# **LETTUCE SEEDLINGS QUALITY: THE EFFECTS OF WHITE AND BLUE LIGHT EXPOSURE**

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## **Abstract**

In the Balkans, the production of globally cultivated green leaf lettuce (*Lactuca sativa* L.) can be slow due to reduced quality and intensity of solar insolation. Our research aimed to investigate the effects of artificial illumination and the duration of light exposure on the growth and quality of two lettuce genotypes seedlings: Genesis and Jukebox. The research was conducted at the Institute for Vegetable Crops Smederevska Palanka, Serbia. Lettuce seeds were sown in 9 styrofoam containers per genotype, filled with commercial substrate. In the phase of three leaves, plants were placed in plant growth chambers under multispectral white (W) LEDs and monochromatic blue (B) LEDs (three containers per LED and genotype), while three containers were kept in the greenhouse and served as control. The plants were cultured in plant growth chambers for 25 days under a 9h/15h and 10h/14h (light/dark) photoperiod, to simulate outdoor conditions. Morphological growth parameters (number of leaves and plant weight) were measured on the 11<sup>th</sup> and 25<sup>th</sup> days. Chlorophyll content was measured on the 4<sup>th</sup>, 11<sup>th</sup>, 18<sup>th</sup> and 25<sup>th</sup> days. Plants grown under W and B LEDs had a significantly higher number of leaves, plant weight, and chlorophyll content than plants grown in a greenhouse. Using energy-efficient white and blue LED lights in lettuce seedling production during 18-25 days positively impacts seedling quality, and with this seedling production, the plants can achieve high yields and quality.

*Key words: Lactuca sativa* L.*,* Genesis, Jukebox, LEDs, vegetables, production, chlorophyll content

## **Introduction**

In recent years, newly adopted human population dietary habits have led to increased consumption of vegetables (Maziero et al., 2017). Due to the increasing population, there is a greater demand for vegetables and products grown on arable land (Kozai, 2016). Although vegetables are commercially produced outdoors in many world regions, indoor cultivation in protected environments plays an important role in fresh vegetable production (Gruda, 2005). Indoor vegetable gardening in greenhouses can reduce threats associated with outdoor gardening and provide the ability to produce vegetables during the cold season. Additionally, the yield of greenhouse vegetables, as well as the visual quality and market value are much higher compared to field-produced vegetables (Bot, 2003; Gruda, 2005). Owing to the previously mentioned, indoor vegetable cultivation has become an important trend in commercial horticulture.

Several studies have indicated that vegetable greenhouse production in northern hemisphere can be limited by low light (Gruda, 2005). Additionally, factors such as greenhouse glazing material, superstructure, and shading can lead to a reduction of up to 50% in ambient photosynthetic daily light (Hanan, 1997). This

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is important as plants' metabolism, growth, and development are dependent on the light spectrums (Muneer et al., 2014; Wollaeger and Runkle, 2015; Hernández and Kubota, 2016; Hamdani et al., 2019).

Light is a fundamental factor that regulates plant growth and development, as it provides energy for photosynthesis and influences signaling pathways that regulate seed germination, phototropism, and flowering time (Huché-Thelier et al., 2016; Urestarazu et al., 2016; Hasan et al., 2017). However, plant growth can be slow under low-light greenhouse conditions such as those found in the Balkans winter. Therefore, setting up appropriate light conditions is crucial for plant cultivation during winter.

Among economic crops, lettuce (*Lactuca sativa* L.) is the main vegetable in plant factories. It is the most cultivated vegetable plant worldwide and one of the most consumed in the human diet (Pérez-López et al., 2013; Kim et al., 2016). Lettuce can be successfully grown in controlled indoor growing systems owing to its small size and relatively short growing period (Xu, 2019).

Because of the importance of light on lettuce growth and development, the effects of different types of industrial light sources, such as fluorescent, ultraviolet (UV), and lightemitting diodes (LEDs), on managing lettuce growth and development have been extensively studied. Numerous studies have shown that LEDs are the best source of light for increasing lettuce productivity and quality improvement (Chen et al., 2023; Hernández-Adasme et al., 2023; Jin et al., 2023; Miao et al., 2023). Despite that, only several studies investigated the effects of light quality on young seedlings development (Yadav et al., 2020; Frąszczak and Kula-Maximenko, 2021; Hernández-Adasme et al., 2022; Wei et al., 2023).

This research aimed to study the effects of different light treatments (L) and exposure length (E) on the development and chlorophyll content in two lettuce genotypes (G) seedlings compared to standard cultivation in protected greenhouse environments, to define procedures for reaching high-quality commercial plant seedlings.

#### **Material and methods**

## *Plant material and experimental design*

The research was performed during December 2023/January-February 2024 at the Institute for Vegetable Crops Smederevska Palanka, Serbia (44°22' N, 20°47' E, 110 m above sea level). This study used commercial green leaf lettuce (*L. sativa* L.) genotypes Genesis (Bejo Zaden, the Netherlands) and Jukebox (Vilmorin SA, France) as plant material.

Seeds were sown in 9 styrofoam containers per genotype, with 104 seeds per container. The containers were filled with the commercial substrate (Pindstrup Mosebrug A/S, Danmark). In the phase of three leaves, six containers with plants per genotype were placed in plant growth chambers (three containers per LED), while three containers per genotype were kept in the greenhouse and served as the control. The containers were placed in a 3-tier metal rack, open on each side. The LEDs were installed on each level at 35 cm above the plant's top and spaced 40 cm apart to avoid interference between treatments. For lighting in the plant growth chambers, we used LEDs: multispectral white light (W, 350-700 nm) and monochromatic blue light (B, 440 nm). Plants were grown in the plant growth chambers for 25 days with 9h/15h and 10h/14h (light/dark) photoperiods to simulate outdoor conditions.

Morphological growth parameters, such as the number of leaves and plant weight, were measured on the 11th and 25th days after the plant removal to the plant growth chambers. Plant weight was measured using an IoT-Line Precision Balance 572-31 technical scale (Kern & Sohn GmbH, Germany). Chlorophyll content was measured on the 4<sup>th</sup>, 11<sup>th</sup>, 18<sup>th</sup>, and 25<sup>th</sup> days after plant removal to plant growth chambers, using a Portable Chlorophyll Meter CM-B (BioBase Industry, Shandong, Co., Ltd). The measurements are expressed in SPAD units. Measurements were performed on three

containers per light condition and on five plants per container. The plants left in greenhouse conditions were analyzed at the same time and in the same way.

#### *Statistical analysis*

To examine the influence of different light treatments on Genesis and Jukebox lettuce seedlings, we used a three-factor ANOVA

analysis model. Duncan's test was performed to determine the significance of mean differences (*p* < 0.05) between the light treatment and length of plant exposure to light treatments using SPSS software version 22.0 (IBM Corporation, New York, NY, USA).

Graphs were made using the SigmaPlot 15.0 program (SigmaPlot, San Francisco, CA, USA).

# **Results and discussion**

Lettuce growth and development under LEDs have been well-studied for more than 20 years. All these studies indicated that light treatment and exposure length significantly influence lettuce metabolism, growth, and development, both positively and negatively (Ruban, 2015; Landi et al., 2020; Ptushenko et al., 2020; Samuolienė et al., 2021). Our results concur with available literature data, indicating that the number of leaves of Genesis and Jukebox lettuce seedlings significantly varied depending on light treatment and exposure length, while genotype influence was insignificant. In addition to the individual influence, the interaction between  $G \times L$  and  $G$  $\times$  E had a statistically significant effect on lettuce number of leaves (Table 1). Plant weight was significantly influenced by the genotype, light treatment, and exposure length. In addition to the individual influence, the interactions between  $G \times L$ ,  $G \times E$ ,  $L \times E$ , and  $G \times L \times E$ had a statistically significant influence on plant weight (Table 1).

Source of variation	df		Leaf number		Plant weight (g)		
		SS	<b>MS</b>	F	SS	<b>MS</b>	F
Genotype (G)	1	0.27	0.27	0.35 <sup>ns</sup>	0.64	0.64	$11.51**$
Light treatment $(L)$	$\overline{2}$	71.23	35.62	$46.44**$	11.86	5.93	$106.82**$
Replications	$\overline{4}$	9.14	2.27	$2.98*$	0.49	0.12	2.21 <sup>ns</sup>
Exposure length (E)	1	317.34	317.34	$413.74**$	19.60	19.60	353.20**
$G \times L$	$\overline{2}$	8.81	4.41	$5.74**$	2.58	1.29	$23.28**$
$G \times E$	1	31.25	31.25	$40.74**$	1.71	1.71	$30.80**$
$L \times E$	2	1.078	0.54	0.70 <sup>ns</sup>	3.61	1.80	$32.48**$
$G \times L \times E$	$\overline{2}$	2.03	1.02	$1.33^{ns}$	1.62	0.81	$14.64**$
Error	164	125.79	0.767		9.10	0.056	
Total	179	566.95	$\overline{\phantom{m}}$	-	51.21		$\overline{\phantom{a}}$
**Significant at $p = 0.01$ level, <sup>ns</sup> Non-significant							

*Table 1. Analysis of variance for leaf number and plant weight of Genesis and Jukebox lettuce seedlings Tabela 1. Analiza varijanse broja listova i mase biljaka sadnica zelene salate sorti Genesis i Jukebox* 

On both the 11<sup>th</sup> and 25<sup>th</sup> days after treatment, genotype Genesis and Jukebox, which were grown under W and B LEDs, exhibited a significantly higher number of leaves and

plant weight compared to plants grown under daily light (DL) in the greenhouse (Figures 1, 2). In studies by Ohashi-Kaneko et al. (2007), Matysiak and Kowalski (2019), and Arif (2022), plants grown under W LEDs had a higher number of leaves compared to B, but similar plant biomass. In our research, the highest plant weight was observed on the 11<sup>th</sup> day after light treatment in plants grown under B LEDs under a 9h/15h photoperiod. A slightly lower plant weight was observed in plants grown under W LEDs, while plants grown under DL in a greenhouse had the smallest weight. From the 11<sup>th</sup> to 25<sup>th</sup> days, plants were grown under an extended photoperiod (10h light/14h dark), as it was following a light regime in the greenhouse. Thereafter, plants' morphological parameters were analyzed again. Both genotypes grown under W and B LEDs had a similar number of leaves (Figure 1). The Jukebox genotype grown under B LEDs had the highest specific weight, while the Genesis genotype grown under W and B LEDs did not show a significant difference regarding plant weight (Figure 2). Similarly, Muneer et al. (2014) found that plant biomass increases in plants grown under B LEDs. Plants grown under DL in the greenhouse had a significantly lower number of leaves and plant weight compared to plants grown under W and B LEDs in the plant growth chamber (Figures 1, 2).



*Figure 1. Average values of plant weight in Genesis and Jukebox lettuce seedlings grown under daily light (DL), multispectral white (W) and monochromatic blue (B) LEDs.* 

*Grafikon 1. Prosečne vrednosti mase biljaka sadnica zelene salate Genesis i Jukebox gajenih pod dnevnom svetlošću (DL), multispektralnim belim LED svetlom (W) i monohromatskim plavim LED svetlom (B).* 



*Figure 2. Average values of the number of leaves in Genesis and Jukebox lettuce seedlings grown under daily light (DL), multispectral white light (W) and monochromatic blue (B) LEDs.* 

*Grafikon 2. Prosečne vrednosti broja listova sadnica zelene salate Genesis i Jukebox gajenih pod dnevnom svetlošću (DL), multispektralim belim LED svetlom (W) i monohromatskim plavim LED svetlom (B).* 

Our research indicates that G, L, and E, as well as their interactions, significantly influence the chlorophyll content in Genesis and Jukebox

lettuce seedlings (Table 2). In other words, the chlorophyll content depends on the genotype, environmental conditions, and their interaction.

<b>Source of variation</b>	Chlorophyll content (SPAD units)						
	SS	df	<b>MS</b>	F			
Genotype (G)	497.26	1	497.26	$42.97**$			
Light treatment (L)	221.58	2	110.79	$9.57**$			
Replications	11.63	$\overline{4}$	2.91	$0.25^{ns}$			
Exposure length (E)	3229.82	3	1076.61	$93.03**$			
$G \times L$	79.78	2	39.89	$3.45*$			
$G \times E$	103.06	3	34.35	$2.97*$			
$L \times E$	951.06	6	158.51	$13.70**$			
$G \times L \times E$	203.31	6	33.88	$2.93**$			
Error	3842.10	332	11.57				
Total	9139.61	359					
**Significant at $p = 0.01$ level, <sup>ns</sup> Non-significant							

*Table 2. Analysis of variance for chlorophyll content in Genesis and Jukebox lettuce seedlings Tabela 2. Analiza varijanse sadržaja hlorofila u listovima sadnica sorti zelene salate Genesis i Jukebox* 

After four days of light treatments, Jukebox lettuce seedlings grown under W LEDs had significantly higher chlorophyll content compared to those grown under B LEDs and DL. However, after 18 days, the highest chlorophyll content was observed in plants grown under B LEDs, slightly lower in plants grown under W LEDs, and the lowest in plants grown under DL. The effect of light treatments on chlorophyll content varied for the Genesis genotype. In the first 18 days, there was no significant difference in chlorophyll content for plants grown under W LEDs, B LEDs, and DL. After 18 days, the chlorophyll content was significantly higher in plants grown under B LEDs compared to W LEDs and DL, with no significant difference between W LEDs and DL. However, after 25 days, plants grown under W and B LEDs had approximately similar chlorophyll content, which was significantly higher than in plants grown under DL in the greenhouse (Figure 3). Similarly, Muneer et al. (2014) and Arif (2022) determined that chlorophyll amount increases under B LEDs.



*Figure 3. Average values of chlorophyll content (SPAD units) in Genesis and Jukebox lettuce seedlings grown under daily light (DL), multispectral white light (W) and monochromatic blue (B) LEDs. Grafikon 3. Prosečne vrednosti sadržaja hlorofila (SPAD jedinice) sadnica zelene salate Genesis i Jukebox gajenih pod dnevnom svetlošću (DL), multispektralnim belim LED svetlom (W) i monohromatskim plavim LED svetlom (B).* 

#### **Conclusion**

In the Balkans agroecological conditions, early seedlings production takes place during December, January, and February. During this period, when the quality and duration of sunlight are suboptimal, the seedlings may not reach their full genetic yield potential as they are deprived of that part of the light quality. In such conditions, the usage of energy-efficient LED lights can fulfil the plants' light requirements during the early stages of seedling production, facilitating smooth and efficient organogenesis

This research was supported by the Ministry of Science, Technological Development, and throughout all growth stages. Therefore, for Genesis and Jukebox seedlings cultivation in Balkan conditions, we recommend exposure to B LEDs for 18 days, and to W LEDs for 25 days, under 9h/15h and 10h/14h (light/dark) photoperiod, to accelerate seedling growth and chlorophyll content. This cultivation method positively impacts seedling quality, and with this seedling production, the plants can achieve high yields and quality.

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# **Author contribution**

Conceptualization, K.L. and A.R; Formal analysis, K.L.; Investigation, K.L., S.A., V.P., B.G. and A.R.; Resources, S.A. and N.Đ.; Supervision, K.L. and A.R.; Visualization,

K.L. and A.R.; Validation, K.L., V.Z. and A.R.; Funding acquisition, N.Đ.; Roles/Writing original draft, K.L. and A.R.; Writing - review & editing, K.L., V.Z. and A.R.

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# **KVALITET RASADA ZELENE SALATE: UTICAJ BELOG I PLAVOG SVETLOSNOG TRETMANA**

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# **Sažetak**

Proizvodnja zelene salate (*Lactuca sativa* L.) na Balkanu može biti spora usled smanjenog kvaliteta i intenziteta sunčeve insolacije. Cilj ovog istraživanja bio je da se ispita uticaj veštačkog osvetljenja i trajanja izlaganja svetlosnim tretmanima na rast i kvalitet sadnica dva genotipa zelene salate: Genesis i Jukebox. Istraživanje je sprovedeno u Institutu za povrtarstvo Smederevska Palanka, Srbija. Zelena salata je zasejana u 9 kontejnera po genotipu, ispunjenih komercijalnim supstratom. U fazi tri lista, biljke su prebačene u komore za rast biljaka i gajene pod multispektralnim belim (W) i monohromatskim plavim (B) LED svetiljkama (tri kontejnera po LED svetlu i po genotipu), dok su tri kontejnera sa biljkama ostavljena u stakleniku i služila su kao kontrola. Biljke su gajene u komorama za rast biljaka 25 dana, sa 9h/15h i 10h/14h (svetlost/mrak) fotoperiodom, čime su simulirani spoljašnji uslovi. Morfološki parametri rasta (broj listova i težina biljaka) mereni su 11. i 25. dana. Sadržaj hlorofila meren je 4., 11., 18. i 25. dana. Biljke gajene pod belim i plavim LED svetiljkama imale su značajno veći broj listova, težinu i sadržaj hlorofila u poređenju sa biljkama gajenim u stakleniku. Upotreba energetski efikasnih belih i plavih LED svetiljki u proizvodnji rasada zelene salate tokom 18-25 dana pozitivno utiče na kvalitet rasada, a biljke dobijene iz ovakvog rasada mogu da ostvare visoke prinose i kvalitet.

*Ključne reči: Lactuca sativa* L.*,* Genesis, Jukebox, LED, povrće, proizvodnja, sadržaj hlorofila

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