

Effect of Different Light Spectra on the Growth of Potato Varieties Under Micropropagation Conditions

Wagdi Ghaleb^{1*} , Hanan Abosalah² , Kheri Lazrag¹ , Masoud Alsheebani¹ , Abdulmonem Daabub¹ ,
Ahmed Shaaban¹ , Elmundr Abughnia¹ 

¹Department of Plant Tissue Culture, Libyan Biotechnology Research Centre, Tripoli, Libya

²Alasmarya Islamic University, Zliten, Libya

Corresponding Email. ghalebwagdi@gmail.com

Abstract

Potato ranks as the third most significant food crop worldwide for human consumption, following rice and wheat. According to worldwide statistics, potato production is increasingly moving to developing countries, with substantial growth in production and the area harvested, particularly in Asia and Africa (notably East Africa) and in Latin America and the Caribbean (LAC). The main objective of this investigation was to ascertain the most suitable light type for plant tissue culture laboratories by exposing plant tissues to four light spectra (red, blue, white fluorescent lamps, and a blend of red and blue fluorescent lamps) and establishing contamination-free tissue cultures for two potato cultivars, *Spunta* and *Agria*, to analyze their responses to various light spectra. The obtained results indicated that the best sterilization treatment was obtained when explants were immersed in a Sodium hypochlorite (NaOCl) 3% for 15 minutes. When the explants were exposed to different light spectra (red light, blue light, white light, red and blue mixture), the results of this study indicated that red light outperformed the other treatments in terms of plant length, number of leaves, number of branches, and number of roots. In addition, it was evident from our results that blue light positively influences the production of chlorophyll (T, a, and b).

Keywords. Micropropagation, Light Spectra, Tissue Culture, and Potato.

Introduction

Potato (*Solanum tuberosum* L) belongs to the family Solanaceae. It is a vegetable crop produced worldwide in temperate and subtropical regions. It is one of the most important vegetable crops due to its high yields, low production costs, and its ability to be grown under a wide range of environmental conditions [1]. According to the International Potato Center, potatoes are the third most important food crop worldwide for human consumption, behind rice and wheat [2]. Global data show that the production of potatoes is moving to emerging nations, particularly in Asia and Africa (particularly East Africa), as well as Latin America and the Caribbean (LAC), where production and harvested areas have increased significantly. The developing world's potato production surpassed that of the developed world for the first time in 2005 [3]. Africa has recorded large increases in cultivated area over the last 20 years [4].

Local output of potatoes in Libya reached 360217 tons in 2019 [5] and 366749 tons in 2020 [6], nearly meeting local consumption but not yet reaching the export stage. Potatoes can be propagated sexually (by botanical seeds, also called true potato seed) or asexually (vegetatively) using tubers. Seeds of potato tubers are utilized for multiplication and production. This approach has several disadvantages, including an elevated risk of viral infections and a low rate of multiplication. The in vitro culture technique is a novel technology that has gained a lot of popularity recently as an alternative method for vegetative growth of many plants [7]. In vitro culture techniques facilitate producing many clones from a single seed or explant, selecting for desirable traits, reducing the space required for field trials, and eliminating plant diseases through careful selection and sterile techniques. The system's ability to produce many plants quickly is one of its key characteristics [8].

Due to the impossibility of using true seed, conservation and exchange of germplasm of this species by means of conventional methods are not feasible in all potato-production regions; the demand for high-quality tubers has been paramount to ensure crop production. Therefore, using biotechnologies to solve this issue is crucial. Plant tissue culture offers alternative methods of propagation by in vitro techniques that provide production and multiplication of plant material with high quality, besides the clonally multiplication biotechnological tool provides available initial indexed material to plant breeding programs, potato certified seeds and facilitates the exchanging and conservation of germplasm; moreover use of these techniques in potato production chain benefits producers directly through providing high-quality propagates [9]. However, plant tissue culture technology is one of the most important modern techniques used in the field of agriculture because of its benefits in reducing agricultural problems and contributes significantly to sustainable agricultural development [10].

Plant tissue culture technique and formation of whole plant in vitro is a modern method used in plant propagation (Asexual propagation), which often leads to producing a Huge number of plants that are identical to the original plant and homogeneity of obtained plants in a short period of time [11,12]. *Agria* and *Spunta*, two cultivars, are commonly used among the local farmers, while their maturation period takes from 115 to 120 days. *Agria* is one of the most recently introduced cultivars to Libya, characterized by its large size and resistance to A (X) virus. Its tubers are very large, oval in longitudinal section, outer color

yellow, and interior dark yellow, with soft, superficial eyes, thick stem standing, large leaves, big white flowers, and resistant to virus A. This cultivar is suitable for making chips [13]. For plants grown in vitro, light is essential to their growth and development. Light directly affects the structure and morphology of plants during seed germination, leaf development, neighbor evasion, stem lengthening, flower initiation, pigment synthesis, and other processes. Recent studies have demonstrated that because plants react differently to different light spectra, the kind of light has a substantial effect on plant growth. Growth rate is only one aspect of this reaction; other aspects include plant quality, the synthesis of chemical compounds within the plant, and even the plant's capacity for environmental adaptation. This mechanism depends critically on both red light (600-700 nm) and blue light (400-500 nm). Because it encourages the growth of leaves and stems and increases the production of chlorophyll, which makes plants denser and stronger, blue light is crucial for vegetative growth. Controlling the amount of blue light has been shown to have an impact on plant morphology in recent studies [14]. Conversely, red light affects stem elongation and is essential throughout the flowering and fruiting phases. Recent research on strawberry plants has demonstrated that the optimal production and quality outcomes are achieved when red and blue light are combined [15]. Understanding these correlations is crucial for modern indoor agriculture, where LED lights can be used to precisely change the light spectrum to meet the needs of a plant at every stage of its growth. This method not only boosts cultivation efficiency but also makes it possible to produce crops with superior quality and traits [16]. Replace with: This study aims to evaluate the response of cultivated potato tissues to light spectra and compare two potato cultivars (*Spunta* and *Agria*) to their light spectra response, and to establish an in vitro cultivation protocol to produce a large number of contamination-free seedlings of *Spunta* and *Agria*.

Methods

This study aimed to determine the most effective type of light for plant tissue culture laboratories by testing four different types of spectra: red fluorescent lamps, blue fluorescent lamps, white fluorescent lamps, and a combination of red and blue fluorescent lamps under 2000 lux light intensity. This study was conducted in the Plant Tissue Culture Laboratory of the Biotechnology Research Center in Tripoli.

Plant material

The samples were taken from the local agricultural company and then moved directly to the Biotechnology Research Center Laboratories. *Spunta* is considered one of the most important cultivars used for the local market; it is drought-tolerant and resistant to viruses (A, Y). Many stems spread over the side with crimson color at the base and leaf hubs. Leaves are relatively small, and the flowers are white and small; while the tubers are large, long, slightly curved, somewhat pointed at the top, and soft; their outer color is pale yellow, their interior color is light yellow, and the buds are very superficial.

Preparation of culture medium

Murashige and Skoog (MS) medium was prepared to provide the necessary plant needs, especially the major nutrients, source of carbon, vitamins, and supplemented with growth regulators that are appropriate for the study. In addition, MS culture media contains 3% sucrose and 0.7% agar, and the pH was adjusted to 5.7 to 5.8 for culturing single nodes of a potato. The media were sterilized in an autoclave at 121 °C and a pressure of 1.02 bar for 15 min to obtain a contamination-free medium. Finally, 25ml of sterile medium was distributed into the jars containers inside the air laminar flow.

Surface sterilization of potato tuber buds

Potato tubers were placed in a dark room for bud' growth. Potato buds, after reaching suitable lengths, were collected and then put under running water for 30 minutes for cleaning purposes. Buds were transferred to a laminar airflow cabinet for sterilization using 70% ethanol for two minutes, followed by sodium hypochlorite with concentrations of 2%, 2.5%, 3%, and 4% for 15 minutes. To ensure high-quality sterilization, stirring must be done from time to time to remove any bacteria or fungi. The final stage of sterilization involved washing the culture with sterilized, double-distilled water three times, each for five minutes, to remove the toxic effects of sodium hypochlorite [17].



Figure 1. Contamination-free- free culture

Culture process

The culture stage begins with the placement of one single bud in special jars containing 25 ml of sterilized MS media. All the culture processes were conducted in the laminar flow cabinet under sterilized conditions for obtaining a culture free of pathogens. Jars were incubated in the growth chamber at 16-hour light /day and 8-hour dark/day (2000 Lux) using white fluorescent lamps, temperature $25 \pm 2^\circ\text{C}$ and relative humidity 40%, while humidity 40%, while culture were incubated for four weeks (Figure 1). Subculture process After obtaining successful establishment of free pathogen cultures, the newly obtained potato cultures were transferred to the subculture stage for multiplication. The subculture process was conducted in the laminar flow cabinet under sterilized conditions, while the subcultured shoots were placed on MS media as one explant per jar. Ten jars for each treatment. Jars were incubated in a growth chamber for 45 days under four types of spectra: white fluorescent lamps (Figure 2), mixed red and blue fluorescent lamps (Figure 3), red fluorescent lamps (Figure 4), and blue fluorescent lamps (Figure 5). The physical measuring involves length of shoot, leaves per shoot, nodes per shoot, roots per shoot, length of roots and chlorophyll content.

Determination of chlorophyll content

One gram of the dry plant sample obtained for each treatment was gently mashed by using a mortar until a soft powder was obtained, then 50 ML of acetone at a concentration of 80% was added to the powder samples for the extraction of chlorophyll. The chlorophyll content of all samples was measured using a spectrophotometer device at a wavelength of 645nm and 663nm. Total chlorophyll content, Chlorophyll (a), and chlorophyll (b) were determined. The chlorophyll assessment was calculated according to the following formula.

$$\text{mg/g chl. a} = [12.7.A_{663} - 2.69.A_{645}] \times v / (1000.w)$$

$$\text{mg/g chl. b} = [22.9.A_{645} - 4.68.A_{663}] \times v / (1000.w)$$

$$\text{mg/g chl. T} = [20.2.A_{645} + 8.02 .A_{663}] \times v / (1000.w) \quad [18]$$



Figure 2. Growing cultures under white light



Figure 3. Growing cultures under mixture red and blue



Figure 4. Growing cultures under red light



Figure 5. Growing cultures under blue light

Experimental design and statistical analysis

The percentage of contamination was determined, and plant shoot and root development were evaluated according to some parameters, including plantlet length, leaves, number, shoot number, roots length, each treatment containing ten replicates for all spectra parameters. The design of the Completely Randomized Design (CRD) was used [19], and each replicate consists of one explant cultivated in a vessel. Means were separated by using Duncan's multiple range test at 5% level of significance.

Results

Surface sterilization

For explants sterilization, the results showed that using of 3% sodium hypochlorite was the best treatment among tested concentrations that decreased the contamination and increasing the percentage of non-

contaminated explants up to 95% and 91% in both *Spunta* and *Agria* respectively, followed by 2.5% sodium hypochlorite treatment which give a percentage of non-contaminated explants free up to 85% and 75% in both *Spunta* and *Agria* respectively, whereas the lowest percentage of non-contaminated explants was found in treatment contain 2% of sodium hypochlorite and the percentage was 68% and 61% in both *Spunta* and *Agria* respectively. However, using 4% sodium hypochlorite has a negative effect on potato buds as it causes the death of buds due to the high concentration of sodium hypochlorite in both *Spunta* and *Agria* (Figure 6). For explants sterilization, the results showed that using of 3% sodium hypochlorite was the best treatment among tested concentrations that decreased the contamination and increasing the percentage of non-contaminated explants up to 95% and 91% in both *Spunta* and *Agria* respectively, followed by 2.5% sodium hypochlorite treatment which give a percentage of non-contaminated explants free up to 85% and 75% in both *Spunta* and *Agria* respectively, whereas the lowest percentage of non-contaminated explants was found in treatment contain 2% of sodium hypochlorite and the percentage was 68% and 61% in both *Spunta* and *Agria* respectively. However, using 4% sodium hypochlorite has a negative effect on potato buds as it cause the death of buds due to the high concentration of sodium hypochlorite in both *Spunta* and *Agria* (Figure 6).

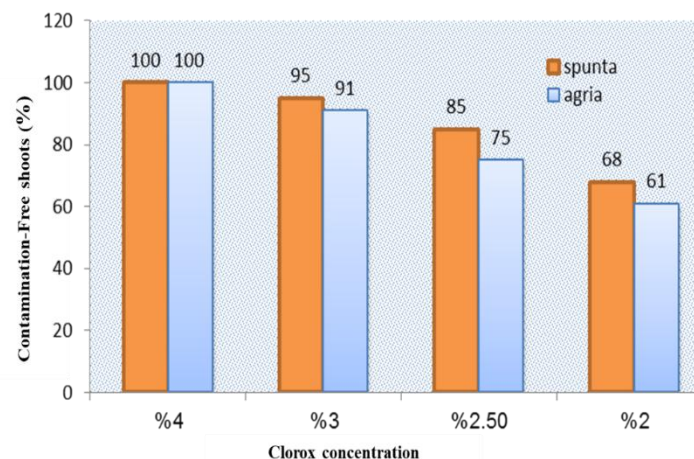


Figure 6. Percentage of the uncontaminated shoots under different concentrations of sodium hypochlorite solution for *Spunta* and *Agria*.

As reported above, four types of spectral light (red light, blue light, white light, and mixed red and blue light) have been examined to observe the effect of these types on plant growth under in vitro culture conditions. The results in (Figure 7) showed that the plantlet length parameter in plants grown under red light treatments was significantly higher compared to other spectral light treatments in both cultivars used, followed by treatment of white light, proving the positive effect of red light on plantlet length. In contrast, the shortest plantlet length was observed in blue light treatment. Furthermore, the longest plantlets were obtained from the *Spunta* under red light treatment, with an average of 28.2 cm, but there was no significant difference between the *Agria* and *Spunta* under red light treatment. The average length for the *Agria* was 27.9 cm. In addition, plantlets grown under white light treatments have no significant differences between the two cultivars used. Furthermore, the shortest plantlets were obtained in *Agria* and *Spunta* under blue light treatment, which averaged 17.8cm and 19cm, respectively. In all three of the potato varieties under investigation, LEDs produced longer plantlets. Additionally, research on potatoes found that plantlets produced under red LEDs were longer than those grown under blue LEDs [20].

Blue light treatment with an average of 6.3 leaves/shoot, but there was no significant difference with *Spunta* under a combination of red and blue light. These findings are consistent with research on strawberries [21]. The obtained result proved that the combination of red and blue light treatment had no positive effect on number of leaves, which means that there was low leaf formation under combination of red and blue light treatment (Figure 8). These results differed from those of a research on grapes, which indicated that red light had no beneficial effect on the number of leaves in the varieties of grapes [22].

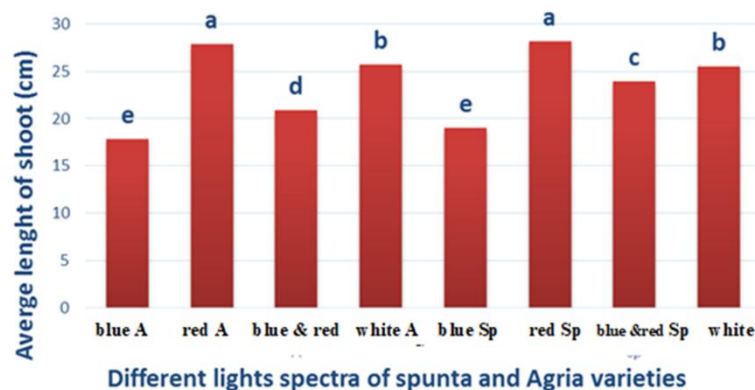


Figure 7. Effect of light spectrum on plant height of potato (Agria and Spunta). Columns with the same letter are not significantly different by Duncan's multiple range test at 5% level of significant.

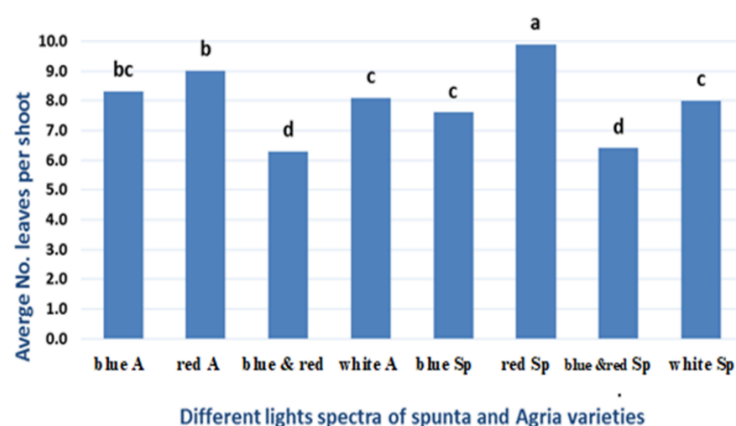


Figure 8. Effect of light spectrum on number of leaves. Columns with the same letter are not significantly different by Duncan's multiple range test at 5% level of significant.

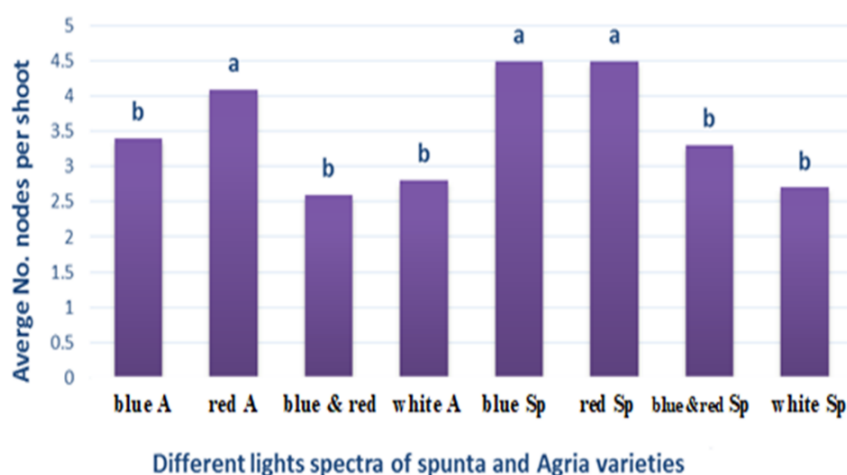


Figure 9. Effect of light spectrum on number of shoots. Columns with the same letter are not significantly different by Duncan's multiple range test at 5% level of significant.

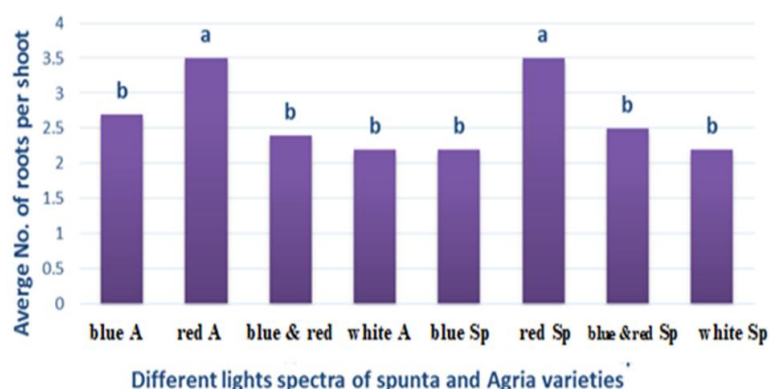


Figure 10. Effect of light spectrum on number roots. Columns with the same letter are not significantly different, by Duncan's multiple range test at 5% level of significant.

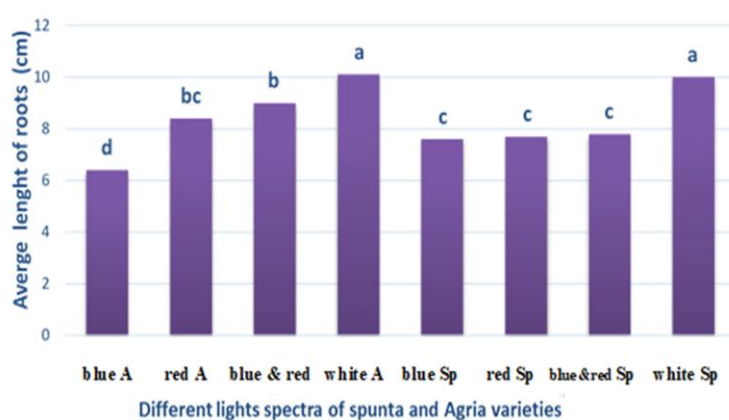


Figure 11. Effect of light spectrum on roots length. Columns with the same letter are not significantly different by Duncan's multiple range test at 5% level of significant.

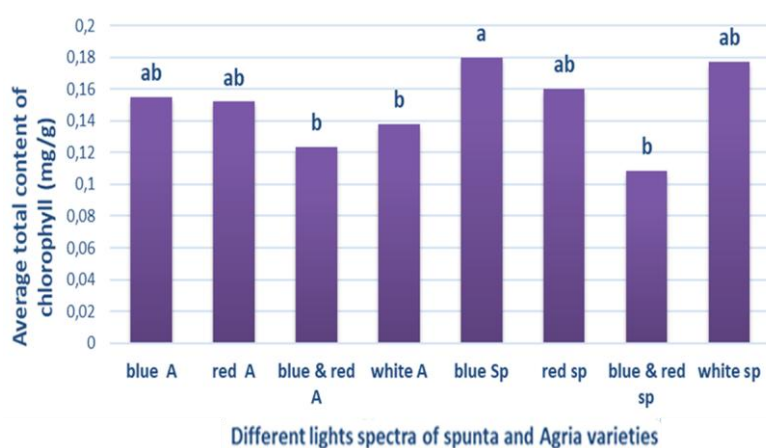


Figure 12. Effect of light spectrum on T- chlorophyll content. Columns with the same letter are not significantly different by Duncan's multiple range test at 5% level of significant.

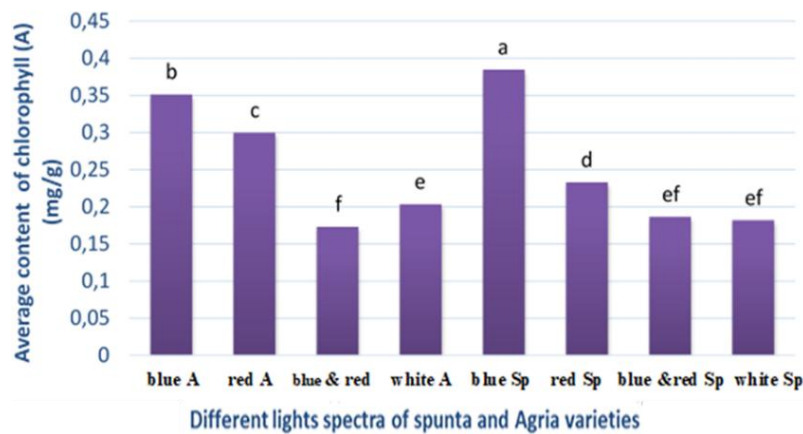


Figure 13. Effect of light spectrum on chlorophyll (a) content. Columns with the same letter are not significantly different by Duncan's multiple range test at 5% level of significant.

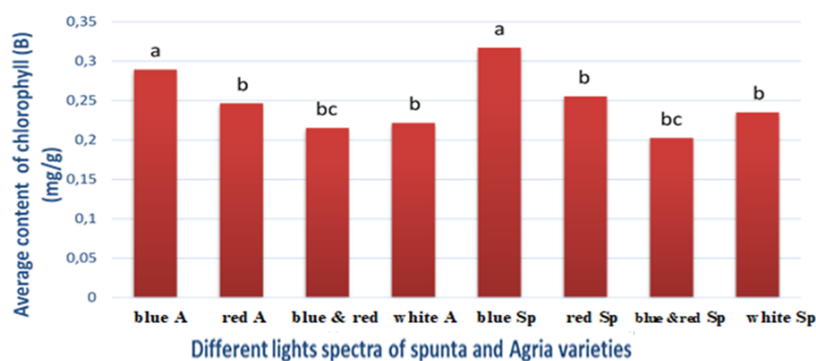


Figure 14. Effect of light spectrum on chlorophyll (b) content. Columns with the same letter are not significantly different by Duncan's multiple range test at 5% level of significant.

Effect of light spectrum on the number of shoots

The result of this experiment showed that the average number of shoots was significantly higher in red light in both cultivars and blue light in the *Spunta*. Plantlets of *Spunta* under red light and blue light have an average number of shoots of 4.5 shoots/plantlet, while the average number of shoots in *Agria* reached 4.1 shoots/plantlet. Results also indicated that no significant differences between *Spunta* and *Agria* in red light treatment, but in blue light treatments, there were significant differences between the two cultivars. The lowest number of shoots was recorded in the *Agria* under a mixture of blue and red light treatments, with an average of 2.6 shoots/plantlet (Figure 9). Moreover, there were no significant differences observed between treatments under white light and a mixture of red and blue light in both cultivars. These findings corroborated those of a study on *Dendrobium kingianum* [23], which discovered that explants grown under red LEDs had the highest percentage of shoot formation and fresh plant weight, which was comparable to the findings of this study [8]. Their research demonstrated that strawberries treated with red LEDs had a higher shoot/root ratio. However, in another experiment, they found that red light had no effect on the number of shoots growing in two groups of cultivars, and in the third cultivar, it was found that the red light significantly outperformed blue light, and there was no difference with the white light [21].

Effect of light spectrum on the number of roots

The average number of roots under red light treatments in both cultivars was significantly higher compared to other light treatments used in the experiment. This proves the positive effect of the red light on plantlets in general. The average number of roots under red light treatment reached 3.5 Roots/plantlet in both *Agria* and *Spunta*, while the lowest number of roots was recorded in the *Spunta* under white and blue light treatment, with an average of 2.2 roots/plantlet. Moreover, there were no significant differences between treatments under white light and blue light, and a mixture of red and blue light in both cultivars. (Figure 10). These findings were consistent with research that reported that the grape varieties' rooting percentage was highest under red light, followed by blue light [22].

Furthermore, another study on gerbera found that the use of auxin-free MS medium under blue light gave the highest number of roots with an average of 10.10 roots/plantlet, but there was no significant difference with red light, which gave an average of 8.9 roots/plantlet [24]. On the other hand, a study on *Withania*

somnifera L. reported that root system or root induction is probably dependent on luminous intensity [25].

Effect of light spectrum on root length

For the length of roots, the results showed that white light gave the longest roots and the average of root length in this treatment was significantly higher than other treatments in both cultivars, with an average of 10.1cm (Figure 11). Followed by *Agria* under a mixture of red and blue light. From the obtained results, we also found that in the *Spunta*, there were no significant differences found between the treatments under red, blue, and mixture red & blue lights. While the shortest plantlet roots were found in the *Agria* under blue light, with an average of 6.4 cm (Figure 12), these findings were in line with research that discovered that *Gerbera* cv. Martinique grown in media without NAA under white light produced the highest roots, and the average root length in this treatment differed considerably from other interactions [24]. Additionally, research on orchids found that plantlets grown under white fluorescent lights had the longest roots [26]. In contrast, a study found different results in their research on *Oncidium*, revealing that the longest roots were seen in plantlets treated with composite red and blue light spectra [27].

Effect of light spectrum on chlorophyll content

Total chlorophyll content

The results showed that the largest amount of T-chlorophyll was obtained in the *Spunta* growing under blue light with an average of 0.180 mg /g. The results also showed that there were no significant differences between plantlets under red and white light treatments in the *Spunta*. Furthermore, the significant differences have not been found in *Agria*, but under blue light and red light, while the lowest levels of T-chlorophyll content were found in the mixture of red & blue treatments in both cultivars, there were no significant differences with *Agria* under white light. (Figure 12) Chlorophyll (a) content: The results showed that chlorophyll (a) content in *Spunta* under blue light was significantly higher than other treatments, with an average of 0.384mg/g followed by blue light in *Agria* (Figure 13). This result expresses the positive effect of blue light on chlorophyll (a) formation and accumulation. According to the results, the lowest content of chlorophyll (a) was observed in the *Agria* under a mixture of red and blue light treatment, with an average of 0.173mg/g. Moreover, significant differences have also been found among all the treatments except white light and the mixture of red and blue lights in the *Spunta*.

Chlorophyll (b) content

The results of chlorophyll (b) content showed a similar trend. Chlorophyll b was significantly higher in both cultivars under blue light treatments. The highest levels of chlorophyll (b) were found in *Spunta* under blue light with an average of 0.317mg/g, while the lowest content of chlorophyll (b) was found in *Agria* under mixture of red and blue light treatment with an average of 0.215mg/g (Figure 14). The results showed that no significant differences have been found among the two cultivars under the same light, no differences have been found among *Spunta* and *Agria* under blue light, and no differences were observed among the two cultivars under red light treatment. The same trend was observed for other light treatments. Furthermore, the positive effect of blue light on chlorophyll formation (T, a, and b) was clearly observed [2]. Our results were also in line with research that reported plantlets grown under blue light gave the highest levels of chlorophyll, followed by red light treatments [26,22]. In their study, they suggested that blue light might be required for chlorophyll synthesis, and this is exactly what has been shown by our results.

Discussion

Although phytochrome signaling can modulate the biosynthesis and signaling pathways of several plant hormones, the effects on gibberellins (GAs) and cytokinins are highly context-dependent. Numerous studies have shown that phytochrome-mediated light perception regulates GA metabolism indirectly through transcriptional networks, mainly via the degradation or stabilization of PIF transcription factors and the subsequent regulation of GA-biosynthetic genes such as GA3ox and GA2ox [28,29]

According to the study's findings, red light performed better than the other treatments in terms of the length, number of leaves, branches, and roots of the plants. Similar results were reported by Hendricks and colleagues in Beltsville, Maryland, who found that several plants' photomorphogenic responses had action spectra that peaked in red light [30]. Plants can absorb both red and blue light easily, and when combined, they create an effective light spectrum for photosynthesis and photomorphogenesis. However, under plant tissue culture conditions, different plant species and cultivars have different ideal red to blue light ratios. 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 [2].

Phytochrome and cryptochrome absorb red light (600–700 nm) and blue light (400–500 nm), respectively. These two light receptors have been found to have several molecular forms with distinct spectrophotometric, biochemical, and physiological properties. It is thought that the associated genes express themselves differently in response to physiological and environmental cues [31,32]. It has also been demonstrated that light quality affected the metabolism and distribution of phytohormones in radish plants and potato plantlets grown in vitro. Exposure to red light preferentially activates the red-absorbing form (Pr) and converts it to the (Pfr), which may then be utilized in biological responses [33].

The red light through the phytochrome pigment and its regulation positively affect the plant in terms of stem elongation, leaf flattening, and the flowering process. Phytochrome also affects the permeability of cells and their membranes, not only on the plasma membrane, but on the other organelles' membranes, such as the nucleus and mitochondria as well, hence the rapid effect on the processes of trophic transformation in cells [34]. Because it stimulates hormones, red light is essential for plant growth. When plants are exposed to red light, their leaves quickly produce more endogenous gibberellins and cytokinins [33]. Furthermore, studies on de-etiolation have shown that red light reduces GA levels by enhancing DELLA protein stability, thereby promoting photomorphogenesis without requiring a direct increase in cytokinin production [35,36]. Collectively, current evidence indicates that red light influences GA and cytokinin pathways indirectly, acting through phytochrome-regulated gene networks and hormone-responsive transcription factors, rather than by directly increasing their endogenous levels in leaf tissues. As for chlorophyll, the positive effect of blue light on chlorophyll formation (T, a, and b) was clearly observed in our results. This was because both types of chlorophyll b show the highest absorbance in the violet-blue region at about 429 and, n, a, 453 nm, respectively [2].

Conclusion

The maximum percentage of contamination-free shoots in both cultivars was obtained by using a surface sterilization regimen of 3% sodium hypochlorite for 15 minutes, according to this study. The study's findings indicate that the *Spunta* under red light treatment produced the longest plantlets, with no discernible difference between the *Spunta* and *Agria*. The cultivars of *Spunta* and *Agria* yielded the shortest plantlets, respectively. The largest number of leaves was obtained in the *Spunta* cultivar under red light treatment, whereas the lowest number of leaves was obtained in the *Agria* under mixed red and blue light. The number of shoots was significantly larger in the *Spunta* under red light and blue, whereas no significant differences with *Agria* under red light. The lowest number of shoots was found in the *Agria* under a mixture of red & blue light. Moreover, there were no significant differences observed between treatments under white light and a mixture of red and blue light in both cultivars. The results showed that the largest number of roots per plantlet was obtained under red light treatment in both cultivars, while the lowest number of roots was recorded in *Spunta* under white and blue light treatments. Moreover, there were no significant differences observed between treatments under white light and blue light, and a mixture of red and blue light in both cultivars. Regarding the length of roots, results showed that white light gave the longest roots, and the average of root length in these treatments was significantly higher than in other treatments in both cultivars, while the shortest plantlet roots were found in *Agria* under blue light. In chlorophyll content, results showed that plantlets grown under blue light treatments contained the largest amount of total chlorophyll (T-chlorophyll), chlorophyll (a), and chlorophyll (b).

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Conflict of interest. Nil

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