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RESEARCH ARTICLE

Effects of red and blue LEDs on *in vitro* growth and microtuberization of potato single-node cuttings

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Abstract The objectives of this study were to investigate the effects of red and blue LEDs on *in vitro* growth and microtuberization of potato (*Solanum tuberosum*) single-node cuttings. Explants were incubated under 6 light treatments: 100% red LEDs (R), 75% red LEDs + 25% blue LEDs (3RB), 50% red LEDs + 50% blue LEDs (RB), 25% red LEDs + 75% blue LEDs (R3B), 100% blue LEDs (B) and white LEDs (W). Most of the growth and physiological parameters were significantly higher in 3RB than W. Enhancement of leaf area and chlorophyll concentrations were obtained in B. Leaf stomata were elliptical with the lowest density in 3RB. However, those in W were round in shape, and those with the smallest size and the highest density were observed in R. Most of the characteristics of microtuberization were also improved in 3RB. The combined spectra of red and blue LEDs increased the number of large microtubers. The fresh weight of individual microtubers in R and W were increased, but not their number. These results suggest that, of the treatments assessed, 3RB is optimal for the *in vitro* growth of potato plantlets and the combination of red and blue LEDs is beneficial for microtuberization.

Keywords blue LED, microtuber, plant tissue culture, potato plantlets *in vitro*, red LED

1 Introduction

Potato (*Solanum tuberosum* L.) is now the third most important food crop after rice and wheat in the world in terms of human consumption according to International Potato Center[1]. However, this crop is vulnerable to virus infection when field propagated using potato tubers from the previous crop, giving rise to yield reduction, quality deterioration and germplasm degradation. It is important to propagate strong virus-free potato plantlets and/or microtubers used for certified seed tuber production[2].

Light is a crucial environmental factor that influences the *in vitro* growth and development of potato plantlets[3], because they are strongly affected by the light spectrum[4]. Light spectral quality prior to cryopreservation can influence potato growth characteristics *in vitro*, and the success of shoot tip cryopreservation and subsequent survival[5]. It was reported that potato plantlets grown *in vitro* under red light were weak and slim with small leaves while those under blue light were short and sturdy with well-developed leaves[6]. Microscopic studies revealed that cells were shorter when cultured under blue light compared to culturing under red light[7]. The phototropic curvature of potato shoots grown *in vitro* was actively induced by unilateral blue light[8]. When the total power consumption was equal, and red and blue lights illuminated simultaneously with lower photosynthetic photon flux density (PPFD) and longer time (16 h photoperiod), the potato plantlets grew better than with higher PPFD, shorter time, or alternate red-blue light illumination[9]. The addition of green LEDs to the combination of red and blue LEDs lights promoted potato plantlet growth[10].

Red and blue light are readily absorbed by plants and the combination of red and blue light provide an efficient light spectrum for photosynthesis and photomorphogenesis[11–13]. However, the optimal ratio of red to blue light differs among plant species and cultivars under plant tissue culture conditions[14–17]. A red to blue LED light ratio of 9:1 was the most beneficial for potato shoot formation from shoot tips being revived after cryopreservation[18]. Nevertheless, little is known about the effects of different red and blue photon flux ratios on the growth and development of potato plantlets *in vitro,* and which ratio is optimal for growth of potato plantlets during propagation or after being transplanted *ex vitro*.

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The axillary buds of single-node cuttings from potato plantlets grown *in vitro* can be induced to tuberize aerially under suitable conditions to produce microtubers[2]. Previous research on microtuberization has concentrated on plant growth regulators, photoperiod, temperature and genotypes[19–21]. It has been reported that red light inhibited and blue light promoted microtuberization[6]. However, the inhibition of tuberization of day neutral potatoes under blue light has also been reported[22]. White and green light increased the fresh weight of microtubers produced by single-node cuttings[23]. However, there is limited information on the effects of different percentages of red and blue photon flux on the microtuberization. Therefore, the objectives of this study were to investigate (1) the growth and morphological characteristics of potato plantlets *in vitro,* and (2) the microtuberization of single-node cuttings exposed to different ratios of red and blue LED light.

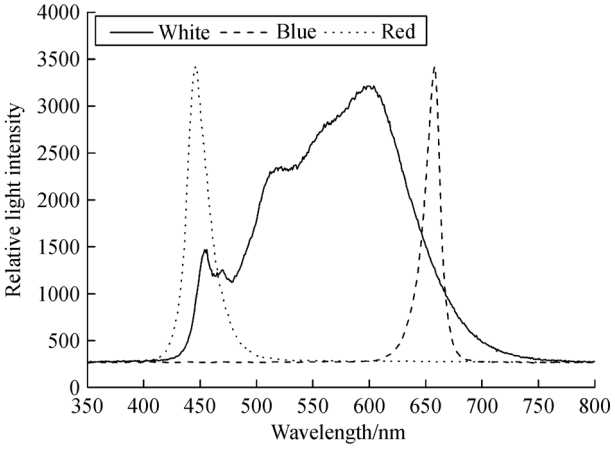

Fig. 1 Spectral distributions of red, blue, and white LEDs in the experiment. Red, red LEDs; Blue, blue LEDs; White, white LEDs.

2 Materials and methods

2.1 Plant materials and growth conditions

Virus-free potato (cultivar Ke Xin 1) plantlets grown *in vitro* were supplied by the Institute of Vegetables and Flowers of the Chinese Academy of Agricultural Sciences. The experiments were performed in Beijing Academy of Agriculture and Forestry Sciences. Single-node cuttings (15 mm long) with one leaf from plantlets grown *in vitro* were inoculated vertically onto standard MS medium[24] containing 30 $g \cdot L^{-1}$ sucrose and 8 $g \cdot L^{-1}$ agar under sterile conditions. The pH of MS medium was adjusted to 5.8 and 40 mL dispensed into 350 mL cylindrical transplant glass culture vessels before autoclaving at 121°C for 15 min. The explants were cultured at 23±1°C with 16 h photoperiod (6:00–22:00) and 70%±5% RH. After 4 weeks, mature potato plantlets were obtained.

2.2 Light treatments

There were 6 light treatments: 100% red LEDs (R); 75% red + 25% blue LEDs (3RB); 50% red + 50% blue LEDs (RB); 25% red + 75% blue LEDs (R3B); 100% blue LEDs (B) and white LEDs (W). The total PPFD was 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the bottom of the vessels (measured with LI-250A; LI-COR Biosciences, Lincoln, NE, USA). The light spectrum of experiments is shown in Fig. 1.

Explants were incubated under the 6 light treatments at 23±1°C with 16 h photoperiod (6:00–22:00) and 70%±5% RH for 4 weeks. Some of the 4-week-old potato plantlets were used to measure the morphological and physiological parameters. The others were kept for another 2 weeks under the same conditions to obtain strong and vigorous plantlets. Single-node cuttings from the 6-week-old potato plantlets were inoculated onto MS medium with 80 $g \cdot L^{-1}$ sucrose and 8 $g \cdot L^{-1}$ agar (induction medium without plant growth regulators) and incubated under the same light treatment at 19±1°C with 8 h photoperiod for 8 weeks. Then microtubers were then harvested for assessment.

A single factor completely randomized block design was adopted. Each experiment was repeated two times. Each treatment included 15 vessels and each vessel contained 9 explants.

2.3 Growth parameters

For each treatment, 30 potato plantlets were sampled for biomass analysis. Plantlets were oven-dried at 85°C for 72 h to achieve constant weight. The fresh and dry weights were measured using an analytical balance. Plantlet height was determined from the bottom to the top of the main stem. Stem diameter of the third node from the bottom of the plantlets was measured with a vernier caliper. The number of nodes were recorded. The projection area of the leaves were obtained with the WinRHIZO (Regent Instruments, Montreal, QC, Canada). Each parameter was measured five times in each treatment. A health index was applied according to the methods of Ma et al.[10].

2.4 Stoma observations

Fully expended leaf samples were taken from the fourth node (counting from the bottom) of 20 plantlets in each treatment for stomata observation. A thin layer of transparent nail polish was brushed evenly onto the lower side of the leaves. After the nail polish dried, transparent adhesive tape was pressed to the leaf surface, stripped and pressed onto a microscope slide to give temporary epidermal fingerprints. The fingerprints were examined under an optical microscope (Olympus CBB

microscope and Ken-a-vision 2100), and polar diameter (PD), equatorial diameter (ED) and density of the stomata measured under 40 × magnification. PD/ED was calculated. Photographs of the stomata were taken with a Canon camera coupled to the microscope.

2.5 Physiological parameters

2.5.1 Chlorophyll concentrations

To determine chlorophyll (Chl) concentrations, third and fourth leaves from 20 plantlets of each treatment were collected, and 0.1 g fresh weight (FW) of leaves put into centrifuge tubes with 30 mL (V) acetone (80%) in the dark for 24 h until all the leaves turned white. Optical density at 665 (A_{665}), 649 (A_{649}) and 470 (A_{470}) nm was measured with a UV-1200 spectrophotometer (UV-1600, Mei Puda Inc., Shanghai, China). Concentrations of Chl a and b, and carotenoids (Car) were calculated according to the following equations[25]:

$$\text{Chl a } (\text{mg}\cdot\text{g}^{-1}) = \frac{(12.72 \times A_{654} - 2.59 \times A_{649})\ V}{1000FW}$$

$$\text{Chl b } (\text{mg}\cdot\text{g}^{-1}) = \frac{(22.88 \times A_{649} - 4.67 \times A_{665})\ V}{1000FW}$$

$$\text{Chl (a + b) } (\text{mg}\cdot\text{g}^{-1}) = \text{Chl a} + \text{Chl b}$$

$$\text{Car } (\text{mg}\cdot\text{g}^{-1}) = \frac{(1000 \times A_{470} - 3.27 \times \text{Chl a} - 104 \times \text{Chl b})\ V}{229 \times 1000FW}$$

2.5.2 Root activity

The root activity were determined according to TTC method[26] and the following equation:

$$\text{Root activity } (\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}) = \frac{M_{TTC}}{FW \times T}$$

where M_{TTC} indicates the reduction value of TTC, FW indicates the fresh weight of root and T indicates the reaction time.

2.5.3 Soluble protein, soluble sugar, and starch content

The concentrations of soluble protein were determined by the Coomassie Brilliant Blue G-250 method[27].

Soluble sugar and starch concentrations were determined by a modified method of Fairbairn[28].

2.6 Microtuber assessments

2.6.1 Tuberization degree

Types of microtubers were scored according to Veramendi et al.[29]. Tuberized single-node cuttings were scored on a 0–4 scale: 0, neither stolon nor microtuber developed; 1, stolon developed; 2, apical or lateral microtuber developed on a shoot; 3, apical or lateral microtuber developed on a stolon; and 4, sessile microtubers. The degree of tuberization was estimated as the sum value for all microtubers divided by the total number of explants.

2.6.2 Tuberization percentage

The percentage of *in vitro* tuberization was calculated according to the number of tuberized single-node cuttings divided by the total number of the inoculated single-node cuttings.

2.6.3 Microtuber yield

After harvest, the fresh weight and diameter of microtubers from each vessel were measured. The number and weight of microtubers per vessel was recorded. The number of large microtubers per vessel ($\geqslant$0.1 g) were also recorded. The microtuber yield per vessel was evaluated as the average number of microtuber per vessel multiplied by the average weight of individual microtubers.

2.7 Statistical analysis

Statistical analyses were performed with SPSS for Windows Version 21.0 (IBM, Armonk, NY, USA). Data were analyzed by oneway analysis of variance. The statistically significant differences of means were tested by Tukey's test ($P < 0.05$).

3 Results

3.1 Growth and morphology of potato plantlets grown *in vitro* under different light treatments

The light treatment had variable effects on the growth of potato plantlets *in vitro* (Table 1). Plant height, stem diameter, leaf area, node number, fresh and dry weight, and health index in 3RB were significantly greater than those in W. The height of plantlets in R were 2.8 times larger than those in B. In contrast, the largest leaf area was observed in B, 1.96 times that of R. In addition, a few of the plantlets in R produced axillary bud outgrowths on the bottom nodes (data not shown).

3.2 Physiological parameters of potato plantlets grown *in vitro* under different light treatments

The pigment concentration of potato plantlets grown *in vitro* varied with light treatment (Fig. 2a). Chl a and Chl (a + b) concentrations in B were significantly greater than in other treatments; the combined spectra of red and blue

Table 1 Effects of red and blue LEDs on the growth of potato plantlets *in vitro*

Light treatment	Stem diameter/mm	Leaf area/cm^2	Node number	Node length/cm	Dry weight/mg	Fresh weight/mg	Health index
R	1.17c	1.00c	8.00a	2.03a	68.37a	927.30a	0.61c
3RB	1.68a	1.14b	6.67ab	1.17b	61.97a	877.31ab	1.19a
RB	1.60ab	1.18b	5.17b	1.19b	30.20b	651.03b	0.79b
R3B	1.53ab	1.10b	4.67c	1.08b	30.20b	559.36b	0.94b
B	1.52ab	2.96a	4.33c	1.06b	32.77b	670.74b	1.06a
W	1.43b	1.04c	4.67c	1.91a	30.33b	399.75c	0.58c

Note: Different letters in each column indicate significant differences ($P < 0.05$).

LEDs showed no significant differences compared to W; R performed significantly lower than W. The concentrations of Chl b and Car showed no significant differences between the treatments. The root activity of the plantlets increased with the enhancement of the ratio of red LEDs (Fig. 2b). Treatments with red LEDs induced significantly more root activity than with W. The soluble protein and sugar concentrations of the plantlets were significantly higher in 3RB than those in other treatments (Fig. 2c and 2d)). The greatest starch concentrations were found in R followed by 3RB (Fig. 2e).

3.3 The stomata observation of potato plantlets grown *in vitro* under different light treatment

The stomata density of the light treatments was not significantly different from W except for 3RB, which was lower by 3.2% (Table 2). Stomata in all treatments were elliptical in shape except for W, which were almost spherical (Fig. 3). Stomata in R with a narrower pore were smaller in size, while those in 3RB were larger in size with a wider pore (Fig. 3). PD, ED and PD/ED did not differ significantly between the combined spectra of red and blue LEDs. PD/ED was the largest in R and smallest in W (Table 2).

3.4 Microtuberization under different light treatment

Tuberization degree and percentage varied with light treatment (Fig. 4a–4b)). The greatest tuberization was observed in 3RB, followed by RB and R3B, and the least in R, B and W. Tuberization percentage in the treatments showed a similar trend to that of tuberization degree.

Microtuber yield was strongly influenced by light treatment (Table 3). Microtuber number per vessel was the greatest in 3RB, 2.6 times that of W, and the least in R, B and W. The average weight of individual microtubers was the greatest in R and W, followed by the mixture spectra of red and blue LEDs. The yield of microtuber per vessel was the greatest in 3RB (544 mg), intermediate in RB and R3B (365 and 370 mg, respectively), and the least in W, B and R (248, 224 and 200 mg, respectively). The number of large microtubers per vessel was highest in the combined spectra of red and blue LEDs (Fig. 4c).

4 Discussion

4.1 Effect of light treatments on the growth and physiology of potato plantlets grown *in vitro*

Various light regimes are sensed and absorbed by plants through their light receptors[30]. Potato plantlets grown *in vitro* and radish plants grown under pure red light have sufficient IAA to sustain stem elongation growth, while the opposite was found for those grown under pure blue light[6,31]. Histological observations showed that stems of potato plantlets grown *in vitro* under pure red light had shorter cells than those grown under blue light[7]. This may explain the height performance in R and B in our experiment. Red light (600–700 nm) and blue (400–500 nm) light are absorbed by phytochrome and cryptochrome, respectively[30]. Multiple molecular forms of these two light receptors have been identified with different spectrophotometric, biochemical and physiological characteristics, and the corresponding genes are believed to be expressed differentially in response to environmental and physiological signals[32,33]. It has also been demonstrated that light quality affected the metabolism and distribution of phytohormones in radish plants and potato plantlets grown *in vitro*[31,34]. It is possible that the outgrowth of axillary buds in R was related to the distribution of hormones in the potato plantlets.

RNA-Seq analysis of grape plantlets grown *in vitro* under red, blue, green and white LEDs revealed that the gene related to Chl synthesis was upregulated[35]. This is consistent with our finding that plantlets in B had the greatest Chl a and Chl (a + b) concentrations.

Leaf area of tissue cultured potato plantlets increased with increasing proportion of red light[3]. However, in our study the smallest leaf area was observed in R and the largest in B. Blue LEDs significantly increased the leaf area of both grape plantlets[35] and upland cotton[16] grown *in vitro*. However, Mortensen and Stromme[36] observed that blue light from high pressure sodium lamps (HPSL)

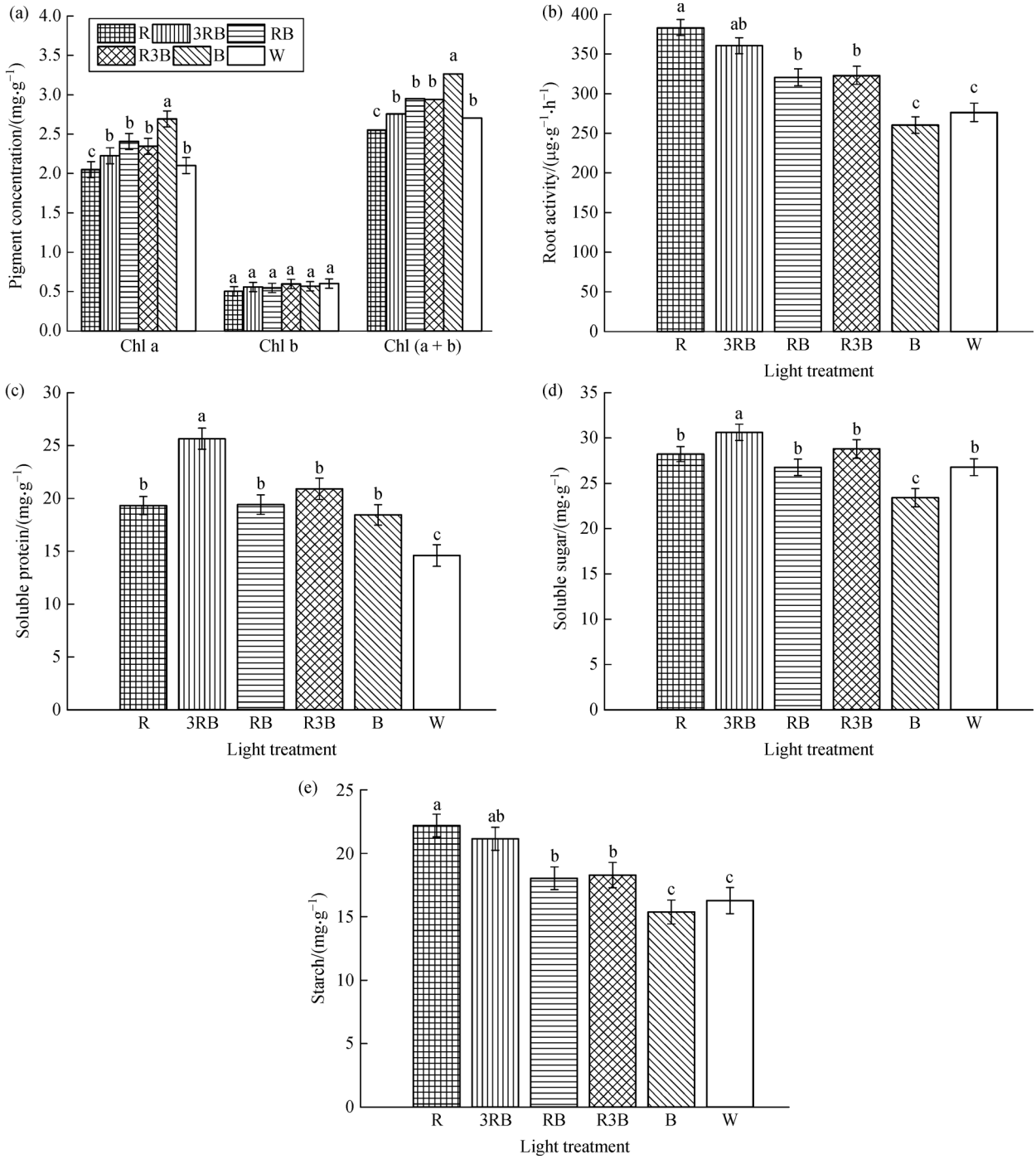

Fig. 2 Effects of red and blue LEDs on the physiological parameters of potato plantlets *in vitro*. Different letters in each bar graph indicate significant differences ($P < 0.05$)

Table 2 Effects of red and blue LEDs on the stomata size and stomata density of lower epidermis of leaves of potato plantlets *in vitro*

Light treatment	PD	ED	PD/ED	Stomata density (number per mm^2)
R	13.63b	3.05b	4.47a	53.61a
3RB	16.43ab	7.15a	2.29b	49.61c
RB	16.55a	7.31a	2.26b	51.96ab
R3B	16.45ab	7.48a	2.20b	51.94ab
B	16.19ab	7.18a	2.25b	50.12b
W	9.43c	7.06a	1.33c	51.26ab

Note: PD, polar diameter; ED, equatorial diameter; PD/ED, the ratio of PD/ED. Different letters in each column indicate significant differences ($P < 0.05$)

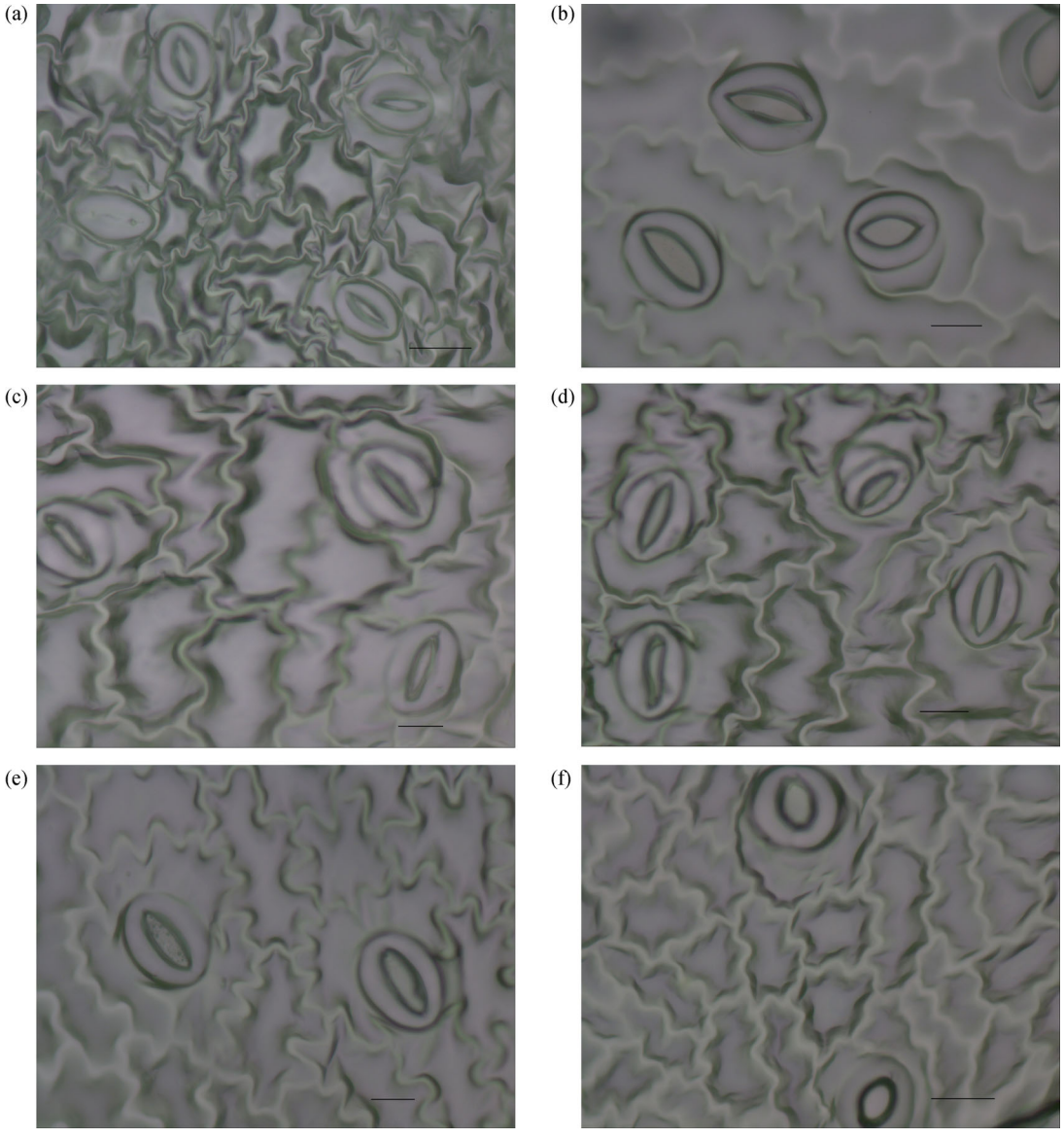

Fig. 3 Effects of red and blue LEDs on the leaf stomata of potato plantlets *in vitro*. Bar = 100 μm. (a) R; (b) 3RB; (c) RB; (d) R3B; (e) B; (f) W.

decreased the chrysanthemum leaf area. It is possible that the light spectrum from different light sources (for example, LEDs, fluorescent light and HPSL) effect plant leaf area differently. This could also be due to the response of different genotypes.

In our study, potato plantlets grown *in vitro* were photomixotrophic, which means that besides leaf photosynthesis, roots absorbed organic matter from the media to support growth and development. The greatest root activity was found in R followed by 3RB. This could explain why the greater fresh and dry weight were recorded in 3RB. Similar results were also observed in upland cotton plantlets grown *in vitro*[16].

Carbohydrate metabolism in plants can be regulated by light quality[37]. Our results were consistent with Li et al.[35], who demonstrated that red light contributes to the synthesis and accumulation of starch, and blue light facilitates synthesis and accumulation of soluble protein. The combination of red and blue LEDs is likely to induce the characteristics of both red and blue LEDs. Thus, in 3RB the plantlets had more carbohydrates and soluble proteins.

4.2 Effect of light treatments on stomata of potato plantlets grown *in vitro*

Stomata with guard cells surrounding each pore regulate their turgor to optimize the influx and efflux of gases and act as hydraulic valves on the surface of above-ground parts of plants[38]. Normal and functional stomata in potato

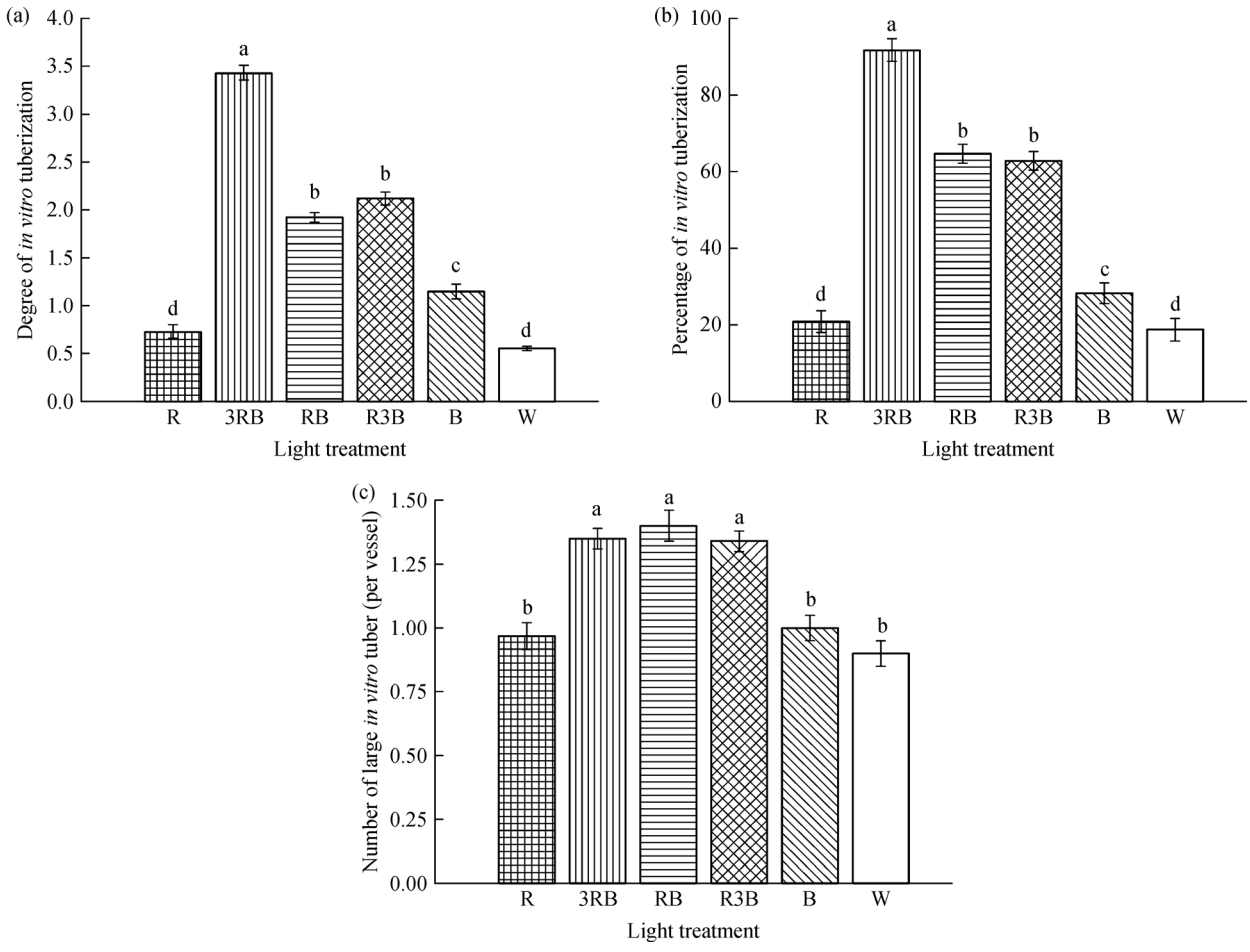

Fig. 4 Effects of red and blues LEDs on the parameters of microtubers. Different letters in each bar graph indicate significant differences ($P < 0.05$)

Table 3 Effects of red and blue LEDs on the yield of potato microtuber

Light treatment	Microtuber weight/mg	Microtuber number per vessel	Microtuber yield per vessel/mg
R	111.0a	1.8c	199.8
3RB	95.5b	5.7a	544.4
RB	89.4b	4.1b	364.9
R3B	84.1b	4.4b	370.0
B	80.1c	2.8c	224.3
W	112.5a	2.2c	247.5

Note: Different letters in the column indicate significant differences ($P < 0.05$).

plantlets grown *in vitro* were elliptical in shape, while dysfunctional stomata were round in shape[39]. The stomata in the present study performed functionally except in W, with its near circular stomata (Fig. 3). This is confirmed by PD/ED values (Table 2); the closer the value is to unity, the closer the shape is to round. Blue light is directly received by phototropins and activates a signal cascade which results in stomata opening rapidly with a red light background[30,33,37,38]. The percentage of blue light in W may have been too low, thus resulting in abnormal stomata. The stomata in R were the smallest (Fig. 3). This can be explained by the absence of blue light, because blue light is important for the development of plant stomata[40,41].The density and size of stomata were closely related to the *in vitro* environment in which plantlets are raised[39]. Potato plantlets with larger and fewer normal stomata have been found to grow vigorously[10,39]. A similar result was obtained in our study: the plantlets in 3RB had the lowest stomata density and largest stomata size and the highest health index. Meanwhile, Kim et al.[15]

and Li et al.[16] reported that plantlets treated with equal red and blue light had larger stomata, a lower stomata density and a faster growth rate.

4.3 Effect of light treatments on *in vitro* tuberization from single-node cuttings

Formation of potato microtubers is a complicated process controlled by many factors. The plant hormone, gibberellic acid (GA), is a key hormone that influences potato tuber induction and initiation[42]. Light spectral quality mediated the induction and development of microtubers by regulating hormone concentrations especially GA. High concentrations of GA prevents potato tuber induction and initiation. Previous research reported that blue light inhibited tuberization of day-neutral potato through increasing GA synthesis and accumulation[22]. This may explain the observed microtuberization in B. IAA is believed to enhance the sink capacity of plant organs[34,43]. The concentration of IAA in potato plantlets increased under red light[31]. Thus, the high IAA concentration may promote assimilates to flow into the tubers. That may be why R had a greater microtuber number (Table 3). It has also been reported that white light can increase potato microtuber fresh weight[23]. A similar result also occurred in W in our study. White light and the combined spectrum provided a range of light wavelengths. The development of microtubers may need a range of wavelengths in the light spectrum. That may be the explanation for why most large microtubers were found in the combined spectrum of red and blue LEDs (Fig. 4c; Table 3). Axillary bud development from single-node cuttings was closely associated with the degree and percentage of *in vitro* tuberization[29]. A higher degree of tuberization was correlated with earlier maturing potato cultivars and higher concentrations of inducing substances[44]. However, Ke Xin 1 is a mid to late maturing cultivar. The highest degree and percentage of *in vitro* tuberization was observed in 3RB, which indicated that this treatment may lead to the accumulation of more inducing substances.

5 Conclusions

The present study indicates that, of the treatments assessed, the optimal ratio of red to blue LEDs for *in vitro* growth of potato plantlets was 3:1. Moreover, this ratio also promoted *in vitro* tuberization giving the greatest tuberization degree and yield. White and red light contributed to increasing the fresh weight of microtubers but did not increase microtuber numbers. The combination of red and blue LEDs enhanced the number of large microtubers. The combined spectrum of 3RB is recommended for *in vitro* micropropagation of potato plantlets and microtuber production systems.

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Compliance with ethics guidelines Lili Chen, Xuzhang Xue, Yadong Yang, Fei Chen, Jie Zhao, Xiquan Wang, Alam Tariful Khan, and Yuegao Hu declare that they have no conflicts of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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