International Journal of Horticultural Science and Technology (2023) Vol. 10, Special Issues, pp. 113-124

International Journal of Horticultural Science and Technology
Journal homepage: http://ijhst.ut.ac.ir

# Impact of Light Quality on *in vitro* Potato Microtubers Formation

**Bayram Emin Develi*, Natalia Miler**

Laboratory of Ornamental Plants and Vegetable Crops, Faculty of Agriculture and Biotechnology, University of Science and Technology, Bydgoszcz, Poland

**ARTICLE INFO**

*Article history*:

Received: 5 January 2023,
Received in revised form: 1 March 2023,
Accepted: 3 March 2023

*Article type:*

Research paper

**Keywords:**

*Solanum tuberosum* L.
light emitting diodes
LEDs
microtuber
*in vitro* culture

**ABSTRACT**

In the present study, the influence of three-wide spectrum light-emitting diodes (LEDs), emitting warm light AP67 (moderate blue and red and low red:far-red), AP673L (high red and high red:far-red) and G2 (high red and low red:far-red) with different colours mixing blue, green, red and far-red was used for micropropagation of three Polish potato cultivars; 'Aruba', 'Oberon' and 'Skawa'. The planlets were grown in a growth chamber with a 16-h photoperiod. The photosynthetic photon flux density was 30 µmol $m^{-2}$ $s^{-1}$. The tissue culture medium for shoot proliferation was a standard MS-based medium. Microtuberization was observed on MS medium supplemented with higher sugar content (40 m g/$L^{-1}$). Light quality affected the number of microtubers (MTs) per plantlet, the volume ($cm^3$) of MTs, the fresh weight (mg) of MTs, and the width (mm) of MTs. Different light qualities did not affect morphological parameters, including length (mm), perimeter (mm), and cross-section area ($mm^2$) of microtubers, whereas the highest number of microtuber were detected under the effect of AP67 light quality. The morphological parameters mostly depended on cultivar. The lowest number of microtubers was observed in the G2 treatment. In conclusion , 'Aruba' and 'Oberon' with AP67 and AP673L, as well as LED lighting systems, were determined as most suitable in terms of the MT count per plantlet. Also, these variables were most suitable for MT volume ($cm^3$), fresh weight (mg), and width (mm).

## Introduction

Potato (*Solanum tuberosum* L.) belongs to the Solanaceae family. Due to its high nutrient content and high yield production, potato ranks as the fourth most important food crop in the world after rice, wheat, and maize for human consumption (AL-Shmary and AL-Taey 2020).

Potatoes are a major source of income in several developing countries (Lutaladio and Castaidi 2009). It is predicted that the world population will increase significantly and this will affect countries where people have difficulties in accessing food, natural resources, water and shelter. With global warming, the decrease in agricultural areas suitable for the climate makes the situation even more difficult. Under these conditions, the potato plant is a good field crop for an ever-increasing global population, as it produces food much faster and in larger quantities in smaller areas than other major agricultural crops (Devaux et al. 2021).

Different techniques and strategies are constantly being used to grow potatoes. The main goals of these studies are to boost production, enhance processing, and also to exist tuber quality in various metrics. (Halterman et al. 2016).

Tuberization of potatoes is a time-consuming and complicated process that can be induced *in vitro*. Microtubers provide significant advantages in storage, transportation, and manufacturing applications due to their small size and weight. It can be sown directly in the field as well as grown in greenhouses and laboratories at any time of year. They have morphological and biochemical characteristics that are identical to conventional tubers. In this approach, microtuber formation is

* Corresponding author's email: emindeveli95@gmail.com

a significant advancement in potato breeding (Kanwal et al. 2006; Mamiya et al. 2020).

Tissue culture techniques are effective and practical for potato seed technology because the plants can be reproduced in vegetative form and develop quickly in tissue culture without requiring specialized media to obtain disease-free microtubers (Mohapatra and Batra 2017). Furthermore, according to Thorpe (2007), the plant tissue culture method provides aseptic conditions in controlled environmental conditions and under certain nutrient media for the production of the new plant as well as metabolites from plant parts. The resultant clones are true-to-type of the selected genotype. In addition, the controlled conditions provide the culture with an environment conducive to their growth also multiplication phases (Askari et al., 2022).

Light is the primary source of energy for plant photosynthesis as well as for the growth of plants (Aliniaeifard et al., 2018; Seif et al., 2021; Ashrostaghi et al., 2022; Lastochkina et al., 2022; Esmaeili et al., 2022). In addition, a wide range of signals and information for morphogenesis also other physiological processes are begun by light spectra (Chen et al. 2004). It is clear that artificial light sources are important in situations where natural light cannot be attained at the level that is needed by plants. In that situation, light-emitting diodes (LEDs) have a number of benefits over conventional lighting sources in agricultural conditions (Lastochkina et al., 2022). LEDs are superior to conventional light sources for use in plant-based applications due to their endurance, compact size, and ability to choose precise wavelengths for a desired plant response. (Massa et al. 2008; Ma et al. 2021). Besides, other important features of LEDs are low power usage, long-term use, low heat output, and making the best use possible by placing the LEDs closer to the plants (Miler et al. 2019). However, fluorescent lamps are versatile because they provide a broad spectrum of light (350-750 nm) applicable to plenty of plant species, despite the fact that, their disadvantages contain unsteady radiation parameters, significant heat emission, and high electricity consumption (Bello-Bello et al. 2017; Hasan et al. 2017)

There are plenty of studies focused on LEDs efficiency in the micropropagation of potatoes which are researched to obtain the effect of spectral conditions in recent years (Li et al. 2018; Pundır et al. 2021). Moreover, most studies have focused on a variety of narrow light spectra. Mainly red and blue lights corresponding to the two areas of the chlorophyll absorption curve, which are necessary absorption of light energy for photosynthesis (Al-Shareefi et al. 2020; Li et al. 2020). However, there was no previous research has been done on the production of potato microtubers with the new technology of LED lights. The current research aimed at studying the influence of light quality during *in vitro* culture on the formation of microtubers in three Polish cultivars of potato, i.e. 'Aruba', 'Oberon' and 'Skawa'.

## Material and Methods

### *Plant material*

Three Polish potato (*Solanum tuberosum* L.) cultivars were used in the experiment, i.e. 'Aruba', 'Oberon', and 'Skawa'. The plant material was provided by Hodowla Ziemniaki Zamarte Sp. - z o.o – Grupa IHAR in the form of *in vitro* plantlets. Plantlets were maintained in an *in vitro* gene bank of the Laboratory of Ornamental Plants and Vegetables, Faculty of Agriculture and - Biotechnology, University of Science and Technology, Bydgoszcz, Poland.

### *Multiplication of plant materials*

The determined number of plantlets was obtained by the multiplication single node method. Plant tissue culture media were based on components of the MS culture medium (Murashige and Skoog 1962). The pH was established to 5.8 with 0.1 M HCI and 0.1 M NaOH, after adding all MS media components.

The medium (40 ml) was poured into 350 ml glass jars and closed with plastic caps. Five explants were inoculated in each jar. The cultures were maintained under uniform lighting conditions. Light was provided by TLD 54 / 36W fluorescent tubes emitting standard cool daylight with a colour temperature of 6200 K (Koninklijke Philips Electronics N.V., Eindhoven, Netherlands), at photosynthetic photon flux density (PPFD) of 65 μmol $m^{-2}$ $s^{-1}$ and 16/8-hour light/dark photoperiod. The temperature was set at 23 ± 0.5 °C.

### *Microtuber formation*

After the multiplication phase, the plantlets were cut into single-node explants, and explants were transferred onto MS basal medium supplemented with a higher amount - 40g/$L^{-1}$ sucrose. The preparation and basal components of the medium were the same as described in the previous section. The PPFD was measured using an FR-10 photometer (Optel, Opole, Poland) at the central area of an empty shelf surface and was established for every tube type at 30 μmol $m^{-2}$ $s^{-1}$ and a 16/8-h light/dark photoperiod was applied. The temperature was set at 23 ± 0.5°C. The cultures were placed under the LED tubes emitting three spectra combinations, including AP67, AP673L and G2 (Valoya, Helsinki, Finland). The LED lamps were tube-shaped with T8 ends belonging to L-series Valoya horticulture lighting (Table 1).

**Table 1.** Applied LEDs spectrum details on the lighting recipes used in the present study

| Colour | Wavelength (nm) | LED Source | | |
|---|---|---|---|---|
| | | **AP67** | **AP673L** | **G2** |
| | | **Light composition** | | |
| **Ultraviolet (%)** | <400 | 0 | 0 | 0 |
| **Blue (%)** | 400-500 | 14 | 12 | 8 |
| **Green (%)** | 500-600 | 16 | 19 | 2 |
| **Red (%)** | 600-700 | 53 | 61 | 65 |
| **Far Red (%)** | 700-800 | 17 | 8 | 25 |
| **PAR (µmol $m^{-2}$ $s^{-1}$)** | 400-700 | 83 | 92 | 75 |
| **CCT (K)** | | 2500 | 2000 | N/A |
| **B:G** | | 1.2 | 1.8 | 25.9 |
| **R:FR** | | 3.3 | 5.5 | 3.1 |

PAR photosynthetically active radiation, CCT correlated color temperature, B:G blue:green light ratio determines the effectiveness of the blue light response, R: FR red:far-red light ratio determines the ratio between active phytochromes (Pfr) and inactive phytochromes(Pr), N/A not applicable

The spectral characteristics of lamps are given in Figure 1. The values were determined using a Lighting Passport Spectrometer (Allied Scientific Pro, Canada).

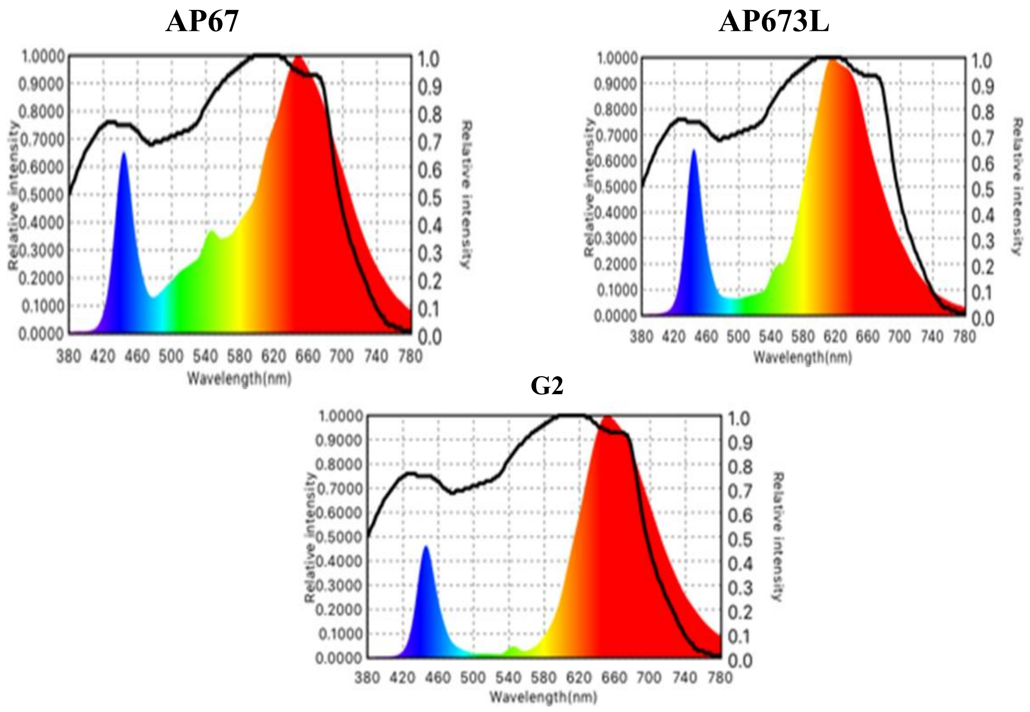



**Fig. 1.** Spectral characteristics of AP67, AP673L, and G2 lamps used in the experiment at the stage of microtuber formation of potatoes

The plants were carefully removed from the jars 25 weeks after subculturing, followed by the measurements. The total number of microtubers and the average number of microtubers per plantlet were determined after the microtubers were harvested. The average fresh weight of each microtuber was estimated. The volume of the microtubers was defined with a 50 ml measuring cylinder partially filled with water, into which individual microtubers were immersed, and the change in the volume reading was recorded.
Microtubers were placed on the calibrated scanner's trays filled with water and scan photos were taken. Based on the scan photos, the microtubers' cross-sectional area, average width, and length, as well as the perimeter was described with the application of WinFolia software (Regent Instruments, Canada).

### *Statistical analysis*

The experiment was organized as a completely uniform layout with five replication (jars) and each jar had five explants. The results were statistically analyzed with a two-way analysis of variance (ANOVA), and the comparisons of means were made with HSD Tukey's Multiple Comparison Test ($P \leq 0.05$) using Microsoft Excel.

## Results

The different light conditions affected the development and morphogenesis of only one cultivar. 'Aruba' produced microtubers under the AP67 light and had a significantly higher number of microtubers than the rest of the treatment groups (Fig. 2). Furthermore, the lowest number of microtubers was obtained under the AP673L spectrum. In the measurements made, it was determined that different light spectra did not have a significant effect on microtuber production in 'Oberon'. Meanwhile, 'Skawa' produced the microtuber only under the G2 light spectra.

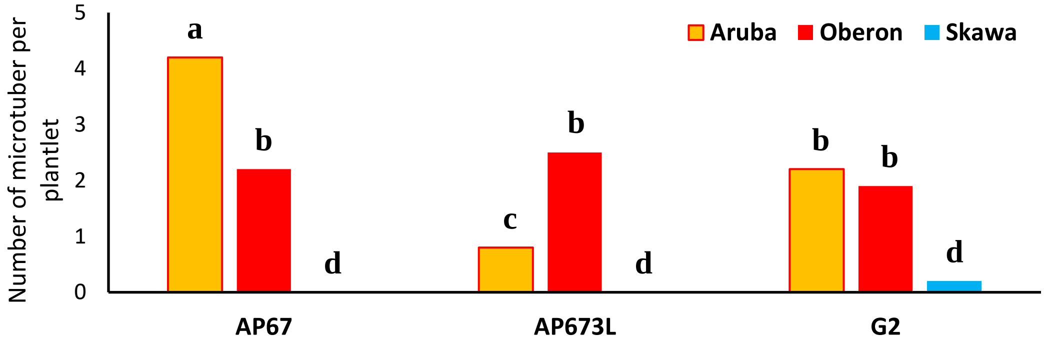



**Fig. 2.** Effect of different light spectra provided by AP67, AP673L, and G2 lamps (detailed information in Figure 1 and Table 1) on the number of microtubers per potato plantlet in the cultivars. Mean values followed by the same letter do not differ significantly at P<0.05 according to Tukey's test

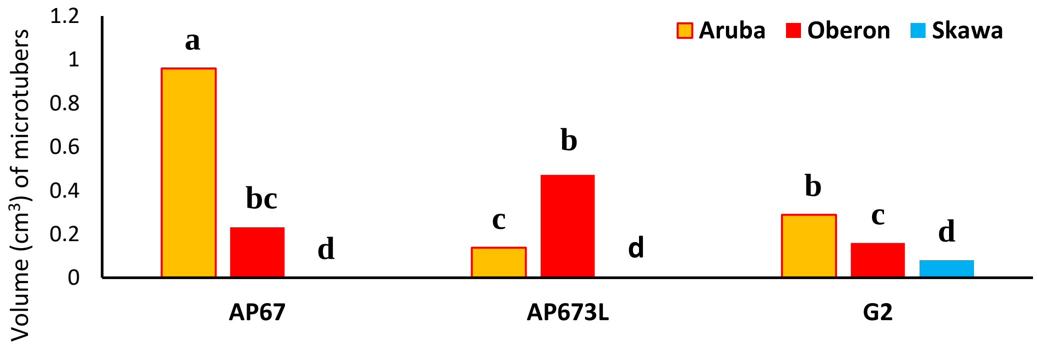



**Fig. 3.** Effect of light spectra provided by AP67, AP673L, and G2 lamps (detailed information in Figure 1 and Table 1) on volume (cm3) of microtubers in cultivars –Means followed by the same letter do not differ significantly at P<0.05 according to Tukey's test

LED light spectra had important effects on both cultivars in the calculation of the volume ($cm^3$) of microtubers (Fig. 3). Measurements were significantly higher in 'Aruba' under AP67 *in vitro*, whereas the largest volume of microtubers were obtained in 'Oberon' after AP67 and AP673L treatments.

Among the tested cultivars, the fresh weight (mg) of the microtubers had significant effects on the different light spectra of LEDs (Fig. 4). The greatest fresh weight (mg) of microtubers of 'Aruba' was reported under AP67 compared to AP673L and G2 treatments. Considering the 'Oberon', no significant effect occurred under any of the light spectra *in vitro*.

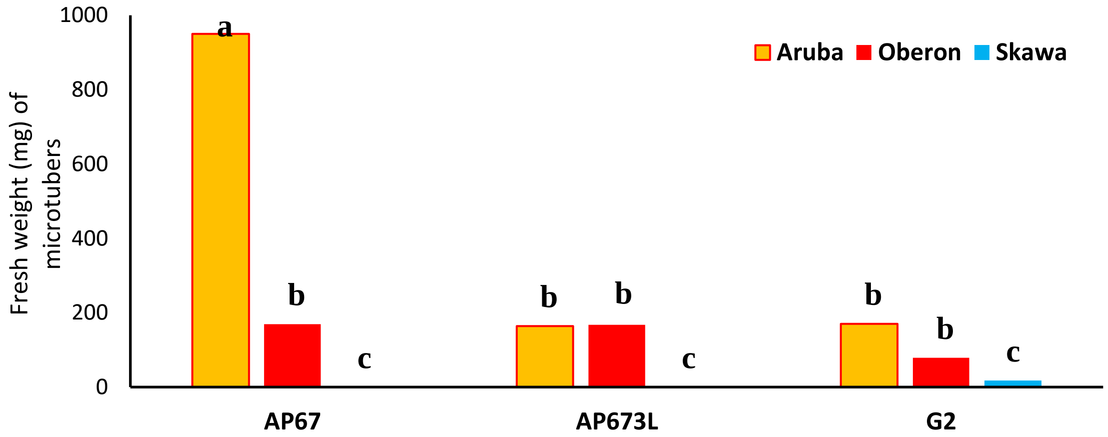
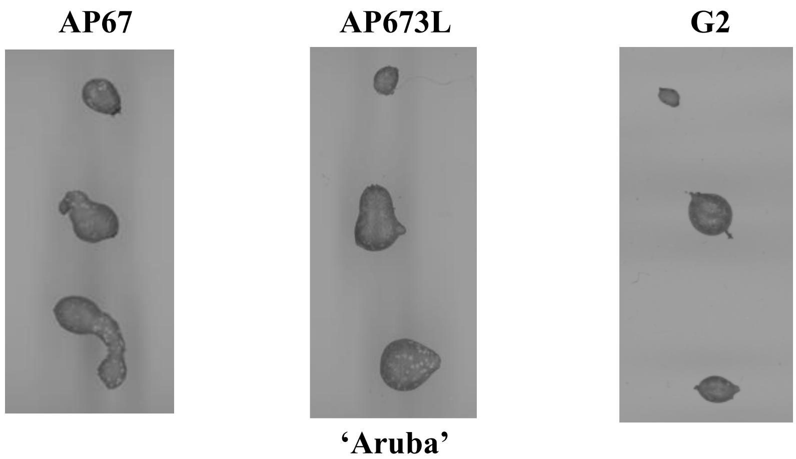



**Fig. 4.** Effect of light spectra provided by AP67, AP673L, and G2 lamps (detailed information in Figure 1 and Table 1) on fresh weight (mg) of the microtubers in the cultivars. Means followed by the same letter do not differ significantly at P<0.05 according to Tukey's test

While calculating the other parameters, the width, length, perimeter, and cross-sectional area of the microtubers were measured using the scanner program (Fig. 5).



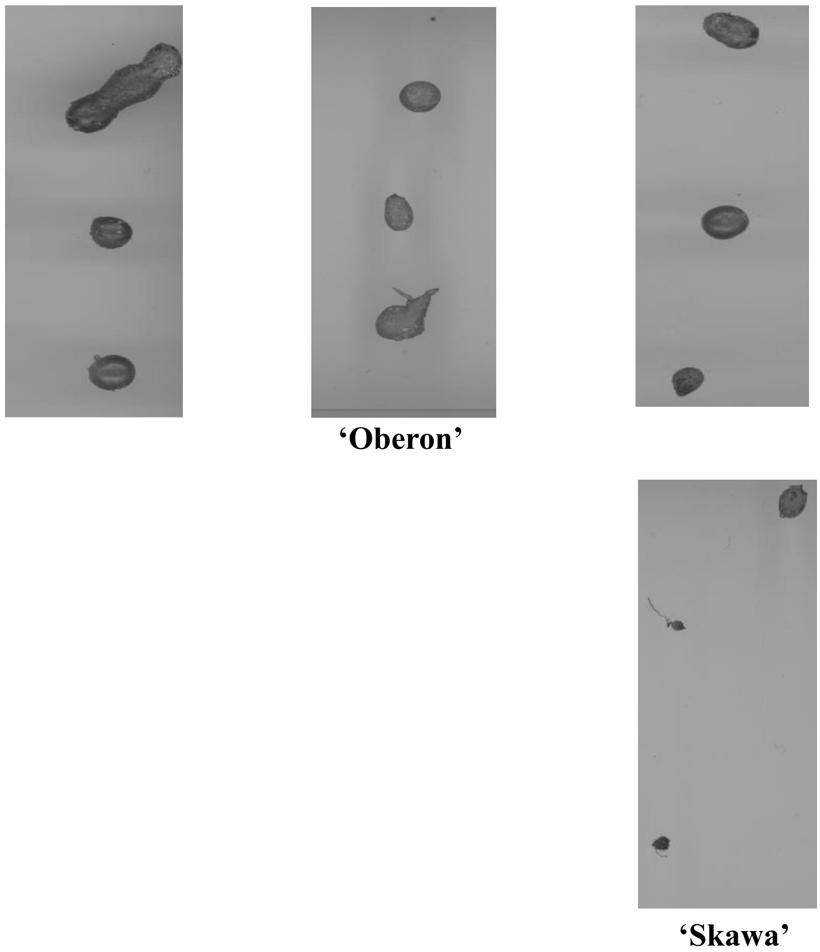



**Fig. 5.** Microtubers of 'Aruba', 'Oberon' and 'Skawa' formed under different light spectra provided by AP67, AP673L, and G2 lamps (detailed information in Figure 1 and Table 1).

Different light qualities after the harvest of 'Aruba' microtubers resulted in significantly higher width (mm) of MTs grown under AP673L, compared to all other LED treatments (Fig. 6). The results of 'Oberon' showed no significant effect emanating from any of the light conditions.

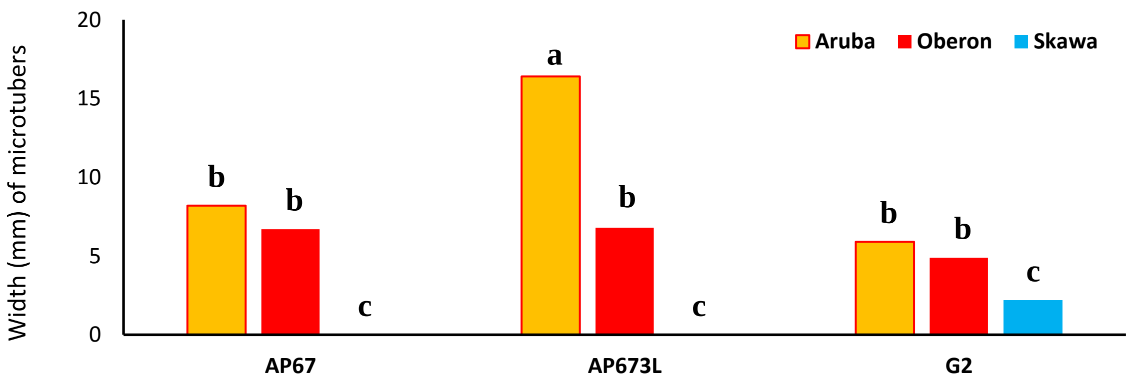



**Fig. 6.** Effect of light spectra provided by AP67, AP673L, and G2 lamps (detailed information in Figure 1 and Table 1) on width (mm) of microtubers –Means followed by the same letter do not differ significantly at $P<0.05$ according to Tukey's test

Light spectra did not significantly affect the length parameter (mm) of the microtubers. (Figure 7). Although the light spectra did not have a significant effect on the values of 'Aruba' and 'Oberon', the results depended on the cultivar.

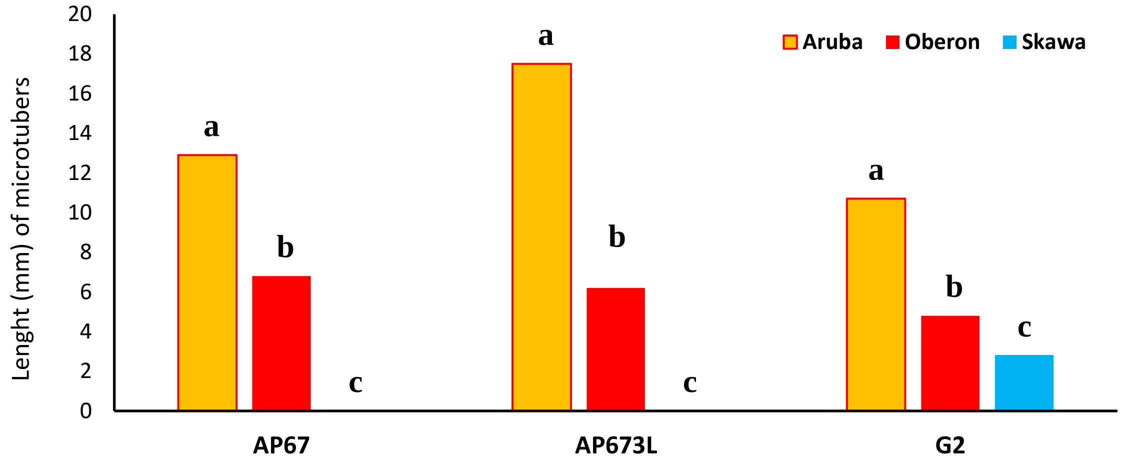



**Fig. 7.** Effect of light spectra provided by AP67, AP673L, and G2 lamps (detailed information in Figure 1 and Table 1) on length (mm) of microtubers –Means followed by the same letter do not differ significantly at $P<0.05$ according to Tukey's test

The LED spectra had no significant effect on the perimeter (mm) of microtubers in neither 'Aruba' nor 'Oberon' (Fig. 8). However, as with many other calculations, the results differed depending on the cultivars.

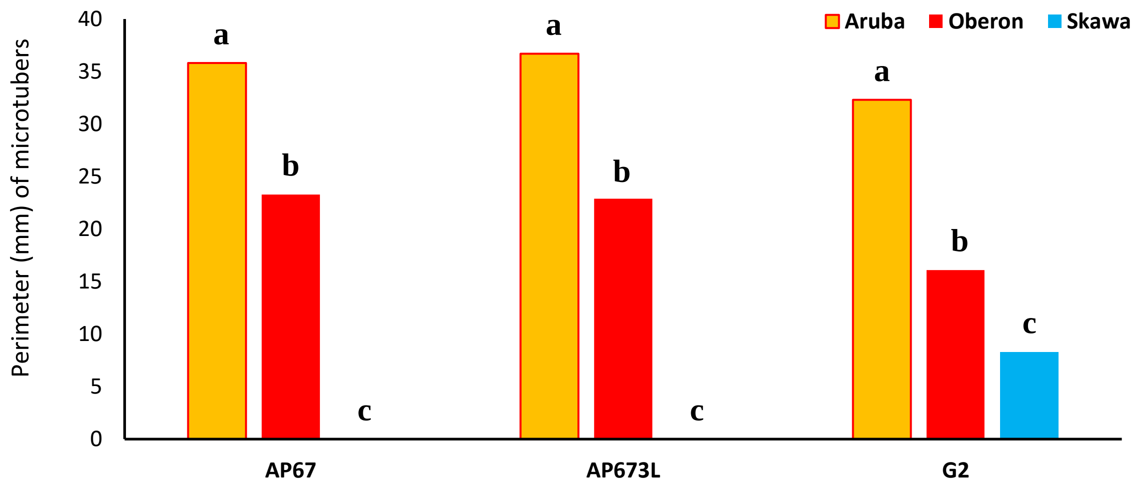



**Fig. 8.** Effect of light spectra provided by AP67, AP673L, and G2 lamps (detailed information in Figure 1 and Table 1) on the perimeter (mm) of microtubers in cultivars. Means followed by the same letter do not differ significantly at $P<0.05$ according to Tukey's test

As with some other measurements, the cross-section area ($mm^2$) of 'Aruba' and 'Oberon' was not affected by the different LED light spectra (Fig. 9). In addition, the significant difference between the results depended on the cultivars.

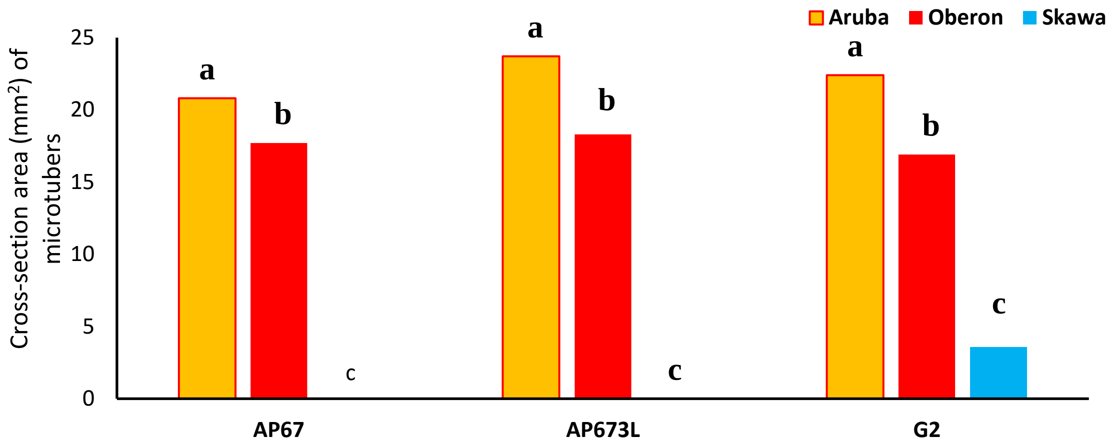



**Fig. 9.** The effect of light spectra provided by AP67, AP673L, and G2 lamps (detailed information in Figure 1 and Table 1) on the cross-section area (mm$^2$) of microtubers in cultivars. Mean values followed by the same letter do not differ significantly at P<0.05 according to Tukey's test

## Discussion

The light spectrum is one of the important environmental factors that influence the morphogenesis of plants. Mohamed Ali and Esmail (2011) observed that white and green lights effectively improved the yield of potato microtubers. In a recent study, AP67 and G2 lamps were predominantly composed of red and far red light and also consisted of green and blue light sources with similar percentages (Tab. 1). Likewise, Eftekhari et al. (2023) reported that red and blue light spectra were appropriately used along with exogenous GABA. They had a positive effect on both the number of saffron (*Crocus sativus* L.) flowers and their yield. Furthermore, Pelacho and Mingo-Castel (1991) reported that the yield of the microtubers was directly proportional to the quality of the potato seedlings. In our experiment, cultivars and LED light spectra had an impact on the number of microtubers per plantlet. According to the results, 'Aruba' had a significantly higher yield under the AP67 and G2 LED light spectra, while the effects of different light spectra on the measured efficiencies of 'Oberon' were not significantly different *in vitro* (Fig. 2). In this case, the different genotypes and LEDs light spectrum used in the research directly affected the results.

Potato microtubers require a different range of wavelengths in the LED light source during the growth period and, as determined, the largest type of microtubers occurred on the spectrum of R (%100 red) and B (%100 blue) LEDs under *in vitro* (Chen et al. 2018).

Furthermore, Aalifar et al. (2019) observed that blue light significantly improved plant parameters compared to other light spectra. Similarly, in our study, the LED light factor significantly affected the volume (cm$^3$) of microtubers. 'Aruba' and 'Oberon' proved to be more efficient than 'Skawa' under all LEDs *in vitro* (Fig. 3). The AP67 (moderate blue and red and low red:far-red) and AP673L (high red and high red:far-red). LED lights were significantly more effective than the G2. These results, along with other articles, were observed to improve the plant morphogenesis of blue and red light spectra.

Specific light treatments were beneficial to the fresh weight of the microtubers of potato plantlets under *in vitro* conditions. Chen et al. (2018) observed that the fresh weight (mg) of different cultivars of microtubers increased when grown under red (100% red) and white LEDs. The current results were also influenced by cultivars and the light spectrum of LEDs. The 'Aruba' and 'Oberon' proved to be more effective under *in vitro* AP67 and AP673L LED lamps (Fig. 4). Their fresh weights (mg) were significantly higher than the 'Skawa'. Based on these results, red and far-red spectrum LEDs light spectrum were effective in increasing the weight of microtubers in various genotypes. On the other hand, trying various hormonal combinations on potato microtubers by Dessoky et al. (2016) reported a low weight of microtubers obtained on MS media without growth regulators. Accordingly, if the appropriate type of LEDs and hormonal supplements in MS media are used, the desired level of microtuber can be obtained. These results showed that the effect of growth regulators and LEDs in microtuber formation should be taken into account.

In this work, light spectra had different effects on the cultivars. The highest width (mm) value was determined as AP673L light for ‘Aruba’ and, in addition, there was no significant difference between light spectra in ‘Oberon’. (Fig. 6). In this respect, ‘Aruba’ was several times wider than other cultivars. Besides, according to Asadi et al. (2017), potato microtubers morphogenesis and subsequent growth of microtubers were affected by diverse LEDs. This may suggest that the several cultivars and different light spectra created variable results.

It has been proven that the red LED light spectrum has a positive effect on potato microtuber production. Omar (2017) used different light spectra on potatoes, using two potato cultivars, and reported that microtubers growing under the light spectrum of red LEDs showed a significant increase in height. Nevertheless, we observed that the LED lights used herein were not significantly effective on the cultivars. However, the cultivar was the only factor affecting microtuber length (mm). ‘Aruba’ proved to be more productive with respect to many results. ‘Oberon’ had equivalent values (Fig. 7). All types of LED spectra had similar effects on cultivars. When considering the research, LEDs may have had various effects on dissimilar genotypes.

Studies have shown that red LED light spectra (100% red) have different effects on plants. Poudel et al. (2008) showed that grape cultivars cultured under red LED light spectra had longer plant height and length. Despite that, Chen et al. (2020) observed that potato plantlets grown using red LED (100 % red) produced the smallest tuber size and weight under greenhouse conditions. Regarding the perimeter (mm) of microtubers in our experiment, there was no significant difference between the effects of LED lights on the cultivar (Fig. 8). These results may be due to the difference between the genotypes and the variety of plants that responded differently to environmental conditions.

LED lights are reportedly effective on microtubers. Chen et al. (2018) indicated that the growth of microtubers may necessitate a variety of wavelengths in the LED light spectra. Many authors have discovered that microtubers can increase in size when exposed to various types of LEDs *in vitro* (Barceló-Muoz et al. 2022; Rahman et al. 2021). In our study, however, no significant difference was determined between the different light spectra on the cultivars (Fig. 9). The current study found that these LEDs had a similar effect on the cultivars in some parameters, which may explain the discrepancies among the results of various studies.

## Conclusion

The current research demonstrated that an appropriately selected LED light source promoted potato plant development and microtuber growth in a variety of parameters. The characteristics of *in vitro*-produced potato microtubers were affected by the light spectrum and cultivar (genotype). LEDs had a significant influence on the number of microtubers produced per plantlet, volume (cm3), fresh weight (mg), and width (mm) of the microtubers. In terms of microtuber production and fresh weight, the AP67 LED spectrum was the most effective, whereas the G2 spectrum was the least effective. In terms of *in vitro* microtuber yield, ‘Aruba’ and ‘Oberon’ outperformed ‘Skawa’. The microtuber size and fresh weight depended on the cultivar.

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