

YUMSUK JOURNAL OF PURE AND APPLIED SCIENCES

EVALUATION OF THE PHOTOPROTECTIVE ROLE OF QUERCETIN TO *LACTUCA SATIVA*

¹Aisha I., ²Zakiyyu I T.,

¹Department of Biological Sciences, Faculty of Sciences, Federal University Dutse, PMB 7156, Jigawa State, Nigeria

²Faculty of Science, Yusuf Maitama Sule University, Kano State, Nigeria

*Corresponding Author e-mail: idrisaisha24@gmail.com

ABSTRACT

Plants respond to different light intensities depending on their genetic make-up, mutation and other environmental conditions. *Lactuca sativa* was studied to analyze how varying light intensity affects the production of flavonoid content. Besides, the study was aimed at evaluating the photoprotective role of quercetin to the plant. The chlorophyll, carotenoid, anthocyanin, flavonoid, antioxidant enzymes, malondialdehyde, soluble sugar and soluble protein contents were quantified using spectroscopic techniques. Quercetin was quantified using high-performance liquid chromatography (HPLC). Sun-exposed plants were having the maximum quercetin content compared with semi-shaded or fully shaded plants. The highest anthocyanin, flavonoid, carotenoid, soluble sugar and soluble protein content were recorded for sun-exposed *L. sativa* while the lowest were recorded for fully shaded plant. The highest oxidative stress was recorded for sun-exposed plants. In contrast, the activities of antioxidant enzymes were lowest under sun-exposed plants, and highest under shaded plants. The result obtained makes it possible to accept the generated hypothesis of the study because the quercetin content was higher when the antioxidant enzymes of the plants were low. This leads to a conclusion that increase in the production of secondary metabolites at high light intensity is due to the production of photo protective metabolites to conquer the light stress.

Keywords: Antioxidant enzymes; Flavonoid; *Lactuca sativa*; Light intensity; Quercetin

INTRODUCTION

In recent decades, the adverse effects of ultraviolet (UV) radiation on living organisms have become a subject of intense research scrutiny. Increased levels of UV radiation due to environmental changes have prompted scientists to explore potential solutions to mitigate its damaging effects. One such avenue of exploration is the utilization of naturally occurring compounds with photo protective properties (Idris *et al.*, 2021). Among these compounds, quercetin, a flavonoid abundant in various plant species, has emerged as a promising candidate for its diverse range of biological activities (Ahmed *et al.*, 2019).

Lactuca sativa, commonly known as lettuce,

serves as an excellent model organism to investigate the photo protective attributes of quercetin. As a common leafy vegetable, lettuce is exposed to varying degrees of solar radiation, including UV light. The potential of quercetin to safeguard plants from the detrimental effects of UV radiation has spurred interest in assessing its role within the context of *L. sativa*'s physiological responses (Bian *et al.*, 2018).

This paper aims to present a comprehensive evaluation of the photoprotective role of quercetin in *L. sativa*. Through a combination of experimental analyses and literature review, we delve into the mechanisms by which quercetin may contribute to mitigating UV-induced damage. Our investigation

encompasses both the direct effects of quercetin on light absorption and its indirect modulation of cellular pathways that respond to oxidative stress. The understanding of quercetin's photoprotective potential in *L. sativa* holds implications beyond the realm of plant biology. Insights gained from this research could inform strategies to enhance crop resilience, ameliorate agricultural practices, and contribute to the broader understanding of how phytochemicals interact with environmental stressors.

MATERIALS AND METHODS

Plant Growth Condition

L. sativa was grown under different lighting conditions. The sun exposed plants were directly under full sunlight, the semi shaded plants were under a net, while the fully shaded plants were under complete shading. All other growth conditions like soil, nutrient and water supply were the same throughout the light treatments. The experiments were conducted in triplicates (Idris 2019).

Determination of the Photosynthetic Pigment Contents

The quantitative determination of the photosynthetic pigment contents presents in the plant were done using UV visible spectrometer according to Lichtenthaler & Buschmann (2001). The plant leaf samples were collected and washed. 0.5 g of leaf sample was ground in 10 ml of ethanol. The solution was centrifuged at 10,000 rpm for 15 minutes at 4 °C. Furthermore, 4.5 ml of ethanol was added to 0.5 ml of the supernatant. 1 ml of the sample was transferred into a cuvette and inserted into a spectrophotometer (Sumanta *et al.*, 2014). Absorbance was recorded at $\lambda = 664.2$ nm (chlorophyll *a*) and $\lambda = 648.6$ nm (chlorophyll *b*), whereas $\lambda = 470$ nm was used for Carotenoid. Lastly, absorbance values were used to calculate individual pigment contents.

Determination of Flavonoid Content

The total flavonoid content (TFC) was determined using the aluminium chloride colourimetry method. The sample solution was prepared; accordingly, were 10 % AlCl_3 was first prepared by dissolving 10 g of AlCl_3 in 90 ml distilled water. The solution was stirred, and then finally filled to 100 ml with distilled water. After

this step, 1 M potassium acetate solution was prepared by dissolving 9.8 g of potassium acetate in 90 ml distilled water, stirred, and then filled up to 100 ml with distilled water. A stock solution of the standard used (quercetin) was prepared by dissolving 1 mg of quercetin in 1 ml distilled water (finally producing 1 mg/ml). The stock solution was further diluted serially, achieving 10, 50, 100, and 200 $\mu\text{g/ml}$.

The extract solution was prepared by mixing 1 ml of the plant extract with 3 ml of methanol. Further, 200 μl of 10 % AlCl_3 and 200 μl of 1 M potassium acetate were added to the extract solution. Finally, 5.6 ml of distilled water was added to the solution. Standard solution and blank solution were prepared as above except that for the standard solution, 1 ml of plant extract was substituted with different standard dilutions while for blank solution 0.25 ml of ethanol replaced 1ml of extract solution (Chang *et al.*, 2002). Additionally, the extract, standard and blank solutions were incubated at ambient temperature for half an hour and then the absorbance was recorded at $\lambda = 420$ nm.

Absorbance's for quercetin standard and for extract solution were recorded. A calibration curve was constructed and TFC was calculated using the curve. The results were expressed as μg quercetin equivalent ($\mu\text{g QE}$).

Determination of the Anthocyanin Contents

The quantitative determination of the total monomeric anthocyanin contents presents in the studied plants were done using the pH differential method according to AOAC method (Lee, 2005). 0.2 g of the leaves was homogenized in 10 ml of distilled water. 1.86 g of potassium Chloride was added to 1000 ml of distilled water and the pH was adjusted to 1.0. After this, 54.43 g of sodium acetate was added to 1000 ml distilled water and the pH was adjusted to 4.5. Test solutions were made by making dilutions of the sample with the buffers, but care was taken so as not to exceed the capabilities of the buffer. Turbid dilutions were filtered. The absorbance of the test solutions at $\lambda = 520$ nm and $\lambda = 700$ nm for both buffers were taken within an hour of test solution preparation (Lee, 2005). The results are expressed as mg per litre cyanidin-3-glucoside (cy-3-glu) equivalent.

Determination of Quercetin Content

The plant extracts were dissolved in 80 % methanol at a concentration of 250 mg/ml and were centrifuged at 13,200 rpm for 5 min. The supernatant was collected and filtered through a 0.45 μ m nylon syringe filter. Standard stock solutions of quercetin were prepared at a concentration of 2 mg/ml in 80 % methanol. The standard solutions of quercetin were serially diluted with 80 % methanol to obtain calibration standard solutions at concentrations of 20, 40, 60, 80 and 100 μ g/ml. All solvents were degassed, and analysis of sample and standard solutions were done in a reverse phase HPLC method, at ambient temperature. 1 % acetic acid and methanol were prepared in a ratio (10:90), (v/v). Injection volume was 10 μ l (Dar *et al.*, 2017). Solvents eluted in an isocratic manner with 1.00 ml flow for every one minute.

Quantitative Determination of Lipid Peroxidation

Lipid peroxidation was estimated by quantifying the malondialdehyde (MDA) content of the studied plants. 0.1 g of leaf sample was ground and homogenized in 1 ml of 0.10 % (w/v) trichloroacetic acid (TCA) and then centrifuged at 10,000 g for 10 minutes. Then, 20 % TCA was mixed with 5 % Thiobarbituric acid (TBA), thereby generating the reaction mixture. MDA standard was serially diluted to generate 10, 20, 40, 60, 80 and 100 μ g/ml. Both sample and standard solutions were mixed with 4 ml of the reaction mixture, boiled at 95 °C for 15 minutes, and then placed on ice. The sample and standard solutions were centrifuged at 10,000 g for 5 minutes, and the absorbance's of the supernatant were recorded at λ = 532 nm (Zhang & Rongfeng, 2013). The MDA content was calculated from the standard curve, and the results were expressed as μ g/ml.

Determination of Antioxidant Enzymes Activity

For superoxide dismutase (SOD), the reaction solution was prepared by mixing 30 ml of 100 mM phosphate buffer with 0.6 ml of 1 mM EDTA- Na_2 , 2 ml of 20 μ M riboflavin, 2 ml of 750 μ M NBT and 2 ml of 130 mM methionine. The sample solution was prepared by mixing 50 ml of crude protein with 1 ml of the reaction mixture.

Two types of control were made, one of which was incubated in light together with the sample mixture while the other control was kept in the dark. All incubations were ended after 15 minutes, and absorbance was immediately recorded at λ = 560 nm. The second control was used for comparison only (Tianzi & Baolong, 2016).

For peroxidase (POD), the reaction mixture was prepared by mixing 28 μ l of 0.2 % guaiacol with 50 ml phosphate buffer. The reaction was heated, then cooled down before adding 19 μ l of 30 % H_2O_2 . 1 ml of the reaction solution was further diluted with 50 μ l of phosphate buffer. The sample solution was prepared by mixing 50 μ l of crude protein with 1 ml of the previously prepared reaction solution in a cuvette and immediately recording the absorbance at λ = 470 nm. Absorbance's were recorded at an interval of 15 seconds for a duration of 1 minute (Tianzi & Baolong, 2016).

For catalase (CAT), the reaction solution was prepared by mixing 77.5 μ l of 30 % H_2O_2 with 50 ml 100 mM phosphate buffer. The sample solution was prepared by mixing 50 μ l of crude protein with 1ml of the previously prepared reaction solution in a cuvette and immediately recording the absorbance at λ = 240 nm. Absorbance's were recorded at an interval of 15 seconds for a duration of 1 minute. For blank solution preparation, 50 μ l of crude protein was replaced with 50 μ l of 100 mM phosphate buffer (Tianzi & Baolong, 2016).

Determination of Total Soluble Sugar (TSS)

The TSS was quantified based on the anthrone colourimetric method. 2 ml of plant extract was centrifuged at 6,000 rpm for 10 min and then an equal volume of chloroform was added to the supernatant and centrifuged at 12,000 rpm for 10 min. Moreover, 4.95 ml of anthrone reagent was added to 50 μ l of the sample solution. After mixing thoroughly, the solution was then boiled in a water bath for 15 minutes. Furthermore, the solution mixture was cooled down and the absorption was recorded at λ = 620 nm. TSS was calculated using the standard curve and the result was expressed in μ g/ml glucose.

Determination of Soluble Protein Content (SP)

This was achieved using the Bradford protein assay method. The standard solution was prepared by serially diluting bovine serum albumin at 1, 2, 3, 4,

and 5 µg/ml, and adding 200 µl of Bradford reagent to 800 µl of each standard. Sample solutions were prepared by adding 200 µl of Bradford reagent to 800 µl of the plant extract. Both sample and standard solutions were incubated at ambient temperature for 5 minutes and then their absorbance's were recorded at $\lambda = 595$ nm. A standard curve was generated and the protein contents were derived using the standard curve (Fanglian, 2011).

Statistical analysis

Data were reported as the mean $\pm$ standard deviation (SD), and all statistical tests were performed using the SPSS 16.0 statistical software (SPSS, Chicago, IL, USA). Data was analyzed using one way analysis of variance (ANOVA), and all analyses were performed at a 95 % confidence level.

RESULTS AND DISCUSSION

In the laboratory analysis, a comprehensive evaluation of various physiological and biochemical parameters was conducted to unravel the intricate interplay between quercetin and the photo protective responses of *L. sativa* under varying light conditions. The examined parameters encompassed chlorophyll content,

carotenoids, anthocyanin, flavonoid, antioxidant enzymes, MDA, soluble sugar, soluble protein contents, and quercetin concentration.

Remarkably, the results revealed a pronounced correlation between light exposure and the accumulation of specific compounds crucial for photo protection and oxidative stress response. Among the studied parameters, the trends in flavonoids, anthocyanin, quercetin, and MDA levels particularly stood out (Figure 1).

Sun-exposed plants exhibited the highest levels of flavonoids, anthocyanin, and quercetin. This outcome underscores the pivotal role of these compounds in shielding plants from the detrimental effects of UV radiation. The heightened synthesis of these antioxidants can be attributed to the heightened light exposure, prompting the plant to enhance its antioxidant arsenal to counteract potential damage. Surprisingly, semi-shaded plants followed sun-exposed plants with moderate levels of flavonoids, anthocyanin, and quercetin. This suggests that even partial exposure to sunlight triggers a significant adaptive response, although the levels might not reach the same magnitude as those in fully sun-exposed plants.

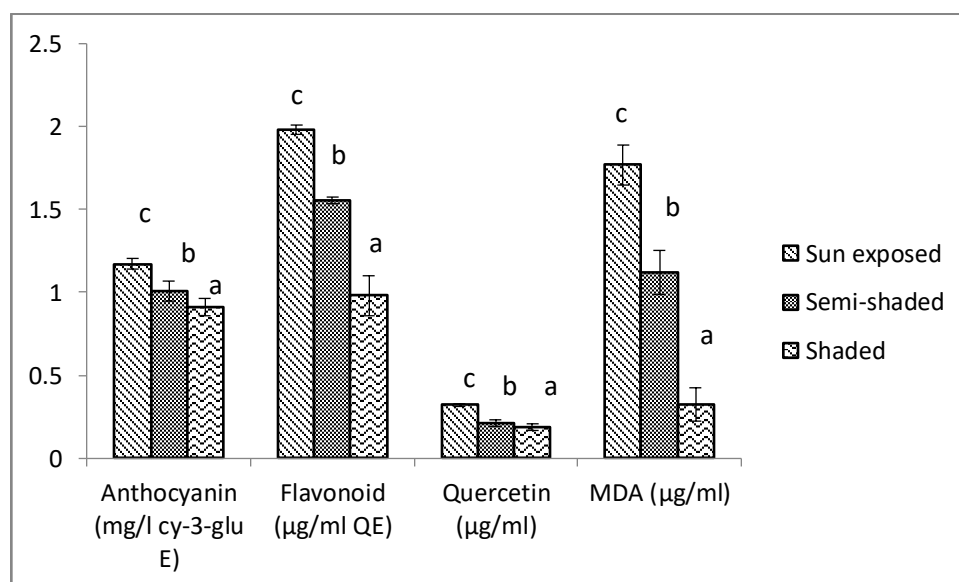


Figure 1: Effect of light intensity on secondary metabolites and MDA content (Different small letters indicate significant differences in secondary metabolites among three light conditions $p < 0.05$).

In contrast, shaded plants exhibited the lowest levels of these photoprotective compounds. This outcome is in line with expectations (Lee *et al.*, 2013; Miliauskiene *et al.*, 2021; Mohamed *et*

al., 2021; Samuoliene *et al.*, 2021; Samuoliene *et al.*, 2022), as shaded conditions inherently offer reduced exposure to UV radiation. As a result, the plant's need to produce high levels of flavonoids, anthocyanin, and

quercetin is diminished, leading to lower levels of these compounds compared to their sun-exposed counterparts.

Moreover, the observed increase in MDA, a marker of lipid peroxidation and oxidative stress (Bian *et al.*, 2016), in sun-exposed and semi-shaded plants further supports the notion that these plants are subjected to higher levels of oxidative stress due to increased light exposure. This, in turn, triggers a stronger antioxidative response characterized by elevated flavonoid and quercetin production.

These findings collectively highlight the dynamic and adaptive responses of *L. sativa* to varying light conditions. The accumulation of flavonoids, anthocyanin, and quercetin, along with the heightened antioxidant enzyme activities observed in sun-exposed and shaded plants, underscore their critical role in defending against UV-induced damage and oxidative stress. Such insights have the potential to contribute to the optimization of agricultural practices and crop breeding strategies, ultimately enhancing plant resilience and productivity in a changing environment (Vaštakait'e-Kairien'e *et al.*, 2022). A similar trend of increasing order of flavonoid with increasing light intensity was observed in *Ginkgo biloba* (Xu *et al.*, 2014), *Labisia pumila* (Karimi *et al.*, 2013), and *Lactuca sativa* (Pérez-López *et al.*, 2018). Studies have indicated the photo protective role of anthocyanin (Arena *et al.*, 2017; Cheng *et al.*, 2018; Edreva, 2005; Debski *et al.*, 2017; Gould, 2004; Hughes, 2009; Neill, 2002; Shourie *et al.*, 2014; Trojak & Skowron, 2017; Yuan *et al.*, 2015). Quercetin is a flavonoid possessing a dihydroxy-B-ring which gives it the photo protective characteristic (Agati *et al.*, 2011; Agati *et al.*, 2013; Brunetti *et al.*, 2013; Edreva, 2005; Pérez-López *et al.*, 2018; Shourie *et al.*, 2014; Zhang *et al.*, 2017).

Some studies also indicated that higher amount of quercetin was recovered under full sunlight (Debski *et al.*, 2017; Karimi *et al.*, 2013; Shourie *et al.*, 2014; Tattini *et al.*, 2005). Biosynthesis of quercetin can be decreased by shading (Cortell & Kennedy, 2006; Downey *et al.*, 2004; Koyama *et al.*, 2012). However, in *Zingiber officinale*, a lower amount of quercetin was recorded under high light intensity because the plant accumulates higher amount of other phenolic compounds (Ghasemzadeh *et al.*, 2010). In *Berberis microphylla*, a lower amount of quercetin was recorded under high light intensity because the plant accumulates higher amount of soluble solids (Arena *et al.*, 2017). Vacuolar quercetin in mesophyll cells of *Catharanthus rosues* increases under high sunlight (Ferrerres *et al.*, 2011). Quercetin biosynthesis may have a contribution to natural selection, dynamic selection, evolutionary response and physiological response in plants (Lesne, 2008). Presence of quercetin, therefore, helps in UV-B tolerance (Jacobs & Rubery, 1988; Jansen *et al.*, 2001).

Studies had indicated an increasing level of MDA with increasing light intensity (Agati *et al.*, 2011; Distelbarth, Nagale, & Heyer, 2013; Gu *et al.*, 2017; Liu *et al.*, 2013; Tang *et al.*, 2015; Tattini *et al.*, 2005; Wang *et al.*, 2017). Other studies indicate a decreasing order of MDA as light intensity increases because the plants accumulate more H₂O₂ under lower light intensity (Ibrahim & Jaafar, 2012; Ibrahim *et al.*, 2014; Li *et al.*, 2016; Ma *et al.*, 2015; Zhu *et al.*, 2017). Other studies had indicated an increase in MDA content under blue light (Yu *et al.*, 2016), red and blue light (Bian *et al.*, 2018) and UV light (Alexieva *et al.*, 2001; Basahi *et al.*, 2014), because the light quality influence the oxidative stress.

The observed variations in the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Table 1) across different light conditions provide valuable insights into the plant's antioxidative defense mechanisms under varying levels of light exposure.

Table 1: Effect of light intensity on the activities of antioxidant enzymes

Light intensity	SOD (U/mg protein)	POD (U/mg protein)	CAT (U/mg protein)
Sun exposed	1.12±0.01 ^a	99.61±1.12 ^a	5.88±0.91 ^a
Semi-shaded	1.42±0.02 ^b	116.33±2.11 ^b	7.43±0.63 ^b
Shaded	1.93±0.05 ^c	121.54±1.91 ^c	11.55±1.1 ^c

Key; Different small letters indicate significant differences in antioxidant activity among three light

conditions ($p < 0.05$).

In shaded plants, the higher activity of antioxidant enzymes suggests an enhanced capacity to cope with oxidative stress. Shaded conditions inherently lead to reduced light exposure, which might prompt the plant to prioritize other defense mechanisms. The elevated levels of these antioxidant enzymes indicate that shaded plants are equipped to manage oxidative stress through efficient scavenging of reactive oxygen species (ROS) and detoxification of harmful molecules.

Conversely, the lower activity of these enzymes in sun-exposed plants aligns with the higher levels of oxidative stress indicators such as MDA observed in these conditions. The decrease in antioxidative enzyme activity in sun-exposed plants might be attributed to the fact that these plants are under constant light stress, causing a depletion of antioxidative resources due to the heightened demand for ROS scavenging.

Semi-shaded plants, exhibiting intermediate levels of antioxidant enzymes activity, seem to reflect a balance between the physiological responses of sun-exposed and shaded plants. This observation suggests that partial exposure to sunlight prompts the plant to trigger a moderate antioxidative response, likely to counteract the oxidative stress induced by UV radiation while conserving energy for other growth processes.

Overall, the activities of antioxidant enzymes serve as indicators of how *L. sativa* adapts its antioxidative defense strategies to the prevailing light conditions. These enzymes play a pivotal role in maintaining redox homeostasis and protecting plant cells from oxidative damage (Bian *et al.*, 2016; Idris 2019; Idris *et al.*, 2021). The variations in their activities across different light conditions underscore the dynamic nature of the plant's response to light exposure and its ability to fine-tune its antioxidative machinery to optimize survival and growth. This knowledge contributes not only to our understanding of plant biology but also has potential implications for crop management strategies aimed at enhancing plant resilience in changing environmental conditions.

It has been reported that a decrease in antioxidant enzymes cause a decrease in hydrogen peroxide concentration in the chloroplast due to the presence of chloroplast flavonoids (Agati *et al.*, 2013). Short

term light stress can increase antioxidant enzymes like SOD, CAT and POD while long term stress can decrease their activity (Chen *et al.*, 2016). High light intensity can lead to an increase in the expression of flavonoid biosynthesis genes (Agati *et al.*, 2012), decrease in expression of photosynthetic genes (Pego *et al.*, 2000) and a decrease in activity of antioxidant enzymes (Fini *et al.*, 2011). The activity of SOD may decrease due to light-induced hydrogen peroxide generation (Peltzer & Polle, 2001).

Other studies also indicate a decreasing order of antioxidant enzymes with increasing light intensity (Chen *et al.*, 2016; Lu *et al.*, 2017; Ma *et al.*, 2015). Studies had reported an increase in antioxidant enzymes with increasing light intensity because few ROS is generated, as the light is used for photosynthesis (Agati *et al.*, 2011; Gu *et al.*, 2017; Li *et al.*, 2016; Liu *et al.*, 2013; Tang *et al.*, 2015; Tattini *et al.*, 2005; Wang *et al.*, 2017). UV light can increase antioxidant enzymes activity (Alexieva *et al.*, 2001; Basahi *et al.*, 2014). Basahi *et al.* (2014) claimed that contradictory results about antioxidant enzyme activity under different light stresses is due to the fact that the levels of enzyme responses depend on the plant species, the developmental stage, the organs, as well as on the duration and severity of the stress. Likewise in *Clidemia acuminata*, the activities of antioxidant enzymes of the plant are not inhibited by high light stress (Yu *et al.*, 2016).

The comparison between the results of quercetin content and the activities of antioxidant enzymes, namely SOD, POD, and CAT, offers a comprehensive view of how quercetin contributes to the antioxidative defense mechanisms of *L. sativa* under varying light conditions. The observed trend of quercetin content aligns with the plant's response to light exposure. Sun-exposed plants exhibited the highest levels of quercetin, suggesting that increased UV radiation prompts the plant to synthesize and accumulate this flavonoid as a protective response. Quercetin's antioxidative properties are well-documented, as it acts as a scavenger of ROS and helps mitigate oxidative damage caused by UV radiation (Idris *et al.*, 2021). The positive correlation between quercetin content and light exposure underscores its role in bolstering

the plant's antioxidative defenses.

The activities of antioxidant enzymes reflect the plant's enzymatic responses to oxidative stress. Shaded plants exhibited higher activities of these enzymes, indicating their need to manage oxidative stress even in reduced light conditions. The elevated activity of these enzymes suggests that shaded plants are equipped to cope with oxidative damage through efficient ROS scavenging and detoxification.

Comparing quercetin content with antioxidant enzyme activities, it's evident that quercetin plays a significant role in reinforcing the antioxidative defense mechanisms. The higher quercetin content in sun-exposed plants aligns with their lower activities of SOD, POD, and CAT. This suggests that the presence of quercetin contributes to alleviating the burden on enzymatic antioxidative pathways. In these conditions, quercetin's direct ROS-scavenging properties might be more prevalent, reducing the reliance on enzymatic defense mechanisms.

Conversely, in shaded plants with higher antioxidant enzyme activities, quercetin content is comparatively lower. This observation implies that these plants may rely more heavily on enzymatic defenses due to reduced quercetin availability. The combination of higher antioxidant enzyme activities and reduced quercetin content indicates a more prominent role for enzymatic antioxidative mechanisms in these conditions.

In semi-shaded plants, the balance between quercetin content and antioxidant enzyme activities suggests a harmonious interaction between quercetin's direct antioxidative role and the enzymatic defenses. Here, the moderate quercetin content complements the intermediate activities of antioxidant enzymes, potentially indicating a cooperative response to oxidative stress.

In summary, the comparison between quercetin content and antioxidant enzyme activities reveals a dynamic interplay between direct and enzymatic antioxidative mechanisms in *L. sativa*'s response to varying light conditions. Quercetin's presence

appears to influence the reliance on enzymatic defenses, with potential implications for how the plant allocates its resources to combat oxidative stress induced by UV radiation. Understanding this interaction contributes to a deeper comprehension of plant adaptation strategies and informs potential avenues for enhancing plant resilience in changing environmental contexts.

The contrasting trends observed between the results of quercetin content, carotenoids, soluble sugars, soluble proteins, and chlorophyll content (Table 2) offer a complex insight into *L. sativa*'s responses to varying light conditions and their interplay with different biochemical compounds.

As previously discussed, quercetin content was found to be highest in sun-exposed plants, suggesting its role in protecting the plant from UV-induced oxidative stress. The higher levels of carotenoids, soluble sugars, and soluble proteins in sun-exposed plants indicate an adaptive response to increased light exposure. Carotenoids, which contribute to photosynthesis and offer photo protection (Müller *et al.*, 2013; Sousa Paiva *et al.*, 2003; Triantaphylides *et al.*, 2008), are likely upregulated in response to higher light availability. Soluble sugars and proteins can act as energy sources and building blocks for stress-responsive proteins, respectively, and their elevated levels in sun-exposed plants may signify their roles in supporting growth and defense mechanisms under high light conditions.

In contrast to the other compounds, chlorophyll content was highest in shaded plants. This can be attributed to a shade-induced response where plants allocate more resources to chlorophyll production to optimize light capture in low light conditions. Many plants growing in shaded environments tend to accumulate a higher amount of chlorophyll than their sun-exposed species (Belgio *et al.*, 2017; Chen *et al.*, 2016; Chuyong & Acidri, 2017; Fu *et al.*, 2017; Gregoriou *et al.*, 2007; Jespersen *et al.*, 2017; Khan *et al.*, 2000; Li *et al.*, 2010; Li *et al.*, 2014; Wang *et al.*, 2017; Yao *et al.*, 2017).

Table 2: Effect of light intensity on pigments and primary metabolites

Light intensity	Sugar ($\mu\text{g/ml}$)	Protein ($\mu\text{g/ml}$)	Car ($\mu\text{g/ml}$)	Chl ($\mu\text{g/ml}$)	a+b
Sun exposed	14.52 $\pm$ 0.93 ^c	6.33 $\pm$ 0.05 ^c	0.79 $\pm$ 0.04 ^c	0.71 $\pm$ 0.06 ^a	
Semi-shaded	11.32 $\pm$ 0.21 ^b	5.10 $\pm$ 0.11 ^b	0.71 $\pm$ 0.03 ^b	0.88 $\pm$ 0.02 ^b	
Shaded	8.21 $\pm$ 0.09 ^a	4.91 $\pm$ 0.17 ^a	0.4 $\pm$ 0.06 ^a	1.10 $\pm$ 0.91 ^c	

Key; Different small letters indicate significant differences among three light conditions ($p < 0.05$).

When considering the entire picture, we can see a coordinated effort by *L. sativa* to adapt to its environment. Sun-exposed plants invest heavily in photo protective compounds like quercetin, carotenoids, soluble sugars, and soluble proteins to counteract the increased oxidative stress and energy demands caused by high light exposure. The synthesis of quercetin and carotenoids, in particular, may function as a first line of defense against excess light condition.

On the other hand, shaded plants prioritize efficient light capture, as indicated by higher chlorophyll content, to maximize their limited light resources. This comes at the cost of reduced synthesis of photo protective compounds like quercetin, leading to lower quercetin content in shaded plants.

Semi-shaded plants exhibit intermediate levels in most parameters, reflecting their status between full sun exposure and complete shade. The balance between different responses may contribute to a more adaptable strategy that allows for moderate photo protection while optimizing light capture.

In summary, the results suggest that *L. sativa* employs a range of strategies to adapt to varying light conditions. Quercetin and other biochemical compounds play crucial roles in this adaptation, with their synthesis and accumulation reflecting the plant's attempt to strike a balance between managing oxidative stress, optimizing photosynthesis, and conserving resources in response to light availability. These findings contribute to our understanding of plant responses to environmental cues and can have implications for optimizing agricultural practices and crop productivity.

CONCLUSION

The comprehensive investigation into the photo protective responses of *L. sativa* to varying light conditions has shed light on the intricate interplay

between biochemical compounds and plant adaptation strategies. Through a meticulous analysis of quercetin content, antioxidant enzyme activities, carotenoids, soluble sugars, soluble proteins, and chlorophyll content, a multifaceted narrative of how *L. sativa* navigates its environment has emerged.

Our findings highlight the pivotal role of quercetin as a key player in the plant's defense against UV-induced oxidative stress. Its content exhibited a direct correlation with light exposure, underlining its significance as a photo protective agent that shields the plant from harmful effects of excess light intensity.

The variations in antioxidant enzyme activities, such as superoxide dismutase, peroxidase, and catalase, further underscore the plant's dynamic antioxidative defense mechanisms. These enzymes act as essential safeguards against oxidative stress, and their activities vary in response to the intensity of light exposure. The interplay between quercetin and antioxidant enzymes reveals a complex orchestration of direct and enzymatic antioxidative responses, contributing to the plant's resilience in fluctuating light environments.

Moreover, the contrasting trends observed in carotenoids, soluble sugars, soluble proteins, and chlorophyll content emphasize the adaptability of *Lactuca sativa* to different light conditions. The synthesis of these compounds demonstrates the plant's ability to fine-tune its resource allocation, optimizing growth and photo protection based on environmental cues.

Therefore, our research not only provides a deeper understanding of *L. sativa*'s responses to light exposure but also underscores the significance of quercetin as a multifunctional compound in photo protection. The intricate interactions between quercetin, antioxidant enzymes, and other biochemical compounds showcase the plant's

capacity for flexible adaptation to environmental stressors. These insights have broader implications for agricultural practices, offering potential strategies to enhance crop resilience and productivity in a changing climate. As we continue to uncover the complexities of plant-environment interactions, further research in this field holds the promise of unveiling novel avenues for sustainable crop management and ecological conservation.

REFERENCES

- Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science*, 196, 67–76.
- Agati, G., Biricolti, S., Guidi, L., Ferrini, F., Fini, A., & Tattini, M. (2011). The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in *L. vulgare* leaves. *Journal of Plant Physiology*, 168(3), 204–212.
- Agati, G., Brunetti, C., Di Ferdinando, M., Ferrini, F., Pollastri, S., & Tattini, M. (2013). Functional roles of flavonoids in photoprotection: New evidence, lessons from the past. *Plant Physiology and Biochemistry*, 72, 35–45.
- Alexieva, V., Sergiev, I., Mapelli, S., & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell and Environment*, 24, 1337–1344.
- Arena, M. E., Postemsky, P. D., & Curvetto, N. R. (2017). Scientia Horticulturae Changes in the phenolic compounds and antioxidant capacity of *Berberis microphylla* G. Forst. berries in relation to light intensity and fertilization. *Scientia Horticulturae*, 218, 63–71.
- Basahi, J. M., Ismail, I. M., & Hassan, I. A. (2014). Effects of Enhanced UV-B Radiation and Drought Stress on Photosynthetic Performance of Lettuce (*Lactuca sativa* L. Romaine) Plants. *Annual Research & Review in Biology*, 4(11), 1739–1756.
- Belgio, E., Trsková, E., Kotabová, E., Ewe, D., Prášil, O., & Kaňa, R. (2017). High light acclimation of *Chromera velia* points to photoprotective NPQ. *Photosynthesis Research*, 135(1–3), 263–274.
- Bian, Z., Cheng, R., Wang, Y., Yang, Q., & Lu, C. (2018). Effect of green light on nitrate reduction and edible quality of hydroponically grown lettuce (*Lactuca sativa* L.) under short-term continuous light from red and blue light-emitting diodes. *Environmental and Experimental Botany*, 153(December 2017), 63–71.
- Chuyong, G. B., & Acidri, T. (2017). Light and moisture levels affect growth and physiological parameters differently in *Faidherbia albida* (Delile) A. Chev. seedlings. *Acta Physiologiae Plantarum*, 39(5), 1–6.
- Cortell, J. M., & Kennedy, J. A. (2006). Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot noir fruit and extraction in a model system. *Journal of Agricultural and Food Chemistry*, 54(22), 8510–8520.
- Dar, F. A., Jain, K., Jain, B., & Modak, M. (2017). Preliminary Phytochemical Analysis and Characterization of Flavonoid Moiety from *Vitex negundo* Leaves Origin in Madhya Pradesh State by HPLC Study. *UK Journal of Pharmaceutical and Biosciences*, 5(5), 60–65.
- Debski, H., Wiczowski, W., Szawara-Nowak, D., Baczek, N., Szwed, M., & Horbowicz, M. (2017). Enhanced Light Intensity Increases Flavonol and Anthocyanin Concentrations but Reduces Flavone Levels in the Cotyledons of Common Buckwheat Seedlings. *Cereal Research Communications*, 45(2), 225–233.
- Distelbarth, H., Nagale, T., & Heyer, A. G. (2013). Responses of antioxidant enzymes to cold and high light are not correlated to freezing tolerance in natural accessions of *Arabidopsis thaliana*. *Plant Biology*, 1, 1–9.
- Downey, M. O., Harvey, J. S., & Robinson, S. P. (2004). The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research*, 10, 55–73.
- Edreva, A. (2005). The importance of non-photosynthetic pigments and cinnamic acid derivatives in photoprotection. *Agriculture, Ecosystems and Environment*, 106(2-3 SPEC. ISS.), 135–146.
- Fanglian, H. (2011). Bradford Protein Assay. *Bio-Protocol*, 1(6), 4–5.

- Ferreres, F., Figueiredo, R., Bettencourt, S., Carqueijeiro, I., Oliveira, J., Gil-Izquierdo, A., Pereira, D. M., Valentão, P., Andrade, P. B., Duarte, P., Barceló, A. R., & Sottomayor, M. (2011). Identification of phenolic compounds in isolated vacuoles of the medicinal plant *Catharanthus roseus* and their interaction with vacuolar class III peroxidase: An H₂O₂ affair? *Journal of Experimental Botany*, 62(8), 2841–2854.
- Fini, A., Brunetti, C., Ferdinando, M. Di, Ferrini, F., & Tattini, M. (2011). Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. *Plant Signaling and Behavior*, 6(5), 709–711.
- Fu, Y., Li, H. Y., Yu, J., Liu, H., Cao, Z. Y., Manukovsky, N. S., & Liu, H. (2017). Interaction effects of light intensity and nitrogen concentration on growth, photosynthetic characteristics and quality of lettuce (*Lactuca sativa* L. Var. youmaicai). *Scientia Horticulturae*, 214, 51–57.
- Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2010). Synthesis of phenolics and flavonoids in ginger (*Zingiber officinale* Roscoe) and their effects on photosynthesis rate. *International Journal of Molecular Sciences*, 11(11), 4539–4555.
- Gould, K. S. (2004). Nature 's Swiss Army Knife : The Diverse Protective Roles of Anthocyanins in Leaves. *Journal of Biomedicine and Biotechnology*, 5, 314–320.
- Gregoriou, K., Pontikis, K., & Vemmos, S. (2007). Effects of reduced irradiance on leaf morphology , photosynthetic capacity , and fruit yield in olive (*Olea europaea* L .). *Photosynthetica*, 45(2), 172–181.
- Gu, J., Zhou, Z., Li, Z., Chen, Y., Wang, Z., Zhang, H., & Yang, J. (2017). Photosynthetic Properties and Potentials for Improvement of Photosynthesis in Pale Green Leaf Rice under High Light Conditions. *Frontiers in Plant Science*, 8(June), 1–14.
- Hughes, M. N. (2009). *The photoprotective role of anthocyanin pigments in leaf tissues*. Wake Forest University.
- Ibrahim, M. H., & Jaafar, H. Z. E. (2012). Primary, Secondary Metabolites, H₂O₂, Malondialdehyde and Photosynthetic Responses of *Orthosiphon stamineus* Benth. to Different Irradiance Levels. *Molecules*, 17, 1159–1176.
- Ibrahim, M. H., Jaafar, H. Z. E., Karimi, E., & Ghasemzadeh, A. (2014). Allocation of secondary metabolites, photosynthetic capacity, and antioxidant activity of *Kacip Fatimah* (*Labisia pumila* Benth) in response to CO₂ and light intensity. In *The Scientific World Journal* (Vol. 2014).
- Idris, A., Alona C. L., Munir G, Zakiyyu IT. (2021). Photoprotective role of quercetin to *Tetracera sarmentosa*. *ASM science journal*. Vol 16, special issue 1. pp 196-210.
- Idris, A. (2019). Evaluation on the photoprotective role of quercetin to selected light adapted and shade tolerant plant species. *University Tun Hussein Onn Malaysia*.
- Jacobs, M., & Rubery, P. H. (1988). Naturally Occurring Auxin Transport Regulators. *Science*, 241(4863), 346–349.
- Jansen, M. A., van den Noort, R. E., Tan, M. Y., Prinsen, E., Lagrimini, L. M., & Thorneley, R. N. (2001). Phenol-oxidizing peroxidases contribute to the protection of plants from ultraviolet radiation stress. *Plant Physiology*, 126(3), 1012–1023.
- Jespersen, E., Brix, H., & Sorrell, B. K. (2017). Acclimation to light and avoidance of photoinhibition in *Typha latifolia* is associated with high photosynthetic capacity and xanthophyll pigment content. *Functional Plant Biology*, 44(8), 774–784.
- Karimi, E., Jaafar, H. Z. E., Ghasemzadeh, A., & Ibrahim, M. H. (2013). Light intensity effects on production and antioxidant activity of flavonoids and phenolic compounds in leaves, stems and roots of three varieties of *Labisia pumila* Benth. *Australian Journal of Crop Science*, 7(7), 1016–1023.
- Khan, S. R., Rose, R., Haase, D. L., & Sabin, T. E. (2000). Effects of shade on morphology, chlorophyll concentration, and chlorophyll fluorescence of four Pacific Northwest conifer species. *New Forests*, 19(2), 171–186.
- Koyama, K., Ikeda, H., Poudel, P. R., & Goto-Yamamoto, N. (2012). Light quality affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape. *Phytochemistry*,

- 78, 54–64.
- Lee, J. (2005). Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *Journal of AOAC International*, 88(5), 1269–1278.
- Lee, M-J, Jung E.S., & Myung-Min O. (2013). Growth and phenolic compounds of *Lactuca sativa* L. grown in a closed-type plant production system with UV-A, -B, or -C lamp. *J Sci Food Agric*. 1-8.
- Lesne, A. (2008). Robustness: confronting lessons from physics and biology. *Biological Reviews*, 83(4), 509–532.
- Li, A., Li, S., Wu, X., Zhang, J., He, A., Zhao, G., & Yang, X. (2016). Effect of Light Intensity on Leaf Photosynthetic Characteristics and Accumulation of Flavonoids in *Lithocarpus litseifolius* (Hance) Chun. (Fagaceae). *Open Journal of Forestry*, 06(05), 445–459.
- Li, H., Jiang, D., Wollenweber, B., Dai, T., & Cao, W. (2010). Effects of shading on morphology, physiology and grain yield of winter wheat. *European Journal of Agronomy*, 33(4), 267–275.
- Li, Q., Deng, M., Xiong, Y., Coombes, A., & Zhao, W. (2014). Morphological and photosynthetic response to high and low irradiance of *aeschynanthus longicaulis*. *Scientific World Journal*, 2014, 1–9.
- Lichtenthaler, H. K., & Buschmann, C. (2001). chlorophylls and Carotenoids : Measurement and Characterization by UV-VIS. In *Current Protocols in Food Analytical Chemistry* (pp. 1–8)
- Liu, S.-L., Ma, M.-D., Pan, Y.-Z., Wei, L.-L., He, C.-X., & Yang, K.-M. (2013). Effects of light regimes on photosynthetic characteristics and antioxidant system in seedlings of two alder species. *Chinese Journal of Plant Ecology*, 36(10), 1062–1074.
- Lu, T., Meng, Z., Zhang, G., Qi, M., Sun, Z., Liu, Y., & Li, T. (2017). Sub-high Temperature and High Light Intensity Induced Irreversible Inhibition on Photosynthesis System of Tomato Plant (*Solanum lycopersicum* L.). *Frontiers in Plant Science*, 08(March), 1–16.
- Ma, X., Song, L., Yu, W., Hu, Y., Liu, Y., Wu, J., & Ying, Y. (2015). Growth, physiological, and biochemical responses of *Camptotheca acuminata* seedlings to different light environments. *Frontiers in Plant Science*, 6, 321.
- Miliauskienė, J.; Karlicek, R.F., Jr.; Kolmos, E. (2021). Effect of Multispectral Pulsed Light-Emitting Diodes on the Growth, Photosynthetic and Antioxidant Response of Baby Leaf Lettuce (*Lactuca sativa* L.). *Plants*, 10, 762
- Mohamed, S.J. Rihan, H.Z. Aljafer, N. & Fuller, M.P. (2021). The Impact of Light Spectrum and Intensity on the Growth, Physiology, and Antioxidant Activity of Lettuce (*Lactuca sativa* L.). *Plants*, 10, 2162.
- Müller, V., Albert, A., Barbro Winkler, J., Lankes, C., Noga, G., & Hunsche, M. (2013). Ecologically relevant UV-B dose combined with high PAR intensity distinctly affect plant growth and accumulation of secondary metabolites in leaves of *Centella asiatica* L. Urban. *Journal of Photochemistry and Photobiology B: Biology*, 127, 161–169.
- Neill, S. O. (2002). The functional role of anthocyanins in leaves. In *PhD Thesis* (Vol. 1994). University of Auckland.
- Pego, J. V, Kortstee, A. J., Huijser, C., & Smeekens, S. C. (2000). Photosynthesis, sugars and the regulation of gene expression. *Journal of Experimental Botany*, 51, 407–416.
- Peltzer, D., & Polle, A. (2001). Diurnal fluctuations of antioxidative systems in leaves of field-grown beech trees (*Fagus sylvatica*): Responses to light and temperature. *Physiologia Plantarum*, 111(2), 158–164.
- Pérez-López, U., Sgherri, C., Miranda-Apodaca, J., Micaelli, F., Lacuesta, M., Mena-Petite, A., Quartacci, M. F., & Muñoz-Rueda, A. (2018). Concentration of phenolic compounds is increased in lettuce grown under high light intensity and elevated CO₂. *Plant Physiology and Biochemistry*, 123, 233–241.
- Samuoliene G, Viršilė A, Miliauskienė J, Haimi PJ, Laužikė K, Brazaitytė A & Duchovskis P (2021). The Physiological Response of Lettuce to Red and Blue Light Dynamics Over Different Photoperiods. *Front. Plant Sci.* 11:610174.
- Shourie, A., Tomar, P., Srivastava, D., & Chauhan,

- R. (2014). Enhanced Biosynthesis of Quercetin Occurs as A Photoprotective Measure in *Lycopersicon esculentum* Mill. under Acute UV-B Exposure. *Brazilian Archives of Biology and Technology*, 57(3), 317–325.
- Sousa Paiva, É. A., Dos Santos Isaias, R. M., Aguiar Vale, F. H., & De Senna Queiroz, C. G. (2003). The influence of light intensity on anatomical structure and pigment contents of *Tradescantia pallida* (Rose) Hunt. cv. *purpurea boom* (commelinaceae) leaves. *Brazilian Archives of Biology and Technology*, 46(4), 617–624.
- Sumanta, N., Haque, C. I., Nishika, J., & Suprakash, R. (2014). Spectrophotometric Analysis of chlorophylls and Carotenoids from Commonly Grown Fern Species by Using Various Extracting Solvents. *Research Journal of Chemical Sciences*, 4(9), 63–69.
- Tang, H., Hu, Y.-Y., Yu, W.-W., Song, L.-L., & Wu, J.-S. (2015). Growth, photosynthetic and physiological responses of *Torreya grandis* seedlings to varied light environments. *Trees*, 29(4), 1011–1022.
- Tattini, M., Guidi, L., Morassi-Bonzi, L., Pinelli, P., Remorini, D., Degl'Innocenti, E., Giordano, C., Massai, R., & Agati, G. (2005). On the role of flavonoids in the integrated mechanisms of response of *Ligustrum vulgare* and *Phillyrea latifolia* to high solar radiation. *New Phytologist*, 167(2), 457–470.
- Tianzi, C., & Baolong, Z. (2016). Measurements of Proline and Malondialdehyde Content and Antioxidant Enzyme Activities in Leaves of Drought Stressed Cotton. *Bio-Protocol*, 6(17), 1–14.
- Triantaphylides, C., Krischke, M., Hoeberichts, F. A., Ksas, B., Gresser, G., Havaux, M., Van Breusegem, F., & Mueller, M. J. (2008). Singlet Oxygen Is the Major Reactive Oxygen Species Involved in Photooxidative Damage to Plants. *Plant Physiology*, 148(2), 960–968.
- Trojak, M., & Skowron, E. (2017). Role of anthocyanins in high-light stress response. *World Scientific News*, 81(2), 150–168.
- Vaštakait'ė-Kairien'ė, V.; Samuolien'ė, G.; Šveikauskas, V.; Laužik'ė, K.; Jurkonien'ė, S. (2022). The Influence of End-of-Day Blue Light on the Growth, Photosynthetic, and Metabolic Parameters of Lettuce at Different Development Stages. *Plants* 11, 2798.
- Wang, C. L., Guo, Q. S., Zhu, Z. B., & Cheng, B. X. (2017). Physiological characteristics, dry matter, and active component accumulation patterns of *Changium smyrnioides* in response to a light intensity gradient. *Pharmaceutical Biology*, 55(1), 581–589.
- Xu, Y., Wang, G., Cao, F., Zhu, C., Wang, G., & El-Kassaby, Y. A. (2014). Light intensity affects the growth and flavonol biosynthesis of Ginkgo (*Ginkgo biloba* L.). *New Forests*, 45(6), 765–776.
- Yao, X. yang, Liu, X. ying, Xu, Z. gang, & Jiao, X. lei. (2017). Effects of light intensity on leaf microstructure and growth of rape seedlings cultivated under a combination of red and blue LEDs. *Journal of Integrative Agriculture*, 16(1), 97–105.
- Yu, W., Liu, Y., Song, L., Jacobs, D. F., Du, X., Ying, Y., Shao, Q., & Wu, J. (2016). Effect of Differential Light Quality on Morphology, Photosynthesis, and Antioxidant Enzyme Activity in *Camptotheca acuminata* Seedlings. *Journal of Plant Growth Regulation*, 36(1), 148–160.
- Yuan, M., Jia, X., Ding, C., Zeng, H., Du, L., Yuan, S., Zhang, Z., Wu, Q., Hu, C., & Liu, J. (2015). Effect of fluorescence light on phenolic compounds and antioxidant activities of soybeans (*Glycine max* L. Merrill) during germination. *Food Science and Biotechnology*, 24(5), 1859–1865. 0243-4
- Zhang, Q., Liu, M., & Ruan, J. (2017). Metabolomics analysis reveals the metabolic and functional roles of flavonoids in light-sensitive tea leaves. *BMC Plant Biology*, 17(1), 1–10.
- Zhang, Z., & Rongfeng, H. (2013). Analysis of Malondialdehyde, Chlorophyll Proline, Soluble Sugar, and Glutathione Content in *Arabidopsis* seedling. *Bio-Protocol*, 3(14), 1–8.
- Zhu, H., Li, X., Zhai, W., Liu, Y., Gao, Q., Liu, J., Ren, L., Chen, H., & Zhu, Y. (2017). Effects of low light on photosynthetic properties, antioxidant

enzyme activity, and anthocyanin accumulation in purple pak-choi (*Brassica campestris* ssp.

Chinensis Makino). *PLoS ONE*, 12(6), 1–17.