

Effect of Light Quality and Quantity on the Accumulation of Flavonoid in Plant Species

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Abstract: Light effects including its intensity, wavelength, and duration are important environmental factors that affects flavonoid accumulation. Ultraviolet (UV) light can induce flavonoid biosynthesis. Under normal condition, flavonoids are produced in response to stress, and they function as UV filters. In this paper, we review how light quality and quantity affect the accumulation of flavonoid in plant species. High light intensity can influence flavonoid accumulation, but in heliophytes, the opposite is true. Some medicinal plants require shady environment for flavonoid accumulation. In monocots, the flavonoid is situated in both epidermis and mesophyll while in dicot, it is found only in the epidermis. This review leads to a conclusion that high variation in flavonoids accumulation in response to light can occur within and between plant species.

Keyword: flavonoid; light quality; light quantity; plant; UV radiation.

1. Introduction

The environment does not have a constant stable condition, it always changes and these changes can lead to various effects in the morphological and physiological characteristics of a leaf including its shape, curling degree, and its surface characteristics. One of the most important environmental factors affecting plants is light [1]. Plants can adapt to different light intensity depending on their environment or depending on the amount of shading they receive. This adaptation would be possible if plants change the distribution of its biomass and its morphology, in order to be able to utilize the amount light they receive, so as to survive [2, 3].

Various studies have indicated the significant effect of light intensity on the production of secondary metabolites like flavonoid glycosides and terpene lactone [4]. Shading was reported to affect flavones (a type of flavonoid) concentration in leaves of *Litocarpus litseifolius* [5]. Moderate shading favors the accumulation of flavonoids in *L. litseifolius* and therefore as the light intensity increases or decreases, the flavone accumulation would be affected. This

secondary metabolites function in protecting the plants against harmful ultraviolet (UV) radiations. When the light intensity increases, the harmful UV radiation increases, and therefore the plant produce more flavonoids to protect itself from the radiation [6]. In some plants like *Ginkgo biloba* [4], and *Erigeron breviscapus* [6], flavonoid accumulation reduces when there is shading and increase when the light intensity increases. Other plants like *L. litseifolius* [5] do not fall under this category because the flavonoid accumulation do not have a linear relationship with light intensity and it produce more flavonoids at about 40% shading and fewer flavonoids at 80% shading. This indicate that *L. litseifolius* requires an optimum light intensity for the accumulation of flavonoids.

The variation in flavonoids accumulation among plant species may be due to the complex metabolism of flavonoids. Also when the photosynthesis is higher, the flavonoid accumulation increases in the leaves. This is true for *Fagopyrum esculentum* [7] which produce flavonoids depending on the L-phenylalanine ammonia lyase (PAL) activity.

In heliophytes, the activity of antioxidant enzymes decreases under lower light intensity.

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This increase reactive oxygen species (ROS). Due to an increase in ROS, more flavonoids would be synthesized in order to scavenge the ROS, and protect the plant. This is true for *L. litseifolius* [5]. When *L. litseifolius* is growing under shading for 60 days, it reduces the production of flavonoids. This may be due to senescence of the cells.

Under 50% irradiance, *Piper aduncum* was reported to accumulate more flavonoids than under 100% irradiance [8]. *Epimedium pseudowushanense* has medicinal effects due to its flavonoid contents. L3 ($54.6 \pm 2.5 \mu\text{molm}^{-2}\text{s}^{-1}$) and L4 ($90.9 \pm 2.5 \mu\text{molm}^{-2}\text{s}^{-1}$) light treatments were the optimum light intensity for the production of flavonoids in *E. pseudowushanense*. Epimedin A and B contents increased as light intensity increases from L1 to L4 but decreased when the light intensity is very high (L5). This shows that different flavonoids production are affected by different light intensities [9]. The optimum light intensity for flavonoid accumulation in *Epimedium sagittatum* ranges from 40 to 60 $\mu\text{molm}^{-2}\text{s}^{-1}$ while in *E. pseudowushanense*, it ranges from 54.6 to 90.9 $\mu\text{molm}^{-2}\text{s}^{-1}$ [9].

The objective of this paper is to review the effect of varying light intensity on the accumulation of flavonoid in plants.

2. Flavonoids

Flavonoids are a group of aromatic compounds derived from Phe and malonyl coenzyme A. They include flavones, flavonols, tannins, chalcones, anthocyanins, and flavandiols which can be found in higher plants [10]. These secondary metabolites are produced by plants for protection against harsh conditions like cold, drought heat, salinity, UV radiation, pathogens, they also serve as detoxifying agents, allelopathic compounds, and signal molecules [11]. Due to this reason, flavonoids are not constantly produce by the plant, but rather, they are produced as response to a harsh condition. Example of such flavonoids (Table 1) includes flavonol, flavones, and anthocyanin [12].

2.1 Flavonoids biosynthesis

The flavonoid biosynthetic pathway is represented in Fig. 1. Variations in flavonoids accumulation may be due to the biosynthesis pathway (Shikimic acid pathway) where

phenolic compounds are produced first in the pathway followed by phenolic acids, hydroxyl cinnamic acids, lignans and then flavonoids respectively [13]. Due to this, [14] hypothesized that lower level of flavonoids at higher light intensity was due to the production of other phenolic compounds more than flavonoids in the Shikimic acid pathway while higher level of flavonoid at lower light intensity is due to the production of more flavonoids than other phenolic compounds in the pathway.

Therefore, a higher quantity of phenolic compounds inhibits flavonoids biosynthesis by inhibiting the activity of phenylalanine ammonia lyase (PAL) enzyme [15]. The enzyme responsible for flavonoids biosynthesis is located in the cell cytosol [16]. Increase in light intensity leads to an increase in flavonoids of medicinal plants [14, 17]. Light affects the activity of PAL, the enzyme that regulates flavonoid biosynthesis [18, 19]. The activity of flavonoid enzyme (PAL) increases at 50% and 70% irradiance as well as under blue net for *P. aduncum* while in *Labisa pumila* Benth leaves, PAL has its highest activity at 630 $\mu\text{molm}^{-2}\text{s}^{-1}$ [17].

When a plant receives enough nutrients, it concentrate more on using phenylalanine for protein synthesis instead of flavonoid accumulation [20]; i.e. there is a decrease in secondary metabolite accumulation when primary metabolites production increases [21]. Lower light intensity favors the biosynthesis of monohydroxy B ring flavonoids while high light intensity influence the biosynthesis of dihydroxy B ring substituted flavonoids [22, 23]. This, therefore, indicates that luteolin and quercetin will be higher at higher light intensity while kaempferol and apigenin will be higher at the lower light intensity. Therefore, luteolin and quercetin play a vital role in protecting plants against UV radiation [24].

Flavonoids can be found in plant leaf palisade and spongy mesophyll cells in accordance with the light intensity [25]. Flavonoids can also be found in plant chloroplast, nucleus, and vacuoles [24]. In leaves that are adapted to high irradiance, flavonoids especially dihydroxy B substituted flavonoids accumulate in the whole leaf depth [26]. In monocots, flavonoids are situated at the epidermis and mesophyll [27] while in dicots, it is restricted to the epidermis only.

Phototropin photoreceptors (PHOT 1 and PHOT 2) are responsible for sensing UV-A,

while UV-RESISTENCE LOCUS 8 (UVR8) sense UV-B light [28].

Table 1 Different types of flavonoid

Class	Group	Description	Examples
Anthoxanthin	Flavone	2-phenylchromen-4-one	Luteolin, apigenin, tangeretin
	Flavonol	3-hydroxyphenyl-2-chromen-4-one	Quercetin, kaempferol, myricetin, fisetin, galangin, isorhamnetin,
Flavanones	Flavanones	2,3-dihydro-2-phenylchromen-4-one	Hesperetin, Naringenin, Eriodictyol, Homoeriodictyol
Flavanonols	Flavanonol	3-hydroxy-2,3-dihydro-2-phenylchromen-4-one	Taxifolin (or Dihydroquercetin), Dihydrokaempferol
Flavans	Flavanols	2-phenyl-3,4-dihydro-2H-chromen-3-ol	Catechin, Gallocatechin, Catechin 3-gallate, Gallocatechin 3-gallate, Epicatechins,
	Theaflavin	3,4,5-Trihydroxy-1,8-bis[(2 <i>R</i> ,3 <i>R</i>)-3,5,7-trihydroxy-2-chroman-6-yl]-6-benzo[7]annulenone	Theaflavin-3-gallate, Theaflavin-3'-gallate, Theaflavin-3,3'-digallate
Anthocyanidins	Anthocyanin	flavylium (2-phenylchromenylium)	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin

These photoreceptors absorb light and activate the transduction signal. Grape berries are non-climacteric fruits that adapt to high light intensity by increasing the expression of flavonoid biosynthesis genes to accumulate more anthocyanins, proanthocyanins and flavonols [23, 29, 30, 31, 32, 33, 34].

When a shaded apple was exposed to light, a sudden up-regulation of flavonoid biosynthetic gene (*MdFLS*) and other anthocyanin and leucoanthocyanidin genes were observed [35], [36]. This leads to increase in accumulation of flavonols and anthocyanins. Mutation leads to situations where-by light do not stimulate the accumulation of anthocyanins, as reported in grape berries [37], bilberry [38], Chinese bayberry [39], and strawberry [40]. This process is regulated by R2R3MYB transcription factors [41].

The genetic constituents of a plant determine its flavonoid content, but the quality and quantity is influenced by environmental factors. These transcription factors were

reported to be present in plants about 500 million years ago [42]. Veraison (removal of the leaf before ripening) leads to up-regulation of MYB transcription factors, thereby increasing the accumulation of flavonoid in berries [33].

2.2 Functions of flavonoids

Flavonoids have a variety of function in a plant. They can act as UV protectors [43], phytoalexins, signal molecules, growth regulators, allelochemicals and detoxifying agents [44], stimulate spore and seed germination, as well as act as pollinator attractants [45]. Lipid peroxidation occurs due to oxidative stress. Flavonoids like quercetin and rutin can protect plant membranes from oxidative damage. In humans, they have antioxidant [46], hepatoprotective [47], antibacterial [48], anti-inflammatory [49], anticancer and antiviral effects [50].

3. Factors affecting flavonoids accumulation

Flavonoids production is affected by the light intensity and density [51]. The quality and quantity of irradiance are important for accumulation of flavonoids [52]. Flavonoid accumulation is also affected by geographical

factors like latitude and altitude [53], temperature, PAR [45], and photoperiod [54].

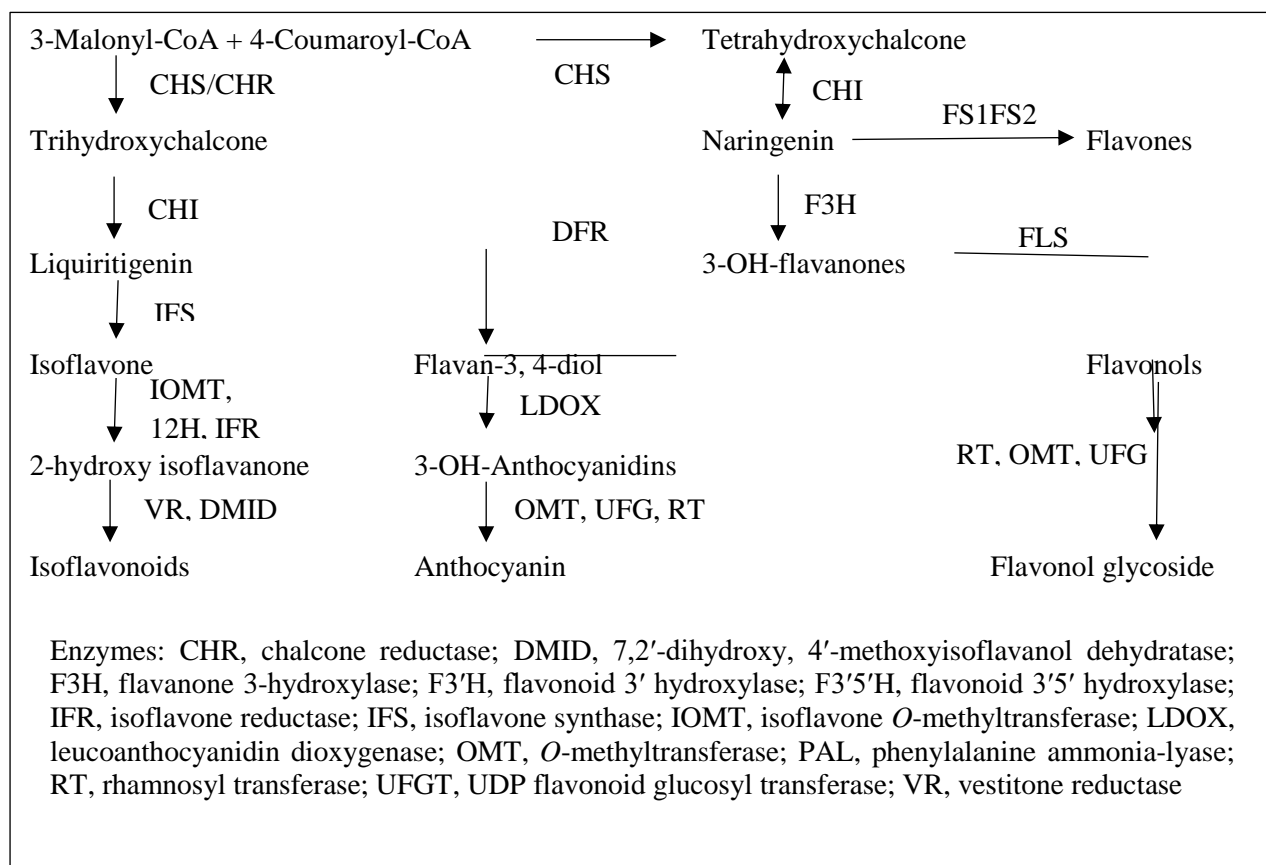


Fig. 1 The flavonoid biosynthetic pathway

3.1 Effect of light intensity on flavonoid accumulation

UV radiation of 320-400nm (UV-A) reach the earth together with some (~0.5%) of 280-320nm (UV-B) [55]. The latter is very little but it has harmful effect on both plant and animals, leading to activation of ROS which depending on the dose damage proteins, DNAs, and photosynthetic pigments in plants [56]. Flavonols are excellent ROS scavengers and due to this, the plant produces more flavonol for better protection [57].

The wavelength of 300-320nm was reported as the spectra for flavonoid production [58]. Kaempferol and quercetin accumulate in higher quantity in grape berries cultivated in New Zealand (at high level of UV radiation) [59]. Quercetin-3-O-galactoside and quercetin-

3-O-glucoside levels increased when harvested grape berry was treated with UV-C radiation [60].

Blue and red nets; and different irradiance were used to study the effect of light on *Piper aduncum*. Red net and 100% irradiance yield the lowest flavonoid content while the highest accumulation of flavonoid was observed under blue net [52]. This is also true for *Prutea cynaroides* [61] but not true for *Zingiber officinale* [14]. The quality and quantity of irradiance is important for accumulation of flavonoid [52]. To study the effect of light on flavonoids synthesis in Ginger varieties, 4 different light intensities (310, 460, 630, and 790 $\mu\text{molm}^{-2}\text{s}^{-1}$) were employed. *Alpinia purpurata* produced the highest amount of flavonoids in the leaves at 310 $\mu\text{molm}^{-2}\text{s}^{-1}$ [14]. This shows that varying light intensities have an

impact on the medicinal content of the studied plant. Light is an important environmental factor that regulates plant growth, development and biosynthesis of secondary and primary metabolites [59, 62]. Flavonoids production depends on the light intensity and density [51]. Due to the facts that medicinal plants exert their effect depending on the flavonoids and phenolic they contain, growing the plant at an optimum light intensity will help in increasing the medicinal effect of the plant. It is important to keep in mind that different plants have a different response to varying light intensity in terms of flavonoids production [63, – 65]. Shade plants have the advantage of lower temperature which influences flavonoid production especially anthocyanin [66 – 68]. Strawberries and *Tanacetum parthenium* were reported to have an increasing accumulation of flavonoids with decreasing light intensity [69], [70]. Various medicinal plants produce flavonoids at low light intensity [14, 63, 71, 72]. Isoflavones and other flavonoids accumulate in higher concentration if the plant is either infected [72, 73] or when the plant is under low nutrient/low light intensity [74, 75]. In an approach to studying the effect of light intensity and quality on the photosynthesis and flavonol accumulation in *Ginkgo biloba*, [4] find out that the studied plant accumulate flavonoids at a low level of UV radiation. [6] recorded that *Erigeron breviscarpus* grown under 100% and 80% light intensity accumulate more flavonoid than those grown under 50% light intensity. The optimum light intensity for the accumulation of the major flavonoid of *Epimedium pseudowushanense* (epimedin c) was $54.6 \pm 2.5 \mu\text{molm}^{-2}\text{s}^{-1}$ [9].

High light intensity favors auxin production; which controls the glycosylation patterns of flavonoids according to the intensity of light [76]. The flavonoid that responds to light has catechol group in the B ring of their skeletal structure [22]. They are found in Nano and micro concentrations in mesophyll cells particularly in the vacuoles and chloroplast and they can reduce ROS.

Blue light leads to the accumulation of more flavonoid in *Saussurea medusa* [77]. As the intensity of white light increases, the concentration of flavonoid also increases. The highest white and black light radiation that can lead to the maximum accumulation of flavonoid in *S. medusa* is 16-hour white light and 8-hour black light or vice versa [77]. UV-B was

reported to increase the level of anthocyanin and other flavonol in grape berries. The flavonol content increased proportionally to the UV radiation [43].

Cluster shading of *Vitis vinifera* leads to a decrease in the accumulation of skin proanthocyanidins and flavonols but rarely affect the accumulation of anthocyanin [78]. Anthocyanin, flavonol and hydroxycinnamic acids accumulate in higher concentration in the leaves of *Vaccinium myrtillus* which was previously exposed to direct sunlight; while polymeric procyanidins were higher in shady plants [79]. Light and temperature affect the accumulation of flavonoid in *Ginkgo biloba* [15]. UV-C increase the level of flavonoid in *Vaccinium corymbosum* L. (blueberries). The effect of UV-C is dose and time related as it diminishes with time [80].

Light intensity has effects on the accumulation of flavonoid in cranberry [81], raspberry [82], Bayberry [39], Tomato [83], and in Bilberry [54]. It also regulate the accumulation of flavonoid in plants belonging to the *Rosaceae* family especially Apples [84], Strawberries [85, 86], Pears [87, 88], and Peach [89, 90]. Light treatment on harvested apples leads to accumulation of flavonols and anthocyanins which leads to the desired red coloration [91]. Therefore, light affects the accumulation of flavonoids even after harvest. Zhang et al. [92] reported that Pears accumulate less anthocyanin if the light intensity is high. This was also true for *Mangosteen* fruit [93]. Flavonoid accumulation was higher in sunny *Phillyrea laticifolius* than in shady plants. Altitude influence the quality of sun radiation i.e. (UV-B) is higher at higher habitats than lower ones [94]. In apples, when the UV radiation was blocked completely, the flower fails to produce anthocyanin, because the gene responsible for anthocyanin accumulation was not activated [95].

When a fluorescent lamp of 312 nm UV radiation was used on sweet cherry, anthocyanin accumulates in higher concentration than when white fluorescent lamp was used. The accumulation of the flavonoid was dose and time-dependent [96]. After 72-96 hours of exposing *Lysimanchia callus* cultures to UV-B radiation, the maximum level of flavonoid accumulates [27]. At higher UV-B radiation dose, flavonoid content of *Acorus calamus* L increases [18]. When UV-B applied to *Brassica napus* which

was pre-treated with UV-A, the accumulation of flavonoid was impeded [97].

The levels of saponarin and lutanarin flavonoids increase in the mesophyll and lower epidermis of Barley leaf. UV-A lead to an increase in the accumulation of flavonoids in the study [98]. The level of flavonoid in baby spinach sown in August was not affected by shading, while that sown in April was affected by shading; with an increase in flavonoid accumulation in un-shaded leaves [71]. This might be due to the fact that increasing levels of UV-B radiation leads to accumulation of flavonoids. Another reason might be due to PAR, which can also increase flavonoid synthesis [99].

Jeong et al. [32] reported that the accumulation of anthocyanin in grape berry skin was affected by shading. Quercetin-3-O-glycoside had absorbance maxima at $355\pm 2\text{nm}$ while luteolin absorb at $348\pm 2\text{nm}$. Their monohydroxy B ring counterparts absorbs maximally at $351\pm 2\text{nm}$ for kaempferol and $337\pm 2\text{nm}$ for apigenin. The monohydroxy B ring absorbs UV wavelength more than dihydroxy B ring but the latter had greater antioxidant activity and responds to light [100]. *Torreya grandis* seedling at 75% shading produces lower levels of flavonoid, but at 100%

and 50% irradiance, the plant produces more flavonoid [101]. Table 2 shows the light requirement for the accumulation of flavonoid in various plants.

3.2 Effect of photosynthetic active radiation (PAR) and photoperiod on flavonoid accumulation

In an approach to studying the relationship of high photosynthetic active radiation (PAR) and ambient UV-B intensity on the accumulation of secondary metabolites, [45] find out that anthocyanin and saponin level increase in *Centella asiatica* leaf under high PAR while under ambient UV-B radiation, saponin and saponin did not increase. The study reveals that saponin predominates older leaves, while saponin predominates younger leaves. The combination of high PAR and ambient UV-B has an effect on flavonol and anthocyanin production in *C. Asiatica*. This might be due to the reason that UVR8 (UV-B photoreceptor) pathway have a relationship with the visible light photoreceptor pathway [102, 103, 104]. Moreover, thicker leaves provide more protection to plant against UV radiation than thinner leaves [105].

Table 2 Effect of light quality, intensity, PAR and photoperiod on the accumulation of flavonoids in selected plants

Plant	Light requirement for maximum flavonoid accumulation	Type of flavonoid	Reference
<i>Erigerium breviscarpus</i>	High light intensity	Total flavonoid content	[6]
<i>Ginkgo biloba</i>	High UV light intensity	Flavonol	[4]
<i>Alpinia purpurata</i>	Low light intensity	Total flavonoid content	[14]
<i>Labisa pumila</i>	High light intensity	Total flavonoid content	[107]
<i>Lithocarpus litseifolius</i>	Moderate shading	Flavone	[5]
<i>Hyptis marrubiodes</i>	White LED	Rutin	[108]
<i>Anacardium othonianum</i>	Blue LED	Flavone	[109]
<i>Berberis microphylla</i>	Moderate shading	Quercetin and catechin	[110]
<i>Berberis microphylla</i>	High light intensity	Rutin and anthocyanin	[110]
<i>Cyclocarya paliurus</i>	Blue LED	Quercetin	[111]
<i>Elephantopus scaber</i>	Moderate shading	Total flavonoid content	[112]
<i>Lactuca sativa</i>	High light intensity	Total flavonoid content	[113]
<i>Aronia sp.</i>	Blue LED	Total flavonoid content	[114]
<i>Pyrus pyrifolia</i>	Blue LED	Anthocyanin	[115]
<i>Tanacetum parthenium</i>	Night time	Total flavonoid content	[116]
<i>Brassica oleracea</i>	12 hour day length	Flavone	[117]
<i>Perilla frutescens</i>	Longer photoperiod	Anthocyanin	[118]
<i>Ipomoea batatas</i>	Long photoperiod	Flavonols	[106]
<i>Centella asiatica</i>	High PAR	Anthocyanin	[119]

Photoperiod also influences the accumulation of flavonoid in response to UV irradiation. In Bilberry, higher levels of anthocyanins were recorded when the day length was 24 hour compared to when it was 12 hour [54]. This is also true for *Vaccinium berries* [53]. Longer days have a longer period of sunlight. Due to this, higher flavonoid content was recorded for *Pomoea batatas* L (sweet potato) leaves while lower flavonoid content was recorded for short photoperiods [106].

4. Conclusion

In conclusion, flavonoid accumulation is strongly affected by the environmental light conditions. In general, higher sun radiation tends to increase flavonoid accumulation in plants especially fruits, but decrease flavonoid accumulation in heliophytes and some medicinal plants. This shows that variation in response to light can be high within and between species. Understanding the flavonoid biosynthetic pathway, its regulation and light signaling machinery in plants will help in selecting plant enriched with the desired health and dietary requirements. Knowledge of the optimal growth condition of a plant will help in cropping strategy of plants especially the endangered species.

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