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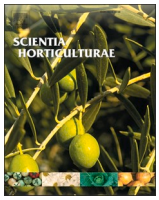
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Research Paper



Interactive effects of far-red light and vapor-pressure deficit on photosynthetic performance and water-use efficiency of cucumber seedlings

Toshio Shibuya *, Nanoka Wada, Ryosuke Endo

Graduate School of Agriculture, Osaka Metropolitan University, 1–1 Gakuen-cho, Naka-ku, Sakai 599-8531, Japan

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ABSTRACT

Exploring the effects of interactions between light quality and other environmental factors on photosynthetic performance is crucial for improving post-transplant performance of transplants grown under artificial lighting. We investigated the effects of far-red (FR) light and vapor-pressure deficit (*VPD*) on the photosynthetic performance and water-use efficiency of cucumber (*Cucumis sativus*) seedlings. Seedlings were grown in controlled-environment chambers under four different combinations of FR light and *VPD*: with or without FR light (FR+ or FR–), under low or high *VPD* (0.8 or 2.3 kPa). We found a synergistic interaction in which FR– combined with increased *VPD* enhanced photosynthetic performance. Analysis of the response curves of net photosynthetic rate (P_n) to intercellular CO_2 concentration indicated that this enhancement was mainly due to non-stomatal factors. Leaf mass per unit area, which was positively correlated with P_n, was higher under these conditions, implying increased mesophyll capacity. Intrinsic water-use efficiency (WUE_i) was higher in leaves that had been acclimatized at FR+ and high *VPD*. Under FR–, particularly at high *VPD*, the increase in photosynthesis was outweighed by an increase in stomatal conductance (g_s), leading to lower WUE_i. This increased g_s may be attributable to improved leaf hydraulic conductance induced by low-FR light. Our results reveal the complex interactions between light quality and evaporative demand through their effects on plant responses, which suggest possible trade-offs between increasing photosynthetic performance and maintaining water-use efficiency across combinations of FR light and *VPD*. Such insights are critical for optimizing growth conditions in controlled environments for transplant production to improve post-transplant growth.

1. Introduction

In the process of growing transplants with artificial lighting, during seedling development, adjusting light quality is essential for effectively controlling their morphology and gas-exchange properties (Gallegos-Cedillo, 2024). During their growth, transplants are moved from a controlled indoor environment to potentially harsh conditions in greenhouses or outdoors. Managing the light and overall environment during their growth indoors can enhance the plants' tolerance of subsequent environmental changes. This, in turn, reduces stress and supports successful post-transplant growth.

Far-red (FR) light has been used to control plant growth and development in horticultural production (Demotes-Mainard et al., 2016). Under illumination that emits minimal FR light, such as that provided by commercial white LEDs and fluorescent lamps, plants exhibit responses similar to those under acclimatization to high-light conditions. This response improves their photosynthetic performance and increases their tolerance to water stress under high-light conditions (Shibuya et al., 2010, 2012, 2015; Hogewoning et al., 2010). Such characteristics are beneficial for plants that will be transplanted outdoors and exposed to intense light (Shibuya et al., 2024a).

Photosynthetic performance is regulated by both stomatal and non-stomatal factors. Non-stomatal factors, particularly mesophyll photosynthetic capacity, play a crucial role in enhancing photosynthetic performance. An increase in leaf mass per unit area (*LMA*) due to low-FR light contributes to this enhancement by allowing for a greater density of chloroplasts, ultimately resulting in more efficient carbon fixation per unit leaf area (Shibuya et al., 2012, 2015). Stomatal factors are also influenced by FR light. For instance, the increased proportion of active phytochrome under low-FR light enhances stomatal conductance (g_s),

* Corresponding author at: Graduate School of Agriculture, Osaka Metropolitan University, 1–1 Gakuen-cho, Naka-ku, Sakai 599-8531, Japan.
E-mail address: tshibuya@omu.ac.jp (T. Shibuya).

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which facilitates greater CO_2 assimilation. However, this increase in g_s can also lead to greater water loss through transpiration, which may negatively impact overall water-use efficiency (WUE) (Boccalandro et al., 2009; Shibuya et al., 2015). Therefore, balancing the trade-offs between increased photosynthesis driven by both stomatal and non-stomatal factors and the need to retain water is crucial during efforts to improve transplant quality by manipulating FR light.

Furthermore, it is vital to explore the interactions between light quality and other environmental factors to effectively control the photosynthetic performance of transplants. Here, we focused on atmospheric humidity, which we measured as vapor-pressure deficit (*VPD*), as the key factor that potentially interacts with FR light. FR light and atmospheric humidity can interact in complex ways to affect plant growth (Shibuya et al., 2019, 2024b, 2024c). Importantly, our previous research showed that plants may perceive shade conditions not only directly through light signals but also indirectly through changes in atmospheric humidity (Shibuya et al., 2024b), which further indicates a complex interaction between FR light and humidity.

Atmospheric humidity affects how plants adjust to evaporative demand by affecting both *LMA* (Itagaki et al., 2014; Li et al., 2019; Amitrano et al., 2021) and stomatal behavior (Buckley, 2005; Peak and Mott, 2011; Shibuya et al., 2017; Grossiord et al., 2020; Salmon et al., 2020). Such responses can improve plants' tolerance to water deficit (Shibuya et al., 2006; Sánchez-Blanco et al., 2004; Bañón et al., 2006) and increase WUE (Shibuya et al., 2017). This may in turn affect both photosynthetic performance and WUE through interactions with the amount of FR light.

To explore these complex interactions, we designed this study to investigate the photosynthetic performance and WUE of cucumber seedlings grown under four combinations of FR light and atmospheric humidity. Our goal was to elucidate how these factors interact to influence transplant quality, with the ultimate goal of informing strategies for optimizing growth conditions in controlled environments.

2. Materials and methods

2.1. Plant material and growing conditions

Cucumber seeds (*Cucumis sativus* L., 'Hokushin') were sown in plastic pots filled with vermiculite. The seedlings were grown in growth chambers (Ikeya Co., Kashiba, Japan) until the first true leaf expanded under four combinations of FR light and *VPD*: with or without FR light (FR+ or FR–), under low (0.8 kPa) or high (2.3 kPa) *VPD* at an air temperature of 28 °C. Temperature and humidity were maintained nearly constant through PID control of a heater and a steam humidifier installed in each chamber. The illumination was provided by LED panels (LED Sun Light Z4; Taiwan Hipoint Co., Kaohsiung, Taiwan) containing blue, green, red, and (in the FR+ treatment) FR light sources. Each light treatment received 30 % blue light, 35 % green light, and 35 % red light (Fig. 1). In the FR+ treatments, the ratio of FR light to red light was set to 1.2, the same proportion as in sunlight. The photosynthetic photon flux density (*PPFD*) was set to 180 μmol m^{-2} s^{-1}, with a photoperiod of 16 h. The PPFD and photoperiod were established within the range of typical conditions for transplant production under artificial lighting, as outlined by Gallegos-Cedillo et al. (2024). The CO_2 concentration was maintained at approximately 400 μmol mol^{-1} using a CO_2 controller (Ace Point Systems Inc., Osaka, Japan) connected to an infrared gas analyzer (CO_2 Engine K30; SenseAir, Delsbo, Sweden). Pots were placed permanently in a plastic tray filled with 5–10 mm of nutrient solution (the A-type recipe of OAT Solution; OAT Agrio Co. Ltd., Tokyo, Japan), which was considered to keep the root-zone moisture content nearly constant throughout the experiment.

The light treatments were applied in two independent chambers, each maintained at either high or low *VPD* throughout the study. To establish separate FR+ and FR– zones within a single chamber, we installed two LED panels and used a reflective aluminum film partition to prevent light mixing between these zones. This setup allowed us to simultaneously apply the light treatments (FR+ or FR–) while maintaining the specified *VPD* conditions.

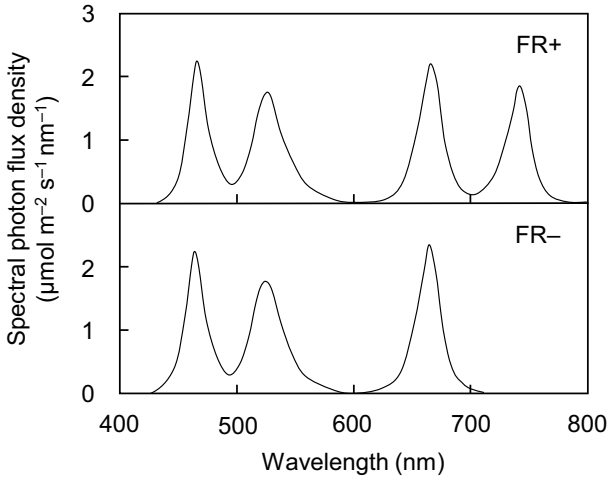


Fig. 1. Spectral photon flux density of light sources containing different proportions of far-red light: FR+, FR light at approximately the same proportion as in sunlight; FR–, light containing no FR light.

2.2. Measuring gas exchange and morphological properties

When the first true leaves had expanded and the second true leaves had begun to emerge, the gas exchange properties of the seedlings that had been acclimatized to each treatment group were evaluated with a photosynthesis measuring system (LI-6800, LI-COR, Inc., Lincoln, NE, USA). The net photosynthetic rate (P_n) and g_s of the first true leaf were measured under a saturating light intensity of 1500 μmol m^{-2} s^{-1} PPFD at atmospheric CO_2 concentrations of 70, 200, 400, and 800 μmol mol^{-1}. We then used these values to determine the relationships between intercellular CO_2 concentration (C_i) and P_n. Illumination during the measurements was provided by the built-in LED light source of the LI-6800 system, consisting of red (peak wavelength = 635 nm) and blue (peak = 465 nm) LEDs at a ratio of 7:3. A light intensity of 1500 μmol m^{-2} s^{-1} was selected to ensure photosynthetic saturation across all treatment conditions. Shibuya et al. (2012) reported that cucumber seedlings approached saturation at approximately 1200 μmol m^{-2} s^{-1}. Moreover, a PPFD of 1500 μmol m^{-2} s^{-1} is commonly used as a saturating irradiance level in P_n–C_i curve measurements to evaluate non-stomatal limitations of photosynthesis (Bernacchi et al., 2013; Salter et al., 2019). Leaf temperature and *VPD* were maintained at 28 °C and 1.5 kPa, respectively. Intrinsic water-use efficiency (WUE_i) was calculated as P_n at 400 μmol mol^{-1} CO_2 (P_{n400}) divided by g_s at the same CO_2 concentration (g_{s400}).

The dry weight and leaf area of the first true leaf of the seedlings that had been acclimatized to each treatment group were measured. We then calculated *LMA* as leaf dry weight divided by leaf area. Imprints of the abaxial surfaces of the first true leaves were obtained by spreading a liquid adhesive (Ekiban A; Taihei Yakuhin Co., Ltd., Tokyo, Japan) onto the leaf surface, allowing it to dry, and then peeling it from the leaf. These imprints were examined at 1000 × magnification under a VHX-1000 digital microscope (Keyence Corp., Osaka, Japan) to determine stomatal density.

2.3. Statistical analysis

The effects of FR light and *VPD* on gas-exchange and morphological traits were analyzed using a generalized linear mixed model (GLMM) with replicate as a random factor. The model was specified as follows:

$$\text{trait} \sim \text{factor}(FR) \times \text{factor}(VPD) + (1|\text{replicate})$$

In matrix notation, the model can be expressed as:

$$y = X\beta + Zu + \varepsilon,$$

where y is the vector of observed trait values, X is the design matrix for fixed effects including the intercept, FR, *VPD*, and their interaction, β is the vector of fixed-effect coefficients, Z is the design matrix for random effects corresponding to replicates, u is the vector of random effects, and ε is the vector of residual errors.

We identified significant differences among treatment means using this model, followed by the Tukey–Kramer method at $P < 0.05$ to detect significant differences between pairs of treatment means. The model's performance was assessed by calculating the Akaike information criterion (AIC), Bayesian information criterion (BIC), as well as Marginal R^2 and Conditional R^2. The random effects variance components were also extracted from the model. Data analysis was performed in R software, with the lme4 package for mixed models and the MuMIn package for R^2 calculations.

To evaluate the contribution of fixed effects, we also fitted a baseline model that included only the random intercept: *trait* ~ 1 + (1|replicate), and compared it to the full model using AIC, BIC, and Conditional R^2.

We analyzed the relationship between P_{n400} and *LMA* by using a generalized linear model based on the average values from the four treatments across five replicates, resulting in a total of 20 data sets.

To assess the variability across biological replicates, the mean and standard error of the mean (SEM) were calculated for each FR × *VPD* treatment using the five independent experimental trials as replicates. This ensures an appropriate estimate of the variability of the treatment means.

The experiment was performed over five separate trials, each referred to as a replicate. In each replicate, we examined the effects of various treatments, which included combinations of two levels of FR light and two levels of *VPD*. For gas-exchange properties, we used three seedlings per treatment in four of the replicates and five seedlings per treatment in one replicate. For *LMA*, we used five seedlings per treatment in four replicates and three seedlings per treatment in one replicate. For stomatal density, we used three seedlings per treatment in three replicates, five seedlings per treatment in one replicate, and one seedling per one replicate.

3. Results

3.1. Summary of model analysis

The results of the linear mixed models for gas-exchange and morphological traits are summarized in Table 1. Comparisons between the full model and the baseline model, which included only random intercepts, reveal that the inclusion of fixed effects (FR × *VPD*) improved model fit across all traits, as evidenced by lower AIC and BIC values in the full model.

The marginal and conditional R^2 values further clarify the contribution of fixed and random effects. For g_{s400}, the marginal R^2 is high (0.878), and equal to the conditional R^2, while the baseline model R^2 is zero, indicates that fixed effects alone explain nearly all the variation, and random effects contribute negligibly. In contrast, P_{n400} shows a lower marginal R^2 (0.267), suggesting that fixed effects alone are insufficient. However, the high conditional R^2 (0.850) and its substantial improvement over the baseline model ($R^2 = 0.563$) indicate that both fixed and random effects contribute meaningfully to the model. Other traits, including WUE_i, *LMA*, and stomatal density, also showed improvements in model fit with the inclusion of fixed effects.

Overall, these results support the appropriateness of using mixed models in this study and demonstrate the robustness of the statistical approach employed.

Table 1
Summary of generalized linear mixed model (GLMM) analysis. The full model structure was *trait* ~ *FR* × *VPD* + (1 | replicate). The baseline model included only the random intercept: *trait* ~ 1 + (1 | replicate). Random effects variance refers to the variance of the random intercept associated with replicate. Values outside parentheses correspond to the full model; values inside parentheses indicate the corresponding metrics for the baseline model.

Trait	Random effects variance	AIC	BIC	Marginal R^2	Conditional R^2
P_{n400}	14.82	295.2 (355.8)	308.3 (362.3)	0.267	0.850 (0.563)
g_{s400}	6.12×10^{-19}	–29.03 (91.92)	–15.83 (98.53)	0.878	0.878 (0.000)
WUEi	93.7	487.3 (599.9)	500.2 (606.4)	0.713	0.844 (0.098)
LMA	5.17	365.8 (408.8)	380.1 (415.9)	0.275	0.667 (0.368)
Stomatal density	30.01×10^{3}	733.8 (801.0)	746.1 (807.2)	0.351	0.708 (0.032)

AIC: Akaike information criterion; BIC: Bayesian information criterion; Marginal R^2: variance explained by fixed effects; Conditional R^2: variance explained by both fixed and random effects.
P_{n400}: net photosynthetic rate at atmospheric CO_2 = 400 μmol mol^{-1}, g_{s400}: stomatal conductance at atmospheric CO_2 = 400 μmol mol^{-1}, *WUEi*: intrinsic water-use efficiency, *LMA*: leaf mass per unit area.

3.2. Gas-exchange properties

P_{n400} was greater in the leaves acclimatized to FR– and high *VPD* than in the leaves acclimatized to FR+ and low *VPD* (Fig. 2A). There was a significant effect of the interaction between FR light and *VPD* on P_{n400}: specifically, P_{n400} in the leaves acclimatized to FR– was 0.95 × the FR+ value under low *VPD* (not significant), but 1.31 × the value (significantly higher) under high *VPD*.

g_{s400} was significantly greater in the leaves acclimatized to FR– and low *VPD* than in the leaves acclimatized to FR+ and high *VPD* (Fig. 2B). The effects of FR light and *VPD* on g_{s400} were both significant, but the interaction was not significant. g_{s400} differed significantly between FR– and FR+ in both *VPD* treatments. g_{s400} in the leaves acclimatized to FR– was 1.39 × the value in the leaves acclimatized to FR+ under low *VPD* and 2.47 × under high *VPD*.

WUE_i was greater in the leaves acclimatized to both FR+ and high *VPD* than in the leaves acclimatized to FR– or low *VPD* (Fig. 2C). The effect of FR light on WUE_i was more pronounced in the leaves acclimatized to high *VPD*. WUE_i in the leaves acclimatized to FR– was 0.67 × the value in the leaves acclimatized to FR+ under low *VPD* and 0.52 × under high *VPD*.

The P_n–C_i curves (Fig. 3) show that under the same internal CO_2 concentration (C_i), the leaves acclimatized to FR– and high *VPD* had greater P_n than those acclimatized not only to FR+ and low *VPD* but also for both the low *VPD* treatments, although the low-*VPD* treatments did not differ. The changes in P_{n400} due to the variability in the photosynthetic capacity of the mesophyll (the P_n–C_i curves) were greater than the possible changes in P_{n400} due to the larger range of changes in g_s (the reverse of the slopes of the straight lines shown in Fig. 3).

3.3. Morphological properties

There was a significant effect of the interaction between FR light and *VPD* on *LMA: LMA* did not differ significantly between the FR-light treatments at low *VPD*, but *LMA* in the leaves acclimatized to FR– was 1.19 × the value in the leaves acclimatized to FR+ at high *VPD*. *LMA* had a significant positive correlation with P_{n400} ($r = 0.60$, $P = 0.005$). The stomatal density was significantly higher under FR– than under FR+ at both low and high *VPD* levels (Fig. 4B), with values 1.44 × and 1.21 ×, respectively, the corresponding FR+ value.

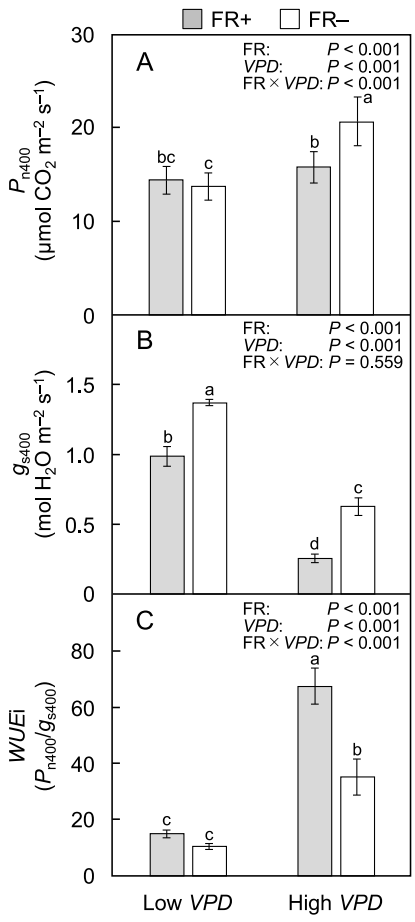


Fig. 2. (A) Net photosynthetic rate at atmospheric CO_2 = 400 μmol mol^{-1} (P_{n400}), (B) stomatal conductance at atmospheric CO_2 = 400 μmol mol^{-1} (g_{s400}), (C) intrinsic water-use efficiency (WUE_i = P_{n400}/g_{s400}) of the first leaves in cucumber seedlings that had been acclimatized to four conditions: with or without far-red (FR) light (FR+ or FR–), under low (0.8 kPa) or high (2.3 kPa) vapor-pressure deficit (*VPD*). Values are means ± SEM (n = 5, based on five independent experiments). For a given variable, bars labeled with the same letters are not significantly different (generalized linear mixed model with replicate as a random factor, followed by a Tukey–Kramer test at $P < 0.05$).

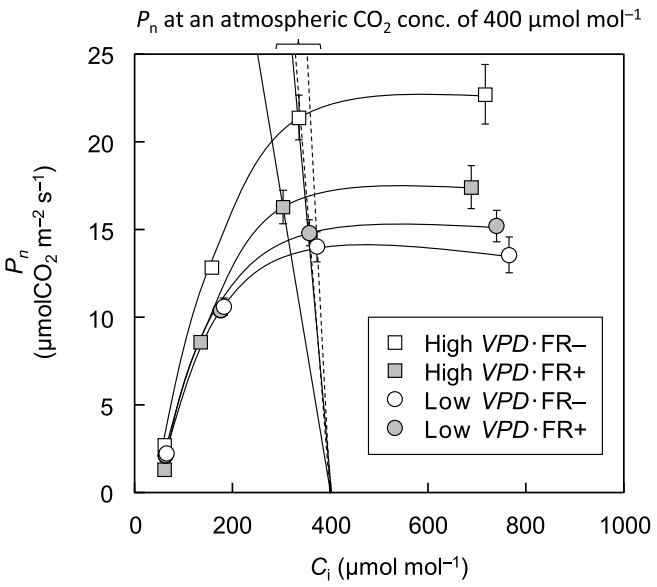


Fig. 3. Relationships between intercellular CO_2 concentration (C_i) and net photosynthetic rate (P_n) of the first true leaves of cucumber seedlings that had been acclimatized under four conditions: with or without far-red (FR) light (FR+ or FR–), under low (0.8 kPa) or high (2.3 kPa) vapor-pressure deficit (*VPD*). Values are means ± SEM (n = 5, based on five independent experiments). The reverse of the slopes of the solid and dashed lines originating from C_i of 400 μmol mol^{-1} represent the response of stomatal conductance to CO_2 diffusion under high *VPD* (solid) and low *VPD* (dashed).

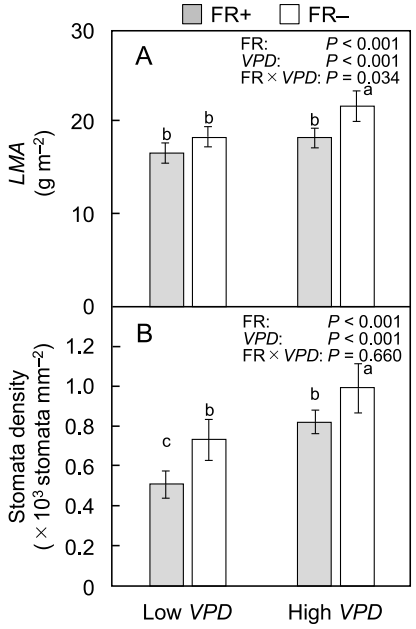


Fig. 4. (A) leaf mass per unit area (*LMA*), and (B) stomatal density of the first leaves in cucumber seedlings that had been acclimatized to four conditions: with or without far-red (FR) light (FR+ or FR–), under low (0.8 kPa) or high (2.3 kPa) vapor-pressure deficit (*VPD*). Values are means ± SEM (n = 5, based on five independent experiments). For a given variable, bars labeled with the same letters are not significantly different (generalized linear mixed model with replicate as a random factor, followed by a Tukey–Kramer test at $P < 0.05$).

4. Discussion

4.1. Gas-exchange properties

The greater photosynthetic performance of the cucumber seedlings acclimatized to FR– illumination agrees with the results in previous studies that investigated the same species (Shibuya et al., 2010, 2012, 2015; Hogewoning et al., 2010). The significant positive correlation between *LMA* and P_{n400} suggests that increased photosynthesis in the leaves acclimatized to FR– and high *VPD* can be attributed to an increase in *LMA*, thereby increasing the photosynthetic tissue per unit leaf area (Frak et al., 2002; De La Riva et al., 2016). Previous studies have also shown that low-FR light increases *LMA* and photosynthetic performance at high light intensities (Shibuya et al., 2010, 2012, 2015; Hogewoning et al., 2010). However, our results suggesting the enhanced photosynthetic performance due to acclimatization under high *VPD* contradicts with previous reports, which showed no change in photosynthetic capacity in cucumber leaves acclimatized to different *VPD*s (Shibuya et al., 2017, 2018). Importantly, these studies did not investigate the interactions with FR light, which may limit the applicability of their findings to our study. In addition to this difference, this inconsistency might also be attributable to the differing *VPD* conditions used in the studies; although photosynthesis was enhanced at a higher *VPD* of 2.3 kPa, it was not enhanced at higher *VPD*s (3.0 to 3.2 kPa). This could be due to the negative effects of excessively high *VPD* outweighing the potential benefits to photosynthesis. Li et al. (2019) reported lower photosynthesis of tomato leaves due to non-stomatal factors at high *VPD*

(2.5 to 3.0 kPa) than at low *VPD* (0.5 to 1.0 kPa). Further studies under a range of *VPD* conditions will be necessary to clarify the relationship between *VPD* and photosynthesis and to identify the optimum *VPD* for enhancing photosynthetic capacity.

The P_n–C_i curves show that the influence of non-stomatal factors on P_{n400} was greater than that of stomatal factors under the study conditions. We attribute this enhancement of photosynthetic capacity primarily to the increase in *LMA*, which leads to a higher mesophyll photosynthetic capacity per unit leaf area. This is consistent with our previous results that the reduction in photosynthesis due to decreased g_s and the subsequently lower C_i can be minimal, depending on the extent of the g_s reduction (Shibuya et al., 2017). Even though g_{s400} was lower in the leaves acclimatized at high *VPD* than in leaves acclimatized at low *VPD,* P_{n400} tended to be greater. This suggests that non-stomatal factors, such as an increase in *LMA* associated with high *VPD*, play a more significant role in boosting photosynthesis under these conditions than the reduction in gas exchange caused by decreased g_s.

Although P_n increased under FR–, this improvement was outweighed by an increase in g_s, resulting in lower WUE_i. Plants generally reduce g_s under high *VPD* to minimize water loss (Buckley, 2005; Peak and Mott, 2011; Grossiord et al., 2020; Salmon et al., 2020). However, under FR–, g_s increased despite high *VPD*. This may be explained by our recent finding that leaf hydraulic conductance is enhanced under low-FR light, allowing sustained stomatal opening even under high evaporative demand (Shibuya et al., 2024a).

As previously mentioned, the increase in P_n under FR− was significantly influenced by non-stomatal factors, while the elevated g_s further contributed to this enhancement. Leaves acclimatized to low-FR light can maintain high g_s possibly due to enhanced leaf hydraulic conductance, allowing them to maintain leaf water content even under high evaporative demand when root-zone water availability is sufficient. Our findings suggest that the low-FR light conditions may have mitigated water stress when root-zone moisture was adequate.

This acclimatization can effectively boost photosynthetic rates; however, the associated high g_s may lead to increased transpiration and consequently faster depletion of root-zone water, potentially outweighing the benefits of increased photosynthesis under conditions of low root-zone moisture. Although specific data on water consumption were not collected in this study, this mitigation is likely dependent on environmental conditions, particularly root-zone water availability. Therefore, further investigation into actual water consumption is essential to fully understand the balance between photosynthetic gain and water loss in practical agricultural settings.

4.2. Morphological properties

The increase in *LMA* observed under FR− and high *VPD* conditions is consistent with previous findings that highlight the role of strong-light acclimatization resulting from exposure to low-FR light (Shibuya et al., 2010, 2012, 2015; Hogewoning et al., 2010). Additionally, our results support the notion that such increases in *LMA* are adaptations to heightened evaporative demand, as suggested by Itagaki et al. (2014), Li et al. (2019), and Amitrano et al. (2021). This correlation underscores the importance of environmental factors in driving physiological responses in the plants studied.

The increase in *LMA* under FR− and high *VPD* conditions can be explained by the restrictive effects these conditions have on leaf expansion. Shibuya et al. (2019) reported that low-FR light combined with high *VPD* limits leaf expansion, promoting thicker and denser leaves, which can lead to higher *LMA*. Conversely, while high-FR light and low *VPD* may also promote leaf thinning, there may be physiological limits to this thinning process.

If such limits exist, the increase in *LMA* under low-FR light and high *VPD* may appear more pronounced, as the capacity for leaf thinning under high-FR light and low *VPD* can be constrained. This suggests that high-FR light and low *VPD* may act antagonistically on *LMA* dynamics, potentially creating the appearance that high-FR light and high *VPD* are synergistically increasing *LMA*. Therefore, understanding *LMA* dynamics must consider these opposing effects. This observation highlights the need for further investigation of the interactions between light quality and atmospheric humidity in controlled environments, supported by a detailed examination of the effect of specific leaf area (the inverse of *LMA*).

The increase in stomatal density observed under low-FR light and high *VPD* is consistent with findings from previous studies (Boccalandro et al., 2009; Casson et al., 2009; Carins-Murphy et al., 2014). Under both *VPD* conditions, g_{s400} was greater in the seedlings exposed to FR– compared to those under FR+. However, under high *VPD,* g_{s400} was lower than that under low *VPD*, which can be attributed to the smaller stomatal apertures that occur at higher *VPD* levels. The increase in g_s due to low-FR light under high *VPD* was greater than the increase in stomatal density, indicating that the stomatal aperture was larger when exposed to low-FR light. This observation is consistent with the hypothesis that higher g_s can be sustained despite high *VPD* conditions due to enhanced leaf hydraulic conductance under low-FR light, as mentioned above. Such increased g_s potentially increases CO_2 uptake even at the expense of decreased WUE (Boccalandro et al., 2009; Shibuya et al., 2015). Our findings indicate that this phenomenon is more pronounced under high *VPD* than under low *VPD* due to complex interactions in morphological and physiological responses.

These results demonstrate that *LMA* and stomatal density were significantly influenced by both FR light and *VPD*, and that morphological acclimation plays a key role in determining photosynthetic performance under these environmental conditions. However, because only two levels of FR light were tested in this study, we cannot determine the optimal FR intensity for improving photosynthetic performance. Moreover, as our findings suggest that the effects of FR may vary depending on *VPD*, further research using a wider range of FR intensities under different *VPD* levels will be necessary to identify conditions that optimize both morphological and physiological acclimation.

5. Conclusion

FR light and atmospheric humidity affected photosynthetic performance in complex ways, with low-FR light and high *VPD* synergistically increasing photosynthesis at intense light. Such traits may be beneficial for plant production under controlled environments, such as during transplant production using artificial light sources, where plants will be moved under intense light. However, under low-FR light, particularly at high *VPD*, the increase in photosynthesis was outweighed by an increase in stomatal conductance, resulting in lower WUE, which indicates a trade-off between photosynthetic performance and water use efficiency. Further research into how different lighting conditions and atmospheric humidity settings affect physiological and morphological properties will be important in optimizing horticultural practices and tailoring the environment to maximize photosynthetic efficiency and growth of both cucumber and other horticultural crop species.

Declarations

Artificial intelligence: We utilized AI technology in the writing process solely to enhance the readability and language of the manuscript.

CRediT authorship contribution statement

Toshio Shibuya: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nanoka Wada:** Methodology, Investigation, Data curation. **Ryosuke Endo:** Writing – review & editing, Validation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Toshio Shibuya reports financial support was provided by Japan Society for the Promotion of Science. Reports a relationship with that includes: Has patent pending to. If there are other authors, they 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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Data availability

Data will be made available on request.

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