



**Full Length Article**

## Spectral Effect on Growth, Dry Mass, Physiology and Nutrition in *Bletilla striata* Seedlings: Individual Changes and Collaborated Response

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### Abstract

*Bletilla striata* (Thunb.) Rchb. f. is a famous understory shade-obligated species with significant values as a source of herb medicine. Dry mass production is the main purpose that most cultural projects of *B. striata* cultivars purchase, but the current cultivation regime of *B. striata* is hard to advance its biomass accumulation. Therefore, this study was conducted to test the spectral effect on growth and dry mass in *B. striata* seedlings and further detect the physiological and biochemical responses accordingly as explanation for the mechanism. In a semi-plant factory condition, *B. striata* seedlings were cultured under three spectra with average photosynthetic photon flux rate of  $74 \mu\text{mol m}^{-2} \text{s}^{-1}$  given by light-emitting diodes (LEDs): R1BG5, 13.9% red, 77% green and 9.2% blue; R2BG3, 26.2% red, 70.2% green, and 3.5% blue; R3BG1, 42.3% red, 57.3% green, and 0.4% blue. Seedlings in the R3BG1 treatment generally showed faster growth and biomass accumulation in both shoot and root parts relative to the R1BG5 treatment. The red-light high spectrum also resulted in faster nitrogen (N) and phosphorus (P) uptake which countered nutrient deficiency in blue-high spectrum. Leaves expanded in area at highest efficiency in red-light high spectrum with even faster rate of biomass accumulation and nutrient uptake; while roots were proliferated faster in substrates but biomass allocation to roots was not modified by spectra so did root P uptake. Overall, *B. striata* should be cultured in red-light high spectrum and taken above-ground organs as the source of dry mass production. © 2020 Friends Science Publishers

**Keywords:** Baiji; Artificial lighting; Fine root morphology; Secondary metabolism

### Introduction

Agroforestry is taken as an available approach to cope with climate change on reducing farmlands (Nair *et al.* 2009). Bamboo plantation accounts for considerable area of subtropical lands and mainly affords for the rural development (Li *et al.* 2015; Lin *et al.* 2017). *B. striata* is one of the most important understory herb species in bamboo agroforestry system of subtropical China (Zhang *et al.* 2019b). Natural *B. striata* population can adapt to farmland soils that are covered by Moso bamboo and supply economic outcomes as not only an ornamental flower but also a pseudo-bulb tuber (He *et al.* 2017; Ru *et al.* 2018). Extracts from *B. striata* have significant activities to counter tumor, inflammation, skin-chilblain, and ulcerative carbuncle (He *et al.* 2017; Chen *et al.* 2018; Liao *et al.* 2019). These functions can be derived from compound extracts from dry mass of *B. striata*, such as, the most widely known, polysaccharides, phenanthrene and chitosan (Huang *et al.* 2019; Zhou *et al.* 2019a; Chen *et al.* 2020). Hence, the growth-based dry mass accumulation is the main goal for

harvest in nearly all projects of artificial culture of *B. striata* resources.

The nature of being short of endosperm causes the lack of inherent nutrient supply during germination of *B. striata* seeds (Zhang *et al.* 2019a). In contrast, the demand for the dry mass production from this species never stopped increasing in recent decades (Li *et al.* 2012). The contradiction between the low rate of natural reproduction and the gap for market demand resulted in the necessity to increase the efficiency of culturing *B. striata* resource (Zhang *et al.* 2019a). Imitation wild cultivation under the understory condition in the agroforestry system is a wide approach to produce *B. striata* dry mass (Zhang *et al.* 2019a). The seed provenance, however, is apt to be messed by mixed cultivation of *B. striata* and other species from the Arethuseae tribe (Zhang *et al.* 2019a). In addition, at least two kinds of *B. striata* leaf spots have been found to cause damage on leaves as irregular expansion along the rachis and across the foliage system (Li *et al.* 2019; Zhou *et al.* 2019b). Seedling reproduction of *B. striata* has made progress in tuber cloning, aseptic seed culture, and rapid in

*in vitro* propagation (Wei *et al.* 2008; Zhang *et al.* 2018a; b). These techniques, however, cannot resolve the issue to promote the post-propagation growth rate of *B. striata*.

Facility cultivation was suggested to be an effective mode to culture and produce large-size *B. striata* plants in an efficient rate (Zhang *et al.* 2019a). It was also indicated that lighting condition is a critical factor that determines the growth of *B. striata* seedlings and either too low or too high light intensities (3,000 lux and 9,000 lux, respectively). However, no further studies can supply results about specific lighting condition that is responsible for the growth of *B. striata* seedlings. Light is one of the main resources that determine economic plant traits (Li *et al.* 2018; Zhao *et al.* 2019; Centofante 2020). Artificial lighting can accelerate dry mass accumulation in economic plants through extended daily photoperiod (Wei *et al.* 2013; 2017; Zhu *et al.* 2016; Li *et al.* 2017; Wang *et al.* 2017; An *et al.* 2018). The development of light-emitting diode (LED) technique enables juvenile plants tested in different spectra created by designed compositions of red, green, and blue wavelengths (Wei *et al.* 2019). The color-dependent spectra can regulate economic plant growth and dry mass accumulation through modifying nutritional and physiological processes (Doerr *et al.* 2019; Graham *et al.* 2019). Therefore, as a shade-obligate species, *B. striata* may probably have different responses to varied spectral manipulations. It is necessary to screen for the spectral quality that can promote the growth and dry mass in *B. striata* seedlings to enlarge the reserve of reactive compounds.

Recent reports confirmed that changes in nutrition and physiology in *B. striata* seedlings were related to the assimilation and accumulation of polysaccharides in *B. striata* (Zhang *et al.* 2018a; b). Hence, the objective of this study was to quantify the spectral effect on nutritional and physiological responses in *B. striata* seedlings. In the current study, three LED spectra were tested for their effects on growth, dry mass accumulation, and nutritional and physiological characteristics in *B. striata* seedlings. We hypothesized that: (i) different spectra may have various effects on growth and dry mass accumulation, and (ii) nutritional and physiological may have relationships with growth and dry mass in *B. striata* seedlings.

## Materials and Methods

### Seedling material and experiment commencement

In early September 2018, *B. striata* capsules were collected from mature individuals in the nursery of understory herb plants (23°9' N, 113°22' E), South China Agricultural University, Tianhe District, Guangzhou City, China. Seeds were peeled off in running water, washed in ethanol (50%, v/v) for 30 min, and sterilized in 0.3% (v/v) potassium permanganate for 30 min. Seeds were dried at room temperature and sent to the Laboratory of Combined Manipulation of Illumination and Fertility on Plant Growth

(Zhilunpudao Agric. S&T Ltd., Changchun, China) (43°58' N, 125°24' E). Seeds were germinated in mashed peat substance at the temperature of 36±1°C with relative humidity (RH) of 85%.

In mid-October 2018 germinated seedlings emerged to the density of about 65 individuals per liter of substance. Juvenile seedlings were carefully transplanted by a tweezer to trayed-cavities (height in 13 cm and top-diameter in 7 cm) which were filled up with commercial substrates of the mixture of peat, perlite, and spent-mushroom residue in the volumetric proportion of 55:25:20 (Mashiro-Dust™, Zhiluntuowei A&F S&T, Inc., Changchun, China). Ten seedlings were transplanted to one cavity and 320 seedlings were transplanted to a tray with 32 cavities with the 4×8 arrangement. Substrates were fully watered before transplant to ensure enough porous moisture for initial root uptake. Totally, a total number of 2,880 seedlings were transplanted to 18 trays.

Three replicates of substrates (10 g DM each) were collected for determination of chemical property, which revealed results as follows: pH of 6.28±0.08, electric conductivity of 297.67±2.49 μS cm<sup>-1</sup>, organic matter of 149.56±17.96 mg g<sup>-1</sup>, ammonium nitrogen (N) of 38.86±1.15 mg kg<sup>-1</sup>, nitrate N of 10.25±0.66 mg kg<sup>-1</sup>, and available phosphorus (P) of 1.18±0.15 mg g<sup>-1</sup>.

### Optical treatment and seedling cultivation

This experiment was conducted as a random block design with three spectral treatments randomly arranged in three blocks as replicates. Tray of seedlings were placed to iron shelves (each size: 2 m × 0.5 m × 1.5 m, height × width × length) that were assigned to supply various lighting spectra. The inner space of each shelf was divided into three chambers (each size: 0.5 m × 0.5 m × 1.5 m, height × width × length) by two iron sheets. A total number of 100 LEDs were embedded in spacing of 2 cm × 2 cm to a panel (each size: 0.1 m × 0.4 m × 1.2 m, height × width × length) which was attached to the down-toward back of upper-chamber-floor. The layout of real-time lighting is shown in Fig. 1. Three spectra were designed with various compositions of wavelengths in red, green and blue lights. Specific properties for each of three spectra are shown in Table 1. The treatments of R1BG5, R2BG3, and R3BG1 termed visible lights with red light ratio from 14%, through 26%, to 42%, respectively. Photosynthetic photon flux rate (PPFD) was designed to 70–80 μmol m<sup>-2</sup> s<sup>-1</sup> which meets the generally optical requirement by economic plants (Li *et al.* 2018; Zhao *et al.* 2019). Light intensity ranged between 2,000 and 3,000 lx which fell in the suggested range for *B. striata* (Zhang *et al.* 2019a).

Seedlings started to receive LED lighting treatment two days after transplant. Thereafter, a week after transplant were seedlings thinned to a density of 16 individuals per tray in the spacing of 14 cm × 14 cm (every two cavities) to avoid the interplay among individuals by leaves overlap.

The sub-irrigation was employed to water seedlings by placing trays in tanks (85 cm × 55 cm × 7 cm, length × width × height) where the water table was maintained to be 3 cm in height to enable continuous root uptake through porous delivery (Fig. 1). Seedlings were fed by exponential nutrient loading using nutritional solution at the rate of 40 mg N seedling<sup>-1</sup> (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O, 10-7-9) over a four-month time. This nutritional regime can make *B. striata* seedlings load more nutrients than they needed for basic growth and reserve additional part within tubers as reserve (Wei *et al.* 2013; Li *et al.* 2017; 2018; Zhao *et al.* 2019). The total amount of nutrient delivery to *B. striata* seedlings was estimated from the field investigation on soils of understory population in bamboo plantations (Zhang *et al.* 2019b). Nutrients were fed once a week and the whole cultural period lasted for four months according to the exponential fertilization model (Xu *et al.* 2019). During experiment, temperature was maintained at 25.4°C by installing the corporation of floor heating and cooling fan while RH was maintained at 85%.

### Seedling sampling and measurements

All seedlings were sampled from each tray and measured for growth by every individual then by the bulk of a tray for dry mass and chemical analysis. Plant height and stem diameter were measured in situ by the method of Zhang *et al.* (2018a). Sampled seedlings were washed by tap water to clean roots free from substrates and rinsed by distilled water in 1 min. Cleaned seedlings were immediately divided into shoot and root parts and measured for fresh weight. Half of samples were measured for dry weight after oven-dried at 60°C for 48 h. These samples were further used for total N and P concentrations through the methods described by Wei *et al.* (2013) and Zhao *et al.* (2019), respectively. The above-ground foliage part of fresh samples were used for determination of chlorophyll and soluble protein contents (Gu *et al.* 2019; Zhao *et al.* 2019) and activities in glutamine synthetase (GS) and acid phosphatase (AP) (Wei *et al.* 2019). Leaves and roots were scanned to obtain their digital images in the quality of about 120 pixels cm<sup>-1</sup> (HP Deskjet 1510 scanner, HP Inc., Palo Alto, CA, USA). Thereafter, leaf images were opened in Photoshop (Adobe, San Jose, CA, USA) and analyzed for the degree of green color index and projected area (Zhu *et al.* 2019). Root images were analyzed using WinRhizo software (Regent Instrument Inc., Calgary, Canada) to obtain data about root length, surface area, diameter and tips number.

### Vector analysis for nutritional status

Monographs of vector directions were graphed using data about whole-plant biomass, nutrient (N or P) concentration and nutrient content. All data were standardized to constants between 0 and 100 to eliminate the effect from multiple units. Nutritional interpretations for each nutritional

symptom were adapted from Salifu and Timmer (2003).

### Statistical analysis

Water content was calculated by the difference between fresh and dry weights. Nutrient (N or P) uptake efficiency was calculated by the whole-plant nutrient uptake (mg plant<sup>-1</sup>) divided by nutrients through fertilizers (Zhao *et al.* 2019). Data were analyzed by analysis of variance (ANOVA) to detect the effect of three spectra on parameters. The multiple comparison was made by Tukey test ( $P < 0.05$  level) using IBM SPSS statistic software. Principle component analysis (PCA) was used to analyze the interplay among measured parameters.

## Results

### Plant growth and biomass accumulation

Plant growth increased with the ratio of red light in spectrum (Table 1). Plant height was higher in the R3BG1 treatment than the other two treatments by 30–37%. However, the R3BG1 treatment only increased RCD compared to the R1BG5 treatment by 15%. In addition, both fresh and dry weights were higher in the R3BG1 than in the R1BG5 treatment while the difference between R3BG1 and R2BG3 treatments was not statistically different. The R3BG1 treatment caused an increase of water content in root and the whole-plant than the R1BG5 treatment (Table 2).

### Nutrient uptake and allocation

Shoot N concentration was lower in the R1BG5 treatment than in the other two treatments ( $F_{2,6}=14.5$ ,  $P=0.0050$ ), but root N concentration was lowest in the R3BG1 treatment ( $F_{2,6}=42.1$ ,  $P=0.0003$ ) (Fig. 2A). Shoot P concentration was highest in the R3BG1 treatment ( $F_{2,6}=362.9$ ,  $P < 0.0001$ ) (Fig. 2B). Root P concentration declined with an increase in red-light ratio in visible light ( $F_{2,6}=737.2$ ,  $P < 0.0001$ ). However, whole-plant P concentration was highest in the R3BG1 again ( $F_{2,6}=111.1$ ,  $P < 0.0001$ ).

Shoot N content was lowest in the R1BG5 treatment ( $F_{2,6}=19.4$ ,  $P=0.0024$ ) (Fig. 3A). Root N content was higher in the R2BG3 treatment than in the R3BG1 treatment by 5.9-fold ( $F_{2,6}=8.9$ ,  $P=0.0161$ ). Whole-plant N content was the lowest in the R1BG5 treatment ( $F_{2,6}=8.4$ ,  $P=0.0180$ ). P content was highest in the R3BG1 treatment in both shoot ( $F_{2,6}=99.4$ ,  $P < 0.0001$ ) and whole-plant ( $F_{2,6}=47.9$ ,  $P=0.0002$ ) (Fig. 3B).

### Vector analysis for nutritional status

Relative to the R1BG5 treatment, the whole-plant of *B. striata* plants in the R2BG3 treatment had higher N concentration and content with unchanged biomass between the two treatments. Therefore, plants in the R2BG3

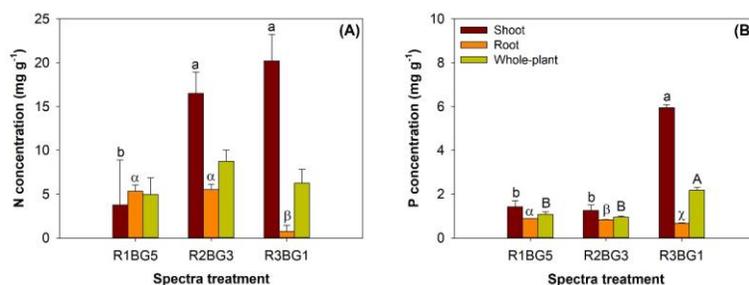
**Table 1:** Spectra of lighting from three types of light-emitting diodes (LEDs) for the culture of *B. striata* seedlings

Light source	PPFD <sup>2</sup> ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Intensity (Lx)	Red (%)	Green (%)	Blue (%)
R1BG5 <sup>2</sup>	69.18	2678	13.9	77	9.2
R2BG3 <sup>3</sup>	77.12	2499	26.2	70.2	3.5
R3BG1 <sup>4</sup>	73.99	2392	42.3	57.3	0.4

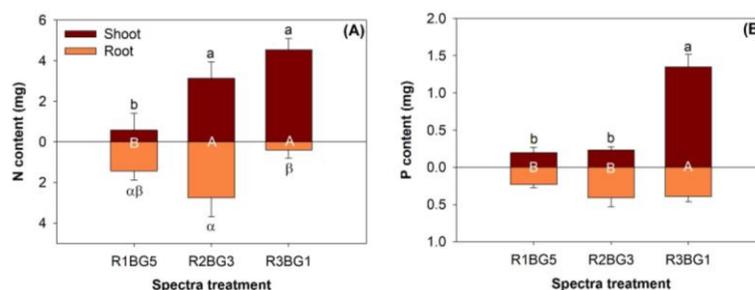
Note: <sup>1</sup> PPFD, photosynthetic photon flux rate; <sup>2</sup> R1BG5, electric current for red and combined green and blue LEDs were controlled to be 10% and 50%, respectively; <sup>3</sup> R2BG3, electric current controlled to be 20% (red) and 30% (green and blue); <sup>4</sup> R3BG1, electric current controlled to be 30% (red) and 10% (green and blue)



**Fig. 1:** Layout of the experiment of spectral effect on *B. striata* seedlings. R1BG5, 13.9% red, 77% green, and 9.2% blue; R2BG3, 26.2% red, 70.2% green, and 3.5% blue; R3BG1, 42.3% red, 57.3% green, and 0.4% blue



**Fig. 2:** Nitrogen (N) (A) and phosphorus (P) concentrations (B) in *B. striata* seedlings exposed to different spectral treatments. R1BG5, 13.9% red, 77% green, and 9.2% blue; R2BG3, 26.2% red, 70.2% green, and 3.5% blue; R3BG1, 42.3% red, 57.3% green, and 0.4% blue. Error bars present standard errors. Lower case letters indicate difference for shoot; roman letters indicate difference for root; capital letters indicate difference for the whole-plant.



**Fig. 3:** Nitrogen (N) (A) and phosphorus (P) contents (B) in *B. striata* seedlings exposed to different spectral treatments. R1BG5, 13.9% red, 77% green, and 9.2% blue; R2BG3, 26.2% red, 70.2% green, and 3.5% blue; R3BG1, 42.3% red, 57.3% green, and 0.4% blue. Error bars present standard errors. Lower case letters indicate difference for shoot; roman letters indicate difference for root; capital letters indicate difference for the whole-plant

treatment can be assessed to load steady-state uptake of N relative to the R1BG5 treatment (Fig. 4A). Relative to the R1BG5 treatment again, the R3BG1 treatment induced all increases in biomass, N content and N concentrations, which was characterized as a counter by the R3BG1 treatment to nutrient deficiency in the R1BG5 treatment (Fig. 4A).

Although P content and biomass were increased in the

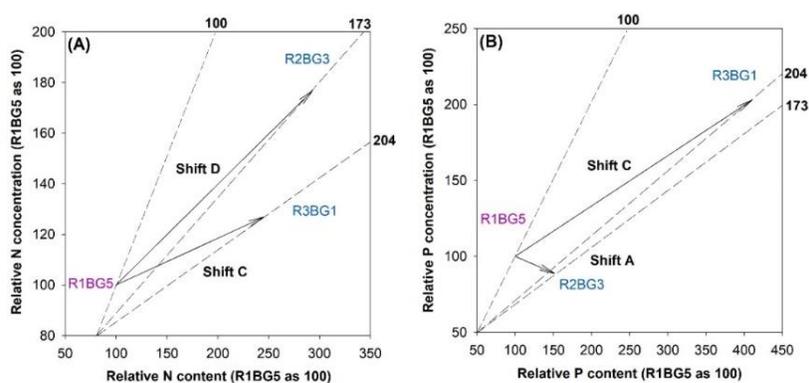
R2BG3 treatment relative to the R1BG5 treatment, P concentration declined in the earlier treatment (Fig. 4B). This was assessed as a symptom of P dilution in R2BG3 treatment relative to the R1BG5 treatment. However, all biomass, P content (concentration  $\times$  biomass; the same below), and P concentration were increased in the R3BG1 treatment compared to the R1BG5 treatment, which was characterized as an alleviation to nutrient deficiency (Fig. 4B).

**Table 2:** Growth, weight and water content in *B. striata* plants exposed to LED spectra treatments of R1BG5, R2BG3 and R3BG1

Seedling variables	R1BG5	R2BG3	R3BG1	$F_{2,6}$	$P$
Plant height (cm)	5.26±0.73b	5.57±0.53b	7.21±0.50a	8.36	0.0184
Stem diameter (cm)	2.17±0.05b	2.44±0.17ab	2.50±0.06a	7.37	0.0242
Shoot fresh weight (g)	0.62±0.05b	0.84±0.18ab	1.04±0.18a	5.42	0.0453
Root fresh weight (g)	1.50±0.27b	2.75±0.88ab	3.22±0.55a	5.62	0.0422
Whole-plant fresh weight (g)	2.11±0.27b	3.58±1.05ab	4.26±0.68a	5.94	0.0377
Shoot dry weight (g)	0.13±0.02b	0.19±0.03ab	0.23±0.03a	7.40	0.0240
Root dry weight (g)	0.26±0.05b	0.49±0.14ab	0.58±0.11a	6.09	0.0359
Whole-plant dry weight (g)	0.40±0.06b	0.68±0.16ab	0.81±0.14a	7.54	0.0231
Root to shoot ratio	2.32±0.43	2.60±0.70	2.24±0.58	1.01	0.4194
Unit-leaf dry weight (mg)	47.5±6.3b	62.5±12.5ab	75.8±8.3a	6.11	0.0357
Shoot water content (g)	0.48±0.03	0.65±0.16	0.81±0.15	4.62	0.0611
Root water content (g)	1.24±0.22b	2.26±0.74ab	2.64±0.44a	5.42	0.0450
Whole-plant water content (g)	1.72±0.23b	2.90±0.89ab	3.46±0.55a	5.56	0.0430

**Table 3:** Foliar physiology and enzyme activity in *B. striata* plants exposed to LED spectra treatments of R1BG5, R2BG3 and R3BG1

Foliar variables	R1BG5	R2BG3	R3BG1	$F_{2,6}$	$P$
Chlorophyll a ( $\text{mg g}^{-1}$ )	0.85±0.17b	1.42±0.28a	1.49±0.03a	9.07	0.0153
Chlorophyll b ( $\text{mg g}^{-1}$ )	0.45±0.10b	1.03±0.08a	1.09±0.20a	17.96	0.0029
Chlorophyll a+b ( $\text{mg g}^{-1}$ )	1.29±0.27b	2.45±0.36a	2.58±0.19a	16.84	0.0035
Soluble protein ( $\text{mg g}^{-1}$ )	2.55±0.19a	2.24±0.11ab	1.93±0.21b	8.40	0.0182
GS ( $\text{A mg}^{-1} \text{ protein h}^{-1}$ )	3.05±0.27a	2.83±0.14ab	2.26±0.27b	8.04	0.0201
AP ( $\mu\text{g NPP g}^{-1} \text{ FW min}^{-1}$ )	7.59±0.45ab	6.78±0.76b	8.70±0.59a	6.71	0.0295



**Fig. 4:** Vector analysis of nutritional status for nitrogen (N) (A) and phosphorus (P) (B) in *B. striata* seedlings exposed to different spectral treatments. R1BG5, 13.9% red, 77% green, and 9.2% blue; R2BG3, 26.2% red, 70.2% green, and 3.5% blue; R3BG1, 42.3% red, 57.3% green, and 0.4% blue. Shift A, nutrient dilution; shift C, nutrient deficiency alleviation; shift D, steady-state uptake

### Foliar physiology and morphology

Contents in chlorophyll a, b, and a+b were lower in the R1BG5 treatment than in the other two treatments (Table 3). In contrast, soluble protein and GS activity were higher in the R1BG5 treatment than in the R3BG1 treatment. Foliar AP activity was higher in the R3BG1 treatment than in the R2BG3 treatment.

Leaf green index decreased with an increase of red-light in the spectrum (Fig. 5A). Leaf green index was lowered in the R3BG1 treatment by 11% than in the R1BG3 ( $F_{2,6}=9.5$ ;  $P=0.0138$ ). In contrast, leaf area was lower in the R1BG5 treatment than in the other two treatments ( $F_{2,6}=21.6$ ;  $P=0.0018$ ) (Fig. 5B). Specific leaf area was higher in the R1BG5 treatment than in the other two treatments ( $F_{2,6}=18.7$ ;  $P=0.0030$ ) (Fig. 5C).

### Root morphology

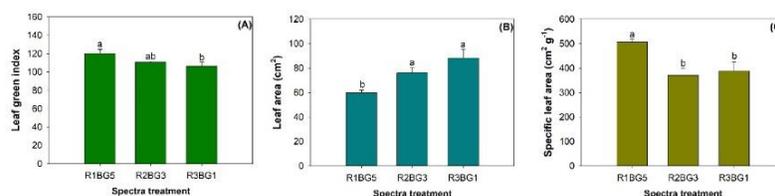
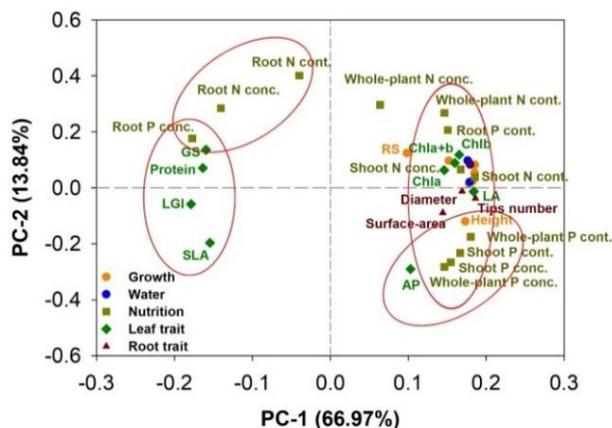
Root morphology showed increasing trends with the increase of red-light ratio in spectrum (Table 4). Root length was lower in the R1BG5 treatment than in the other two treatments, while surface area, diameter, and tips number were all higher in the R3BG1 treatment than in the R1B5 treatment.

### Principle component analysis (PCA)

The first two PCs accounted for 80.83% of total variation whereas the first PC accounted for 66.97% and the second 13.84%. In the first axis, most of growth, water content, nutrition, and root variables generally showed contrasting relationship with leaf traits (Fig. 6). For example, height,

**Table 4:** Root morphology and growth in *B. striata* plants exposed to LED spectra treatments of R1BG5, R2BG3 and R3BG1

Root variables	R1BG5	R2BG3	R3BG1	$F_{2,6}$	$P$
Root length (cm)	759.66±219.38b	1305.20±250.80a	1538.45±122.06a	10.23	0.0117
Surface area (cm <sup>2</sup> )	166.24±63.92b	282.51±91.65ab	390.05±55.41a	6.49	0.0315
Diameter (mm)	2.27±0.12b	2.56±0.26ab	2.87±0.20a	5.99	0.0372
Tips number	2.24±0.11b	2.54±0.19ab	2.91±0.16a	12.29	0.0076

**Fig. 5:** Spectral effect on leaf green index (A), leaf area (B), and specific-leaf area (C) in *B. striata* seedlings exposed to different spectral treatments. R1BG5, 13.9% red, 77% green, and 9.2% blue; R2BG3, 26.2% red, 70.2% green, and 3.5% blue; R3BG1, 42.3% red, 57.3% green, and 0.4% blue. Error bars present standard errors. Different letters indicate difference among spectra treatments**Fig. 6:** Eigenvalues from principle component (PC) analysis on growth, dry mass accumulation, water content, nutrition uptake, leaf traits, and root morphology in *B. striata* seedlings exposed to different spectral treatments. Red-edge circles indicate the tendency of correlation between parameters with contrasting eigenvalues

RCD, tips number, shoot N content, shoot P content, and whole-plant P content all showed negative relationship with leaf GS, protein content, and green index. No apparent relationship was indicated among variables along the second axis. Eigenvalues about nutrition in shoot in the fourth quadrant had negative relationship with those in the second quadrant about nutrition in root. P content and concentration in shoot and the whole-plant showed contrasting relationships with N and P concentrations in the root.

## Discussion

Our results showed a general trend of increasing growth traits with the increase of red-light ratio in spectrum. Our results concurred with the response from seedlings of forest timber species (Apostol *et al.* 2015; Li *et al.* 2018; Zhao *et*

*al.* 2019), but showed contrasting trend to the increase of red-light ratio in spectrum for vegetable crops (Hogewoning *et al.* 2010; Ying *et al.* 2020). It was found that the blue-high light was responded by the increase of photosynthesis and thereafter more dry mass accumulation was noted. However, the dry mass in our study was also higher in red-light high spectrum. However, the across-species study revealed that plant growth response to spectra was a species-specific trait (Ying *et al.* 2020). Higher-red ratio in spectrum can benefit the increase of stomatal ratio (Hogewoning *et al.* 2010), which was supported by our evidence of higher water content because higher transpiration results from more stomata per leaf area (Larcher *et al.* 2015). Our results also indicated that biomass accumulation in shoot and root parts of *B. striata* increased at the same rate without significant response of root to shoot ratio (R/S). Biomass allocation was null to spectra variation in Li *et al.* (2018) as well. However, R/S was also reported to be higher in blue-high spectrum (Riikonen 2016; Zhao *et al.* 2019). Although tuber is the most important organ to produce secondary metabolisms by *B. striata* plants (Zhang *et al.* 2018a, b; 2019a), spectrum cannot act as the factor that regulated biomass allocated to roots to support the expansion of tubers therein.

Both N and P uptake in *B. striata* plants showed contrasting trends in shoot and root parts, whereas, with the increase of red-light ratio in spectrum, shoot nutrient concentration increased but that in root decreased. Our study highly concurs with indoor lettuce (*Lactuca sativa* L.) plants (Pennisi *et al.* 2019). In accordance to our study, shoot N concentration was also found to increase in red-light high spectrum in *Dalbergia odorifera* (Li *et al.* 2018). In contrast, stem P concentration was higher in red-low spectrum in *Larix principis-rupprechtii* (Zhao *et al.* 2019). The increase of N concentration with red-light ratio in spectrum synchronized with chlorophyll contents but not soluble contents and GS activity. The red-light high spectrum was also found to induce higher foliar N

concentration and GS in beech (*Fagus sylvatica* L.) seedlings (Astolfi *et al.* 2012). Bian *et al.* (2018) further revealed that the red-light high spectrum depressed the N assimilation of nitrite reductase activity which was positively correlated with GS activity. Therefore, it can be speculated that in *B. striata* plants the red-light high spectrum promoted N uptake and assimilation in roots and the transport of N as the form of amino acid upwards to shoot. However, P was assimilated by AC in leaves of *B. striata* plants due to the increase of AC activity with the increase of red-light ratio in spectrum.

At the whole-plant scale, although N status was induced to be steady-state uptake in the R2BG3 spectrum, P status was induced to be diluted at the same time. This was formed because P concentration increase rate was slower than that for N when biomass accumulation kept at the same pace for the two elements. Thus, P uptake rate was slower than N in several understory plant species (Li *et al.* 2017; 2018; An *et al.* 2018). Compared to the R1BG5 treatment, the R3BG1 treatment countered the dilution of both N and P relative to the R1BG5 treatment. These results were formed because of dry mass increase at the whole-plant scale chromized with the uptake of both N and P in the red-light ratio. However, results from Li *et al.* (2018) disagreed to our study that nutrient uptake was slower than biomass accumulation in red-light high spectrum. Thus, when exposing to lighting spectra, the biomass accumulation rate of *B. striata* is fast enough to catch up the speed of nutrient uptake, which was not common for other species.

Leaf green index decreased with the increase of red-light ratio which resulted in a negative relationship with leaf chlorophyll content and nutritional concentration. The green color index given by histogram resulted from the synthesis of color indices of every unit pixel. The negative relationship between leaf green index and N concentration was also reported in agricultural crops (Rabara *et al.* 2017; Zhu *et al.* 2019). This characteristic of green color index can be used to fast predict inherent N status. Our results of increasing leaf area with red-light ratio in spectrum concur with Borowski *et al.* (2015) but contradict Clavijo-Herrera *et al.* (2018). The red-light in the spectrum benefited the projected area to receive lighting. However, the investment to leaf area by the leaf biomass in our study decreased with red-light ratio, which concurred with Clavijo-Herrera *et al.* (2018). The sufficiency of leaf dry mass investment to area expansion varied across species depending upon the speed of dry mass accumulation. According to our results, *B. striata* is the species with fast leaf biomass accumulation in response to spectrum with higher red ratio, which had higher speed than that of leaf area expansion.

Root morphology in *B. striata* was higher in red-light high spectrum, which concurred with Xu *et al.* (2019). Greater root length and surface area accorded with root dry mass accumulation which together resulted from higher photosynthetic production and allocation downwards to roots in red-light high spectrum. Promoted root morphology

also supported high-efficient nutrient uptake and resulted in higher nutrient concentration in red-light high spectrum.

## Conclusion

Using across-wavelengths spectrum with composing red, green, and blue lights in different ratios, we conclude that *B. striata* seedlings obtained optimum dry mass production and growth outcome in the red-light high spectrum, i.e. the lights with 42.3% red, 57.3% green, and 0.4% blue wavelengths. Growth, biomass accumulation, water content, N and P uptakes, chlorophyll content, and foliar and root morphologies were all higher in red-light high spectrum. Within these series of responses, leaves expanded in area at highest efficiency in red-light high spectrum with even faster rate of biomass accumulation and nutrient uptake; while roots were proliferated faster in substrates but biomass allocation to roots was not modified by spectra so did root P uptake. Therefore, during intensive cultural period with the purpose to fast harvest dry mass from *B. striata* seedlings, above-ground organs should be a better choice than the below-ground ones.

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## References

- An BY, HX Wei, LL Li, P Guo (2018). Nutrient uptake and utilization and antioxidants of fruits in red raspberry (*Rubus idaeus* L.) cultivar 'Autumn Bliss' in response to fertilization under extended photoperiod. *Not Bot Horti Agrobot* 46:440–448
- Apostol KG, RK Dumroese, JR Pinto, AS Davis (2015). Response of conifer species from three latitudinal populations to light spectra generated by light-emitting diodes and high-pressure sodium lamps. *Can J For Res* 45:1711–1719
- Astolfi S, C Marianello, S Grego, R Bellarosa (2012). Preliminary Investigation of LED Lighting as growth light for seedlings from different tree species in growth chambers. *Not Bot Horti Agrobot* 40:31–38
- Bian ZH, RF Cheng, Y Wang, QC Yang, CG Lu (2018). Effect of green light on nitrate reduction and edible quality of hydroponically grown lettuce (*Lactuca sativa* L.) under short-term continuous light from red and blue light-emitting diodes. *Environ Exp Bot* 153:63–71
- Borowski E, S Michalek, K Rubinowska, B Hawrylak-Nowak, W Grudzinski (2015). The effects of light quality on photosynthetic parameters and yield of lettuce plants. *Acta Sci Pol-Hortorum Cultus* 14:177–188
- Centofante AR (2020). Light quality on the morphoanatomy and physiology of *Campomanesia pubescens* (DC.) O. Berg. seedlings. *Sci Hortic* 259:11
- Chen ZY, LZ Cheng, YC He, XL Wei (2018). Extraction, characterization, utilization as wound dressing and drug delivery of *Bletilla striata* polysaccharide: A review. *Intl J Biol Macromol* 120:2076–2085
- Chen ZY, Y Zhao, MK Zhang, XF Yang, PX Yue, DK Tang, XL Wei (2020). Structural characterization and antioxidant activity of a new polysaccharide from *Bletilla striata* fibrous roots. *Carbohydr Polym* 227; Article 115362

- Clavijo-Herrera J, E van Santen, C Gomez (2018). Growth, water-use efficiency, stomatal conductance and nitrogen uptake of two lettuce cultivars grown under different percentages of blue and red light. *Horticulturae* 4; Article 4030016
- Doerr OS, BF Zimmermann, S Kogler, H Mibus (2019). Influence of leaf temperature and blue light on the accumulation of rosmarinic acid and other phenolic compounds in *Plectranthus scutellarioides* (L.). *Environ Exp Bot* 167; Article 103830
- Graham T, N Yorio, P Zhang, G Massa, R Wheeler (2019). Early seedling response of six candidate crop species to increasing levels of blue light. *Life Sci Space Res* 21:40–48
- Gu YY, FH Zhang, YL Zeng, L Zhang, XF Tan, HP Cao, Z Li (2019). Physiological responses of tung tree (*Vernicia fordii*) saplings to different red, white and blue light-emitting diodes. *Intl J Agric Biol* 22:569–577
- He X, X Wang, J Fang, Z Zhao, L Huang, H Guo, X Zheng (2017). *Bletilla striata*: Medicinal uses, phytochemistry and pharmacological activities. *J Ethnopharmacol* 195:20–38
- Hogewoning SW, G Trouwborst, H Maljaars, H Poorter, W van Ieperen, J Harbinson (2010). Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *J Exp Bot* 61:3107–3117
- Huang YB, FL Shi, LM Wang, Y Yang, BM Khan, KL Cheong, Y Liu (2019). Preparation and evaluation of *Bletilla striata* polysaccharide/carboxymethyl chitosan/carbomer 940 hydrogel for wound healing. *Intl J Biol Macromol* 132:729–737
- Larcher L, I Hara-Nishimura, L Sternberg (2015). Effects of stomatal density and leaf water content on the O-18 enrichment of leaf water. *New Phytol* 206:141–151
- Li P, G Zhou, H Du, D Lu, L Mo, X Xu, Y Shi, Y Zhou (2015). Current and potential carbon stocks in Moso bamboo forests in China. *J Environ Manage* 156:89–96
- Li W-P, S-S Tian, G-Y Lu, D Lv, Y-N Wang, F-S Jiang, Z-S Ding (2012). Rapid propagation of *Bletilla striata* by synthetic seeds technology, China. *J Chin Mater Med* 37:3386–3390
- Li X, J Jiang, S Cheng, K Zhang, Z Zhang, T Zheng, L Liu, T Hsiang, Z Sun, Y Zhou (2019). First report of leaf spot of *Bletilla striata* caused by *Fusarium asiaticum* in China. *Plant Dis* 103:1766–1767
- Li XW, QX Chen, HQ Lei, JW Wang, S Yang, HX Wei (2018). Nutrient uptake and utilization by fragrant rosewood (*Dalbergia odorifera*) seedlings cultured with oligosaccharide addition under different lighting spectra. *Forests* 9; Article f9010029
- Li XW, Y Gao, HX Wei, HT Xia, QX Chen (2017). Growth, biomass accumulation and foliar nutrient status in fragrant rosewood (*Dalbergia odorifera* TC Chen) seedlings cultured with conventional and exponential fertilizations under different photoperiod regimes. *Soil Sci Plant Nutr* 63:153–162
- Liao ZC, R Zeng, LL Hu, KG Maffucci, Y Qu (2019). Polysaccharides from tubers of *Bletilla striata*: Physicochemical characterization, formulation of buccoadhesive wafers and preliminary study on treating oral ulcer. *Intl J Biol Macromol* 122:1035–1045
- Lin MY, IF Hsieh, PH Lin, S Laplace, M Ohashi, TH Chen, T Kume (2017). Moso bamboo (*Phyllostachys pubescens*) forests as a significant carbon sink? A case study based on 4-year measurements in central Taiwan. *Ecol Res* 32:845–857
- Nair PKR, BM Kumar, VD Nair (2009). Agroforestry as a strategy for carbon sequestration. *J Plant Nutr Soil Sci* 172:10–23
- Pennisi G, F Orsini, S Blasioli, A Cellini, A Crepaldi, I Braschi, F Spinelli, S Nicola, JA Fernandez, C Stanghellini, G Gianquinto, LFM Marcellis (2019). Resource use efficiency of indoor lettuce (*Lactuca sativa* L.) cultivation as affected by red: blue ratio provided by LED lighting. *Sci Rep* 9; Article 14127
- Rabara RC, G Behrman, T Timbol, PJ Rushton (2017). Effect of spectral quality of monochromatic LED lights on the growth of artichoke seedlings. *Front Plant Sci* 8; Article 190
- Riikonen J (2016). Pre-cultivation of Scots pine and Norway spruce transplant seedlings under four different light spectra did not affect their field performance. *New For* 47:607–619
- Ru Y, X Chen, J Xu, L Huang, M Jiang, L Guo, Z Lin, B Qiu, KY Wong (2018). Hypoglycemic effects of a polysaccharide from *Tetrastigma hemsleyanum* Diels & Gilg in alloxan-induced diabetic mice. *Chem Biodivers* 15; Article e1800070
- Salifu KF and VR Timmer (2003). Optimizing nitrogen loading of *Picea mariana* seedlings during nursery culture. *Can J For Res* 33:1287–1294
- Wang Z, Y Zhao, HX Wei (2017). Chitosan oligosaccharide addition affects current-year shoot of post-transplant Buddhist pine (*Podocarpus macrophyllus*) seedlings under contrasting photoperiods. *iForest* 10:715–721
- Wei HX, P Guo, HF Zheng, XY He, PJ Wang, ZB Ren, C Zhai (2017). Micro-scale heterogeneity in urban forest soils affects fine root foraging by ornamental seedlings of Buddhist pine and Northeast yew. *Urban For Urban Green* 28:63–72
- Wei HX, J Ren, JH Zhou (2013). Effect of exponential fertilization on growth and nutritional status in Buddhist pine (*Podocarpus macrophyllus* Thunb. D. Don) seedlings cultured in natural and prolonged photoperiods. *Soil Sci Plant Nutr* 59:933–941
- Wei HX, HT Zhao, X Chen (2019). Foliar N:P stoichiometry in *Aralia elata* distributed on different slope degrees. *Not Bot Horti Agrobot* 47:887–895
- Wei K, Y Liu, J Qin, X Feng (2008). Study on screening of explants of tissue culture in *Bletilla striata*. *Mod Chin Med* 10:13–14
- Xu YY, YY Liang, M Yang (2019). Effects of composite LED light on root growth and antioxidant capacity of *Cunninghamia lanceolata* tissue culture seedlings. *Sci Rep* 9; Article 9766
- Ying QL, Y Kong, C Jones, YB Zheng (2020). Responses of yield and appearance quality of four Brassicaceae microgreens to varied blue light proportion in red and blue light-emitting diodes lighting. *Sci Hortic* 259; Article 108857
- Zhang BQ, S Sarsaiya, XW Pan, LL Jin, DL Xu, BH Zhang, GJ Duns, JS Shi, JS Chen (2018a). Optimization of nutritional conditions using a temporary immersion bioreactor system for the growth of *Bletilla striata* pseudobulbs and accumulation of polysaccharides. *Sci Hortic* 240:155–161
- Zhang BQ, L Song, LD Bekele, JS Shi, Q Jia, BH Zhang, LL Jin, GJ Duns, JS Chen (2018b). Optimizing factors affecting development and propagation of *Bletilla striata* in a temporary immersion bioreactor system. *Sci Hortic* 232:121–126
- Zhang M, QS Shao, ET Xu, ZA Wang, Z Wang, LH Yin (2019a). *Bletilla striata*: A review of seedling propagation and cultivation modes. *Physiol Mol Biol Plants* 25:601–609
- Zhang XP, GB Gao, ZZ Wu, X Wen, H Zhong, ZK Zhong, FY Bian, X Gai (2019b). Agroforestry alters the rhizosphere soil bacterial and fungal communities of moso bamboo plantations in subtropical China. *Appl Soil Ecol* 143:192–200
- Zhao J, X Chen, HX Wei, J Lv, C Chen, XY Liu, Q Wen, LM Jia (2019). Nutrient uptake and utilization in Prince Rupprecht's larch (*Larix principis-rupprechtii* Mayr.) seedlings exposed to a combination of light-emitting diode spectra and exponential fertilization. *Soil Sci Plant Nutr* 65:358–368
- Zhou D, G Chen, YP Ma, CG Wang, B Lin, YQ Yang, W Li, K Koike, Y Hou, N Li (2019a). Isolation, structural elucidation, optical resolution, and antineuroinflammatory activity of phenanthrene and 9, 10-dihydrophenanthrene derivatives from *Bletilla striata*. *J Nat Prod* 82:2238–2245
- Zhou H, H Long, YH Li, J Xu, YP Shi, Q Gao (2019b). First report of leaf spot on *Bletilla striata* caused by *Fusarium solani* in China. *Plant Dis* 103:2955–2956
- Zhu H, SJ Zhao, JM Yang, LQ Meng, YQ Luo, B Hong, W Cui, MH Wang, WC Liu (2019). Growth, nutrient uptake, and foliar gas exchange in pepper cultured with un-composted fresh spent mushroom residue. *Not Bot Horti Agrobot* 47:227–236
- Zhu KY, HC Liu, HX Wei, JH Zhou, QC Zou, GY Ma, JQ Zhang (2016). Prediction of nutrient leaching from culture of containerized Buddhist pine and Japanese maple seedlings exposed to extended photoperiod. *Intl J Agric Biol* 18:425–434