



Morphological and Photosynthetic Responses of Kale (*Brassica oleracea* var. *sabellica*) Grown Under Different Light Conditions

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ABSTRACT

Kale is known for its anticancer properties and is rich in nutrients. Light plays a major role in plant growth. This study determined the effects of different light conditions on kale in terms of morphological, yield, and physiological responses. The treatments used in this study were Ambient Light (AL) and 16:8 light-dark period schemes on Red LED (RL), Green LED (GL), and Blue LED (BL). The treatments were applied six days before harvest. Kale grown under GL and BL became the tallest, four times taller than the kale treated with AL. However, in terms of leaf length and width, AL resulted in the most significant values, compared to seedlings grown under controlled lighting. Regarding the yield response, no significant differences were noted on fresh weight, dry weight, and estimated yield. In terms of physiological responses, RL, GL, and BL increased the average vapor pressure deficit. In terms of the average photosynthetic rate, BL performed better than AL. However, AL caused a higher stomatal conductance compared to LED lights. The average transpiration rate showed improvements under RL. These results suggested that GL and BL are most suited for plant height development while AL enhance the number, length, and width of leaves. Moreover, when grown under BL, the photosynthetic rate increased. On the other hand, exposure to AL improved stomatal conductance. The increase in photosynthetic rate led to the production of more secondary metabolites such as glucosinolate.

Introduction

Kale (*Brassica oleracea* var. *sabellica*), or Chinese broccoli, is a popular leafy vegetable in the Cruciferae family. It is a good source of vitamins A and C. Its antioxidant content and anticancer properties have made it a medicinal plant that cures several diseases (Ishida et al., 2014). Kale leaves are rich in flavonoids. Its extracts have suitable functional properties, such as anti-colitis, anti-ulcer, and anti-genotoxicity (Huang et al., 2022; Ming et al., 2019; Goncalves et al., 2012; Lemos et al., 2011). LEDs can potentially improve lighting for plant

development (Ishida et al., 2014). LEDs are efficient for experimental wheat cultivation in optimizing their growth conditions, manipulating metabolism, yield, and quality through modification of light quality and quantity (Monorstori et al., 2018). Several studies have reported that LED exposure resulted in optimal growth in plants. LED causes a rapid increase in height, greater fresh and dry weight, greater leaf area, crude fiber, and vitamins (Bantis et al., 2018; Rahman et al., 2021; Chen et al., 2022). Tomato seedlings grown under supplementary light resulted in increased values of shoot and root

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fresh weights, net photosynthetic rate, and total chlorophyll content (Zhang et al., 2022). Other studies on physiological responses on LED resulted in higher chlorophyll content and net photosynthetic rate, compared to those grown without supplementary light in cucumber (Yan et al., 2021).

Plant responses to light are modulated by signal transduction through various photoreceptors. These photoreceptors are known to perceive, interpret, and transduce light signals even at very low intensity via distinct intracellular signaling pathways that regulate photo responsive nuclear gene expression, leading to adaptive changes at the metabolic, cellular, and the whole organismic level (Li et al., 2011). This provides versatile options to control vital plant processes through a tailored light spectrum, which can be fitted with the absorption of plant photoreceptors (Olle and Virsile, 2013). Moreover, solid-state LEDs provide many advantages, such as longevity, safety, emitting lower heat, and enhanced light quality (Dąbrowski et al., 2015; Yeh and Chung, 2009).

The inclusion of healthy foods in our diets is becoming more and more important. This has led to an increase in the consumption of dietary vegetables due to their phytochemical contents like glucosinolates. However, light plays a major role in plant growth, physiological responses, the production of vitamin C and antioxidants in kale. These compounds are not essential for plant life but play a significant role in plant survival (Pagare et al., 2015).

Accordingly, the current study sought to apply red, blue, and green light-emitting diodes (LED) to examine the morphology, physiology, and yield in kale (*Brassica oleracea* var. *sabellica*). This may have benefits for the production of glucosinolate, a vital compound in kale.

Material and Methods

Experimental area and field layout

This study was conducted at the Center for Studies in Biotechnology CTU, Barili. An area of 8x18 meters was considered for placing 120 pots to represent the three replications, i.e. 40 pots per replication. The dimension of each pot was 19 cm × 15.5 cm × 13.5 cm.

Plant materials and treatments

Kale plants (*Brassica oleracea* var. *sabellica*) were grown from seeds. Kale seeds were sown on a 1:2:1 mixture of vermicompost, sand, and garden soil. Kale seedlings were grown under greenhouse conditions for 30 days after planting, which is the best time to yield more tender and sweeter products. These were then transferred

into a controlled environment for six days following an exposure duration (Ma et al., 2012). The treatments included different lights, i.e. T0-ambient light ($40.79 \mu\text{mol m}^{-2} \text{s}^{-1}$; $25.92 \text{ }^\circ\text{C}$), T1- red LED ($0.43 \text{ m}^{-2} \text{ s}^{-1}$; $20.87 \text{ }^\circ\text{C}$), T2- green LED ($0.51 \text{ m}^{-2} \text{ s}^{-1}$; $19.94 \text{ }^\circ\text{C}$), and T3- blue LED ($2.21 \text{ m}^{-2} \text{ s}^{-1}$; $23.54 \text{ }^\circ\text{C}$). Plants grown under controlled lights received the 16:8 light-dark period scheme at $20 \text{ }^\circ\text{C}$, following a procedure outlined by Tahera et al. (2019).

Data collection

Multiple parameters were gathered in this experiment. The increase in plant height (cm) was measured by subtracting the plant height after exposure to the light treatment from the initial plant height. The increase in leaf count was measured by subtracting the leaf count after the light treatments from the initial leaf count. Leaf length increase (cm) was the initial length of each sample plant from the base to the tip of the leaf, subtracted from the length developed under the lighting treatment. Leaf width increase (cm) was the initial width at the midpoint of the leaf blade minus the leaf width after exposure to LED lights. Fresh weight was the weight of harvested plants from 5 samples using a digital scale. Dry weight was the weight of oven-dried plants (at $70 \text{ }^\circ\text{C}$ for 48 h) from 5 samples using a digital scale. Computed yield t/ha was calculated by measuring the crop moisture content multiplied by the average number of crops per hectare, divided by 10,000. All photosynthetic parameters, i.e. photosynthetic rate of plants, transpiration rate, stomatal conductance, and vapor pressure deficit at leaf temperature, were measured using a portable photosynthesis system Li-Cor PPS 6800 (Mj Riches et al., 2020). These were done by clamping the PPS on the outer leaf chamber of 36-day-old plant leaves.

Data analysis

The experiment was laid out in a Randomized Complete Block Design (RCBD) with four treatments replicated three times. Data from the experiment were analyzed using one-way analysis of variance (ANOVA) via SPSS. Tukey's test ($P < 0.05$) was used for obtaining multiple comparisons among the mean values of the treatment groups.

Results

Morphological and yield responses

Fig. 1A indicates that the green and blue light emitting diode (LED) significantly improved the growth of kale after three days as it produced the tallest plant at 4.5 ± 0.21 SE cm and 4.67 ± 0.33 SE

cm, respectively. These results were four times higher than the control. The results were consistent after six days at harvest. This indicated that the green and blue LEDs can actuate the rapid growth response in the plant. This finding is essential because researchers have been investing time in improving plant height, which led to improved access to light by plants and determined plant resistance to lodging and crowding (Niu et al., 2021).

The leaf count is essential for plant development because it is where photosynthesis takes place. In this study, ambient light significantly caused more leaves than supplementary light at 2 ± 00 SE after three days and on the sixth day (Fig. 1B). The same treatment enhanced the leaf length of kale at 2.40 ± 0.13 SE cm on the third day of exposure

(Fig. 1C). However, plants grown on controlled conditions rapidly enhanced leaf length the following day and were already comparable with ambient light on the sixth day. In the case of leaf width, similar results were gathered where ambient light showed significant leaf width three days after exposure at 0.83 ± 0.41 SE cm, compared to samples exposed to supplementary light (Fig. 1D). The data indicated a positive effect of controlled lights on kale performance after 3 days where a rapid increase in the growth can be observed under controlled conditions, having comparable growth with the ambient light on the sixth day. In terms of yield response, the results showed no difference between treatments in fresh weight, dry weight, and computed yield (Fig. 2).

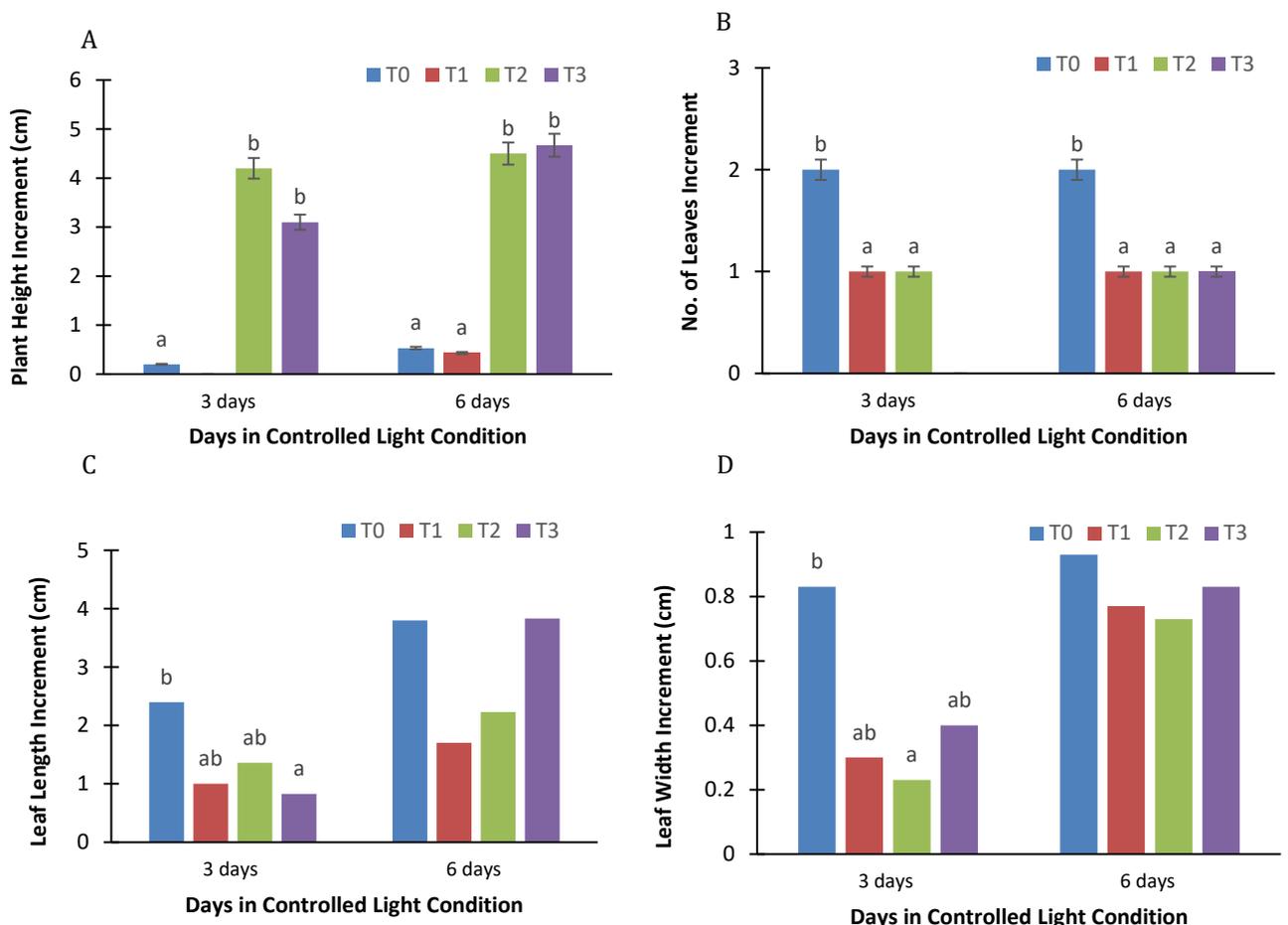


Fig. 1. Morphological responses of kale grown in T0- ambient light, T1- red LED, T2- green LED, and T3- blue LED: (A) increase in plant height, (B) increase in leaf count, (C) increase in leaf length, (D) increase in leaf width. Different small superscript letters indicate significant differences by Tukey's test ($p \leq 0.05$).

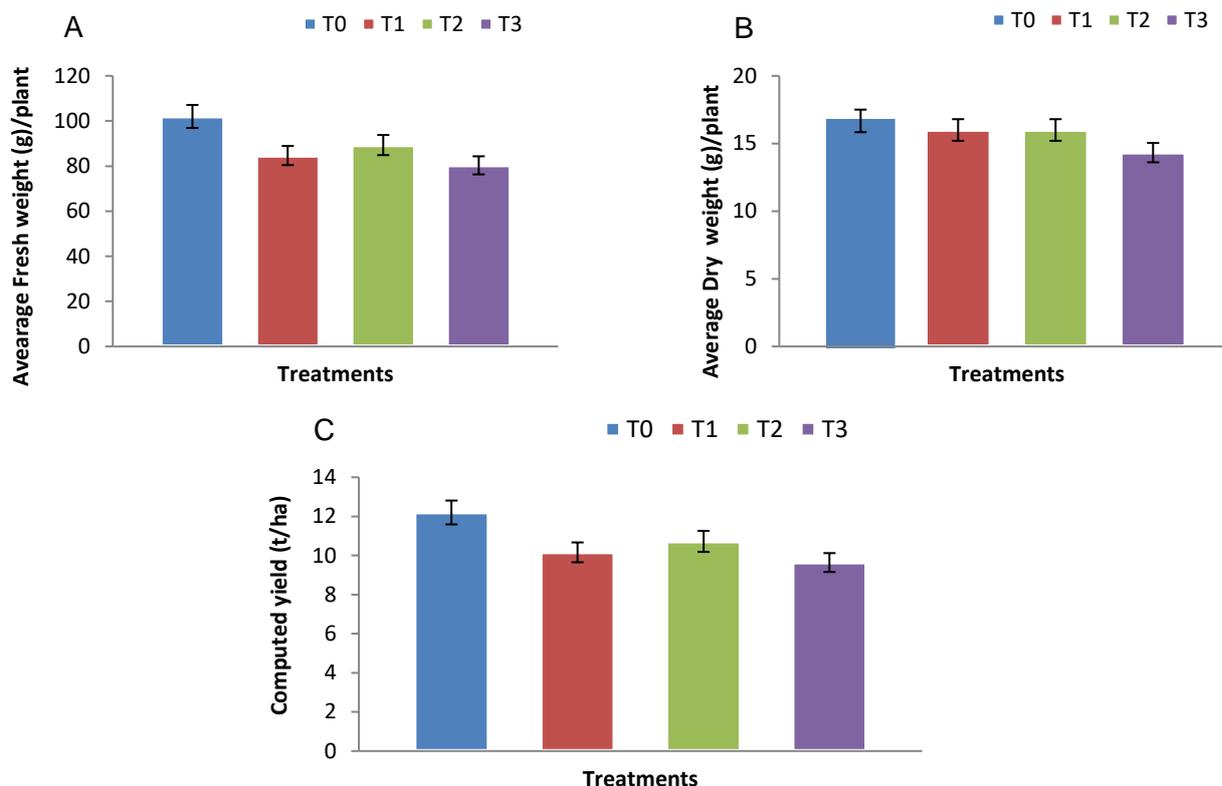


Fig. 2. Yield response of kale grown in T0- ambient light, T1- red LED, T2- green LED, and T3- white (blue) LED: (A) average fresh weight, (B) average dry weight, (C) computed yield.

Physiological responses

Kale plants grown under red, green, and blue LED had a significant difference in the average vapor pressure deficit at 1.09 ± 0.05 SE kPa, 0.99 ± 0.02 SE kPa, and 1.20 ± 0.11 SE kPa, respectively (Fig. 3D). This indicated that the ambient light showed 17.5% lower VPD than the other treatments. Blue LED enhanced the average photosynthetic rate of kale at 5.05 ± 0.28 SE $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3B). This resulted in a 40% increase in values by the supplemental light. On the other hand, red LED caused the highest transpiration rate at 0.0028 ± 0.0008 SE $\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3C). Ambient light resulted in 14% lower transpiration among the treatment groups. However, ambient light produced the greatest stomatal conductance at 1.22 ± 0.22 SE $\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 3A). The results of this study suggested the importance of LED lights on the physiological enhancement of plants. Farmers should also consider this in the future to produce healthier plants for consumers.

Discussion

Light is essential for plant development and

protection. This provides the energy needed for the photosynthetic process. Elevated PPFD ($100 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$) increased net photosynthetic rate resulting from the mean dry weight (mg/plant) and percentage of increase in dry matter by 0.26 and 0.11 times, respectively (Pascual et al., 2012). As such, artificial light confirmed the role and importance of light quality and the ability to strategically manipulate plant growth and development. The green and blue LEDs in this study led to the tallest plants. It was found that these penetrate the plant canopy better than the red and ambient light in terms of promoting plant development and in increasing the plant height (Klein, 1992; Snowden, 2015). Plants reportedly adapted to their light environment to maximize light interception and growth (Hogewoning et al., 2010). Che'e (1986) reported that blue light conditions in the *Vitis* hybrid 'Remaily seedless' resulted in greater shoot production. This exerts a positive and coordinated influence on both genomes, nuclear and plastid in the chloroplast environment for cellular development (Richter and Wessel, 1985).

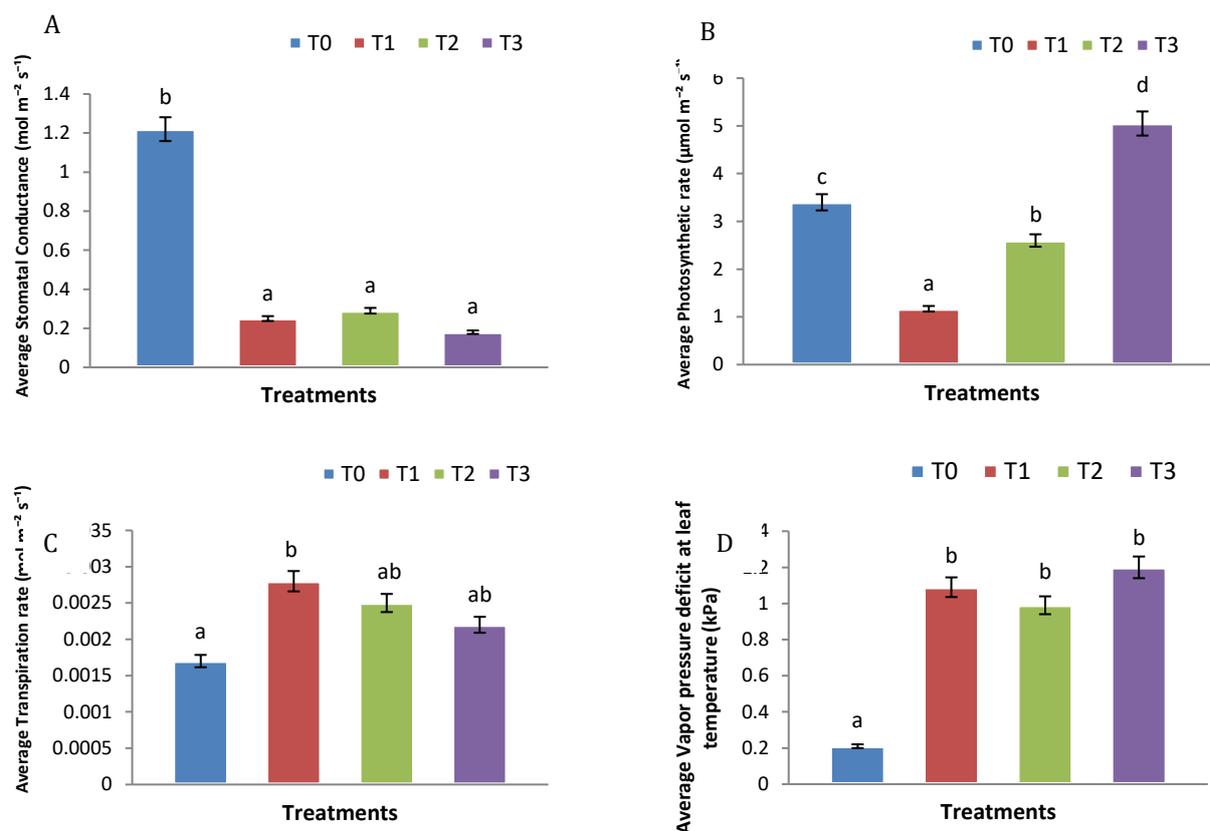


Fig. 3. Photosynthetic responses of kale grown under T0- ambient light, T1- red LED, T2- green LED, and T3- blue LED: (A) average stomatal conductance, (B) average photosynthetic rate, (C) average transpiration rate, (D) average vapor pressure deficit. Different small superscript letters indicate significant differences determined by Tukey's test ($p \leq 0.05$).

As for leaf count, Moss (1969) reported that administering 8 hours of natural daylight on plants caused them to grow more leaves, increase stem elongation, and staminate flowers. Moreover, the leaf count and leaf area indicated a strong positive correlation with light intensity (Zervoudakis et al., 2012; Nguyen et al., 2019).

Vapor pressure deficit

Vapor pressure deficit influences plant photosynthesis and the hydraulic behavior associated with stomatal regulation. Higher values of vapor pressure deficit can cause plants to close their stomata and limit water loss to facilitate water retention within the xylem. It is an essential determinant of global water resources and plant water relations which could be important for the dynamics of vegetation because of global, temperature-driven concerns (Grossiord et al., 2020). In a study by Amitrano et al. (2021), plants under high vapor pressure deficit caused more phytochemical levels, especially in a red cultivar of lettuce. This

corresponds with findings by Will et al. (2013) that vapor pressure deficit is a driving force for transpiration in plants. Transpiration can be approximated by VPD and stomatal conductance, wherein an increase by 20 °C in temperature can increase the VPD by 45%.

Photosynthetic rate

Regarding the photosynthetic rate, blue light was the best light for normal leaf chlorophyll development and net photosynthetic rate (Runkle, 2013; Chen et al., 2014). In this study, the photosynthetic rate confirmed previous findings by Randall and Lopez (2014) where enhanced net photosynthesis was measured in leaf samples irradiated with blue LEDs. This was supported by Zanoria et al. (2021) in that the net photosynthetic rate was higher under blue light at an irradiance intensity of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In a study by Klaiber et al. (2013), significant positive correlations were observed between aliphatic glucosinolates and photosynthetic pigments. Glucosinolates may be affected by carbon dioxide

enrichment as photosynthetic rates increase (Coley et al., 2002).

Transpiration rate

Transpiration rate is the ratio of the mass of water transpired to the mass of dry matter produced. According to Manivannan et al. (2017), red LED significantly increased plant growth, photosynthetic parameters like transpiration, and nutrient content, compared to the conventional cool white fluorescent lamp treatment. Plants transpire more when higher amounts of dry matter are produced (Barber and Martin, 1976). This is affected by common factors such as temperature and light intensity. Evaporation and diffusion occur faster at higher temperatures and increase when the stomata open wider to allow more carbon dioxide into the leaf for photosynthesis.

Stomatal conductance rate

Stomata play a crucial role in plant adaptation to changing environmental conditions as they control water loss and carbon dioxide intake. In this research, the changes in stomatal conductance influence stomatal movements affected by environmental factors such as light quality, light intensity, and photoperiod. Kim et al. (2004) reported that stomatal conductance was higher in plants grown under controlled lighting. The uniqueness of spectral quality during growth affects the diurnal pattern of stomatal conductance. Matthews et al. (2018) showed that stomatal conductance is influenced by the intensity and pattern of lighting. The acclimation of stomatal conductance to the lighting can be an important strategy for maintaining carbon fixation and overall plant water status. In addition, light has a profound effect on the growth and development of plants (Folta and Maruhnich, 2007).

Conclusion

Light-emitting diodes caused an increase in plant height and photosynthetic responses in kale. Green LED and blue LED are most suitable for plant height development while ambient light enhanced the leaf count, length, and width. Red, green and blue LED intensified the vapor pressure deficit while red LED boosted the transpiration rate. Moreover, when grown under blue LED, the photosynthetic rate was enhanced. On the other hand, exposure to ambient light improved stomatal conductance. An increase in photosynthetic rate implicated an increase in the biosynthesis of secondary metabolites such as glucosinolate. The latter is a sought-after

compound in kale.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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