Addition of White Light to Monochromatic Red and Blue Lights Alters the Formation, Growth, and Dormancy of In Vitro-grown *Solanum tuberosum* L. Microtubers

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Abstract. Monochromatic light and wide-band white light both affect plant growth and development. However, the different effects between monochromatic light and addition white light to monochromatic light on the formation, growth, and dormancy of microtubers have not been fully explored. Therefore, we evaluated these effects using in vitro potatoes grown under pure blue and red lights and a combination of blue light and red light supplemented with white light, respectively. Current results suggested that light spectra influenced microtuber formation, growth, and dormancy by regulating potato plantlet morphogenesis, affecting the synthesis and transportation of photosynthetic metabolites, and altering the accumulation and distribution of biomass in various plant tissues. Monochromatic lights and the combined spectra had differing effects. For instance, monochromatic red light induced the growth of more microtubers, whereas addition white light to red light decreased number but increased weight of microtubers. Meanwhile, monochromatic blue light facilitated tuber growth, whereas addition white light to blue light decreased microtubers weight but increased microtuber number. In addition, composite lights of addition white light to monochromatic red and blue lights both extended the dormancy period, and monochromatic blue light shortened the dormancy period of microtubers >300 mg. Therefore, in microtuber agricultural production, specific light conditions may be applied at different growth stages of in vitro potatoes to increase the number of effective microtubers (>50 mg) and to satisfy storing requirement of seed microtubers.

Potato tuber is rich in starch, proteins, and other important nutrients, making potato (*Solanum tuberosum* L.) one of the most important staple food and vegetable crops (Abelenda et al., 2019). However, potato crops are susceptible to viral and fungal infections, resulting in yield loss and crop quality deterioration (Chen et al., 2018). The production of virus-free microtubers in sterile environments may reduce the risk of viral infection (Park et al., 2009). The morphogenesis of potato plantlets and the subsequent growth of microtubers are influenced by light (Halerman et al., 2016). However, the artificial light environments for the production of seed microtuber in the sterile environments have not been fully explored.

It has been reported that monochromatic red and blue lights regulate the formation and growth of microtuber (Aksenova et al., 1989; Chen et al., 2018; Fixen et al., 2012). However, studies have yielded inconsistent or contradictory results regarding the effects of red light and blue light on microtuber formation and growth, which may be attributed to differences in the variety, intensity, and peak wavelength of lights used, resulting in a puzzling application in microtuber production. The research has reported that white light is conducive to the growth of cattleya hybrid (Cybularz-Urban et al., 2007), lettuce (Hunter and Burritt, 2004), and spinach (Toledo and Ueda, 2003) and beneficial for photosynthesis because more light could penetrate through the canopy to lower leaves compared with red and blue lights (Park et al., 2013). In addition, white light-emitting diode (LED) can also increase the fresh weight of individual microtuber (Chen et al., 2018). However, little attention is paid to the studies of addition white light to monochromatic light on microtuber formation and growth, and further research is required.

The yield of microtubers is directly determined by the quality of potato plantlets,

and increased biomass in potato plantlets helps to induce high yield and quality of microtubers (Pelacho and Mingo-Castel, 1991). Furthermore, the carbohydrates produced by potato plantlets are an important material source for the growth of plantlets and microtubers. Tuberization is also known to be regulated by the availability of carbohydrates—in particular, sucrose, the transported form of sugar, required for starch synthesis (Abelenda et al., 2019). In addition, the distribution and transportation of sugar among leaves, stems, and microtubers regulates microtuber formation and growth. However, few studies have investigated this process in detail.

A shorter dormancy period is vital for potato production at sites restricted by short growing seasons (Eremeev et al., 2008), a longer dormancy is used to limit sprout growth during long-term storage of seed tubers for the subsequent season (Mölmann and Johansen, 2019). Therefore, the quantification of microtuber dormancy is necessary for potato production. Tuber dormancy is related to the size and dry matter content of the microtubers, thus determining storage behavior and sprouting potential (Haverkort et al., 1991). The environmental conditions to which the mother plant is exposed often affect seed dormancy (Zhao, 1995). When *Arabidopsis* is grown under a high ratio of red-to-far-red light (R/FR), seeds are able to germinate in the dark; however, when grown under a lower R/FR, seeds only germinate under light (Zhao, 1995). The preceding studies show that microtuber dormancy is related to the light environment of mother plant. However, how light spectra influence on microtuber dormancy period after harvesting has not been reported. The sugar content at harvest is one of the important parameters determining the maturity and sprouting vigor of seed potato (Rees and Morrell, 1990) and has a strong effect on crop performance (Donnelly et al., 2003). Whether light spectra influence on sugar content of microtuber and then change the dormancy period is not yet well known.

The preceding issues led us to hypothesize that the addition of white light to monochromatic red and blue lights could alter the formation, growth, and dormancy of microtubers, so we grew potato plantlets under four light spectra provided by LEDs to evaluate the formation, growth, and dormancy of potato microtubers. To assess the effects of the lights, the number, weight, and dormancy period of microtubers were observed. Biomass accumulation and distribution and carbohydrate levels of the potato plantlets and microtubers throughout microtuber production were also measured to assess the regulatory mechanism for microtuber formation, growth, and dormancy induced by different light spectra.

Materials and Methods

Plant materials and growth conditions. The experiment was conducted in a tissue culture chamber at Nanjing Agricultural

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University, Nanjing, China. *Solanum tuberosum* L. cv. Favorita is one of the major varieties in China and is sensitive to light variation and was therefore used in this experiment. Potato plantlets were cut into 1- to 1.5-cm segments with a piece leaf and then inoculated into Murashige and Skoog medium (8% sucrose, 0.9% agar) in tissue culture bottles (the internal diameter and height of the bottle are 63 mm and 85 mm, respectively). There were five stem segments in each bottle and 200 bottles in total. These in vitro shoots were irradiated with white fluorescent lamps for the first 3 d, and then 180 bottles selected from the 200 bottles were divided into four groups for exposure to light spectra provided by LEDs (Opt-run Biotechnology Co., Nanjing, China): blue light with a peak wavelength of 460 nm (B_{460}), composite light of white light and B_{460} ($B_{460}W$), red light with a peak wavelength of 660 nm (R_{660}), and composite light of white light and R_{660} ($R_{660}W$). The total photosynthetic photon flux density (PPFD) of all light treatments was set to $65 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, where the PPFD of white light accounted for $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the treatments of $B_{460}W$ and $R_{660}W$. Each treatment consisted of 45 bottles. The spectral distributions of white, blue, and red lights are shown in Fig. 1 and were measured by a spectroradiometer (OPT-2000; ABDPE Co., Beijing, China). The in vitro plantlets were grown under an 8-h light/16-h dark photoperiod, day/night temperatures of $22 \pm 2^\circ\text{C}/18 \pm 2^\circ\text{C}$, a relative humidity of $75 \pm 5\%$, and a CO_2 concentration identical to that of the outdoor atmosphere.

Morphology and dry weight determination. Potato plantlets from three bottles of each treatment were randomly selected to measure plant height, stem diameter, leaf number, and microtuber number using standard methods. Plant height was measured using a ruler from the main stem base to the top of the plantlets. Using a vernier calipers (601-01; Links Inc., Harbin, China) to measure the diameter of the internode between the lower two leaves as stem diameter. All leaves, stems, and microtubers were dried at 80°C to a constant weight, cooled to room temperature, and weighed with

an electronic balance (LS 120 A, Precisa Gravimetrics AG, Dietikon, Switzerland) as dry weight. After weighing, the dry samples were used for determination of the carbohydrate.

Carbohydrate measurements. The soluble sugar and starch content were measured using the anthrone method, and sucrose content was determined using the resorcinol method (Fairbairn, 1953; Martin et al., 2000).

Dormancy period determination. The microtubers induced by different lights were classified and then placed into darkness at a temperature of $22 \pm 2^\circ\text{C}$ after harvesting on day 90 of growth. Microtubers were checked every day for sprouts, and each group contained nine samples. Germination dates were recorded, and the elapsed time between harvest and germination was recorded as the dormancy period.

Statistical analysis. After 20, 40, 60, 80, and 90 d of light irradiation, we randomly took the bottles first and then randomly took the plantlets from nine bottles per treatment at 09:00 on each date for analysis and measurement. Each measurement was repeated three times. The effects of light spectra were compared by analysis of variance using SPSS, version 20.0 (IBM, Armonk, NY) followed by Duncan's test at $P < 0.05$ level. The correlations of between sugar contents and dormancy period of microtuber were determined on treatment means by Pearson's correlation coefficient. The figures were mapped using Origin 2016 software (OriginLab, Northampton, MA).

Results

Microtuber number, weight, and size distribution. As indicated in Table 1, the number of microtubers per bottle and per plantlet, and effective microtubers (those >50 mg) per bottle and per plantlet were higher under R_{660} than under $R_{660}W$, whereas those parameters were higher under $B_{460}W$ than under B_{460} . The effective microtuber rate under B_{460} was 100% and the highest. These results showed that red light favored a larger number of microtubers compared with blue light, and monochromatic red light seemed to be more effective in inducing microtubers than composite light of addition white light to monochromatic red light.

The microtuber weight per bottle, average microtuber weight, and effective average microtuber weight were higher under B_{460} than under $B_{460}W$, R_{660} , and $R_{660}W$. Furthermore, the weight of microtuber was higher under B_{460} than under $B_{460}W$. These indicators were the lowest under R_{660} . These results indicate that monochromatic blue light favored the growth of microtubers. Furthermore, B_{460} clearly helped induce larger tuber-shaped potatoes, whereas $B_{460}W$ reduced the percentage of microtubers >300 mg compared with B_{460} , confirming that blue light is important to induce large tuber-shaped potatoes and that the effect of blue light can be disturbed by white light. However, the percentages of microtubers >300 mg increased by $R_{660}W$

compared with R_{660} . Under $B_{460}W$, R_{660} , and $R_{660}W$, 11.2%, 4.8%, and 5.0% of microtubers were <50 mg, respectively. Conversely, virtually no microtubers <50 mg occurred under B_{460} .

Microtuber formation and growth changes. Microtubers were induced on day 60, and the number of microtubers was greatest under B_{460} , followed by R_{660} . Thus, the addition of white light to B_{460} and R_{660} reduced the rate of induction of microtubers on day 60. However, the number of microtubers and effective microtuber increased more rapidly under R_{660} , $R_{460}W$, and $B_{460}W$ than under B_{460} after day 60; the most microtubers were obtained under R_{660} ; and more effective microtubers were observed under $B_{460}W$ than under B_{460} on day 90 (Fig. 2A and B). The microtuber formation rate, rates of effective microtubers, microtuber weight per bottle, and average microtuber weight under B_{460} were consistently higher than under the other treatments from days 60 to 90 (Fig. 2C, D, and F), indicating that monochromatic blue light is beneficial to microtuber growth. The average microtuber weight increased more rapidly under $R_{660}W$ than under R_{660} from days 80 to 90 (Fig. 2E and F).

Plantlet morphology changes. As indicated in Fig. 3A, a large number of leaves developed under B_{460} and $B_{460}W$, fewer leaf numbers were induced by $R_{660}W$ than B_{460} and $B_{460}W$. However, there were no leaves on plantlets grown under R_{660} treatment throughout the growth period (Fig. 4), suggesting that blue light is vital for leaf formation of plantlets. Additionally, the leaves under $B_{460}W$ and $R_{660}W$ withered on day 60, but leaves under B_{460} did not wither until the day 90 (Fig. 3A), implying that leaves were more prone to senescence when adding white light to monochromatic blue light. Plant heights under R_{660} and $R_{660}W$ were always higher than those under B_{460} and $B_{460}W$ from days 20 to 60, respectively. Interestingly, the plant heights under $B_{460}W$ and $R_{660}W$ were consistently higher than those under B_{460} and R_{660} after 60 d, respectively (Fig. 3B). Differences in stem diameter of different light treatments were similar to those of plant height (Fig. 3C).

Biomass accumulation and distribution changes in different organs. The dry weights of leaves and stems were heavier under B_{460} than under $B_{460}W$ on day 20 but were less than weights from days 40 to 90 (Fig. 5A, C, E, G, and I). The dry weights of leaves and stems under R_{660} were not much different with $R_{660}W$ on day 20 but was consistently less than from days 40 to 90 (Fig. 5A, C, E, G, and I). The dry weight of stems observed under B_{460} was consistently lowest compared with other treatments from days 40 to 90 (Fig. 5C, E, G, I). Additionally, the average proportion of dry weight in microtubers under B_{460} , R_{660} , $B_{460}W$, and $R_{660}W$ on day 60 was 59.4%, 13.6%, 3.6%, and 3.1%, respectively (Fig. 5F), demonstrating that white light temporarily inhibits dry matter in the microtubers. On day 90, the proportion of dry weight in the microtubers under these treatments was 80.0, 75.2, 67.6, and 65.0%,

Fig. 1. Spectral distribution of white, blue, and red lights. PPFD = photosynthetic photon flux density.

Table 1. Effects of different light spectra on the number, weight, and size distribution of microtubers.

Parameters			Light treatments			
			B ₄₆₀	B ₄₆₀ W	R ₆₆₀	R ₆₆₀ W
MT no. (no./bottle)			5.8 b	6.7 ab	10.0 a	7.7 ab
EMT no. (no./bottle)			5.8 b	6.3 ab	9.4 a	7.3 ab
MT no. (no./plantlet)			1.2 b	1.4 ab	2.0 a	1.5 ab
EMT no. (no./plantlet)			1.2 ab	1.3 ab	1.9 a	1.5 ab
AMT weight (g)			0.54 a	0.39 b	0.23 c	0.34 b
EAMT weight (g)			0.54 a	0.43 ab	0.32 bc	0.35 abc
MT weight (g/bottle)			3.13 a	2.80 ab	2.30 bc	2.62 b
MT formation rate (%)			100.0 a	100.0 a	100.0 a	100.0 a
EMT rate (%)			100.0 a	90.5 a	95.2 a	95.7 a
MT weight	<0.05 g	No.	0.0 b	0.7 a	0.6 a	0.3 ab
		Rate (%)	0.0 b	11.2 a	4.8 ab	5.0 ab
	0.05–0.1 g	No.	0.0 b	0.7 b	2.3 a	1.0 ab
		Rate (%)	0.0 b	8.9 b	19.0 a	15.6 ab
	0.1–0.2 g	No.	0.6 b	0.3 b	3.9 a	2.0 ab
		Rate (%)	9.5 c	4.4 c	40.5 a	31.4 b
	0.2–0.3 g	No.	0.8 ab	1.3 ab	1.9 a	0.7 ab
		Rate (%)	14.3 ab	24.5 a	22.0 a	10.0 ab
	>0.3 g	No.	4.4 a	4.0 a	1.4 b	0.3 ab
		Rate (%)	76.2 a	71.2 a	13.7 b	57.8 ab

B₄₆₀ = 460-nm blue light; B₄₆₀W = the combined spectra of white light and 460-nm blue light; R₆₆₀ = 660-nm red light; R₆₆₀W = the combined spectra of white light and 660-nm red light; MT number = microtuber number; EMT number = effective microtuber number; AMT weight = average microtuber weight; EAMT weight = effective average microtuber weight; MT weight = microtuber weight per bottle; MT formation rate = microtuber formation rate; EMT rate = effective microtuber rate. Microtuber with a weight of >50 mg was defined as effective microtuber. Different lowercase letters in the same row indicate statistically significant differences ($P \leq 0.05$).

Fig. 2. Effects of different monochromatic lights on (A) microtuber (MT) number, (B) effective microtuber (EMT) number, (C) MT formation rate, (D) effective MT rate, (E) MT weight per bottle, and (F) average MT (AMT) weight during different growth stages. Microtuber with a weight of >50 mg was defined as effective microtuber. Error bars indicate standard deviations. B₄₆₀ = 460-nm blue light; B₄₆₀W = the combined spectra of white light and 460-nm blue light; R₆₆₀ = 660-nm red light; R₆₆₀W = the combined spectra of white light and 660-nm red light. k = 1000.

respectively (Fig. 5J). The proportion of dry weight in microtubers and the whole plant weight were consistently greater under B₄₆₀ than the other lights (Fig. 5F, H, and J). These results showed that pure blue light favored distribution of the dry matter in microtuber and that the composite lights with the addition white light to blue or red reserved a greater proportion of dry matter in stems and decreased the distribution rate of dry matter in microtubers.

Carbohydrate content changes in different organs. The data show that the starch content in leaves decreased sharply under B₄₆₀ from days 20 to 40 and then remained stable, whereas it increased sharply under B₄₆₀W and R₆₆₀W from days 40 to 60 (Fig. 6A). The starch content in stems under B₄₆₀ and R₆₆₀ followed a similar trend (Fig. 6D). Under B₄₆₀, B₄₆₀W, and R₆₆₀, the starch content in microtubers initially increased and then decreased from days 60 to 90, but

the pattern was reversed under R₆₆₀W (Fig. 6G). The highest starch content was under B₄₆₀ and the second highest was under R₆₆₀, whereas the addition of white light to red and blue lights reduced the starch content in microtubers on the 80th days (Fig. 6G).

The soluble sugar in leaves increased throughout the growing period under B₄₆₀, B₄₆₀W, and R₆₆₀W and was consistently lower under B₄₆₀ and B₄₆₀W than R₆₆₀W (Fig. 6B). The soluble sugar content in stems

Fig. 3. Effects of different light spectra on leaf number (A), plantlet height (B), and stem diameter (C) of potato plantlets during different growth stages. Error bars indicate standard deviations. B₄₆₀ = 460-nm blue light; B₄₆₀W = the combined spectra of white light and 460-nm blue light; R₆₆₀ = 660-nm red light; R₆₆₀W = the combined spectra of white light and 660-nm red light.

Fig. 4. Images of potato plantlets grown on day 20 under different light spectra. B₄₆₀ = 460-nm blue light; B₄₆₀W = the combined spectra of white light and 460-nm blue light; R₆₆₀ = 660-nm red light; R₆₆₀W = the combined spectra of white light and 660-nm red light. Scale bar is 1 cm.

decreased in all treatments from days 20 to 40 (Fig. 6E). The soluble sugar was consistently lower under B₄₆₀ than under R₆₆₀, and the addition of white light to red and blue lights reduced soluble sugar content in stems from days 20 to 40 (Fig. 6E). The soluble sugar content in microtubers initially decreased and then increased under R₆₆₀, B₄₆₀W, and R₆₆₀W and increased under B₄₆₀ from days 60 to 90 (Fig. 6H).

The sucrose content in leaves under B₄₆₀ reached minimum and maximum values at days 40 and 80, respectively. The sucrose content in leaves was always the highest under R₆₆₀W (Fig. 6C). In all treatments, the sucrose content in stems first decreased and then increased from days 20 to 90 and was significantly higher under B₄₆₀ than the other treatments on day 80 (Fig. 6F). The sucrose content in microtubers decreased from days 60 to 90 under B₄₆₀W and R₆₆₀W, but the starch content in microtubers was initially stable, then increased under B₄₆₀ and R₆₆₀ from days 80 to 90 (Fig. 6I).

Microtuber dormancy. As shown in Table 2, dormancy periods were influenced by light spectra. The dormancy periods under B₄₆₀ and R₆₆₀ were significantly shorter than under B₄₆₀W and R₆₆₀W, and the dormancy period of microtubers >300 mg under B₄₆₀ was significantly shorter compared with the other treatments, indicating that monochromatic 460-nm blue light decreased the dormancy period for large microtubers. As shown

in Table 3, correlation analysis revealed no significant relationship between average microtuber weight and dormancy period, and a correlation coefficient of $r = -0.206$ was recorded. However, there was a negative and highly significant correlation between microtuber starch content and dormancy period.

Discussion

Red light increases microtuber formation and blue light favors microtuber growth. Light wavelengths, as environmental signals, stimulate various plant life activity, and light quantity of different wavelengths, as major energy sources, affects photosynthesis and thus alters plant growth (Li et al., 2018; Seabrook, 2005). Red and green lights have been shown to promote microtuber growth (Mohamed Ali and Esmail, 2011). In the current study, R₆₆₀ facilitated microtuber formation in the middle and late growth stages (Fig. 2A and B). However, Aksenova et al. (1989) found that red light inhibited microtuber formation, which is inconsistent with the present study. The regulatory effects of light spectra on growth can be partly attributed to the regulation of phytohormone levels in plants (Kaufman, 1993). Endogenous hormones in potato are affected by light quality (Fixen et al., 2012). It has been reported that gibberellic acid (GA₃) and abscisic acid (ABA) are closely related to tuber formation and that GA₃ inhibits tuber

formation whereas ABA promotes it (Lovell and Booth, 2010; Roumeliotis et al., 2012). GA₃ concentrations decrease in leaves of grape grown under red light (Wang et al., 2017), and ABA concentrations induced by red light increased in hypocotyl of cucumber (Su et al., 2014). In the present study, red light facilitated microtuber formation, which might be correlated with decreased levels of GA₃ and increased ABA concentrations in plantlets. Interestingly, composite light with the addition of white light to R₆₆₀ slightly reduced the effect of R₆₆₀, which may be caused by a decrease in PPFD of red light (R₆₆₀ = 65 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; 45 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ < R₆₆₀W < 65 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and an increase of blue light in R₆₆₀W. Compared with R₆₆₀, B₄₆₀ induced significantly fewer microtubers (Table 1), indicating that blue light does not favor the induction of microtubers compared with red light. Composite light with the addition of white light to B₄₆₀ induced more microtubers than B₄₆₀ (Table 1), perhaps through a decrease in PPFD of blue light and an increase of red light in B₆₆₀W. Thus, red light is beneficial for the formation of microtubers, whereas blue light reverses the active effect.

Nevertheless, B₄₆₀ had beneficial effects on microtuber growth (Fig. 2E and F). Similar effects of blue light on microtuber growth were reported by Chang et al. (2009). Compared with R₆₆₀ treatment, which was characterized by no leaves on plantlets (Fig. 4), greater microtuber weights were observed under B₄₆₀, B₄₆₀W, and R₆₆₀W (Table 1). These results can likely be attributed to the large number of leaves grown under these lights, confirming that blue light is vital to form leaves of potato plantlets and red light has an adverse effect on leaf germination. Leaves are the main site of photosynthesis, and a large number of leaves has greater photosynthetic potential (Arp, 2010), creating more opportunities for plantlet metabolism and microtuber growth. Additionally, leaves withered under B₄₆₀W and R₆₆₀W on day 60, whereas they withered under B₄₆₀ on day 90 (Fig. 3A), which is closely related to research demonstrating senescence of leaves

Fig. 5. Effects of different light spectra on dry weight (DW) and proportion of DW in leaves, stems, and microtubers grown for (A, B) 20, (C, D) 40, (E, F) 60, (G, H) 80, and (I, J) 90 d. B₄₆₀ = 460-nm blue light; B₄₆₀W = the combined spectra of white light and 460-nm blue light; R₆₆₀ = 660-nm red light; R₆₆₀W = the combined spectra of white light and 660-nm red light.

induced by red light but delayed by blue light (Borras et al., 2003; Causin et al., 2006; Wang et al., 2010; Zhang and Zhou, 2013). The longer photosynthesis lasts, the more carbohydrate that is produced (Shaaf et al., 2019), and thus the higher microtuber weight per bottle was obtained under B₄₆₀ (Table 1). Likewise, composite light with the addition of white light to B₄₆₀ decreased microtuber weight, but the addition of white light to R₆₆₀ increased microtuber weight compared with R₆₆₀ treatment, which had the lowest microtuber weight (Table 1). This may result from the positive effect of blue light and the adverse effect of red light on microtuber weight. Therefore, we can conclude that blue light favors microtuber growth, and red light can reverse the positive effect of blue light on microtuber growth.

The accumulation and transportation of substances determine microtuber growth. Assimilates in leaves, as the main products of metabolism, are associated with increased photosynthetic activity and yield in sink organs (Kang et al., 2013; Malone et al., 2006). Blue light is essential for the development of photosynthetic organs (Wang et al., 2015). A similar result was observed in the present study (Figs. 3A and 4), which provides a good material foundation for the

growth of the microtubers grown under B₄₆₀. The transportation of carbohydrates depends on transportation distance and resistance (Lalonde et al., 2010). Shorter plant height and thicker stems with larger cross-sectional phloem area were found under blue light (Fig. 3B and C), suggesting that carbohydrates could be transported to tubers over a short distance and with low resistance and thus that rapid carbohydrate transportation facilitates microtuber growth under blue light. In addition, the distribution of biomass and metabolites determines the growth of various organs (Gautier et al., 1997; Moreira et al., 1997). It has reported that blue light causes a higher proportion of metabolites distributed in the stems of strawberry and sweet pepper plants (Xu et al., 2006; Yang et al., 2012). Similar effects caused by monochromatic blue light on sucrose in stems on days 60 to 90 was also observed (Fig. 6C). However, the lowest stem dry weight and the heaviest microtubers were observed under B₄₆₀ from days 40 to 90 (Fig. 5C, E, G, and I). Hence, we deduced that the higher concentration sucrose in stems was not used for growth of the stems themselves but is transferred into microtubers from the leaves (Fig. 6F and I). In contrast, the sucrose content in leaves

was consistently highest under R₆₆₀W (Fig. 6C), and the sucrose content in stems maintains relatively stable levels on days 20 to 80 (Fig. 6F); however, the sucrose, soluble sugar, and starch contents in microtubers continuously declined (Fig. 6I), indicating that the transportation of sucrose in this treatment is inhibited from source to sink. Furthermore, higher starch contents were observed in the leaves of B₄₆₀W and R₆₆₀W (Fig. 6A), suggesting that, except for the composite light of R₆₆₀W, the addition of white light to blue light also inhibits transportation of the carbohydrates, resulting in a portion of these metabolites being temporarily reserved as starch in leaves (Fig. 6A). Consequently, lighter microtubers under B₄₆₀W and R₆₆₀W compared with B₄₆₀ were observed (Table 1). Sugar content in leaves reflects the supply potential of carbohydrate (Farrar et al., 2000; Kang et al., 2013). As leaves formed, more carbohydrate was produced under R₆₆₀W (Fig. 6A–C), and the dry weight of leaves and stems irradiated with R₆₆₀W was greater than the dry weight of stems under R₆₆₀ from days 40 to 60 (Fig. 5C and E), ensuring sufficient supply potential of carbohydrate, and thus higher microtuber weight per bottle under R₆₆₀W than R₆₆₀ (Table 1).

Fig. 6. Effects of different light spectra on starch, soluble sugar, and sucrose contents in leaves (A–C), stems (D–F), and microtubers (G–I) at various growth stages. B₄₆₀ = 460-nm blue light; B₄₆₀W = the combined spectra of white light and 460-nm blue light; R₆₆₀ = 660-nm red light; R₆₆₀W = the combined spectra of white light and 660-nm red light. Error bars indicate standard deviations.

Table 2. Effects of different light spectra on microtuber dormancy period.

Light treatments	Dormancy period of microtuber (d)		
	100–200 mg	200–300 mg	>300 mg
B ₄₆₀	50.3 b	51.7 b	44.7 c
B ₄₆₀ W	71.7 a	70.3 a	70.3 a
R ₆₆₀	51.7 b	50.3 b	50.3 b
R ₆₆₀ W	71.7 a	70.3 a	70.3 a

B₄₆₀ = 460-nm blue light; B₄₆₀W = the combined spectra of white light and 460-nm blue light; R₆₆₀ = 660-nm red light; R₆₆₀W = the combined spectra of white light and 660-nm red light. Different lowercase letters in the same column indicate statistically significant differences ($P \leq 0.05$).

Addition of white light extends microtuber dormancy. Potato size was closely related with dry matter content, thus determining sprouting potential (Haverkort et al., 1991), while sprouting potential seem to be highly related to the maturity of the potato microtubers (Park et al., 2009). In the current study, the dormancy periods of microtubers >300 mg exposed to B₄₆₀ were

significantly shorter than under other treatments (Table 2). This confirmed that sprout production was correlated with dry matter and maturity of tubers (Wijewardana et al., 2018). In addition, sugar is an energy source for sprouting, and its content at harvest is an important factor in determining the maturity and sprouting vigor of seed potato (Rees and Morrell, 1990). In the

present study, the starch, soluble sugar, and sucrose contents in microtubers grown under B₄₆₀ and R₆₆₀ were higher at harvest than under B₄₆₀W and R₆₆₀W, so shorter dormancy periods were observed, which provides further evidence for previous results (Park et al., 2009). A highly significant negative correlation between sucrose and starch contents and dormancy period suggested that the addition of white light extended the dormancy period by decreasing sucrose and starch contents in microtubers (Table 3).

Conclusions

Light spectra influenced the formation, growth, and dormancy of microtubers by regulating potato plantlet morphogenesis, affecting the synthesis, transportation, and storage of photosynthetic metabolites. Red light facilitated the induction of more microtubers,

Table 3. Pearson correlation coefficients among sugar content in microtubers, average microtuber weight, and dormancy period after harvesting.

Parameters	Soluble sugar	Sucrose	Starch	Total sugar	AMT wt
Dormancy period	-0.435	-0.672*	-0.844**	-0.844**	-0.206

AMT weight = average microtuber weight. **, *Significant at $P = 0.01$ or 0.05 , respectively.

but blue light favored microtuber growth. Furthermore, blue light shortened the dormancy period of large microtubers, and addition of white light extended the dormancy period by decreasing sucrose and starch content in microtubers. Therefore, in potato agricultural production, specific light conditions may be applied at different growth stages of *in vitro* potatoes to increase the number of effective microtubers (>50 mg) and satisfy storage requirements of seed microtubers.

Literature Cited

- Abelenda, J.A., S. Bergonzi, M. Oortwijn, S. Sonnewald, M. Du, R.G. Visser, U. Sonnewald, and C.W. Bachem. 2019. Source-sink regulation is mediated by interaction of an FT homolog with a sweet protein in potato. *Curr. Biol.* 29:1178–1186.
- Aksenova, N.P., T.N. Konstantinova, and M.K. Chailakhyan. 1989. Morphogenetic effect of blue and red light in the illumination of underground and above-ground potato organs in *in vitro* cultivation. *Doklady Akademicheskikh Nauk SSSR*. 305:508–512.
- Arp, W.J. 2010. Effects of source-sink relations on photosynthetic acclimation to elevated CO_2 . *Plant Cell Environ.* 14:869–875.
- Borras, L., G.A. Maddonni, and M.E. Otegui. 2003. Leaf senescence in maize hybrids: Plant population, row spacing and kernel set effects. *Field Crops Res.* 82:13–26.
- Causin, H.F., R.N. Jauregui, and A.J. Barneix. 2006. The effect of light spectral quality on leaf senescence and oxidative stress in wheat. *Plant Sci.* 171:24–33.
- Chang, H., Y.P. Wang, D. Wang, and F. Zhang. 2009. Effects of light quality on microtuber induction of *Solanum tuberosum*. *Chin. J. Appl. Ecol.* 20:1891–1895.
- Chen, L., X.Z. Xue, Y.D. Yang, F. Chen, J. Zhao, X.Q. Wang, T.K. Alam, and Y.G. Hu. 2018. Effects of red and blue LEDs on *in vitro* growth and microtuberization of potato single-node cuttings. *Front Agr. Sci. Eng.* 5:197–205.
- Cybularz-Urbán, T., E. Hanus-Fajerska, and A. Swiderski. 2007. Effect of light wavelength on *in vitro* organogenesis of a *Cattleya* hybrid. *Acta Biol. Cracov. Ser. Bot.* 49:113–118.
- Donnelly, D.J., W.K. Coleman, and S.E. Coleman. 2003. Potato microtuber production and performance. *Amer. J. Potato Res.* 2:103–115.
- Eremeev, V., A. Lohmus, P. Laeaeniste, J. Joudu, L. Talgre, and E. Lauringson. 2008. The influence of thermal shock and pre-sprouting of seed potatoes on formation of some yield structure elements. *Acta Agr. Scand. B Soil Plant Sci.* 58:35–42.
- Fairbairn, N.J. 1953. A modified anthrone reagent. *Chem. Ind.* 4:86.
- Farrar, J., C. Pollock, and J. Gallagher. 2000. Sucrose and the integration of metabolism in vascular plants. *Plant Sci.* 154:1–11.
- Fixen, K.R., S.C. Thomas, and C.B.S. Tong. 2012. Blue light inhibition of tuberization in a day-neutral potato. *J. Plant Growth Regul.* 31:342–350.
- Gautier, H., C. Varlet-Grancher, and N. Baudry. 1997. Effects of blue light on the vertical colonization of space by white clover and their consequences for dry matter distribution. *Ann. Bot.* 80:665–671.
- Halterman, D., J. Guenther, S. Collinge, N. Butler, and D. Douches. 2016. Biotech potatoes in the 21st century: 20 years since the first biotech potato. *Amer. J. Potato Res.* 93:1–20.
- Haverkort, A.J., M. Waart, and M. Vande. 1991. Field performance of potato microtubers as propagation material. *Potato Res.* 34:353–364.
- Hunter, D.C. and D.J. Burritt. 2004. Light quality influences adventitious shoot production from cotyledon explants of lettuce (*Lactuca sativa* L.). *In Vitro Cell. Dev. Biol. Anim.* 40:215–220.
- Kang, G.Z., G.Q. Liu, X.Q. Peng, L.T. Wei, C.Y. Wang, Y.J. Zhu, Y. Ma, Y.M. Jiang, and T.C. Guo. 2013. Increasing the starch content and grain weight of common wheat by overexpression of the cytosolic AGPase large subunit gene. *Plant Physiol. Biochem.* 73:93–98.
- Kaufman, L.S. 1993. Transduction of blue-light signals. *Plant Physiol.* 102:333.
- Lalonde, S., M. Tegeder, M. Throne-Holst, W.B. Frommer, and J.W. Patrick. 2010. Phloem loading and unloading of sugars and amino acids. *Plant Cell Environ.* 26:37–56.
- Li, R.N., W.W. Huang, X.X. Wang, X.Y. Liu, and Z.G. Xu. 2018. Effects of yellow, green, and different blue spectra on growth of potato plantlets *in vitro*. *HortScience* 53:541–546.
- Lovell, P.H. and A. Booth. 2010. Effects of GA on growth, tuber formation and carbohydrate distribution in *Solanum tuberosum*. *New Phytol.* 66:525–537.
- Malone, J.G., V. Mittova, R.G. Ratcliffe, and N.J. Kruger. 2006. The response of carbohydrate metabolism in potato tubers to low temperature. *Plant Cell Physiol.* 47:1309–1322.
- Martin, A.B., Y. Cuadrado, H. Guerra, P. Gallego, O. Hita, L. Martin, A. Dorado, and N. Villalobos. 2000. Differences in the contents of total sugars, reducing sugars, starch and sucrose in embryogenic and nonembryogenic calli from *Medicago arborea* L. *Plant Sci.* 154:143–151.
- Mohamed Ali, F.H. and M.A. Esmail. 2011. The impact of *in vitro* light quality on potato microtuberization from single-node cuttings. *Acta Hort.* 923:73–77.
- Mölmann, J.A. and T.J. Johansen. 2019. Sprout growth inhibition and photomorphogenic development of potato seed tubers (*Solanum tuberosum* L.) under different LED light colours. *Potato Res.* 1–17.
- Moreira, D.A., M.H. Silva, and P.C. Debergh. 1997. The effect of light quality on the morphogenesis of *in vitro* cultures of *Azorella vidalii* (Wats.) Feer. *Plant Cell Tissue Organ Cult.* 51:187–193.
- Park, S.W., J.H. Jeon, H.S. Kim, S.J. Hong, C. Aswath, and H. Joong. 2009. The effect of size and quality of potato microtubers on quality of seed potatoes in the cultivar ‘Superior’. *Scientia Hort.* 120:127–129.
- Park, Y.G., H.J. Oh, and B.R. Jeong. 2013. Growth and anthocyanin concentration of *perilla frutescens* var. *acuta* Kudo as affected by light source and DIF under controlled environment. *Hort. Environ. Biotechnol.* 54:103–108.
- Pelacho, A.M. and A.M. Mingo-Castel. 1991. Jasmonic acid induces tuberization of potato stolons cultured *in vitro*. *Plant Physiol.* 97:1253–1255.
- Rees, T. and S. Morrell. 1990. Carbohydrate metabolism in developing potatoes. *Amer. Potato J.* 67:835–847.
- Roumeliotis, E., R.G. Visser, and C.W. Bachem. 2012. A crosstalk of auxin and GA during tuber development. *Plant Signal. Behav.* 7:1360–1363.
- Seabrook, J.E.A. 2005. Light effects on the growth and morphogenesis of potato (*Solanum tuberosum*) *in vitro*: A review. *Amer. J. Potato Res.* 82:353–367.
- Shaaf, S., G. Bretani, A. Biswas, I.M. Fontana, and L. Rossini. 2019. Genetics of barley tiller and leaf development. *J. Integr. Plant Biol.* 61:226–256.
- Su, N., Q. Wu, and J. Cui. 2014. Effects of monochromatic LED light quality on endogenous hormone content in leaves and hypocotyl of *Cucumis sativus* L. *Chin. J. Appl. Environ. Biol.* 20:45–49.
- Toledo, M.E.A. and Y. Ueda. 2003. L-ascorbic acid metabolism in spinach during postharvest storage in light and dark. *Postharvest Biol. Technol.* 1:47–57.
- Wang, H.B., S. Wang, X.T. Wang, X.B. Shi, B.L. Wang, X.C. Zheng, Z.Q. Wang, and F.Z. Liu. 2017. Effects of light quality on leaf senescence and endogenous hormones content in grapevine under protected cultivation. *Ch. J. Appl. Ecol.* 11:3535–3543.
- Wang, H., Y.P. Jiang, K. Shi, Y.H. Zhou, and J.Q. Yu. 2010. Effects of light quality on leaf senescence and activities of antioxidant enzymes in cucumber plants. *Sci. Agr. Sinica* 43:529–534.
- Wang, X.Y., X.M. Xu, and J. Cui. 2015. The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of *Cucumis sativus* grown under weak light. *Photosynthetica* 53:213–222.
- Wijewardana, C., K.R. Reddy, M.W. Shankle, S. Meyers, and G. Wei. 2018. Low and high-temperature effects on sweet potato storage root initiation and early transplant establishment. *Scientia Hort.* 240:38–48.
- Xu, K., Y.P. Guo, S.L. Zhang, W.S. Dai, and Q.G. Fu. 2006. Effects of different light quality on growth and development of fengxiang strawberry. *J. Fruit Sci.* 23:818–824.
- Yang, Z.Q., X. Zhao, T.X. Su, Z.L. Zhou, K. Zhu, and X.D. Peng. 2012. Effects of light quality on dry matter production and distribution index of sweet pepper in greenhouse. *J. Ecol.* 31:1117–1122.
- Zhang, H.S. and C.J. Zhou. 2013. Signal transduction in leaf senescence. *Plant Mol. Biol.* 82:539–545.
- Zhao, D.L. 1995. Effects of light on seed dormancy and germination. *Chin. J. Biol.* 7:24–25.