0.05$) was the lowest.

Variation in relative expression of CPT biosynthesis related genes

Gene expression of *TSB*, *TDC1* and *TDC2* in leaves of *C. acuminata* as affected by light internisty was significantly varied among treatments ($P < 0.05$, Fig. 3). Generally, relative expressions of *TSB*, *TDC1*, and *TDC2* were consistent with the enzyme activity of TSB and TDC. The relative expressions of three studied genes (*TSB*, *TDC1* and *TDC2*) showed an increasing tendency with light intensity reduction on the 20th and the 40th day (Fig. 3). Interestingly, the expression level of *TDC2* was higher than *TDC1* under different light intensities (Fig. 3B, C). The relative expression of three genes was also consistently maximum in 25 % irradiance treatment and lowest in 100 % irradiance treatment between days 20 and 40. However, on day 60, relative expressions of three genes in 50 % irradiance treatment were highest ($P < 0.05$), followed by 25 % ($P < 0.05$) and 75 % irradiance treatments ($P < 0.05$), with 100 % irradiance treatments ($P < 0.05$) being lowest. The correlations between total CPT content and relative expression of *TSB, TDC1* and *TDC2* were 0.890, 0.980, and 0.956 respectively, which indicated that their expression levels are notably correlated with total CPT content in leaves of *C. acuminata* seedlings.

The relative expression of related genes biosynthesis of CPT was positively correlated with CPT content when growing under 50 irradiance (Fig. 4.), it was $P = 0.048$ in *TSB*, $P = 0.016$ in *TDC1* and $P = 0.010$ in *TDC2*, respectively.

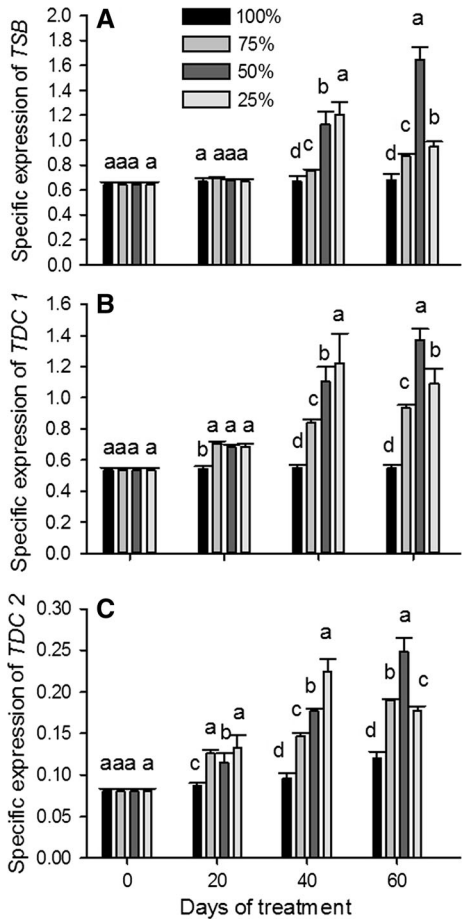

Fig. 3 Variation in relative gene expression (TSB, TDC1, and TDC2) in *C. acuminata* Decne leaves growing under different levels of irradiance. The values presented are the mean ± SE. *Different letters* indicate significant differences between irradiance treatments ($P < 0.05$); $n = 5$. 100 % level = 1500 ± 30 μmol m^{-2} s^{-1}

Discussion

Light intensity is an important environmental factor influencing tree growth; light compensation and saturation points differ among various tree species (Zhang et al.

2007). Significant effects on leaves and total biomass production of *C. acuminata* seedlings were observed with different irradiance levels. An irradiance level of 75 % resulted in highest total leaves biomass after 60-day treatment, compared with those growing in excessive high light (100 % light irradiance) and in deep shade (25 % light irradiance) (Table 2). This is consistent with other studies, which showed that growth of plants can be severely reduced after exposure to light intensity in excess of that required to saturate photosynthesis (Maxwell and Johnson 2000). Whereas, when grown in a low irradiance environment, plants become extremely stressed, unable to adjust quickly to low light and thus are less likely to survive (Hiroki and Ichino 1998; Paquette et al. 2007).

CPT, as natural secondary metabolite, is involved in response to variety of biotic and abiotic stresses (Dai et al. 2004). The metabolism of CPT follows a complex pathway, and some environmental factors such as temperature, water status, light condition, and nitrogen have all been demonstrated to affect CPT accumulation (Feng et al. 2002a, b; Dai et al. 2004; Sun and Yan 2008). The present study demonstrated that lower light intensity was favorable to CPT accumulation in leaves of *C. acuminata* seedlings. Irradiance treatments (50 %) produced highest CPT content after 60 days, compared with 25 % ($p < 0.05$) and 75 % irradiance treatments ($p < 0.05$) (Table 3). Similar observation was reported by Dai et al. (2004), they reported substantial changes in CPT contents with different light intensity. This result indicates that CPT production is sensitive to light intensity and lower light intensity has promoted CPT biosynthesis until light levels inhibited plant growth. The explanation for lower CPT content in plants grown under very low irradiance (such as 25 % irradiance) plant growth is subjected to stress and would suffer damage and concurrent decreases in primary metabolism levels. This would consequently lead to decrease in materials for secondary metabolism and will lead to decrease in biosynthesis of secondary metabolites, such as CPT.

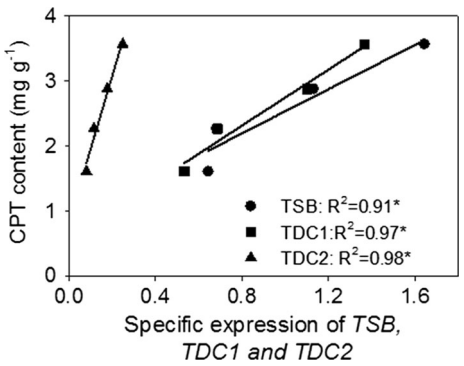

Fig. 4 Relations between the relative expression of the related genes of TPC biosynthesis (*TSB*, *TDC1*, and *TDC2*) and TPC content in leaves of *C. acuminata* Decne growing under 50 % irradiance. The *solid lines* represent the best-fit linear regressions for each gene: $*P < 0.05$

CPT accumulation in leaves of *C. acuminata* seedlings is the consequence of the coordinated interaction of multiple enzymes involved in CPT biosynthesis. TSB is a key enzyme related to CPT biosynthesis which catalyzes irreversible reaction from anthranilate to tryptophan. Abundant TSB was reported by previous research studies in leaves and shoots, especially in cambium, primary xylem, and primary phloem. In *C. acuminata* during early seedling development TSB was the maximum corresponding to peak accumulation of CPT, which is consistent with the idea that Trp biosynthesis and secondary TIA pathway is coordinately regulated (Lorence and Nessler 2004). In our study, TSB activity, concordant with CPT content, significantly differed ($P < 0.05$) among different light intensity treatments (Fig. 2A), which indicated that light intensity has affected CPT content in leaves of *C. acuminata* through regulation of enzyme activity related to CPT biosynthesis. Several studies on *Catharanthus roseus* cultures and *C. acuminata* reported that TDC catalyzes the irreversible reaction from tryptophan to tryptamine. The activity of this enzyme was correlated with TIA accumulation in suspension cell lines treated with biotic and abiotic elicitors (Eilert 1987) or transferred to alkaloid production medium (Mérrillon et al. 1986). In present study, TDC activity was higher under lower irradiance treatments (50 and 25 % irradiance), which is consistent with Feng et al. (2008). Thus, TDC activity increased with the attenuation of the stress, and consequently led to increase in CPT biosynthesis and accumulation.

Encode genes of biosynthetic enzymes active in secondary metabolism have changed their expression patterns in response to light with varying secondary metabolite accumulation in plant (Liu et al. 2006). High TSB expression is often found in parallel with high levels of CPT content (Lu and McKnight 1999). Two genes which encode first decarboxylating enzyme (TDC) in CPT biosynthesis pathway during seedling development, expression of *TDC1* enhanced with alkaloid buildup during treatment. *TDC2* was only induced in *C. acuminata* leaves under adversity (Lorence and Nessler 2004). Our study showed that expression level of *TDC2* was higher than *TDC1* under different light intensities (Fig. 3B, C). Significant differences between expression of *TSB* and *TDC1*, enzyme activity and CPT content were observed in response to 50 % irradiance. Moreover, relative expression of related genes of TPC biosynthesis (*TSB*, *TDC1*, and *TDC2*) was significantly correlated with the TPC content in leaves of *C. acuminata* Decne growing under 50 % irradiance (Fig. 4). It is revealed that *TSB* and *TDC* have played significant function in regulatory control of CPT

biosynthetic pathway. 50 % irradiance was most effective treatment to enhance CPT biosynthesis and buildup.

In leaf-producing plantations of *C. acuminata*, the purpose was to obtain not only higher CPT content but also higher yield (higher leaf biomass). In terms of CPT yield per plant (equal to CPT content multiplied by leaves biomass) (Dai et al. 2004), our study demonstrated that an irradiance of 50 % was optimal for leaf-harvest plantation (Table 4), which suggested that light level was more effective in maintaining balance between biomass production and secondary metabolite content in order to obtain higher yields of health-promoting substances per area from plantation. In order to achieve highest CPT yield per area from medicinal plantation, it may be important to manipulate field growing conditions such as light intensity.

Conclusion

Significant changes in biomass accumulation and allocation were found in *C. acuminata* seedlings grown under variable light intensities. The treatment of 75 % irradiance has achieved highest dry biomass, which indicated that *C. acuminata* seedlings have high plasticity of morphological acclimation to light intensity. In contrast, highest CPT content and CPT yield were obtained under 50 % irradiance. These results indicated that an irradiance of 50 % was conducive to CPT biosynthesis and accumulation in leaves of *C. acuminata* seedlings, because CPT accumulation per area depends on both biomass production and CPT content. The enzyme activity, expression of CPT biosynthesis-related genes *(TSB, TDC1* and *TDC*2) and total CPT content were concordant under different light intensity treatments, as supported by significant correlations between studied genes' expression levels and CPT content. These results indicated that 50 % sunlight was effective in increasing the production of camptothecin in leaves of *C. acuminata* seedlings. It is revealed that carefully manipulating light intensity would be an effective means for maximum production of health-promoting substances per unit area.

Author contribution statement YYH, WWY, JSW and YYQ conceived and designed research. XHM recorded the growth data. WWY, YL and XHD analyzed the TSB and TDC activity and CPT content. YYH performed the expression analysis of CPT biosynthesis-related genes. YYH and WWY wrote the manuscript. LLS, XHD, JSW and YYQ assisted in drafting script of research paper. All authors have read and approved the manuscript.

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