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Response of Potato Growth in *Vitro* to Led Spectral Exchange

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ABSTRACT

This study was conducted to explore the growth behavior of plants tissue culture in *vitro* using an optical spectrum system exchange. In this investigation, the 3rd generation in *vitro* plantlets of potato cv. "Diamond" were employed. The experiment included 5 treatments. The adventitious shoots of *S. tuberosum* (0.5–1 cm in length) were exposed in jars containing approximately 30 ml of the Murashige and Skoog (MS) salt medium. The cultures were kept under following LED lights: White, Yellow, Green, Red and Blue respectively for 4 days switch within every light, compared to continuous white light for the same period (20 days). Comparing to the white light every 20 days found that the third subculture was significantly higher than the second and the first, in most optimal spectrum LED treatment color, also most averages of growth treatment was higher under red and blue LED light. the contents of amino acids and proteins exhibited accumulation trend with variant degree depend on type of light used. Generally, the combined light had the highest accumulation of amino acids and proteins compared to monochromatic light. For monochromatic light, the blue light recorded the most accumulation of amino acids and proteins compared to other lights. Also, plantlets exposed to red and blue light had the maximum levels of pigments. High accumulation of α -tocopherol content in the order, blue>red>green>yellow>white. Blue light and plants subjected to multiple light cycles were found to have the most accumulation in anthocyanin.

Keywords: Led spectrum, tissue culture and potato plants



INTRODUCTION

Potato (*Solanum tuberosum* L.) plants is the most common widely consumed vegetable on the planet (ABBA, 2014) and a major source of carbs, fiber, and potassium for human use (El-Sherpiny et al., 2022; Abd El-Hady and Shehata, 2019; Hamaiel et al., 2021; Abd El-Hady et al., 2021). In Egypt, the potato occupies a considerable position in every vegetable crop, accounting for almost 20% of the total area dedicated to vegetable production. Meanwhile, the total developed potato territory was 176,670 fed., yielding 4,896,476 ton. (FAO, 2018).

Tissue culture is one of the most essential procedures for cultivating plant cells, tissues, or organs in an aseptic environment in order to research or genetically manipulate the plant (Espinosa-Leal et al., 2018). The explant is a portion of the plant that has been separated from its intact body and placed in or on a nutritional medium suited for its growth and propagation (Molnár et al., 2011). The nutritional medium has two purposes for the cultured plant cells' growth: first, it serves as a replacement for the natural plant's surrounding elements, and second, it serves as a supply of nutrients (Chimdessa, 2020). The ability of the plant cell to grow a whole plant from any explant, which is known as cellular totipotency of the plant cells (Fehér, 2019), is what the plant tissue culture relies on. The tissue culture nutritional media must meet all cell growth needs, which vary depending on the plant tissue employed (Bhatia, 2015). Energy sources, vitamins, amino acids, and growth regulators such as plant growth hormones are all included in standard medium (Leva and

Rinaldi, 2012). The pH of the media must be adjusted to meet the cell's development requirements, which is usually 5.7. It is possible to employ both liquid and solid media. Finally, the physical conditions include temperature, which should be kept constant during the experiment and should vary from 20 to 30 degrees Celsius (Arab et al., 2014; San Pedro et al., 2017).

In comparison to traditional lighting sources such as incandescent and fluorescent bulbs, LEDs have a longer life cycle and are more solid, making them more versatile for installation and manipulation (Chang et al., 2012). LEDs are used in advanced indoor horticulture systems to improve plant productivity, crop output, and nutrient content in plant products. Lighting systems are the primary source of light in indoor growing systems or enclosed planting circumstances; thus their features should be improved to achieve the greatest lighting results for efficient plant growth. LEDs have these properties, making it possible to give the necessary radiant light near plants while still conserving energy.

In *vitro* propagation, although fluorescent lights are considered the traditional source of light, it is costly and usually produces a wide range of wavelengths unsuitable for plant growth. By providing optimal illumination conditions, maximum chemical buildup can be achieved. Therefore, the purpose of our investigation was to compare the interchange of traditional light [545–610, nm] with yellow LEDs (585-600nm), red LEDs (660 nm), blue LEDs (460 nm), and green LEDs (520-555 nm) during potato micropropagation.

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MATERIALS AND METHODS

The present experiment was conducted between August and November in 2020. The 3rd generation *in vitro* plantlets of potato (*Solanum tuberosum*) cv. "Diamond" used in this study were conducted at the Plant Tissue Culture Laboratory, Scientific and Technological Applications Unit, Al-Azhar University Assiut branch, Assiut, Egypt to explore the behavior of plants tissue culture *in vitro* using an optical spectrum system exchange.

As the experimental substances, adventitious shoots from the axillary buds of *S. tuberosum* were gotten from the Technological Applications Unit (TAU), Al-Azhar Univ., Egypt.

Optical spectra system traits were executed in an incubation room at 80 ± 5% (RH), (16 h light per 8.0 h dark as photoperiod) and 25 ± 2 °C. In this investigation, the optical spectra sources were as follows:

1. Traditional light FL (fluorescent light as Control): (wavelengths at 400–700, nm).
2. R: Red LEDs (wavelength at 660, nm)
3. B: Blue LEDs (wavelength at 460, nm).
4. Y: Yellow LEDs (wavelength at 585-600, nm).
5. G: Green LEDs (wavelength at 520-555, nm).

Table A shows the description of potato diamond variety. MS medium (Murashige and Skoog, 1962) supplemented with 3 % (w/v) sucrose, 1.5 mg/L BAP (Benzyl Amino Purine) hormone for growth with 0.1 mg/L IBA (Indole-3-Butyric Acid) hormone for induce rooting, 100 mg/L myo-inositol, and 100 mg/L Ferrum were used in the shoot induction culture media. The pH was set between 5.7 and 5.8. The medium was then solidified with 2.5 g L⁻¹ Gelrite™ (Sigma-Aldrich, Merck Egypt: Agitech) before being autoclaved for 15 minutes at 121 °C and 124 kPa.

Table A. Description of potato Diamond variety:

Variety	Diamond
Maturity	Med Late 110-115 day
Shape	Oval
Size	Big
Color	Yellow cortex, creamy flesh
Productivity	12-15 tuber/plant
Dry matter	23.6%

The experiment included 5 treatments were conducted as factorial based on completely randomized design (CRD) with five replications and each replication included five jars with 3 plants per each one. The

Table 1. Effect of optical spectra exchange on *in vitro* explant development of potato plant in the three subcultures studied periods.

Char. Treat	Multiplication rate	Shoot length cm	Number of leaves	Plant fresh weight g	Number of roots	Root length cm	Number of micro tubers	Weight of micro tubers g
Subcultural 1	(Control)	13.15	3.48	9.62	21.90	1.36	1.02	0.90
	Whit light	±1.939	±1.070	±3.042	±0.115	±0.799	±0.586	±1.111
	Optical Spectra Exchange	31.02 ±0.165	11.71 ±0.738	21.62 ±0.563	24.51 ±0.399	2.67 ±0.297	2.16 ±0.139	9.63 ±0.208
Subcultural 2	(Control)	29.97	6.93	15.87	33.52	2.62	2.18	1.70
	Whit light	±2.168	±2.360	±3.195	±1.301	±1.206	±1.559	±1.353
	Optical Spectra Exchange	48.68 ±0.234	15.02 ±0.488	24.04 ±0.267	37.64 ±3.296	5.44 ±0.182	3.07 ±0.317	12.65 ±0.306
Subcultural 3	(Control)	62.11	14.15	20.48	67.50	5.50	5.35	1.90
	Whit light	±5.775	±5.164	±4.743	±1.32	±3.916	±2.542	±1.353
	Optical Spectra Exchange	82.13 ±1.077	29.25 ±0.639	36.33 ±0.946	75.21 ±0.210	12.40 ±0.225	6.95 ±0.408	13.33 ±0.481

The most significant aspect of a micropropagation procedure is its effectiveness, which is determined by the

adventitious shoots of *S. tuberosum* (0.5–1 cm in length) were exposed in jars containing approximately 30 ml of the MS salt medium. the cultures were kept under following LED lights: White, Yellow, Green, Red and Blue respectively for 4 days switch within every light, compared to continuous white light for the same period (20 days).

At the end of the intervals the parameters investigated were, multiplication rate (MR) were calculated as the difference in mean shoot number between the mean numbers of shoots derived before and after subculture from one culture at the end of each passage, shoot length cm (SL), number of leaves (NL), number of roots (NR), root length cm (RL), plant fresh weight g (PFW), number of micro tubers (NMT) and weight of micro tubers g (WMT).

The fractions of pigments (chlorophyll a, chlorophyll b and carotenoids) were estimated using the spectrophotometric method recommended by (Lichtenthaler, 1987). Protein content was measured depending on (Lowery, 1951), and the Folin solution was used as a guide for protein detection. Total amino acid in leaves was determined according to (Moore and Stein, 1948). Anthocyanin pigments were determined according to (Krizek et al., 1993). α - Tocopherol was estimated following to (Kivcak and Mert, 2001).

ANOVA was used to analyses the data, which corresponded to a completely randomized design (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The following experiment was conducted to get the advantages of each light, alternate between lights every 4 days for 20 days beginning with white light ending with the blue light comparing with white light .

Data presented in Table 1 that the optical spectrum LED lighting effects on *in vitro* plantlets were varied depending on cultivation period. Comparing to the white light every 20 days found that the third subculture was significantly higher than the second and the first, in most optimal spectrum LED treatment color, also most averages of growth treatment was higher under red and blue LED light.

Light quality pronouncedly influences the success of tissue culture system. The control of the light quality that part of the spectrum zones are involved in various operations enables production of potatoes plants with desired parameters (Bantis et al., 2016).

number of explants obtained from a single donor plant, such as micro shoots and single-nodes (the So-called

propagation ratio). Previous research has found that light quality has a species-specific effect on this parameter (Gupta and Jatothu, 2013).

As a result, identifying a single light spectrum suited for the propagation of all plant species is difficult. Bello-Bello *et al.* (2016) found that blue and red LED light alone inhibited the proliferation of *Vanilla plan folia* Andrews, whereas white LED, a combination of blue and red LED, and FL offered the best propagation ratio. The propagation ratio of *Panax vietnamensis* was observed to be two times higher under red:blue LED light in a 60:40 ratio than under FL 11.21 and 5.8, respectively (Nhut *et al.*, 2015). FLs, white LED, and blue LED light, on the other hand, improved the propagation ratio of *G. jamesonii* 'Rosalin' (Gök *et al.*, 2016). The favorable influence of LED light on the in-vitro growth and development of the examined potato cultivars was confirmed by these findings. Red and blue lights, according to (Folta and Maruhnich, 2007), cause faster plantlet growth than white light, but green light, which is absorbed by phytochromes and cryptochromes, influences events that limit vegetative development. In light of the aftereffects of bud number per plantlet and plantlet length, Omar (2017) found that different light sources altered potato plantlet development: red and green LEDs were the most and least prescribed for plantlet advancement, respectively. The stems of potato plantlets developed in vitro under pure red light exhibited shorter cells than those grown under blue light, according to histological examinations (Wilson *et al.*, 1993). This could explain R and B's height performance in our experiment. Phytochrome and cryptochrome absorb red (600–700 nm) and blue (400–500 nm) light, respectively (Smith, 1993).

During the in vitro rooting, the light quality possesses a differential influences on the root system, with the major effect being a raise in the quantity and length of plant roots per explant (Kwon *et al.*, 2015). LEDs affected on the number of roots /explant and root length as for potato plants as conventional FL light. In addition to, with chinese foxglove grown under blue LEDs. Our results are in harmony with those of Manivannan *et al.*, (2015) who obtained a larger number of roots.

The formation of potato microtubes is a complex process that is influenced by a variety of circumstances. Gibberellic acid (GA), a plant hormone, is a vital factor in potato tuber induction and initiation (Roumeliotis *et al.*, 2012). The formation and growth of microtubes were mediated by light spectral quality, which regulated hormone concentrations, particularly GA. Potato tuber induction and initiation are hampered by high levels of GA. Blue light suppressed tuberization of day-neutral potatoes by increasing GA production and accumulation, according to previous study (Fixen *et al.*, 2012). This could explain the micro tuberization seen in blue light. Plant organs' sink capacity is thought to be enhanced by IAA (Kara *et al.*, 1997). Under red light, the content of IAA in potato plantlets rose (Segeeva *et al.*, 1994). As a result, the increased concentration of IAA may encourage assimilates to flow into the tubers. That could explain why Red Light had a higher amount of microtubes. Micro tuber formation may necessitate a wide variety of light wavelengths. That could explain why the combined spectrum of red and blue LEDs contained the majority of big micro tubers.

The present work in Tables 2 we used red, blue, green, yellow, and white LEDs in cycles to detect their role for morphological and developmental changes, as well as physiological changes also occurred accordingly.

Table 2. Effect of optical spectra exchange on physiological parameters of potato plant.

Char. Treat	Soluble protein mg/g DW	Amino acids mg/g DW	α -Tocopherol	Anthocyanins μ mol/ gm FW	Chlorophyll a mg/g FW	Chlorophyll b mg/g FW	Carotenoids mg/g FW
(Control)	9.12	2.12	555.10	4.16	1.010	0.375	0.820
Whit light	± 0.639	± 0.260	± 19.744	± 0.291	± 0.125	± 0.045	± 0.101
Optical Spectra Exchange	11.44	4.94	849.76	5.89	1.887	0.438	0.944
	± 0.846	± 0.365	± 30.228	± 0.435	± 0.139	± 0.032	± 0.070

In the present study, the contents of amino acids and proteins exhibited accumulation trend with variant degree depend on type of light used. Generally, the combined light had the highest accumulation of amino acids and proteins compared to monochromatic light. For monochromatic light, the blue light recorded the most accumulation of amino acids and proteins compared to other lights. The following pattern emerged in the augmentation of amino acids and proteins: blue>red>green>yellow>white light. According to Kowallik (1982), blue light increases dark respiration and organic acids produce amino acids during dark respiration, resulting in increased protein synthesis. Blue was good for protein accumulation in potato plantlets in vitro, according to Chang *et al.*, (2009), and shorter wavelengths of the same spectrum were favourable for protein accumulation, according to Johkan *et al.*, (2010), which was consistent with our findings. Blue light at 445 nm was found to be more advantageous for protein accumulation in potato

plantlets in vitro than blue light at 465 nm, according to Li *et al.*, (2018).

This is in line with our findings that plantlets exposed to red and blue light had the highest levels of chlorophyll a, chlorophyll b, and carotenoids. The Chl content of potato plants exposed to the individual or combined spectra of R and B was reported to be much higher than that of plants exposed to monochromatic and fluorescent light, similar to our findings (Kim *et al.*, 2004 and Wang *et al.*, 2015).

In the present study, the accumulation of tocopherol in response to different light intensities is a major cause of chlorophyll biosynthesis stability. The data of present work denoted high accumulation of α -tocopherol content in the order, blue>red>green>yellow>white and further accumulation was denoted for the combined light treatments. The activation of combined light treatments and more especially blue light for various metabolic

products produce vigorous plantlets, higher tuberization rate as well as high tuber numbers.

Anthocyanins, a key component of secondary metabolites, were found to be increased by the LEDs. Anthocyanins are water-soluble glycosides and acyl glycosides of anthocyanidins, a phenolic chemical class found in nature (Lian *et al.*, 2019). Blue light and plants subjected to multiple light cycles were found to have the most accumulation. Anthocyanins are induced by visible, ultraviolet, and various forms of LED lights in general (Hasan *et al.*, 2017). For *Gynura procumbens*, similar increases in phenolics, flavonoids, and anthocyanins were observed under various LEDs, with the biggest effect occurring when calli was exposed to blue light rather than red or white light (Lian *et al.*, 2019).

Plant photosynthetic pigments are responsible for light energy absorption, transport, and transition. Plants' basic element for photosynthesis is chlorophyll. The photosynthetic rate is directly influenced by the chlorophyll content of leaves. Chl a, Chl b, and carotenoids were found to be elevated in monochromatic light green, red, and blue, but considerably more so in the blue color. For the combined light, more improvements in photosynthetic pigments were reported. The gene involved in Chl production was increased in grape plantlets grown in vitro under red, blue, green, and white LEDs, according to RNA-Seq research (Lekham *et al.*, 2016).

CONCLUSION

This study confirmed that the various optical spectrum lights possesses positive impacts on the growth and development of in vitro potato plant growth. The utilization of LED lights may be very beneficial to solve many issues that occur frequently in the in vitro culture. So, incubation of micro-propagation plants in the white, yellow, green, red and blue spectra, respectively, for a period of 4 days, in each of them, stimulates morphological and physiological characteristics and increases the formation of micro-tubers in potatoes.

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استجابته نمو نبات البطاطس في المزارع تحت النسيجية الى التبادل بين الاطيفاء الضوئية لمصابيح الليد
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الملخص

أجريت دراسة بهدف استكشاف سلوك نمو نباتات زراعة الأنسجة تحت نظام التغير في الأطياف الضوئية معملياً. تم استخدام براعم بطيئة بطول (1.0-0.5 سم) من المزارع تحت نسيجية الثالثة لنبات البطاطس صنف "دياموند"، وتم وضعها في برطمانات تحتوي على 30 مل من بيئه MS. اشتملت التجربه على 5 معاملات في 5 مكررات كل مكرره تحتوي 5 برطمانات بكل منها ثلاث نباتات. تم الإحتفاظ بالمزارع تحت كل ضوء (أبيض، أصفر، أخضر، أحمر و أزرق) بصورة متتاليه لمدة 4 يوم بالتبادل بينهم مقارنة مع الضوء الابيض لمدة 20 يوم. أشارت النتائج بحدوث تأثير نتيجة التعرض للأطياف الضوئية اعتماداً على فترة التعرض للضوء مقارنة مع التعرض للضوء الابيض لمدة 20 يوم حيث وجد زياده معنويه كبيره بتقدم المزارع النسيجية من المرحلة الأولى حتى الثالثه. جميع صفات النمو سجلت أعلى القيم تحت الضوء الأحمر والأزرق. بينما أظهرت محتويات الأحماض الأمينية والبروتينات اتجاه تراكم بدرجات متفاوتة تعتمد على نوع الضوء المستخدم. بشكل عام سجل الضوء المركب (الأبيض) أعلى تراكم لمحتوى الأحماض الأمينية والبروتينات مقارنة بالضوء أحادي اللون. بالنسبة للتبادل بين الأضواء، سجل الضوء الأزرق أعلى معدل لتراكم الاحماض الأمينية و البروتينات مقارنة مع الأضواء الأخرى. كذلك تحتوي النباتات المعرضة للضوء الأحمر والأزرق على أعلى محتوى من كلوروفيل أ وكلوروفيل ب والكاروتينات. تراكم مرتفع لمحتوى α -tocopherol بالترتيب في الأزرق < الأحمر < الأخضر < الأصفر < الأبيض. وُجد أن الضوء الأزرق والنباتات التي تعرضت لدورات ضوئية متعددة هي الأكثر تراكمًا في الأنثوسيانين.