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Light-NPK Synergy Increases Biomass, Photosynthetic Pigment and Nitrogen Content in *Agastache rugosa* (Fisch. & C.A.Mey.) Kuntze

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ABSTRACT

Agastache rugosa (Fisch. & C.A.Mey.) is a medicinal herb native to subtropical and temperate climates which is highly valued for its essential oils and phytochemicals in the pharmaceutical, cosmetic, and food industries. This study examined the adaptive strategies of *A. rugosa* in response to varying light and NPK levels. The treatments were four NPK levels which were low (NPK1, 40 mg kg^{-1}), moderate (NPK2, 80 mg kg^{-1}), high (NPK3, 120 mg kg^{-1}) and very high (NPK4, 160 mg kg^{-1}) nested under two light levels namely high-light (HL, 0% shade) and low-light (LL, 50% shade). We uncovered a resource allocation mechanism that optimises growth and photosynthetic efficiency through a multidimensional analysis involving biomass, photosynthetic pigment, and nitrogen contents. High-light promoted greater biomass and pigment content across NPK treatments, with the most pronounced effects under low NPK levels, indicating enhanced nitrogen use efficiency. This suggested that strategic management of light levels could compensate for nutrient deficiencies in this valuable herb. High-light increased leaf nitrogen content in a non-linear way, implying shifts in resource allocation, which are crucial for optimising fertilisation. Principal component analysis exhibited distinct clustering patterns, highlighting the dominant effect of light on overall physiology, with NPK levels introducing a secondary gradient of variation. Our results demonstrated the complex interplay between light and NPK availability in shaping plant responses, challenging simplistic notions of productivity and proving the importance of considering multiple environmental factors in tandem. These insights advance our understanding of plant adaptation to environmental changes and offer valuable guidance for optimising cultivation practices in medicinal herb production.

Keywords: *Agastache rugosa*, biomass, chlorophyll, light, nitrogen, nutrient.

INTRODUCTION

As sessile organisms, plants have evolved remarkable adaptive strategies to cope with fluctuating environmental conditions. Among the myriad factors affecting growth and development, light and nutrient availability stand out as key determinants of plant productivity (Evans and Clarke, 2019). Interaction between these two factors shapes plant responses at multifaceted levels from molecular signaling pathways to whole-plant resource allocation (Heerah et al., 2019; Brüllhardt et al., 2020; Tibocha-Bonilla et al., 2020). *Agastache rugosa* (Fisch. & C.A.Mey.), a medicinal herb, presents an ideal model for studying these complex interactions due to its adaptability and economic value (Hou et al., 2022). Plants can adjust their physiology in response to light and nutrient variability, highlighting their distinctive nature in environmental sensing and response mechanisms (Sakuraba and Yanagisawa, 2018).

The relationship between light and nutrient utilisation has been extensively studied in many plant systems. High-light conditions can enhance nitrogen use efficiency, potentially compensating for nutrient deficiencies in some species (Bernacchi et al., 2007; Esmaeili et al., 2022). Moreover, the allocation of resources between biomass accumulation and photosynthetic apparatus development is a dynamic process, influenced by both environmental cues and internal signaling networks (Wu et al., 2019; Li et al., 2021; Chen and Ham, 2022). These findings have significant implications for understanding plant adaptation to changing environments and for optimising cultivation practices in agriculture. Chlorophyll a/b ratio, for instance, has emerged as a key indicator of photosynthetic adaptation to different light availability, indicating adjustments in the light-harvesting complexes (Catoni et al., 2015; Kunugi et al., 2016). Similarly, nitrogen partitioning between different plant organs and metabolic routes offer clues on plant strategies for maximising growth under limiting resources (Yin et al., 2019; Qiang et al., 2023).

Despite the growing body of research on plant responses to environmental factors, there remains a significant gap in our understanding of how medicinal herbs like *A. rugosa* adapt to varying light and NPK levels. *A. rugosa*, valued for its essential oils and phytochemicals (Haiyan et al., 2016; An et al., 2018), used in pharmaceutical, cosmetic, and food industries (Yamani et al., 2014; Anand et al., 2018; Kim, 2020; Lee et al., 2020; Hou et al., 2022), has traditionally been cultivated in subtropical and temperate zones. Field cultivation of *A. rugosa* in tropical environments poses unique challenges due to the high-light intensity and low native soil fertility (Amissah et al., 2024; Terán et al., 2024). Most available studies have focused on artificial lighting using LEDs and hydroponic systems in plant factories (Su et al., 2016; Kim et al., 2018a; Kim et al., 2018b; Lam et al., 2019; Park et al., 2020). Only two studies have reported the effects of different nitrogen forms and rates on field-grown *A. rugosa* (Gardner, 2019; Ohk et al., 2000), in its native habitats. This study aimed to address this gap by investigating the interactive effects of light and NPK on the growth, photosynthetic pigment and nitrogen content of *A. rugosa* grown in the tropics. We seek to unveil the adaptive strategies of *A. rugosa* under changing environments by employing multivariate statistical approach. We hypothesised that high-light and NPK improves biomass, photosynthetic pigment, and nitrogen content in the plant. Our findings provide insights into the broader mechanisms of plant adaptation to environmental variability and inform improved cultivation practices that can enhance the yield and quality of this valuable crop.

MATERIALS AND METHODS

Plant Materials

A. rugosa seeds were bought from a local supplier (WHT Wellgrow Seeds, Malaysia). The seeds were surface-sterilised for 1 min with 70% ethanol, followed by 5% sodium hypochlorite solution for 10 min, then rinsed thrice with distilled water. The seeds were sown in 200-cell trays containing a 1:1 mixture of blonde peat (Pindstrup Mosebrug A/S, Denmark) and perlite. The seed cell trays were placed on capillary mats under white fluorescent light/dark (16/8 h) photoperiod (Park et al., 2020), in a room maintained at 20 - 25 °C and 50 - 70% relative humidity. The substrate was kept moist but not drenched. Seeds were allowed to germinate and grow under these conditions.

Experimental Setup

This experiment was conducted under two north-south oriented polytunnels (10 m long x 6 m wide x 2 m high) with both end-walls and side-walls (1.5 m high) at Farm 10, Faculty of Agriculture, Universiti Putra Malaysia from September to December 2020. The polytunnels were enclosed with a single layer of transparent polyethylene film (280 microns thick). The end-walls were open, and side-walls were rolled down during the experimental period. To simulate low-light conditions, we covered externally one tunnel roof with a single layer of 50% black polyethylene net. External roof-shading decreases thermal radiation heat loads, reducing the difference in air temperature between the two tunnels (Abdel-Ghany et al., 2015; Mahmood et al., 2018). This would help reduce bias in interpreting the effects of light. Photosynthetic photon flux density (PPFD), air temperature (T_{Air}), and relative humidity (RH) data were recorded using a light meter (LI-189, LI-COR, Lincoln, NE, USA) and dataloggers (Figure 1). Six-week-old seedlings of uniform size with four to five true leaves were transplanted to black polythene pots (15 cm diameter x 25 cm high) containing ≈3.5 kg pot^{-1} (≈24 cm depth) of 3:2:1 potting mix (University Agriculture Park, Malaysia). The pots were carefully arranged in the polytunnels spaced 30 cm apart.

Four nutrient levels based on NPK (16:16:16) which were low (NPK1, 40 mg kg^{-1}, 1.26 g), moderate (NPK2, 80 mg kg^{-1}, 2.52 g), high (NPK3, 120 mg kg^{-1}, 3.78 g) and very high (NPK4, 160 mg kg^{-1}, 5.04 g) were nested under two light levels namely high-light (HL, 0% shade) and low-light (LL, 50% shade). NPK levels were arranged in a randomized complete block design with four replicates. Light treatments were started at transplanting or 0 days after transplanting, while nutrients were split-applied 6, 13, 27, 41, 55, and 69 days after transplanting. Granular compound fertilizer (YaraMila, Norway) was used as source of nutrients. Plants were watered after each nutrient application using drip irrigation. Irrigation frequency was adjusted depending on the weather and the stage of plant growth. On sunny days, the drip irrigation was turned on at 0800 and 1700, each for 15 min. The average flow rate was 20 mL/min.

Leaf Greenness and Photosynthetic Pigments

Fully expanded leaves (the third or the fourth from the apex) were used for the leaf greenness tests. Leaf greenness was determined using a chlorophyll meter (SPAD-502, Minolta Co. Ltd., Japan), expressed as SPAD values (Wicharuck et al., 2024). Leaf disks were then collected from the exact spots of the SPAD measurements by using a single hole paper punch (6 mm diameter). Leaf disks were placed in prelabeled 20-mL amber screw cap glass vials, and immediately transported to the laboratory. Photosynthetic pigments were extracted from the leaf disks with dimethyl sulfoxide (DMSO) following a modified protocol (Hiscox and Israelstam 1979; Parry et al. 2014). Samples were added with 10 mL of DMSO and incubated at 65°C in an oven for 4 h until the disks became transparent. Absorbance was read at 649, 665, 480 and 510 nm under dimmed light in a multiplate spectrometer equipped with a cuvette port (Multiskan GO, Thermo Scientific, USA). Chlorophyll (Chl) and carotenoid (Car) were expressed as nanomoles per centimeter square (nmol/cm^2) in the following equations (Lichtenthaler and Wellburn, 1983; Hendry and Price, 1993; Garg, 2012):

$$\begin{aligned}
\text{Chl a (nmol cm}^{-2}\text{)} &= [(12.19\,E_{665} - 3.45\,E_{649}) \times V \times 1.119] / A \\
\text{Chl b (nmol cm}^{-2}\text{)} &= [(21.99\,E_{649} - 5.32\,E_{665}) \times V \times 1.102] / A \\
\text{Chl (nmol cm}^{-2}\text{)} &= \text{Chl a} + \text{Chl b} \\
\text{Chl a/b} &= \text{Chl a} / \text{Chl b} \\
\text{Car (nmol cm}^{-2}\text{)} &= [(7.60E_{480} - 1.49\,E_{510}) \times V \times 1.863] / A
\end{aligned}$$

Where E_x = absorbance of extract at 649, 665, 480 or 510 nm
V = final volume of extract in mL
A = total area of leaf disks in cm^2

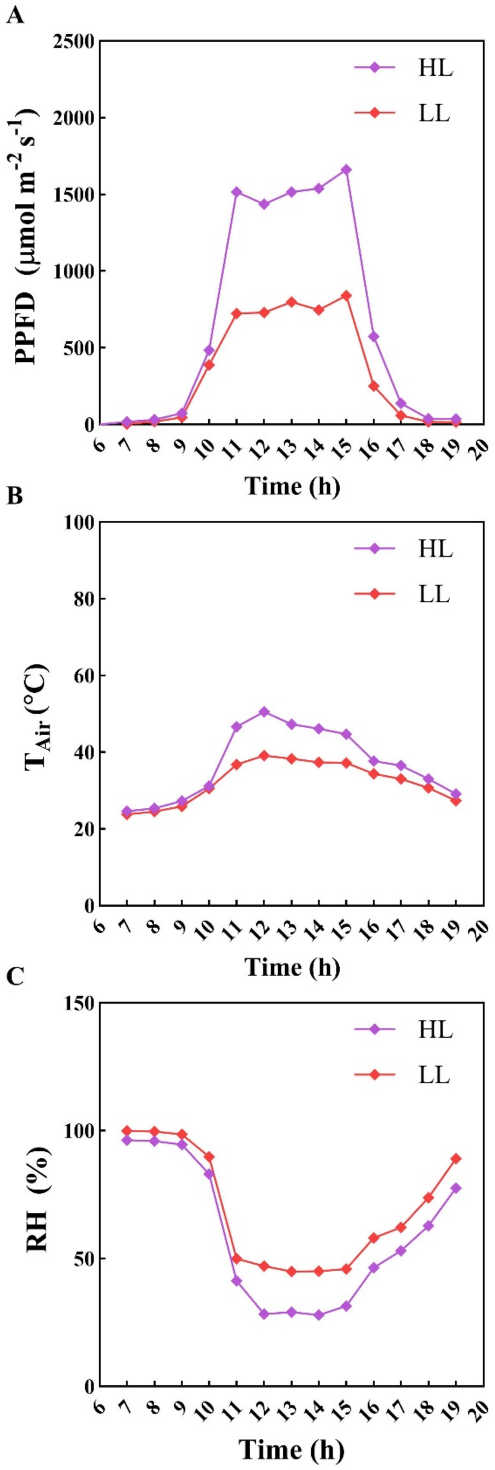

Figure 1. Diurnal variations of microclimatic parameters in tunnels set for high-light (HL, 0% shade) and low-light (LL, 50% shade) levels, averaged over September to December 2020. Abbreviations: PPFD, photosynthetic photon flux density; T_{Air}, air temperature; RH, relative humidity.

Biomass and Total Nitrogen Determination

Aboveground parts (stems and leaves) were harvested 84 days after transplanting in the morning and transported to the laboratory. At 84 days after transplanting, the plants are considered to reach physiological maturity (An et al., 2018). Samples were oven-dried at 105 °C for 30 min, followed by 80 °C for 2 days (Memmert UNB-500, Germany). Shoot dry weight (DWS) was recorded. Dried fully expanded leaves were ground in a stainless-steel grinder (Nima NM-8300, Japan) to pass a 300-mesh sieve (Motsara and Roy, 2008). Ground samples at 0.25 g each were liquefied through wet acid digestion with 5 mL of sulfuric acid and hydrogen peroxide. The resulting digested solutions were tested for total nitrogen content following the spectrometer methods of Koistinen et al. (2019) with modifications. Total nitrogen content was expressed as percentages (Mills and Jones Jr 1996).

Statistical Analysis

Data were analysed using SAS® version 9.4 by the general linear model (PROC GLM). Wherever necessary, data was transformed before analysis through Box-Cox transformations to ensure the normality of residuals was satisfied. A combined analysis of variance was performed for the four NPK levels (NPK1–NPK4) nested under light levels (HL and LL), where light and NPK treatments were considered as fixed effects. This allowed us to assess the interaction between the two factors, as their combined effect on the measured traits was of key interest (Bowley, 1999; Moore and Dixon, 2015). The means of significant ($p < 0.05$) main effects and interactions were separated with the least significant difference (LSD) test (Vargas et al., 2015). Pearson's correlation test and principal component analysis (PCA) were used to explore the relationship among variables and group them based on their similarities. Graphs, heatmap and biplot after PCA were developed using OriginPro® 2024b with the "Correlation Plot" and "Factor Analysis" packages (www.originlab.com).

RESULTS

Shoot Biomass

Light and nutrient interactions significantly affected ($p < 0.05$) shoot dry weight (Figure 2). Shoot dry weight generally increased with nutrient levels, favoring high-light conditions, with HL-NPK4 showing the highest values (DWS: 11.51 g plant^{-1}). The lowest shoot dry weight occurred under LL-NPK1 (DWS: 5.83 g plant^{-1}) and HL-NPK2 (DWS: 6.18 g plant^{-1}), but there was no significant difference between these two groups.

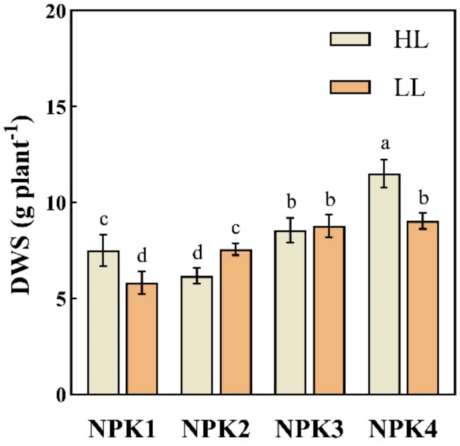

Figure 2. Shoot biomass of *A. rugosa* under different light and nutrient levels. Abbreviations: HL, high-light (0% shade); LL, low-light (50% shade); NPK1, low-nutrient (40 mg kg^{-1}); NPK2, moderate-nutrient (80 mg kg^{-1}); NPK3, high-nutrient (120 mg kg^{-1}); NPK4, very high-nutrient (160 mg kg^{-1}); DSW, shoot dry weight. The vertical bars are mean ± SD (n = 4). Different letter(s) above bars indicate significant differences according to LSD. LSD = Least significant difference; SD = Standard deviation.

Leaf Greenness and Photosynthetic Pigments

Light and nutrient levels affected ($p < 0.05$) leaf greenness and chlorophyll content of *A. rugosa* (Figure 3). Plants grown under high-light (HL) conditions had 10% higher SPAD values, indicating increased leaf greenness, compared to those under low-light (LL) levels (Figure 3A). Chlorophyll a content was consistently higher in HL conditions across all nutrient levels. Within HL condition, chlorophyll a generally increased with nutrient level, peaking at NPK3 (Chl a: 53.09 nmol cm^{-2}; Figure 3B) before slightly decreasing at NPK4, but there were no statistical differences among HL-NPK2, HL-NPK3 and HL-NPK4. Chlorophyll b level followed a similar trend to chlorophyll a, with HL conditions yielding higher values. The highest chlorophyll b concentration was observed in the HL-NPK3 treatment (Chl b: 11.88 nmol cm^{-2}; Figure 3C). Chlorophyll a/b ratio exhibited a decreasing trend as nutrient levels increased from NPK1 to NPK3, with a slight increase at NPK4. The highest chlorophyll a/b ratio was observed under NPK1, while the lowest ratio occurred in the NPK3 treatment (Chl a/b: 4.51; Figure 3D). Total chlorophyll content mirrored the trends seen in individual chlorophyll components. HL conditions resulted in higher total chlorophyll across all nutrient levels.

In HL conditions, total chlorophyll increased with nutrient level up to NPK3 (Total Chl: 64.96 nmol cm^{-2}; Figure 3E), then slightly decreased at NPK4. Carotenoid content under HL conditions also increased with nutrient levels, reaching a maximum at NPK3 (Total Car: 37.17 nmol cm^{-2}; Figure 3F) before declining slightly at NPK4. Interestingly, there was minimal variation for chlorophyll components and carotenoid content across nutrient levels under LL conditions.

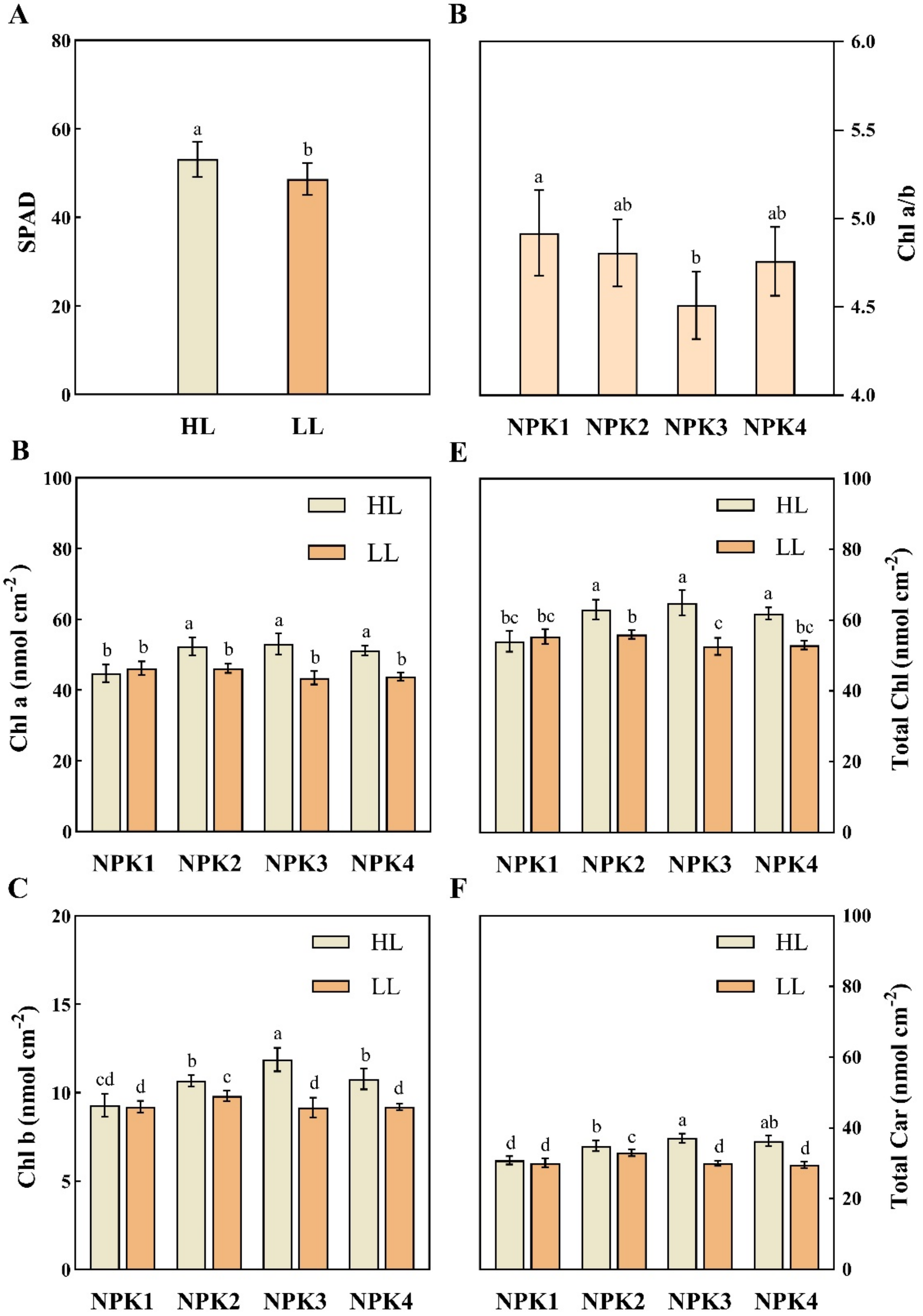

Figure 3. Leaf greenness and chlorophyll content of *A. rugosa* under different light and nutrient levels. Abbreviations: HL, high-light (0% shade); LL, low-light (50% shade); NPK, low-nutrient (40 mg kg^{-1}); NPK2, moderate-nutrient (80 mg kg^{-1}); NPK3, high-nutrient (120 mg kg^{-1}); NPK4, very high-nutrient (160 mg kg^{-1}); SPAD, soil plant analysis development; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a/b, chlorophyll a to b ratio; Chl, chlorophyll; Car, carotenoid. The vertical bars are mean ± SD (n = 4). Different letter(s) above bars indicate significant differences according to LSD. LSD = Least significant difference; SD = Standard deviation.

Total Nitrogen Content

Light and nutrient levels affected ($p < 0.05$) total nitrogen content in the leaves of *A. rugosa* (Figure 4). In high-light (HL) conditions, nitrogen content was consistently higher compared to low-light (LL) conditions across all nutrient levels. The most striking difference was observed under NPK1, where HL leaves contained about 5% nitrogen, nearly three times the content found in LL leaves. Interestingly, the response to increasing nutrient levels was not linear. In HL conditions, nitrogen content decreased from NPK1 to NPK2, then showed a gradual increase through NPK3 and NPK4. In contrast, under LL conditions, nitrogen content was lowest in NPK2, peaked in NPK3, and slightly decreased in NPK4. The highest nitrogen content in LL conditions (about 1.6% in NPK3) was still lower than the lowest content observed in HL conditions (about 2% in NPK2 and NPK3).

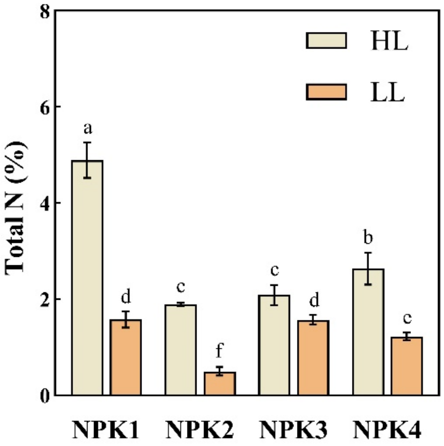

Figure 4. Total nitrogen content in the leaves of *A. rugosa* under different light and nutrient levels. Abbreviations: HL, high-light (0% shade); LL, low-light (50% shade); NPK1, low-nutrient (40 mg kg^{-1}); NPK2, moderate-nutrient (80 mg kg^{-1}); NPK3, high-nutrient (120 mg kg^{-1}); NPK4, very high-nutrient (160 mg kg^{-1}); N, nitrogen. The vertical bars are mean ± SD (n = 4). Different letter(s) above bars indicate significant differences according to LSD. LSD = Least significant difference; SD = Standard deviation.

Correlations Among Measured Traits

Correlation analysis revealed intricate relationships between biomass, photosynthetic pigments, and leaf nitrogen content in *A. rugosa* under different light and nutrient levels (Figure 5). Notably, leaf greenness (SPAD) showed strong positive correlations with chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll, and total carotenoid content (all $p < 0.001$). These pigments also had robust intercorrelations, particularly between Chl a and Chl b ($r = 0.88$, $p < 0.001$). Interestingly, chlorophyll a to b ratio (Chl a/b) showed negative correlations with most other traits, markedly so with Chl b ($r = -0.52$, $p < 0.01$) and total carotenoids ($r = -0.38$, $p < 0.05$). Shoot dry weight (DWS) showed a weak positive correlation with SPAD ($r = 0.38$, $p < 0.05$) but did not correlate with other traits. Total nitrogen content exhibited minimal correlations with all measured traits.

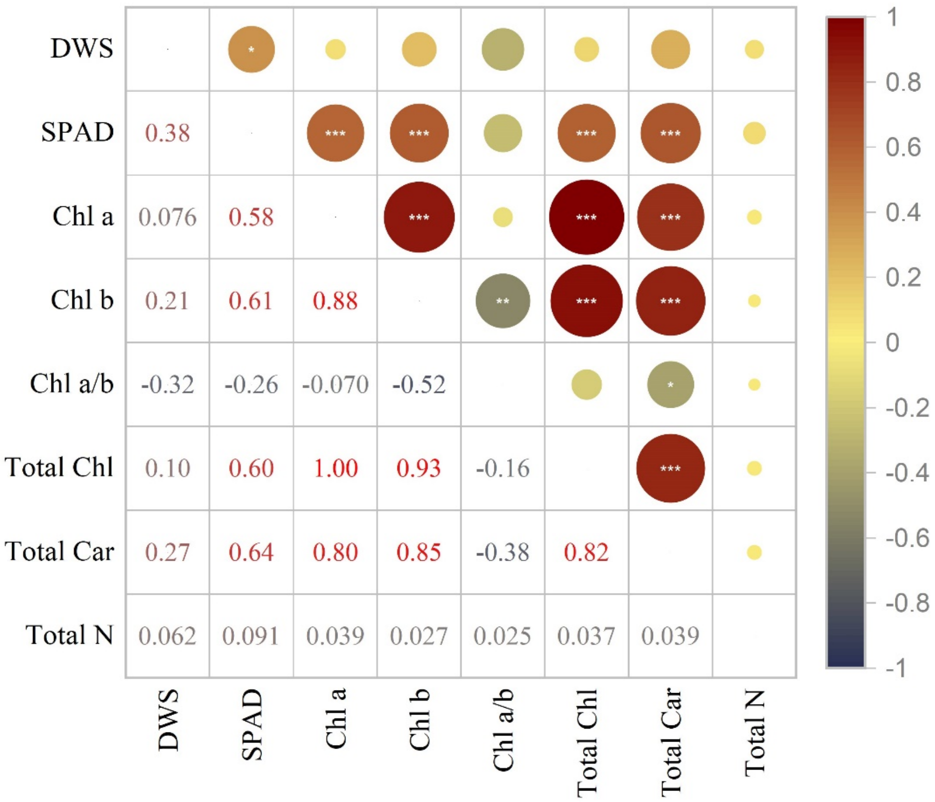

Figure 5. Heatmap showing correlations between traits related to biomass, photosynthetic pigments and leaf nitrogen content in *A. rugosa* grown under different light and nutrient levels. Abbreviations: DWS, shoot dry weight; SPAD, soil plant an alysis development; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a/b, chlorophyll a to b ratio; Chl, chlorophyll; Car, carotenoid; N, nitrogen (∗∗∗$p < 0.001$; ∗∗$p < 0.01$; ∗$p < 0.05$).

Principal Component Analysis of Measured Traits

The PCA biplot reveals distinct clustering patterns for different light and nutrient treatments in *A. rugosa* (Figure 6). The first two principal components explained a substantial 70.1% of total variance (Dim1: 53.9%, Dim2: 16.2%). Notably, high-light treatments (HL-NPK1 to HL-NPK4) clustered predominantly in the positive quadrants of Dim1, associating strongly with chlorophyll concentra (Chl a, Total Chl) and carotenoids (Total Car). Conversely, low-light treatments (LL-NPK1 to LL- NPK4) were largely distributed in the negative quadrants of Dim1, showing a closer relationship with Chl a/b ratio. Remarkably, nutrient levels induced a gradient along Dim2, with higher nutrient treatments (NPK3 and NPK4) generally positioned higher on this axis, correlating positively with total nitrogen content and leaf greenness (SPAD). The shoot dry weight (DWS) vector opposed the chlorophyll-related vectors, indicating a potential trade-off between biomass accumulation and chlorophyll concentration.

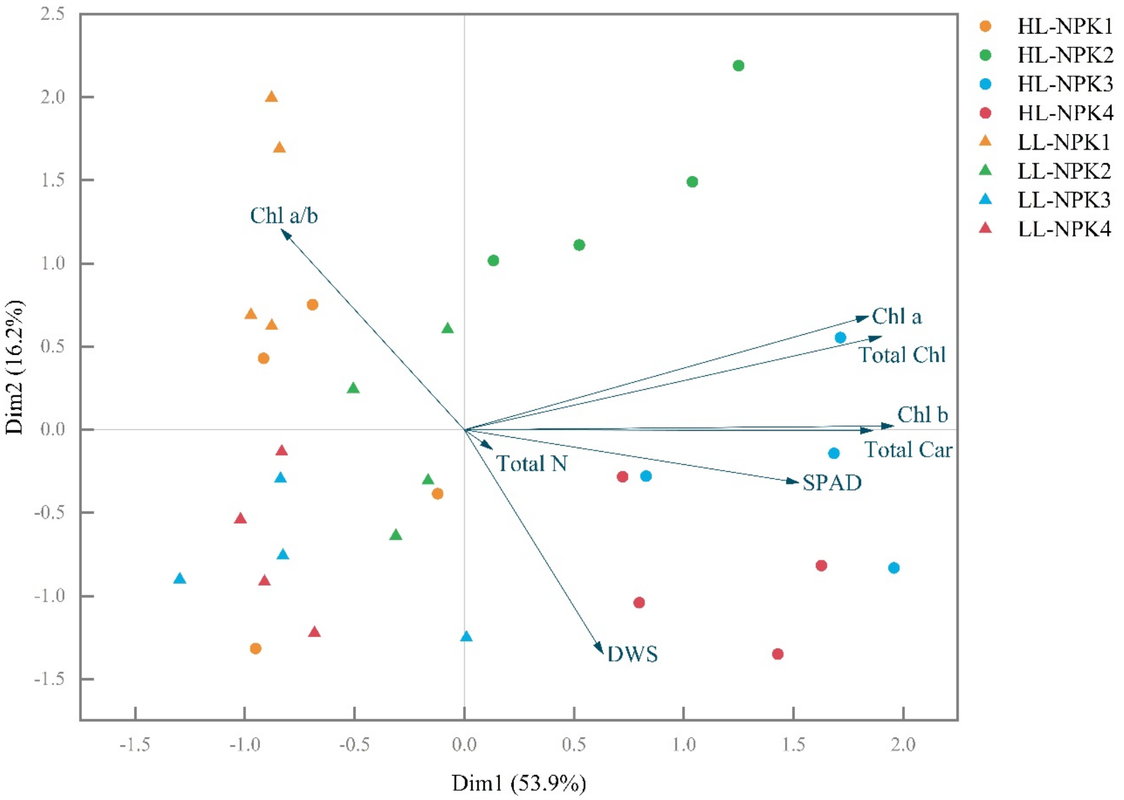

Figure 6. Principal component analysis (PCA) biplot displaying the relationship among traits related to biomass, photosynthetic pigments and leaf nitrogen content in *A. rugosa* under different light and nutrient levels. Abbreviations: HL, high-light (0% shade); LL, low-light (50% shade); NPK, low-nutrient (40 mg kg^{-1}); NPK2, moderate-nutrient (80 mg kg^{-1}); NPK3, high-nutrient (120 mg kg^{-1}); NPK4, very high-nutrient (160 mg kg^{-1}); DWS, shoot dry weight; SPAD, soil plant analysis development; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a/b, chlorophyll a to b ratio; Chl, chlorophyll; Car, carotenoid; N, nitrogen pigments and leaf nitrogen content in A. rugosa grown under different light and nutrient levels. Abbreviations: DWS, shoot dry weight; SPAD, soil plant analysis development; Chl a, chlorophyll a; Chl b, chlorophyll b; Chl a/b, chlorophyll a to b ratio; Chl, chlorophyll; Car, carotenoid; N, nitrogen ($***p < 0.001$; $**p < 0.01$; $*p < 0.05$).

DISCUSSION

The interplay between light and NPK levels in *A. rugosa* revealed a sophisticated adaptive strategy that optimises resource allocation and photosynthetic efficiency. The PCA analysis highlighted this by revealing distinct clustering patterns that highlighted the dominant effect of light on overall plant physiology. Our results showed that high-light conditions promoted greater biomass accumulation and pigments content across all NPK treatments, with the most pronounced differences observed at low-nutrient levels. This demonstrated that *A. rugosa* possesses a remarkable ability to leverage light energy even when nutrient supply is scarce, potentially via enhanced nitrogen use efficiency or by reallocating internal nitrogen reserves. The capacity of the plant to maintain higher photosynthetic performance in nutrient-limited and high-light conditions indicated compensatory mechanisms that maximise resource utilisation. These mechanisms possibly involve physio-biochemical adaptations, such as enhanced root-shoot nutrient transport or modified expression of genes involved in nutrient acquisition and assimilation. The increased chlorophyll and total carotenoid contents at high-light and moderate to high-NPK levels, implied a synergistic effect where abundant light allows the plant to optimise its utilisation of available nutrients for pigment synthesis and photosynthetic apparatus development. This suggested a coordinated response between light sensing and nutrient metabolism pathways, possibly involving regulatory networks that integrate environmental signals to modulate resource allocation. The phytochrome-interacting factors (PIFs) and elongated hypocotyl 5 (HY5) transcription factors possibly play important roles in coordinating these responses by regulating the expression of chlorophyll biosynthetic genes such as *HEMA1* and *POR* (Yuan et al., 2017; Shi et al., 2018). The increase in carotenoid content might be mediated through the activation of biosynthetic genes like *PSY* and *PDS*, whose expression is often coordinated with chlorophyll synthesis through common regulatory elements (Lu et al., 2018). These findings aligned with Poorter et al. (2019), who found that plants often exhibit enhanced physiological responses to high-light intensity in multiple traits. The robust relationship between increased light availability and improved nutrient utilisation demonstrated the ability of *A. rugosa* to adapt its metabolic processes to optimise growth in varying environmental conditions. Moreover, the strong linear correlations between leaf greenness (SPAD) and chlorophyll content (a, b, and total) further supported the reliability of using SPAD measurements as a non-destructive proxy for leaf chlorophyll content (Wicharuck et al., 2024).

Notably, our results revealed a non-linear response in leaf nitrogen content to increasing NPK levels, under high-light conditions. The gradient observed along Dim2 of the PCA biplot, linked to nutrient levels, reflected this complex integration of light and nutrient signaling in regulating physiological responses. The surprising decrease in leaf total nitrogen content from low- to moderate-NPK levels, followed by a gradual increase, suggested a unique nutrient management strategy in *A. rugosa*. This non-linear response probably reflected a shift in resource allocation priorities as nutrient levels changes. At low-NPK levels, the plant might prioritise rapid growth and biomass accumulation to leverage on available light energy at the expense of maintaining high nitrogen concentrations. This could be an adaptive response to overcome nutrient deficiencies, as has been observed in other species where high-light conditions can enhance nitrogen use efficiency (Hirose, 1998; Esmaeili et al., 2022). The plant might be able to remobilise internal nitrogen reserves or increase the uptake and assimilation of nitrogen to support growth in these conditions. As NPK levels increased from low to moderate, the plant might initially invest more resources into expanding leaf area and photosynthetic machinery rather than maintaining high nitrogen content per unit leaf mass. This shift could enable the plant to maximise light capture and carbon fixation, as shown by the higher chlorophyll and carotenoid contents under high-light and moderate- to high-NPK conditions. However, as NPK levels continued to rise, the plant appeared to adjust its resource allocation strategy again, gradually increasing leaf nitrogen content. This might reflect an optimisation of nitrogen distribution, where the plant balances investment between photosynthetic proteins and other growth-related processes such as structure formation to achieve the highest overall productivity, as posited in the optimal nitrogen partitioning theory (Hikosaka and Terashima, 1996; Yin et al., 2019). This adaptive response echoed the findings of Tegeder and Masclaux-Daubresse (2018) stating the importance of flexible nitrogen allocation strategies in plant adaptation. This nitrogen allocation patterns observed in *A. rugosa* is potentially driven by the integration of multiple signaling pathways that sense and respond to changes in light and nutrient availability. One potential mechanism might involve the cross-talk between light signaling mediated by phytochromes and nitrogen signaling regulated by Target of Rapamycin (TOR) kinase complex (Wu et al., 2019; Mankotia et al., 2024). The interplay between these pathways would allow *A. rugosa* to adjust nitrogen allocation based on the prevailing light and nutrient conditions. Under low NPK levels, the plant might have enhanced the activity of nitrogen uptake and assimilation genes through TOR signaling, while also promoted growth and biomass accumulation via phytochrome-PIF/HY5 pathways to maximise light capture. As NPK levels increased, the plant might have shifted resource partitioning to invest more in leaf nitrogen content via the TOR-mediated upregulation of nitrogen metabolic enzymes and transporters. Future research investigating the expression patterns of key regulatory genes, as well as metabolomic and proteomic analyses, could help elucidate the intricate signaling networks underlying the adaptive nitrogen management strategies of this herb.

Our correlation analysis revealed a negative relationship between chlorophyll a/b ratio and most other measured traits, particularly chlorophyll b and carotenoid contents. This inverse relationship suggested an adjustment of the photosynthetic apparatus in response to different light and nutrient levels.

As noted by Catoni et al. (2015), changes in chlorophyll a/b ratios often reflect adaptations in the light-harvesting complexes (LHC), with lower ratios often associated with low-light adapted leaves. These adjustments probably involve modifications in the composition and organisation of the photosynthetic machinery, particularly LHCII proteins, which bind majority of chlorophyll b (Sato et al., 2015). The dynamic regulation of these protein complexes enables plants to optimise light capture under changing environmental conditions. The negative correlation with carotenoids might imply a protective mechanism, where increased carotenoid synthesis could help prevent photooxidative damage when chlorophyll b levels are lower. Furthermore, the weak positive correlation between shoot dry weight and SPAD values, combined with minimal correlations between nitrogen content and other traits, suggested an intricate resource allocation tactic that prioritise different physiological processes depending on environmental conditions. This selective allocation of resources indicated the presence of complex regulatory networks that monitor and respond to internal resource status and external environmental signals. These networks possibly involve multiple hormonal signaling pathways, including cytokinins and auxins, which are known to affect both biomass accumulation and chlorophyll biosynthesis (Kobayashi et al., 2012; Schaller et al., 2015; Cortleven et al., 2016). These findings echoed the work of Krapp (2015), emphasising the regulation of nitrogen assimilation and its effect on plant growth and development. The trade-offs between these various physiological traits underscore the challenges plants face in optimising resource use under varying environmental conditions (Evans and Clarke, 2019). Having said that, this multidimensional response focuses on the importance of considering both light and nutrient levels in tandem when optimising cultivation practices or predicting plant responses to environmental changes (Figure 7).

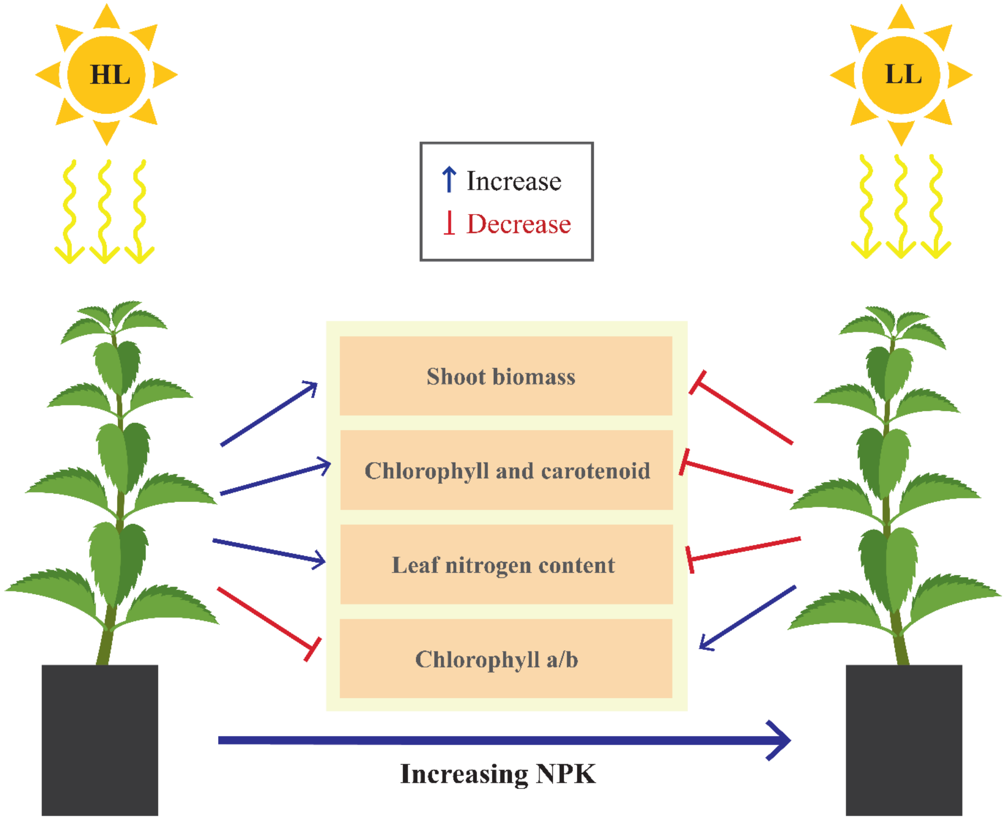

Figure 7. Schematic diagram illustrating the changes in biomass, photosynthetic pigment and nitrogen contents in *A. rugosa* under different light and nutrient levels. Abbreviations: HL, high light; LL, low light; NPK, nutrient.

CONCLUSION

Our study revealed some adaptive strategy of *A. rugosa* to light and nutrient that optimises resource allocation and photosynthetic efficiency. The synergistic effects of high-light and NPK treatments on biomass accumulation, photosynthetic pigment, and nitrogen content indicate the plant's ability to leverage environmental resources. Most notably, the non-linear response in leaf nitrogen content to increasing NPK supply, particularly under high-light, suggests a dynamic resource management strategy that prioritises different physiological processes as environmental conditions change. The trade-offs between biomass accumulation, chlorophyll content, and nitrogen allocation underscore the challenges plants face in optimising resource utilisation. These findings not only advance our understanding of plant adaptation to environmental changes but also provide valuable insights for optimising cultivation practices in medicinal herb production. Future research should examine the molecular mechanisms underlying these adaptive responses, potentially uncovering new avenues for enhancing crop yield and quality in the face of climate change.

ETHICAL APPROVAL

A. rugosa is not native to Malaysia. Seeds were bought from a local supplier (WHT Wellgrow Seeds, Malaysia).

AUTHORS CONTRIBUTION

KAR: Conceptualisation, Methodology, Experimentalize, Data analysis, Writing-original draft preparation. PEMW: Conceptualisation, Methodology, Revision, Supervision. AM: Revision, Supervision. LSY: Revision, Supervision. All authors have read and revised the manuscript, provided helpful discussions, and approved its final version.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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