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Influences of Light-Emitting Diodes (LEDs) and Culture Media on Micropropagation of Native Hemp (*Cannabis sativa*) Population from Türkiye

Cennet YAMAN[1]✉, Güngör YILMAZ[1,2], Serkan URANBEY[3]
[1]Yozgat Bozok University, Faculty of Agriculture, Department of Field Crops, 66200 Yozgat, Türkiye, [2]Yozgat Bozok University, Institute of Hemp Research, 66200 Yozgat, Türkiye], [3]Ankara University, Faculty of Agriculture, Department of Field Crops, 06100 Yozgat, Türkiye.
[1]https://orcid.org/0000-0002-2364-8171, [2]https://orcid.org/0000-0003-0070-5484, [3]https://orcid.org/0000-0002-0312-8099
✉: cennet.yaman@yobu.edu.tr

ABSTRACT
Light-emitting diodes (LEDs) have historically been the standard for supplemental lighting in industrial hemp (*Cannabis sativa*) cultivation in a greenhouse, but there is a lack of scientific understanding in *C. sativa* regarding different media under various LEDs in vitro conditions. The aim of this study was to determine the top-performing LEDs and nutrient media for micropropagation of *C. sativa*. In this study, two different spectra (white and red LEDs) and different media [MS, B5, MS+B5, ½ MS, ½ B5, ½ MS + ½ B5, and control (without medium)] were used, maintaining constant light photoperiod. The diversity of light spectra and nutrient media showed statistically significant differences for the growth parameters of *C. sativa*. Red LED provided an extraordinarily high shoot length of 7.37 cm, whereas white LED exhibited the highest root length with 6.57 cm. Also, nutrient media were found to be effective on shoot growth in full-strength media, while on root growth in half-strength media. Among the plant growth parameters, the number of shoots and nodes per plant is of great importance for clonal propagation. B5 medium under red LED provided an extraordinarily high shoot number with 4.64, whereas MS medium under white LED exhibited the highest node number with 5.87. Each LED source required the respective medium adjustment for the best results of the desired parameters. In this respect, the study may be advantageous for the selection of the appropriate nutrient medium and led spectrum in micropropagation of *C. sativa*.

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Türkiye'deki Yerel Kenevir (*Cannabis sativa*) Popülasyonunun Mikroçoğaltımı Üzerinde Işık Yayan Diyotların (LED'ler) ve Kültür Ortamlarının Etkileri

ÖZET
Işık yayan diyotlar (LED'ler), tarihsel olarak serada endüstriyel kenevir (*Cannabis sativa*) yetiştiriciliğinde ek aydınlatma için standart olmuştur, ancak *C. sativa*'da in vitro koşullarında çeşitli LED'ler altında farklı ortamlar konusunda bilimsel anlayış eksikliği vardır. Bu çalışmanın amacı, *C. sativa*'nın mikro çoğaltılması için en iyi performans gösteren LED'leri ve besin ortamlarını belirlemektir. Bu çalışmada, sabit ışık fotoperiyodu korunarak iki farklı spektrum (beyaz ve kırmızı LED'ler) ve farklı ortamlar [MS, B5, MS + B5, ½ MS, ½ B5, ½ MS + ½ B5 ve kontrol (ortamsız)] kullanıldı. Işık spektrumlarının ve besin ortamlarının çeşitliliği, *C. sativa*'nın büyüme paarametreleri için istatistiksel olarak anlamlı farklılıklar gösterdi. Kırmızı LED, 7,37 cm ile olağanüstü yüksek bir sürgün uzunluğu sağlarken, beyaz LED 6,57 cm ile en yüksek kök uzunluğunu sergiledi. Ayrıca, besin ortamlarının tam güçteki ortamlarda sürgün büyümesinde, yarı güçteki ortamlarda ise kök büyümesinde etkili olduğu bulunmuştur. Bitki büyüme parametreleri arasında, bitki başına sürgün ve nod sayıları klonal çoğaltım için büyük önem taşımaktadır. Kırmızı LED altında B5 ortamı 4.64 ile olağanüstü yüksek bir sürgün sayısı sağlarken, beyaz LED altında

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Anahtar Kelimeler
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Cannabis sativa
Kırmızı LED
Beyaz LED
Kültür ortamı
Mikro çoğaltma

MS ortamı 5.87 ile en yüksek nod sayısını sergilemiştir. İstenilen parametrelerin en iyi sonuçları için her bir LED kaynağı ilgili ortam ayarlamasını gerektirmiştir. Bu açıdan, çalışma *C. sativa*'nın mikroçoğaltımında uygun besin ortamının ve LED spektrumunun seçilmesi için avantajlı olabilir.

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INTRODUCTION

Cannabis sativa L. which is commonly known as cannabis, hemp and marijuana, an annual dioecious species belonging to Cannabaceae family is an important multipurpose crop cultivated for industrial, medicinal and nutritional uses since ancient times (Bonini et al., 2018, Akıncı & Karaman, 2024): its stem has fiber serving as feedstock for textile, paper, and mechanical industries and in the construction sectors (Ceyhan et al., 2022); its seeds are rich in nutrients as a source of nutritious oil and protein; Its flowers, leaves and roots contain commercially serious bioactive compounds that have a wide spectrum of biological activity (Esposito et al., 2021; Liu et al., 2022). However, in the 20th century, its cultivation was legally restricted in many countries due to the psychoactive effect of one of its compounds, Δ-9- Δ-9-tetrahydrocannabinol (THC) (Liu et al., 2022). In the commercial production of industrial hemp, the threshold THC amounts in its flowering plant parts range from 0.1% (several Australian states, Columbia, Mexico, Switzerland and Uruguay), to 0.2% (Europe), to 0.3% (Brazil, Canada, China and USA) of dry weight according to the legal permission of each country's governments (Anonym, 2016; Adhikary et al., 2021). Due to these legal restrictions, research and development studies on *C. sativa* have been slow.

The cultivation of this economically valuable plant is increasing day by day. Traditionally, the fiber type (commonly called hemp, with Δ^9-THC < 0.3%) of *C. sativa* is predominantly monoecious (hermaphrodite), mostly cultivated by seeds, as this characteristic produces more uniform material, and its cultivation has been highly mechanized (Chandra et al., 2017). In contrast, drug type (commonly called cannabis, with Δ^9-THC > 0.3%) of *C. sativa* is often propagated using clonal methods (Caplan et al., 2018, Campbell et al., 2019) to reduce the high levels of phenotypic diversity exhibited in seedling populations and to produce high-quality, genetically and phenotypically uniform crops that meet consumer preferences and comply with government regulations (Lata et al., 2010). Although more expensive than seed, this approach is efficiently used by some *C. sativa* producers to commercially produce a more uniform crop containing CBD (Cannabidiol) and other non-psychoactive cannabinoids (Fike et al., 2020; Mark et al., 2020). Also, many scientists are trying to produce new varieties that can serve the purpose due to the economic importance of *C. sativa*. The predominantly annual dioecious nature of *C. sativa* (Clarke et al., 2016) is an important obstacle to slowing down traditional breeding efforts. Therefore, clonal multiple propagation practices offer great service for the production of uniform crops of male and female individuals to be used for the breeding program of *C. sativa*.

Micropropagation by tissue culture is an effective method for mass propagation of disease-free plants in a highly controlled environment using aseptic techniques and has been actively used for many commercial plants for a long time (Abdalla et al., 2022; Xue et al., 2023; Ioannidis et al., 2023; Miladinova-Georgieva et al., 2022, Özyalın & Yaman, 2023). Successful in vitro propagation of plants depends on explant source (He et al., 2023; Papafotiou et al., 2023), basal medium type (Suraya et al., 2021; Meyad et al., 2023), and climatic conditions such as light, temperature, humidity, and others (Yadav et al., 2020; Wojtania et al., 2023). Moreover, the type and concentration of basal media have a significant effect, both on shoot multiplication and rooting, as well as their promotion of the formation of strong plants with well-developed leaves (Garcia et al., 2011; Page et al., 2020).

Light, which is a physical factor, plays an important key role in processes of morphogenesis, photosynthesis, and primary and secondary metabolism of plants, which ensures the survival of the in vitro plant cultures (Azmi et al., 2016; Khurshid et al., 2020). The use of light-emitting diodes (LEDs) among the light sources is widely used in clonal reproduction techniques from *C. sativa*, especially in greenhouse conditions (Namdar et al., 2019), due to the many advantages, such as lower heat emission and more efficient energy utilization, as well as significantly longer lifetimes. However, the effects of LED spectra on *C. sativa* have not yet been previously elucidated in vitro culture. Therefore, the aim of the present study was to determine the effects of different media under various LED spectra on in vitro shoot proliferation and rooting in order to improve the in vitro *C. sativa* micropropagation system.

MATERIAL and METHOD

Material

The seeds of *Cannabis sativa* L., dioecious genotype Narlısaray, a native population of Türkiye, were obtained from the Hemp Research Institute of Yozgat Bozok University.

Surface Disinfection and Germination Condition

The seeds were washed with running tap water. After initial washing, the seeds were treated with 70% ethanol for 1 min. Lastly, the surface of seeds was disinfected by shaking in a solution containing 0.5% NaOCl (15% v v^{-1} commercial bleach) and 0.1% Tween 20 (v v^{-1}) for 15 min. Finally, the seeds were thoroughly rinsed 4-5 times with de-ionized sterile water and cultured in ½ × Murashige & Skoog basal salts (MS, Murashige and Skoog, 1962) supplemented with 1.5 % sucrose. The pH of the medium was adjusted to 5.7±0.1 with 0.1 N NaOH or 0.1 N HCl before the addition of 0.6% (w v^{-1}) plant agar (Duchefa), and then autoclaved at 121 °C for 20 min. The seeds were maintained in a growth chamber at room temperature (25 ± 1 °C) under 16/8 h light/dark photoperiod, 60% relative humidity, and cool white, fluorescent light (36 W/830 G13 1214 mm) intensity of about 40 µmol $m^{-2}s^{-1}$ PPFD for 15 days. After two weeks, the apical shoots with a single node excised from germinated seedlings (healthy zygotic embryos) were used as an explant source.

Culture Media

The apical shoots were cultured onto seven different media prepared separately in a 100 ml culture flask. Two different basal nutrient media (MS basal salts+vitamins and Gamborg B5 vitamins (B5, Gamborg et al., 1968)) and their different concentrations (full and half-strength) and their combinations were used as the medium for the culture: MS, B5, MS+B5, ½ MS, ½ B5, ½ MS + ½ B5, and control (without medium). All media were supplemented 3% (w/v^{-1}) sucrose and 0.64% (w/v^{-1}) plant agar.

All cultures were incubated in growth rooms for 5 weeks at 24 ± 2 °C temperature under two different spectra (red and white) with 16/8 h light/dark photoperiod by light-emitting diodes (LEDs) fluorescent tubes (Lumilux, L 36W/865, cool daylight, Osram, Germany): white LED and red LED (λ = 650 nm), relative humidity 60 %. All media were separately tested at white and red LEDs to evaluate their effects on shoot proliferation (Figure 1).

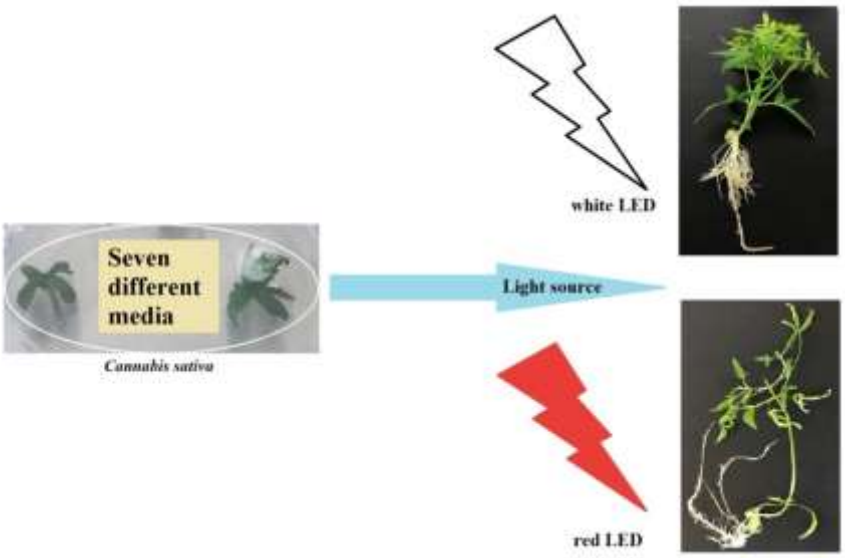

Figure 1. In vitro culture of *Cannabis sativa* on seven different nutrient media under two different LEDs
Şekil 1. İki farklı LED altında yedi farklı besin ortamında Cannabis sativa'ın in vitro kültürü

Analysis of Growth Parameters

Three weeks after the beginning of the culture, the apical segments of the in vitro-grown shoots were cut to form new shoots from the nodal parts. After this treatment, the in vitro shoots were continued to culture for another two weeks. In total, explants were cultured for 5 weeks. Then, the observations were recorded as shoot vitality, root formation, number of shoots per plant, number of roots per plant, number of nodes per plant, number of leaves per plant, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight.

Experimental Design and Statistical Analysis

The experimental design was completely randomized with four replicates using five explants in each culture flask. In the experiment, seven different culture media, including a control under two different (white and red) light sources, a total of 14 treatments, were used. These data were analyzed by One-Way analysis of variance (ANOVA) using the SPSS statistical program. The mean values were given with standard error (±SE) and compared with Duncan's multiple range test at $P< 0.05$ for significant differences. The mean values of the applications were compared using the Duncan test. Percentages were subjected to arcsine transformation before statistical analysis (Snedecor and Cochran 1967). Hierarchical cluster analysis (HCA) was used to evaluate all parameters and to select the best combination of LED spectra and different culture media for *in vitro* culture plantlets of *C. sativa*. HCA findings based on between-groups linkage and squared Euclidean distance are presented as a dendrogram.

RESULTS

Effect of different LEDs and basal media on the growth parameters of *C. sativa*

In this study, the plant growth of *C. sativa* was very critically affected by light and nutrient media, which are environmental factors. The red LEDs produced the highest mean shoot vitality, shoot length, number of shoots per plant and number of roots per plant whereas the white LEDs promoted higher root formation, root length, number of nodes per plant, number of leaves per plant, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight (Table 1).

Table 1. Effect of different LEDs on the growth parameters of *C. sativa* in vitro condition.
Çizelge 1. Farklı LED'lerin in vitro koşullarda C. sativa'nın büyüme parametreleri üzerine etkisi.

Growth parameters	Red LED	White LED
Shoot vitality (%)	88.5	78.6
Root formation (%)	61.5	67.5
Shoot length (cm)	7.37	4.85
Root length (cm)	4.45	6.57
Number of shoots per plant	3.51	2.62
Number of roots per plant	2.70	2.33
Number of nodes per plant	2.24	3.61
Number of leaves per plant	11.06	12.73
Shoot fresh weight (g)	1.41	1.75
Shoot dry weight (g)	0.23	0.26
Root fresh weight (g)	0.24	0.82
Root dry weight (g)	0.03	0.08

The explants were separately cultured on various media (MS, B5, MS+B5, ½ MS, ½ B5, ½ MS + ½ B5, and control) supplemented with no plant growth regulators. The effect of these media on the growth parameters of *C. sativa* is given in Table 2. MS medium displayed a stronger effect on parameters related to shoot growth. However, ½ B5 and ½ MS + ½ B5 media showed a higher benefit on root growth characteristics.

Table 2. Effect of different nutrient media on the growth parameters of *C. sativa* in vitro condition.
Çizelge 2. Farklı besin ortamların C. sativa'nın in vitro büyüme parametreleri üzerine etkisi.

Growth parameters	Control		MS		B5		MS+B5		1/2 MS		1/2 B5		1/2MS+1/2 B5	
Shoot vitality (%)	16.7±7.5	c	100.0	a	100.0	a	100.0	a	70.8±9.6	b	97.2±2.8	a	100.0	a
Root formation (%)	27.8±14.1	b	83.3±6.1	a	63.9±13.9	a	55.6±11.1	ab	54.2±9.6	ab	83.3±4.2	a	83.4±7.5	a
Shoot length (cm)	1.00±0.5	c	7.76±1.1	a	7.38±0.9	ab	7.04±0.7	ab	5.42±0.2	b	7.50±0.3	ab	6.65±0.8	ab
Root length (cm)	0.73±0.4	d	6.43±0.7	abc	5.31±0.6	bc	5.05±0.3	c	7.67±0.4	a	6.04±1.3	abc	7.34±0.5	ab
Number of shoots per plant	0.88±0.4	c	3.68±0.2	ab	4.10±0.2	a	3.53±0.3	ab	2.74±0.4	b	3.39±0.3	ab	3.17±0.4	ab
Number of roots per plant	0.96±0.4	b	2.64±0.2	a	2.70±0.3	a	3.11±0.4	a	2.42±0.3	a	3.09±0.2	a	2.67±0.2	a
Number of nodes per plant	0.75±0.3	c	4.27±0.7	a	3.70±0.4	ab	2.57±0.1	b	3.05±0.5	ab	2.45±0.3	b	3.71±0.5	ab
Number of leaves per plant	1.00±0.5	d	15.47±0.3	a	14.94±0.5	ab	12.21±0.5	c	12.64±0.7	bc	13.09±1.7	abc	13.92±0.5	abc
Shoot fresh weight (g)	≤0.01		2.06		2.53		1.84		1.08		1.69		1.87	
Shoot dry weight (g)	≤0.01		0.38		0.33		0.24		0.19		0.28		0.30	
Root fresh weight (g)	≤0.01		0.67		0.48		0.16		1.12		0.66		0.63	
Root dry weight (g)	≤0.01		0.07		0.05		0.02		0.10		0.06		0.06	

Data expressed as the mean value ± SE (n = 6). Different lowercase (a–d) within column for each parameter indicate significant differences between means (Duncan test; $p \leq 0.05$).

Effect of basal media under different LEDs on the growth parameters of *C. sativa*

The treatments with the different basal media under different LEDs such as red LED or white LED promoted significant responses on shoot and root growth of *C. sativa* (Table 3).

Shoot vitality was affected by a different basal media and LEDs (Table 3). High shoot vitality (33.3-100%) occurred under both LEDS in all media except control media with white LEDs

Rooting was seen from the cut ends of the shoots all media tested under red and white LED. Root formation ratio was fluctuated between 0 % and % 100% (Table 3). The highest frequency of root proliferation (100%) was achieved on ½ MS+½ B5 medium (100%) under the white LED. Whereas B5 medium with white LED (94.4%) followed this combination. No root formation occurred in control medium with white LED.

Table 3. Shoot vitality, root formation, shoot length, root length in *C. sativa* seedlings grown in different nutrient media under LEDs of different colors.

Çizelge 3. Farklı renkteki LED'ler altında farklı besin ortamlarında yetiştirilen C. sativa fidelerinde sürgün canlılığı, kök oluşumu, sürgün uzunluğu, kök uzunluğu.

Color LEDs*, media	Shoot vitality (%)		Root formation (%)		Shoot length (cm)		Root length (cm)	
Red LED, control	33.3±0.0	d	55.5±9.0	cd	2.00±0.1	g	1.47±0.4	g
Red LED, MS	100.0±0.0	a	83.3±10.4	abc	10.10±0.4	a	4.88±0.2	d
Red LED, B5	100.0±0.0	a	33.3±0.0	d	9.33±0.0	b	4.07±0.1	e
Red LED, MS+B5	100.0±0.0	a	33.3±0.0	d	8.47±0.1	c	4.42±0.1	de
Red LED, ½ MS	94.4±5.6	ab	75.0±3.2	bc	5.56±0.4	f	6.81±0.3	b
Red LED, ½ B5	91.7±4.8	b	83.3±10.4	abc	7.69±0.4	de	3.23±0.4	f
Red LED, ½ MS+½ B5	100.0±0.0	a	66.7±0.0	cd	8.41±0.2	cd	6.29±0.2	bc
White LED, control	0.0±0.0	e	0.0±0.0	e	0.0±0.0	h	0.0±0.0	h
White LED, MS	100.0±0.0	a	83.3±9.6	abc	5.43±0.0	f	7.98±0.2	a
White LED, B5	100.0±0.0	a	94.4±5.6	ab	5.43±0.2	f	6.55±0.0	b
White LED, MS+B5	100.0±0.0	a	77.8±11.1	bc	5.61±0.4	f	5.68±0.2	c
White LED, ½ MS	50.0±0.0	c	33.3±0.0	d	5.28±0.0	f	8.53±0.1	a
White LED, ½ B5	100.0±0.0	a	83.3±0.0	abc	7.30±0.3	e	8.84±0.2	a
White LED, ½ MS+½ B5	100.0±0.0	a	100.0±0.0	a	4.89±0.1	f	8.40±0.1	a

Data expressed as the mean value ± SE (n = 3). Different lowercase (a–h) within column for each parameter indicate significant differences between means (Duncan test; $p \leq 0.05$). *red LED (λ = 650 nm); White LED.

Shoot length was also influenced by different basal media under the different LEDs. The maximum shoot length (10.10±0.4 cm) was obtained on MS medium under red LEDs. B5 medium x red LED combination also showed high shoot length (9.33±0.0). Whereas the shoot length was drastically decreased on the control (0.0 %) under white LED. Different media and LED types also affected root length, and the highest root length was observed in ½ B5 medium (8.84±0.2 cm) and ½ MS (8.53±0.1 cm) and ½ MS+½ B5 (8.40±0.1 cm), and MS medium (7.98±0.2 cm) under the white LED. Because there is no root formation as indicated in root formation, the lowest root length was observed in the control medium under both red and white LEDs (1.47±0.4 cm and 0.0±0.0 cm, respectively).

A significant effect of all basal media under different color LEDs was observed on the number of shoots per plant, number of roots per plant, number of nodes per plant, and number of leaves per plant (Table 4).

The number of shoots per plant was varied in response to the different media and light sources (Table 4). The red LED exhibited the highest number of shoots per plant in B5 medium (4.64±0.1) and MS medium (4.11±0.2) and in ½ MS+½ B5 (4.00±0.3), and MS+B5 (3.83±0.5). Under white LED, only ½ B5 medium (4.07±0.2) was statistically in the same group as these media under red LED.

The number of roots per plant a significantly affected by different LEDs and basal media (Table 4). The greatest number of roots per plant was observed in MS+B5 (3.56±0.6) medium under the white LED.

All treatments showed a significant effect on the number of nodes per plant (Table 4). The highest number of nodes per plant was observed in MS medium under the white LED (5.87±0.1).

LED colors had a serious effect on the number of leaves per plant (Table 4). Statistically, the highest values were recorded under the white LED in ½ B5 medium (16.84±0.4) and MS medium (16.06±0.2), and in B5 medium (15.94±0.3).

The shoot and root activities of *C. sativa* seedlings grown under the red and white LEDs varied in response to the different media (Table 5).

Shoot fresh weight both treatments showed a significant effect (Table 5). A high level of shoot fresh weight was

observed in B5 medium under the white LED (3.37±1.1 g) which was statistically significantly different from other applications.

Table 4. Number of shoots per plant, number of roots per plant, number of nodes per plant, number of leaves per plant in *C. sativa* seedlings grown in different nutrient media under LEDs of different colors.

Çizelge 4. Farklı renklerdeki LED'ler altında farklı besin ortamlarında yetiştirilen C. sativa fidelerinde bitki başına sürgün sayısı, bitki başına kök sayısı, bitki başına düğüm sayısı, bitki başına yaprak sayısı.

Color LEDs*, media	Number of shoots per plant		Number of roots per plant		Number of nodes per plant		Number of leaves per plant	
Red LED, control	1.75±0.1	e	1.92±0.3	c	1.50±0.0	f	2.00±0.0	f
Red LED, MS	4.11±0.2	ab	2.53±0.3	abc	2.67±0.3	c	14.89±0.4	b
Red LED, B5	4.64±0.1	a	3.23±0.1	ab	2.79±0.0	c	13.93±0.3	bc
Red LED, MS+B5	3.83±0.5	ab	2.67±0.3	abc	2.36±0.2	cde	13.30±0.2	c
Red LED, ½ MS	3.56±0.2	bc	2.51±0.3	abc	1.84±0.1	ef	10.98±0.0	d
Red LED, ½ B5	2.70±0.2	cd	3.17±0.3	ab	1.98±0.3	def	9.34±0.3	e
Red LED, ½ MS+½ B5	4.00±0.3	ab	2.83±0.4	abc	2.55±0.2	cd	13.00±0.8	c
White LED, control	0.0±0.0	f	0.0±0.0	d	0.0±0.0	g	0.0±0.0	g
White LED, MS	3.25±0.3	bc	2.75±0.1	abc	5.87±0.1	a	16.06±0.2	a
White LED, B5	3.56±0.1	bc	2.17±0.3	bc	4.61±0.3	b	15.94±0.3	a
White LED, MS+B5	3.22±0.2	bc	3.56±0.6	a	2.78±0.1	c	11.11±0.4	d
White LED, ½ MS	1.92±0.5	de	2.33±0.6	bc	4.25±0.1	b	14.29±0.2	b
White LED, ½ B5	4.07±0.2	ab	3.00±0.1	abc	2.92±0.3	c	16.84±0.4	a
White LED, ½ MS+½ B5	2.33±0.2	de	2.50±0.1	abc	4.86±0.1	b	14.83±0.2	b

Data expressed as the mean value ± SE (n = 3). Different lowercase (a–g) within column for each parameter indicate significant differences between means (Duncan test; $p \leq 0.05$). *red LED (λ = 650 nm); White LED.

Shoot dry weight varied in response to the different media and LED colors (Table 5). The highest shoot dry weight was obtained in MS medium (0.38±0.0 g) under both red LED and white LED, followed by B5 medium (0.37±0.0 g), ½ B5 medium (0.36±0.0 g), and ½ MS+½ B5 (0.35±0.0 g) under the red LED.

Root fresh weight LED colors had a significant effect on root fresh weight (Table 5). The higher level of root fresh weight was observed under the white LED in ½ MS medium (1.48±0.8 g), followed by ½ MS+½ B5 medium (1.08±0.1 g), ½ B5 medium (1.07±0.1 g), MS medium (1.05±0.1 g), B5 medium (0.83±0.0 g), and also those did not differ statistically from the red LED in ½ MS medium (0.76±0.2 g).

Table 5. Shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight in *C. sativa* seedlings grown in different nutrient media under LEDs of different colors.

Çizelge 5. Farklı renkteki LED'ler altında farklı besin ortamlarında yetiştirilen C. sativa fidelerinde sürgün taze ağırlığı, sürgün kuru ağırlığı, kök taze ağırlığı, kök kuru ağırlığı.

Color LEDs*, media	Shoot fresh weight (g)		Shoot dry weight (g)		Root fresh weight (g)		Root dry weight (g)	
Red LED, control	0.0±0.0	d	0.0±0.0	d	0.00±0.0	c	0.0±0.0	d
Red LED, MS	2.18±0.1	b	0.38±0.0	a	0.29±0.1	bc	0.04±0.0	bcd
Red LED, B5	1.69±0.2	bc	0.29±0.0	abc	0.13±0.1	c	0.02±0.0	cd
Red LED, MS+B5	1.91±0.1	bc	0.26±0.0	abc	0.08±0.0	c	0.01±0.0	d
Red LED, ½ MS	1.31±0.1	bc	0.22±0.0	bc	0.76±0.2	abc	0.08±0.0	abcd
Red LED, ½ B5	1.30±0.1	bc	0.2±0.0	c	0.25±0.1	bc	0.03±0.0	bcd
Red LED, ½ MS+½ B5	1.48±0.0	bc	0.24±0.0	abc	0.19±0.1	bc	0.02±0.0	bcd
White LED, control	0.0±0.0	d	0.0±0.0	d	0.0±0.0	c	0.0±0.0	d
White LED, MS	1.94±0.5	bc	0.38±0.1	a	1.05±0.4	ab	0.10±0.0	ab
White LED, B5	3.37±1.1	a	0.37±0.0	a	0.83±0.0	abc	0.09±0.0	abc
White LED, MS+B5	1.76±0.2	bc	0.22±0.1	bc	0.25±0.0	bc	0.03±0.0	bcd
White LED, ½ MS	0.86±0.2	cd	0.16±0.0	c	1.48±0.8	a	0.12±0.1	a
White LED, ½ B5	2.07±0.3	b	0.36±0.0	ab	1.07±0.4	ab	0.10±0.0	ab
White LED, ½ MS+½ B5	2.27±0.1	b	0.35±0.0	ab	1.08±0.1	ab	0.10±0.0	ab

Data expressed as the mean value ± SE (n = 3). Different lowercase (a–d) within column for each parameter indicate significant differences between means (Duncan test; $p \leq 0.05$). *red LED (λ = 650 nm); White LED.

Root dry weight LED colors had a significant effect on root dry weight. similar to root fresh weight, the greater root dry weight occurred under the white LED in ½ MS medium (0.12±0.1 g), followed by ½ MS+½ B5 medium (0.10±0.0 g), ½ B5 medium (0.10±0.0 g), MS medium (0.10±0.0 g), B5 medium (0.09±0.0 g), and also those did not differ statistically from the red LED in ½ MS medium (0.08±0.0 g).

Hierarchical cluster analysis

A hierarchical cluster analysis (HCA) was also used to identify the possible nearest and similarity of all growth parameters analyzed of *C. sativa* in vitro culture (Figure 2). According to the dendrogram results of HCA, four major clusters were observed. These clusters contain: Cluster I: White LED, control. Cluster II: White LED, ½ MS; Red LED, control. Cluster III: White LED, B5; White LED, ½ MS+½ B5; White LED, MS; White LED, MS+B5; White LED, ½ B5. Cluster IV: Red LED, B5; Red LED, ½ MS+½ B5; Red LED, MS; Red LED, ½ B5; Red LED, ½ MS; Red LED, MS+B5.

Regarding the clustering seen in HCA, we can speculate about the responses of in vitro growth similarities of different media used in this study under LED colors. Among the treatments, the white LED inhibited the growth of *C. sativa* in a medium that did not contain any basal medium. So, the White LED control clustered distinctly from the other treatments.

White LED, ½ MS, and Red LED, control clustered together, showed similar low impact on shoot vitality, root formation, number of shoots per plant, number of roots per plant, shoot fresh weight, and shoot dry weight in terms of analyzed parameters.

As cluster III strikingly indicates, the media under white LED, cluster IV covers the media under red LED. This shows that the white LED and red LED have significantly different effects on the in vitro growth of *C. sativa*.

For micropropagation from the nodal segment as an explant, the number of nodes on in vitro growing shoots is as important as shoot vitality. When examined from this point of view, the MS medium under the white LED was the most effective treatment. According to the HCA results, we can speculate that treatments in cluster III were higher than those in cluster IV.

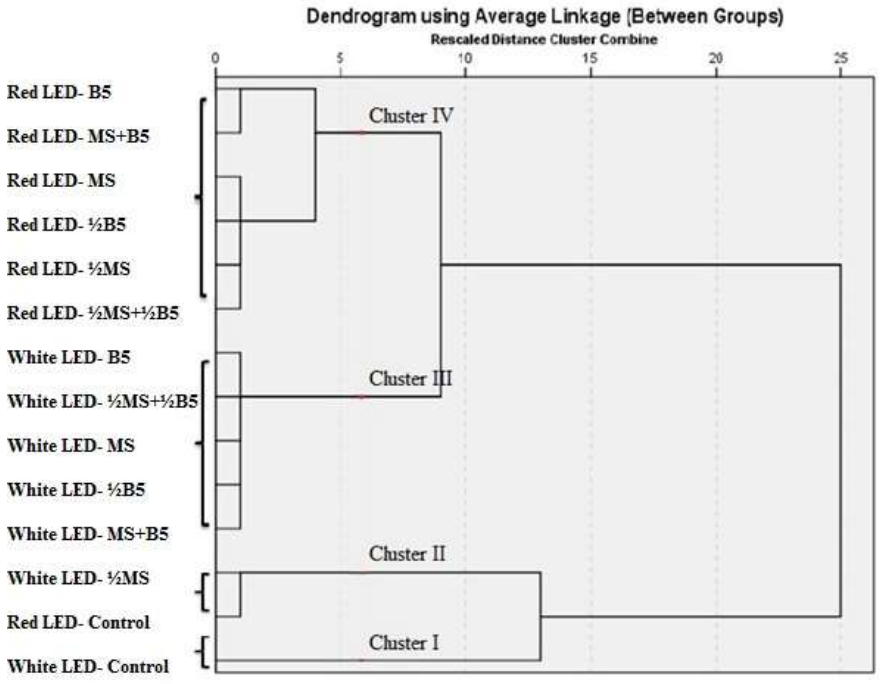

Figure 2. Dendrogram of the nutrient media under the red LED and the white LED for the twelve growth parameters of *C. sativa*.

Şekil 2. C. sativa'nın on iki büyüme parametresi için kırmızı LED ve beyaz LED altında besin ortamlarının dendrogramı.

DISCUSSION

In this study, the effects of light and culture medium were investigated in order to produce mass micro production from *C. sativa* under in vitro conditions. Miler et al. (2019) indicated that the success of an in vitro culture system

is related to the light source and basal medium type; it is important to identify the best spectra and suitable culture medium that provide the production of plants with desired characteristics. The most desirable growth parameters for clonal micropropagation are a great number of nodes and shoots on the plant, which is effective in the rapid reproduction of the plant.

Light source has a serious impact on the regulation of plant metabolism and morphology, as well as many other factors necessary for healthy plant growth (Davis & Burns, 2016; Yuanchun et al., 2021). Different light sources with various colors have actively been used in greenhouse and in vitro plant cultivation (Hung et al., 2016; Bantis et al., 2018). Spectral quality can have a profound effect on the growth, development, and physiology of plants. Especially, the spectra during in vitro culture are of serious importance not only for healthy morphological characteristics but also for the efficiency of adventitious root formation and shoot vitality (Iacona & Muleo, 2010). Among various light sources, red LEDs are mostly used for plant growth because wavelengths around 660 nm are highly effective for chlorophyll absorption, which in turn is effective for leaf growth, carbohydrate accumulation, and anatomical development through optimum photosynthetic efficiency (Mengxi et al., 2011; Nam et al., 2022).

There is not enough detailed information about the effects of light sources on the plant regeneration of *C. sativa* in vitro culture conditions. Recently, many studies have been carried out to determine the effect of light on *C. sativa*, especially increasing its phytochemical content and clonal propagation for field cultures and usually greenhouses by cutting from seed or from the mother plant (Hawley et al., 2018; Livadariu et al., 2019; Namdar et al., 2019; Jenkins & Livesay, 2021; Islam et al., 2021a; Islam et al., 2021b; Wei et al., 2021; Morello et al., 2022; Schilling et al., 2023). However, this study is almost the first report on the effect of (white and red) LEDs for *C. sativa* multiplication in vitro. *C. sativa* growth in vitro under different LED colors (red and white) resulted in higher shoot vitality, shoot length, number of shoots, and number of roots when the explants were subjected to red LED. Our findings indicated that red LED had a positive effect on shoot activity. Many studies reported that red color light stimulated shoot elongation in plants (Casal, 2013; dos Reis Oliveira et al., 2020). Similarly, Shulgina et al. (2021) recorded that red LED had the tallest plants and the greatest microshoots of *Stevia rebaudiana* Bertoni grown in vitro compared to all other LED treatments. Gnasekaran et al. (2021) indicated that the red LED exhibited the greatest number of roots, number of shoots, and shoot length from *Zingiber officinale* var. *rubrum* Theilade compared to the white LED.

On the other hand, early works showed that the white light compared to red light increased the chlorophyll a and b content, and accordingly, it influenced the number of leaves, number of nodes and fresh/dry weight of the plant (Matysiak & Kowalski, 2019; Gnasekaran et al., 2021; Nacheva et al., 2021). In this study, the white LED had the maximum node production per plant, which is important for clonal micropropagation. Similar to this study, Nacheva et al. (2021) reported that red light limited node formation. The high root length may have occurred as Silva-Navas et al. (2016) reported that white light, together with cytokinin, induces cell elongation by stimulating the accumulation of some bioactive agent on the light side of the root.

Under in vitro conditions, the types of nutrient medium have a serious effect due to their macro and micro elements. Previous literature reported that each nutrient medium had different effects on the vegetative growth of many plant species (Joo et al., 2019; Page et al., 2021).

In comparison to B5 vitamins, MS basal salts are widely used not only in monocots but also in dicotyledons. Phillips and Garda (2019) explained the composition of these media in detail, but the major difference between these two media includes the amount and relative proportions of the various salts, and more particularly, the amount and type of nitrogen. MS has higher total nitrogen levels in both nitrate and ammonium forms. This may contribute to good plant regeneration. However, sometimes low levels of ammonium ions can be the best medium for optimal growth of plant.

In the current study, these two different basal salts and their combinations were scanned: MS, B5, MS+B5, ½ MS, ½ B5, ½ MS+ ½ B5. Of these media, MS medium gave a high response on shoot vitality, shoot length, number of nodes per plant, and number of shoots, which are important for clonal micropropagation. Many previous studies have also mentioned that the MS medium is suitable for micropropagation of *C. sativa* (Lata et al., 2009; Wang et al., 2009; Chaohua et al., 2016; Lata et al., 2016). In a recent study, Page et al. (2021) reported that multiple commercial cultivars of *C. sativa* produced the best results in MS and DKW (Driver & Kuniyuki, 1984) media, while resulting in very poor growth in WPM (Lloyd & McCown, 1980), B5, and BABI (Greenway et al., 2012) media, and DKM medium offered more stable plant growth than MS for multiple subcultures of plants.

In addition, these findings showed that the root formation and root elongation were the best in half-strength nutrient media, but the maximum root number was obtained from full-strength nutrient media. This was supported by many scientists that half-strength media gave the best rooting response (Chaohua et al., 2016; Smýkalová et al., 2019; Wróbel et al., 2022). The results showed that ½ B5 and ½ MS+ ½ B5 media can be preferable for rooting of *C. sativa*.

Plant growth response to varying light quality across white and red colors behaved in a manner very similar to that shown by many scientists (Matysiak & Kowalski, 2019; Shulgina et al., 2021; Gnasekaran et al., 2021;

Nacheva et al., 2021). In vitro conditions, apart from the vigor of plants attained by LED treatments, the micropropagation quantity is always considered necessary for any breeding studies, particularly for micro-shoot production of commercial plants. In the current study, culture media under LED treatments had obvious effects on the coefficients of shoot multiplication from *C. sativa*. The control treatment without basal medium inhibited the plant growth from apical meristem explants for *C. sativa* under white LEDs, but it stimulated a slight plant development under red LEDs.

However, as mentioned in the results section, MS medium was found to be effective in parameters related to shoot growth in general. Again, the MS medium showed a strong effect with slight differences under different LEDs. Under red LED, MS and B5 media showed the highest shoot vitality, shoot length, and shoot number of *C. sativa*, while the highest node and leaf numbers were recorded in MS medium under white LED. For shoot weight, both fresh and dry, white LED was found to be effective in both B5 and MS media.

In the parameters related to rooting, the highest values were generally detected under white LED. But the effect of nutrient media changed under different LEDs according to the investigated parameters. The highest values were obtained from ½ MS+½ B5 for root formation, ½ B5 for root length, MS+B5 for root number per plant, and ½ MS for root weight, both fresh and dry. Considering the in vitro propagation of *C. sativa*, ½ B5 was the greatest medium for rooting, which is statistically in the same group for these parameters.

CONCLUSION

This study is the first report about the effects of various media under different spectra of light-emitting diode (LED) light quality on the shoot growth and rhizogenesis of the *C. sativa* tissue culture seedlings. All treatments significantly but differentially influenced the vegetative growth of in vitro *C. sativa* plantlets. Because it is important to produce genetically identical and disease-free plants in breeding studies, shoot and node numbers per plant are equally valuable. From this study, we can draw the following conclusions: Firstly, LED spectra and basal medium type/concentration significantly affect both shoot multiplication and rooting, as well as their promotion of the formation of strong plants with well-developed leaves of *C. sativa* in vitro conditions. Secondly, red LED combined with MS medium greatly promotes the micro shoot through increasing shoot number and elongation, but white LED combined with MS medium improves healthier shoot growth by increasing both node number and leaf number. Thirdly, white LED considerably promotes the rooting capability of *C. sativa* in vitro culture. The ½ B5 and ½ MS+½ B5 media significantly stimulate root formation and elongation, as well as they provide the highest root number in addition to the MS+B5 medium. This study may give a general background on light spectral quality affecting hemp morphogenesis. More detailed studies are required to standardize the effects of LEDs on the growth and development of hemp cultivars and genotypes.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

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