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GROWTH, PHYSIOLOGICAL AND STOMATAL CHARACTERISTICS OF RED OKRA (*ABELMOSCHUS ESCULENTUS* L. MOENCH) SEEDLINGS AT DIFFERENT LIGHT INTENSITIES

HUIMIN LI*, JIE YANG AND FEI WENG

[1]*Suzhou Polytechnic Institute of Agriculture, Suzhou Jiangsu 215008, China*
**Corresponding author's email: hmli0621@163.com*

Abstract

Okra is a significant vegetable crop, extensively cultivated in tropical and temperate regions. Its antioxidant potential and medicinal properties have been well-documented. The study uses red okra (*Abelmoschus esculentus* L. Moench) of the cultivar 'Yanjiao 1' and uses a one-factor randomized block design. The seedlings, flattened after the cotyledons, were exposed to rare earth plant light consisting of blue light (B), red light (R), blue plus red light (BR37), purple light (PL) and fluorescent light (CK, control) to examine the impact of diverse light qualities on growth, root activity, pigments, photosynthetic products, flavonoid, soluble protein and stomatal characteristics. This investigation analyses the effects of varying light qualities on different growth-related parameters. The study revealed that the BR37 treatment had the greatest impact on the fresh and dry mass, stem length, root length, and stem diameter of red okra seedlings. Furthermore, root activity of red okra seedlings in B, BR37, R and P treatments was greater than CK, with the highest root activity observed under the BR37. Red okra leaves exhibited a similar trend in pigments levels among different light quality. The largest values were observed under the B, followed by the BR37. The highest levels of soluble sugars and free amino acids were found under the R light, while starch and sucrose levels were highest under the BR37 light. The flavonoid content in red okra seedlings was highest under BR37 and B. Soluble protein of red okra seedlings was the biggest under the BR37 light. The adaxial and abaxial surfaces of stomatal length and width in red okra were highest under treatment with BR37. Stomatal density under various light qualities yielded similar trends with the highest density achieved under BR37 treatment. It is evident that BR37 significantly enhances the growth, root activity, pigment, photosynthetic products, flavonoid, soluble protein, and stomatal development of red okra seedlings. Thus, BR37 can serve as an artificial light source for cultivating red okra seedlings.

Key words: Red okra; Light quality; Pigments; Flavonoid; Stomatal characteristics.

Introduction

Red okra (*Abelmoschus esculentus* L. Moench) is a widely consumed vegetable, which can be eaten either raw or cooked. It holds immense significance in the culinary world. (Akbar *et al*., 2021; Khanzada *et al*., 2021). The red okra's young pods are commonly used as food, due to the rich content of protein, carotenoids, vitamin C, sugar, as well as minerals, flavonoid, free amino acids, and other nutrients and biologically active ingredients and is favored by the people (Irshad *et al*., 2018; Husen *et al*., 2019). Okra is now distributed in all parts of the world, to the most common in the tropics and subtropics, China's north and south have been cultivated all over the country. It is a short-day plant, especially sensitive to light conditions. In northern of China, due to climate, light and other factors, okra is mainly used in facility cultivation mode. Therefore, the weak light weather in early spring has become a limiting factor in cultivating robust seedlings, and the use of artificial light sources to regulate facility seedling and cultivation has become a necessary means of light regulation (Li *et al*., 2016).

Light acts both as an energy source controlling photosynthesis and as a trigger signal affecting plant growth and development (Lee *et al*., 2021). Using different wavelengths of light to irradiate plants, it has been found that plants are particularly sensitive to ultraviolet A (320-380 nm), ultraviolet B (280-320 nm), blue (380-500 nm), red (620-700 nm), and far-red (700-800 nm) light. Among these, blue light plays an important role in the formation of chlorophyll, the development of chloroplasts and the opening of stomata (Im *et al*., 2010). The use of artificial lights to compensate for the lack of natural light or to replace natural light in facility cultivation has become an important means of environmental control of plant growth and development. At present, incandescent lamps, fluorescent lamps, high-pressure sodium lamps, low-pressure sodium lamps, metal halide lamps, dysprosium lamps, etc. are mainly used as artificial light sources in the long term, Of these, high-pressure sodium lamps and dysprosium lamps are light sources with high luminous efficacy and effective light synthesis efficiency, but their emission spectrum cannot be matched with the absorption spectrum of plant photosynthesis, and cannot be irradiated to the plant in close proximity to the plant, and the light stimulation of plant growth is not very efficient, and the thermal radiation is more and less efficient, power consumption and high cost. Therefore, the introduction of new light sources with good luminescence performance, energy saving, low heat production and long-life span is of great significance to improve the utilization of electric energy and space and reduce the cost in plant nursery facilities. The rare-earth plant growth lamp is to use the selection of rare-earth three-color phosphor proportioning and application to tune the spectrum required for plant growth, and its light efficiency distribution is uniform, twice as much as other energy-saving lamps of the same wattage, three times as much as general fluorescent lamps, and has a long service life (Li *et al*., 2016). In recent years, the research on regulation of plant growth using artificial light sources has gradually attracted the attention of many scholars. Researchers have carried out several related light-regulated plant growth application research, and some of its results have confirmed that light quality on the growth, physiological characteristics, and development of plants and show a significant promotional effect (Yorio *et al*., 2001; Puspa *et al*., 2008; Fan *et al*., 2013; Li *et al*., 2017; Gao *et al*., 2022; Li *et al*., 2023), but it was also found that the photobiological effects produced by different plant species or varieties are significantly different. Therefore, to artificial light sources suitable for okra seedlings and optimize the facility okra artificial seedling and cultivation system, it is necessary to use high-quality artificial light sources to carry out research in light source environment regulation technology. In the present study, using fluorescent light (CK) as control, blue light (B), purple light (PL), red light (R), and blue plus red light (BR37), We investigated the effects of light quality on red okra seedling growth, photosynthetic pigments and stomatal characteristics, and screened light sources and related technologies suitable for the growth of red okra seedlings, with a view to providing theoretical basis and technical support for the selection of light sources in red okra factory nursery.

Material and Methods

Plant materials: This study was carried out at the Suzhou Polytechnic Institute of Agriculture between January and December 2022. Seeds of the same size and plumpness from the red okra strain Yanjiao 1 (Fujian Longhai Municipal Agricultural Seeds Co., Ltd., China) were selected and sown into peat+perlite (2V: 1V) culture bowls. The culture bowls were then placed inside an incubator with a constant temperature and light source. The seedlings were removed from the incubator once their cotyledons have flattened and subjected to different light sources for further cultivation. Technical term abbreviations are explained upon first usage for clarity.

Light treatments: In a randomized block design, the transplanted seedlings were placed under different types of light: blue (B), blue-red composite (BR37), red (R), and purple (PL) light (40 W, Shanghai Heming Lighting Electrical Appliances Co., Ltd., Shanghai, China), as well as fluorescent light (with T5-28 W, Philips Lighting Industry Co., Ltd., Yangzhou, China, serving as the control group). The seedlings were irradiated under consistent light intensity. For 25 days of treatment, the light intensity was set at 120 mol $m^{-2}\cdot s^{-1}$ and the photoperiod were 12 $h\cdot d^{-1}$. The distance between the seedlings and various light sources was maintained at 10-15 cm, the temperature was kept at 26-28℃, and the relative humidity was maintained at 55-60%. Seedlings were randomly allocated to each treatment and the number of lights maintaining the same light intensity. The spectral-energy distribution of the lights was measured (Table 1).

Measurements: Thirty seedlings from each treatment were random extraction for biomass analysis. Determination of root activity using triphenyl tetrazolium chloride (TTC) reduction method. Pigment content was measured with Ethanol extraction method for determination. Measure the soluble sugar and starch content using the anthrone method; Measure sucrose content using m-diphenol method; Determine the content of free amino acids using the hydrated ninhydrin colorimetric method; The content of flavonoid was determined using the method of Toor & Savage (2005); The soluble protein content was determined using coomassie brilliant blue staining method. Methods to observe the stomata using the nail polish and transparent tape (Zeng *et al*., 2012; Li *et al*., 2017; Li *et al*., 2023).

Statistical analyses: Statistical analyses were carried out with SPSS 22.0, using analysis of variance and the Tukey's multiple comparison test ($p<0.05$) to examine the data. The measurements were repeated three times.

Results

The morphology: Fresh and dry mass as well as stem length, stem diameter and root length of red okra seedlings under BR37, B, R treatments were increased to different degrees compared to CK (Table 2), and all of them were highest in the BR37 treatment, with the increases of 40.86%, 38.89%, 27.47%, 46.24% and 39.29% respectively compared to CK. The fresh and dry mass as well as stem length and root length of B treatment were significantly increased by 18.74%, 22.22%, 19.53%, 23.96% and 18.19% respectively, compared to those of CK. The fresh mass as well as stem diameter and root length of R treatment were significantly increased by 14.67%, 27.43% and 15.25%, respectively, compared to that of CK, of which the dry mass and stem length did not differ significantly from that of CK. The PL treatment of all those indexes were not significantly different from CK treatment. These can be seen that B, BR37 and R treatments could promote the growth of red okra seedlings to different degrees compared with CK, especially BR37 was more prominent.

Root activity: Root activity of red okra seedlings under B, BR37, R, and P treatments were all considerably higher than CK (Fig. 1), where seedlings under BR37 had the greatest root activity, followed by R, BR37, B and PL treatments which were 66.35%, 28.49%, 18.74% and 7.46% greater than CK, respectively, where BR37 and R lights were substantial bigger than B and PL lights, however there was no significant difference between treatments B and PL. These showed that BR37, R, B and PL treatments increased root activity of okra seedlings, and BR37 treatment was the most effective.

The pigments: Chlorophylls a, b, a+b and carotenoids in red okra leaves showed basically the same trend among light quality treatments (Fig. 2). They were all the largest under the B treatment, followed by the BR37 treatment, and both showed significantly higher than those of CK treatment. However, the R treatment and CK had no significant differences. PL treatment in red okra leaves showed the lowest. It was concluded that B and BR37 lights considerably increased the pigments in red okra seedlings.

Table 1. Spectral distribution of lights.

Light treatment	Light spectral distribution	Peak value λ p (nm)	Half-wave width Δ λ(nm)	Light intensity (μ mol $m^{-2}\cdot s^{-1}$)
CK	Fluorescent lamp	380～760	～	120
B	Blue	660/460	5～10	120
BR	Blue plus red (37)	660/460	5～10	120
R	Red	660	5～10	120
PL	Purple	380	5～10	120

Table 2. Effects of different lights on the morphology of red okra seedlings.

Light treatment	Fresh mass (g)	Dry mass (g)	Stem length (cm)	Stem diameter (cm)	Root length (cm)
CK	4.43 ± 0.21c	0.54 ± 0.06c	15.87 ± 0.35c	10.10 ± 0.26c	3.41 ± 0.10c
B	5.26 ± 0.19b	0.66 ± 0.06b	18.97 ± 0.21b	12.52 ± 0.21b	4.03 ± 0.17b
BR37	6.24 ± 0.33a	0.75 ± 0.05a	20.23 ± 0.25a	14.77 ± 0.15a	4.75 ± 0.09a
R	5.08 ± 0.11b	0.61 ± 0.02bc	16.63 ± 0.15c	12.87 ± 0.25b	3.93 ± 0.03b
PL	4.25 ± 0.18c	0.51 ± 0.01c	14.12 ± 0.26d	10.79 ± 0.15c	3.15 ± 0.17c

Note: FL: Fluorescent lamp; B: 100% blue light; BR37: 30% blue light and 70% red light; R:100% red light; PL: 100% purple light. Values are the mean ± standard deviation. Different letters within the column indicate significant differences at $p<0.05$ according to Tukey's test (n=3). The same as below

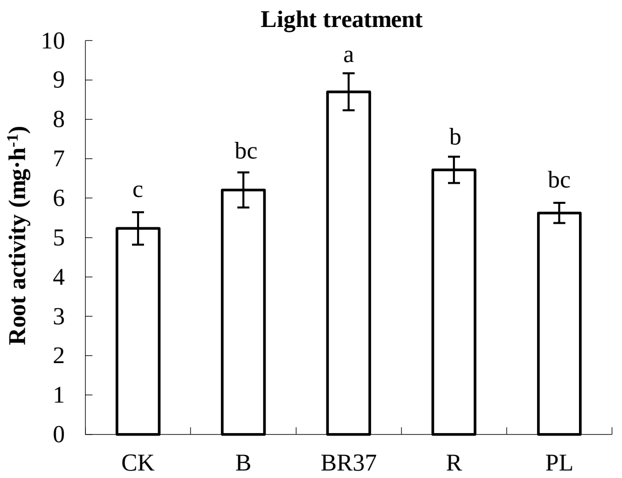

Fig. 1. Effects of different light treatments on pigment content of red okra seedlings.
Note: FL: Fluorescent lamp; B: 100% blue light; BR37: 30% blue light and 70% red light; R:100% red light; PL: 100% purple light. Different letters within the column indicate significant differences at $p<0.05$ according to Tukey's test (n=3). The same as below.

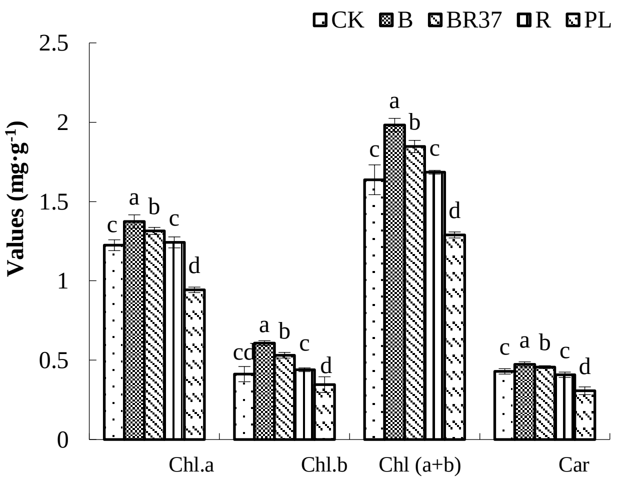

Fig. 2. Effects of different light treatments on pigment content of red okra seedlings.

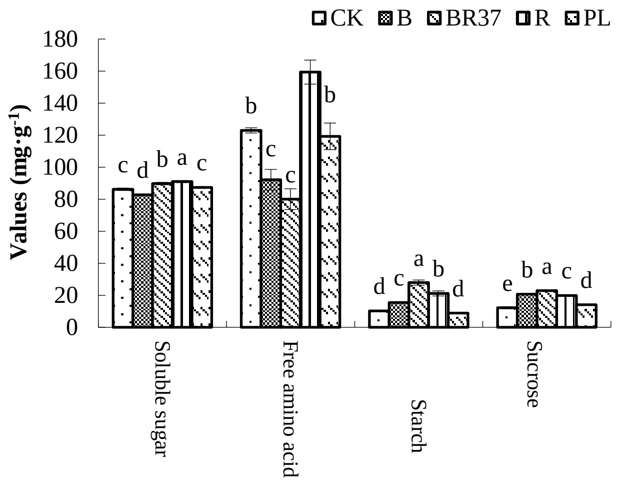

Fig. 3. Effects of different lights on the photosynthetic products of red okra seedlings.

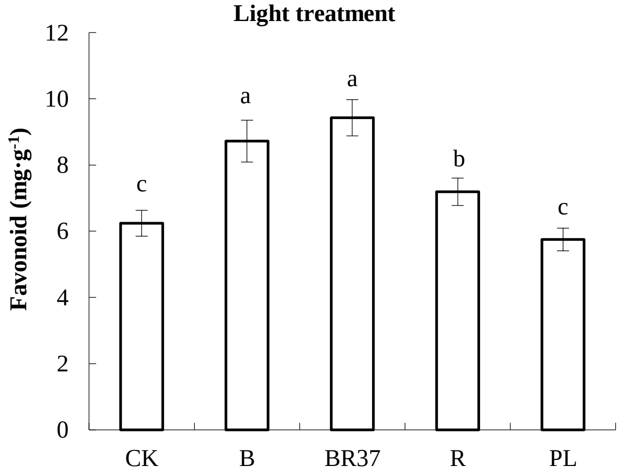

Fig. 4. Effects of different lights on the flavonoid of red okra seedlings.

The photosynthetic products: The soluble sugar content was highest under the R light, flowed by BR37, both were significantly higher than the others, the lowest was B light (Fig. 3). Free amino acids were highest under the R light, flowed by

CK and PL, but they had no significant different. The content of starch was highest under the BR37 light, flowed by R, both were significantly higher than the others, the lowest was PL light. Sucrose was highest under the B37R light, flowed by B, both were significantly higher than the others, the lowest was CK. It showed that BR37 and R lights effectively increased the content of photosynthates in leaves of red okra seedlings.

The flavonoid: The content of flavonoid in leaves of red okra seedlings was highest under BR37 and B treatments, which were significantly higher than CK treatments by 50.96% and 39.74%, followed by R treatment, which was higher than CK treatments by 15.22%, but was not significantly different from CK treatments, and was lowest under P treatment (Fig. 4). It was concluded that BR37 and B increased the flavonoid content in the leaves of red okra seedling, especially BR37 performed most prominently.

The soluble protein: The soluble protein content of red okra seedlings leaves was greatest in BR37 treatment, which was considerably greater than CK by 14.07%, while the rest of the treatments (B, R, and P) were not significantly different from CK (Fig. 5). It showed that BR37 increased the synthesis of soluble protein of red okra seedlings.

The leaf stomata: Stomatal length of the adaxial and abaxial in red okra were greatest under BR37 treatment, followed by PL treatment, both were significantly higher than that of CK treatment then B and CK treatments, but there was no significant difference among B, R and CK treatments (Fig. 6). The results showed that the stomatal length of red okra was greatest under BR37 treatment.

The stomatal width of the adaxial and abaxial in red okra was greatest under BR37 treatment, followed by B and R treatment, which were significantly higher than CK treatment, and there was no significant difference between R and B treatments (Fig. 7). And the stomatal width of the adaxial and abaxial in red okra was greatest under PL treatment, which were significantly lower than CK treatment. The results indicated that stomatal width of red okra in adaxial and abaxial epidermis was greater under BR37 treatment.

The trends of stomatal density in the adaxial and abaxial surfaces of red okra under different light qualities were consistent, both of which were greatest under the BR37 treatment, followed by B, CK, and R, which were higher than PL treatments, but there were not significantly different among them, and was lowest under PL treatment (Fig. 8). The results showed that stomatal density of adaxial surface in red okra under different light quality treatments was less than that of abaxial surface and it was greatest under BR37 treatment.

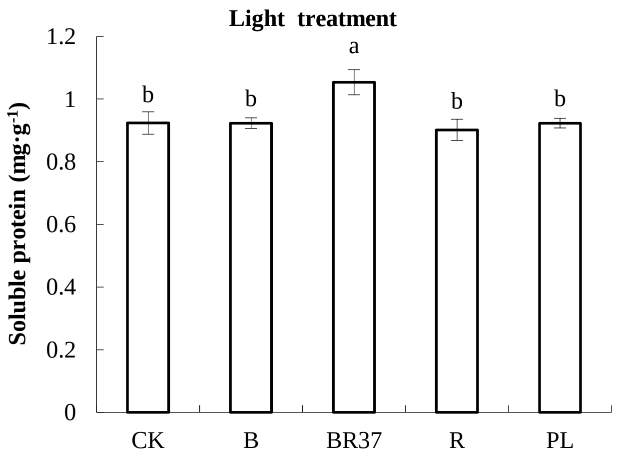

Fig. 5. Effects of different lights on the soluble protein of red okra seedlings.

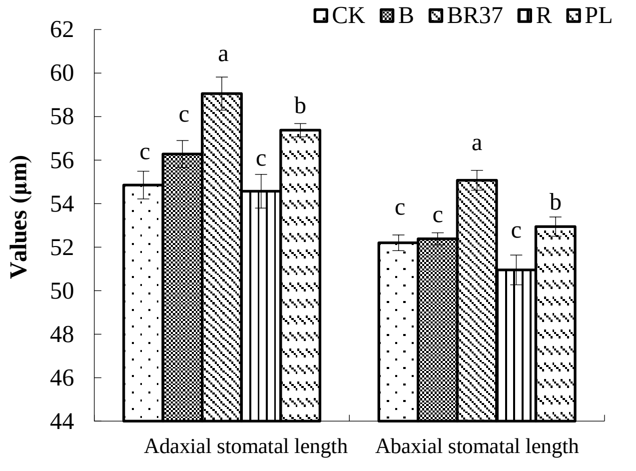

Fig. 6. Effects of different lights on the stomatal length of leaves in red okra seedlings.

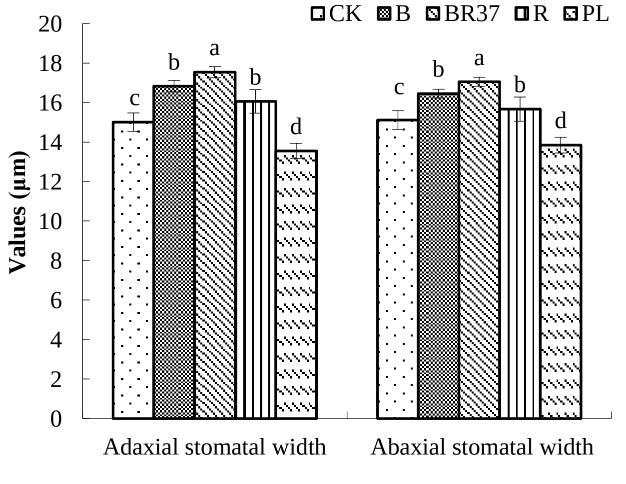

Fig. 7. Effects of different lights on the stomatal width of leaves in red okra seedlings.

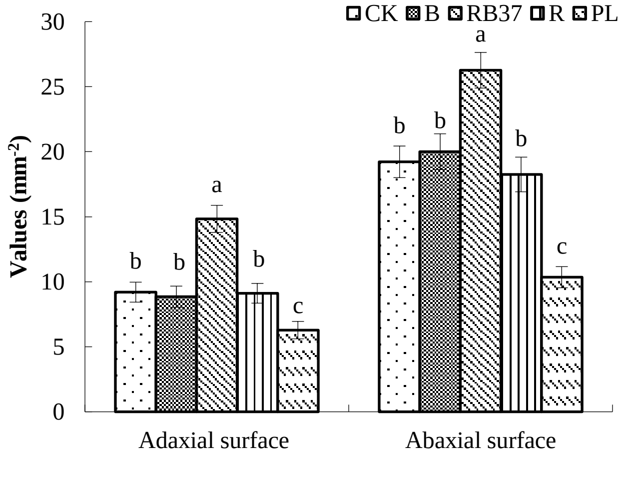

Fig. 8. Effects of different lights on the stomatal density of red okra seedlings.

Discussion

The relationship between light quality and plant growth: The study showed that BR 37 had the highest mass, stem length, stem diameter, root length, and root activity. Meanwhile, the former research has been found that many crops grew well under certain blue plus red, such as tomato, non-heading Chinese cabbage, mesembryanthemum crystallinum, radish and spinach (Yorio *et al*., 2001; Fan *et al*., 2013; Jie *et al*., 2017; Li *et al*., 2023). The combined absorption spectra of blue light receptors (cryptochromes and phytotropins) and red/far-red light receptors (phytotropins) overlap with those of photosynthetic pigments, which may be produced by triggering changes in the interactions between pigment receptors and other proteins, which in turn cause alterations in the subcellular localization of light-signaling proteins, intra-ionic homeostasis, other cellular activities, or gene expression, and ultimately, changes in plant development (Smith, 2000; Wang & Deng, 2003). Blue plus red light can positively affect plant growth and development, mainly because the spectral energy distributions of blue light receptors (cryptochrome and phytotropin) and red-light receptors (phytotropin) coincide with the peak regions of chlorophyll absorption spectra (Yang, 2008), but the exact proportion of suitable light sources may vary depending on the species of the plant (Li *et al*., 2010). In the present study, it was concluded that blue plus red light BR37 favors the growth of red okra seedlings. To summaries, plant growth was enhanced with composite light quality.

The relationship between photosynthetic metabolites and plant quality: Light of certain wavelengths can enhance the production of bioactive compounds in plants, and various wavelengths have different effects on the phytochemical pathways during plant development (Nam *et al*., 2018). The present study found that the content of soluble sugars and free amino acids were highest under the R light. The content of starch and sucrose were highest under the BR37 light. However, soluble sugar, sucrose, and starch contents were the highest under R in upland cotton plantlets and seedlings (Li *et al*., 2010; Li *et al*., 2017). Li *et al*. (2023) found that soluble sugar and sucrose was highest under BR1:1, starch content was the greatest under R of tomato. The soluble protein content of red okra seedlings leaves was greatest in BR37 treatment. Under the blue-white combined light treatment, however, leaf pigment, soluble sugars and free amino acids were significantly higher in green onions (Gao *et al*., 2022). The present study showed that that the flavonoid content in red okra seedlings was highest under BR37 and B treatments, chlorophylls a, b, a+b and carotenoids values were observed under B treatment, followed by the BR37 treatment. Nam *et al*., (2018) revealed that BL, RL and FL increased the accumulation of different individual flavonoids in common buckwheat. Light stimulated the biosynthesis of flavonoids of buckwheat sprouts. Li *et al*., (2023) found that BR1:1 was advantageous for pigments accumulation in tomato. B had the highest soluble sugar concentration, BR had the highest soluble protein concentration, R treatment was averse to pigment accumulation. Concentrations of photosynthesis pigments and chlorophyll biosynthetic precursors were greater under BR (Fan *et al*., 2013). Pigmentation, plant quality and nutritional value are influenced by light-signaling genes. The results demonstrated that BR37 improved the levels of flavonoids, soluble proteins, pigments and photosynthetic products.

The relationship between light quality and stomatal development: Stomata are important channels for gas exchange between plant leaves and the outside world. Plants photosynthesize in the light, through the stomata to absorb CO2, so the stomata must be open, but the stomata open and inevitably transpiration occurs, stomata can be adjusted according to changes in environmental conditions to adjust the size of their own degree of openness (Outlaw, 2003). The present study revealed that stomatal length and width on both adaxial and abaxial surfaces of red okra were observed to be greatest when treated with BR37. Stomatal density under various light qualities yielded similar trends with the highest density achieved under BR37 treatment. Different plants stomata size, number, and distribution of different. Most plants possess stomata in the upper and lower epidermis of the leaves. However, the number of stomata in the upper and lower epidermis of the leaves varies between different plant species (Li *et al*., 2010). The long axis, short axis and arc length were larger and stomatal openings were larger in okra under blue-red composite light and blue light (Li *et al*., 2016). However, Heo *et al*. (2002) found that the number of stomata was higher in marigolds and sage under a mixture of fluorescent lamps and LEDs, the stomatal openings of Salvia officinalis were smaller under blue light, whereas those of Calendula officinalis did not vary much under blue light. Puspa *et al*., (2008) found that the number of stomata in grapes was higher under blue light along with higher chlorophyll values, and that photosensitive pigments had a direct effect on stomatal development. Li *et al*., (2023) showed that B and BR1:1 was good for the stomatal opening in tomato. Kim *et al*., (2004) found that seedlings grew better under BR1:1, but the stomatal number was less and seedlings under fluorescent light had poorer growth but higher stomatal number, stomatal openings were larger under the BR1:1. In the present study, we concluded that BR37 light increased the number of stomata and the opening of stomata.

Conclusion

The physiological and biochemical indexes of okra seedlings were characterized by different responses after irradiation with different light qualities, among which the BR37 light was effective in inducing robust seedling growth and significantly improved the fresh mass, dry mass, root activity, the contents of pigments, photosynthetic products, flavonoid, soluble protein, and the characteristics of the stomata, including the stomata density, the stomatal length and diameter. Therefore, BR37 light can be used as the preferred proportion of artificial light source in red okra nursery or cultivation. The results of this study provide a theoretical basis and technical support for future light regulation measures in okra factory nursery and cultivation technology.

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