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Research article

Temperature-dependent growth and hypericin biosynthesis in *Hypericum perforatum*

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A R T I C L E I N F O

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A B S T R A C T

Hypericum perforatum is a perennial herb that produces the anti-depression metabolite hypericin (Hyp). While several efforts to increase Hyp production have been made, the effects of temperatures on growth and Hyp biosynthesis are still limited. In this study, the growth morphophysiological traits, Hyp biosynthesis and their related genes expression, as well as major bioactive compounds accumulation and antioxidant capacity were assessed by exposing *H. perforatum* seedlings to three different temperatures (15, 22 and 30 °C). The results showed that aerial parts biomass was greater at 15 °C with 1.3 and 1.6-fold increase compared to at 22 and 30 °C, in large part because of greater increase in chlorophyll content, stem number and leaf area on a per plant basis. Hyp content in the aerial parts was greater 1.9 and 5.6-fold on a per plant basis compared to 22 and 30 °C treatments, and the contents of other bioactive compounds (flavonoids and phenolics) as well as antioxidant capacity in the aerial parts, on dry weight and per plant basis, also exhibited significant increases with the temperatures decrease. The mRNA expressions of eight genes (*psbA*, *psbB*, *psbC*, *psbD*, *ycf3*, *ycf4*, *ycf5* and *matK*) related to photosynthesis and two genes (Polyketide synthase, *PKS*; Phenolic oxidative coupling protein, *Hyp-1*) involved in Hyp biosynthesis were also up-regulated at 15 °C. The findings are useful in guiding cultivation and regulating Hyp biosynthesis in *H. perforatum*.

1. Introduction

Hypericum perforatum L.(family Hypericaceae), popularly named as St. John's Wort, is an herbaceous perennial plant widely distributed in Asia, Europe, Northern Africa and the United States (Couceiro et al., 2006; Sun et al., 2019). Aerial parts have been used worldwide for the treatment of mild to moderate depression (Haas et al., 2017; Velingkar et al., 2017), as well as other biological agents including anti-oxidant, anti-viral and wound healing (Shakya et al., 2017; Radulovic et al., 2018), largely relying on its chemical constituents such as naphthodianthrones (*e.g.* Hyp, pesudohypericin and phlorodianthrones), flavonoids (*e.g.* quercetin, amentoflavone and isoquercitrin) and phenolics (*e.g.* chlorogenic acids, caffeic and coumaroylquinic acids) (Kwiecien et al., 2018; Barnes et al., 2019). Of these, Hyp is the highly valued polycyclic dianthroquinone responsible for the anti-depression and anti-viral agents (Rahnavrd, 2017).

Many approaches to improve the production of Hyp, flavonoids and phenolics have been made by investigating into the effects of germplasm (Couceiro et al., 2006; Morshedloo et al., 2015), harvest stages (Southwell and Bourke, 2001; Couceiro et al., 2006; Ionescu et al., 2018; Sun et al., 2019), UV-B radiation (Germ et al., 2010; Brechner et al., 2011), drought stress (Gray et al., 2003; Alibas and Kacar, 2016), nutrients (Briskin et al., 2000; Cui et al., 2010b) and temperatures (Zobayed et al., 2005; Couceiro et al., 2006; Odabas et al., 2010; Skyba et al., 2012; Brunáková et al., 2015). An alternative approach to increase Hyp and other bioactive compounds could involve biotechnology-based manipulations including elicitations (*e.g.* acetic acid, jasmonic acid, methyl-jasmonate acid, salicylic acid and chitosan) (Walker et al., 2002; Conceicao et al., 2006; Tocci et al., 2010; Gadzovska et al., 2013; Brasili et al., 2014; Valletta et al., 2016), *in vitro* culture and bioreactor (Zobayed et al., 2004; Gadzovska et al., 2013; Cui et al., 2010a). In this context, much effort has been exerted to

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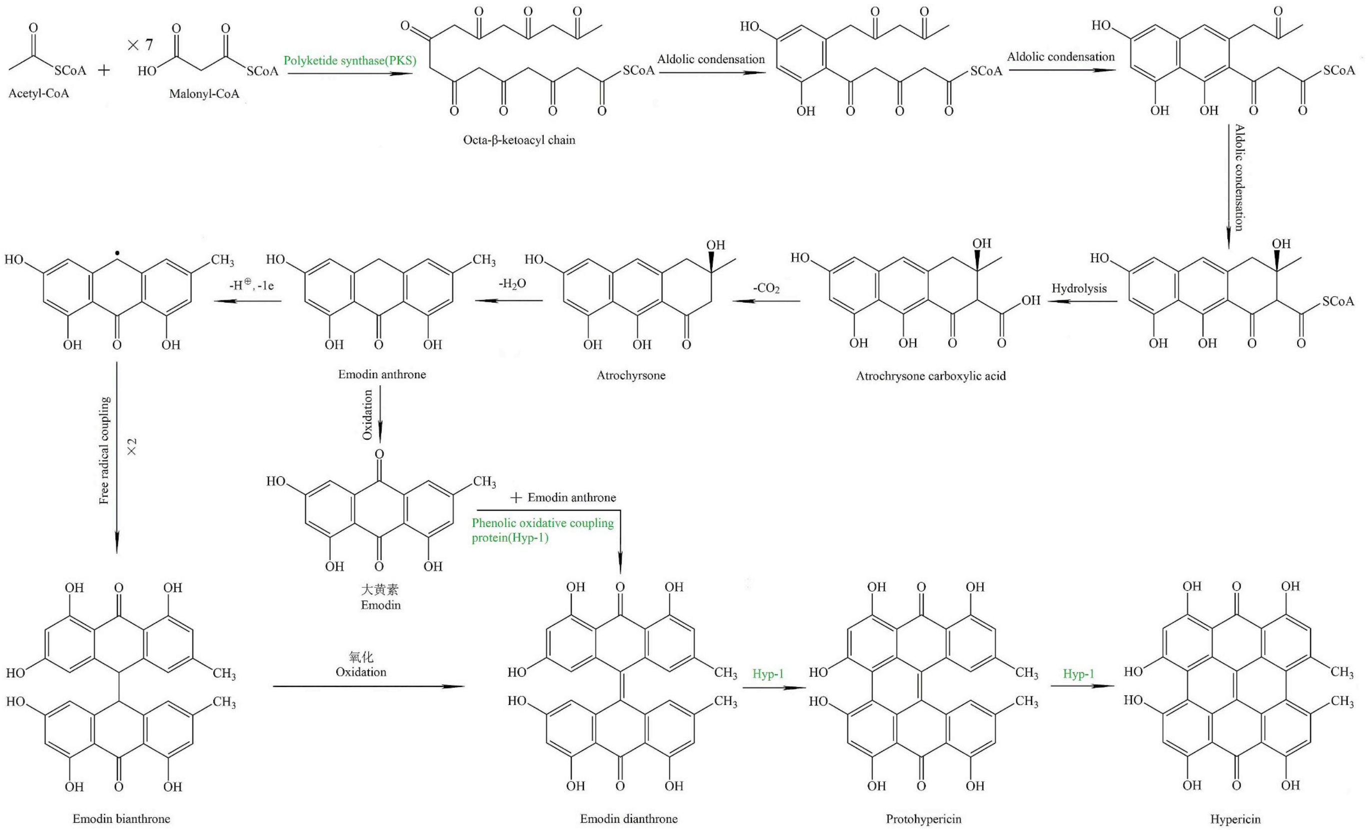

Fig. 1. Schematic representation of biosynthetic pathway leading from acetyl- and malonyl-CoA to Hyp. Enzyme abbreviations are as follows: PKS, Polyketide synthase; Hyp-1, Phenolic oxidative coupling protein.

dissect the Hyp biosynthetic pathway (Fig. 1) (Bok et al., 2009; Kusari et al., 2009; Chiang et al., 2010), while the improvement of Hyp production in *H. perforatum* by regulating abiotic and biotic factors was limitedly conducted based on the above pathway.

For the effect of temperatures on major bioactive compounds accumulation in *H. perforatum*, Bruňáková et al. (2015) have demonstrated that *H. perforatum* is a freezing-tolerant plant and a 1.6-fold increase of naphtodianthrones content on a dry weight (DW) basis was observed when plants exposure to −4 °C for 48 h. Zobayed et al. (2005) have investigated that the Hyp and pseudohypericin contents on a fresh weight (FW) basis generally decreased with the decrease of temperatures from 35 to 15 °C, while the hyperforin content showed higher levels at 20 and 15 °C compared to 25 and 30 °C. Couceiro et al. (2006) reported that on a FW basis the contents of Hyp and pseudohypericin were higher at 30 °C than at 25 °C, while the hyperforin content was higher at 25 °C than at 30 °C grown in a field or a greenhouse. Tocci et al. (2013) found that chemical compositions in aerial parts at two altitudes (68 and 453 m above sea level) showed a similar metabolic pattern with small differences including chlorogenic acid, quercetrin, luteolin, rutin and isoquercetin, while contents of quercetrin and hyperosid respectively had a 1.6-fold increase and 3.5-fold decrease at the 453 m compared to the 68 m site. Unlike previous investigations that plants were grown at the different temperatures and bioactive compounds (Hyp, pseudohypericin and hyperforin) were analyzed on a FW basis (Couceiro et al., 2006), in this study, effects of three temperatures (15, 22 and 30 °C) on plant growth, Hyp biosynthesis and their related genes expression, as well as major bioactive compounds (flavonoids and phenolics) accumulation and antioxidant capacity were systematically investigated on both DW and per plant basis.

2. Methods

2.1. Plant materials

H. perforatum mature seeds were collected from Kangxian County (33°16′20″N, 105°31′50″E) located in Gansu, P.R. China in July 2016. Seeds were rinsed with running water for 10 min, and successively immersed in 70% ethanol for 2 min, 0.1% $HgCl_2$ for 1 min. After each treatment, seeds were rinsed with sterile water for 3 times. Sterilized seeds were inoculated on MS basal medium (pH5.8) supplemented with 4.0 g/L agar + 20.0 g/L sucrose + 1.0 mg/L 6-BA + 0.5 mg/L NAA + 1.0 g/L activated carbon at 22 °C with 24 h/d photoperiod (500 μmol $m^{-2}s^{-1}$ flu). After inducing 45 days (See Fig. S1), the stem internodes were cut and inoculated on the above mentioned medium and photoperiod at three different temperatures including: 15, 22 and 30 °C, with each treatment 40 flasks (3 seedlings per flask). Seedlings were harvested after 45 days (See Fig. S2). 10 flasks (30 seedlings) were used for measurements of physiological characteristics, bioactive compounds (Hypericin, flavonoids and phenolics) as well as antioxidant capacity; the others were immediately frozen in liquid nitrogen and kept at −80 °C for genes expression.

2.2. Physiological measurements

2.2.1. Growth characteristics

The seedlings were taken out from the flasks, rinsed with running water to remove the medium, and then used to measure leaf area, plant height, root length, number of stem internodes and root as well as dry weight of aerial parts.

Table 1

Primer sequences used to amplify *ACT*, *psbA*, *psbB*, *psbC*, *psbD*, *ycf3*, *ycf4*, *ycf5*, *matK*, *PKS* and *Hyp-1*.

Genes	Sequences (5′ to 3′)	Amplicon size (bp)	Accession no.
ACT	Forward: ATCCTCCGTCTTGACCTTGC Reverse: ACGATTTCCCGTTCTGCTGT	104	CP002685.1
psbA	Forward: GCCTATGGGGTCGTTTCTGT Reverse: CATCAATATCTACTGGCGGCG	150	JX663284.1
psbB	Forward: AACAAGTCGGAGTAACCGTCG Reverse: GCCAAAAGTAAACCACCCCC	185	JX662199.1
psbC	Forward: TCTGGTCCGGGGAAGC Reverse: AGCAGATCCGACGTTAGCC	200	JX662583.1
psbD	Forward: TATGGGGTCCTGAAGCACAAG Reverse: CCCGTGGAGAGCAACAAAAG	80	JX662323.1
ycf3	Forward: TGTCTGCTCAATCCGAAGGAAA Reverse: AAAAGGGTTTCTTTCTAGTGCTTGG	185	JX664461.1
ycf4	Forward: GGCCTTCTATGGTATCGCCG Reverse: CGGAAAGCCCCAACGAAAAA	133	JX663242.1
ycf5	Forward: TCTCGGATTCGTCAGAAAAGGG Reverse: CCGAGGAAAGCCAACGAGTA	81	GQ435774.1
matK	Forward: AGTTGCATTTTCGCCACGAT Reverse: CAGCCAGTACCGAAGGGTTT	193	DQ168438.1
PKS	Forward: GGGGAAGGCCGATTAGTGAG Reverse: TTCTCCGCTATGTCCTTGGC	190	EF186675.1
Hyp-1	Forward: GGCACGGTCACCAAAATCAC Reverse: TGTCACGCAACACATCACCT	130	AY148090.1

2.2.2. *Determination of chlorophyll content*

Total chlorophyll content was determined as the description of Li et al. (2009). The aerial parts (0.1 g) of seedlings were grind to homogenate in ethanol (10 mL), and then the homogenate was centrifuged at 1000 r/min and 25 °C for 10 min. The supernatant was increased to 25 mL with ethanol. The absorbance was measured using a spectrophotometer (721G, Shanghai Instrumental analysis Co., Ltd, P.R. China) at 665 nm (chlorophyll *a*) and 649 nm (chlorophyll *b*) against a blank sample containing no substrate.

2.3. *Determination of bioactive compounds and antioxidant capacity*

2.3.1. *Preparation of extracts*

Air-dried aerial parts were grind into powder, 0.1 g sample was soaked in 20 mL ethanol and placed at 25 °C for 72 h, then extracted samples were centrifuged at 8000 r/min and 4 °C for 10 min. The supernatant was increased to 20 mL with ethanol then kept at 4 °C for determination.

2.3.2. *Hyp HPLC quantification*

Hyp content was quantified as the description of Chi and Franklin (1999). The extracts were filtered with a 0.22 μm durapore membrane and then analyzed (20 μL) at 588 nm by HPLC (Eclipse XDB-C8, 150 mm × 4.6 mm, 5 μm; Column temperature 60 °C; Agilent 1100 series, Santa Clara, California, USA) and isocratically eluted with 0.03 mol/L KH_2PO_4 (adjusted to pH 7.0 with 0.5 mol/L K_2HPO_4): methanol (30:70, v/v) at a flow rate of 1.0 mL/min. The quantification was based on peak area comparison with a reference standard using Hyp (56690; Sigma, USA) (See Fig. S3).

2.3.3. *Determination of flavonoids and phenolics content*

Flavonoids content was determined based on $NaNO_2$-$AlCl_3$-NaOH method (Ma et al., 2014; Sun et al., 2019). Extracts (400 μL) were added into ddH_2O (2 mL) and 5% $NaNO_2$ (0.3 mL), after the mixture agitating for 5 min, added 10% $AlCl_3$ (0.3 mL) reacted for 1 min, then added 1.0 mol/L NaOH (2 mL) to stop the reaction. Absorbance was determined at 510 nm, flavonoids content was expressed as mg of catechin equivalent (CE).

Phenolics content was determined based on Folin-Ciocalteu method (Beato et al., 2011; Sun et al., 2019). Extracts (50 μL) were added into 10% Folin-Ciocalteu reagent (2 mL) and 7.5% Na_2CO_3 (1 mL), after the mixture agitating for 5 min and then reacting at 37 °C for 1 h in the dark. Absorbance was determined at 760 nm, phenolics content was expressed as mg of gallic acid equivalent (GAE).

2.3.4. *Determination of antioxidant capacity*

DPPH (1, 1-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) were selected to determine the antioxidant capacity due to their widely used by many researchers for rapidly evaluating antioxidant capacity of natural plant extracts (Arnao, 2000; Li et al., 2018). The specific processes are as follows.

DPPH radical scavenging assay was determined as the description of Nencini et al. (2011). Briefly, extracts (100 μL) was added into 10^{-4} mol/L DPPH methanol solution (3 mL), then the mixture was agitated and reacted at 25 °C for 30 min in the dark. Absorbance was determined at 515 nm, the capacity to scavenge DPPH radicals was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_0 - A)/A_0] \times 100$$

where "A_0" and "A" were the absorbance of DPPH without and with sample, respectively.

FRAP assay was determined as the description of Benzie and Strain (1996). Briefly, extracts (50 μL) or standard sample ($FeSO_4 \cdot 7H_2O$, 500 μmol Fe (II)/g) were added into FRAP reagent (3 mL), then the mixture was agitated and reacted at 37 °C for 30 min in the dark. Absorbance was determined at 593 nm, the FRAP value was calculated on the basic of ($FeSO_4 \cdot 7H_2O$, 500 μmol Fe (II)/g) as follows:

$$\text{FRAP value (μmol Fe(II)/g)} = [(A - A_0)/(A_{FeSO4 \cdot 7H2O} - A_0)] \times 500\ \text{(μmol Fe(II)/g)}$$

where "A_0" and "A" were the absorbance of FRAP without and with sample, respectively; $A_{FeSO4 \cdot 7H2O}$ was the absorbance of $FeSO_4 \cdot 7H_2O$.

2.4. *qRT-PCR analysis*

Total RNA samples were isolated from aerial parts using a Plant RNA Kit (R6827, Omega). The quality of total RNA was evaluated by agarose gel electrophoresis (See Fig. S4). Expression levels of 10 genes (*psbA*, *psbB*, *psbC*, *psbD*, *ycf3*, *ycf4*, *ycf5*, *matK*, *PKS* and *Hyp-1*) were analyzed by qRT-PCR. *ACT* (*Action*) gene was used as a reference control. The primer sequences for amplification of the 10 genes are shown in Table 1.

cDNA synthesis and qRT-PCR analysis were respectively performed using FastKing RT Kit and SuperReal PreMix (Tiangen Biotech Co. Ltd.). Reverse transcription was performed on the following protocols: 42 °C for 15 min and then 95 °C for 3 min, one cycle. PCR amplification was performed on the following protocols: one cycle at 95 °C for 15 min, and 35 cycles at 95 °C for 10 s, 60 °C for 20 s and 72 °C for 30 s. Melting curve analysis was performed after a 34 s incubation at 72 °C (See Fig. S5). The $2^{-\triangle\triangle Ct}$ method (*Ct*, Cycle threshold value of target gene) was used to calculate the relative gene expression amount according to the following formula (Willems et al., 2008):

$$\triangle Ct_{Test\ gene} = Ct_{Test\ gene} - Ct_{Reference\ gene}$$

$$\triangle Ct_{Control\ gene} = Ct_{Control\ gene} - Ct_{Reference\ gene}$$

$$-\triangle\triangle Ct = -(\triangle Ct_{Test\ gene} - \triangle Ct_{Control\ gene})$$

$$\text{Relative gene expression fold (Test gene/Control gene)} = 2^{-\triangle\triangle Ct}$$

2.5. *Statistical analysis*

All determinations were carried out in triplicate. Statistical analysis was performed using SPSS 19.0 One-Way analysis of variance

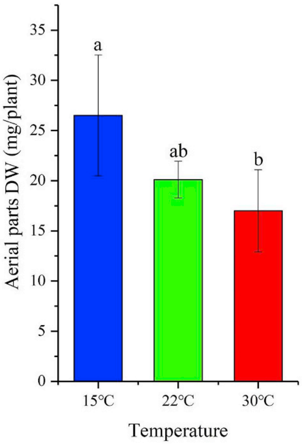

Fig. 2. Aerial parts dry weight of *H. perforatum* at different temperatures. Values are on a per plant basis. Different lowercase letters indicate significant difference at $P < 0.05$ level. The same below.

(ANOVA), Duncan multiple comparison tests were used for statistical comparisons and $P < 0.05$ was accepted as a significant difference.

3. Results

3.1. Temperature-dependent plant growth

Aerial parts dry weight was greater at 15 °C with 1.3 and 1.6-fold increase compared to 22 and 30 °C, respectively (Fig. 2). Chlorophyll content at 15 °C, on FW and per plant basis, were significantly greater at 15 °C than that of 22 and 30 °C, with 1.6 and 2.7-fold increase respectively compared to 22 and 30 °C on a plant basis. The stem number and leaf area on a per plant basis exhibited a similar trend with aerial parts dry weight as well as chlorophyll content, with 1.2, 1.4-fold (stem number) and 1.2, 2.6-fold (leaf area) increase compared to 22 and 30 °C; even though other growth parameters including plant height, root length, number of stem internode and root showed greater at 22 °C compared to 15 and 30 °C (Table 2).

3.2. Low temperature increases Hyp accumulation

Hyp content at 15 °C was increased up to 1.4 and 3.6-fold compared to 22 and 30 °C on a DW basis (Fig. 3A), while on a per plant basis, Hyp content at 15 °C was higher 1.9 and 5.6-fold than 22 and 30 °C (Fig. 3B).

3.3. Low temperature increases flavonoids and phenolics content

Both flavonoids and phenolics contents were significantly decreased with temperatures increase from 15 to 30 °C (Fig. 4). The flavonoids contents at 15 °C were 1.3, 4.2-fold and 1.7, 6.7-fold on DW and per plant basis, respectively compared to 22 and 30 °C (Fig. 4A and B). The phenolics contents at 15 °C were 1.5, 4.5-fold and 1.9, 7.0-fold on DW and per plant basis, respectively compared to 22 and 30 °C (Fig. 4C and D).

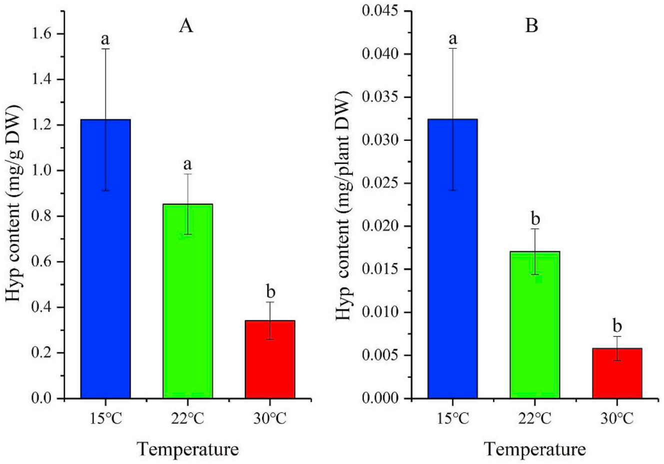

Fig. 3. Hyp content in aerial parts of *H. perforatum* at different temperatures. Images A and B represent Hyp content on dry weight and per plant basis, respectively.

3.4. Low temperature increases antioxidant capacity

DPPH scavenging activity (89%) and FRAP value (2299 μmol Fe (II)/g) at 15 °C were respectively higher 1.4, 3.8-fold and 1.4, 3.6-fold than that of 22 and 30 °C (Fig. 5A and C). On a per plant basis, the DPPH scavenging activity and FRAP value at 15 °C were also significantly higher than that of 22 and 30 °C, with increasing up to1.9-, 5.9-fold and 1.9-, 5.6-fold, respectively (Fig. 5B and D).

3.5. Low temperature enhances gene expression related to plant growth

Eight genes (*psbA*, *psbB*, *psbC*, *psbD*, *ycf3*, *ycf4*, *ycf5* and *matK*) related to photosynthesis were selected from NCBI (searching for *Hypericum perforatum* L.) to detect their mRNA expression levels at different temperatures. The relative expression levels at 15 °C were enhanced up 0.7 (*psbB*) to 3.8 (*ycf4*) -fold, while that of 30 °C were dropped down 0.1 (*psbB*) to 1.0 (*psbC*) -fold compared to 22 °C (Fig. 6).

3.6. Low temperature enhances gene expression related to Hyp biosynthesis

Two genes (*PKS* and *Hyp-1*) related to Hyp biosynthesis were selected to detect their mRNA expression levels at different temperatures. The relative expression levels of *PKS* and *Hyp-1* at 15 °C were respectively enhanced up 2.8 and 2.4-fold, while that of 30 °C were dropped down 0.5 and 0.9-fold compared to 22 °C (Fig. 7).

4. Discussion

Low temperatures is one of the important environmental stresses affecting plant growth and development as well as primary and secondary metabolism (Yang et al., 2016, 2018; Fu et al., 2018). Although plants differ in their sensitivity to low temperature, for *H. perforatum* low temperature can cause noticeable alterations in various physiological and biochemical processes that can potentially improve low-

Table 2
Chlorophyll content and growth characteristics in aerial parts of *H. perforatum* at different temperatures (mean ± SD, n = 30).

Temp. °C	Chlorophyll content		Aerial parts				Rhizome	
	mg/g FW	mg/plant FW	Height (cm)	Stem No./plant	Stem internodes/plant	Leaf area (cm^2/plant)	No./plant	Length (cm)
15	2.95 ± 0.22^a	0.26 ± 0.02^a	6.92 ± 1.38^b	2.03 ± 0.67^a	8.10 ± 0.88^c	7.72 ± 3.12^a	2.87 ± 0.63^b	4.80 ± 1.69^b
22	1.99 ± 0.19^b	0.16 ± 0.02^b	9.95 ± 1.49^a	1.67 ± 0.61^b	10.63 ± 1.27^a	6.41 ± 1.70^b	3.60 ± 0.77^a	7.33 ± 2.03^a
30	1.96 ± 0.17^b	0.10 ± 0.01^c	4.87 ± 0.98^c	1.47 ± 0.57^b	9.30 ± 1.80^b	3.00 ± 0.76^c	1.83 ± 0.99^c	2.06 ± 1.40^c

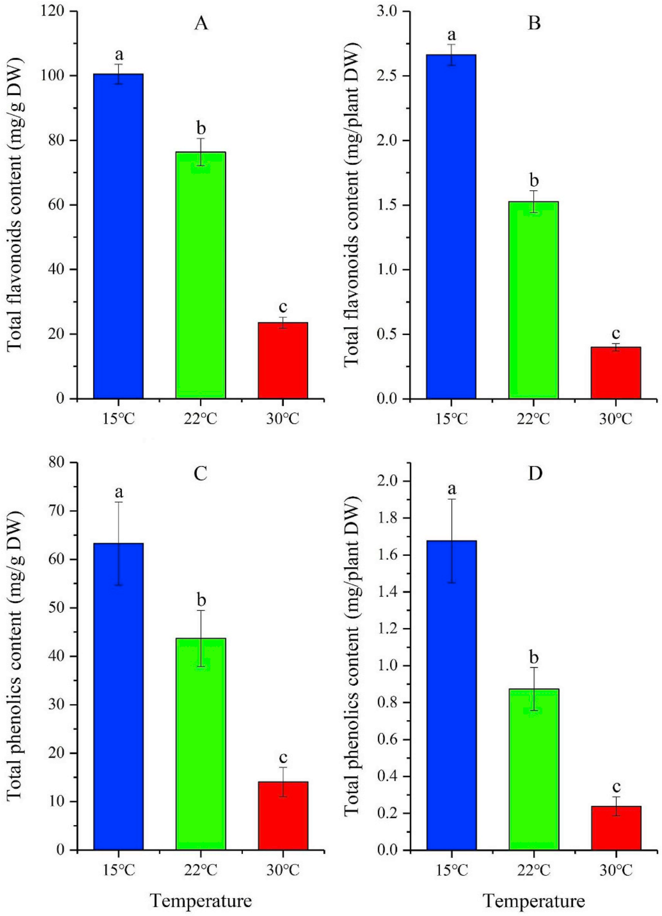

Fig. 4. Flavonoids and phenolics content in aerial parts of *H. perforatum* at different temperature. Images A, B and C, D respectively represent flavonoids and phenolics contents, on dry weight and per plant basis.

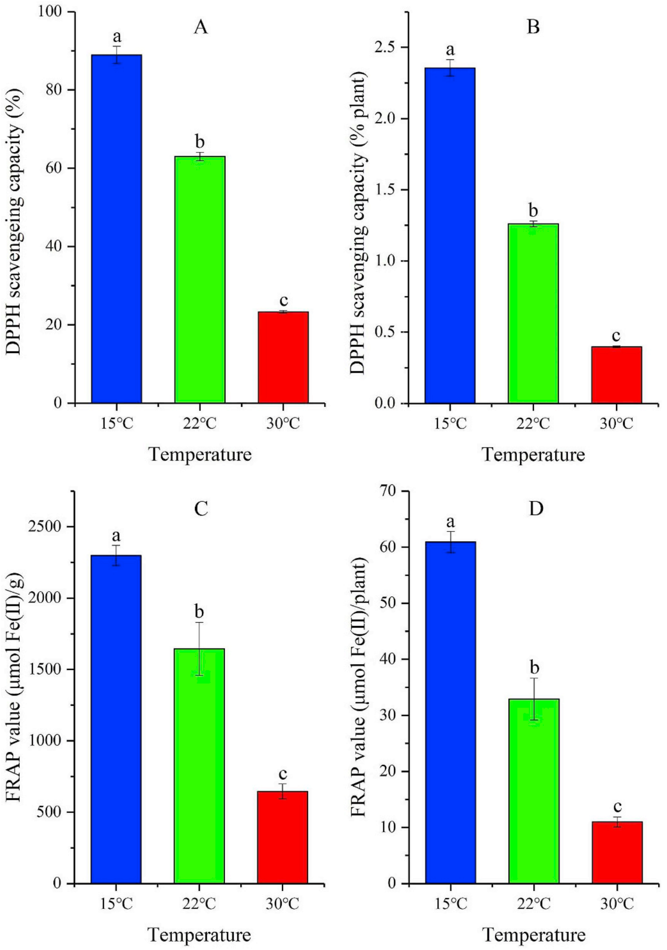

Fig. 5. Antioxidant capacity of extracts from aerial parts of *H. perforatum* at different temperatures, evaluated by DPPH (A and B) and FRAP (C and D) assays.

temperature tolerance (Zobayed et al., 2005; Skyba et al., 2012; Brunáková et al., 2015), meanwhile these responses to low temperature are associated with changes in the expression pattern of genes related to plant growth, oxidative stress and Hyp biosynthesis (Velada et al., 2014). In this study, we found that low temperature significantly enhanced the plant growth, accumulations of Hyp, flavonoids and phenolics as well as genes expression related to photosynthesis and Hyp biosynthesis in *H. perforatum*.

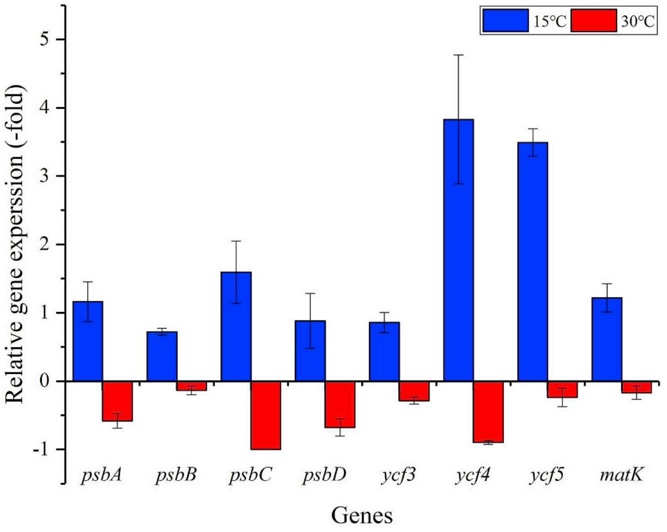

Fig. 6. The expression level of eight selected genes related to photosynthesis in aerial parts of *H. perforatum* at different temperatures, as determined by qRT-PTR. Histograms show the relative gene expression level at 15 and 30 °C compared to 22 °C. The same below.

Greater increase of aerial-parts biomass at low temperature (15 °C) compared to at 22 and 30 °C presents a similar trend as the contents of chlorophyll pigments (See Fig. 2 and Table 2). In large part because of chlorophyll pigments playing key roles in photosynthesis that successively convert light energy into chemical energy that is stored in carbohydrate molecules into biomass (Whitmarsh and Govindjee, 1999). In this study, the relative expression levels of eight selected genes that are closely related to photosynthetic proteins were enhanced up to 3.8-fold (*ycf4*) at 15 °C compared to at 22 and 30 °C (See Fig. 6). Four genes related to photosystem II (PSII) reaction include: *psbA* and *psbD* that encode D1 and D2 proteins, which are the center proteins of PSII via the donor of primary electron as well as several subsequent electron acceptors (Stoppel and Meurer, 2013; Kong et al., 2014); *psbB* that encodes PSII chlorophyll-binding protein CP47, which is closely attached to the *psbA/psbD* heterodimer and transfers excitation energy from the outer light-harvesting complexes (Stoppel and Meurer, 2013); *psbC* that encodes Chl α and β-Car binding proteins CP43, which serves as proximal antenna for PSII, providing a conduit for excitation energy transfer from the exterior antennae of the photosystem to the reaction center core, additionally, CP43 together with CP47 builds up the inner light-harvesting complex (Stoppel and Meurer, 2013; Liu et al., 2014). The four genes (*psbA*, *psbB*, *psbC* and *psbD*) response to environmental stresses (*e.g.* temperatures, drought and toxic) to improve

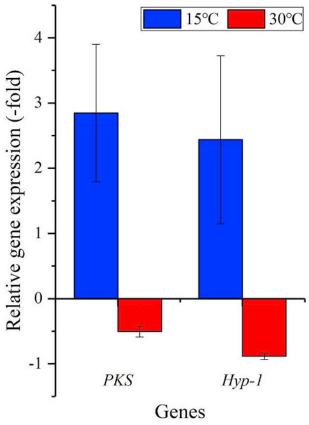

Fig. 7. The expression levels of *PKS* and *Hyp-1* related to Hyp biosynthesis in aerial parts of *H. perforatum* at different temperatures, as determined by qRT-PTR.

photosynthesis have been reported to be differentially expressed in many plants (Shao et al., 2010; Bi et al., 2016; Wang et al., 2018). The other four genes related to photosynthesis include: *ycf3* and *ycf4* that are essential for photosystem I (PSI) complex, the ycf3 protein that acts as a chaperone-like factor to guide the PSI subunits assemble (Albus et al., 2010; Nellaepalli et al., 2018); *ycf5* encoding a protein that is required during biogenesis of c-type cytochromes at the step of heme attachment (Tsuruyal et al., 2006); and *matK* that encodes an intron maturase, which contributes to chloroplast introns as splicing factor (Qu et al., 2018).

Increase in Hyp content is consistent with expression levels of *PKS* and *Hyp-1* genes in aerial parts at 15 °C compared to at 22 and 30 °C in the present investigation (See Figs. 3 and 7). As described in Fig. 1, polyketide synthase (PKS) encoded by the *PKS* gene catalyzes one acetyl-CoA and seven malonyl-CoA to form octa-β-ketoacyl chain; phenolic oxidative coupling protein (Hyp-1) encoded by the *Hyp-1* gene successively catalyzes emodin, emodin anthrone and protohypericin to finally form Hyp (Timsina et al., 2012; Karppinen et al., 2016; Sliwiak et al., 2016). The expression levels of *PKS* and *Hyp-1* at 15 and 30 °C in our present study are in accord with previous investigations that the *PKS* and *Hyp-1* were over-expressed at cold stress (4 °C) and down-expressed heat stress (35 °C) (Velada et al., 2014). Additionally, the PKSs involved in hyperforin, Hyp, flavonoid and xanthone biosyntheses are isobutyrophenone, octaketide, chalcone (CHS) and benzophenone (BPS) synthases, respectively (Beerhues, 2011). The over-accumulation of CHS and BPS in the mesophyll of leaves may play critical roles in flavonoid and xanthone biosyntheses in *H. perforatum* (Belkheir et al., 2016).

5. Conclusion

From the above investigations, low temperature enhances growth, Hyp biosynthesis and their related genes expression as well as accumulations of major bioactive compounds (flavonoids and phenolics) in *H. perforatum* seedling. The adaptation mechanism of this plant to low temperature will be examined via multi-omics (*e.g.* genomics, proteomics and metabolomics) in additional studies. These findings would provide a strong foundation for guiding cultivation at filed or greenhouse, as well as regulating Hyp biosynthesis in *H. perforatum*.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.plaphy.2019.04.012.

Author contributions

Y.Y. Yao, P. Sun and Z. Zhang performed the experiments and analyzed the data; T.L. Kang and Z.H. Liu contributed the materials; L. Jin and M.F. Li designed the experiments, wrote and revised the paper.

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