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RESEARCH PAPERS

LED Lighting Influences Germination, Growth, and Biochemical Indices of Snapdragon

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Abstract—*Antirrhinum majus* L. has many applications in genetic studies and the medicinal and ornamental plant industries. However, its seeds have a low germination percentage and speed, and the growth of its seedlings is weak, which has caused many problems for researchers and flower growers. Plant germination, development, and flowering are affected by light quality. Recently, light-emitting diodes (LEDs) have been used for multiple purposes in plant production. Accordingly, the influence of light quality on the germination and growth of snapdragons was assessed in this study. Hence, different light treatments included natural daylight (C), white LED (W), blue LED (B), red LED (R), and a mix of 50% blue and 50% red (BR). Germination indices were investigated for up to 21 days. Morphological and biochemical indices were analyzed in seven-day and 30-day-old seedlings, respectively. According to the results, the positive effects of BR treatment were evident in all germination indices, particularly in the germination speed. Morphological indices such as fresh weight, dry weight, shoot height, and root length were the highest in R and BR. Also, growth indices such as the contents of photosynthesis pigments, total soluble carbohydrates, total soluble protein, and total phenolic increased remarkably under the BR treatment.

Keywords: *Antirrhinum majus*, germination speed, LED technology, light quality, protein, snapdragon, total phenolic

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INTRODUCTION

Seed germination and seedling production depend on genetic, endogenous, and environmental factors [1]. Light, as an ecological factor, is distinguished by spectrum (quality or color), intensity (quantity or amount), and photoperiod (daily duration of light) [2]. Germination light requirements divide plants into three photoblastic groups: positive (light-requiring), negative (light-inhibited), and neutral (neutral to light) [3].

Antirrhinum majus or snapdragon belongs to the Plantaginaceae family and has long been considered a model plant for genetic studies, an ornamental cut flower, and an edible flower [4, 5]. Although it is a perennial herb, it is used as an annual flower for scientific studies and the flower industry [4]. Seeds are mainly used to propagate this plant. However, snapdragon seeds are positive photoblastic, possess a low germination percentage, and the average germination time of snapdragon seeds is long [6]. Priming techniques such as prechilling and KNO_3 have improved the germination time. However, they have not been efficient [7]. Also, the primary growth of snapdragon seedlings is slow. Hence, the seedlings may be subject to various environmental stresses [8]. The use of F1 hybrids has been able to solve these issues to some extent. However, slow seed germination, the low yield of productive seedlings against the high price of F1 seeds, and slow growth have created many problems for researchers and flower growers [9]. Therefore, it is imperative to employ some special techniques to improve germination quality, produce healthy, stocky seedlings, and establish seedlings.

In the cultivation of flowering plants, uniform seedlings production with a high number and in a short period is an economic attainment for the producers of seedlings [10]. In addition, the high quality of seedlings and traits such as thick stems and a healthy root system induce early flowering, which is desirable for ornamental plant growers [11]. Natural light is a limiting factor during propagation [12]. Accordingly, today, controlled environments with supplemental light (SL) instead of greenhouses and natural light are utilized for year-round production [13, 14].

Newly, LEDs (light-emitting diodes) have replaced traditional light sources such as high-pressure sodium (HPS), metal halide, and fluorescent lights (FL) [2, 14]. The LEDs have several advantages as follows: It is an economic advantage for commercial growers due to their long operating life, low heat output, and adjustable light intensity and quality [15], allowing researchers to investigate morphological reactions by altering

the light spectrum [16], and has made it possible for growers to pursue their production purposes by executing light recipes [17].

There is evidence that lighting quality is associated with seed germination, seedling quality, and subsequent flowering [18]. The application of red and blue lights is prevalent due to easy absorption by green tissues [19]. Germination responses of plant species to various light spectra are species-dependent. Studies have revealed that red LED light promotes germination in some species, such as *Brassica oleracea* [20]. On the other hand, the application of blue LED light has promoted the germination of *Lens culinaris*, *Vicia faba* [21], and *Stevia rebaudiana* [22] but inhibited the germination of *Trifolium subterraneum* [23].

Although snapdragon has many uses, few study has been conducted on the effect of light quality on improving germination and seedling quality [24, 25]. Hence, we investigated the influence of various light qualities on the germination and growth indicators of snapdragon seedlings. Our result provided recommendations for growers of snapdragon flowers for commercial and scientific purposes. In addition, this study added findings to the current knowledge in the field of plant responses to light quality.

MATERIALS AND METHODS

Plant materials. This study was conducted in a greenhouse at the University of Mohaghegh Ardabili, Ardabil (38°25′ N, 48°30′ E, 1351 m above the sea level), Iran. Snapdragon (*Antirrhinum majus* 'Legend White') F1 seeds were obtained from Takii Seed Company and placed in Petri dishes (4 Petri dishes for each treatment, 25 seeds per Petri dish) after surface sterilization. Then, the samples were transferred to the light boxes. Germination parameters were examined when radicles more than 2 mm in length appeared and recorded for 21 days. After that, morphological indices were measured in seven-day seedlings. Then, seedlings were transferred to containers filled with peat moss and perlite mixed (1 : 1) in plastic pots (2 kg) and subjected to the same light treatments. We measured biochemical parameters on the last day of the test (30th day) (Fig. 1).

Light treatments and experimental conditions. For this experiment, five light boxes (5 treatments) were considered, as follows: 100% white LED light (W, 420–680 nm), 100% blue LED light (B, 460–470 nm), 100% red LED light (R, 620–630 nm), combined blue and red LED light (BR, 50 : 50%), and normal condition without LED light as a control (C). All boxes were in the greenhouse. The day/night temperature regimes and RH of the experiment environment were 20/16°C and 60%, respectively. PPFD measured at the top of the Petri dishes and plants was 20 μmol/m^2 s in all light boxes (treatments). The photoperiod of all treatments was 16 hours per day. The 16 w lamps (1200 × 24 mm) were obtained from Pars Shahab Co. Ltd. Iran. Each light had 96 sets of LED. The nominal voltage, operating current, and typical frequency were AC 85-220 v, 100 mA, and 60.50 Hz, respectively.

Germination parameters. Germination parameters were calculated with Sanoubar methods [26] as follows:

$$\text{Germination percentage (GP)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds per assay}} \times 100.$$

$$\text{Speed of germination (SG)} = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \ldots \frac{n_i}{d_i}.$$

Where n is the number of germinated seeds and d is the number of days.

$$\text{Mean Daily Germination (MDG)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of days}},$$

$$\text{Germination Energy (GE)} = \frac{\text{Percentage of germinated seeds at the starting day of germination}}{\text{Total number of seeds per assay}}$$

$$\text{Germination Rate Index (GRI)} = \frac{\text{GP}_1}{d_1} + \frac{\text{GP}_2}{d_2} + \frac{\text{GP}_3}{d_3} + \ldots \frac{\text{GP}_i}{d_i}.$$

Morphological parameters. Ten seedlings were considered for the measurement of morphological parameters in each replicate. Morphological traits included fresh weight (FW), dry weight (DW), shoot height (cm), and root length (cm). FW (g) was determined immediately after removing the seedlings from Petri dishes using a Micro Balance (Semi-Micro Analytical Balances GR-200, A&D Company, JAPAN). The seedlings were oven-dried at 40°C for two days to determine DW (g). Measurement of length (shoot and root) was with a ruler.

Biochemical parameters. Six lower leaves of the shoot were used to measure the biochemical indices. Photosynthetic pigments were extracted according to the methods of Lichtenthaler, Wellburn, and Buschmann [27]. The absorption was measured using a spectrophotometer (SP-UV 200, Spectrum Instruments Limited, Australia) at 470, 645, and 662 nm against 100% acetone. The concentration of pigments was determined using the following formulas:

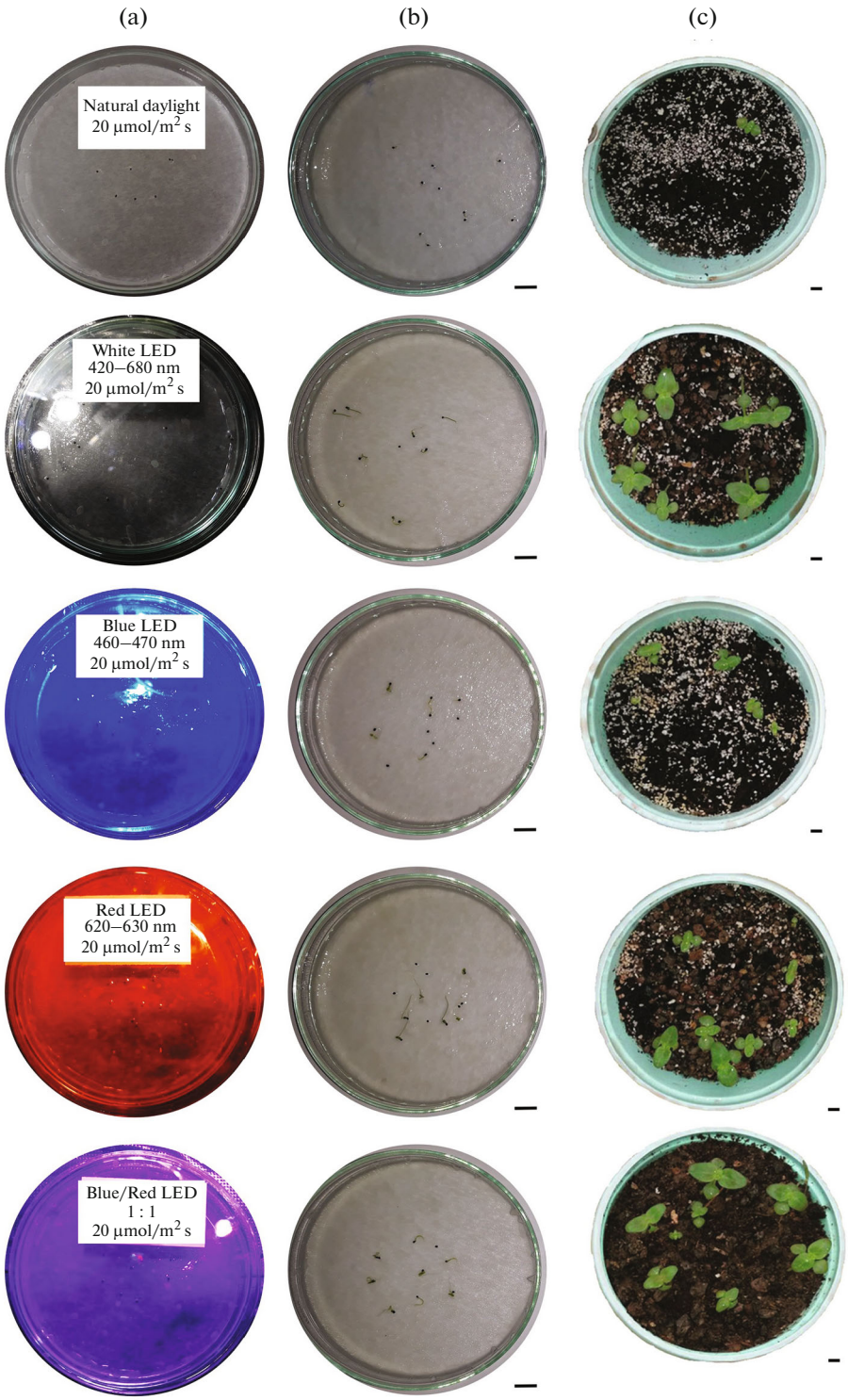

Fig. 1. Snapdragon seedlings under light treatments. (a) Seeds on the first day of germination; (b) seven-day seedlings; (c) thirty-day seedlings. Scale bars 1 cm in all figures.

$$\text{Chlorophyll } a = C_a\,(\mu g/mL) = 11.24A_{661.6} - 2.04A_{644.8},$$

$$\text{Chlorophyll } b = C_b\,(\mu g/mL) = 20.13A_{644.8} - 4.19A_{661.2},$$

$$\text{Total carotenoids} = C_{(x+c)}\,(\mu g/mL) = \frac{(1000A_{470} - 1.90C_a - 63.14C_b)}{214}.$$

Total soluble carbohydrates were extracted and measured using the anthron method [28]. Two grams

of leaf were homogenized and extracted with 80% ethanol (twice) and 70% ethanol (four times). The supernatants were collected, combined, and then evaporated ethanol at 50°C to concentrate them. The extract (1 mL) was reacted with 5 mL of anthron-sulfuric acid reagent (2 grams of anthron in 1 liter of cold 95% sulfuric acid) for 10 minutes at 90°C. The absorbance of samples was measured at 630 nm (Spectrophotometer, SP-UV 200, Spectrum Instruments Limited, Australia), and the glucose equivalents were calculated with a glucose standard curve.

The leaf tissue (100 mg) was homogenized for total soluble protein in a phosphate buffer solution (pH = 7). Then, the samples were centrifuged at 10000 rpm for 10 min at 4°C. 100 μL of the supernatant was added to Coomassie solution (5 mL) and then vortexed. The sample absorbance was measured at 595 nm, and total soluble protein was calculated using BSA standard curve, according to the Bradford assay [29].

Extraction and measurement of total phenolic content were performed utilizing the Wang procedure [30] and the Folin-Ciocalteu method [31], respectively. The leaf extract (20 μL), distilled water (1580 μL), and Folin-Ciocalteu reagent (100 μL) were mixed. Then 1500 μL of sodium carbonate was added to the mixtures and maintained in darkness for two h. The absorbance was read at 765 nm, and phenolics content was calculated with the Gallic acid standard curve.

Statistical analysis. The present experiment was a completely randomized design (CRD) with four replications (four Petri dishes containing 25 seeds and four plastic pots containing 10 seedlings for each treatment). Data were analyzed using SPSS software (version 16; SPSS Inc, USA). Duncan's multiple-range tests at 5% probability were used to compare differences among mean values. The experiment was repeated at least twice with similar results.

RESULTS

The results of the light quality experiment on snapdragon seeds indicated that different light qualities affect germination parameters. LED treatments notably influenced the germination percentage. Compared to the control, the germination percentage increased by 89, 24, 47, and 76% with W, B, R, and BR lights, respectively. There was no significant difference between the W and BR light treatments (Fig. 2a). The impact of LED treatments on germination speed was astonishing. W and BR treatments increased the germination speed by 479 and 468%, respectively, compared to the control. Both treatments had no significant differences, however. The R (378%) and B (313%) treatments had better performance in increasing the germination speed than the control (Fig. 2b). The positive effects of LED light were recorded in the mean daily germination in BR light treatment, which showed an increase of 76, 86, 273, and 139%, respectively, compared to the control, W, B, and R light (Fig. 2c). Light treatments were significantly different in terms of germination energy index. The highest germination energy was acquired when seeds were under BR light (3.16). W treatment (2.72) also improved germination energy. There were no significant differences between blue (1.84) and control (1.8) (Fig. 2d). Similarly, to the germination percentage results, LED light treatments enhanced the germination rate index. Among them, W (26.91) and BR (26.33) lights proved to be the most effective (Fig. 2e).

The light quality affected seedling growth and morphology. In this study, seedling FW ranged from 0.0094 to 0.0211 g. Compared to the control, R and BR LEDs provided the highest FW (Fig 3a). All LED light treatments increased DW compared to the control. However, LED treatments did not indicate a significant difference (Fig 3b). In the presence of R LED, the shoot elongated to a maximum of 1.85 cm. Shoots length was shortest at the control (0.695 cm) and B LED (0.618 cm). Compared to the control, both W LED (1.322 cm) and BR LED (1.1 cm) had longer shoots (Fig. 3c). Light quality made a significant difference in root length. R LED treatment significantly increased root length (2.21 cm). In seven-day seedlings, the root length of snapdragon seedlings grown under BR, W, B LED, and the control was 1.696, 1.675, 1.342, and 0.960 cm, respectively (Fig. 3d).

Light spectrum affected the biochemical parameters of snapdragon seedlings during growth (Fig. 4). Generally, the content of chlorophyll *a*, *b*, and carotenoids were higher under LED light than under the control condition. Chlorophyll *a* concentration raised significantly under BR (562.35 μg/g FW) and W (539.69 μg/g FW) lights (Fig. 4a). Chlorophyll *b* concentration was the highest in plants grown in BR light (328.41 μg/g FW) (Fig. 4b). BR (126.82 μg/g FW) and W (125.42 μg/g FW) treatments led to an increase in the content of total carotenoids, but they did not reveal significant differences (Fig. 4c). The impact of different qualities of light on total soluble carbohydrates was significant (Fig. 4d). The combination of blue and red light (BR) led to a substantial increase (137.93 mg/g FW) in carbohydrate content compared to the control (63.675 mg/g FW). Compared to the control, other LED treatments increased carbohydrates more efficiently. Light quality also changed the total soluble protein (Fig. 4e). BR treatment produced the highest protein content (24.2958 mg/g FW), which made it 327% higher than the control (5.6916 mg/g FW). Furthermore, there was an increase of 237, 227, and 191% compared to the control with the W, B, and R treatments. LED treatments revealed a significant effect in increasing the content of phenols compared to the control (7.3925 mg/g FW). The highest content of phenolics was observed in BR (17.9675 mg g^{-1}FW), B (17.505 mg/g FW), W (12.1175 mg/g FW), and R (10.6050 mg/g FW), respectively. The BR and B did

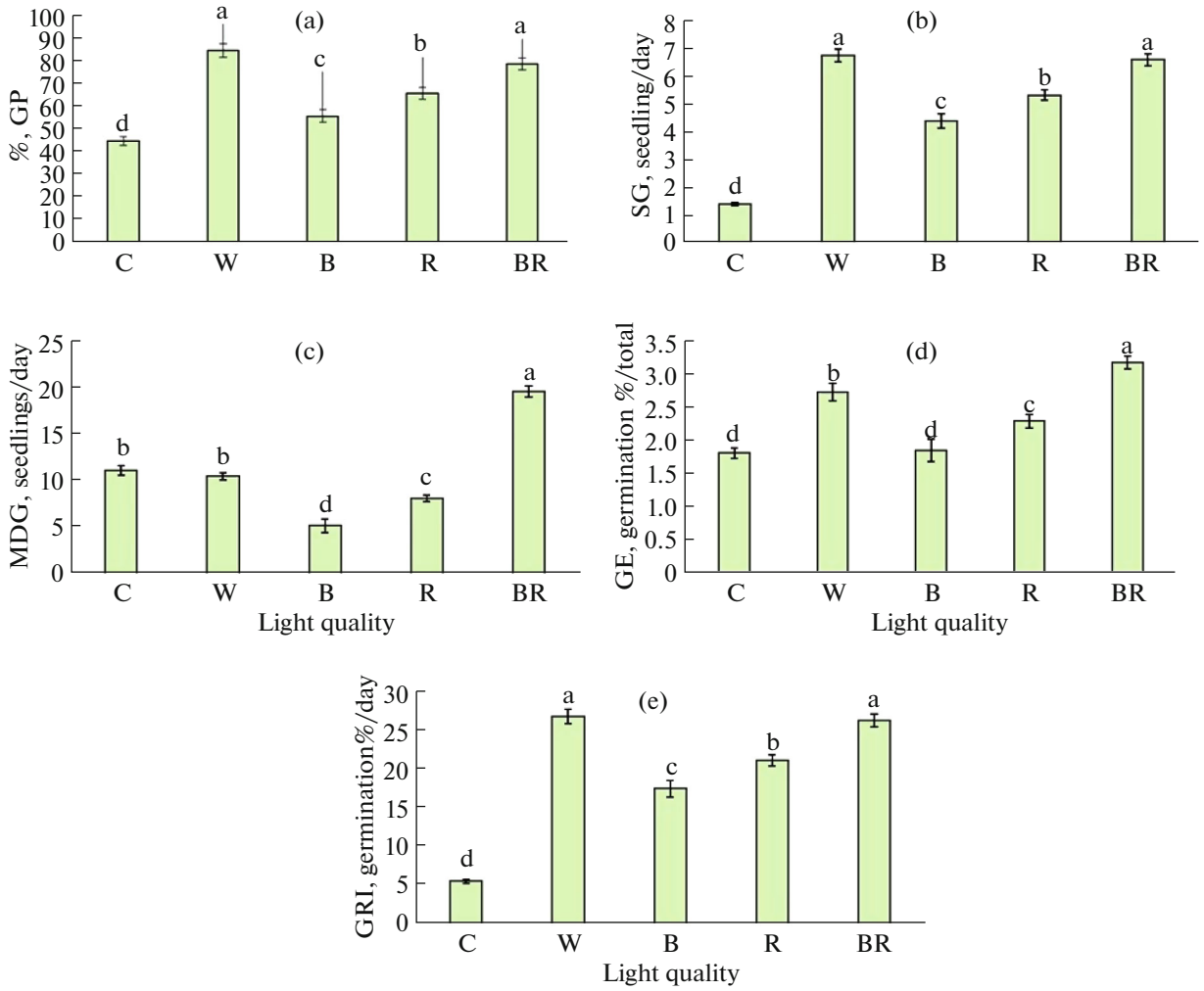

Fig. 2. Germination parameters. (a) Germination percentage (GP); (b) speed of germination (SG); (c) mean daily germination (MDG); (d) germination energy (GE); (e) germination rate index (GRI). Different letters indicate significant differences at $P \leq 0.05$ (Duncan's test). Error bars represent ±SE, $n = 4$.

not indicate significant differences in the content of phenolics (Fig. 4f).

DISCUSSION

Snapdragon has long been known in scientific studies and is considered an ornamental, edible, and medicinal flower. Despite its widespread use, snapdragon flower seeds have a low germination speed and growth in the early stages of the seedling. Despite numerous studies on snapdragon flowers in many distinct aspects, few studies have focused on improving their germination and growth characteristics. Therefore, in this study, we investigated the impacts of light qualities to enhance seed germination indices and seedling growth.

Our study demonstrated that LED illumination had stimulating effects in improving seed germination indices. The combination of blue and red light (BR) considerably increased all germination parameters (GP, SG, MDG, GE, and GRI) compared to the control. The remarkable effects of BR LED were especially evident in the SG. Like BR LED, W LED increased many germination indices, although it had no significant impact on MDG. Generally, LED treatments improve seed germination, as reported in *Momordica charantia* L. [2], *Lens culinaris*, *Vicia faba* [21], *Artemisia absinthium*, *Artemisia vulgaris*, *Atriplex halimus*, *Chenopodium quinoa*, *Salicornia europaea*, *Sanguisorba minor*, *Portulaca oleracea*, *Rosmarinus officinalis* [26], *Stevia rebaudiana* Bertoni [22], and *Brassica oleracea* L. [20]. However, the germination responses are highly dependent on the environment, light quality, and plant species, and contradictory impacts are also observed in some reports [32].

Morphological indices were mainly affected by R and BR treatments. R light led to the elongation of shoots and roots. However, the seedlings grown in BR

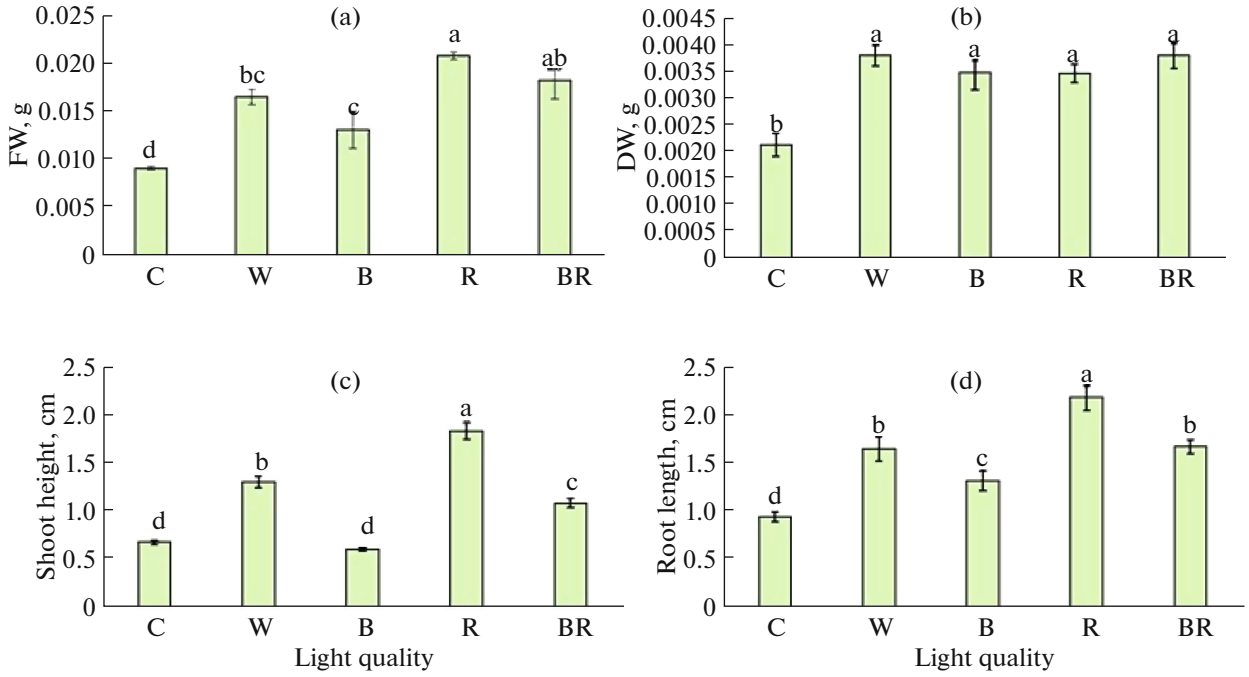

Fig. 3. Morphological parameters. (a) Fresh weight (FW); (b) dry weight (DW); (c) shoot height; (d) root length. Different letters indicate significant differences at $P \leq 0.05$ (Duncan's test). Error bars represent ±SE, $n = 4$.

light had compact morphology (short roots and shoots). Overall, blue and red light are responsible for stimulating the phytochromes of plants [1]. The combination of these two lights in many species has increased the net rate of photosynthesis and plant growth, while red light has often led to the elongation of shoots and roots [22, 33–35]. It appears red light can facilitate shoot growth by increasing the primary root's growth and absorption of water and nutrients. Blue light can also create a balance in plant architecture by stimulating shoot growth [1].

Biochemical indices increased under the impact of BR. Chlorophyll and carotenoids enable photosynthesis in plants by absorbing blue and red light spectrums [36]. In our study, we found that these pigments accumulated under BR light. The emission peak of blue and red lights corresponds to the absorption peak of photosynthetic pigments. Therefore, these two light combinations can help improve photosynthesis [34]. Also, studies have revealed that blue light is involved in inducing the formation of chlorophyll and the development of chloroplasts [22]. There are conflicting reports on the effects of LED on photosynthetic pigments [22, 37]. In some studies, BR light has raised the content of photosynthetic pigments, as reported in *Lactuca sativa* L. cv. Banchu Red Fire [36] and *Stevia rebaudiana* Bertoni [22].

BR treatment increased the total carbohydrate content significantly. Previous studies have shown that monochromatic red light increased the soluble sugar content in *Momordica charantia* L. [2], *Cunninghamia lanceolate* [38], and *Dendranthema grandiflorum* Kitam 'Cheonsu' [33]. Blue light has also led to the improvement of the photosynthetic properties of plants compared to red light [2]. Also, the positive effects of the combination of red and blue light on photosynthesis, biosynthesis of carbohydrates, and plant growth have been mentioned in studies [35, 38].

Although proteins can influence photosynthetic performance [35], the impact of light quality on total protein content has been paid less attention in reports. However, in our study, soluble protein content was strongly affected by BR treatment, which is similar to the Xu results in *Cunninghamia lanceolate* [38]. It has been found that blue light is involved in the synthesis of proteins, as reported in bitter gourd [2]. Blue light has a higher photon energy and can supply more energy for macromolecule synthesis, such as proteins. Also, studies have demonstrated that blue light increases pyruvate kinase protein within the glycolysis pathway [38].

In plants, phenols play various biological roles as compounds produced from secondary metabolism [39]. The influence of light quality on the secondary metabolism of many plant species has been determined. The use of blue light has increased the content of phenolics in *Lactuca sativa* L. 'Banchu Red Fire' [36], *Fagopyrum esculentum* [34], *Ocimum basilicum* L. [35], *Pisum sativum* L. [39], *Brassica oleracea* var. alboglabra Bailey [40], *Stevia rebaudiana* Bertoni [22], and *Cunninghamia lanceolate* [38]. This study indicated that BR treatment significantly increased the phenolics con-

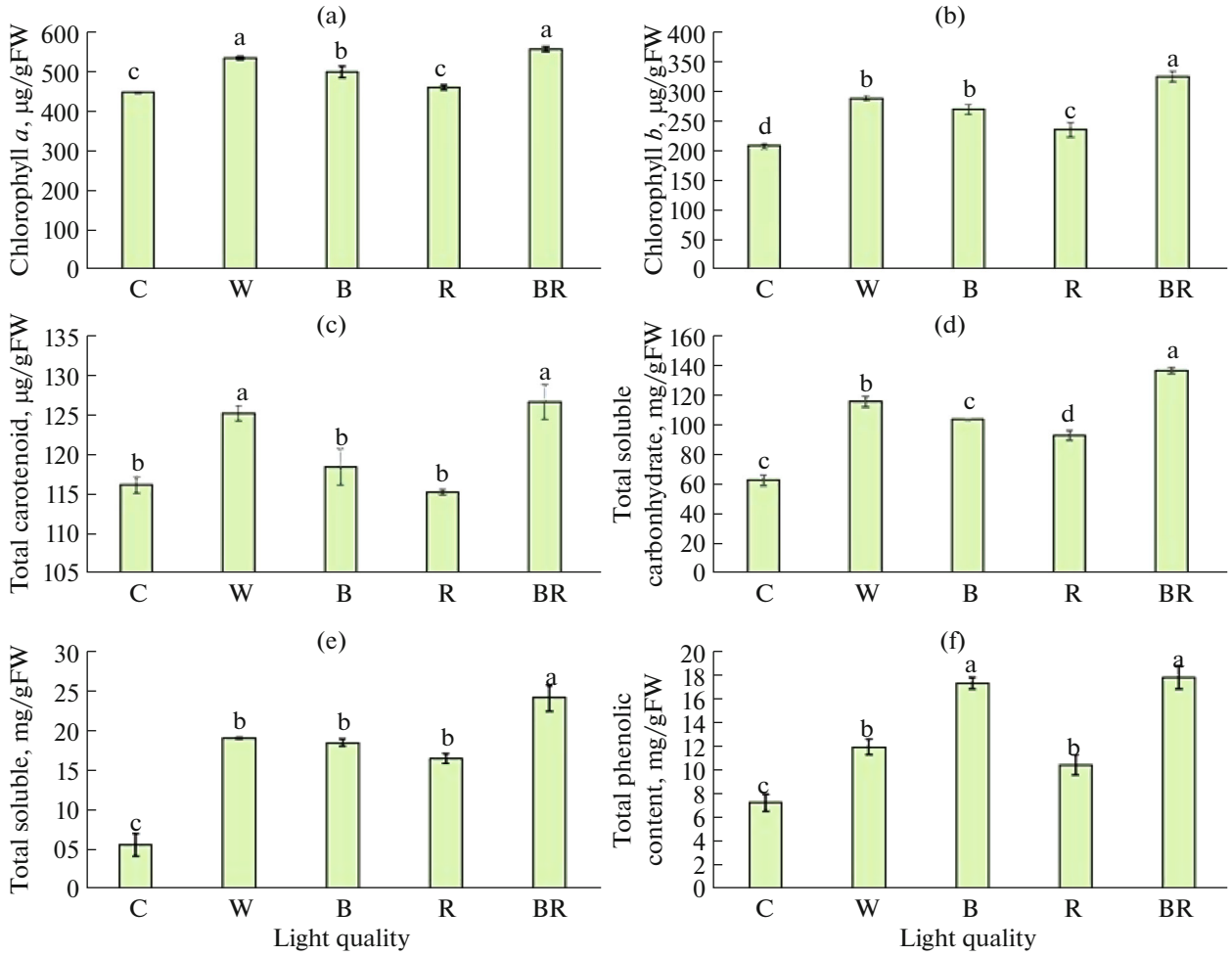

Fig. 4. Biochemical parameters. (a) Chlorophyll *a*; (b) chlorophyll *b*; (c) total carotenoids; (d) total soluble carbohydrates; (e) total soluble proteins; (f) total phenolic content. Different letters indicate significant differences at $P \leq 0.05$ (Duncan's test). Error bars represent ±SE, $n = 4$.

tent. It is reported that light can increase the production of malonyl CoA and coumaroyl CoA. These two compounds are involved in phenolic compound synthesis [40].

Recently, the use of LEDs has been expanded as a unique technology in plant production with desirable characteristics. However, rare reports have been documented about the effect of LED on the germination and initial growth of seedlings. Our study is the first report on the impact of light quality on seed germination and morphological and biochemical indicators of snapdragon seedlings. Our results revealed that the combination of red and blue light with a ratio of 50:50 not only increased the germination rate surprisingly but also had a significant effect on the initial growth, the appearance quality of the seedlings, and the content of phenols.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research involving people as objects of research.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

AUTHOR CONTRIBUTIONS

Funding acquisition, Esmaeil Chamani; Methodology, Roghayeh Nabipour Sanjbod; Project administration, Esmaeil Chamani; Software, Roghayeh Nabipour Sanjbod; Supervision, Younes Pourbeyrami Hir and Esmaeil Chamani; Writing—original draft, Roghayeh Nabipour San-

jbod; Writing—review and editing, Younes Pourbeyrami Hir, Esmaeil Chamani and Asghar Estaji.

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