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The influence of LED light spectra on the *in vitro* growth of grapevine plants

M. Dubceac[1]* and C. Losmanschii[2]

[1]Scientific-Practical Institute of Horticulture and Food Technologies, Republic of Moldova

[2]Institute of Applied Physics, Republic of Moldova

*Corresponding author e-mail: dubceacm@gmail.com

ABSTRACT

In this study, the influence of different LED light spectra on the rooting, growth, and development processes of grapevine shoots under *in vitro* culture conditions were investigated. Photometric measurements were conducted for three types of LED-based artificial light sources (Osram, Germany), with variations in parameters such as PPFD, DLI, and color temperature (T). The study results indicated that the development of both the aboveground and underground parts of the vitroplants was most strongly stimulated by illumination with the "white- warm" spectrum, where T ≤3200 K, $PPFD_{max}$ = 30 µmol·s^{-1}·m^{-2}, and DLI_{max} = 2.04 mol·m^{-2}·d^{-1} "white-warm". Presented the weakest results, on the studied morphological parameters, illumination with the "white-cool" spectrum, characterized by T = 6000K, PPFD=63 µmol·s^{-1}·m^{-2}, and DLI_{max}=4.05 mol·m^{-2}·d^{-1}. "Fito" light spectrum, characterized by parameters T ≤1700 K, $PPFD_{max}$ =25 µmol·s^{-1}·m^{-2}, and DLI_{max} =1.64 mol·m^{-2}·d^{-1}, Overall, the type of illumination had a moderate impact on grapevine plants cultivated under controlled *in vitro* conditions.

Keywords: *Vitis vinifera L.*, *in vitro,* microplants, propagation, LED light spectrum, PPFD, DLI.

INTRODUCTION

In vitro culture represents a method of plant propagation carried out under strictly controlled laboratory conditions and is used for various purposes, such as the rapid multiplication of plants, their sanitation to eliminate diseases, the preservation of genetic diversity, and conducting studies on plant physiology and genetics. The development of plants in this controlled environment is influenced by both the composition of the nutrient substances in the growth medium and the conditions within the culture chamber, such as temperature and light. Light is one of the most significant environmental factors that affect how a plant develops, influencing the appearance and growth rate of different parts of the plant through morphological and physiological changes, in a sequence largely determined by the plant's genetic inheritance. The effects of light are caused by the total amount of energy that reaches a plant and how much light it utilizes (George and Davies, 2008).

In laboratory-controlled conditions, the intensity of plant photosynthetic processes is significantly influenced by essential characteristics of light, especially Photosynthetic

Active Radiation (PAR), Photosynthetic Photon Flux Density (PPFD), and Daily Light Integral (DLI). PAR refers to the range of wavelengths in sunlight that spans from 400 to 700 nanometers (nm), (Gensler, 1984), within which plant photosynthetic pigments absorb light energy to perform photosynthesis. In other words, PAR is the 'useful light' for plants in their growth and development process. On the other hand, PPFD is a measure that indicates how many photosynthetic photons reach the canopy level of plants. This measure quantifies how much light energy reaches the plants and is available for photosynthesis in a specific area or cultivation surface. DLI is the parameter that represents the total amount of photosynthetic light delivered to the plants each day (Faust *et al.*, 2018).

Previous studies have shown that red light plays a significant role in the production of photosynthetic substances, the regulation of carbohydrate metabolism, stem and shoot elongation, as well as in determining internode length and rooting frequency, along with biomass accumulation. On the other hand, blue light is essential for chlorophyll synthesis and the accumulation of photosynthetic pigments. It also influences the development of stomata and has an impact on the accumulation of soluble proteins and the carbon photosynthetic metabolism. It has also been demonstrated that the presence of monochromatic red or blue light alone does not meet the normal requirements for plant development; only a combination of red and blue light seems to be suitable for some crops, as indicated by the mentioned research (Moon *et al.,* 2006; Silva *et al.,* 2014).

In recent research, it has been proven that for plants cultivated in the laboratory, it is essential to use a light source that closely mimics natural sunlight. This light source should encompass a cultivar of wavelengths, ranging from ultraviolet, violet, blue, green, yellow, orange, red, and even far-red (Park *et al.,* 2023).

Because research on the influence of light spectra on the growth and development of grapevine plants in *in vitro* cultures has been limited until now (Poudel *et al.,* 2007; Fallah and Kahrizi, 2016; Sadigova *et al.,* 2021), the results of experiments conducted in this direction are particularly valuable both from a scientific and practical standpoint. Therefore, this study aims to investigate the impact of spectral light variables on the processes of rooting, growth, and development of grapevine plants under *in vitro* culture conditions.

MATERIALS AND METHODS

Plant material. As biological material, grapevine plants of the native cultivar 'Fetească regală', tested negative for viral, phytoplasma, and bacterial canker diseases, were used.

In vitro culture. Plant material was collected during the active growth period. The initial explants were sterilized with 10% calcium hypochlorite for 10 minutes, followed by rinsing three times with sterile distilled water under a laminar flow hood for 30 minutes. Sterilization was followed by inoculating the explants onto agar-solidified nutrient medium in 14 cm-high test tubes, with the top covered with aluminum foil. To stimulate the growth and development of plantlets, the MS medium was supplemented with phytohormones (IAA - 1 ml/l; 2iP - 1 ml/l) and vitamins. Agar was used for solidification at a concentration of 4.75 g/l, and the pH was adjusted to 6.2. Sucrose at a concentration of 18 g/l was used as a carbon source.

Growth chamber environment. The test tubes containing inoculated explants were transferred to the growth chamber, equipped with racks and equipment to maintain a stable temperature of 25°C and a photoperiod of 16/8 hours throughout the entire year.

Description of LED light sources used in the study. For this study, three types of LED-based artificial lighting (Osram, Germany) were used, all with the same electrical power consumption (P = 36 W) but with different color temperatures. The white light source with

a white-cool spectrum has the following manufacturer specifications: color temperature T = 6000 K. For the white-warm spectrum light source, the parameters are T = 2500 K, and the third type of lighting source, labeled as "Fito" has the following specifications: P = 36 W, T = 1700 K. Each shelf was illuminated with 3 bars, installed at 50 cm. The emission spectra of each lighting source were recorded using an MDR-23 monochromator, with its photomultiplier connected to a UNIPAN RS232 lock-in amplifier, ADC, and subsequently linked to a computer for signal digitization. Lux measurements were performed using a CEM DT-1308 digital luxmeter. The parameters of PPFD and DLI were recorded using a MQ-200X PAR meter. DLI measurements were conducted for the 18-hour duration of the light day.

Photometric measurement method and experimental setup. Given that, for any type of wave, the value of a physical quantity at a given point in space is inversely proportional to the square of the distance from the source of the field, referred to as the inverse square law, photometric measurements were conducted on a rectangular surface with dimensions of 1650 mm x 880 mm. To study the distribution of photometric parameters, the surface was divided into zones with dimensions of 150 mm x 80 mm, thus creating a grid (Figure 1a). The lighting fixtures were installed at a height of 50 cm, the same as on the laboratory shelves. The surface of the grid was made from material with a low reflection coefficient, with the 'X' representing the area where sensors were placed for photometric measurements (Figure 1b).

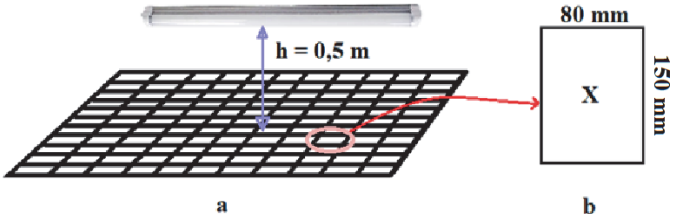


Figure 1. Arrangement for photometric measurements, a - placement of the lighting fixture and the grid-like surface, b - Measurement cell and the area where photometric measurement sensors were placed.

Statistical analysis of data. This study involved three distinct analytical replications. For each of these replications, a total of 90 samples were prepared, subsequently divided into three subsamples, each containing 30 samples. To determine the leaf lamina area, specialized software called 'Pet□le' was used. The collected data were statistically analyzed using a univariate analysis of variance method, with a significance level set at 0.05 or 5%. For the qualitative assessment of parameters, the Chaddock scale was applied, where values between 0.1 and 0.3 were considered indicative of weak influence, between 0.3 and 0.5 of moderate influence, and between 0.7 and 0.9 of significant influence. The study was conducted between the years 2021-2023.

RESULTS AND DISCUSSIONS

In this study, photometric measurements were conducted to assess the performance of three different types of lighting fixtures used in the experiment. These measurements included the measured temperature, photosynthetic photon flux density, and daily light integral for each lighting fixture. These data were collected to evaluate the impact of the lighting spectrum on photometric parameters and its potential effect on plant development. The maximum measurement values are presented in Table 1.

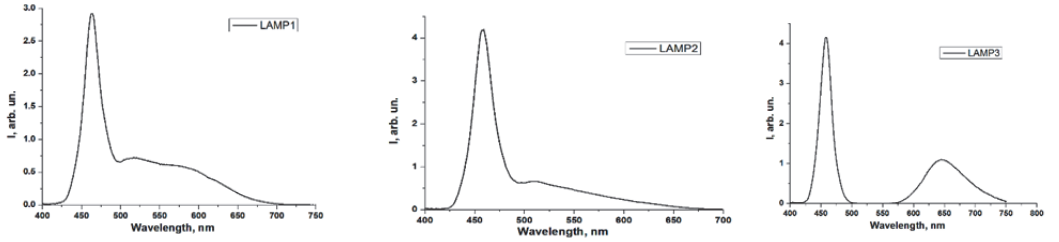
Table 1. Photometric measurement results

№	Lighting source	T measured, K	PPFDmax, μmol·s⁻¹·m⁻²	DLImax, mol·m⁻²·d⁻¹
1	White-cool	5950±50K	63	4.05
2	White-warm	3200±50K	30	2.04
3	"Fito"	≤1700 K	25	1.64

The white-cool lighting fixture provided the highest measured color temperature (6000K) and the highest values for PPFD (63 μmol·s⁻¹·m⁻²) and DLI (4.05 mol·m⁻²·d⁻¹). In contrast, the "Fito" lighting fixture had the lowest temperature (1700K) and the lowest values for PPFD (25 μmol·s⁻¹·m⁻²) and DLI (1.64 mol·m⁻²·d⁻¹). The white-warm lighting fell in an intermediate range, with T ≤ 1700 K, PPFD (25 μmol·s⁻¹·m⁻²), and DLI (1.64 mol·m⁻²·d⁻¹).

Analysis of the emission spectra of lighting fixtures used in the experiment. The photochemical reactions crucial for plant development occur when plants are exposed to rays from the visible spectrum of light (PAR). Plants primarily absorb light from the blue and red spectra. Chlorophylls, the pigments responsible for photosynthesis, absorb rays in the violet-blue region with greater intensity, but carbon fixation, which is a crucial step in photosynthesis, occurs more intensely in the red spectral region. In addition to PAR, plants also utilize a portion of the light from the light spectrum, such as far-red light.

Photometric measurements yielded the emission spectra of the lighting fixtures used in the experiment (Figure 2). Photometric measurements of the surface distribution of PAR parameters indicate a non-uniform distribution of values, which correlates with the inverse square law.



a *b* *c*

Figure 2. Emission spectra of the used lighting fixtures: a - spectrum corresponds to the white-warm lighting fixture, b - spectrum corresponds to the white-cool lighting fixture, and c - spectrum corresponds to the "Fito" lighting fixture.

In Figure 2a, a peak in the blue range is observed at a wavelength of 465 nm. The rest of the spectrum, ranging from 480 to 675 nm, including the green, yellow, orange, and red range, represents approximately 20-25% of the intensity of the blue range. This indicates that the lighting has a significant concentration of blue light compared to other wavelengths in the spectrum. In Figure 2b, we can observe a peak in the blue light spectrum at a wavelength of 460 nm. Similarly, light from the green to red spectral range is present. However, for the wavelength range between 550 nm (green) and 600 nm (yellow light), the intensity of the light is much lower compared to Figure 2a. In comparison to the lighting fixtures in Figures 2a and 2b, in Figure 2c, which represents the "Fito" lighting fixture, we can observe a complete absence of wavelengths from 500 nm to 575 nm, indicating the absence of blue-green, green, and yellow light in the spectrum emitted by this lighting fixture. This lighting fixture focuses on the colors blue and red, where the blue peak is at λ=465 nm, and a weaker

second peak in the red at λ=650 nm, with a broader spectrum in these colors, including the near-infrared spectrum, between 575 nm and 750 nm.

Analysis of surface distributions of PPFD parameter. In plant production, each plant species requires the optimal PPFD density to be determined (Banerjee and Roychoudhury, 2016; Miller *et al.*, 2019). The light intensity level in in vitro cultures is lower than in greenhouse cultivation. In *in vitro* culture conditions, the low light levels serve as sufficient informational signals to determine plant morphogenesis, and this effect is amplified by the presence of sugar in the growth medium, which provides the plant with the necessary energy. Additionally, these conditions of low light result in more efficient energy utilization, and plants do not overheat when grown in closed containers (George and Davies, 2008). Light levels can vary depending on the species (Alvarenga *et al.*, 2015; Silva *et al.*, 2017), but for most herbaceous plants cultivated in this environment, the optimal light level falls in the range of 30 to 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, although it varies from 7 to 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ depending on the species and their specific requirements. Light intensity significantly impacts how plants grow and develop their branches (Liu *et al.*, 2002). This influence affects both the physiological and morphological aspects of plants, which are closely linked to how plant genes are activated in response to environmental signals. Comparing the datasets for PPFD for the three lighting sources (Figure 3) on the same measuring surface (1650 mm x 880mm), the following observations can be made: In Figure a, for white-cool lighting, the highest PPFD values were recorded, ranging from 14 to 63 $\mu mol \cdot s^{-1} \cdot m^{-2}$. There is also a significant variation in PPFD across the entire measured surface. The lowest PPFD values were recorded for the "Fito" variant, with values ranging from 5.4 to 25 $\mu mol \cdot s^{-1} \cdot m^{-2}$. In Figure b, for white-warm lighting, the maximum recorded PPFD values were 30 $\mu mol \cdot s^{-1} \cdot m^{-2}$. This suggests that white-cool lighting provides a greater amount of light available for photosynthesis compared to white-warm and "Fito" lighting on the same measuring surface. Furthermore, all three datasets exhibit non-uniform variation of PPFD across the measured surface, which may indicate non-uniform distribution of light emitted by the lighting sources. This aspect can be significant in the context of plant growth studies as it can influence the development and yield of plants based on their position on the shelf relative to the light sources.

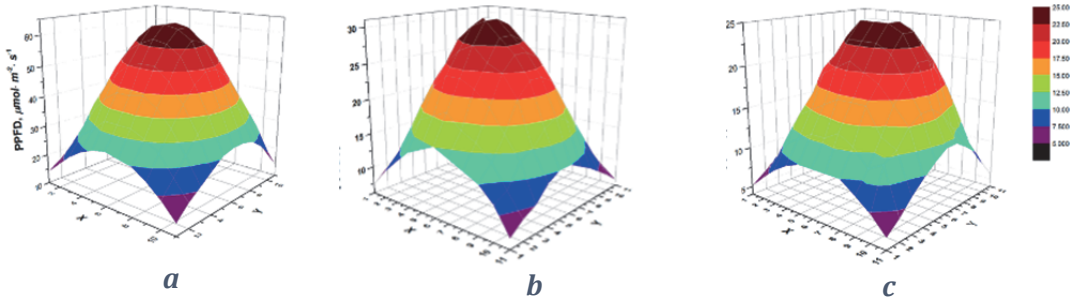


Figure 3. Surface distributions of the PPFD parameter: a - white-cool; b- warm white; c- ”Fito”

Analysis of the DLI parameter surface distributions. Many studies have shown that an adequate DLI could promote the growth and development of plants, such as the germination rate of photoblastic seeds, biomass of shoots and roots (Currey and Lopez, 2012; Walters and Curry, 2018; Solis-Toapanta and Gómez, 2019), stem diameter (Baumbauer *et al.*, 2019), leaf surface area and leaf number (Kelly, 2020), and differentiation of floral buds (Graver et *al.*, 2018; Lee et *al.*, 2019;). However, prolonged high-light DLI or long photoperiod can cause damage to the photosynthetic system and inhibit photosynthetic production (Velez-Ramirez *et al.*, 2017). It is known that excessive

light intensity can induce light stress in plants. Understanding the effects of these excesses still requires extensive research. High-light intensity stress leads to photodamage to the photosynthetic apparatus and degradation of photosynthetic proteins (Robinson, 2001). Plants respond to this stress by reducing the level of chlorophyll (Banerjee and Roychoudhury, 2016), accumulating anthocyanins, and causing bleaching or yellowing of leaves (Singh *et al.,* 2017), thereby resulting in reduced yield (Fu *et al.*, 2012). In artificial light conditions, DLI is entirely supplied by lamps and varies by adjusting light intensity or photoperiod. Therefore, an optimal DLI that can meet the growth and development demands of plantlets in vitro needs to be identified for each culture.

Comparing the daily light integral (DLI) distribution across the three lighting sources on the measurement surface (Figure 4), we can make the following observations: the dataset for the white-cool lighting fixture has the highest DLI values, reaching a maximum of 5.54 $mol \cdot m^{-2} \cdot d^{-1}$, while the dataset for the white-warm lighting indicates a maximum DLI value of 2.04 $mol \cdot m^{-2} \cdot d^{-1}$, and the dataset for the "Fito" lighting fixture reaches a maximum of 1.64 $mol \cdot m^{-2} \cdot d^{-1}$. The data for all lighting fixtures represent different illumination level distributions and vary depending on the measurement locations.

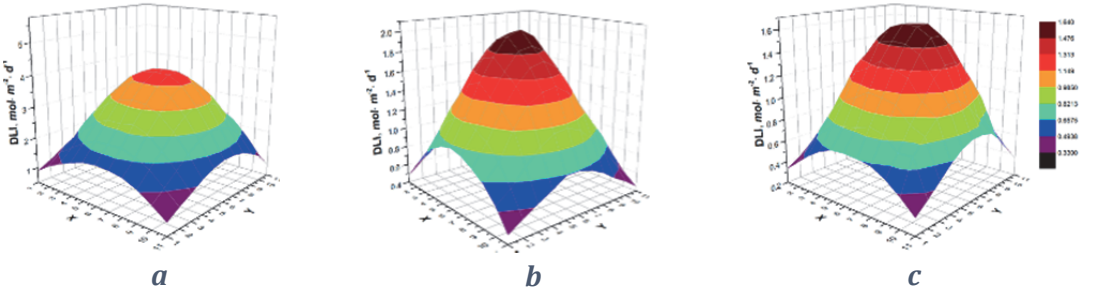


Figure 4. Surface distribution of the DLI parameter: a - white-cool; b – white- warm; c - "Fito"

Impact of lighting spectrum on rooting and morphogenesis in grapevine in vitro plantlets. The results obtained in this study indicate the significant influence of different lighting spectra on the process of rooting and shoot morphogenesis in grapevine plants cultivated *in vitro.* After 14 days of culture, it was observed that the "Fito" spectrum lighting initiated the most intense process of root formation, resulting in the rooting of 20 out of the total 30 explants inoculated, representing a success rate of 66.6%. In contrast, the white-cool and white-warm lighting systems had a less pronounced effect, with a rooting rate of only 40% (Figure 5).

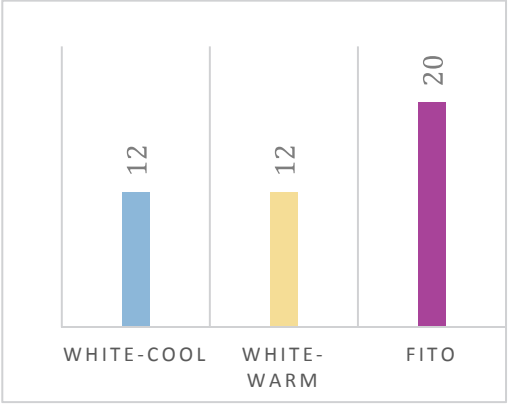


Figure 5. The number of grapevine explants that developed roots after 14 days from inoculation, depending on the type of lighting

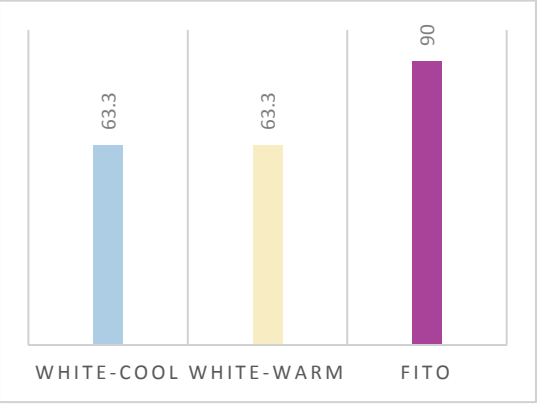


Figure 6. The rooting rate (%) of microplants in vitro, depending on the type of lighting, after 30 days

At 30 days of culture, a significant evolution of the results highlighted in the case of the "Fito" lighting led was observed regarding the rooting rate of 90%, while in the case of the other two lighting type, a rate of 63.3% was recorded (Figure 6). However, regarding the other morphological parameters studied (Table 2), the plants grown under the influence of "Fito" lighting recorded the lowest values, compared to the other types of lighting analyzed.
These plants exhibited shoots with uneven internodes, a reduced number of leaves, and small leaf sizes.

Table 2. The effect of the type of lighting *in vitro* on the morphological parameters of the 'Fetească regală'

Lighting source	Rooted Plants (%)	Callus (%)	Internodes (no)	Leaves (no)	Plant Height (cm)	Root Length (cm)	Leaf Area (cm^2)
White-cool	63.3 ± 0.4^{b}	0	6.36 ± 3.1^{ab}	6.04 ± 2.8^{ab}	$6.04\pm3.^{b}$	13.63 ± 9.3^{a}	2 ± 0.87^{a}
White-warm	63.3 ± 0.49^{b}	36.6±0.49	7.68 ± 2.8^{b}	7.84 ± 3.8^{b}	6.6 ± 2.7^{b}	20.68 ± 13.5^{b}	1.27 ± 0.6^{b}
"Fito"	90 ± 0.18^{a}	0	5.4 ± 1.6^{a}	6.1 ± 2.0^{a}	4.2 ± 1.8^{a}	11.67 ± 7.4^{a}	1.12 ± 0.48^{a}
Statistical analysis of data	*28%*			*28%*	*39%*	*7%*	*34%*

**letters a,b indicate the significance of differences compared with control at P < 0.05.*

Analyzing the data collected over 30 days of culture, it can be observed that the three lighting systems studied have different influences on both the process of rooting and the morphogenesis of newly formed shoots (Table 2). Under white-warm lighting, there was a promotion of both above ground and underground growth of the plantlets, characterized by the development of shoots with the most internodes (7.68), the most leaves (7.84), and the longest roots (20.68 cm).
Regarding the white-cool spectrum lighting, it favored the development of leaves with the largest leaf area (cm^2). This lighting, characterized by the highest values for T=6000K, PPFD 63 $\mu mol \cdot s^{-1} \cdot m^{-2}$, and DLI=4.05 $mol \cdot m^{-2} \cdot d^{-1}$, resulted in the development of leaves with an average surface area of 2 cm^2, which is 1.57 times larger compared to the leaves of plants grown under white-yellow light, and 1.78 times larger than those under ”Fito” lighting. This morphological leaf development was evident through well-developed shoots with regular internodes (±1 cm) and healthy leaves.

CONCLUSIONS

The results obtained indicate that plants have varying lighting requirements depending on their developmental stages. To stimulate the process of rhizogenesis in grapevine explants, it was found that the "Fito" spectrum with parameters T≤1700, PPFD (25 $\mu mol \cdot s^{-1} \cdot m^{-2}$), and DLI (1.64 $mol \cdot m^{-2} \cdot d^{-1}$) proved to be optimal. For the growth of in vitro plants characterized by the largest leaves and internodes, the most effective lighting was provided by the white-cool spectrum, with a color temperature of 6000K, PPFD (63 $\mu mol \cdot s^{-1} \cdot m^{-2}$), and DLI (4.05 $mol \cdot m^{-2} \cdot d^{-1}$). The growth of both aerial and underground parts of the vitroplants was stimulated by the white-warm light, where T≤3200 K, PPFD (30 $\mu mol \cdot s^{-1} \cdot m^{-2}$), and DLI (2.04 $mol \cdot m^{-2} \cdot d^{-1}$) Grapevine seedlings grown under controlled in vitro conditions were moderately affected by the type of lighting used.

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