Regulation of Different Lights on Energy Acquisitions, Microtuber Formation, and Growth of In Vitro-Grown Solanum tuberosum L.

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Abstract: Limited research has been conducted on the regulation of light quality on heterotrophy in vitro-grown potato plantlets. Here, we investigated the effect of light quality on photosynthetic and heterotrophic abilities as well as microtuber formation and growth of potato plantlets (Solanum tuberosum L. cv. Shepody). Potato plantlets pre-cultivated under white light for 30 days were then transferred to grow under blue (B), green (G), red (R), yellow (Y), and white (W) lights, and parameters including dry weight, photosynthetic pigment, medium solute consumption, δ13C value, root activity, and sucrose transport (SUT) gene expression of these plantlets were measured. The results showed that B, G, and W were conducive to the rapid induction of microtubers, while R, and especially Y, delayed microtuber formation. Higher photosynthetic ability was observed in the W treatment, whereas the opposite effect was seen in the monochromatic light treatments. Microtuber growth was primarily dependent on heterotrophy, and B was conducive to microtuber growth. The delay in microtuber formation was related to the high expression of StSUT4 in the root, and better microtuber growth was associated with higher root activity, more medium solute consumption, and a higher expression level of StSUT1 in the roots.

Keywords: energy acquisition; formation; growth; microtuber; in vitro-grown potato

1. Introduction

Potato (Solanum tuberosum L.) is a nutrient-rich food crop commonly grown worldwide in more than 150 countries and regions [1]. Being a homoyzogous tetraploid with a highly heterozygous genome, potato exhibits extreme instability in agronomic traits during sexual reproduction [2]. Recently, Chinese scientists have successfully bred the first generation of highly homozygous diploid potato inbred lines and the hybrid potato cultivar ‘Youshu1’ [3], opening up new avenues for germplasm innovation in potatoes. Currently, the main cultivated varieties in agricultural production are still homozygous tetraploid potatoes, propagated through asexual reproduction for seed potato production. However, potato tubers are susceptible to viral infections, leading to symptoms such as declining plant vigor, reduced tuber size, and deformities, significantly impacting potato production [4]. The production of virus-free microtubers in sterile environments may reduce the risk of viral infection, and accelerated breeding cycles may help avoid viral re-infection due to adverse production conditions [5]. Virus-free microtubers in sterile environments with a lower CO2 environment need to obtain energy for growth and development through a combination of photosynthesis and the direct uptake of exogenous sugars from a culture medium, thereby showcasing a mixotrophic mode, which is distinct from plants grown in open environments [6]. Coordinating light exposure and exogenous...
sugar supply is crucial to ensure optimal plantlet growth and efficient energy utilization in the production of virus-free microtubers.

Light, as a vital environmental factor and energy source, governs plant growth throughout the lifecycle [7]. In potato microtuber production, existing research indicates that the successful growth of plantlets and microtubers is closely associated with light [8]. Light quality is one of the important characteristics of the light environment for plants. In response to changes in light environments, plants can sensitively perceive changes in light quality through photoreceptors, and then adjust their morphology and metabolic activities [9]. There have been some reports on the regulatory effects of light quality on potato plantlets, and plantlets grown under monochromatic red light typically exhibit longer and more stem internodes, slender plant stature, abundant yet small leaves [10], and abnormal chloroplast development [11], resembling characteristics of the shade avoidance response. Plantlets grown under monochromatic green and yellow lights also show similar morphological features [12]. Li et al. [5] discovered that potato plantlets cultivated under monochromatic red, green, and yellow lights throughout the entire growth period developed extremely small leaves or even no leaves. Conversely, blue light demonstrated comparable benefits to white light in stimulating the formation and growth of microtubers. Well-developed chloroplasts and a higher photosynthetic rate of potato plantlets under blue and white lights were also observed [13]. Chen et al. [14] concluded that an increased leaf area of potato plantlets under blue and white lights correlated with elevated expression of genes involved in expansin, xyloglucan glucosyltransferase, actin, and tubulin. Relatively consistent results on the influence of light quality on the growth and morphology of potato plantlets have been obtained, while there are various inconsistent results regarding the effects of light quality on microtuber formation and growth. Chen et al. [15] and Jiang et al. [10] observed that blue light was more conducive to microtuber formation than red light, whereas Li et al. [5] indicated that red and green lights were more favorable for the induction of microtubers compared to blue and yellow lights, with yellow light being unfavorable for both the induction and growth of microtubers. Shan et al. [16] found that the induction of microtubers by light quality was genotype-specific. Nevertheless, many studies obtained the same results that blue light was more beneficial for tuber growth [5,10,15,17].

The culture medium is essential for maintaining the normal growth of plantlet. In addition to providing water and minerals, it is also the main carbon source for plantlet growth [18]. Exogenous sugars can be incorporated into metabolism and synthesized into amino acids, fatty acids, or other metabolites [18,19], and their levels in a culture medium are positively correlated with osmotic pressure. A certain level of osmotic pressure in a culture medium is necessary for microtuber formation and growth [20,21]. A previous study showed that high concentrations of sucrose in the culture medium were necessary for microtuber formation [22], while also being a major factor in inhibiting photosynthesis of potato plantlets [23]. An 8% sucrose concentration is considered an appropriate sugar concentration for potato microtuber formation [5,24]. In many heterotrophic plants, sucrose and other nutrients are transported into cells through phloem sap [25,26], and this process requires a sucrose transporter (SUT) [27,28]. Also, increasing evidence suggests that the transport of metabolites from the apoplast to plant vacuoles is not always coordinated solely through solute movement via a single channel or transporter, and in some cases, alternative or parallel transport mechanisms exist [29–31]. In potato leaves, the highly expressed SISUT1 is an important characteristic for the efficient transport of sucrose from the leaves to the outside; furthermore, when the expression of SISUT4 in potatoes is inhibited, it promotes early tuberization and increases tuber yield [32].

Since the application of LED technology, significant progress has been made in understanding the regulatory effects of light quality on the formation and growth of virus-free microtubers. Although research results show diversity due to variations in cultivars and cultivation conditions, they have provided valuable foundational data for potato production. The importance of sucrose in the culture medium has been evaluated in terms of
sugar concentration, osmotic pressure, transport, and utilization. However, precise assessment of how much sugar in the culture medium is utilized by potato plantlets is rarely conducted. There are currently no reports on the heterotrophic ability of potato plantlets to utilize sugars in the culture medium under different lights. However, investigating the photosynthetic and heterotrophic abilities of potato plantlets under various light conditions holds immense importance in the quest to optimize the combination of light and sugar environments for the production of virus-free microtubers.

Based on the issues discussed above, we measured and analyzed the relevant indicators related to photoautotrophy, heterotrophy, and microtuber formation and growth of in vitro-grown potato plantlets under different light quality conditions, aiming to reveal the mechanisms by which light quality regulates microtuber formation, position, and the growth of in vitro-grown potato plants from an energy acquisition perspective. The current results can provide a reference for the selection of the supplemental light spectrum and the input of exogenous sugar in the production of microtubers.

2. Materials and Methods
2.1. Plant Materials and Growth Conditions

The experiment was conducted in a growth chamber, where the temperature, humidity, and light conditions were artificially controlled. The experiment was conducted using the Shepody variety (Solanum tuberosum L. cv. Shepody), which was developed in Canada in 1980 and is primarily used for making French fries. The variety is characterized by rapid degeneration, high susceptibility to tuber diseases, and a reliance on potato in vitro growth and virus-free breeding. Virus-free potato seedlings were cut into 1–1.5 cm segments with a leaf per piece and inoculated into tubes containing 10 mL of MS medium (Hope Bio-Technology Co., Qingdao, China) supplemented with 8% sucrose and 0.8% agar, and the pH value of the culture medium was adjusted to 5.8. The tubes had a transparent design with an internal diameter of 22 mm and a height of 96 mm. After placing the segments on the Murashige and Skoog medium, the tubes were promptly sealed with a layer of aseptic membranes and a layer of cling film. Subsequently, the sealed tubes were incubated in a W environment for 3 days with an intensity of 10 ± 5 μmol m⁻² s⁻¹. After a three-day acclimation to low-light conditions, the plantlets were transferred to a W environment with an intensity of 75 ± 5 μmol m⁻² s⁻¹ for a culture period of 30 days. The plantlets were grown under a photoperiod of 16 h of light and 8 h of darkness, with day and night temperatures maintained at 22 ± 1 °C/20 ± 1 °C, respectively. The relative humidity was set at 80 ± 5%, and the CO₂ concentration in the growth chamber matched that of the outdoor atmosphere.

After 30 days of plantlet cultivation, the cling film was removed from the tubes. Subsequently, the plantlets were randomly divided into five groups to be exposed to monochromatic light provided by LEDs (Opt-run Biotechnology Co., Nanjing, China) at four different wavelengths for 8 h a day: blue light with a peak wavelength of 460 nm (B), green light with a peak wavelength of 520 nm (G), yellow light with a peak wavelength of 590 nm (Y), and red light with a peak wavelength of 660 nm (R). The remaining group was exposed to W and served as the control group. The light intensity of all treatments was the same at 90 ± 5 μmol m⁻² s⁻¹. The growth chambers maintained a relative humidity of 60 ± 5%. There was airflow in the cultivation container at a rate of 1.6 L/d. The day temperature/dark temperature during the light period was 20 ± 1 °C/18 ± 1 °C. The experiment was repeated three times.

2.2. Measurements of Morphology and Dry Weight

During the 0–60 d, 10 randomly selected samples were taken to count leaf numbers and measure leaf area. Leaf number only included the number of mature, fully expanded leaves. Leaf areas were measured using Image J software (CC 2017, NIH, LOCI, University of Wisconsin) after taking photos of plantlets. Dry weights of the tuber, root, shoot, and
whole plantlets were measured for 10 plants under each growth condition after drying at 85 °C in an oven to a constant weight using an electronic balance (AUY 120, SHIMADZU, Taguig, Philippines). The increase in dry weight of different organs was calculated as the difference in dry weight of different organs between the end and the beginning of each designated period (light treatment period including 0–15 d, 15–30 d, 30–45 d, and 45–60 d). The induction rate of microtubers was calculated by dividing the number of plantlets with microtubers by the total number of plantlets. The distance from the site of root initiation to the position of the microtuber was recorded as the microtuber position.

2.3. Measurement of Solute Consumption in the Culture Medium

We utilized the method of drying and weighing to measure solute consumption by the plantlets in the culture medium. By comparing the measurements with theoretical calculations, the error rate of solute consumption measured using this method was found to be less than 5.2% (Table S1). Specifically, the culture medium was dried at 70 °C until it reached a constant weight, weighed using an electronic balance (AUY 120, SHIMADZU, Philippines). The solute consumption in the culture medium (MS-consumption) was calculated as the difference in dry weight of the culture medium between the end and the beginning of each designated period (light treatment periods including 0–15 d, 15–30 d, 30–45 d, and 45–60 d).

2.4. Measurements of Root Activity and Photosynthetic Pigment

After 30 days of different light treatments, 0.05 g root tips of three plants were randomly collected under different light treatments to measure root activity using the triphenyl-tetrazoliumchloride method as described by Li et al. [33]. Chlorophyll (Chl) and carotenoids (Car) were extracted from the third fully expanded leaf of five plants with a mixture containing acetone, ethanol (Lingfeng Chemical Reagent Co., Shanghai, China), and water (4.5:4.5:1, v/v/v). The Chl and Car contents were determined using the method of Ma et al. [34].

2.5. Evaluation of Photoautotrophic and Heterotrophic Contributions of Plantlets

After 60 days of exposure to different lights, six plants from each treatment were selected and dried at 85 °C until they reached a constant weight. The dried plants were then ground and sieved through a 100-mesh screen to determine the δ^{13}C values of the plant samples. The measurement method for the δ^{13}C value of plantlets followed the protocol used by Gu et al. [35] using an Isoprime100 stable isotope ratio mass spectrometer (Isoprime, Cheadle Hulme, UK) and vario MICRO cube elemental analyzer (Elementar, Langenselbold, Germany). We used the formula of Equation (1) reported by Wolf et al. [36] and Yakir et al. [37] to estimate the contribution rates of photosynthesis and heterotrophy. The δ^{13}C value for photoautotrophic plants is −26‰, which is consistent with C3 photosynthesis in a normal atmosphere. The δ^{13}C value for the sucrose standard is −10.4‰, as provided by the International Atomic Energy Agency.

\[ P_N = \frac{-10.4(1 - P_N) - \delta^{13}C_{(sample)}}{26} \]  

(1)

In the equation, \( P_N \) represents the proportion contributed to the δ^{13}C value of the sample by photosynthesis, while \( 1 - P_N \) represents the proportion contributed to the δ^{13}C value of the sample by the carbon source in the medium.

2.6. Quantitative Real-Time Polymerase Chain Reaction

After 15 days of different light treatments, roots and leaves of the plants grown under different lights were sampled for RT-qPCR analysis. Total RNA was extracted using the RNA Easy Fast Plant Tissue RNA Extraction Kit (DP452, Tiangen Biotech Co., Ltd., Beijing, China). The concentration and quality of the total RNA were assessed using a Nano
Spectrophotometer (NanoDrop one, Thermo Fisher Scientific, Waltham, WA, USA). Subsequently, a one-step method was utilized to eliminate genomic DNA from the total RNA and perform reverse transcription into cDNA with the KR118 kit (Tiangen Biotech Co., Ltd., Beijing, China). RT-qPCR was carried out using the FastReal Fast Fluorescent Quantitative PCR Kit (FP217, Tiangen Biotech Co., Ltd., Beijing, China) on the StepOnePlus Real-Time PCR System (Thermo Fisher). The primer sequences of the target and reference genes used in the RT-qPCR are provided in Table S2. The relative gene expression levels in roots and leaves were calculated using the $2^{-\Delta\Delta CT}$ method. Each experiment was conducted with three biological replicates and three technical replicates.

2.7. Statistical Analysis

The effects of different lights were compared by analysis of variance using Statistical Product and Service Solutions, version 20.0 (IBM, New York, NY, USA) followed by Duncan’s test at $p \leq 0.05$ level. The correlations between the dry weight of the root, stem, leaf, whole plant of the plantlets, and solute consumption were determined on treatment means by Pearson’s correlation analysis.

3. Results

3.1. Microtuber and Leaf Growths of Potato Plantlets

At 30 d under different lights, the phenotype of the plantlets displayed that the W-treated plants were the shortest with well-developed root systems and leaf development, and microtuber formation occurred in plantlets grown under B, G, and W, but not under Y and R. By day 45, microtuber formation was observed in all treatments, with no significant difference in the number of tubers. However, the microtuber weight in the B treatment was significantly greater than in the other treatments (Figure 1a–g). Interestingly, the location of microtubers was different, and the microtubers in the B, Y, and R treatments were closer to the roots, while the microtubers in the G and W treatments were located further away from the roots (Figure 1c). Compared to W, plantlets under all monochromatic lights had fewer numbers of leaves, with treatment B exhibiting a faster leaf aging rate, leading to a significant decrease in effective leaf area during the later stages. Within the first 15 days of light treatments, the largest leaf area was observed in the G treatment, while a moderate size of leaf area was observed in the treatments of W, B, and R, and a smaller leaf area was observed in the Y treatment. However, after 30 days of light treatments, the leaf areas of plantlets under monochromatic lights were all smaller than those of plantlets under W (Figures 1h and S1).
Figure 1. Microtuber and leaf growths of potato plantlets grown under different lights. Images of potato plantlets grown under different lights for 0 d (a), 30 d (b), and 60 d (c); number (d), and dry weight (e) of microtubers per plantlet grown under different lights at 60 d; microtuber position (f); induction rate per potato plantlet grown under different lights from 15 to 60 d (g); and leaf area (h) per plantlet grown under different lights from 0 to 60 d. Different lowercase letters on the bars indicate statistically significant differences (p ≤ 0.05). Scale bar is 2 cm in (a–c). B, blue light; G, green light; Y, yellow light; R, red light; and W, white light.

3.2. Increase in Dry Weight of Different Organs in Potato Plantlets

During the initial 0–15 days after light treatment, no tuber formation occurred in the potato plantlets under all treatments. Compared to W, the dry weight of plantlets under B significantly decreased, while there were no significant changes in the other treatments. Under B, more dry matter was allocated to the leaves, whereas W promoted both leaf and root development. In contrast, the two treatments of Y and R allocated more resources to stem growth (Figures 2a–f and S2).

By day 30, there were no significant differences in plantlet and root dry weight among the treatments. Numerically, the total dry weight of the plantlets was highest under G and lowest under Y. The dry weight of leaves in the W and R treatments was significantly greater than that of the Y treatment. During 15–30 d, microtubers began to occur in all treatments, but the dry weight of microtubers of the G and W treatments was significantly greater than that of the Y and R treatments, and the proportion of stem dry weight of the Y and R treatments remained significantly greater than in the other three treatments. Compared to day 15, by day 30, the leaf dry weight in treatments B, G, and Y was decreasing, while the leaf dry weight in treatments R and W was still increasing, especially in treatment R. The root and stem dry weight in treatment W were both decreasing (Figures 2a–f and S2).
On day 45 of different light treatments, the dry weight of plantlets under W was still the greatest, significantly greater than that of plants under Y. The dry weight of microtubers in the Y treatment was still smaller than in other treatments, but the dry weight of microtubers in the R treatment had caught up with that of the others. Compared to day 30, the dry weight of microtubers in all treatments had increased in size. The stem, leaf, and root dry weights in the B, Y, and R treatments were decreasing, but the stem dry weight in Y treatment and the leaf dry weight in W treatment were still increasing (Figures 2a–f and S2).

On day 60 of different light treatments, the dry weight of plantlets under the B treatment became the largest, significantly greater than that of the G, Y, and W treatments. Compared to day 45, the proportion of stem and leaf dry weights in all treatments was decreasing in relation to the whole plant, and especially the stem dry weight in the Y treatment shows a significant decrease. Accordingly, the microtuber dry weight was still increasing, with the highest increase observed in the Y treatment and a lesser increase in the W and G treatments. At this stage, it was observed that the root dry weights of the B, G, Y, and R treatments began to increase, whereas the root dry weight in the W treatment remained unchanged (Figures 2a–f and S2).

Figure 2. The effects of different lights on the dry matter accumulation in various organs of potato plantlets. Dry weights of leaf, stem, root, and tuber under B (a), G (b), Y (c), R (d), and W (e), and dry weight distribution ratio of different organs (f) at different growth days. B, blue light; G, green light; Y, yellow light; R, red light; and W, white light.
3.3. Photosynthetic Pigment Content in Leaves

During the 0–60 growth days, Chl and Car contents in the leaves of plantlets under different lights showed an initial increase followed by a decreasing trend (Figure 3a,b). Specifically, under B and G, the Chl in leaves decreased rapidly during the 30–60 growth days and 45–60 growth days, respectively, and the changes in Chl and Car contents in the R and W treatments were relatively stable, especially in the W treatment. The differences in pigment content were mainly observed in the initial and later stages of growth. Compared to other treatments, the W and R treatments exhibited higher Chl content, while the B treatment had the lowest Chl content. The Car content was lower in the G treatment compared to the other treatments. Compared to the Chl a/b values before treatment, the Chl a/b values in all treatments significantly decreased, but showed signs of recovery in the later stages. In the early stages after treatment, the G and Y treatments had higher Chl a/b values, while in the later stages after treatment, the B and G treatments had higher Chl a/b values (Figure 3c). Except for the B treatment, where there was a rapid decline in the Chl/Car ratio on the 30th day of treatment, the other treatments showed a very slow declining trend in the Chl/Car ratio (Figure 3d).

![Figure 3](image)

**Figure 3.** Photosynthetic pigments of potato plantlets grown under different lights. The contents of Chl (a + b) (a), Car (b), Chl a/b (c), and Chl/Car (d) in the leaves of potato plantlets grown under different lights during growth periods. Chl, chlorophyll; Car, carotenoids. B, blue light; G, green light; Y, yellow light; R, red light; and W, white light.

3.4. MS-Consumption and Root Activity of Plantlets

We calculated MS-consumption to assess the heterotrophic ability of plantlets under different lights. During the 0–15 d period after light treatment, the data showed that there was no significant difference in MS-consumption among different light treatments, where the B treatment exhibited the lowest MS-consumption numerically. During the 15–30 d period after light treatment, the MS-consumption was the highest in the B treatment, followed by the G treatment, slightly lower in the R treatment, and the lowest in the Y and W treatments, approaching zero. During the 30–45 d period after light treatment, there was no significant difference in MS-consumption among the different light treatments.
During the 45–60 d period after light treatment, MS-consumption was highest in the Y treatment, significantly greater than the G and W treatments, but not significantly different from the B and R treatments. Differently, MS-consumption in the G treatment approached zero, while in the W treatment, it was significantly less than zero (Figure 4a). Overall, during the 0–60 d period, the highest MS-consumption was observed in the B treatment, while the lowest MS-consumption was in the W treatment, with no significant differences in MS-consumption among the other three treatments (Figure 4b). Furthermore, the root activities of plantlets grown under monochromatic lights were all higher than those of plantlets grown under W. The difference in root activity between the W treatment and the B/G/Y treatments was statistically significant (Figure 4c).

![Figure 4](image_url)

**Figure 4.** MS-consumption and root activity of plantlets grown under different lights. MS-consumption of plantlets during different growth stages (a), MS-consumption of plantlets throughout growth period (b), and root activity at 30 days after light treatments (c) under different lights. Different lowercase letters on the bars indicate statistically significant differences (p ≤ 0.05). MS-consumption, solute consumption in the culture medium; R-activity, root activity. B, blue light; G, green light; Y, yellow light; R, red light; and W, white light.

### 3.5. The Photoautotrophic and Heterotrophic Performance of Plantlets

We conducted further analysis of the δ¹³C values in the plantlets to assess the contributions of carbon from the culture medium and photosynthesis to their dry matter accumulation under different light conditions. The data revealed that over 84% of the dry matter in all treatments originated from the culture medium. Among the treatments, the W treatment exhibited the highest contribution of dry matter from photosynthesis, at 15.46%. Correspondingly, the dry matter derived from the culture medium was significantly higher in the monochromatic light (B, G, Y, and R) treatments. There were no significant differences in carbon contribution ratios from photosynthesis and the culture medium among the different monochromatic lights. When comparing the values, the contributions from photosynthesis can be arranged in descending order as R > B > Y > G. Additionally, the W treatment showed the highest dry matter accumulation from photosynthesis, with no significant difference compared to the R treatment. There were also no significant
differences in dry weight derived from photosynthesis among the monochromatic light treatments. However, there was a notable difference in the dry weight derived from the culture medium of plantlets grown under different lights. The B treatment obtained the highest amount of dry weight from the culture medium, followed by the R treatment, while the G and W treatments acquired the lowest amounts of dry weight from the culture medium (Table 1).

Table 1. The photoautotrophic and heterotrophic performance of plantlets under different lights.

<table>
<thead>
<tr>
<th>Light Treatments</th>
<th>$\delta^{13}$C (%)</th>
<th>$P_n$ (%)</th>
<th>$1 - P_n$ (%)</th>
<th>Dry Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Photosynthesis</td>
<td>Culture Medium</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>-11.78 a</td>
<td>8.66 b</td>
<td>91.14 a</td>
<td>12.23 b</td>
</tr>
<tr>
<td>G</td>
<td>-11.51 a</td>
<td>7.14 b</td>
<td>92.86 a</td>
<td>7.63 b</td>
</tr>
<tr>
<td>Y</td>
<td>-11.69 a</td>
<td>8.27 b</td>
<td>91.73 a</td>
<td>9.47 b</td>
</tr>
<tr>
<td>R</td>
<td>-11.99 a</td>
<td>10.16 b</td>
<td>89.84 a</td>
<td>12.61 ab</td>
</tr>
<tr>
<td>W</td>
<td>-12.81 b</td>
<td>15.46 a</td>
<td>84.54 b</td>
<td>17.44 a</td>
</tr>
</tbody>
</table>

Note: $P_n$, proportion contributed to the $\delta^{13}$C value of the sample by photosynthesis; $1 - P_n$, proportion contributed to the $\delta^{13}$C value of the sample by the culture medium. Different lowercase letters in same column indicate statistically significant differences ($p \leq 0.05$). B, blue light; G, green light; Y, yellow light; R, red light; and W, white light.

3.6. The Expression of StSUT1 and StSUT4 in Leaves and Roots

After 15 days of light treatments, we measured the relative expression levels of StSUT1 and StSUT4 in the root and leaves to assess the abilities of microtuber formation and sugar transport of plantlets under different lights. The expression level of StSUT1 in the roots in the monochromatic light treatments was significantly higher than that in the W treatment, while the expression level of StSUT1 in the leaves in W treatment was significantly higher than that of all monochromatic light treatments, and the expression level of StSUT1 in leaves was the least in the Y treatment and significantly less than that in the W and G treatments (Figure 5a,b). The expression level of StSUT4 in the roots was significantly higher under Y and R treatments compared to the other treatments, while, in the leaves, the expression level of StSUT4 was highest under the B treatment among all the treatments, and significantly greater than that of plantlets grown under G treatment (Figure 5c,d).

Figure 5. The relative expression of StSUT1 (a) and StSUT4 (c) in roots, and StSUT1 (b) and StSUT4 (d) in leaves of potato plantlets grown under different lights. Different lowercase letters on the bars
indicate statistically significant differences ($p \leq 0.05$). B, blue light; G, green light; Y, yellow light; R, red light; and W, white light.

3.7. Correlation Analysis between Plantlet Growth and MS-Consumption

To evaluate the relationships among dry weights of different organs and MS-consumption of plantlets, we conducted a correlation analysis. The analysis revealed a negative correlation between microtuber weight and stem/leaf dry weight. Additionally, there was a significant positive correlation between microtuber weight and MS-consumption, but negative correlations between stem and leaf dry weights and MS-consumption were observed. Interestingly, there was also a negative correlation between root and stem dry weights, but a positive correlation between root dry weight and MS-consumption (Table 2).

Table 2. Correlation analysis between dry weight of root, stem, leaf, whole plant of plantlets, and solute consumption.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Root</th>
<th>Stem</th>
<th>Leaf</th>
<th>Tuber</th>
<th>MS-Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight</td>
<td>Root</td>
<td>-0.519 *</td>
<td>-0.073</td>
<td>0.356</td>
<td>0.463 *</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>-0.073</td>
<td>0.172</td>
<td>-0.671 **</td>
<td>-0.581 **</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
<td>0.356</td>
<td>-0.671 **</td>
<td>-0.518 *</td>
<td>-0.555 *</td>
</tr>
<tr>
<td>MS-consumption</td>
<td>0.463 *</td>
<td>-0.581 **</td>
<td>-0.555</td>
<td>0.898 **</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Note: Shown are the Pearson’s correlation coefficient values between them. * and ** indicate significant correlations at the $p < 0.05$ and $p < 0.01$ level, respectively. MS-concentration, solute concentration in the culture medium.

4. Discussion

4.1. Short-Wavelength Light Benefit for Rapid Formation of Microtuber

Light quality plays a significant role in the photosynthesis, morphogenesis, and substance metabolism of potato plantlets, as well as the formation and growth of potato microtubers [4,38]. In this study, it was observed that short-wavelength B and G, as well as W, containing both B and G components, could rapidly induce microtuber formation. Conversely, compared with these three lights, microtuber formation under R and Y was delayed by around 5 and 15 days, respectively (Figure 1g). Previous studies also found that B was conducive to the rapid formation of microtubers in comparison to R [10]. Similarly, Li et al. [5] reported that Y did not promote microtuber formation and growth, while G induced rapid microtuber formation, which is consistent with our observations. However, inconsistent with our findings, Li et al. [5] noted that R induced rapid microtuber formation, but B did not. The difference in results may be attributed to the variation in experimental conditions. In our study, we subjected the plantlets to a 30-day pre-treatment with W to induce leaf formation before applying different lights, whereas Li et al. [5] exclusively used R throughout the entire growth period. The above results indicate that microtuber induction by Y and G is less influenced by the light conditions under which the plantlets were grown prior to treatment. In contrast, microtuber formation by B and R appears to depend on the vegetative growth of plantlets.

Potato tuberization is a complex physiological process regulated by various factors including gene expression [20]. Genes associated with tuber formation are closely linked to plant hormone signaling pathways [39], and several studies have observed that gibberellic acid (GA) inhibits the formation of potato tubers [40,41]. The sucrose transporter StSUT4 from potato influences flowering, tuberization, and the shade avoidance response, with a reciprocal regulation observed between StSUT4 and GA [32]. GA induces StSUT4 expression of wild-type plants, while StSUT4 probably acts upstream of GA [32]. In this study, plantlets under G showed lower expression levels of StSUT4 in both leaves and
roots, indicating reduced endogenous GA content in plantlets under G, which was favorable for the rapid induction of microtubers (Figures 1g and 5c). Similar to the G treatment, the B and W treatments also facilitated rapid formation of microtubers. In these two treatments, there was a lower expression of StSUT4 in the roots, but in the leaves, StSUT4 expression was significantly higher in the B treatment compared to the G treatment. Conversely, the R and Y treatments, which exhibited higher StSUT4 expression in the roots, resulted in delayed microtuber formation. Furthermore, there is some evidence that plants grown under R have higher GAs levels compared to those grown under B [42–45]. This suggests that StSUT4 in the roots plays a more crucial role than in the leaves in the rapid induction of microtubers, and StSUT4 might act downstream of photoreceptors detecting light quality in potato plantlets and upstream of GA to influence microtuber induction.

4.2. Microtuber Growth Is Positively Associated with the Heterotrophic Ability of the Plantlet

Light quality did not significantly affect microtuber number, but B favored tuber growth compared to other four lights (Figure 1e), which is consistent with findings from several recent studies [5,10,15,16]. The current study also suggested that, under different lights, microtuber growth was significantly negatively correlated with stem and leaf growth, while being significantly positively correlated with MS-consumption (Table 2). Microtuber formation and growth require a substantial amount of energy supply [8], and plantlets obtain the energy from both photosynthesis and the sucrose of the culture medium for their growth and development. Exogenous sugars in the culture medium play a crucial role in potato microtuber growth. Studies have shown that more than 90% of the sugars in microtubers are derived from exogenous sources [36]. The current study revealed that over 84% of the dry weight in the whole plantlets originated from the culture medium, and the potato plantlets’ ability to derive energy from the culture medium was influenced by light quality (Table 1). In comparison to the W treatment, plantlets exposed to monochromatic lights exhibited enhanced heterotrophic ability and could acquire 5%–9% more sugars in the culture medium. Moreover, the B treatment demonstrated the highest MS-consumption, which likely played a significant role in inducing large microtubers under B, whereas lower heterotrophic ability under W might be associated with the inhibition by higher photoautotrophic ability (Figure 6).

![Figure 6. Diagram of substance flow and control in formation and growth of potato microtubers under different lights. B, blue light; G, green light; Y, yellow light; R, red light; and W, white light.](image-url)

Leaves are the primary site of photosynthesis, and compared to monochromatic light treatments, the W treatment exhibited better leaf morphology, with a consistently higher leaf number, leaf area, and leaf dry weight (Figures 1a–c,h, 2e–f and S1). In contrast, when microtubers formed, the leaf number, leaf area, and Chl content in the B treatment showed a rapid decrease (Figures 1a–c,h, 3a and S1), with the stems and leaves completely...
withered at harvest time (Figure 1c,h), thus inhibiting photosynthetic autotrophic ability and then stimulating the highest heterotrophic ability for normal growth (Table 1 and Figure 6). The preference for stem growth over leaf growth in the G, Y, and R treatments (Figure 2b–d,f) resulted in a lower photoautotrophic ability compared to the W treatment. In contrast, the W treatment exhibited a more balanced growth pattern between leaves and stems (Figure 2e,f). Consequently, the plantlets had to seek another way to acquire sugars from culture medium for microtuber growth in the G, Y, and R treatments (Figure 4a and Table 1).

The roots are responsible for the absorption of exogenous sugars. Our results suggested that high root activity enabled plantlets under monochromatic lights to acquire sugars from the culture medium. After microtuber formation, the dry weight of roots in each treatment was almost the same, but the root activity was positively correlated with MS-consumption (Figures 2a–e and 4b,c), indicating that the increased heterotrophic abilities of plantlets under monochromatic lights are highly dependent on enhancing root activity during the microtuber growth stage. In addition, exogenous sucrose or sucrose produced through photosynthesis can be transported from the phloem to other organs, and this phloem loading of sucrose requires the mediation of SUTs [46], with SUT1 playing a critical role [27, 28, 47]. SUT1 serves as a high-affinity transporter essential for phloem loading and long-distance transport in solanaceous species [32, 48]. He et al. [49] found that blue light upregulated the expression of StSUT1 and StSP6A, thus promoting more assimilates to be distributed to the microtubers. In our study, the expression levels of StSUT1 in both leaves and roots were highly consistent with the photoautotrophic capability and heterotrophic utilization of sugars in the culture medium of plantlets under W and monochromatic lights, respectively. Under monochromatic light treatments, where heterotrophic activity was stronger, the expression level of StSUT1 in the roots was higher than that under the W treatment. Conversely, under W treatment where photoautotrophic activity was stronger, the expression level of StSUT1 in the leaves was significantly higher compared to monochromatic light treatments (Figure 5a,c), ensuring a high degree of matching between sugar supply and transport abilities from leaves or roots.

Although there was no significant difference in the overall proportion of carbon sources obtained from photosynthesis and the culture medium in plantlets under different monochromatic lights, the actual weight of sugar acquired from the culture medium varied at different stages (Table 1 and Figure 4a). The results indicated that the peak demand for sugar in the culture medium by plantlets typically occurred within the initial 30 days post-microtuber formation. The period of maximum energy uptake for plantlets subjected to the B and G treatments was observed between 15 and 45 days, whereas for Y and R treatments, it occurred between 30 and 60 days. By comparing the growth potential of the microtubers and the utilization of sugar in the culture medium, it can be deduced that prolonging the light exposure duration for the B, Y, and R treatments may enhance further microtuber development. Conversely, under the G treatment, the dry weight of microtubers showed minimal change after 45 days of light exposure, with negligible MS-consumption. This suggests that while G was conducive to microtuber formation, it hindered the reproductive process, leading to inhibited microtuber growth. Conversely, the B treatment seemed to extend the reproductive phase of the microtubers, suggesting the potential for higher potato yields. In contrast, the Y and R treatments exhibited continued growth potential, indicative of a normal delay in reproductive development (Figures 2a–e and 4a).

In addition, we observed that plantlets in the W and Y treatments released energy into the culture medium from day 15 to 30, as well as under the W treatment from day 45 to 60 (Figure 4a). This phenomenon has been observed in previous studies [50–52]. Durand et al. [53] reported that around 20% of the carbon allocated to Arabidopsis roots was released into the culture medium before the establishment of strong sink organs, but this proportion decreased to below 4% once robust sink organs were formed. In this study, we also observed a limited increase, even a decrease, in the dry weight of plantlets including
microtubers under Y during the 15–30 day period of light treatments and W during the 15–30 and 45–60 day periods of light treatments compared to the other stages (Figure 2c,e), implying weak sink demand in these stage. Furthermore, during the 15–30 day period of light treatments, both the W and Y treatments showed increasing photosynthetic pigment content, and an even higher level of photosynthetic pigment content in the W treatment was maintained in the later stages, indicating that these two treatments with an increasing foundation for photosynthesis could provide energy for limited increase in microtubers in corresponding stages (Figures 3a–b, 4a and S2). The above analysis suggests that once potato plantlets acquire photoautotrophic capabilities, they prioritize the utilization of carbohydrates produced through photosynthesis to meet their energy requirements. They may even release excess energy into the culture medium when the carbohydrates generated from photosynthesis are sufficient. When the photosynthetic ability is insufficient for photoautotrophic growth, the plants actively absorb sugars from culture medium and resort to heterotrophic nutrition to fulfill their growth and development needs, establishing a carbon supply model where autotrophy takes priority over heterotrophy as a supplementary carbon source.

Interestingly, our study found that microtubers in the G and W treatments were located farther from the roots, while those in the B, Y, and R treatments were positioned closer to the roots. Previous research has indicated that the distribution of assimilates is influenced by the source–sink distance, with shorter distances favoring the transport of energy from source to sink [5]. Therefore, we speculate that the higher position of microtubers in the G and W treatments may be related to the elevated photosynthetic ability during the microtuber induction period compared to other three treatments, as we already knew that plantlets under W consistently exhibited better photoautotrophic ability (Table 1). In the case of the G treatment, during the initial 15 days, plantlets showed sustained high levels of leaf area and Chl content, even surpassing those observed in the W treatment, and thus the strong photosynthetic foundation likely contributed to the elevated positioning of microtubers in the G treatment than that in the W treatment. Taken together, the trade-off between photoautotrophy and heterotrophy of potato plantlets at the tuber induction stage determined the positioning of microtubers under different lights.

5. Conclusions

Light quality affected potato microtuber formation, position, and growth. B, G, and W were conducive to the rapid induction of microtuber of plantlets after 30 days of precultivation under W, while R, especially Y, delayed microtuber formation. This delay effect was related to the high expression of StSUT4 in the roots induced by R and Y. The microtuber position at a greater distance from the roots under G and W, as opposed to closer to the roots under B, Y, and R, was associated with the photosynthetic ability of plantlets before microtuber formation. Higher photosynthetic ability was observed in the W treatment, whereas the opposite effect was seen in the monochromatic light treatments throughout growth period. Microtuber growth was primarily dependent on heterotrophy, with sugar obtained from the culture medium. B was conducive to microtuber growth, as it was associated with higher root activity, microtuber position, and high expression of StSUT1 in the roots, facilitating the acquisition of more sugar from the culture medium. Conversely, the W treatment had the opposite effect, inhibiting microtuber growth. Plantlets under G had restricted microtuber growth due to decreased sugar utilization from the culture medium, both in terms of the quantity and the duration, while the smaller microtubers induced by R and Y may be attributed to delayed tuberization and a shorter period of microtuber growth. Therefore, in virus-free microtuber production, it is recommended to use blue light irradiation to produce larger microtubers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14061232/s1, Figure S1: Leaf number of potato plantlets grown under different monochromatic lights at different stages; Figure S2: The effects of
different lights on the dry matter accumulation in various organs of potato plantlets; Table S1: Estimation of the error in measuring solute consumption in the culture medium using the drying and weighing method; Table S2: The primer sequences of target and reference genes used in RT-qPCR analysis.

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**Abbreviations**

B: blue light; Chl, chlorophyll; DW, dry weight; DWL, increase in dry weight; G, green light; GA, gibberellic acid; MS-consumption, solute consumption in the culture medium; Ps, proportion contributed to the δ13C value of the sample by photosynthesis; R, red light; R activités, root activity; SU T, sucrose transporter; W, white light; Y, yellow light; and 1 – Ps, proportion contributed by the carbon source in the medium.

**References**


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