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Light quality affects flavonoid production and related gene expression in *Cyclocarya paliurus*

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ABSTRACT

Understanding the responses of plant growth and secondary metabolites to differential light conditions is very important to optimize cultivation conditions of medicinal woody plants. As a highly valued and multiple function tree species, *Cyclocarya paliurus* is planted and managed for timber production and medical use. In this study, LED-based light including white light (WL), blue light (BL), red light (RL), and green light (GL) were used to affect leaf biomass production, flavonoid accumulation and related gene expression of one-year *C. paliurus* seedlings in controlled environments. After the treatments of 60 days, the highest leaf biomass appeared in the treatment of WL, while the lowest leaf biomass was found under GL. Compared to WL, the total flavonoid contents of *C. paliurus* leaves were significantly higher in BL, RL, and GL, but the highest values of selected flavonoids (kaempferol, isoquercitrin and quercetin) were observed under BL. Furthermore, the greatest yields of total and selected flavonoids in *C. paliurus* leaves per seedling were also achieved under BL, indicating that blue light was effective for inducing the production of flavonoids in *C. paliurus* leaves. Pearson's correlation analysis showed that there were significantly positive correlations between leaf flavonoid content and relative gene expression of key enzymes (phenylalanine ammonia lyase, PAL; 4-coumaroyl CoA-ligase, 4CL; and chalcone synthase, CHS) in the upstream, which converting phenylalanine into the flavonoid skeleton of tetrahydroxy chalcone. It is concluded that manipulating light quality may be potential mean to achieve the highest yields of flavonoids in *C. paliurus* cultivation, however this needs to be further verified by more field trials.

1. Introduction

Plants, especially woody plants, are natural sources of bioactive compounds including flavonoids with commercial use as pharmaceutical substances. Flavonoids are a group of phenolic constituents naturally occurring in fruits, seeds, flowers and leaves of plants and vary greatly among different compounds and subgroups [1]. Flavonoids have many biological functions in plants, such as protect plant against UV light [2], attract pollinators [3], and increase resistance to environmental changes and wounding [4,5]. Flavonoids have been reported to possess a lot of beneficial bioactivities in human health, such as anti-allergenic, anti-inflammatory, anti-bacterial, anti-carcinogenic, and anti-viral activities [1,6,7]. Quercetin and isoquercitrin have been found to promote human health through reducing lipid peroxidation and radical oxygen species (ROS) production [6,8]. Another flavonoid, kaempferol, was reported to have remarkable potential to lower the risks of heart disease and improve free-radical-scavenging activity [7].

Flavonoids are biosynthesized from the key precursors of malonyl-CoA and phenylalanine, which are produced by the TCA cycle and a shikimate pathway [9]. Phenylalanine links secondary metabolism and primary metabolism [7], and is converted into cinnamic acid by phenylalanine ammonia-lyase (PAL) (Fig. 1). Cinnamic acid is changed to p-coumaric acid by cinnamate-4-hydroxylase (C4H) [10], while the p-coumaric acid is then converted into 4-coumaroyl-CoA mediated by 4-coumaroyl CoA ligase (4CL) [7]. Chalcone synthase (CHS) catalyzes the conversion of 4-coumaroyl-CoA into the flavonoid skeleton of tetrahydroxy chalcone [11]. Finally, the tetrahydroxy chalcone is turned into different flavonoid molecules by various enzymes, such as chalcone isomerase (CHI), flavanone 3-hydroxylase (F3′H), dihydroflavonol 4-reductase (DFR), flavonol synthase (FLS) and anthocyanidin synthase (ANS) [12].

Flavonoid accumulation and biosynthesis in plants were affected by many environmental factors, such as light, temperature, water and soil type [13–15]. Light intensity and quality are important environmental factors for plant growth and development [13,16]. Specifically, changes in light quality strongly affect plant morphological, biochemical, and physiological parameters [16]. For example, red light is reported to contribute to photosynthetic apparatus development and may increase

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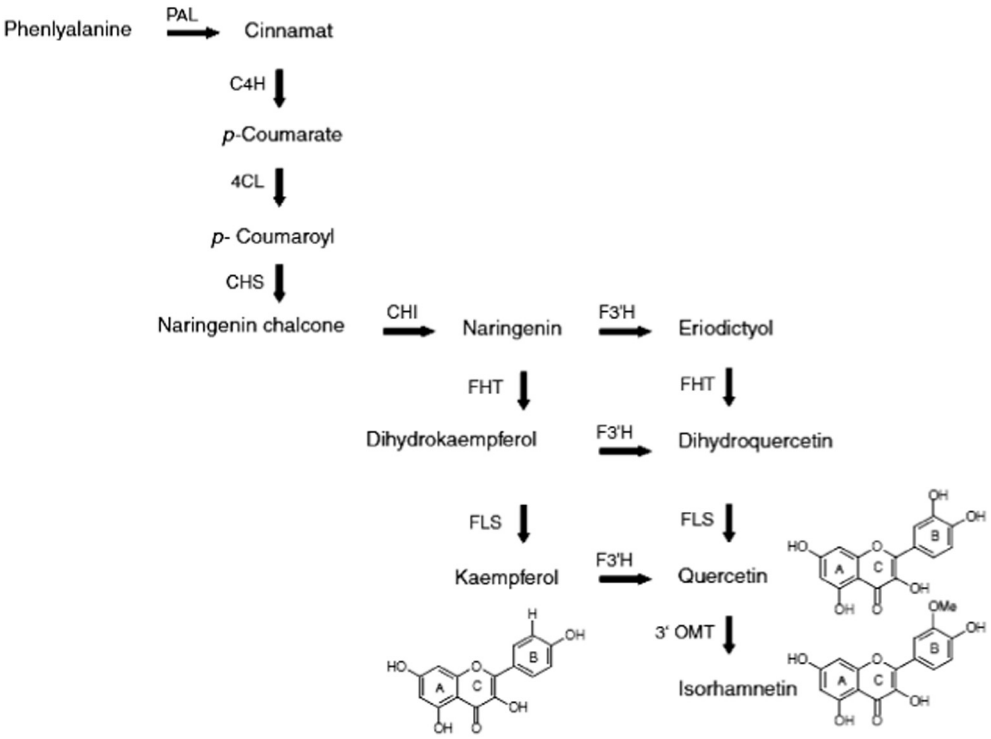

Fig. 1. Biosynthetic pathway of individual flavonoids in plants. PAL: phenylalanine ammonia lyase; C4H: cinnamate-4-hydroxylase; 4CL: 4-coumaroyl CoA-ligase; CHS: chalcone synthase; CHI: chalcone isomerase; FHT: flavanone 3β-hydroxylase; F3′H: flavonoid 3′-hydoxylase; FLS: flavonol synthase; 3′OMT: 3′-Omethyltransferase (quoted from Schmidt et al. 2010).

starch accumulation by inhibiting the translocation of photosynthate out of leaves [17–19]. Blue light is important for chloroplast development, chlorophyll formation, photosynthesis, and chemical composition of plants [20]. Green light shows distinct effects on seedling germination, leaf expansion, leaf hyponasty, stem elongation, photosynthesis, and biomass accumulation [21–23]. Moreover, research of light conditions in recent years suggested that the impacts of light quality on the production of flavonoids in plants are species specific [24]. For example, enhanced blue light strongly improved the production of epidermal flavonoids in pepper plants [25], whereas UV light specifically induced the biosynthesis of flavonoids in young berries of *Cabernet Sauvignon* [26]. Gerhardt et al. [27] also found that the flavonoid accumulation and composition in *Brassica napus* can be altered by the amount of UV-B (290–320 nm) and far-red light. Meanwhile, Fu et al. [28] found that accumulation of a total of 12 flavonoids in tobacco leaves was higher under ultraviolet (350–400 nm), blue light (455–490 nm), and green light (515–540 nm), compared with yellow light (580–600 nm), and red light (610–710 nm). It has been suggested that flavonoid compounds accumulated in the epidermis can protect plants from the damage caused by specific light wavelengths [29,30].

Cyclocarya paliurus (Batal) Iljinskaja belongs to the Juglandaceae family and is widely distributed in mountainous regions of sub-tropical China [31]. Leaves of this plant are traditionally used in China as a medicine or nutraceutical tea because of its unique taste [32]. Many studies have demonstrated that *C. paliurus* possesses a variety of bioactivities, including hypoglycemic activity [33], antihypertensive activity [34], anti-HIV-1 [35], antioxidant activity [36], and anticancer [37]. Therefore, cultivation of *C. paliurus* for its leaf production has been done in recent years for its medical applications. For example, some studies have been carried out to determine the impact of light intensity, genotype and fertilization on growth and phytochemical accumulation in *C. paliurus* [32,38]. Fang et al. [32] have also reported that both isoquercitrin and quercetin contents in *C. paliurus* leaves were greater than those from some fruits (*Malus pumila*, *Prunus armeniaca*, *Fragaria ananassa*, etc.), medicinal plants (*Eugenia jambolana* Lam, *Azadirachta indica*, *Terminalia arjuna*, etc.) and vegetables (*Pisum sativum*, *Brassica oleracea*, *Spinacia oleracea*, etc.) [39,40], while the content of kaempferol in *C. paliurus* leaves was found to be comparable with that of spinach [41]. In previous studies, Deng et al. [38] and Liu et al. [42] also found that flavonoid accumulation in *C. paliurus* leaves significantly increased under higher light intensities, but no knowledge of light quality on flavonoid accumulation was available for this plant. Furthermore, it remains unclear how the genes of flavonoid biosynthesis in *C. paliurus* respond to different light conditions.

The objectives of this study were to investigate the inter-relationship of light quality on leaf biomass and flavonoid accumulation in *C. paliurus* seedlings, to clarify how the genes of flavonoid biosynthesis pathway respond to different light conditions, and to determine the light quality that optimizes flavonoid production. Findings from the study are expected to be of great value for better understanding the responses of plant secondary metabolite accumulation to differential light environment, and to provide a theoretical basis for improving growing conditions for higher production of functional flavonoids in *C. paliurus* cultivation.

2. Materials and Methods

2.1. Plant Materials and Experimental Procedures

The experiment was carried out during the 2016 growing season in Nanjing Forestry University (31° 59′ N, 119° 18′ E). Seeds of *C. paliurus* were collected from a superior tree (based on tree age, stem form and growth vigor) in its location at Jinzhongshan (24° 36′ N, 104° 57′ E), Guangxi province, China in late October 2015 and then subjected to exogenous gibberellin A3 (GA3) treatments and stratification treatments to break seed dormancy in early January 2016 following the method of Fang et al. [31]. After a 3-month stratification treatment, the germinated young seedlings were then transplanted into nonwoven containers (8.0 cm diameter, 10.0 cm height) filled with a substrate mixture of perlite: fowl manure: peat: soil (2: 2: 4: 2, v/v/v/v). The substrate was a loam with pH 6.44, organic matter content of 73.3 g kg^{-1}, total N content of 72.35 g kg^{-1}, total P content of 2.19 g kg^{-1}, and total K content of 9.55 g kg^{-1}. Seedlings were covered with one layer of shading net at 2 m height and kept well-watered once