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ORIGINAL ARTICLE

In Vitro Response of Two Grape Varieties to White, Blue, and Red LED Treatments

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Abstract

The use of artificial lighting increases productivity and quality and is becoming more important in agriculture, especially in closed areas where natural light is insufficient. In in vitro propagation, the source, color, and quality of the artificial light are as important for healthy plant growth as the preparation of the nutrient medium and the presence of a healthy donor plant. In this study, the effects of light quality (white, red, and blue LEDs) on plant growth, chlorophyll content, electrolyte leakage (EL), MDA, and antioxidant enzyme activity were investigated in 'Michele Palieri' and 'Italia' grape varieties under in vitro conditions. Blue LED treatment supported shoot elongation, fresh-dry weight, chlorophyll a/b ratio, and the SPAD index, while a positive effect of white LED treatment was observed in terms of increasing chlorophyll content and carotenoids. The varieties showed variable responses to antioxidant enzyme reactions. In the 'Michele Palieri' grape variety, red LED light was more effective for increasing total soluble protein content, white and blue LEDs were more effective in terms of SOD enzyme activity, and white LED light was more effective for increasing APX enzyme activity. In the 'Italia' grape variety, blue LED treatment was effective in increasing total soluble protein content, white LED light was effective in increasing SOD enzyme activity, and red LED treatment was effective in increasing APX enzyme activity. As a result, it was revealed that different quality LED light treatments have different effects on yield and quality among grape varieties.

Keywords Antioxidant enzyme activity · 'Italia' · 'Michele Palieri' · Chlorophyll content · MDA

Introduction

Light is one of the environmental factors necessary for healthy plant growth and development in plant production (Hernández and Kubota 2016). The use of artificial lighting is gaining more importance in agriculture because it increases yield and quality, as well as enabling cultivation especially in indoor areas where natural light is insufficient. Light-emitting diodes (LEDs), a type of artificial lighting, are currently used in plant production and research. Light-emitting diodes can be tuned to a specific spectral range, have a long life, are durable and small in size, and, most importantly, have alternative usage possibilities, e.g., because they stay cool.

The LEDs used in plant production have been used to regulate various morphological and physiological characteristics and photosynthetic abilities of in vitro grown plantlets, including growth characteristics, plant nutrient content, antioxidant enzyme activities, total flavonoid content, phenolic acids, leaf structure and anatomy, and chlorophyll content fluorescence, especially by adjusting specific wavelengths (Wang et al. 2009; Simlat et al. 2016; Zhao et al. 2020).

Light-emitting diodes, which allow accurate control of changes in the spectral composition of light, have begun to be frequently preferred to improve plant production and quality due to their effectiveness in different physiological processes from photosynthesis to secondary metabolism (Dueck et al. 2016; Lazzarin et al. 2021).

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It has been stated that using a suitable light source is necessary for plant regeneration and in vitro metabolite synthesis to be successful in tissue culture, and that providing appropriate light intensity is one of the most fundamental problems (Gupta and Agarwal 2017; Samuolienė et al. 2017). Studies have found that the anatomical, morphological, physiological, and transcriptomic properties (leaf properties, shoot properties, rhizogenesis, organogenesis, photosynthetic properties, etc.) of plants grown in in vitro culture are regulated according to the spectral distributions of LEDs (Gupta and Jatothu 2013).

In addition, LEDs are preferred for improving plant growth, nutritional quality, and yield, because they provide optional light quality. A systemic approach needs to be developed for more efficient use of LEDs in plant production. The development of a species-specific light recipe to optimize LED lighting has great potential for increasing yield and quality in plants (Al Murad et al. 2021).

Studies investigating the reactions of varieties to different LED lights, especially in in vitro conditions, are limited, and no studies comparing varieties have been found. This study aimed to determine plant growth and development parameters, chlorophyll quality, and the physiological and enzymatic activity of white, red, and blue LEDs in 'Michele Palieri' and 'Italia' grape varieties in vitro, and to determine their reactions on a variety basis.

Materials and Methods

Plant Materials, in Vitro Propagation, and Light Treatment

In the current study, 'Michele Palieri' and 'Italia' table grape varieties were propagated in vitro. To obtain the explants used in the study, cuttings of 'Michele Palieri' and 'Italia' varieties were obtained from the vineyard of Tokat Gaziosmanpasa University Agricultural Application and Research Center. Cuttings of 'Italia' and 'Michele Palieri' grape varieties were kept in storage at +4 °C until the shooting process. The cuttings were kept in water in the climate chamber to form shoots, and micro-cuttings containing one active bud taken from the formed shoots were used as explants. Surface-sterilized explants were prepared to have a 0.5 cm long axillary bud. To prepare the MS basic nutrient medium, 3% sucrose, powdered ready MS medium (Murashige and Skoog 1962; Sigma, M5519 C), and 0.5 mgL^{-1} BAP (6-benzylaminopurine, B3408, Sigma) were used. After adjusting the MS medium to pH 5.8, 7 gL^{-1} agar was added to the medium. The nutrient medium was sterilized in an autoclave at 121 °C and 1.05 atm pressure for 15 min before use. For planting, 15 ml of nutrient medium was placed in test tubes and one explant was planted in each tube. After the micro-cuttings had been planted, they were brought to the growth chamber, which was set at 24 ± 2 °C and had a photoperiod of 16/8 (light/dark). After 3 weeks, micro-cuttings obtained from micro-shoots were subcultured. MS medium was prepared with 3% sucrose, 0.5 mgL^{-1} BA, and 7 gL^{-1} bacto agar. Micro-cuttings were brought to the plant growth room with a temperature of 24 ± 2 °C and a photoperiod of 16/8 (light/dark) for 4 weeks. The light sources used in the experiment were white, blue, and red LEDs. White LED light had a photosynthetic photon flux (PPF) of 4320 lumen (lm), blue LED light had a wavelength of 450 nm and a PPF of 34 µmol/s (Planktekno brand, PLO90018D120ECC model), and red LED light had a wavelength of 660 nm and a PPF of 34 µmol/s (Planktekno brand, PLO90018D120ECC model).

Determination of Growth Traits

Due to the intensive use of rootstocks in viticulture today, explants were not rooted in the study and only shoot data were taken. The shoot lengths of the plantlets were measured in millimeters with a digital caliper. The fresh-dry weights of the shoots of the plantlets were measured in grams on a digital scale with a sensitivity of 0.001 g, and the dry weights were measured after the shoots had been dried in an oven at 65 °C for 72 h.

Determination of SPAD Index and Chlorophyll Content

The SPAD index, determined by chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan), of each leaf was measured to reveal the dynamic effects of leaf chlorophyll content after 4 weeks of LED treatments.

In order to determine the concentration of chlorophyll-a (chl a), chlorophyll-b (chl b), total chlorophyll (total chl), chlorophyll a/b (chl a/b), and carotenoids, leaf samples belonging to the varieties 'Italia' and 'Michele Palieri' grown under in vitro conditions were studied: 0.1 g of fresh leaf sample was weighed on a precision scale and 10 ml of 80% (v/v) acetone was added. It was kept in a dark place without sunlight for 24 h. The absorbances of the prepared samples were measured at 480, 645, and 663 nm wavelengths (A480, A645, A663) on a UV-Vis spectrophotometer (Model T60U, PG Instruments). The amounts of chl a, chl b, total chl, chl a/b, and carotenoids in fresh leaves were calculated as indicated in the formulas below, and the analysis results were expressed as mg/g fresh weight (FW) in the plant (Witham et al. 1971).

$$\text{Chl a } (mgg^{-1}) = [(12.72 \times A663 - 2.59 \times A645) \times v]/(1000 \times W),$$

$$\text{Chlb}(\text{mgg}^{-1}) = [(22.88 \times \text{A645} - 4.67 \times \text{A663})]/(1000 \times \text{W}),$$
$$\text{Total Chl } (\text{mgg}^{-1})\text{FW} = [(20.2 \times \text{A645}) + (8.02 \times \text{A663})] \times \text{v}/(1000 \times \text{W}),$$
$$\text{Clh(a/b)} = (\text{Chla}/\text{Chlb})$$
$$\text{Carotenoid } (\text{mg.g}^{-1}) = (1000 \times 470 - 2.05\,\text{Chla} - 114.8 \times \text{Chlb})/245,$$

where v is the total volume of the acetone extract (mL) and W is the FW (g).

Determination of Electrolyte Leakage (EL, %), MDA (Lipid Peroxidation), and Enzymatic Activity

To determine electrolyte leakage (EL) in 'Italia' and 'Michele Palieri' grape varieties under different LED treatments, 0.3 g of leaf sample was placed in a test tube and then 15 ml of pure water was added before incubation for 24 h in a shaker at 100 rpm under room conditions. After incubation, the electrical conductivity (EC1) of the solution was measured using an EC meter. Then, the same samples were autoclaved at 121 °C for 20 min. When the sample temperature dropped to 25 °C, the electrical conductivity (EC2) value of the solution was measured again. The EL in the leaves was calculated as EC1/EC2 and expressed as percentage (%).

Malondialdehyde (MDA) content was determined according to the method of Heath and Paker (1968). Homogenization of the leaf sample was carried out by adding 1% TCA to 0.4 g of leaf sample. The homogenate was centrifuged (15000 rpm/15 min) and 0.5 ml of supernatant was taken to continue the process. Then 1 ml of 20% TCA (containing 0.5% TBA w/v) was added to the supernatant and incubated in a water bath at 95 °C for 30 min, whereafter the reaction was terminated with a cold-water bath. The samples were then centrifuged (15 min at 1000 rpm). Supernatant absorbance values were measured separately at 532 and 450 nm. Following error corrections, the MDA content of the samples was calculated by using an extinction coefficient of 155 mMcm^{-1}.

Total protein content as well as APX and SOD activities were determined by the method of Mohammadi et al. (2021), and care was taken to not disrupt the cold chain during the analysis. For this purpose, 0.5 g of leaf sample from each treatment was homogenized, phosphate buffer containing 1% PVP and 2 mM Na_2EDTA was used, and the homogenate was prepared with this mixture. The mixture obtained by homogenization was centrifuged at 5000 rpm for 20 min at 4 °C to prepare supernatants. Total soluble protein content was determined by the Bradford (1976) method: 20 µL of the supernatants from each treatment were mixed with 1 ml of Bradford solution, and the absorbance of the reaction mixture was read at 595 nm using a UV-Vis spectrophotometer within 5 min. The results were reported as mgg^{-1} FW. The method of Nakano and Asada (1981) was used to determine APX activity. The reaction mixture was prepared from 50 µL of crude enzyme extract, 25 µL of H_2O_2 (0.05 M), and 10 µL of phosphate buffer (pH 6.8, 0.1 M), and this mixture was incubated at room temperature for 5 min. Then, 25 µL of sulfuric acid solution (5% w/v) was added and the absorbance was recorded at 290 nm at 15-s intervals for 2 min. The APX activity was determined as Umg^{-1} protein, and SOD activity was determined based on the photoreduction of nitroblue tetrazolium according to Sirhindi et al. (2016). The absorbance of the prepared samples was measured at 560 nm and expressed as Umg^{-1} protein.

Statistical Analyses

The experiment was set up with three replications and 15 plants in each replication. After the data obtained in the experiment had been analyzed with variance analysis (ANOVA), the significance of the differences between the treatment means was determined with the Duncan multiple comparison test (at $p \leq 0.05$ and $p \leq 0.01$ levels).

Table 1 Effects of LED treatments on growth traits

Variety	Light treatment	Shoot length (mm)	Fresh weight (g)	Dry weight (g)
'Italia'	White LED	43.195 A-a	0.656 B-a	0.073 B-a
	Red LED	39.693 A-a	0.780 A-a	0.116 A-a
	Blue LED	46.932 A-a	0.790 A-a	0.083 B-a
'Michele Palieri'	White LED	44.075 A-a	0.583 AB-a	0.036 B-b
	Red LED	39.760 A-a	0.470 B-a	0.040 B-b
	Blue LED	38.752 A-a	0.726 A-a	0.080 A-a

*The difference between the means shown in capital letters in the same line for each variety is not significant ($p < 0.05$ level)
**The differences between the means shown in lower-case letters in the same column for each variety are not significant ($p < 0.05$ level)

Fig. 1 Images of the development of the varieties after the treatments. **a** 'Italia' white LED; **b** 'Italia' red LED; **c** 'Italia' blue LED; **d** 'Michele Palieri' white LED; **e** 'Michele Palieri' red LED; **f** 'Michele Palieri' blue LED

Result and Discussion

Growth Traits

In the present study, the influence of light quality on the growth traits of 'Italia' and 'Michele Palieri' grape cultivars cultured in vitro was investigated (Table 1). Images of the development of the varieties after the treatments are given in Fig. 1. Except for shoot length, the other two growth parameters showed statistically significant differences under different LED treatments. In terms of fresh weight, the increasing effect of blue LED light was observed in both varieties. In 'Michele Palieri' grape variety, the decreasing effect of red LED light on fresh weight was remarkable compared to white and blue LED treatments. When dry weight was examined (Table 1), the increasing effect of blue and red LED treatments were observed. Red LED treatment had an increasing effect on dry weight in 'Italia' grape variety compared to other treatments.

Li et al. (2017) investigated the growth and development parameters under different LED lights in the 'Manicure Finger' grape variety and stated that shoot length showed a decreasing effect in blue light and an increasing effect in red light. Poudel et al. (2008) stated in their study with three grape varieties that red LED treatment increased shoot length in all varieties. In our study, no effect of LED treatment on shoot length was observed. It can be postulated that the reason for this variability in shoot length is due to the different synergistic interactions between blue/red receptors and phytochromes, which reveal the stimulating or inhibitory properties depending on the species and variety (Kim et al. 2004). It has been reported that red and blue LED applications increase biomass in many plant species (such as *Carpesium triste, Rehmannia glutinosa, Achillea millefolium, Densribium*, blueberry, sugar cane, and *Chrysanthemum*) in tissue culture (Kim et al. 2004; Poudel et al. 2008; Lin et al. 2011; Alvarenga et al. 2015; Manivannan et al. 2017; Hung et al. 2016; Zhao et al. 2020). In our study, the positive effect of blue LED treatment on fresh and dry weight is parallel to the literature. Plants, which can detect changes in light quality through photoreceptors, can regulate their growth development through these signaling pathways (Ward et al. 2005). This is because in the visible spectrum (400–700 nm), plants predominantly absorb blue (400–500 nm) and red (600–700 nm) wavelengths, and these regions account for 90% of photosynthetic pigment absorption (Terashima et al. 2009). In photosynthesis, leaves absorb light in the blue (430 nm) and red (660 nm) spectral range (Curran 1980). Red light regulates the functioning of the plant photosynthetic system and the transport of assimilates (Baroli et al. 2008), and blue light is reported to play a role in stomatal opening (Gruszecki et al. 2010). Mengxi et al. (2011) stated that under in vitro conditions, red and blue LED treatments alone or in combination provided improvements in fresh and dry weights of *Oncidium* plants. Similarly, it has been stated that blue and red light sources can be used effectively for healthy plant growth in tissue culture (Wheeler et al. 1991). In general, our results clearly show that blue LED light can be incorporated into in vitro conditions.

Table 2 Effects of LED treatments on SPAD index and chlorophyll content

Variety	Light treatment	Spad	Clh a	Chll b	Total Chl	Chl a/b	Carotenoid
'Italia'	White LED	24.75 A-a	0.60 A-b	0.24 A-b	0.85 A-b	2.43 B-b	0.29 A-b
	Red LED	24.48 A-a	0.32 B-b	0.08 B-b	0.40 B-b	4.43 A-a	0.17 B-b
	Blue LED	27.76 A-a	0.28 B-b	0.06 B-a	0.34 B-a	5.16 A-a	0.14 B-b
'Michele Palieri'	White LED	26.15 B-a	1.30 A-a	0.37 A-a	1.67 A-a	3.52 B-a	0.51 A-a
	Red LED	27.00 B-a	0.63 B-a	0.19 B-a	0.83 B-a	3.19 B-a	0.31 B-a
	Blue LED	31.76 A-a	0.47 B-a	0.10 C-a	0.57 B-a	4.61 A-a	0.20 C-a

Chl a chlorophyll a, *Chl b* chlorophyll b, *Total Chl* total chlorophyll, *Chl a/b* chlorophyll a/b
*The difference between the means shown in capital letters on the same line for each variety is not significant ($p < 0.05$ level)
**The differences between the means shown in lower case letters in the same column for each variety are not significant ($p < 0.05$ level)

SPAD Index and Chlorophyll Content

In the present study, the influence of light quality on the SPAD index and chlorophyll content of 'Italia' and 'Michele Palieri' grape varieties cultured in vitro was investigated (Table 2). There was no statistical difference in the SPAD index of the 'Italia' grape variety. There was a statistical difference in the 'Michele Palieri' grape variety, and a positive effect of the blue LED treatment was observed. In addition, there was no statistical difference between the varieties in terms of this parameter. When chl a and total chl parameters were examined, statistical differences were observed, and positive effects of white LED treatment were observed in both varieties compared to blue and red LED treatments. There were also statistical differences in terms of chl b and carotenoid parameters, and in both species, red and blue LEDs had a reducing effect compared to white LED. When chl a/b was examined, effects of red and blue LEDs were observed in the 'Italia' grape variety, while blue LED light came to the fore statistically in the 'Michele Palieri' grape variety. Carotenoid content showed statistical differences, and it was observed that in the 'Michele Palieri' grape variety, red and white LED treatments had a more positive effect than the blue LED treatment, and in the 'Italia' grape variety, white LED had a more positive effect than the other two LED treatments.

White LED increased the accumulation of chlorophyll and carotenoids in both cultivars but did not increased chlorophyll a/b. Zhao et al. (2020) stated that white light positively affected the chlorophyll content and carotenoid content in the *Carpesium triste* Maxim plant under in vitro conditions compared to blue or red light. In a similar study, it was mentioned that blue LED treatment of *Gerbera Jamesonii* plants had a decreasing effect on the chl a and chl b contents in in vitro conditions (Meng et al. 2019). In a study conducted with the *Camptotheca acuminata* plant, chl a, chl b, total chl, and carotenoid contents were found to have higher values in the white LED treatment compared to the blue and red LED treatments, and chl a/b values gave the highest values in the blue LED treatment, which is consistent with our own study. It was observed that parallel results were obtained. While white LED light significantly increased the chlorophyll and carotenoid content, these parameters had lower values in blue and red LED treatments. These decreases in chlorophyll concentration may also be due to excessive-irradiance-induced pigment damage (Shao et al. 2014). Chlorophyll a/b values increased under red and blue LEDs, and this increase was associated with a decrease in chlorophyll b in blue and red LEDs in both grape varieties. It can be said that the high level of chlorophyll a/b in blue light is caused by changes in the organization of light-harvesting and electron transport components resulting from a significant decrease in chl b content under blue LED treatment (Yu et al. 2017). Increases in chlorophyll a/b values indicate strong light adaptation, higher electron transport ability of chlorophyll, and higher activation of the Calvin cycle enzymes (Evans 1988).

Carotenoids act as accessory pigments in the antenna systems to collect light energy and improve photosynthetic efficiency; therefore, they are important components of the reaction center complex. Matsuda et al. (2008) reported that the simultaneous increase in carotenoid content would offset the increased photosynthetic production. In the study, it was observed that white LED treatments gave the highest carotenoid content in both varieties. Blue LED was observed to have a more positive effect on the 'Michele Palieri' grape variety compared to red LED treatment. It is stated that the absorption of light in the wavelength of the blue region of the spectrum is at a high level, and this is provided by carotenoids, which are the auxiliary photoreceptors of chlorophyll (Lanoue et al. 2021; Liu et al. 2018). The study shows that white LED treatments are more effective, especially in terms of chlorophyll content.

Electrolyte Leakage (EL, %), MDA (Lipid Peroxidation, Mmol), and Enzymatic Activity

In this study, different LED lights did not create a statistical difference in electrolyte leakage (EL) and MDA except for in the 'Italia' × white LED treatment (Table 3). Elec-

Table 3 Effects of LED treatments on electrolyte leakage, malondialdehyde (MDA)

Variety	Light treatment	Electrolyte leakage (%)	MDA (mmol)
'Italia'	White LED	13.978 A-b	11.437 A-b
	Red LED	24.505 A-a	17.979 A-a
	Blue LED	25.773 A-a	18.546 A-a
'Michehe Palieri'	White LED	38.953 A-a	22.198 A-a
	Red LED	32.883 A-a	18.223 A-a
	Blue LED	35.833 A-a	20.765 A-a

*The difference between the means shown in capital letters on the same line for each variety is not significant ($p < 0.05$ level)
**The differences between the means shown in lower-case letters in the same column for each variety are not significant ($p < 0.05$ level)

Table 4 Effects of LED treatments on enzymatic activity

Variety	Light treatment	Total soluble protein	SOD	APX
'Italia'	White LED	2.24 C-a	385.523 B-a	5.09 B-a
	Red LED	2.65 B-b	180.373 C-b	8.97 A-a
	Blue LED	3.00 A-a	403.650 A-a	0.14 C-a
'Michehe Palieri'	White LED	1.94 C-b	381.380 A-a	1.16 A-b
	Red LED	3.36 A-a	246.260 C-a	0.05 B-b
	Blue LED	2.02 B-b	273.533 B-b	0.05 B-b

*The difference between the means shown in capital letters on the same line for each variety is not significant ($p < 0.05$ level)
**The differences between the means shown in lower-case letters in the same column for each variety are not significant ($p < 0.05$ level)
***SOD (superoxide dismutase, EC: 1.15.1.1.), APX (ascorbate peroxidase, EC: 1.11.1.11)

trolyte leakage occurs when electrolytes leak from the cell membrane due to weakening of the membrane as the stress increases, which is the first place where damage occurs in plants experiencing abiotic and biotic stress. MDA (lipid peroxidation) is an analysis method used in the biochemical evaluation of stress factors in many stress studies. Lipid peroxidation, referred to as MDA, is known as the final product of cell membrane reactive damage to cellular mechanisms (Ali et al. 2005; Liu et al. 2006). In general terms, it is reported that MDA content affects the stress resistance of the plant, and the extent of damage increases as its amount increases (Liu et al. 2018). The results of our study revealed that LED treatments applied to both grape varieties did not cause stress-induced membrane damage in plants.

In the present study the influence of light quality on the enzymatic activity of 'Italia' and 'Michele Palieri' grape cultivars cultured in vitro was investigated. All parameters showed statistically significant differences from different LED treatments (Table 4). Total soluble protein content showed variable responses under different LED treatments. It had the highest value in the red LED treatment in the 'Michele Palieri' grape variety, and blue LED treatment had an enhancing effect in both varieties. SOD activity had the highest value in the blue LED treatment of the 'Michele Palieri' grape variety. Red LED treatment led to a decrease in this parameter in both varieties. APX activity had the highest value in the red LED treatment in the 'Italia' grape variety and showed a decreasing trend under blue LED treatment in both varieties.

Superoxide dismutase is quantified by determining the concentrations of H_2O_2 and O_2 and is central to the defense mechanism of plants. It is the first enzyme of the detoxification process and catalyzes the dismutation of superoxide into hydrogen peroxide and oxygen (Raychaudhuri and Deng 2000, Molassiotis et al. 2006). It is found in all living things (from microorganisms to humans), in aerobic organisms, and mostly in intracellular organelles (chloroplast, cytosol, mitochondria, peroxisome, apoplast) that produce reactive oxygen species (Pereira et al. 2003). Ascorbate peroxidase protects cells against H_2O_2 not only under stress conditions but also under normal conditions. It is found in intracellular organelles such as chloroplasts, cytosol, mitochondria, peroxisomes, and apoplasts. The observed increase in APX activity in different plant species under abiotic stresses suggests a possible role for APX in the removal of H_2O_2 from the cell (Davis and Swanson 2001, Bueno and Piqueras 2002). In the study, it was observed that the varieties showed different responses in antioxidant enzyme reactions according to different LED light sources (Table 4). In the 'Michele Palieri' grape variety, red LED light was more effective in increasing the total soluble protein content, white LED and blue LED were more effective in increasing SOD activity, and white LED was more effective in increasing APX activity. In Italia grape variety, total soluble protein content was positively affected by blue LED, SOD enzyme activity by white LED and APX enzyme activity by red LED. Antioxidant enzymes not only protect plants against biotic or abiotic stress conditions but also

positively regulate plant growth, differentiation, and yield (Genkov and Ivanova 1995; Blazquez et al. 2009; Shafi et al. 2015). Shohael et al. (2006) stated that light quality can affect antioxidant enzyme metabolism. In the study, both grape varieties showed variable responses to different light sources in terms of antioxidant enzyme activity. Mastropasqua et al. (2012) reported that the APX enzyme activation of oat leaves was higher in the blue spectrum, and Nascimento et al. (2013) similarly stated that blue light positively affected the antioxidant activity of *Kalanchoe pinnata*. In addition, in a study conducted on pea seedlings and rice husks, red light affected antioxidant enzyme activity. In a study examining antioxidant enzyme activity under different LED light sources in *Camptotheca acuminata* seedlings, it was stated that blue and red LED light increased SOD activity (Yu et al. 2017). The abovementioned studies have indicated that antioxidant enzyme activities in plants grown with different light sources or spectra may result in variability or show complex responses, and that these results are due to different morphogenetic, photosynthetic, and antioxidant responses that may vary among plant species and varieties due to spectral light changes (Yu et al. 2017).

Conclusion

The study attempted to examine the responses of 'Italia' and 'Michele Palieri' grape varieties grown under in vitro conditions to white, blue, and red LED treatments. The study showed that the effects of blue LED treatment on plant development, biomass increase, and SPAD index and of white LED treatment on chlorophyll and carotenoid content were positive in both varieties. In addition, EL and MDA values, which are the most important indicators of stress in plants, showed that these treatments did not cause any harm to the plant. Antioxidant enzyme analyses showed that the reactions of the varieties varied depending on the treatments. Growing healthy plants under in vitro conditions is possible by providing the desired nutrient medium for the plant as well as ideal environmental conditions. White light played an important role in ensuring the development of micro-cuttings, since it also contains the blue and red main light colors. At the end of this study, it was concluded that in cultivations where the use of artificial light sources is mandatory or in cases where sunlight is insufficient, the best LEDs of basic colors or their combinations should be determined according to the plant type or even variety. The study also showed that in in vitro propagation where white light is used, using different LED light sources during the appropriate period and interval within the photoperiod will ensure healthy production.

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Author Contribution H. Coşar Tutumlu: data collection, data analysis, writing; N. Topcu Altıncı: visualization, conceptualization, methodology, data collection, data analysis, supervision, writing—original draft, review, and editing.

Data availability Data will be made available upon request to the corresponding authors.

Conflict of interest H. Coşar Tutumlu and N. Topcu Altıncı declare that they have no competing interests.

References

Al Murad M, Razi K, Jeong BR, Samy PMA, Muneer S (2021) Light emitting diodes (LEDs) as agricultural lighting: impact and its potential on improving physiology, flowering, and secondary metabolites of crops. Sustainability 13(4):1985. https://doi.org/10.3390/su13041985

Ali MB, Hahn EJ, Paek KY (2005) Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micro propagated Phalaenopsis plantlet. Environ Exp Bot 54:109–120

Alvarenga ICA, Pacheco FV, Silva ST, Bertolucci SKV, Pinto JEBP (2015) In vitro culture of Achillea millefolium L.: quality and intensity of light on growth and production of volatiles. Plant Cell Tiss Organ Cult 122:299–308. https://doi.org/10.1016/j.envexpbot.2004.06.005

Bantis F, Ouzounis T, Radoglou K (2016) Artificial LED lighting enhances growth characteristics and total phenolic content of Ocimum Basilicum, but variably affects transplant success. Sci Hortic 198:277–283. https://doi.org/10.1016/j.scienta.2015.11.014

Baroli I, Price GD, Badger MR, von Caemmerer S (2008) The contribution of photosynthesis to the red-light response of stomatal conductance. Plant Physiol 146(2):737. https://doi.org/10.1104/pp.107.11092

Blazquez S, Olmos E, Hernández JA, Fernández-García N, Fernández JA, Piqueras A (2009) Somatic embryogenesis in Saffron (Crocus sativus L.). Histological differentiation and implication of some components of the antioxidant enzymatic system. Plant Cell Tiss Organ Cult 97:49–57. https://doi.org/10.1007/s11240-009-9497-y

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1006/abio.1976.9999

Bueno P, Piqueras A (2002) Effect of transition metals on stress, lipid peroxidation and antioxidant enzyme activities in tobacco cell cultures. Plant Growth Regul 36:161–167

Curran P (1980) Multispectral remote sensing of vegetation amount. Prog Phys Geogr 4(3):315–341

Davis DG, Swanson HR (2001) Activity of stress-related enzymes in the perennial weed leafy spurge (Euphorbia esula L.). Environ Exp Bot 46:95–108. https://doi.org/10.1016/S0098-8472(01)00081-8

Dueck T, van Leperen W, Taulavuori K (2016) Light perception, signalling and plant responses to spectral quality and photoperiod in natural and horticultural environments. Environ Exp Bot. https://doi.org/10.1016/j.envexpbot.2015.06.012

Evans JR (1988) Acclimation by the thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and thylakoid proteins. Funct Plant Biol 15(2):93–106

Genkov T, Ivanova I (1995) Effect of cytokinin-active Phenylurea derivatives on shoot multiplication, peroxidase and superoxide dismutase activities of in vitro cultured Carnation. Bulg J Plant Physiol 21(1):73–83

Gruszecki WI, Luchowski R, Zubik M, Grudzinski W, Janik E, Gospodarek M, Goc J, Gryczynski Z, Gryczynski I (2010) Blue-light-controlled photoprotection in plants at the level of the photosynthetic antenna complex LHCII. J Plant Physiol 167(1):69–73. https://doi.org/10.1016/j.jplph.2009.07.012

Gupta SD, Agarwal A (2017) Light emitting diodes for agriculture. LED supplementary lighting, pp 27–36

Gupta SD, Jatothu B (2013) Fundamentals and applications of light-emitting diodes (leds) in in vitro plant growth and morphogenesis. 7:211–220. https://doi.org/10.1007/s11816-013-0277-0

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125(1):189–198

Hernández R, Kubota C (2016) Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. Environ Exp Bot 121:66–74. https://doi.org/10.1016/j.envexpbot.2015.04.001

Hung CD, Hong CH, Kim SK, Lee KH, Park JY, Nam MW, Choi DH, Lee HI (2016) LED light for in vitro and ex vitro efficient growth of economically important highbush blueberry (Vaccinium corymbosum L.). Acta Physiol Plant 38:1–9. https://doi.org/10.1007/s11738-016-2164-0

Kim SJ, Hahn EJ, Heo JW, Paek KY (2004) Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. Sci Hortic 101(1–2):143–151. https://doi.org/10.1016/j.scienta.2003.10.003

Lanoue J, Thibodeau A, Little C, Zheng J, Grodzinski B, Hao X (2021) Light spectra and root stocks affect response of greenhouse tomatoes to long photoperiod of supplemental lighting. Plants 10(8):1674. https://doi.org/10.3390/plants10081674

Lazzarin M, Meisenburg M, Meijer D, Van Leperen W, Marcelis LFM, Kappers IF, Dicke M (2021) LEDs make it resilient: effects on plant growth and defense. Trends Plant Sci 26(5):496–508. https://doi.org/10.1016/j.tplants.2020.11.013

Li CX, Xu ZG, Dong RQ, Chang SX, Wang LZ, Rehman MKU, Tao JM (2017) An rna-seq analysis of grape plantlets grown in vitro reveals different responses to blue, green, red led light and white fluorescent light. Front Plant Sci. https://doi.org/10.3389/fpls.2017.00078

Lin Y, Li J, Li B, He T, Chun Z (2011) Effects of light quality on growth and development of protocorm-like bodies of Dendrobium officinale in vitro. Plant Cell Tiss Organ Cult 105:329–335. https://doi.org/10.1007/s11240-010-9871-9

Liu P, Yang YS, Xu GD (2006) Physiological response of rare and endangered seven-son-flower (Heptacodium miconioides) to light stress under habitat fragmentation. Environ Exp Bot 5:32–40. https://doi.org/10.1016/j.envexpbot.2005.04.003

Liu Y, Wang T, Fang S, Zhou M, Qin J (2018) Responses of morphology, gas exchange, photochemical activity of photosystem II, and antioxidant balance in Cyclocarya paliurus to light spectra. Front Plant Sci 9:1704. https://doi.org/10.3389/fpls.2018.01704

Manivannan A, Soundararajan P, Park YG, Wei H, Kim SH, Jeong BR (2017) Blue and red light-emitting diodes improve the growth and physiology of in vitro-grown carnations green beauty and purple beauty. Hortic Environ Biotechnol 58:12–20. https://doi.org/10.1007/s13580-017-0051-2

Mastropasqua L, Borraccino G, Bianco L, Paciolla C (2012) Light qualities and dose influence ascorbate pool size in detached oat leaves. Plant Sci 183:57–64. https://doi.org/10.1016/j.plantsci.2011.11.009

Matsuda R, Ohashi-Kaneko K, Fujiwara K, Kurata K (2008) Effects of blue light deficiency on acclimation of light energy partitioning in PSII and CO_2 assimilation capacity to high irradiance in spinach leaves. Plant Cell Physiol 49:64–70. https://doi.org/10.1093/pcp/pcn041

Meng XY, Wang Z, He SL, Shi LY, Song YL, Lou XY, He D (2019) LED-Supplied red and blue light alters the growth, antioxidant status, and photochemical potential of in vitro-grown Gerbera jamesonii plantlets. Hortic Sci Technol 37(4):473–489. https://doi.org/10.7235/HORT.2019004

Mengxi L, Zhigang X, Yang Y, Yijie F (2011) Effects of different spectral lights on Oncidium PLBs induction, proliferation, and plant regeneration. Plant Cell Tiss Organ Cult 106:1–10. https://doi.org/10.1007/s11240-010-9887-1

Mohammadi MHZ, Panahirad S, Navai A, Bahrami MK, Kulak M, Gohari G (2021) Cerium oxide nanoparticles (CeO_2-NPs) improve growth parameters and antioxidant defense system in Moldavian Balm (Dracocephalum moldavica L.) under salinity stress. Plant Stress 1:100006. https://doi.org/10.1016/j.stress.2021.100006

Molassiotis AN, Sotiropoulos T, Tanou G, Kofidis G, Diamantidis G, Therios E (2006) Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitol or sorbitol. Biol Plant 50:331–338. https://doi.org/10.1007/s10535-006-0046-9

Nakano Y, Asada K (1981) Hydrogen peroxidase in spinach chloroplasts. Plant Cell Physiol 22(5):867–880

Nascimento LBS, Leal-Costa MV, Coutinho MA, Moreira NS, Lage CLS, Barbi NS, Costa SS, Tavares ES (2013) Increased antioxidant activity and changes in phenolic profile of Kalanchoe pinnata (lamarck) persoon (crassu-laceae) specimens grown under supplemental blue light. Photochem Photobiol 89:391–399. https://doi.org/10.1111/php.12006

Pereira MD, Herdeiro RS, Fernandes PN, Eleutherio Eleutherio ECA, Panek AD (2003) Targets of oxidative stress in yeast sod mutants. Biochimi Biophys Acta (BBA) Gen Subj 1620:245–251. https://doi.org/10.1016/S0304-4165(03)00003-5

Poudel RP, Kataoka I, Mochioka R (2008) Effect of red-and blue-light emitting diodes on growth and morphogenesis of grapes. Plant Cell Tissue Organ Cult 92:147–153. https://doi.org/10.1007/s11240-007-9317-1

Raychaudhuri SS, Deng XW (2000) The role of superoxide dismutase in combating oxidative stress in higher plants. Bot Rev 66(1):89–98. https://doi.org/10.1007/BF02857783

Samuolienė G, Viršilė A, Brazaitytė A, Jankauskienė J, Sakalauskienė S, Vaštakaitė V, Novičkovas A, Viškelienė A, Sasnauskas A, Duchovskis P (2017) Blue light dosage affects carotenoids and tocopherols in microgreens. Food Chem 228:50–56. https://doi.org/10.1016/j.foodchem.2017.01.144

Shafi A, Chauhan R, Gill T, Swarnkar MK, Sreenivasulu Y, Kumar S, Kumar N, Shankar R, Ahuja PS, Singh AK (2015) Expression of SOD and APX genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in Arabidopsis under salt stress. Plant Mol Biol 87:615–631. https://doi.org/10.1007/s11103-015-0301-6

Shao QS, Wang HZ, Guo HP, Zhou AC, Huang YQ, Sun YL, Li MY (2014) Effects of shade treatments on photosynthetic characteristics, chloroplast ultrastructure, and physiology of Anoectochilus roxburghii. PLoS ONE 9(2):e85996. https://doi.org/10.1371/journal.pone.0085996

Shohael AM, Ali MB, Yu KW, Hahn EJ, Islam R, Paek KY (2006) Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in Eleutherococcus senticosus somatic embryos in bioreactor. Process Biochem 41:1176–1185. https://doi.org/10.1016/j.procbio.2005.12.015

Simlat M, Ślęzak P, Moś M, Warchol M, Skrzypek E, Ptak A (2016) The effect of light quality on seed germination, seedling growth and selected biochemical properties of stevia Rebaudiana bertoni. Sci Hortic 211:295–304. https://doi.org/10.1016/j.scienta.2016.09.009

Sirhindi G, Mir MA, Abd-Allah EF, Ahmad P, Gucel S (2016) Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity, and gene expression in Glycine max under nickel toxicity. Front Plant Sci 7:591. https://doi.org/10.3389/fpls.2016.00591

Terashima I, Fujita T, Inoue T, Chow WS, Oguchi R (2009) Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. Plant Cell Physiol 50(4):684–697. https://doi.org/10.1093/pcp/pcp034

Wang H, Gu M, Cui J, Shi K, Zhou Y, Yu J (2009) Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of calvin cycle genes and carbohydrate accumulation in Cucumis sativus. J Photochem Photobiol B Biol 96(1):30–37. https://doi.org/10.1016/j.jphotobiol.2009.03.010

Ward JM, Cufr CA, Denzel MA, Neff MM (2005) The Dof transcription factor OBP3 modulates phytochrome and cryptochrome signaling in Arabidopsis. Plant Cell 17(2):475–485. https://doi.org/10.1105/tpc.104.027722

Wheeler RM, Mackowiak CL, Sager JC (1991) Soybean stem growth under high-pressure sodium with supplemental blue lighting. Agron J 83(5):903–906. https://doi.org/10.2134/agronj1991.00021962008300050024x

Witham FH, Blaydes DF, Devlin RM (1971) Experiments in Plant Physiology. Van Nostrand Reinhold Co. New York. P. 55-56

Yu W, Liu Y, Song L, Jacobs DF, Du X, Ying Y, Shao Q, Wu J (2017) Effect of differential light quality on morphology, photosynthesis, and antioxidant enzyme activity in Camptotheca acuminata seedlings. J Plant Growth Regul 36:148–160. https://doi.org/10.1007/s00344-016-9625-y

Zhao J, Thi LT, Park YG, Jeong BR (2020) Light quality affects growth and physiology of Carpesium triste Maxim. cultured in vitro. Agriculture 10(7):258. https://doi.org/10.3390/agriculture10070258