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Effect of Light Quality on Micropropagation and Some Morphological Properties of Cadaman Avimag (*Prunus persica × P. davidiana*) Rootstock

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Abstract
Cadaman Avimag rootstock is widely used for almonds and peaches. The purpose of this study was to investigate the effect of light quality on micropropagation, and some morphological and physiological properties of this rootstock. Single node explants were cultured on WPM and MS media containing three levels of BA (0, 1 and 2 mg L^{-1}) and three levels of NAA (0, 0.05 and 0.1 mg L^{-1}). WPM medium containing 2 mg L^{-1} BA and 0.1 mg L^{-1} NAA was selected for different light quality treatments including fluorescent, red, and red+blue lights. Results showed that light quality had a significant effect on shoot proliferation, length and diameter, node number, shoot fresh and dry weights, leaf thickness, stomata number, and chlorophyll concentration. Red light resulted in higher shoot diameter, but crunchiness and lack of proper shoot growth. Moreover, red light produced the lowest amount of chlorophyll in the explants. Leaf thickness and its structural layers under the red light were the lowest among different treatments. Combined red+blue light in many factors resembled fluorescent light but induced more chlorophyll and a larger stomata size. The quality of light influenced the growth of this rootstock *in vitro*, and affected the stem and leaf vegetative traits.

Keywords: Cadaman, tissue culture, micropropagation, light quality, *Prunus*, rootstock

Abbreviations: BA: 6-benzyladenine; LED: light emitting diode; MS: Murashige and Skoog medium; NAA: Naphthalene acetic acid; PPFD: photosynthetic photon flux density; WPM: woody plant medium; UV: ultra violet.

Introduction

In modern pomology, the use of vegetative rootstock is of great importance due to some desirable characteristics such as resistance to adverse growth conditions as well as grafting compatibility (Basile and DeJong, 2019). Cadaman Avimag (Cadaman) rootstock, as one of the most important stone fruit rootstock, is derived from the crossing of *Prunus persica* × *P. davidiana*. It is resistant to some viruses such as Plum pox virus (Polak and Oukropec, 2010; Salava et al., 2013) and some nematodes (Pinochet et al. Et al., 1996). Cadaman rootstock has better growth potential, higher production, and superior fruit quality for trees grafted on it, compared with the other peach and almond rootstocks (Hernandez-Dorrego et al.,

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1999; Bussi et al., 2002; Font i Forcada et al., 2009, 2012; Orazem et al., 2011).

In fruit trees, micropropagation is used as an effective method for reproduction of some of difficult-to-root rootstocks (Borkowska et al., 2008; Couto et al., 2004). Benefits of the method include mass-propagation of pathogen-free plants, high reproduction coefficient, and high production in small space. However, use of this method comes with several difficulties, such as selection of internal contamination of explants, suitable culture medium, appropriate concentration of plant growth regulators, optimal conditions for growth and reproduction, and surface disinfection of the specimens as well as high expenses of tissue culture technologies and equipment (Pierik, 1997; Papafotiou and Martini, 2009; Massa et al., 2008). Providing proper light for success and optimal growth of plants under *in vitro* conditions is expensive. Light is one of the most important environmental factors in the growth of explants. Light quality (wavelength) is effective in bud growth regulation, apical dominance, new shoots growth, and lateral buds differentiation (Muleo and Morini, 2006, 2008; Moradnezhad et al., 2017).

The effects of light quality, and different light sources in the growth chamber have been investigated in several studies. Quality of light has an impact on shoot proliferation, shoot and internode length, rooting percentage, and root number and length of woody plants (Appelgren, 1991; Muleo & Morini, 2008; Iacona and Muleo, 2010; Mousavifattah and Sarikhani, 2016; Miler et al., 2019; Sayed et al., 2020). However, the inconsistency in the reports has led to the effects of light quality on woody plants remain obscure. Traditional sources of light for growth chamber used in plant microprogagation research are fluorescent white cool, metal halide and incandescent; which fluorescent white cool is more common (Pierik, 1997; Pimputkar et al., 2009). Light source in the growth chamber is of great importance economically and physiologically (Bourget, 2008). The amount of energy consumed varies between different light sources, while each light source produces a specific spectrum of light (Rajapakse and Shahak, 2008). The plant response to light spectra is broadly classified into growth and photomorphogenic responses. The growth response is triggered by photosynthetic active radiation, which comprises a waveband of 400–700 nm. The photomorphogenic response is generally triggered by blue wavelength (400 to 500 nm), UV (250 to 380 nm), and interaction between red (600-700 nm) and far-red (700-800 nm) (Hernandez and Kubota, 2014; Taiz et al., 2015; Huché-Thélier et al., 2016). Many light sources used in the growth chamber produce a wide range of light spectrum. Due to the need of plants for a particular spectrum, other light spectra turn into heat. In addition to energy loss, it necessitates a cooling system in the growth chamber.

Light emitting diodes (LEDs) are a group of strong semiconductor solids designed to produce a narrow spectrum of light. LEDs have advantages such as long life, specific wavelength, extremely high light output efficiency, low power consumption and adjustable light intensity. In addition, their heat generation is negligible compared to other sources of light (Bourget, 2008; Hernandez and Kubota, 2015). In the past decades, LEDs have been used commercially in greenhouse or plant factory (Massa et al., 2008; Nelson and Bugbee, 2013; Lin et al. 2013; Chen et al., 2014), and in growth chambers for plant propagation (Lian et al., 2002; Kim et al., 2004; Li et al., 2013; Choi et al., 2015; Mousavifattah and Sarikhani, 2016; Miler et al., 2019). Various ratios of blue and red light are used for *in vitro* culture. Shoots elongate and soften under red, while they become short under blue light (Kim et al., 2004; Ding et al., 2010). Although some aspects of light quality have been studied, the

effect of light quality on shoot and leaf morphology is unknown.

LEDs have a high potential for use as an optimum light source in the growth chamber because of their many benefits and low heat. The aim of this study was to investigate the effect of light quality on micropropagation of Cadaman rootstock and some morphological characteristics of this rootstock under *in vitro* culture.

Material and methods

This study was carried out in the tissue culture laboratory of the Department of Horticultural Science, Bu-Ali Sina University, on Cadaman Avimag (*Prunus percica* × *P. davidiana*) rootstock. First, the samples micropropagation was investigated to find the appropriate medium for micropropagation under cool white fluorescent light, and then under different light quality treatments.

Shoots were prepared in spring from new growth of mother plant, and transferred to the laboratory. After removal of the leaves, they were washed with running tap water and detergent for 10 min. Later, the shoots were disinfected with 1% sodium hypochlorite containing 0.05% Tween-20 for 10 min. They were, then, rinsed three times for 2, 5 and 10 min with sterile distilled water, respectively. Single-node explants were cultured on MS (Murashige and Skoog, 1962) and WPM (Lloyd and McCown, 1981) basal media containing different concentrations of plant growth regulators, 3 % sucrose and 0.7 % agar; while pH was adjusted on 5.7±0.1 using 0.1 N HCl or NaOH. Then, after dissolving agar, using an autoclave at 110 °C, for 1 min, 30 mL of culture medium was placed in 200 mL of glass jar, and sterilized in an autoclave under 1.2 atmospheric pressure, at 121 °C, for 15 min.

Effect of medium, BA and NAA on shoot proliferation

In order to investigate the proliferation, the MS and WPM media containing BA (0, 1 and 2 mg L^{-1}) and NAA (0, 0.05 and 0.1 mg L^{-1}), 3% sucrose and 0.7% agar, were used. The culture medium was prepared and divided as the above-mentioned conditions. The experiment was carried out in a factorial experiment with three main factors (culture medium, NAA concentration, and BA concentrations) based on a completely randomized design with three replications. The culture glass was incubated in a growth chamber, under cool white fluorescent light, with intensity of about 75 µmol m^{-2} s^{-1}, and 16: 8 day and night photoperiod at 24± 1 °C. The explants were subcultured in same medium at the end of the fourth week; but at the end of the eighth week, the growth indices were analyzed including shoot number, node number, branch length, and internode length.

Effect of light quality on shoot proliferation and growth

WPM medium containing 2 mg L^{-1} BA and 0.1 mg L^{-1} NAA, 3% sucrose and 0.7% agar, was used for light treatment. Single node explants were cultured in jars and were treated under three light treatments including fluorescent, red (peak wavelength 660 nm), and equal combination of red and blue (peak wavelength 440 nm) lights. Philips 40W cool white fluorescent tubes (with peak wavelengths of 400, 440, 490, 545, 585 and 610 nm base on official site of Philips) were used as control and its light intensity was measured above jar surfaces using a light meter (YK-2005LX, Lutron, Taiwan). For other treatments, the light intensity adjusted on 75±3 µmol m^{-2} s^{-1} using base on lumen to lux and then to PPFD (base on light source and conversion coefficients) conversion method (Ashdown, 2019) by changing number of LEDs. The blue and red lights were prepared using 1W power LEDs with beam angle of 90 ° (Chanzon, China) uniting with a 12V and 20A power supply.

In order to prevent light interference in different light treatments, the growth chamber space was partitioned completely

separated. The temperature of the growth chamber was set at 24± 1 °C; while the photoperiod was set on 16 h light and 8 h dark for all light treatments. At the end of the sixth week, various traits were measured including fresh and dry weights, shoot number and length, node number, internode length, shoot diameter, and leaf area.

Effect of light quality on leaf characteristics

In order to evaluate leaf morphology under light treatment, the explants were subcultured in MS and WPM media containing 3% sucrose and 0.7% agar without plant growth regulators; and were kept in the growth chamber for 6 weeks under the mentioned light treatments. At the end of the sixth week, leaf chlorophyll content was measured by Porra et al. (1989) method using a spectrophotometer (Carry 100, Varian, USA), and expressed as mg g^{-1} FW. Leaf thickness as well as layers thickness and number of stomata per unit area were also measured by light microscopy.

The effect of light quality on rooting

Healthy shoots grown under *in vitro* conditions were selected from the other experiment with three or four leaves and lengths about 2-3 cm, and then transferred to the rooting medium. For this purpose, 1/2MS medium containing 2% sucrose and 0.7% agar was used, while the shoots were kept in the growth chamber under the mentioned light treatments. Six weeks later, rooting percentage, root number, and root length were recorded.

Growth characteristics

The specimens were carefully divided into separated shoots by forceps. The shoot length and diameter were measured by caliper. Root and leaf length were measured using a ruler. For fresh and dry weights, samples were first weighted (FW) and then placed in a paper bag, and then transferred to an oven for 72 h, in 60 °C for dry weight (DW). A digital Sartorius weighing scales with an accuracy of 0.1 mg was used to weight the samples.

Preparation of samples for light microscopy

The specimens were sectioned manually using standard free-hand sectioning technique as described by Ruzin (1999). The samples were bleached by immersing in 1% sodium hypochlorite for 3 to 5 min. They were, then washed with distilled water and immersed in 10% acetic acid for one min and were washed again with distilled water. The cross sections were stained using a double staining protocol (Ruzin, 1999), including 1% (w/v) methylene blue for wood and cork tissues, and 1% (w/v) alum carmine for cellulose (Sarikhani et al., 2014). Finally, the sections were observed under the Leica microscope and ligenin deposition was observed by comparing sections. Thickness of leaf structural layers was measured using graded slides and Image-J software (ver. 1.6).

Stomata properties

The lower epidermis of the leaf was gently separated using leaf epidermal peeling method by breaking the leaf and spreading a few drops of distilled water on the slide. Epidermal and stomata cells were counted using a Leica microscope. The diameter of stomata was measured by a graded slide. Due to the elliptical shape of most stomata (except for fluorescent light that had a circular shape), the length of the longer portion of the stomata was measured. In fact, the stomata guard cells were measured on both sides of the axis; where the smaller diameter was measured perpendicular to the previous diameter. Stomata index was calculated using the following equation: [no. of stomata / (no. of stomata+ no. of epidermis cells)] (Royer, 2001).

Data analysis

In the experiment, each glass was considered as a test unit. The remaining error was extracted and checked for normality by the Minitab (ver. 16.2.1.0)

software. Data were normalized using [Y = Log (x)] equation, by the SPSS (ver. 20) software, and then analyzed by the SAS (ver. 9.4) software. The means were compared using the Duncan's multiple range test at 5% level.

Results

Effect of medium, BA and NAA on shoot proliferation

Interaction effect of culture medium, BA concentration, and NAA concentration was significant on shoot and internode number per explant and shoot and internode length at 1 and 5% levels; respectively (ANOVA tables not shown). The highest number of shoots was obtained in the WPM medium containing 2 mg L^{-1} BA and 0.1 mg L^{-1} NAA; which was significantly different from other treatments. The lowest number of shoots was observed in the MS medium containing 1 mg L^{-1} BA but without NAA; which showed no significant differences with some other treatments (Table 1).

The highest shoot length was observed in MS medium containing 0.1 mg L^{-1} NAA and without BA. It revealed no significant difference with MS medium containing 0.05 and 0 mg L^{-1} NAA without BA. However, the lowest shoot length was observed in MS medium containing 2 mg L^{-1} BA and 0.1 mg L^{-1} NAA; which indicated no significant difference from both WPM containing 2 mg L^{-1} BA and all concentrations of NAA (Table 1).

The highest number of nodes was observed in WPM medium containing 2 mg L^{-1} BA and 0.1 mg L^{-1} NAA; that was significantly different from the other treatments. Nevertheless, the lowest number of nodes was observed in MS medium treatment without BA and NAA; which had no significant difference from other treatments as shown in Table 1.

Table 1. Effect of type of medium, BA and NAA concentrations on shoot proliferation of Cadaman explant and its properties, eight weeks after culture.

Medium	BA concentration (mg L^{-1})	NAA concentration (mg L^{-1})	No. of shoot per explant	Shoot length (cm)	No of nodes per shoot
WPM	0	0	-	1.08b	1.1f
		0.05	-	1.11b	1.2f
		0.1	-	1.11b	1.2f
	1	0	1.6def	0.72cd	1.3e
		0.05	1.7def	0.71cd	1.3e
		0.1	2.0de	0.67cd	1.4cd
	2	0	2.8c	0.51ef	1.4cd
		0.05	4.9b	0.43f	1.6b
		0.1	6.1a	0.5ef	1.8a
MS	0	0	-	1.45a	1.1g
		0.05	-	1.49a	1.1g
		0.1	-	1.53a	1.3e
	1	0	1.2f	1.1b	1.4cd
		0.05	1.6def	0.97b	1.4cd
		0.1	1.3ef	1.05b	1.7b
	2	0	1.9def	0.79c	1.4cd
		0.05	2.2cd	0.61de	1.4cd
		0.1	2.8c	0.21f	1.4cd

Mean values followed by the same letter in each column are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Interaction of culture medium and BA concentration showed that the highest internode length (2.09 mm) was observed in MS medium without BA; while the lowest internode length was detected in WPM medium containing 2 mg L^{-1} BA. They showed no significant difference with WPM medium containing 1 mg L^{-1} BA and

MS medium containing 2 mg L^{-1} BA. Interaction of culture medium and NAA concentration showed the highest internode length (1.48 mm) in MS medium without NAA, which was not significantly different from 0.05 mg NAA in the same medium. The lowest internode length was observed in WPM medium containing 0.1 mg L^{-1} NAA; that was not significantly different from other NAA concentrations in this medium (Fig. 1).

Leaves had higher growth in MS medium than WPM medium. Application of BA and NAA had negative effect on leaf area (base on physical observations). MS medium without growth regulators was the most suitable medium for proper leaf growth.

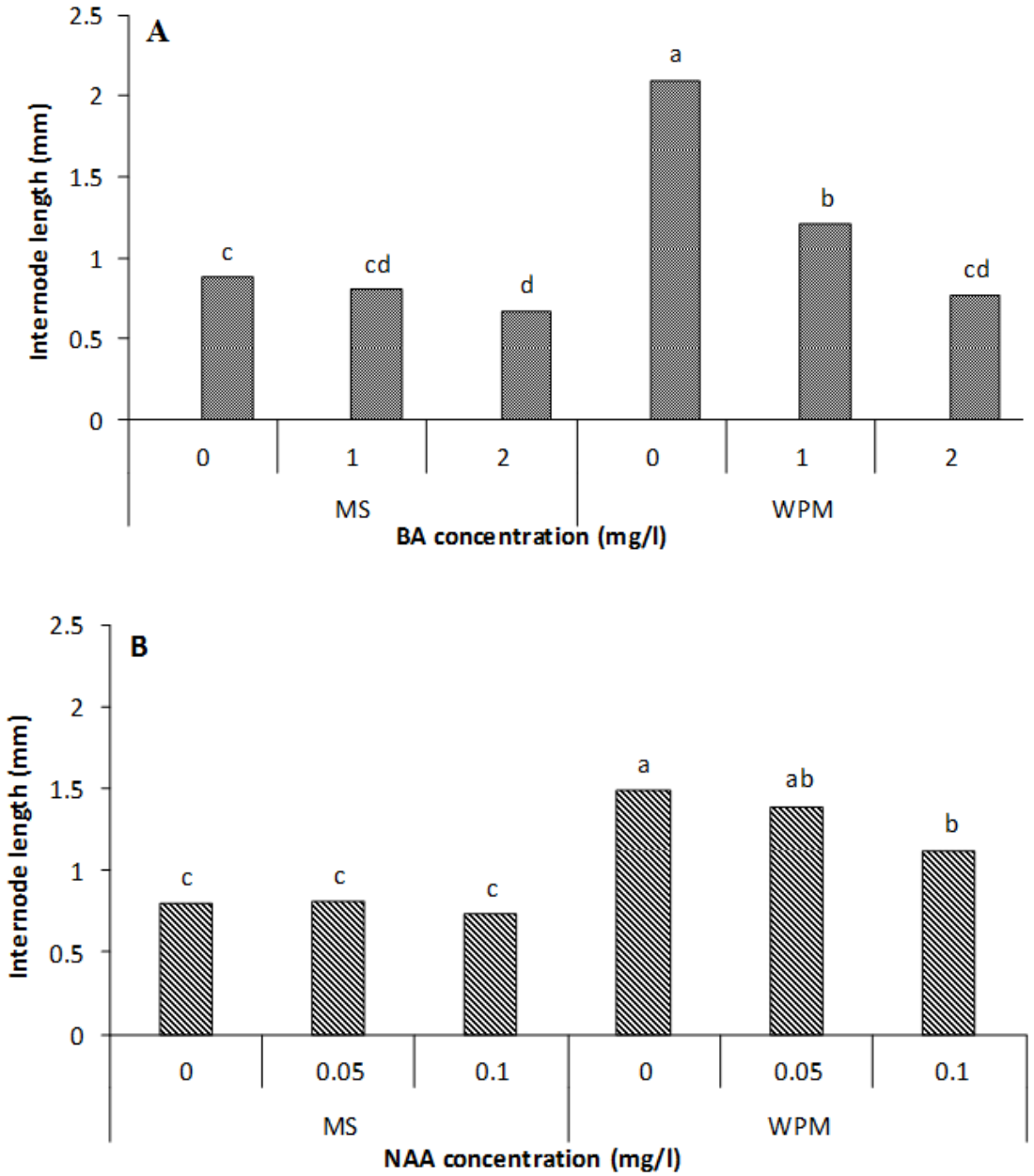

Fig. 1. Effect of medium and BA (A) and medium and NAA (B) concentrations on internode length. Mean values followed by the same letter are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Effect of light quality on shoot growth characteristics

The effect of light quality on shoot number per explant, shoot length, shoot diameter, explant dry weight, number of nodes, and internode length were significant at 1% level. The effect of light quality on explant dry weight was significant at 5% level. The highest and lowest number of shoots per explant was observed in the fluorescent and red lights; correspondingly, which were not significantly different from those of the red+blue light. The highest shoot length was observed in the red+blue light but the lowest shoot length was observed in the fluorescent light. The highest shoot diameter (3.43 mm) was seen in red light and the smallest one in florescent light. The highest number of nodes was found in fluorescent light which was not significantly different from the red+blue light. Meanwhile, the lowest number of nodes was observed in red light. The longest internodes were seen in red light; while the shortest ones were found in fluorescent light. The highest explant fresh weight was obtained in red light (443.5 mg) but the lowest one in fluorescent light; which revealed no significant difference with red+blue light treated explant. The highest dry weight of explant (50.2 mg) was observed in florescent light treatment but the lowest one (37.7 mg) was seen under red light. Percentage of dry mater increased in red+blue light in comparison with those of florescent light; however, the lowest one was observed under red light (Table 2).

The effect of light quality on explant fresh and dry weights was significant at 5 and 1% level; respectively. The explants under red light had the highest weight, which were significantly different from fluorescent and red+blue light. However, in this treatment the lowest explant dry weight was also observed compared with the other light treatments. This change in red light position from the highest explant fresh to the lowest dry weight indicates the percentage of abundant water in these explant tissues (Table 2).

Table 2. Effect of light quality on Cadaman explants proliferation and growth, six weeks after culture on WPM medium containing 2 mg L^{-1} BA and 0.1 mg L^{-1} NAA.

Light quality	No. of Shoot per explant	Shoot length (mm)	Shoot diameter (mm)	No. of nodes per explant	Internode length (mm)	Explants fresh weight (mg)	Explants dry weight (mg)	Explants dry matter (%)
Red	3.83^{b}	9.76^{b}	3.43^{a}	19.7^{b}	1.89^{a}	443.5^{a}	37.7^{c}	8.61^{c}
Red+blue	3.91^{b}	14.31^{a}	2.55^{b}	31.7^{a}	1.70^{b}	300.2^{b}	40.5^{b}	16.24^{a}
White cool	5.74^{a}	7.26^{c}	2.38^{b}	32.7^{a}	1.28^{c}	340.0^{b}	50.2^{a}	14.65^{b}
Significance	**	**	**	**	**	*	**	**

Mean values followed by the same letter in each column are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

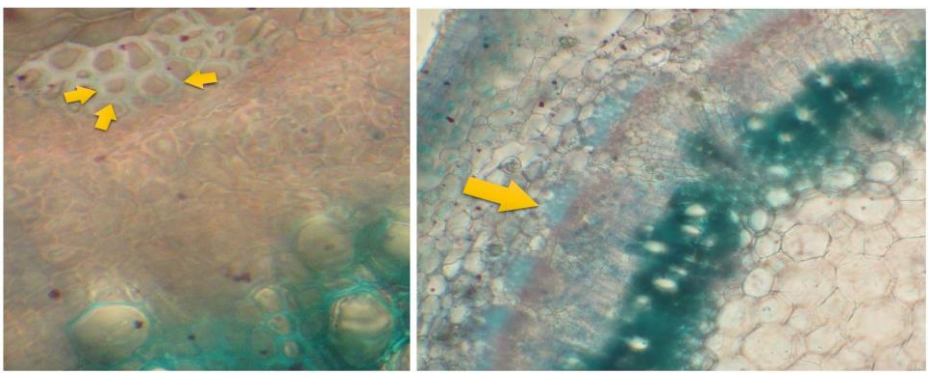

Fig. 2. The cross-section of the stem under fluorescent light treatment, the yellow arrow shows the location of lignin accumulation, and thickening of the cell wall.

Effect of light quality on rooting and root growth

Most of the explants (87%) had no rooting. No rooting was observed among the explants treated in the red light either. However, the explants under fluorescent light had the highest rooting percentage.

Effect of light quality on leaf growth and structure

The effect of light quality on leaf thickness, lower epidermis thickness, spongy parenchymal thickness, palisade parenchymal thickness and upper epidermis thickness were all significant at 1% level. The highest leaf thickness (148.6 µm) was observed in the fluorescent light treatment with no significant differences from the red+blue light. The lowest leaf thickness (109.6 µm) was observed in the red light (Fig. 3; Table 3). The highest thickness of the lower epidermis (17.1 µm) was observed in the red+blue light with no significant differences from the fluorescent light. The lowest epidermis thickness (15.8 µm) was found in the red light. The highest thickness of the spongy parenchyma (71.5 µm) was related to the red+blue light; while the lowest value was observed in the red light (47.6 µm). The highest palisade parenchymal thickness (41.7 µm) was observed in fluorescent light treated explants; but the lowest value (27.5 µm) was observed in red light, which had no significant difference from the red+blue light. The highest upper epidermis thickness (26.2 µm) was observed in fluorescent light and the lower ones (18.6 µm) in red light treated explants (Table 3).

Table 3. Effect of light quality on leaf anatomical properties of Cadaman explants, six weeks after culture on MS medium without plant growth regulators.

Light quality	Leaf thickness (µm)	Lower epidermis thickness (µm)	Spongy parenchyma thickness (µm)	Palisade parenchyma thickness (µm)	Upper epidermis thickness (µm)
Red	109.6^{c}	15.8^{b}	47.6^{c}	27.5^{b}	18.6b
Red+blue	143.1^{a}	17.1^{a}	71.5^{a}	29.8^{b}	24.6a
White cool	148.6^{a}	16.9^{a}	61.7^{b}	41.7^{a}	26.2^{a}
Significance	**	**	**	**	**

Mean values followed by the same letter in each column are not significantly different at $P \leq 0.05$ by Duncan's multiple range tests.

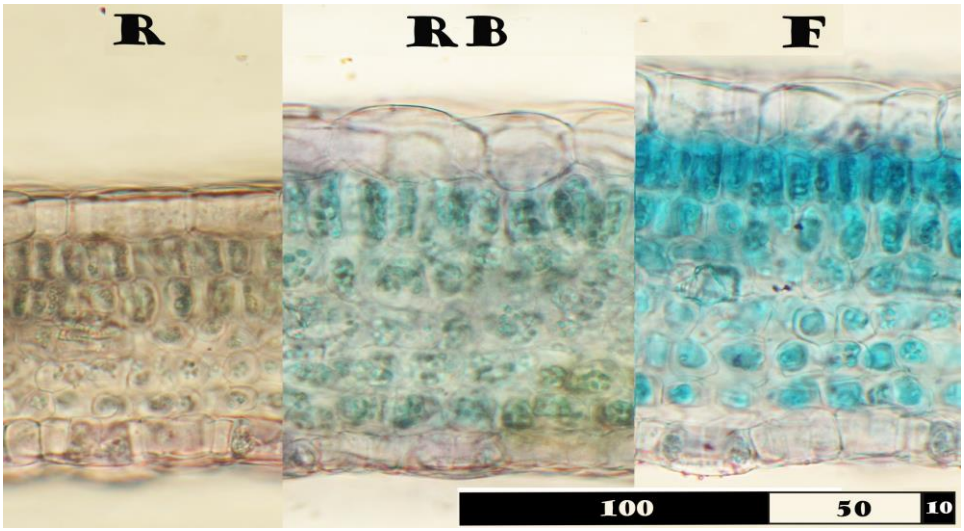

Fig. 3. Comparison of leaf thickness under different light treatments. R) Explant leaf under red light shows lower thickness and compact cell layers. RB) Explant leaf under red+blue light shows medium cell layers and close to the fluorescent light. F) Explant leaf under fluorescent light shows the highest thickness. The index in micrometer was designed with Autodesk AutoCAD 2016 software.

The light intensity used in this study (75 μmol m^{-2} s^{-1}) seems to be the appropriate light intensity to investigate the effects of light quality on leaf growth. According to Fan et al. (2013), when light intensity increased from 50 to 300 $\mu molm^{-2}s^{-1}$, parenchyma ladder cells and leaf blade thickness increased. However, under the highest amount of photosynthetic photon flux (550 μmol m^{-2} s^{-1}), mesophilic cells were smaller than those under 300 or 450 μmol m^{-2} s^{-1} light intensities.

Effect of light quality on the morphology of stomata

The effect of light quality on stomata length, stomata width, number of stomata per unit area, and stomata production were significant at 1% level. The highest stomata length (28.5 μm) was observed in the red+blue light treatment; while the lowest stomata length (20.8 μm) was seen in the red light treatment (Table 4). The highest stomata width (26.6 μm) was observed in explants under the fluorescent light treatment, which was not significantly different from the blue light treatment. The lowest stomata width (15.9 μm) was observed in the red light treatment. The highest number of stomata per unit area was produced under the red light and the lowest number of stomata was found in the red+blue light, which was not significantly different from the blue light. The highest index of stomata output was observed in the red light; while the lowest value was observed in the hybrid light, which was not significantly different from the blue light (Table 4).

Effect of light quality on chlorophyll concentration

Type of media, light quality, and their interaction had a significant effect on chlorophyll a, b, and total chlorophyll concentration at 1% level. The highest concentrations of chlorophyll a, b, and total were observed in MS medium under red+blue light; while the lowest ones were observed in WPM medium under red light treatment, which had no significant difference from MS medium under red light treatment (Table 5).

Table 4. Effect of light quality on stomata properties of Cadaman explants, six weeks after culture on MS medium without plant growth regulators.

Light quality	Stomata length (μm)	Stomata width (μm)	Stomata number (no/mm^2)	Stomatal index
Red	20.8^{c}	15.9^{c}	447.3^{a}	15.26^{a}
Red+blue	28.5^{a}	21.6^{b}	245.2^{c}	7.78^{c}
White cool	25.2^{b}	26.6^{a}	397.0^{b}	12.45^{b}
Significance	**	**	**	**

Mean values followed by the same letter in each column are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Table 5. Effect of light quality on chlorophyll concentration of Cadaman explants, six weeks after culture on MS and WPM medium without plant growth regulators.

Medium	Light quality	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)
MS	Red	0.168^{f}	0.057^{ef}	0.226^{fg}
	Red+blue	1.215^{a}	0.373^{a}	1.589^{a}
	White cool	0.710^{b}	0.225^{b}	0.935^{b}
WPM	Red	0.143f	0.041f	0.175g
	Red+blue	0.441d	0.147c	0.589d
	White cool	0.263e	0.075de	0.339ef
Significance		**	**	**

Mean values followed by the same letter in each column are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

Discussion

Comparison between the two MS and WPM media used in the study showed that more shoots and nodes are produced in WPM medium, which is consistent with the results of Dejampour et al (2017) on peach×almond hybrids, Choudhary et al. (2015) on almond, and Couto et al. (2004) on Cadaman rootstock. Couto et al (2004) reported that decreasing the salt concentration of the medium increased the number of shoots but decreased their length. Choudhary et al. (2015) also reported that MS medium showed less shooting than WPM, and it required more time for shoot growth.

In this study, BA effect on shoot number was in agreement with the results of Isikalan et al. (2008) and Dzhampour et al. (2017). Gurel and Gulsen (1998) reported that BAP is essential for shoot proliferation, but its high concentration results in vitrification and callus formation. Low concentration of auxin was found to be essential in the development of shoots, which was in line with the results of Gurel and Gulsen (1998) and Dejampour et al (2017). Findings of the present study revealed that both the highest shoot proliferation and the highest number of nods were obtained in WPM medium at high BA and low NAA concentrations.

In addition to plant growth regulators, meduim composition and its salt concentration has great effect on Cadaman rootstock proliferation on MS medium. In fact, decreasing salt concentration of medium resulted in shorter shoot (Couto et al. 2004). The highest percentage of Nonpareil almond shoot proliferation is reported from MS medium containing 1 mg L^{-1} BA (Isikalan et al. 2008); while the best rooting is obtained in 1/2MS (Isikalan et al. 2008; Choudhary et al. 2015). Gurel and Gulsen (1998), by examining the micropropagation of Texas and Nonpareil almond cultivars, indicated that low BAP concentration was essential for shoot development. MS medium has a good effect on the growth of explants because of its high nitrogen content. Since nitrogen is an important part of amino acids, vitamins, nucleotide acids, and proteins; its availability can be a suitable factor for explants growth. The explants cultured in MS medium began to grow at a higher rate, and therefore they had better vegetative growth than those grown in WPM medium. Leaf chlorosis was observed in both media at the end of the fourth week. It seems that the cause of chlorosis in the two media is different. The faster growth of the explants in MS medium causes them to absorb the elements present in the medium and to employ it on leaf and stem development. These explants started to grow at a high rate initially, but reached a slow growth phase after consuming the available nutrients. In this case, the explants showed symptoms of nutrient deficiency, especially of macro elements, such as nitrogen.

Here we showed that elongation of shoots is lower under red light in comparison to those of red+blue. Based on these results, it can be reported that for some traits the monochromatic red light can be better than red+blue light (Aliniaeifard et al. 2018), principally explants under red+blue light show higher shoots elongation, possibly due to the synergistic effect among different photoreceptors (Smith et al., 2017). Compared to the florescent light, shoot proliferation was significantly reduced in red and red+blue lights. These results were consistent with those of Kim et al. (2004), Muleo and Morini (2006 and 2008), and Font i Forcada et al. (2012). It has been suggested that blue light impedes apical dominance and increases proliferation. In contrary, red light reduces lateral bud formation, apical dominance, and terminal bud growth (Muleo and Morini, 2001). Muleo and Morini (2006) reported that the number of passive lateral meristems on the main branch in UV-A and blue light was higher than other light qualities. The findings of present study are in consistent

with the results obtained by Heo et al. (2002), Kim et al. (2004) and Gu et al. (2012); who reported significant effect of light quality in shoot proliferation.

It seems that stem elongation can be stimulated or inhibited by plant species affected by light-receptor interactions (Kim et al., 2004; Fukuda et al. 2016). The present results showed that stem length can be controlled or enhanced by both light quality and the ratio between different light qualities. It was in line with the results of Heo et al. (2002), Kim et al. (2004) and Gu et al. (2012), but contradictory with the results of Lian et al. (2002), Muleo and Morini (2008), and Iacona and Muleo (2010). Light quality plays important role in plant growth regulators biosynthesis and accumulation such as gibberellin and cytokinin (Fukuda et al. 2016). However, the role of light quality in plant development has not yet been fully elucidated. The effect of light quality varies greatly depending on plant species, growth stage and environmental conditions such as photon flux, composition of medium, and ventilation (Hahn et al. 2000; Kim et al., 2004).

In the cross-section of the stem of the explants from the new growth section, the red light samples had very little lignin accumulation, but the red+blue light and fluorescent explants produced thick lignin deposition on the cell wall (Fig. 2). This indicated that the stems were more woody in the fluorescent explants than the red ones. This finding is in line with the results of Kim et al. (2004) and Ding et al. (2010); who reported emergence of soft shoots under red light. However, in the red+blue light treatment, a higher dry matter percentage was observed, which was in line with the results of Gu et al. (2012) and Lin et al. (2013). It seems that the explants under red light have more brittle stems and greater diameter because of the presence of more water. Albeit, it requires further investigation.

There are various reports of the effect of light quality on rooting. According to Fuernkranz, et al. (1990), in a study in *Prunus serotiana*, white light inhibits rooting, blue light decreases rooting speed, and yellow light increases rooting. Iacona and Muleo (2010), by studying the Colt cherry rootstock, reported the highest root growth and development in the explants treated with red+blue light; while the red and far red light treatments reduced the number of roots compared to blue light. Red light increased root length compared to blue light. In the *Anthurium andraeanum* micro-environment, light quality had a significant effect on rooting and root growth. Gu et al. (2012) reported that treating explants by monochromatic light produced fewer roots than combined light. They also reported higher fresh weight of roots under red+blue and fluorescent lights in comparison with those of monochromatic light.

Liu et al. (2014) reported that under blue and red+blue light, leaf number was higher than red and fluorescent light. Blue light increases leaf area as well (Wang et al., 2015). Liu et al. (2014) reported that under blue light, leaf area, leaf thickness and dry weight were higher than fluorescent and red+blue light. In the present study, the lowest leaf thickness was observed under red light treatment, which is consistent with the results of Macedo et al. (2011). Macedo et al. (2011) reported that light spectrum could inhibit the parenchymal and the upper epidermis thickness, which is in line with the findings of this study. They also reported that red light reduces the thickness of the spongy parenchyma compared with other light treatments.

The stomata are pores in the leaf surface that regulate gas exchange with the environment. Stomata development is influenced by environment. Light is one of these environmental factors affecting this phenomenon (Casson et al., 2014). It has been found that illumination reduces the number of stomata compared to the dark (Macedo et al., 2011). The red+blue light

treated explants that had the highest growth, the smallest stomata number, and the largest stomatal size. It has been reported that stomata conductance increased in red+blue light compared to red monochromatic light (Hogewoning et al., 2010; Savvides et al., 2012; van Ieperen et al., 2012).

Stomata density, epidermal cell density, stomata aperture per leaf area, and stomata conductance were significantly higher for red+blue combination light treatment compared with red light treatment (Savvides et al., 2012; van Ieperen et al., 2012). Li et al. (2013) reported larger stomata under red+blue light with higher blue ratio compared to the higher red ratio. According to the results of this study, the stomata were open in red light, which is consistent with the results of Hogewoning et al. (2010), Savvides et al. (2012), van Ieperen et al. (2012), and Li et al. (2013). The number of stomata under the red+blue light was lower than the red light, and the index of stomata formation under the red light was higher than the red+blue light, which is consistent with the results of Kim et al. (2004). The diameter of stomata in the red+blue light was more than the red light, which is in agreement with the results of Kim et al. (2004). The number of stomata per unit area under fluorescent light was higher than red+blue light.

Blue light is crucial for chlorophyll biosynthesis, enzyme synthesis, and the maturation of chloroplasts and chlorophylls (Muleo and Marini, 2003). Iacona and Muleo (2010) by studying the effect of light quality on chlorophyll content of cherry rootstock, concluded that the highest amount of chlorophyll was produced under the combination of blue and red light and the lowest chlorophyll content was detected under the far red light. Samuoliene et al. (2010) also reported higher levels of chlorophyll under red+blue light than red, which was attributed to the simultaneous presence of both red and blue light qualities. Blue and red lights are essential for the synthesis of thylakoid membrane polypeptides. According to the obtained results, the highest concentration of chlorophyll was observed under red+blue light, which is in agreement with the results of Samuoliene et al. (2010), Mousavifattah and Sarikhani (2016); Iacona and Muleo (2010) and Muleo and Marini (2003) and Hosseini et al. (2019). Liu et al. (2014) stated that LED of any light quality produced more chlorophyll a, b, and total than fluorescent light. Hosseini et al. (2019) reported higher chlorophyll concentration in basil plant under red+blue light in comparison with those of red or blue monochromatic light. It seems that both red and blue lights are essential for chlorophyll biosynthesis (Taiz et al. 2015).

Conclusion

The management of a favorable lighting environment for plant tissue culture especially for micropropagation has attracted much attention in the last decade. In the present study, WPM medium containing high BA and low NAA levels showed the high proliferation. It seems that MS medium without growth regulators is suitable for rooting of Cadaman explants. Light quality had a significant effect on number of shoots, branch length, shoot diameter, number of nodes, internode length, and fresh and dry weight of shoots. In general, it can be concluded that LED light can replace fluorescent light in the growth chamber for woody plant micropropagation. However, for better performance, it is advisable to use the appropriate red+blue combination. Furthermore, light quality can affect the developmental path of the explants, and lead the plant to proliferation or vegetative growth.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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