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Irradiation and nitrogen regulate growth and physiology in *Horsfieldia hainanensis* seedlings

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Abstract

Preventing extinction is one of the greatest challenges facing the global community. Nursery stock breeding is an effective means to restore endangered species, such as *Horsfieldia hainanensis* Merr., with difficulty in natural regeneration period. In this study, we investigated the optimum combination of irradiance and nitrogen for the cultivation of *H. hainanensis* seedlings by comparing twenty treatments with different combinations of irradiances (100, 67.5, 45.7, 15.6 % of full natural irradiance) and five levels of N supply (0, 1.8, 3.6, 5.4, 7.2 g plant^{-1}). We found that the growth and photosynthetic efficiency of seedlings under full irradiance were significantly inhibited compared with shaded seedlings. Under full irradiance, a lack of N resulted in reduced chlorophyll (Chl) synthesis, causing lower photosynthetic efficiency and an imbalance in metabolism. Proper shading (67.5 and 45.7 % of natural irradiance) and N addition (1.8 - 5.4 g plant^{-1}) promoted root development, increase Chl content and photosynthesis, and ultimately the accumulation of larger amount of biomass. The biomass of the shaded seedlings was mainly distributed to aboveground tissues, while seedlings exposed to stronger radiation accumulated greater root biomass. Therefore, the best seedling management for this species is a combination of 67.5 % of natural irradiance and moderate N supply (4.6 g plant^{-1}).

Keywords: chlorophyll, endangered species, irradiance, nitrogen supply, photosynthesis, seedling cultivation, shading.

Introduction

Sun radiation is important for plant growth and survival (Zhou *et al*. 2017), however, plants vary in their ability to adapt to different irradiances. When the irradiance is greater than the photosynthetic system can safely use, plants become photoinhibited (Yang *et al*. 2018) resulting in changes in plant morphology, growth, and physiology (Okunlola and Adelusi 2014). Under excessive irradiance, leaves turn yellow, and plants become stunted, slender, and may die. The intensity and quality of radiation directly determine the efficiency of photosynthesis, as well as photosynthetic enzyme activities and photosynthetic organelle growth (Li *et al*. 2014). During the onset of photosynthesis, the kinetics of chlorophyll (Chl) fluorescence yields important information about the characteristics of radiation absorption, transmission, and dissipation, and the distribution of energy. In particular, the fluorescence signal of photosystem II (PS II) is a strong indicator of the effect of environmental stress on the photosynthetic apparatus (Ivanov *et al*. 2015).

Photosynthesis occurs in the thylakoids of chloroplasts, in which photosynthetic pigments (Chl and carotenoids) absorb radiation energy and convert it into chemical energy (Chen *et al*. 2018). Nitrogen, as an important component of both the chloroplasts and Chl, directly affects the photosynthetic capacity and efficiency of plants. Therefore, the N content of plants plays a key role in photosynthesis (Ji *et al*. 2015). At reasonable N content, the net photosynthetic rate (P_N), stomatal conductance, total Chl content, and other indicators increase with increasing N content (Wang *et al*. 2019). However, under excess N, nutrient imbalance occurs, reducing the ability of the plant to photosynthesize (Li *et al*. 2018). Insufficient N to meet the needs of plant growth also reduces P_N, resulting in a reduction in plant yield (Cohen *et al*. 2019).

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Abbreviations: Chl - chlorophyll; GS - glutamine synthetase; P_N - net photosynthetic rate; PS II - photosystem II.

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Given its importance to plant growth, it is imperative to study the effects of N on photoinhibition in shade-tolerant plants, especially in endangered species.

Horsfieldia hainanensis Merr. (family *Myristicaceae*) is an endangered species and a symbol of the tropical moist rainforest. It is distributed throughout southern and western China (Dang 2009). It is shade tolerant (Jiang *et al*. 2018a), with a dense crown, straight trunk, and colorful fruit (Hu 2010), and is of great utility in urban landscapes. It also has economic importance in the wood industry (Jiang *et al.* 2016), and in a Chinese traditional medicine, where its bark is used to heal wounds and facilitate postpartum recovery. However, *H. hainanensis* is increasingly subject to severe habitat loss due to landscape changes and climate change (Jiang *et al*. 2018b), and it has been identified as a second-grade threatened species by the Chinese government (State Council 1996). *H. hainanensis* grows in dense, humid forest valleys and hills at elevations of 400 - 450 m (Dang *et al*. 2009). *H. hainanensis* forests normally have canopy densities of 20 - 80 %, and the natural regeneration of seedlings is difficult, with relatively low survival rates. We speculate that *H. hainanensis* is sensitive to irradiance. Therefore, determining the optimum irradiance and N supply for *H. hainanensis* seedlings, and transplanting them back to their original habitats after cultivating the seeds, is important for wildlife conservation, as well as for research on shade-tolerant mechanisms and for the development of forestry projects. Previous studies on *H. hainanensis* focused on chemical components (Xue 2012), cultivation (Hu 2010), community structure (Jiang *et al.* 2016), and organ sections. Studies of the influences of irradiance and N supply on the growth and photosynthesis of *H. hainanensis,* however, are lacking. Therefore, we performed this study to answer the following questions: *1*) Does full natural irradiance cause photoinhibition in *H. hainanensis* seedlings? *2*) Can photoinhibition of *H. hainanensis* seedlings be relieved by N fertilizer application? *3*) Whether the distribution patterns of biomass of seedlings under strong irradiance and shade are consistent? 4) What is the optimum combination of irradiance and N supply for photosynthesis and growth of *H. hainanensis*?

Materials and methods

Growth conditions: The study was conducted outdoors in the nursery of Guangxi University (Nanning, Guangxi China; 108°17'E, 22°50'N). The mean temperature was 22.19 °C during the test period, with a maximum temperature of 38.9 °C in June and a minimum temperature of 12 °C in December. The average number of days with sunshine was 125.08, the number of days with monthly average daily precipitation greater than or equal to 0.1 mm was 9.7, and the average relative humidity was 78.61 %. Seedlings were grown in acid lateritic soil (Table 1 Suppl.) that had been sterilized with 50 % carbendazim (a fungicide) while drying, pulverized, and sieved through a 0.850 mm mesh screen.

Experimental design: The study was conducted from June 2016 to February 2017. One-year-old seedlings of *Horsfieldia hainanensis* Merr. were provided by the China Academy of Forestry (Qinzhou, Guangxi, China). Each seedling was planted in a black 23 cm diameter, 20 cm high pot containing 3.5 kg of prepared soil (sterilized with 5 % formalin for 5 d, air-dried for 5 d, ground and sieved through a 2-mm screen) on June 6, 2016. On June 13, 2016, 100 healthy seedlings (height: 21.4 ± 1.1 cm, diameter: 5.33 ± 0.26 mm) were selected for the experiments.

The seedlings were grown at four irradiances and five N supplies (total of twenty treatments), each treatment included ten replicates. The four irradiances were 100, 67.5, 45.7, and 15.6 % of natural irradiance. The five N concentrations were 0, 1.8, 3.6, 5.4, and 7.2 g plant^{-1}. The shading was performed using a shading net made of polyethylene, high-density polyethylene, polybutylene, polyvinyl chloride, and polyethylene propylene. The transmittance (ratio of irradiance under the shading net to the irradiance under full natural irradiance) and irradiances were measured using a portable photosynthesis system (model *LI-6400XT*; *LI-COR Biosciences*, Lincoln, NE, USA). The N content was chosen according to the nutrient balance method: fertilizer supply = (crop demand - soil nutrient supply) / fertilizer use ratio (Guoqi 2001). The soil and leaf N content of three representative seedlings, measured before the start of the experiments, were substituted into the nutrient-balance formula to obtain a median of approximately 3.6 g plant^{-1}, which was then chosen as the middle point of the N concentration range tested, increasing or decreasing by half of median value twice in turn. There were 20 treatments in the experiment, with ten random repeats per treatment.

Shading and N supply began on June 13, 2016. The seedlings were placed in four shading nets measured 10.0 × 5.0 × 2.5 m in width, length, and height, respectively. Plants were fertilized every 25 d for a total of ten times. N was supplied as a urea (N content: 46.67 %). The appropriate amount of urea, dissolved in 1 dm^3 of tap water, was supplied to each potted plant. A microcomputer-controlled automatic sprinkler irrigation system was used to keep the potted soil at a consistent moisture, and other management (weeding, loosening of soil) was the same for all plants. Table 1 Suppl and Table 2 Suppl describe the specific treatments.

Plant growth: Seedling height and stem diameter were measured at the beginning of the experiment on June 13, 2016, and again at the end of the experiment in February 2017. The height of each seedling from the surface of the potted soil to the top bud was measured using a ruler with a precision of 0.1 cm. The stem diameter of the main stem was measured parallel to the pelvic orifice using a vernier caliper with a precision of 0.01 mm.

At the end of the experiment, three representative seedlings were selected from each treatment. The roots, stems, and leaves were collected, labeled, dried in an oven at 105 °C for 30 min, further dried at 80 °C until reaching a constant mass, and then weighed to a precision of 0.001 g.

A hand-held laser leaf-area meter (*CI-203*, *CID Bio-Science*, Camas, WA, USA) was used to measure the leaf area of three representative seedlings from each treatment. Total root length, total root volume, total root surface area, total root mean diameter and total root tip number were all measured by *Epson Expression 10000XL* (*Epson,* China) scanner and *WinRHIZO* root analysis system.

Chlorophyll content and net photosynthetic rate: After the experiment, three mature leaves were selected from three plants exhibiting similar growth rates from each treatment group, the primary veins of these leaves were removed and the Chl in the leaves was extracted using an acetone-ethanol mixture (Lightenthaler 1987). Briefly, 0.1 g of fresh leaf tissue was added to 10 cm^3 of ethanol-acetone mixture (1:1) and placed in darkness with intermittent oscillations until the leaf tissue was completely white. The absorbance of each sample was measured at 665, 649, and 470 nm using an UV spectrophotometer (*UV-2450*, *Shimadzu*, Kyoto, Japan).

From 09:00 to 11:00 on 25 February, 2017, the P_N of mature intact leaves was measured using a portable photosynthesis meter (*LI-6400XT*; *LI-COR Biosciences*, Lincoln, NE, USA). The reference cell consisted of a CO_2 concentration of 400 µmol mol^{-1} and the temperature in the sample cell was 35 °C. Three leaves from each of three plants were selected for each measurement (Yang *et al.* 2017).

Soluble sugar content: The content of soluble sugars was measured according to Fairbairn 1953. Briefly, 0.5 g of fresh leaf or root tissue were cut into pieces and put into a test tube, with 5 cm^3 of distilled water. The tissue was extracted in a boiling water bath for 30 min, and then the supernatant was collected. The volume of the solution was adjusted to 10 cm^3 by distilled water after repeating twice. The extract was added to an anthrone-sulfuric acid solution and the absorbance was measured at 630 nm.

Glutamine synthetase activity: Determination of glutamine synthetase (GS) activity was according to the method described by Silveira *et al*. (2003). The 1.0 g of the fresh leaves were fully ground in an ice bath and extracted with 5 cm^3 of 100 mM HEPES buffer, pH 7.6, containing 0.2 mM EDTA, 5 mM dithiothreitol, and 10 mM $MgCl_2$ at 0 - 4 ° for 5 min. The enzyme extract (0.5 cm^3) was added to the scale test tube, to which 0.6 cm^3 of Tris-HCl buffer (250 mM, pH 7.0), 0.2 cm^3 ATP (30 mM, pH 7.0), 0.2 cm^3 of $MgSO_4$ (500 mM), and 0.2 cm^3 of 1 M hydroxylamine hydrochloride neutralized by 1 M HCl was added. The absorbance was measured at 540 nm after incubation at 30 °C for 30 min.

Statistical analysis: Data were converted into the means ± standard errors using *Microsoft Excel 2010* (*Microsoft*, Redmond, WA, USA) *R v. 1.1.453*. *SPSS v. 17* (*SPSS Inc.*, Chicago, IL, USA) was used to perform two-way *ANOVA* and principal component analysis to analyze the effects of irradiance, N supply, and their interactions on growth and physiological parameters of *H. hainanensis* seedlings. The significant differences were checked with Duncan's test at the 0.05 probability level.

Principal component of seedling growth, irradiance level, N fertilizer dosage accessed from PCA analysis were used for determining the regression coefficients of the regression model. The analysis of regression model was

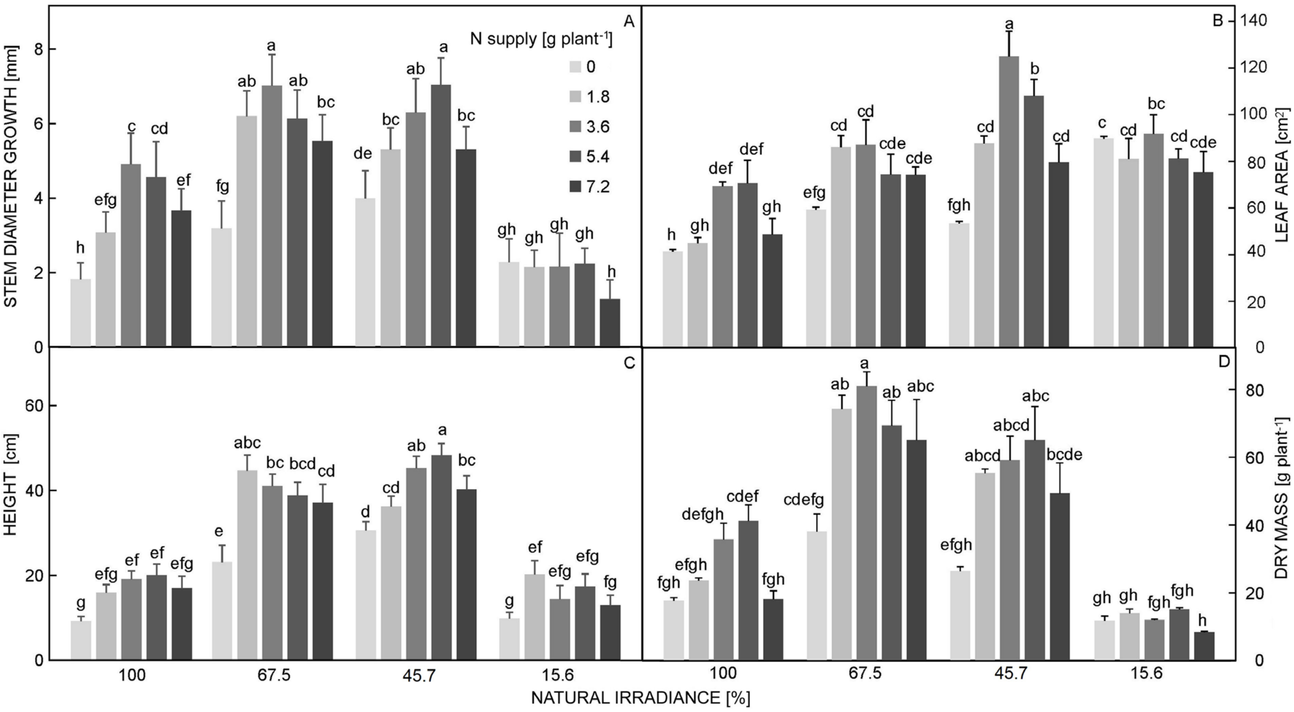

Fig. 1. Effects of N supply on growth indices of *H. hainanensis* seedlings grown under four irradiances. Means ± SEs. Significant differences among treatments are indicated by different letters ($P < 0.05$).

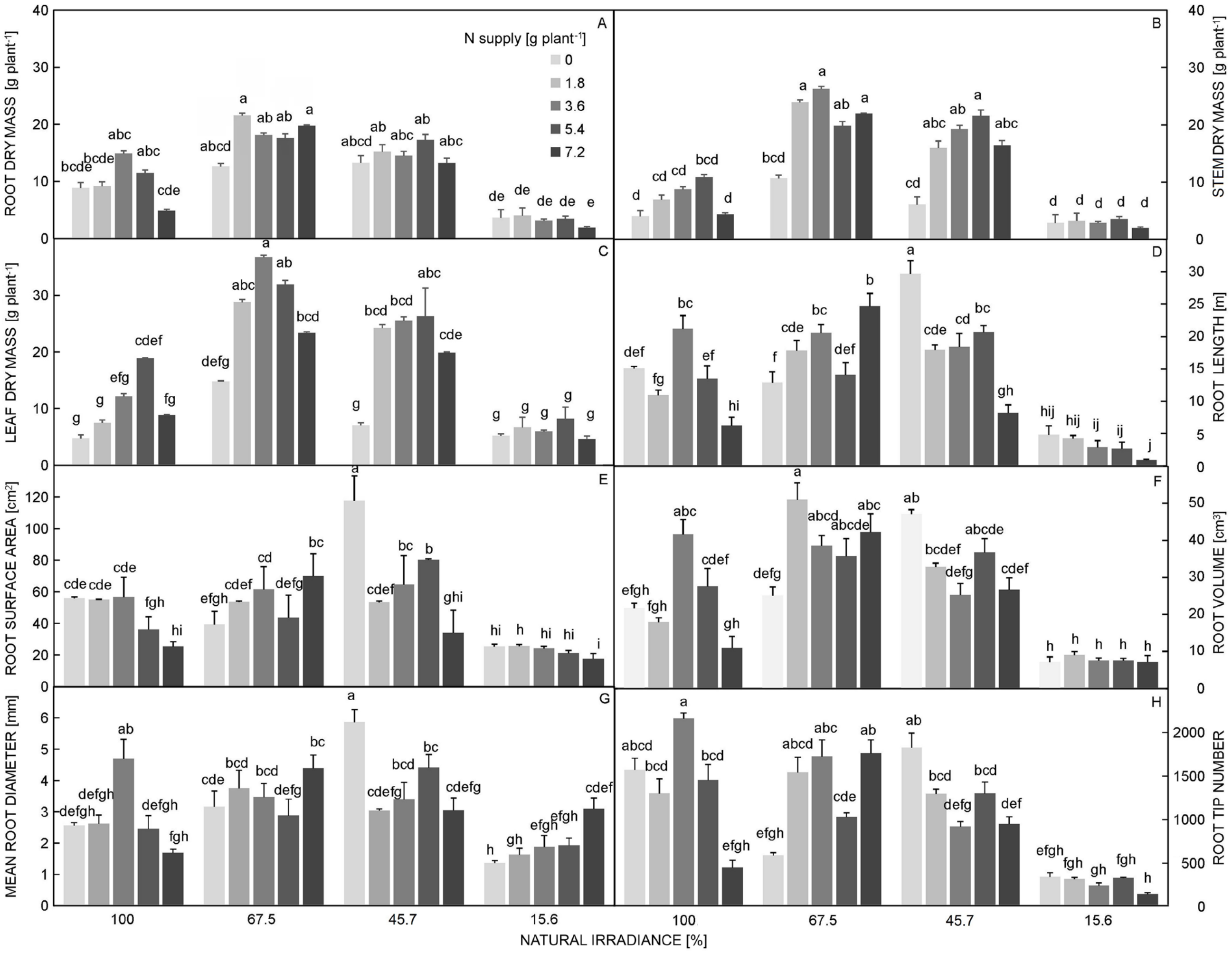

Fig. 2. Effects of irradiance and N supply on dry mass of plant organs and other root characteristics of *H. hainanensis* seedlings. Means ± SEs. Significant differences among treatments are indicated by different letters ($P < 0.05$).

carried out with using the *SAS* statistical package (*version* 8.1, SAS Institute, Cary, NC, USA).

Results

The growth of the seedlings, including stem diameter, height, leaf area, and total dry mass, increased and then decreased with the degree of shading ($P < 0.05$; Fig. 1). The height, stem diameter and total dry mass of overshaded (15.6 % of natural irradiance) seedlings were lower than those without shading (100 % of natural irradiance), while leaf area was not higher. Under the same irradiance conditions, N fertilizer application significantly increased total dry mass, leaf area, plant height, and stem diameter. However, under excess N content (7.2 g plant^{-1}), seedling growth tended to decrease, with seedlings in 15.6 % of natural irradiance and 7.2 g plant^{-1} combination treatment displaying the least growth. In addition, the effect of N supply on seedling growth differed under the different irradiances. Under full nature irradiance and 15.6 % of natural irradiance, the maximum value of three of the four parameters, including height, stem diameter, leaf area and total dry mass were greatest at N supply 5.4 g plant^{-1}, while under 67.5 % of natural irradiance, the peak of plant stem diameter, leaf area and dry mass appeared at N supply 3.6 g plant^{-1}.

Irradiance and N supply interactions had different effects on dry mass of plant organs. In general, leaves were most affected, followed by stems and roots (Fig. 2). For most of the treatments, the underground biomass (root dry mass) was higher than the aboveground biomass (leaf dry mass) under full natural irradiance, while the aboveground biomass was higher than the underground biomass under shading. However, with the increase of shading degree, the three components of N fertilized seedlings increased first and then decreased, and the lowest dry mass was observed in the 15.6 % of natural irradiance. N had a deeper effect on the aboveground components than on the underground components. Specifically, at the same irradiance, different N treatments had no significant effect on the root dry mass of seedlings. Interestingly, under 100 and 15.6 % of natural irradiance, there was no significant difference in stem diameter and leaf dry mass

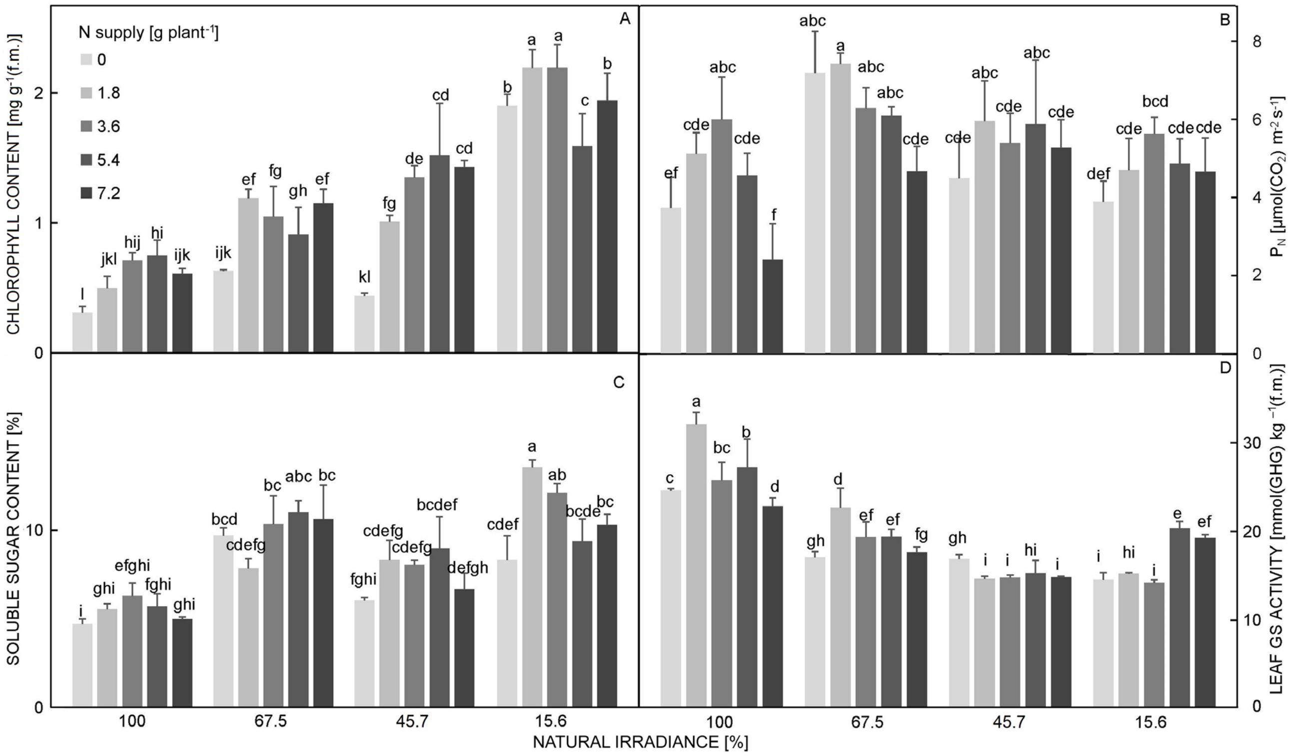

Fig. 3. Effects of irradiance and N supply on total Chl content, net photosynthetic rate, soluble sugar content, and the glutamine synthetase activity. Means ± SEs. Significant differences among treatments are indicated by different letters ($P < 0.05$).

between different N treatments, while under 67.5 and 45.7 % of natural irradiance, stem diameter and leaf dry mass increased firstly and then decreased with increasing N. However, when the N fertilizer amount reached 7.2 g plant^{-1}, it was not conducive to the accumulation of dry mass of all the components of the seedlings.

The influence of irradiance on root characteristics were similar: increased first and then decreased with the increase in shade, with the lowest values under 15.6 % of natural irradiance (Fig. 2*D-H*). However, the effect of N on each root characteristic was different. Under full irradiance, the total root length, total root volume, root diameter, and root tip number increased with the increase of N supply and then decreased. When the seedlings were placed under a shade net with 67.5 % of natural irradiance, the five root characteristics showed consistent changes. With the increase of N, they first increased, then decreased, and finally increased again. However, under 45.7 % of natural irradiance, total root length, total root volume and root diameter decreased with N increase, while root surface area and root tip number fluctuated. In 15.6 % of natural irradiance conditions, except for the increase of mean root diameter, the other four root characteristics decreased with increasing N application, but did not reach significant differences. Similarly, excessive addition of N fertilizer was also detrimental to root growth, especially when 7.2 g plant^{-1} of N was added under 15.6 % of natural irradiance.

Regardless of N treatment, the total Chl content, P_N, and soluble sugar content of seedlings grown under full natural irradiance were significantly lower than those of shaded seedlings (Fig. 3). Under shade, the average total Chl content, P_N, and soluble sugar content increased by 132.61, 33.49 and 63.58 %, respectively. For total Chl content, it increased significantly with the increase in shading, and reached the maximum at 15.6 % of natural irradiance. The P_N rate was greatest under 67.5 % of natural irradiance and then decreased with increasing or decreasing irradiance. N fertilizer application increased the total Chl content and soluble sugar content in leaves. Under 100 and 45.7 % of natural irradiance, the total Chl content of seedlings under the four treatments with N fertilizer increased significantly compared to those without N fertilizer, while under 15.6 % of natural irradiance, only seedlings under 1.8 g plant^{-1} and 3.6 g plant^{-1} treatments increased. Interestingly, under 100 and 67.5 % of natural irradiance, there was no significant change among the four N treatments, while under 45.7 and 15.6 % of natural irradiance, total Chl content showed an increasing trend and then decreased. Under 100, 67.5, and 45.7 % of natural irradiance, N fertilizer had little effect on content of soluble sugars. However, at high shading to only 15.6 % of natural irradiance, applying 1.8 and 3.6 g plant^{-1} of N significantly increased soluble sugar content. Excessive N treatment was detrimental to P_N and soluble sugar content. Notably, under 100 and 67.5 % of natural irradiance, the P_N value of N supply 7.2 g plant^{-1} was lower than in plants that received no N fertilizer. The glutamine synthetase (GS) activity in leaves was significantly greater in seedlings grown under full natural irradiance than in shaded seedlings (Fig. 3). Adequate N supply enhanced GS activity, but excessive N had no significant effect, but under low irradiance (15.6 %), the more N, the higher GS activity was.

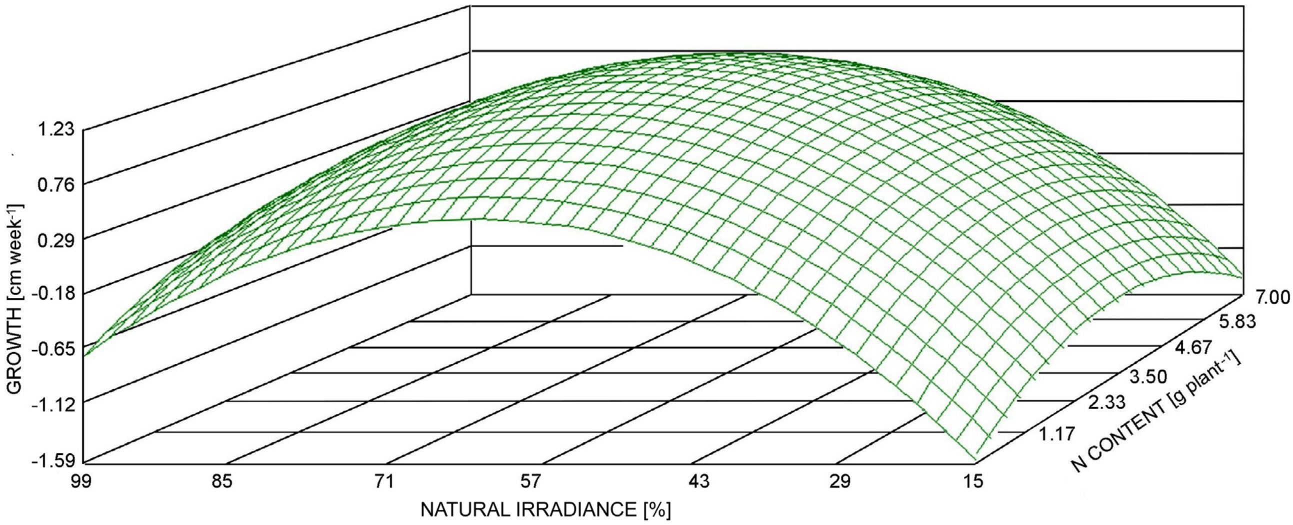

Fig. 4. Effect of irradiance and N supply on the overall growth of *Horsfieldia hainanensis* seedlings.

The extraction and analysis table of variance decomposition principal components of *H. hainanensis* seedlings under different irradiance and N supply (Table 3 Suppl.), presented the cumulative variance contribution rate of two common factors was 88.133 %. The principal component can be expressed as a linear combination of each variable through the feature vector (Table 4 Suppl.). Quadratic response surface regression analysis was carried out on the overall growth of the seedlings of *H. hainanensis* (Table 3 Suppl.). The overall probability of the model was significant ($P < 0.05$), and the linear term and quadratic term were also very significant ($P < 0.05$). The total coefficient of determination of the model R^2 was 0.8835 (Table 5 Suppl.).

Principal component of seedling growth (Y_B), irradiance level (X_1), N fertilizer dosage (X_2), irradiance level as X-axis, N fertilizer dosage as Y-axis, the regression model of the effect of irradiance and N coupling on the overall growth of *H. hainanensis* seedlings was as follows: $Y_B = -3.15106 + 11.80539 * X_1 + 0.3459 * X_2 - 9.43713 * X_1^2 + 0.03059 * X_1X_2 - 0.04817 * X_2^2$ was drawn (Fig. 4). Each cell on the surface represents a value of 0.02 for irradiance and 0.35 for N application. Each point on the curved surface represents the corresponding value of the overall growth of *H. hainanensis* seedlings on each treatment. The higher the point on the curved surface was, the more beneficial the treatment was to the overall growth. Under the same irradiance, the overall seedling growth of *H. hainanensis* increased firstly and then decreased with the increase of N application. Under the same N supply, the overall growth of *H. hainanensis* seedlings increased first and then decreased with the increase of shading degree. As a whole, the shape of the surface was a positive convex surface, which had an irradiance - N coupling level that maximized the overall growth value of seedlings. According to the surface diagram, the overall growth was the highest when irradiance = 0.65 %, N = 4.6 g plant^{-1}. In the coupling effect of irradiance and N, the dual effects of high shading and high N inhibited the growth of seedlings.

Discussion

Plant height, stem diameter, leaf area, and seedling biomass were greater under suitable shading (67.5 and 45.7 % of natural irradiance) and N fertilization than under full natural irradiance and no N application (Fig. 1). However, the excessive shading and high N supply inhibited seedling growth, which is consistent with the results of a previous study on *Liriope spicata* (Wang *et al*. 2006). As the most important abiotic factor affecting plant growth, sun radiation provides the energy for photosynthesis, thereby affecting organic matter production and ultimately determining biomass accumulation (Cardona *et al*. 2018). Photosynthesis occurs in the thylakoids of chloroplasts, where radiation energy is absorbed by photosynthetic pigments (Hall and Rao 1999) and converted into chemical energy. As a component of both chloroplasts and Chl, N directly affects the photosynthetic capacity and efficiency of plants. Therefore, N content plays a key role in photosynthesis (Barraclough *et al*. 2010). Irradiance requirements differ among plant species, and unsuitable irradiance can inhibit plant growth. Too high irradiance results in photoinhibition (Poulson and Thai 2015), consistent with our results showing the inhibition of *H. hainanensis* seedling growth under full natural irradiance. Under the stress caused by full natural irradiance, N supply was beneficial for growth, leading to increases in both leaf area and total dry mass. This result was consistent with another study that found that addition of N fertilizer improved the efficiency of transmission and non-photochemical quenching of excessive radiation, reducing photoinhibitory damage to the photosynthetic apparatus of leaves, and maintaining a higher Chl content and improved integrity of PS II (Kato *et al*. 2003). Shading improved by 35 % the apparent radiation use efficiency (Querné *et al*. 2017), which explains the higher biomass and better growth seedlings under 45.7 and 15.6 % of natural irradiance in this study. However, irradiance and N interactions had different effects on the dry mass of plant components. In general, the growth of shoots fluctuated more than the growth of roots. Moreover, the proportion of aboveground and underground biomass of shade seedlings was opposite to that under full irradiance, probably because shaded seedlings allocate more assimilates to the aboveground tissues (Poorter *et al*. 2012).

The morphological characteristics of *H. hainanensis* root system were consistent with growth under the influence of irradiance and N supply. It is well known that root characteristics play a decisive role in water and nutrient uptake. Previous studies have shown that applying N fertilizer can significantly increase root length and root volume (Fan *et al*. 2010). Proper shade and N application were beneficial to the increase of root tip number and root length of seedlings, thus increasing root volume and surface area to absorb more nutrients (Pan *et al*. 2016), while severe shade inhibited root development (Costa *et al*. 2002). Therefore, the root characteristics further verified the reliability of seedling growth results.

Under different irradiances, the utilization efficiency of the same N supply is different. Under full natural irradiance and 15.6 % of natural irradiance, seedlings total dry mass and leaf area increased gradually with increasing N application, and their greatest values appeared at 5.4 g $plant^{-1}$ N. However, there was a highest growth rate under 45.7 % irradiance and 3.6 g $plant^{-1}$ N. In other words, there is a correlation between the utilization of irradiance and N. Within a range of N < 5.4 g $plant^{-1}$, a high level of irradiance combined with a high concentration of N is more conducive to improving its utilization, and when the irradiance is low, increasing N supply also helps seedling growth. This conclusion is supported by the results of Mooney *et al*. (1978); they found that N content in the leaves was highly correlated with photosynthetic capacity. In addition, the optimum N content increased with the increase in daily sum of photosynthetically active radiation (Field 1983).

However, excess N fertilization (7.2 g $plant^{-1}$) was not beneficial to the formation of leaf area or the accumulation of leaf biomass. The protective effect of N against photoinhibition may be due to hypertrophic leaf growth, which would be conducive to dissipation of excessive radiation energy. Meanwhile, excessive N likely decreases the xanthophyll cycle pool size (on a Chl basis), which would decrease the ability to dissipate excess absorbed radiation energy (Cheng 2003).

Under full irradiance, *H. hainanensis* seedlings were photoinhibited, with reduced P_N, decreased total Chl content, and lower soluble sugar content. By contrast, suitably shaded seedlings exhibited significantly increased P_N, and increased accumulation of both total Chl and soluble sugar (Fig. 3). These results agree with previous studies that showed that shading increased the amount of nutrients allocated to shoot and leaf growth, promoted Chl accumulation (Zhou *et al*. 2017), and enhanced the photosynthetic rate (Huang *et al*. 2015).

The interaction between high irradiance and N deficiency cause damage to seedlings (Cohen *et al*. 2019). The combined stress of excessive irradiance and N deficiency results in a low leaf Chl content, reduced efficiency of radiation absorption, transmission, and transformation, so that the synthesis of sugars is relatively small. Meanwhile, the combination of the lower photosynthetic capacity and excessive radiation reduces the photochemical quantum efficiency of photosystem II. As a result, the excessive energy in the thylakoids cannot be used for chemical synthesis, triggering an imbalance in plant metabolism (Zavafer *et al*. 2017). In this study, the increased irradiance increased GS as plants with higher GS activity have higher photorespiration ability (Kumagai *et al*. 2011). Seedlings grown under full natural irradiance had significantly increased GS activity in leaves compared to shading seedlings, presumably to increase their photorespiration. During this process, the stomata are closed and the CO_2 released by photorespiration is re-fixed, which can protect the photosynthetic reaction center from destruction by excessive radiation energy. Similarly, a study of the effects of irradiance and N on coffee leaves showed that GS activity was greater under full natural irradiance than under shade, and decreased with decreasing N content (Pompelli *et al*. 2010). Therefore, we concluded that application of N fertilizer increased GS activity to alleviate photodamage. These observations were consistent with a study showing that a decrease in Chl content, in response to N deficiency, inevitably led to a decrease in photosynthetic capacity (Grassi *et al*. 2001). Therefore, plants under the combined stress of excessive irradiance and limited N may be less able to harvest energy for photochemistry (Ort and Baker 2002).

A study on the combined effects of irradiance and N content in *Arabidopsis thaliana* suggested that N may help enhance the photoprotective defenses of plants (Cohen *et al*. 2019), because plants grown under full natural irradiance with high supply of N showed an increase in sugar content, which alleviated the effects of photodamage by stimulating the quenching of reactive oxygen species produced by cellular metabolic processes, including the photosynthetic reactions (Miller *et al*. 2010). In this case, to avoid the double hazard of high irradiance and N deficiency, moderate addition of N under full natural irradiance may improve the use of radiation and Chl content and increase their soluble sugar content. In suitable shading, the addition of 1.8 g $plant^{-1}$ N significantly improved plant growth, but the effect of addition of more N was not obvious. However, under excess N fertilization (7.2 g $plant^{-1}$), although the total N content in leaves increased significantly, the Chl content, soluble sugar content, and P_N decreased. Excessive N supply resulted in decreases in P_N and biomass, possibly due to oxidative stress caused by nutrient imbalances in plants (Iqbal *et al*. 2015). Under high N fertilization, variation in N uptake contributes more to the variation in photosynthetic efficiency than to N-use efficiency (Bertin 2001).

Conclusions

We investigated the influence of irradiance and N supply on *H. hainanensis* seedling growth. *H. hainanensis* seedlings were photoinhibited when grown under full natural irradiance. Moreover, when the seedlings were also under N deficiency, GS activity was low, photorespiratory capacity was diminished, and resistance to photo-oxidative damage was low. The P_N and Chl content of seedlings increased under adequate N fertilization; photosynthetic efficiency improved, the soluble sugar content increased, and organic matter synthesis increased, which ultimately promoted the development of plant roots, stems, and leaves. It is worth noting that positive effect of N supply is affected by irradiance and in particular, the increase of irradiance is beneficial to the improvement of N utilization. However, fertlization with N can have negative effects when it is excessive. We concluded that shading was beneficial to alleviate the harm of irradiance inhibition on seedlings. The maximum growth of seedlings was under the shading 0.65 % of natural full irradiance and N supply 4.6 g $plant^{-1}$. The results will provide scientific basis for the future cultivation of *H. hainanensis* seedlings.

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