https://doi.org/10.48048/tis.2024.7373

Metabolomic and Growth Responses of *Houttyunia cordata* to Varying Light Intensities and Subsequent Effects on Fermented Products

Siriwat Boonchaisri[1,2,*], Chutima Mokoed[1], Phanumat Phuangchaona[1] and Ekawit Treenate[3]

[1]Division of Biology, School of Sciences, University of Phayao, Phayao 56000, Thailand
[2]Traditional Lanna Medicine Research Unit, School of Sciences, University of Phayao, Phayao 56000, Thailand
[3]Department of Chemistry, Faculty of Sciences, Maejo University, Chiang Mai 50200, Thailand

([*]Corresponding author's e-mail: siriwat.bo@up.ac.th)

Received: 7 August 2023, Revised: 23 August 2023, Accepted: 28 August 2023, Published: 30 January 2024

Abstract

Suitable light intensities were studied to enable sustainable and efficient cultivation of *Houttuynia cordata* with a high yield of bioactive compounds which later can be a good quality material for fermentation. To achieve this goal, the evaluation of physiological adaptation and elucidation of the metabolic alterations of *Houttuynia cordata* in response to different light intensities were conducted. Four-month-old *H. cordata* was exposed to 3 different light intensities: high light, control, and low light (100, 50 and 20 % natural irradiances, respectively). Physiological responses were measured using leaf gas exchange. The same leaf samples were either methanolic extracted for LC-QTOF-MS analysis to generate metabolite fingerprints or fermented for 3 months prior to quercetin quantification. High light treatment had the greatest effects on both the physiology and metabolisms of *H. cordata* compared to control and low light treatments. Compared to the control, the key results observed in high-light-grown *H. cordata* were a significant increase in photosynthesis, a decrease in relative chlorophyll contents, the enrichment of the top 3 metabolic pathways (phenylpropanoid biosynthesis, pyruvate metabolism and fatty acid degradation) and increasing quercetin concentrations in both fresh and fermented leaves. Metabolite fingerprints of high light-grown *H. cordata* were distinctively different from those of the other 2 treatments according to PCA and hierarchical clustering analysis. In conclusion, *H. cordata* responded to high light intensity by enriching the phenylpropanoid biosynthetic pathway to elevate sunscreen pigments. Additional acetyl-CoA generated from pyruvate metabolism and fatty acid degradation was a key supporting factor in the above response. As high quercetin concentration was observed in both fresh and fermented leaves, this will lead to quality improvement of the fermented products.

Keywords: Cultivation, Light intensities, Herbs, Metabolomics, Physiology, Fish mint, *H. cordata*

Introduction

Houttuynia cordata Thunb. is a perennial herbaceous plant with heart-shaped leaves and slender creeping stems. It is native to Southeast Asia [1] and has been recognized in folk medicine for treating several diseases such as lung abscess, phlegm, dysentery, enteritis and now cancers [2]. The phytochemical compositions of *H. cordata* extract comprised of alkaloids, flavonoids, fatty acids, polyphenolic acids, sterols and volatile oils [3]. Reported pharmacological activities of *H. cordata* include anti-hypertension, anti-edema, detoxicant, anti-pyretic, diuretic activities, anti-cancer, anti-diabetics and immunostimulating activity. *H. cordata* extract also demonstrated anti-viral activity against SARS-CoV2 infection with IC_{50} of 0.98 mg/mL [4]. Results from molecular docking and network pharmacology showed promising inhibitory effects of compounds from *H.cordata* on RNA-dependent RNA polymerases (RdRps) of SARS-CoV2 [5].

Due to pharmacological properties of *H. cordata*, a range of dietary supplements have been commercially invented. These products have a high potential for usage in the healthcare industry. The fermentation of *H. cordata* aboveground parts using natural microbes have substantially augmented the abundance of various flavonoid components in *H. cordata* [6] and significantly improved several diabetes-related symptoms such as reduced blood glucose levels and alleviated oxidative stress induced by inflammation [7]. Increased demand for raw materials is expected but the available resources of *H. cordata* may not be sufficient to satisfy the market needs. Therefore, standard operating procedures for suitable cultivation of *H. cordata* should be developed in accordance with Good Agricultural Practice guidelines

(GAP) to enable sustainable and efficient cultivation of *H.cordata* with a high yield of bioactive compounds which later can be a good quality material for fermentation.

Light is one of the factors that determine plant growth and development. In general, growers attempt to imitate the sciophilous habit of *H. cordata* by growing the plants under low light intensity, either using shading screens or lending cover from other tree canopies. However, decreasing light intensities from 100 to 40 % of full sunlight resulted in an approximately 10 % increase in aboveground biomass, but it came at the expense of a 20 - 50 % reduction in flavonoid content (especially the 3 key flavonoids: Quercetin, quercitrin and rutin) and decreased abundances of several volatile oils. Besides, the light intensities as low as 20 % of natural light caused almost all measurable parameters (biomass, flavonoid content, essential oil concentrations and nutritional components) to decrease [8]. Therefore, it is important to optimize the light intensity to achieve appropriate cultivation conditions.

With the advancement of current analytical instruments, an emerging technology, namely, metabolomics becomes a powerful tool to rebuild metabolic networks that allows us to gain the deepest insight into the global plant metabolome [9].

Therefore, this project will be the first report attempting to perform a comprehensive analysis of global metabolites in *H. cordata* to elucidate the mechanisms of light response in this herbal plant when most previous literature only reported the responsive outcomes of the selected treatments without investigating the underlying mechanisms. Moreover, we also explored a possible connection between the effects of lighting conditions and the qualities of the fermented products which had limited information in the literature. At present, the biotransformation of *H. cordata* through fermentation processes has been adopted by several manufacturers in Northern Thailand. These fermented products are claimed by them to confer greater health advantages than the raw materials, owing to the activities of microorganisms that dismantle plant structures to access soluble nutrients for their growth, consequently releasing a multitude of valuable by-products. Finally, basic physiological measurements: leaf gas exchange, chlorophyll *a* fluorescence and relative chlorophyll contents were also carried out the findings from this project provide basic knowledge which could further help to enhance the qualities of the fermented products by improving cultivation technique with respect to plant shading.

Materials and methods

Plant material and light treatments

H. cordata Thub. plants were generously provided by Rincome Group Company Limited, Doi Saket District, Chiang Mai Province. Seedlings were propagated from rhizomes in 20×10×10 cm^3 potting bags filled-up with an equal mixture of silt soil and cow dung. Once seedlings emerged, they were kept under a weak illumination and watered every 2 days. When the seedlings reached a 2-leaf stage, 90 uniform seedlings were transferred to a greenhouse to receive different light treatments. Although all plants were exposed to natural sunlight, they were randomly divided into 3 groups based on the extent of shading resulting from the placement of a black high-density polyethylene netting positioned 1 m above their canopies. This arrangement aimed to create 3 distinct light intensities for the treatments. The first group (control) experienced 50 % of the sunlight intensity (mimicking *H. cordata*'s sciophilous nature [10]), the high light group received full sunlight intensity, and the low light group received 20 % of the natural light intensity. The 50 and 20 % natural irradiance were achieved by installing 1 and 2 layers of the net, respectively, while the full intensity of natural light was attained by growing the plants without netting cover [8]. The plants were grown to reach maturity under selected light treatments for 4 months when they were measured (growth and physiology) and then harvested either for Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry (LC-QTOF-MS) analysis or transformation to fermented products.

Growth and physiological measurements

Plant height was measured by a measuring tape and leaf number was manually counted by a researcher. Relative chlorophyll concentrations were a good indication of chlorophyll content and were measured, using Chlorophyll Meter SPAD-502Plus (Konica Minolta Sensing Singapore Pte Ltd., Singapore) Measurements were taken at the base, middle and near leaf tip and then averaged for each individual leaf [11]. Photosynthetic rate (P_N) was measured, using a portable gas exchange system (LC Pro-SD, ADC BioScientific Ltd., Herts, UK) at a constant PPFD at 1,200 μmol/m^2/s illuminated by a built-in LED panel [12]. All growth and physiological measurements were randomly conducted on 10 different plants for each treatment so that a total sample was 30.

Leaf harvest and sample extraction

The youngest fully expanded leaves (60 days after plant) were harvested from each plant with 4 biological replicates per treatment. Harvested samples were immediately frozen in liquid nitrogen to cease biochemical reactions following by a storage at −80 °C until further analysis. The samples were freeze-dried, weighed (approximately 70 g each) and extracted in 2 mL of 50 % v/v of methanol, using a ball mill (SpeedMill Plus, Analytik Jena, Jena, Germany) [9]. After the extracts were vortexed for 1 min and centrifuged at 15.00 rpm for 15 min, clear supernants were transferred to fresh microtubes and further filtered through a 0.2 µm Nylon syringe filter (Chrom Tech, Inc., MN, USA) prior to injection to LC-QTOF-MS.

Fermentation of *H. cordata*

Aboveground portions of approximately 2,000 g were harvested, washed with milli-Q water and air-dried for 6 h. The sampling materials were mixed with 10 mL of microorganism seeds commercially used by the above manufacturer and 1 L of 10 % w/v sucrose solution in an airtight container. Fermentation was carried out in 4 replicates per treatment. Fermentation was allowed to ripen for 3 months before the fermented leaves approximately 70 g/sample were harvested for LC-QTOF-MS analysis according to the above-mentioned method.

Liquid chromatography-quadrupole time of flight-mass spectrometry-based metabolomics

The methanolic extracts from either fresh or fermented leaves were prepared as mentioned above. The analyses were conducted similarly to the method described in [9] with a slight modification. Five microliters of the sample were injected in an LC-QTOF-MS system consisting of an Agilent 1,100 series HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a ZORBAX Eclipse plus column (3.5 µm, 150×4.6 mm^2 i.d.) (Agilent Technologies, Palo Alto, CA). The chromatographic separation was operated at 35 °C and a flow rate of 0.3 mL/min. The mobile phase consisted of solvent A (milli-Q water) and solvent B (acetonitrile) both acidified with 0.1 % v/v formic acid. The elution program started from 98:2 solvent A:Solvent B, linearly increased to 0:100 solvent A:Solvent B within 18 min and finally hold at this gradient for 2 min. MS analysis was conducted, using a PE SCIEX API 4,000 triple quadrupole tandem mass spectrometer (Applied Biosystem, Foster city, CA) coupled with electrospray ionization interface (ESI). The MS was operated in a negative electrospray ionization mode and analytes were scanned for the masses from 50 to 1,000 amu. The analysis was performed with 4 biological replications for either fresh or fermented samples. Since the amount of quercetin was regarded as a key commercial marker to represent *H. cordata* quality, the standard solution of authentic quercetin was prepared. Six different concentrations of quercetin were prepared to obtain calibration curves to aid in the quantification of quercetin contents presented in *H. cordata* extracts.

Data processing

Data of mass spectra were pre-processed by a free online software XCMS online in order to align of every mass signal across all the samples. Normalization of each peak intensity within the mass spectra was performed with sample dry weight, resulting in a multi-dimensional data matrix which included sample information, retention time (RT), mass-to-charge ratio (m/z; represent 'metabolites') and the normalized peak intensity. The differential metabolites that contributed to grouping separation were putatively identified in combination with public databases such as METLIN (http://www.metlin.scipps.edu/). Finally, the enriched pathways altered by the light treatments were evaluated, using a mummichog-based pathway analysis [13]. Any significant differences in growth and physiological parameters among the 3 light intensities were tested with one-way ANOVA, using SPSS version 26.0. Tukey's post hoc test was subsequently applied to test the significant differences between each pair of the treatment at $p < 0.05$.

Results and discussion

Effects of light intensities on growth and physiology of *H. cordata* plants

After *H. cordata* were grown from seedlings to maturity for 3 months, it is clear that different light intensities had no effects on plant height (**Figure 1(A)**) but decreasing light intensity resulted in significantly fewer numbers of leaves per plant (**Figure 1(B)**). The constant height (13.70 - 15.31 cm) of *H. cordata* grown under different light intensities was not agreed with previous studies which reported a negative correlation between plant height and light intensities in several plants [14]. Our results suggested that light intensity had very limited effects on the cell elongation of *H. cordata*. Along with this notion, a previous researcher [15] recognized a gradual decrease in sensitivity to light for cell elongation in *Vicia*

faba when the plants began aging. Presumably, *H. cordata* at 8 - 10 leaf stage in this study was not sensitive enough to discriminate different light treatments and thus demonstrated a similar degree of cell elongation.

As previously noted, the leaf count decreased with diminishing light intensity. Specifically, *H. cordata* plants cultivated under high light, control light, and low light conditions exhibited leaf counts of 10.20, 8.20 and 5.10 leaves/plant, respectively. This result agreed well with many previous experiments that reported fewer numbers of leaves in several perennial plant species similar to *H. cordata* when they were grown under relatively low light conditions (such as *Lactuca sativa* L. [16] and *Salvia offinalis* L. [17]). Evidence from a study from rose leaves revealed the interactions between light intensities and a complex hormonal regulation between 2 antagonistic hormones: A growth-stimulating hormone, cytokinin (CK) and an inhibitory hormone, abscisic acids (ABA) in controlling auxiliary bud outgrowth. It appears that low light intensity induced a down-regulation of genes encoding CK degradation (RhCKX1) but reduce expression of genes encoding CK synthesis, transport and signalling [18]. In contrast, relatively low light intensity (89 µmol/m^2/s) not only caused a significant increase in ABA concentration in rose leaves but also demolished CK stimulatory effects on bud outgrowth leading to a lesser number of leaves [19].

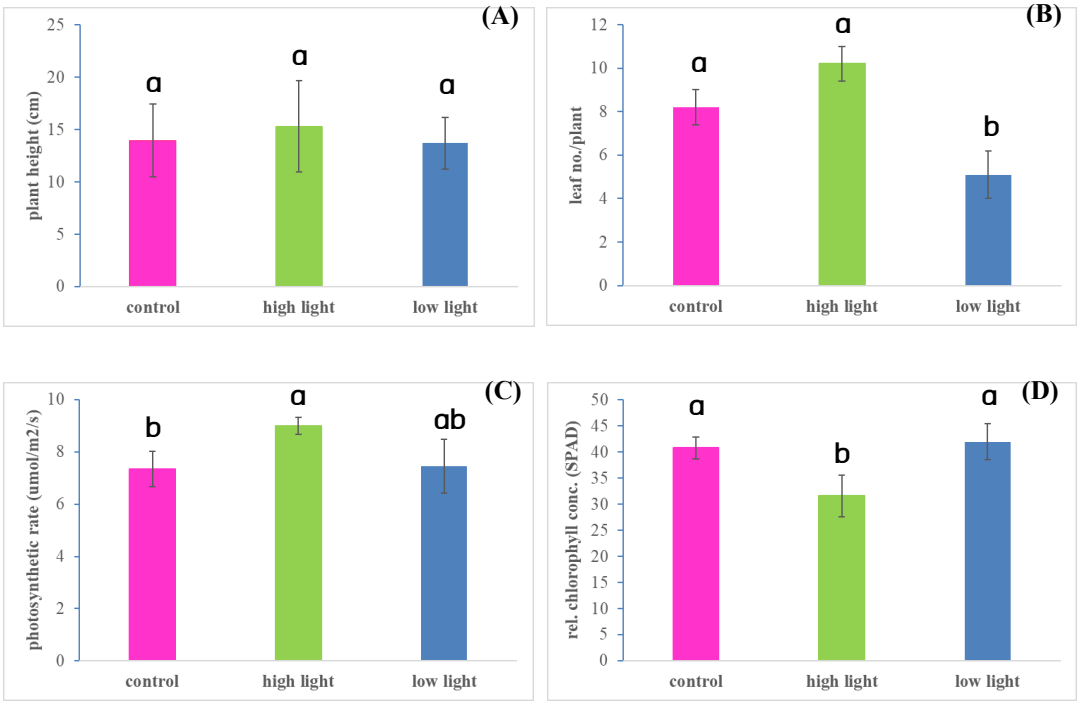

Figure 1 Physiology and growth of *H. cordata* grown under different light treatments. (A) plant height; (B) leaf numbers; (C) photosynthetic rate (P_N), and (D) relative chlorophyll concentrations. Data shown as mean ± SD of 10 replications. Different lowercase letters indicate significant ($p < 0.05$) differences among means.

As shown by [17], there was a direct association between the number of leaves and plant biomass. They pointed out that a greater number of leaves, in turn, represents a greater accumulation of net photosynthetic products which are a major contributor to plant structures. In the present study, *H. cordata* grown under high light treatment showed the highest photosynthetic rate (P_N) (8.99 µmol/m^2/s) (**Figure 1(C)**) compared to those under control and low light treatments (7.34 and 7.44 µmol/m^2/s, respectively). Interestingly, the highest P_N induced by high light treatment corresponded well with the observable highest number of leaves and the tendency of greatest aboveground biomass at this specific light treatment (data not shown). For the relative chlorophyll concentration, the highest value of 41.93 units was measured from *H. cordata* grown under low-light treatment (**Figure 1(D)**). The concentrations decreased with increasing light intensities from control to high light treatment (40.81 and 31.58 units, respectively). Reducing chlorophyll content in response to increasing light intensity was well-documented as a result of chlorophyll degradation in several plant species such as *Anoectochilus formosanus* [20] and *Salvia officinalis* L. [21],

both were medicinal plants. The degradation of chlorophyll contents was considered an efficient method for plants to reduce light absorption and thus lower the damaging effects of photooxidation [18].

Taken together, the strategies of *H. cordata* to cope with different light intensities was by modulating chlorophyll contents to attain appropriate light absorption so that a good balance between photodamage and photosynthesis could be achieved. Once prevented from a severe photo stress, *H. cordata* seemed to benefit from a high light-stimulated photosynthetic dark reaction by slightly promoting leaf formation (leaf number) and plant biomass.

Metabolomics responses of *H. cordata* to different light intensities

Univariate and multivariate analyses

A total of 427 mass peaks from LC-ESI-QTOF-MS were annotated to generate a metabolites fingerprint. Statistically significant differences between light treatments were calculated, using one-way ANOVA, by taking abundances (the normalized peak intensities) of each metabolite as input. Several significantly different metabolites were detected and the top 15 metabolites (15 smallest *p*-value) were selected for putative identification (**Table 1**). A collective comparison of the top 15 metabolites was conducted and visualized by heatmap so that the effects of different light intensities on the responsive metabolites could be easily understood. A careful observation discovered that an alteration in abundances of the top 15 metabolites of *H. cordata* grown under the high light treatment was so distinct from the other 2 light treatments (**Figure 2(A)**).

Table 1 Characteristics of the top 15 significantly different metabolites between 3 light treatments: Control, high light and low light.

No.	Metabolites	m/z	RT (min)	Types*	*p*-value
1	L-Gluconic acid	195.0471	3.66	O	0.0037
2	Aesculin	339.0701	3.82	P	0.0020
3	cis-Zeatin-7-N-glucoside	380.1463	8.88	P	0.0037
4	Epicatechin-3-gallate	441.0823	3.99	P	0.0045
5	Glucohesperalin	464.0861	10.78	O	0.0008
6	Isorhamnetin 7-glucoside	477.0956	11.23	P	0.0001
7	6-Caffeoylsucrose	503.1310	10.99	P	0.0019
8	Oxidized glutathione	611.1402	10.41	O	0.0024
9	p-Coumaroyl vitisin A	705.1517	9.05	P	0.0002
10	PG(20:4/16:1)	767.4846	11.03	F	0.0037
11	PE(20:3(6,8,11)-OH(5) /22:2(13Z,16Z)	836.5734	11.22	F	0.0002
12	PS(20:3(6,8,11)-OH(5) /22:2(13Z,16Z)	880.5651	11.21	F	0.0001
13	Cinnamoyl-CoA	896.1808	11.28	P	0.0014
14	Oolonghomobisflavan A	927.1705	10.78	P	0.0001
15	3-Oxooctanoyl-CoA	928.1755	10.78	F	0.0001

*Type of metabolites: F = metabolites involved with fatty acid metabolism, P = metabolites derived from phenylpropanoid pathway and O = others.

Interestingly, the 15 putative metabolites seemed to belong to 2 major categories: 1) The metabolites derived from the general phenylpropanoid pathway and 2) Those involved with fatty acids (**Table 1**). Detailed analysis of heatmap demonstrated that the abundances of 3 glycerophospholipids (phophatidylglycerol (PG(20:4/16:1)), phophatidylethanolamine (PE(20:3(6,8,11)-OH(5)/22:2(13Z,16Z)) and phophatidylserine [PS(20:3(6,8,11)-OH(5)/22:2(13Z,16Z)]) were clearly decreased in *H. cordata* grown under high light treatment whereas substantial accumulation of 5 phenylpropanoid related metabolites (such as p-coumaroyl vitisin A, cinnamoyl-CoA and epicatechin-3-gallate) as well as 3 other metabolites (gluconic acid, glucohesperalin and oxidized glutathione) were instead induced by the high

light treatment (**Figure 2(A)**). Owing to its distinct abundance pattern, *H. cordata* cultivated under the high light treatment formed a separate cluster, clearly distinguished from those grown under control and low light treatments (see the horizonal linkage lines above heatmap, **Figure 2(A)**). The input variables were expanded to include all 427 metabolites for Principal Component Analysis (PCA), a similar grouping persisted, wherein the high light treatment remained distinctly separated from the other treatments. (**Figures 2(B) - 2(C)**). The similarity between the outcomes obtained from 2 different analyzing methods strongly confirmed that high light intensity had the greatest effects on the metabolism of *H. cordata* compared to those exposed to control and low light treatments. Our results were consistent with previous findings [8].

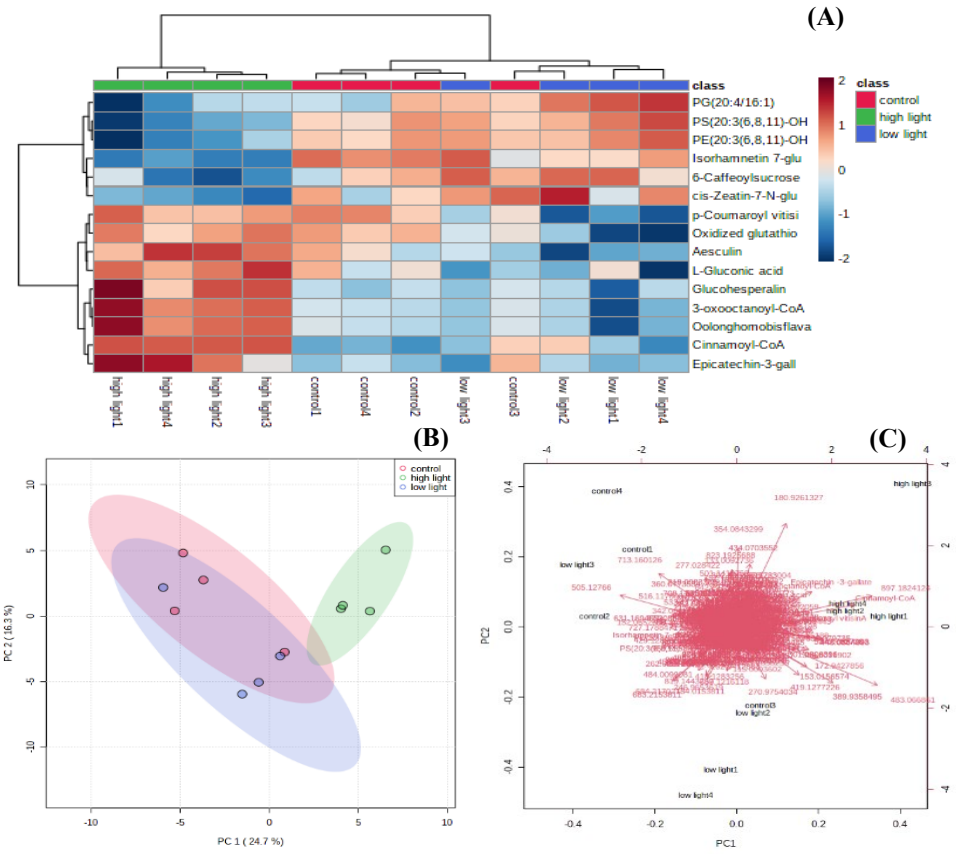

Figure 2 Multivariate analysis of metabolite fingerprints of *H. cordata* grown under different light treatments. (A) heatmap comparing the relative concentrations of the putatively identified metabolites that are significantly different (FDR < 0.05) between 3 light intensity treatments. Rows represent putative metabolites and columns represent an individual sample. Color scale indicates the relative concentrations based on the mean of each significantly different metabolite; (B) PCA score plots demonstrated sample classification; (C) Biplot concurrently exhibited the coordination of both sample classification and all loading metabolites.

Alteration in metabolisms of *H. cordata* induced by a high light intensity

Pathway analysis was performed based on a pair-wise comparison between metabolite fingerprints obtained from control and high light treatment (control vs. high light) to identify the metabolic pathways affected by the light treatments. It should be noted that pathway analysis of control in comparison to the low light treatment (control vs. low light) was not performed as the metabolite fingerprints acquired from both treatments were not significantly different (**Figure 2**). The outcome of pathway analysis showed that eleven pathways were altered as a result of high light intensity. However, based on small *p*-value and large enrichment factor, there were top 3 enriched pathways that were distinguished from the others; namely: Phenylpropanoid biosynthesis followed by pyruvate metabolism and fatty acid degradation, respectively (**Figure 3**).

The enrichment of phenylpropanoid biosynthesis induced by high light intensities was consistent with a number of previous studies. Up-regulation of phenylpropanoid biosynthesis is considered a hallmark

response of several plants to acclimatize to high-light environments [22]. The study by [23] found that many phenylpropanoid genes of Arabidopsis were highly expressed when grown under high light intensity and the observable up-regulation was highly correlated with significant accumulations of coniferin, syringin as well as several flavonoids. It is possible that significant accumulations of the 5 phenylpropanoid related metabolites observed in this project (**Figure 2(A)** and **Table 1**) were a consequence of increasing phenylpropanoid gene expression in response to high light treatment so that a greater extent of protective pigments would be produced.

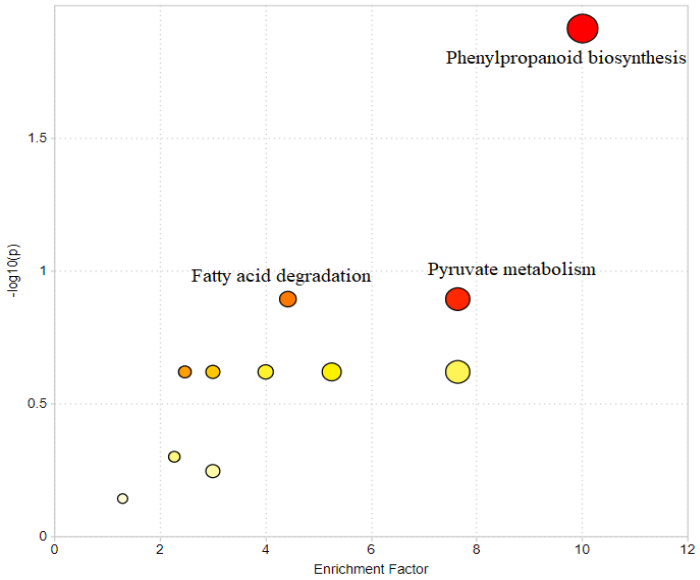

Figure 3 Summary of pathway analysis showing the enriched pathways in fresh leaves of *H. cordata* exposed to control vs. high light treatment. Pathways are plotted according to significance (y-axis) and enrichment factor (x-axis). Larger circles represent greater pathway enrichment and darker colors represent higher significant levels. The most contributing pathways to a metabolic alteration coordinate toward the top right corner.

Enhancement in pyruvate metabolism and fatty acid degradation suggested that acetyl-CoA was on increasing demand in the high-light-grown *H. cordata* because both metabolic pathways produce acetyl-CoA as a chief product. Theoretically, the increase in acetyl-CoA utilization agreed well with the enriched phenylpropanoid biosynthesis. This is because acetyl-CoA is required to form malonyl-CoA which then reacts with a central intermediate of a general phenylpropanoid pathway, 4-Coumaryl-CoA to form a core structure of phenylpropanoid subsequent products, flavonoids. Along with our hypothesis, the successful promotion of flavonoid (2S)-naringenin in *E. coli* by biosensor-based metabolic engineering demonstrated that this was achieved by the coordinated regulation of metabolic flux leading to a simultaneous increase in malonyl-CoA concentration and acetyl-CoA levels. [24]. However, the enriched phenylpropanoid biosynthesis will inevitably lead to increasing synthesis of the amino acid, phenylalanine, an initial precursor of the pathway. To fulfill this requirement, an upstream precursor of acetyl-CoA production, phosphoenolpyruvate will be diverted to produce phenylalanine. Therefore, an additional source of acetyl-CoA generated from β-oxidation of fatty acid degradation becomes essential to maintain a high rate of phenylpropanoid biosynthesis. Furthermore, the up-regulation of fatty acid degradation could explain a significant reduction in the 3 glycerophospholipids observed in the high-light-grown *H. cordata* (**Figure 2(A)**). β-oxidation of fatty acid degradation and biosynthesis of glycerophospholipids basically compete for a common precursor, phophatidic acid (PA). On one hand, PA was an immediate precursor of PG and PE formation catalyzed by phosphatidylglycerol phosphatase and ethanolaminephosphotransferase, respectively. Subsequent interconversion of PE resulted in PS and *vice versa* [25]. On the other hand, PA via diacylglycerol (DAG) intermediate will form triacylglycerol (TAG). The latter is one of the major sources of free fatty acids to feed the fatty acid degradation [26]. A schematic diagram depicting the interrelationship between the top 3 enriched pathways and the corresponding consequences on the differential metabolites is shown in **Figure 4**.

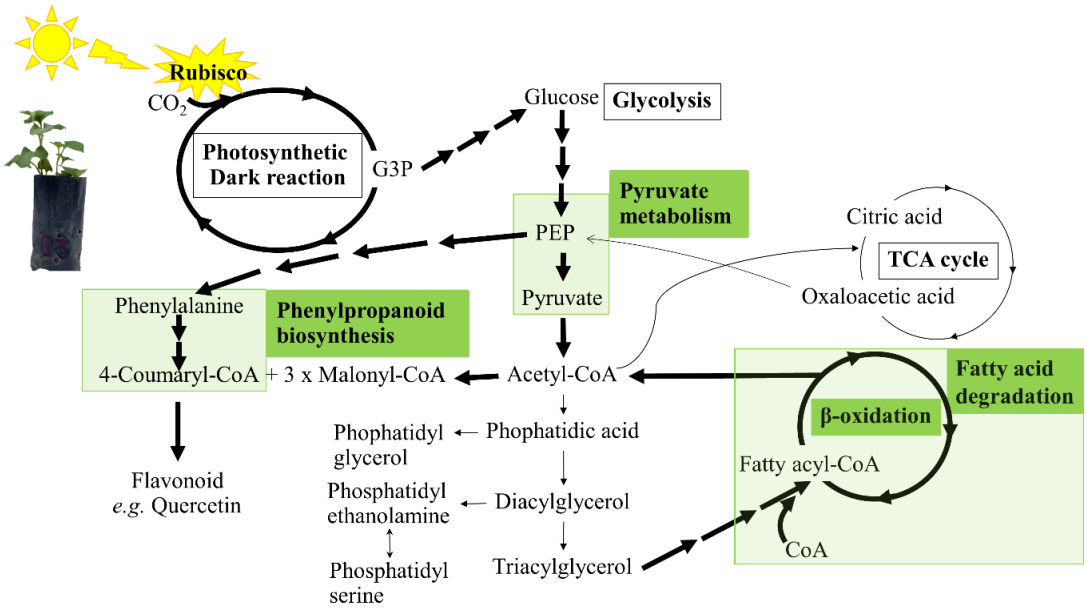

Figure 4 A schematic diagram depicting the interrelationship between the top 3 enriched pathways.
Note that: Bold arrows = direction of metabolic fluxes induced by high light treatment, white boxes = general metabolic pathways and green boxes = the top 3 enriched pathways up-regulated by high light treatment. The diagram was created based on [27].

Influence of different light intensities on qualities of fermented *H. cordata*

In the market, the quality of fermented *H. cordata* was determined from quercetin contents. Moreover, the quality of fermented products was expected to be influenced by the composition of raw materials. A good example of this phenomenon is observed in the Chinese liquor fermentation process, where the use of 2 distinct barley cultivars (*heilaoya* and *dulihuang*) resulted in varying flavor profiles. This serves as a compelling example of how the initial ingredients can effectively regulate the characteristics of fermented foods [28]. Therefore, the concentration of quercetin was evaluated from either fresh or fermented leaves to reveal the influence of different light intensities on the qualities of the starting materials and the fermented products, respectively. In fresh leaves, the high light treatment stimulated a significantly higher ($p < 0.05$) quercetin concentration (40.03 mg/kg) compared to the control and low light treatments (11.03 and 14.86 mg/kg, respectively) (**Table 2**). Increasing quercetin concentration was speculated to be a result of the enriched phenylpropanoid biosynthesis as we already discussed above and that led to the up-regulation of flavonoid biosynthesis. Similarly, an outstanding accumulation of quercetin in the fermented leaves was also induced by high light intensity, leading to a concentration as high as 1,063.24 mg/kg which was 2 folds higher than those found in the control and low light treatments (577.28 and 469.90 mg/kg, respectively) (**Table 2**). Consistent characteristics of both fresh and fermented leaves in response to different light intensities underscored a robust correlation between the original concentration of quercetin in the raw materials and the resultant concentrations found in fermented *H. cordata*.

When the fresh and fermented samples were compared, quercetin concentrations of fermented leaves were approximately 50 times higher than that measured from the fresh leaves. This phenomenon was likely a result of a plant structural breakdown caused by the actions of microorganisms to obtain the soluble and fermentable fiber for their growth. Subsequently, several phenolic compounds which mechanically bound to cell walls were unleased and then converted to quercetin. This hypothesis was supported by previous findings by [29] who detected significant increases in α-amylase, β-glucosidase and xylanase content during the fermentation of maize grain, indicating increasing hydrolytic activities of natural microorganisms in the fermentation process. Furthermore, a recent review [30] firmly pointed out that tricin is unlikely the only flavonoid to incorporate into the lignin polymer of the cell wall, but the incorporation of other flavonoids including quercetin, is highly possible.

Table 2 Quercetin concentrations in fresh and fermented leaves from *H. cordata* grown under different light intensities.

Fresh leaves		**quercetin (mg/kg DW*)**		**SD**
Treatment	control	11.03[a]	±	4.09
	high light	40.03[b]	±	14.68
	low light	14.86[a]	±	5.81
Fermented leaves		**quercetin (mg/kg DW)**		**SD**
Treatment	control	577.28[a]	±	56.04
	high light	1,063.24[b]	±	220.33
	low light	469.90[a]	±	90.19

Values (mean ± SD of 10 replications) within a column of each sample type superscripted with different lowercase letters are significantly ($p < 0.05$) different.
*mg/kg DW = milligram of quercetin per kilogram of sample dry weight

In summary, it is clear that *H. cordata* could grow in a broad range of light intensities without serious alterations in its morphology. Unchanged characteristics of the plants across all light treatments imply that a significant portion of the increased photosynthates induced by the high light treatment was channeled towards the biosynthesis of secondary metabolites (as evidenced by the up-regulation of phenylpropanoid biosynthesis) rather than being allocated to the production of primary metabolites, which form the fundamental building blocks of plant structures. Therefore, a greater amount of sunscreen pigments together with several bioactive compounds including quercetin were produced in response to high-light treatment. Enrichment of pyruvate metabolism and fatty acid degradation served as an additional source of acetyl-CoA to support the elevated phenylpropanoid biosynthetic pathway.

Based on our findings, a full natural irradiance is recommended for manufacturers who aim to cultivate *H. cordata* for making high-quality fermented products. In fact, our recommendation is counterintuitive because growers generally never cultivate *H. cordata* at such intense levels of irradiance due to the sciophilous nature of the plant. Our views agree with Li *et al.* [8] who proposed that appropriate light intensities for growing *H. cordata* could be either low or high depending on the purpose of growers, whether the cultivation aims to produce a rich source of food nutrition or to stimulate a variety of bioactive compounds, respectively.

Conclusions

This study provides valuable insights into the metabolomic responses and growth characteristics based on the length, the number of leaves, photosynthesis, and chlorophyll contents of *H. cordata* under different light intensities. The findings suggest that increased photosynthesis and enhancement of 3 metabolic pathways stimulated by high light intensity during cultivation modulate the metabolite composition of *H. cordata*. Furthermore, these results emphasize that optimizing light conditions during cultivation increased quercetin concentration which resulted in an increase of phytochemicals when *H. cordata* is being fermented. Further research in this area can aid in the commercial production of high-quality *H. cordata* products.

Acknowledgements

This research project was supported by the Thailand Science Research and Innovation Fund and the University of Phayao, Thailand to S.B (Grant No. FF64-RIM016).

References

[1] K Oginuma, H Sato, Y Kono, S Chen and H Setoguchi. Intraspecific polyploidy of *Houttuynia cordata* and evolution of chromosome number in the *Saururaceae*. *Chromosome Bot.* 2007; **2**, 87-91.
[2] M Kumar, SK Prasad and S Hemalatha. A current update on the phytopharmacological aspects of *Houttuynia cordata* Thunb. *Phcog. Rev.* 2014; **8**, 22-35.
[3] J Fu, L Dai, Z Lin and H Lu. *Houttuynia cordata* Thunb: A review of phytochemistry and pharmacology and quality control. *Chin. Med.* 2013; **4**, 101-23.

[4] KH Chiow, MC Phoon, T Putti, BK Tan and VT Chow. Evaluation of antiviral activities of *Houttuynia cordata* Thunb. extract, quercetin, quercetrin and cinanserin on murine coronavirus and dengue virus infection. *Asian Pac. J. Trop. Med.* 2016; **9**, 1-7.
[5] AB Gurung, MA Ali, J Lee, MA Farah, KM Al-Anazi and F Al-Hemaid. Identification of SARS CoV-2 inhibitors from extracts of *Houttuynia cordata* Thunb. *Saudi J. Biol. Sci.* 2021; **28**, 7517-27.
[6] RH Kwon and BJ Ha. Increased flavonoid compounds from fermented *Houttuynia cordata* using isolated six of *Bacillus* from traditionally fermented *Houttuynia cordata*. *Toxicol. Res.* 2012; **28**, 117-22.
[7] W Sakuludomkan, R Yeewa, S Subhawa, C Khanaree, AI Bonness and T Chewonarin. Effects of fermented *Houttuynia cordata* Thunb. on diabetic rats induced by a high-fat diet with streptozotocin and on insulin resistance in 3T3-L1 adipocytes. *J. Nutr. Metabol.*2021; **2021**, 6936025.
[8] A Li, S Li, X Wu, H Lu, M Huang, R Gu, L Wei and A He. Influence of light intensity on the yield and quality of *Houttuynia cordata*. *Plant Prod. Sci.* 2015; **18**, 522-8.
[9] S Boonchaisri, S Rochfort, T Stevenson and DA Dias. LC-MS untargeted metabolomics assesses the delayed response of glufosinate treatment of transgenic glufosinate resistant (GR) buffalo grasses (*Stenotaphrum secundatum* L.). *Metabolomics* 2021; **17**, 28.
[10] RJV Alves, D Medeiros, RL Moura, LCT Gusmao, NG Silva, K Antunes, C Matozinhos and MA Blanco. First report of naturalization of *Houttuynia cordata* Thunb. 1783 (Piperales:Saururaceae) in South America. *Check List* 2013; **9**, 642-4.
[11] J Uddling, J Gelang-Alfredsson, K Piikki and H Pleijel. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynth Res.* 2007; **91**, 37-46.
[12] MD Sarkar, MJ Rahman, J Uddain, M Quamruzzaman, MOK Azad, MH Rahman, MJ Islam, MS Rahman, KY Choi and MT Naznin. Estimation of yield, photosynthetic rate, biochemical, and nutritional content of red leaf lettuce (*Lactuca sativa* L.) grown in organic substrates. *Plants* 2021; **10**, 1220.
[13] S Li, Y Park, S Duraisingham, FH Strobel, N Khan, QA Soltow, DP Jones and B Pulendran. Predicting network activity from high throughput metabolomics. *PLoS Comput. Biol.* 2013; **9**, e1003123.
[14] W Oh and KS Kim. Light intensity and temperature regulate petiole elongation by controlling the content of and sensitivity to gibberellin in *Cyclamen persicum*. *Horticulture Environ. Biotechnol.* 2014; **55**, 175-82.
[15] RD Butler. The effect of light intensity on stem and leaf growth in broad bean seedlings. *J. Exp. Bot.* 1963; **14**, 142-52.
[16] JH Kang, SK Kumar, SLS Atulba, BR Jeong and SJ Hwang. Light intensity and photoperiod influence the growth and development of hydroponically grown leaf lettuce in a closed-type plant factory system. *Horticulture Environ. Biotechnol.* 2013; **54**, 501-9.
[17] G Zervoudakis, G Salahas, G Kaspiris and E Konstantopoulou. Influence of light intensity on growth and physiological characteristics of common sage (*Salvia officinalis* L.). *Braz. Arch. Biol. Tech.* 2012; **55**, 89-95.
[18] H Roman, T Girault, F Barbier, T Péron, N Brouard, A Pěnčík, O Novák, A Vian, S Sakr, L Lothier, JL Gourrierec and N Leduc. Cytokinins are initial targets of light in the control of bud outgrowth. *Plant Physiol.* 2016; **172**, 489-509.
[19] A Corot, H Roman, O Douillet, H Autret, MD Perez-Garcia, S Citerne, J Bertheloot, S Sakr, N Leduc and S Demotes-Mainard. Cytokinins and abscisic acid act antagonistically in the regulation of the bud outgrowth pattern by light intensity. *Front. Plant Sci.* 2017; **8**, 1724.
[20] Z Ma, S Li, M Zhang, S Jiang and Y Xiao. Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of *Anoectochilus* plants. *HortScience* 2010; **45**, 863-7.
[21] S Rezai, N Etemadi, A Nikbakht, M Yousefi and MM Majidi. Effect of light intensity on leaf morphology, photosynthetic capacity, and chlorophyll content in Sage (*Salvia officinalis* L.). *Horticultural Sci. Tech.* 2018; **36**, 46-57.
[22] NQ Dong and HX Lin. Contribution of phenylpropanoid metabolism to plant development and plant-environment interactions. *J. Integr. Plant Biol.* 2021; **63**, 180-209.
[23] MR Hemm, SD Rider, J Ogas, DJ Murry and C Chapple. Light induces phenylpropanoid metabolism in Arabidopsis roots. *Plant J.* 2004; **38**, 765-78.
[24] S Zhou, SF Yuan, PH Nair, HS Alper, Y Deng and J Zhou. Development of a growth coupled and multi-layered dynamic regulation network balancing malonyl-CoA node to enhance (2S)-naringenin biosynthesis in *Escherichia coli*. *Metab. Eng.* 2021; **67**, 41-52.

[25] F Gibellini and TK Smith. The Kennedy pathway-de novo synthesis of phosphatidylethanolamine and phosphatidylcholine. *IUBMB Life* 2010; **62**, 414-28.
[26] A Cagliari, R Margis, FDS Maraschin, AC Turchetto-Zolet, G Loss and MM Pinheiro. Biosynthesis of triacylglycerols (TAGs) in plants and algae. *Int. J. Plant Biol.* 2011; **2**, e10.
[27] KEGG: Kyoto Encyclopedia of Genes and Genomes, Available at: https://www.genome.jp/pathway/map00940, accessed February 2023.
[28] C Liu, S Feng, Q Wu, H Huang, Z Chen, S Li and Y Xu. Raw material regulates flavor formation via driving microbiota in Chinese liquor fermentation. *Front. Microbiol.* 2019; **10**, 1520.
[29] RK Salar, M Certik and V Brezova. Modulation of phenolic content and antioxidant activity of maize by solid state fermentation with *Thamnidium elegans* CCF 1456. *Biotechnol. Bioproc. Eng.* 2012; **17**, 109-16.
[30] JCD Río, J Rencoret, A Gutiérrez, T Elder, H Kim and J Ralph. Lignin monomers from beyond the canonical monolignol biosynthetic pathway: Another brick in the wall. *ACS Sustain. Chem. Eng.* 2020; **8**, 4997-5012.