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Research Article

Secondary metabolites, hormonal homeostasis, and antioxidant enzymes of *Moringa oleifera* in response to white or violet Light Emitting Diodes (LEDs) combined with cytokinins under tissue culture conditions

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Abstract

The present study was carried out between 2021 and 2022 at the tissue culture lab and experimental station of the vegetable and floriculture department, faculty of agriculture, Mansoura University. Using mature seeds, *Moringa oleifera* is propagated on a large scale and in rapid succession by in vitro culture. Two types of cytokinin: 6-benzylaminopurin (BAP), and thidiauron (TDZ) singly at three different concentrations (0, 1, or 2 mg/L), were used, as well as light-emitting diode systems (LEDs) (white as control and violet; the combination of red and blue; 1:1). After incubation for 30 days, the obtained results showed that the survival percentage increased by the treatments with the maximum value (85%) by MS medium supplemented with 2 mg/L TDZ, under violet LEDs illumination. However, the addition of thidiazuron (TDZ) to the medium did not propagate shoot, and this treatment recorded 100% callus formation other than BAP. The maximum number of axillary shoots per explant and the number of leaves recorded in the MS medium contained 2 mg/L BAP under violet LEDs. Compared to the control values, all the used treatments generally caused surprisingly stimulating the determined antioxidant enzymes and compounds (peroxidase, polyphenol oxidase &catalase and phenols, flavonoids, anthocyanin& ascorbic acid). Concerning the phytohormone content of the hormone-free medium (control), IAA, kinetin, and zeatin increased. Meanwhile, GA_3 and ABA decreased with the used treatments.

Keywords: Benzyl aminopurine; light-emitting diode systems (LEDs); *Moringa oleifera*; secondary metabolites; thidiazuron; tissue culture

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Introduction

More than 25% of medications, according to Touaibia and Chaouch (2017), are either directly or indirectly derived from plants. Therefore, interest in utilizing medicinal plants as a source of wholesome raw materials has grown during the last several years. According to research, the use of micropropagation to generate high-demand biomass in large quantities demonstrates the importance of converting medicinal plants into crops and resolving the supply difficulties of therapeutic natural substances (Moraes *et al*., 2021; ALHaithloul *et al*., 2022).

Developing plant cell cultures may open up new opportunities for the commercially viable commercial production of even uncommon or exotic plants, their cells, and the substances they generate. Organogenesis and embryogenesis occur in response to auxins and cytokinins. Generating secondary metabolites for medicinal purposes is becoming more accustomed to tissue culture. Future development of consumer goods that are secure, efficient, and of the highest caliber will be supported by cultural systems' integrated methods (Lemma *et al*., 2020; El-Sheshtawy *et al*., 2021). Additionally, plant tissue culture has also provided fresh insights into plant biology and developed into a crucial technology for the development of crop species (Khan *et al*., 2021).

Moringa oleifera is a highly prized plant that is grown all over the world and used in a variety of food compositions. It also has industrial and medicinal uses. Thanks to its nutrient-rich leaves, pods, seeds, and blooms, this plant is becoming more and more well-known. The miracle tree, *Moringa oleifera,* is a great source of numerous key nutritional components and offers a number of health advantages. The Moringa seed powder has various health advantages and may be used to filter contaminated water quickly and inexpensively (Babar *et al*., 2022).

Additionally, *Moringa oleifera* is abundant in phytosterols like stigmasterol and sitosterol, substances linked to the creation of estrogen, which promote the development of the mammary glands to produce milk (Gopalakrishnan *et al*., 2016). As a result, it is considered the tree of life and is a subject of research in a number of fields, including livestock, agriculture, animal nutrition, medicine, and industry (Lin *et al*., 2018; Abu-Shahba *et al*., 2022). Additionally, moringa leaves include calcium, beta-carotene, potassium, protein, and vitamin C, an excellent source of carotenoids, flavonoids, and phenolics, all-natural antioxidants (Marrazzo and O'Leary, 2020).

Some investigations have been conducted on *Moringa* in vitro propagation using various explants, such as nodal segments (Saini *et al*., 2012), indirect organogenesis (Mathur *et al*., 2014), multiplication using immature seeds (Stephenson and Fahey, 2004), and regeneration of axillary cotyledons as well as buds (Steinitz *et al*., 2009). Artificial lighting is the only source of illumination used in plant tissue cultures. In order to promote photomorphogenic reactions and photosynthetic metabolism, the lighting should emit light from the right electromagnetic spectrum areas (Cavallaro *et al*., 2022).

Plant tissue culture lighting systems have generally been updated or created based on LED technology throughout the last several decades. This has led to several improvements in light quality. Still, the same problem that arose in the 1960s with the introduction of Grolux (Sylvania) fluorescent tubes continues to exist with their widespread implementation (Barceló-Muñoz *et al*., 2021). using LEDs as a primary illumination source in plant tissue culture without impairing the growth of the cultures kept up using the previously established methods in various labs. Finally, the comparison's findings show that one of the key aspects that the light source of choice affects is the temperature (Barceló-Muñoz *et al*., 2021).

New light sources with high photoelectric conversion efficiency and low energy usage include light-emitting diodes (LEDs). Consequently, they can meet the demands of plant culture systems for energy savings (Yu *et al*., 2020). Furthermore, as modern lighting technology, LEDs have become a substitute source of light for plants because of their wavelength specificity, the narrow width of their bands, compact size, sturdy construction, long lifespan, and low heat production (Ahmadi *et al*., 2021).

Batista *et al*. (2018) recently described how the various wavelengths affect plant metabolism. Along with its function in photosynthetic activities, light may also operate as an external regulator in a number of physiological and morphogenic processes that change the structure and phytochemical levels of the plant. Compared to conventional fluorescent lights, light-emitting diodes (LEDs) have lately gained popularity in agriculture, particularly in commercial micropropagation operations, leading to higher production and lower prices (Vendrame *et al*., 2022).

Thus, the objective of this study is to develop a plant tissue culture protocol for direct organogenesis in *M. oleifera* using cotyledons as explants through optimization of a number of variables, including plant growth regulators (two types of cytokinin: benzyl aminopurine (BAP) and thidiazuron (TDZ)) singly at three different concentrations (0, 1 or 2 mg/L) for each as well as light-emitting diode.

Materials and Methods

Plant materials and chemicals used

Moringa oleifera seeds were obtained from the nursery plantation of Mansoura University and selected for apparent uniformity of size and shape. The chemicals used were supplied from Sigma Chemical Company.

Time course of the experiment

Surface sterilization of *Moringa* explant

Seeds of *Moringa oleifera* were washed for 1 hour under running tap water with 4 drops of liquid soap. Next, the seeds were immersed for 30 seconds in 70 % ethanol and transferred to a solution of 25% (v/v) clorox commercial bleach solution (6% NaOCL) for 20 min, followed by rinsing three times in sterile distilled water. Afterward, the seed coat was removed inside the laminar flow hood before being cultured.

Cultural media and conditions

Sterile explants were transferred into jars containing 25 ml of MS (Naik and Chand 2006), basal medium containing 3% sucrose as a carbon source (Table 1) and supplemented with two types of cytokinin: 6-benzylaminopurine (BAP) and thidiazuron (TDZ) singly at three concentrations (0, 1or 2 mg/L) for each cytokinin. The medium was solidified with 0.7% plant agar. The pH of the medium was adjusted to 5.75 before adding agar and autoclaved at 121 °C, 1.1 kg / cm^2 for 25 minutes.

Table 1. Components of basal media

Constituents	Concentration
Macroelements	
NH_4NO_3	1650 mg/L
$CaCl_2.2H_2O$	440 mg/L
KNO_3	1900 mg/L
KH_2PO_4	170 mg/L
$MgSO_4.7H_2O$	370 mg/L
Microelements	
H_3BO_3	6.2 mg/L
$MnSO_4.2H_2O$	16.9 mg/L
$ZnSO_4.7H_2O$	8.6 mg/L
Kl	0.83 mg/L
$NaMoO_4.2H_2O$	0.25 mg/L
$CuSO_4.5H_2O$	0.025 mg/L
$COCl_2.6H_2O$	0.025 mg/L

Na_2 EDTA ($2H_2O$)	37.3 mg/L
Myo-inositol	80.0 mg/L
Glycine	2.0 mg/L
Nicotinic acid (B5)	0.5 mg/L
Pyridoxine – Hcl(B6)	0.5 mg/L
Thiamine – Hcl (B1)	0.1 mg/L

For 30 days for each treatment, the cultures were cultured in growth chambers at a temperature of 25±1 °C with a 16-hr light/ 8 hr dark treatment using two separate light-emitting diodes: white LEDs as the control and a mix of red and blue LEDs at wavelength nm. Twenty explants were used in each treatment.

The research involved nine treatments with 20 replicates in a completely randomized design (CRD):

(1) C (white LEDs as control)
(2) 1 mg/L BAP as 6-benzylaminopurine (BAP) + white LEDs.
(3) 2 mg/L BAP as 2 mg/L 6-benzylaminopurine (BAP) + white LEDs.
(4) 1 mg/L TDZ as thidiazuron (TDZ) + white LEDs.
(5) 2 mg/L TDZ as thidiazuron (TDZ) + white LEDs.
(6) C (violet LEDs as control).
(7) 1 mg/L BAP as 6-benzylaminopurine (BAP) + violet LEDs.
(8) 2 mg/L BAP as 2 mg/L 6-benzylaminopurine (BAP) + violet LEDs.
(9) 1 mg/L TDZ as thidiazuron (TDZ) + violet LEDs.
(10) 2 mg/L TDZ as thidiazuron (TDZ) + violet LED.

Data recorded and analytical methods

Sampling takes place after 30 days from incubation for the determination of growth parameters (Survival percentage %, Shoots number/ explant, shoot length (cm), leaves number/ shoot, callus volume (cm^3), shoot and/or callus fresh and dry weights), antioxidant enzymes and compounds (peroxidase (POX), polyphenol oxidase (PPO), catalase (CAT) and phenols, flavonoids, anthocyanin, ascorbic acid). In addition, phytohormones were also determined (in the case of BAP application only, as TDZ did not propagate shoots).

The following data were recorded:

1- Survival percentage % = (Number of the grown explants/ All number of the cultured explants) × 100.
2- Shoots and Leaves number per explant.
3- Shoots length (cm).
4- Callus formation percentage was calculated from the present formula:

Callus formation % = (Number of explants formed callus/ All number of the cultured explants) × 100.

5- Callus fresh and dry weight (g).
6- Callus size (cm^3) was calculated by water displacement. As the callus was laid down in a beaker, the increase in the volume pointed to the callus volume.

Quantitative determination of some secondary metabolites

Determination of total phenolic and flavonoid compounds

Half a gram of dried organs (leaves in case of treatments 1, 2, 3, 6, 7, 8) and (calli in case of treatments 4, 5, 9 and 10) previously mentioned was homogenized with 80% methanol (v/v), and a clear solution was obtained by centrifuging the mixture for 20 minutes at 10.000 g. The Folin-Ciocalteau (FC) test was performed to calculate the total phenolic compounds, and the spectrophotometer recorded absorbance at 765 nm (Galicia *et al*., 2009). An $ALCl_3$ reagent was also used for the methanolic extract to identify flavonoid components. With the use of a spectrophotometer described by Zhishen *et al*. (1999), the resultant color was measured at 510 nm.

Determination of total anthocyanin content

The anthocyanin content was initially determined using an ammonia HCl test (Egbuna *et al*., 2018). More specifically, 2 mL of the extract, 2 mL of 2N HCl, and 2 mL of ammonia were added. Anthocyanin is present because the color shift from pink-red to blue-violet shows its existence. The pH differential approach, which relies on absorbance measurements at pH 1.0 and 4.5 and structural changes in chemical forms of anthocyanin, was used to calculate the total anthocyanin concentration. Separately, crude extracts were diluted with 0.4 M sodium acetate buffer (pH = 4.5) and 0.025M hydrochloric acid-potassium chloride buffer (pH = 1). The buffers were used to dilute each sample, resulting in an absorbance measurement that ranged from 0.2 to 1.4. A UV-Vis spectrophotometer was used to test the mixture's absorbance at 700 nm and λvis-max (UV1601; Shimadzu, Kyoto, Japan). Based on a calculation of mg of anthocyanins per dry mass.

Ascorbic acid analysis

500 µg of fresh organs (extracted in 10 mL of 6% (w/v) trichloroacetic acid (TCA). The extract was then mixed with 2 mL of 2% (w/v) dinitro-phenylhydrazine, 1 drop of 10% (w/v) thiourea dissolved in 70% (v/v) ethanol, and 2 mL of 2% (w/v) thiourea. The mixture was then heated to a boil for 15 minutes in a water bath. After bringing the samples to room temperature and chilling them, 5 mL of 80% (v/v) H_2SO_4 was added at zero degrees. According to the Mukherjee and Choudhuri (1983) technique, the absorbance was measured at 530 nm using a spectrophotometer to ascertain the amount of ascorbic acid (ASA).

Antioxidant enzyme activity

The protein content of the extract was calculated by grinding 0.5 g of fresh organs in 10 ml of a 50 mM KH_2PO_4 buffer (pH 7.8), then centrifuging the homogenate at 10.000 g for 15 min at 5 °C. The Kar and Mishra (1976) technique was used to assess the peroxidase (POX) activity by using benzidine and a spectrophotometer to measure the absorbance at 470 nm. Kar and Mishra (1976) approach measured polyphenol oxidase (PPO) activity. Using potassium phosphate as a buffer and H_2O_2 as a substrate, as described by Chen *et al*. (2000), a catalase activity test (CAT) was conducted. The absorbance was measured at 240 nm.

Estimation of phytohormones

Indole acetic acid (IAA), gibberellic acid (GA_3), abscisic acid ABA, kinetin, and zeatin endogenous hormone concentrations were measured by Knegt and Bruinsma (1973). First, 5 grams of fresh material was extracted in 80% cold MeOH for a whole night at 4 °C in the dark. Next, the extract was evaporated to an aqueous phase using a rotary evaporator after filtering through Whatman No. 1 paper. The residue was once again dissolved in 0.1 M phosphate buffer (pH 8.0) and incubated at -18 °C for 24 hours. At 4 °C, the extract was centrifuged at 17000 g. The resulting volume was increased entirely to 30 mL by adding two grams of sample PVP as phenol binding and filtering through Whatman No. 1. The filter was then rinsed with phosphate buffer (0.1 M, pH 8.0). Then, partition extract was run twice against diethyl ether (1:2 volume), with the organic phase being discarded each time. The pH of the aqueous phase was then readjusted to 2.5 using 5 N HCl, twice with diethyl ether, and then the aqueous phase was discarded. Finally, the organic phase evaporated to dryness and dissolved in 5 mL of water. 1 N acetic acid was then used to adjust the pH to 2.5. Next, endogenous plant hormones (IAA, GA_3, ABA, kinetin, and zeatin) were separated using a C18 sep-pack cartridge reversed-phase, following the method of (Lee *et al.,* 1989), using high-performance liquid chromatography (HPLC) (Thermo Fisher Scientific, Germany).

Statistical analysis

Randomization was used in the experimental design, and statistical analysis was done using SPSS (Statistical Package for the Social Science Version 28.0) (Gomez and Gomez, 1984). The quantitative analysis

uses Levene's sample parametric distribution and two-way ANOVA with Fisher's test's post hoc test variance analysis. The agreed-upon margin of error was defined at 5%, and the confidence interval was set at 95%.

Results

Changes in variables in vitro culture parameters

The survival percentage

In this study, under white and violet LEDs illumination, all the used treatments caused a marked increase in the survival percentage of micro-propagated *Moringa olifera*, with the highest value (85%) by MS medium with 2 mg/L TDZ, under violet LEDs illumination. In contrast, the lowest value was detected in the case of the control medium (41.6%) under white LEDs illumination (Table 2, Plates 1 and 2).

Number of shoots/ explants

The obtained results herein showed that the number of axillary shoots per explant was induced only by BAP (1 or 2 mg/L) after 30 days of micropropagation. However, the application of 2 mg/L BAP resulted in the highest number of induced shoots per explant after incubation under white or violet LEDs illumination, with an average of 4.531 shoots per explant in white light and 4.667 shoots per explant in violet light. In the present study, BAP was found to be the only effective in inducing multiple shoots (Table 2, Plates 1 and 2).

Leaves number and shoot length

Data herein cleared that the addition of thidiazuron (TDZ) to the medium did not propagate shoot; however, the maximum number of leaves was estimated in *Moringa* plants *in vitro* grown in MS medium contained 2 mg/L BAP, under violet LEDs (31.667). Compared to all other treatments, the highest shoot length (8.125 cm) was significantly obtained with hormone-free medium (control), under incubation in white LEDs and 7.95 cm in violet LEDs (Table 2, Plates 1 and 2).

Callus formation percentage %

The tabulated data in the current study showed no callus formation with a hormone-free medium (control) under two different light conditions (white and violet LEDs). In contrast, adding 1 mg/L BAP and 2 mg/L BAP results in 13.637% and 20% callus formation percentages, respectively, when incubated in white and violet LEDs compared to control (Table 2, Plates 1 and 2).

Callus size (Cm^3) and shoot and/or callus fresh and dry weight

In this study, the largest callus size was 7.65 cm^3 in MS medium containing 2 mg/L TDZ, under violet light, then 7.633 cm^3 by the same treatment under white light. On the other hand, the maximum fresh weight of callus was 4.779 g in MS medium containing 2 mg/L TDZ under white light, then 3.448 g in the same medium under violet light. Consequently, the maximum dry weight of callus was 3.403 g in MS medium containing 2 mg/L TDZ under white light, then 2.751 g in the same medium under violet light (Table 2, Plates 1 and 2).

Table 2. Effect of two different visible light emitting diodes (white and violet) LEDs on a variable *in vitro* culture parameters of micro-propagated *Moringa oleifera* after 30 days from transplanting

Light quality	Plant growth regulators		Survival %	No. of Shoots/ explant	Shoot length (cm)	No. of Leaves	Callus formation %	Callus size (Cm3)	Shoot and/or callus fresh weights (g/explant)	Shoot and/or callus dry weights (g/explant)
	Cytokinin type	Concentration (mg/L)								
White LEDs	Control	0	41.60%	1.03^{b}±0.47	8.13^{a}±0.47	13^{c}±0.47	0%	NO	0.67^{e}±0.04	0.39^{e}±0.04
	BAP	1	46.67%	2.5^{b}±0.62	7.33^{ab}±1.783	25.67^{c}±5.73	0%	NO	0.92^{e}±0.12	0.49^{e}±0.06
		2	63.64%	4.53±0.96	6.93^{ab}±1.62	28.33^{b}±5.94	13.64%	4.75^{b}±0.22	1.09^{e}±0.14	0.63^{de}±0.08
	TDZ	1	72.22%	NO	NO	NO	100%	6.87^{a}±0.89	2.98^{bc}±0.45	1.77^{bc}±0.25
		2	80%	NO	NO	NO	100%	7.63^{a}±1.02	4.77^{a}±0.69	3.40^{a}±0.48
Violet LEDs	Control	0	45.45%	1.03^{b}±0.89	7.95^{a}±1.62	16.67^{d}±5.29	0%	NO	0.72^{e}±0.64	0.41^{e}±0.45
	BAP	1	54.54%	2.74^{b}±0.83	7.22^{ab}±1.58	24^{c}±5.17	9.09%	5.08^{b}±0.95	2.11^{cd}±0.62	1.33^{bcd}±0.43
		2	77.27%	4.67^{a}±0.93	5.83^{b}±1.52	31.67^{a}±5.67	20%	5.08^{b}±0.91	2.60^{d}±0.58	1.05^{cde}±0.41
	TDZ	1	72.73%	NO	NO	NO	100%	6.95^{a}±0.93	2.84^{bc}±0.57	1.99^{b}±0.41
		2	85%	NO	NO	NO	100%	7.65^{a}±0.96	3.44^{b}±0.59	2.75^{a}±0.4

BAP**:** 6-benzylaminopurine, TDZ: thidiazuron**,** LEDs: light-emitting diode systems
The values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation, with the Fishers test at $p \leq 0.05$.

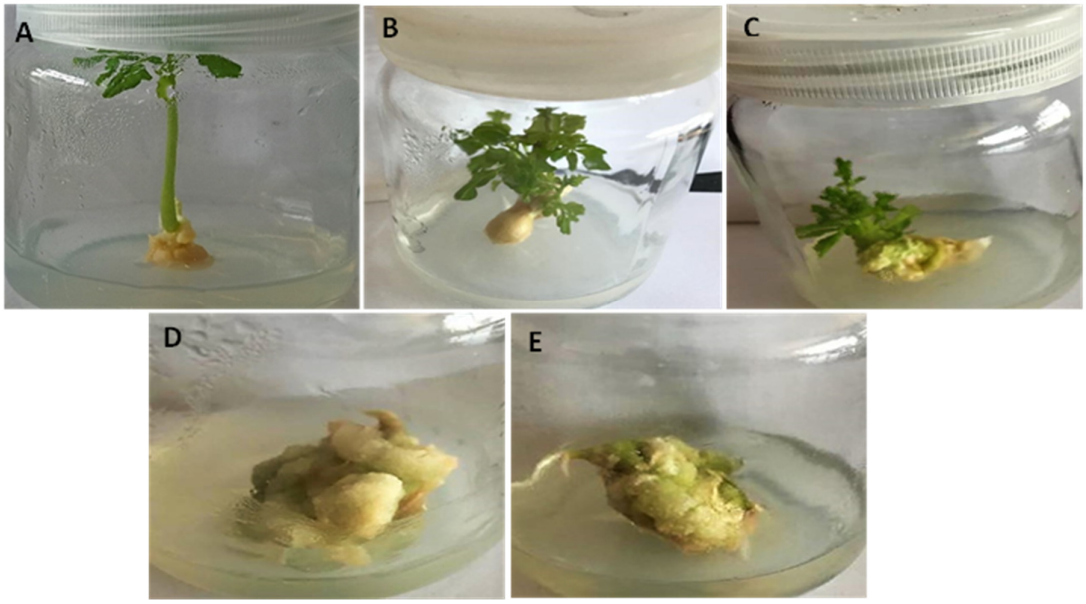

Plate 1. *In vitro* propagation of *Moringa oleifera* seeds under white LEDs
A) culturing on MS-free media. B) Culturing on MS medium supplemented with 1 mg/L BAP. C) Culturing on MS medium supplemented with 2 mg/L BAP. D) Culturing on MS medium supplemented with 1 mg/L TDZ. E) Culturing on MS medium supplemented with 2 mg/L TDZ.

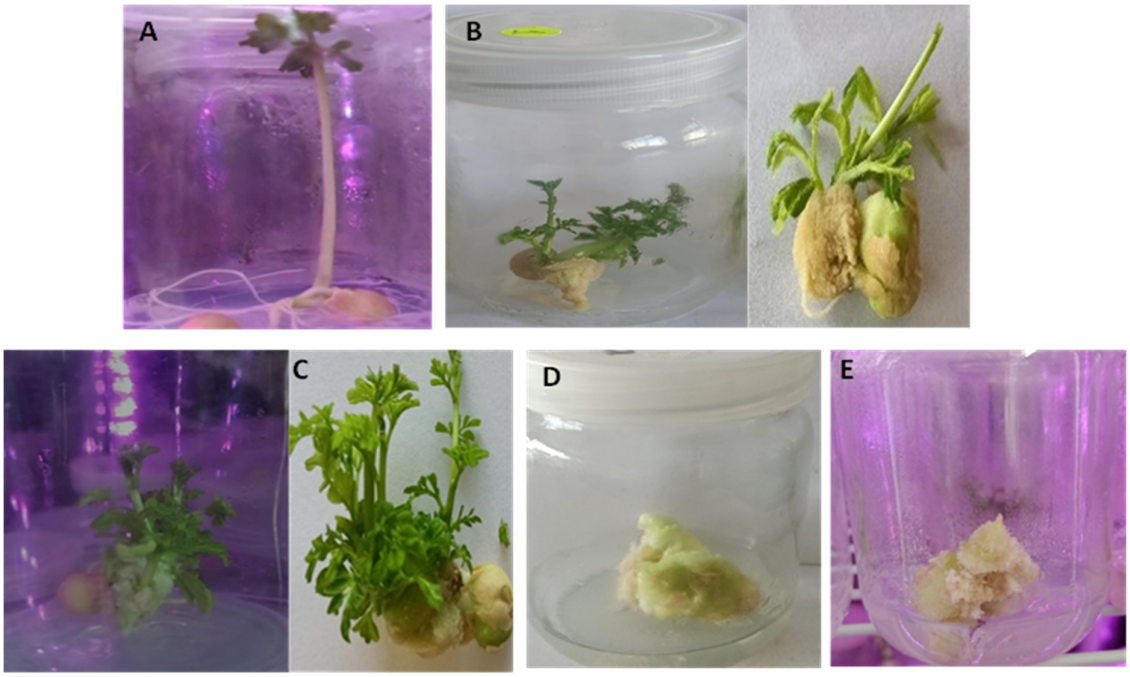

Plate 2. *In vitro* propagation of *Moringa oleifera* seeds under violet LEDs
A) culturing on MS-free medium. B) Culturing on MS medium supplemented with 1 mg/L BAP. C) Culturing on MS medium supplemented with 2 mg/L BAP. D) Culturing on MS medium supplemented with 1 mg/L TDZ. E) Culturing on MS medium supplemented with 2 mg/L TDZ.

Changes in antioxidant enzymes (POX, PPO, CAT activities)

In MS medium supplemented with 1, 2 mg/L BAP or 1, 2 mg/L TDZ *Moringa oleifera* plants, the antioxidant enzymes (POX, PPO, CAT) activities increased significantly compared to the white and/or violet LEDs conditions. The MS medium supplemented with 2 mg/L BAP or 2 mg/L TDZ under white and or violet LEDs conditions show significant increases in POX activities of 60.09%, 44.59%, 232.16%, 210.14%, respectively, compared to LEDs conditions (Figure 1A). On the contrary, the most pronounced increases were observed in PPO when MS medium supplemented with 2 mg/L BAP was used under white and/or violet LEDs conditions. As a consequence of these results, some critical observations may be made on the activity of CAT in *Moringa oleifera* applied with BAP or TDZ in the presence of LEDs, as shown in Figure 1. Our results show that allowing *Moringa oleifera* under LEDs with MS medium containing 2 mg/L BAP or 2 mg/L TDZ caused a significant increase CAT.

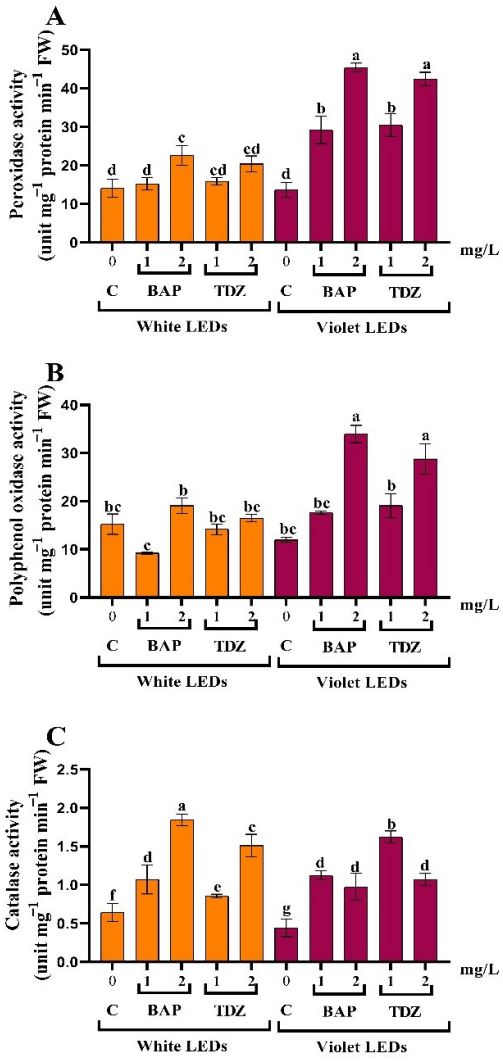

Figure 1. Effect of two different visible light emitting diodes (White and Violet) LEDs on antioxidant enzymes activity

A) peroxidase, B) poly phenoloxidase, and C) catalase of micro-propagated *Moringa oleifera* after 30 days. The values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation, with $p \leq 0.05$. BAP**:** 6-benzylaminopurine, TDZ: thidiazuron**,** LEDs: light-emitting diode systems

Changes in non-antioxidant compounds (phenols, flavonoids, anthocyanins, ascorbic acid)

The results obtained in this study showed that the phenolic content from *Moringa oleifera* tissues (leaves or calli) increased by the used treatments, especially in the case of the two used concentrations of TDZ, under the two different light conditions. Also, it was important to maintain that violet light had the upper hand in that regard at the same concentration as the used growth substances. In detail, the highest significant value of phenols (142.31%) was with MS medium contained 2 mg/L TDZ under violet light followed by (96.29%) with MS medium fortified with 1 mg/L TDZ in the same light treatment, while under the white light it recorded value of 59.38% and 7.05% at the same previous media, respectively (Figure 2 A).

All the treatments used generally increased flavonoid contents. A significant general enhancement in flavonoid production was detected at about (112.92%) in MS medium with 2 mg/L TDZ under violet light. The minimum is about 14.59 in the control medium under white light (Figure 2 B).

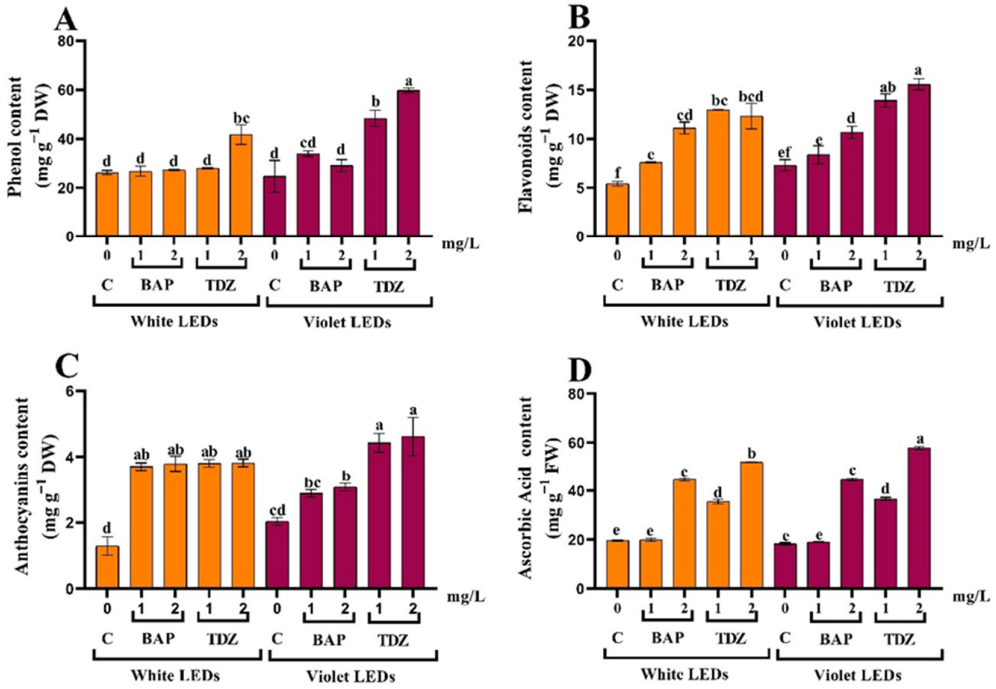

Figure 2. Effect of two different visible light emitting diodes (White and Violet) LEDs on non-antioxidant compounds

A) phenol, B) flavonoids, C) anthocyanins, D) ascorbic acid of micro-propagated *Moringa oleifera* after 30 days. The values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation at $p \leq 0.05$. BAP**:** 6-benzylaminopurine, TDZ: thidiazuron**,** LEDs: light-emitting diode systems

The findings of the present investigation stated that all the used treatments generally increased anthocyanin production in the micro-propagated *Moringa oleifera* significantly under two different light conditions (white and violet LEDs), especially violet LEDs, in relation to hormone-free medium (control). The highest value of anthocyanins is about 195.83% with MS medium fortified with 2 mg/L BAP under white light, and the lowest is about 41.65% under MS medium fortified with 1 mg/L TDZ under violet light (Figure 2 C).

The data recorded in this study revealed that the response of the micropropagation of *Moringa oleifera* to the different treatments under two different light conditions (white and violet LEDs) was more or less similar to those of the other determined antioxidant compounds. Thus, the maximum value of ascorbic acid is about 216.23% with MS medium supplemented with 2 mg/L TDZ under violet light, followed by 4.38% with the same MS medium but under violet LEDs illumination with 1 mg/L BAP (Figure 2 D).

Changes in phytohormones (Gibberellic acid (GA_3), Indole3- acetic acid (IAA), Abscisic acid (ABA), Kinetin, and Zeatin)

A perusal of the data recorded in the present study cleared that, compared to control values, all the used treatments decreased the determined GA_3 in the propagated *Moringa oleifera* under two different light conditions (white and violet LEDs); the magnitude of response was more pronounced in case of the lowest BAP concentration (2 mg/L). So, the amount of GA_3 was the maximum value (154.91 and 146.633 µg/100g) in the control medium under white and violet light, respectively. The minimum value was 84.707 µg/100g with MS medium fortified with 2 mg/L BAP under white LEDs (Figure 3 A). The data of this investigation cleared that applying the two levels (1 and 2 mg/L) of BAP nearly increased IAA content in propagated *Moringa oleifera* under the two different light conditions (white and violet LEDs). The maximum value of IAA (48.117

µg/100g) was by MS medium supplemented with 2 mg/L BAP under white light. In contrast, the minimum value was 28.51 µg/100g by MS medium supplemented with 1 mg/L BAP under violet light (Figure 3 B). A reverse situation to those of IAA was observed in corresponding to abscisic acid content (ABA) in this study; thus, its content decreased by the application of the two levels (1 and 2 mg/L) of BAP, in propagated *Moringa oleifera* under two different light conditions (white and violet LEDs). With the highest values (4.943 and 3.37) in the control medium under white and violet LEDs, respectively. On the other hand, the least value was 1.453 µg/100g with MS medium supplemented with 1 mg/L BAP under white LEDs (Figure 3 C).

In the present study, the trend of changes in kinetin and zeatin; by the application of the two levels (1 and 2 mg/L) of BAP, in propagated *Moringa oleifera* under two different light conditions (white and violet LEDs) resembles that recorded in the other determined promotor; IAA. Thus, the minimum value of kinetin and zeatin was 51.464 and 99.53 µg/100g, respectively, in the control medium under white light conditions. The maximum value of kinetin was 123.1 µg/100g with MS medium supplemented with 2mg/L BAP under violet light. In contrast, zeatin was 125.553 µg/100g with MS medium supplemented with 1 mg/L BAP under white light (Figure 3 D, E).

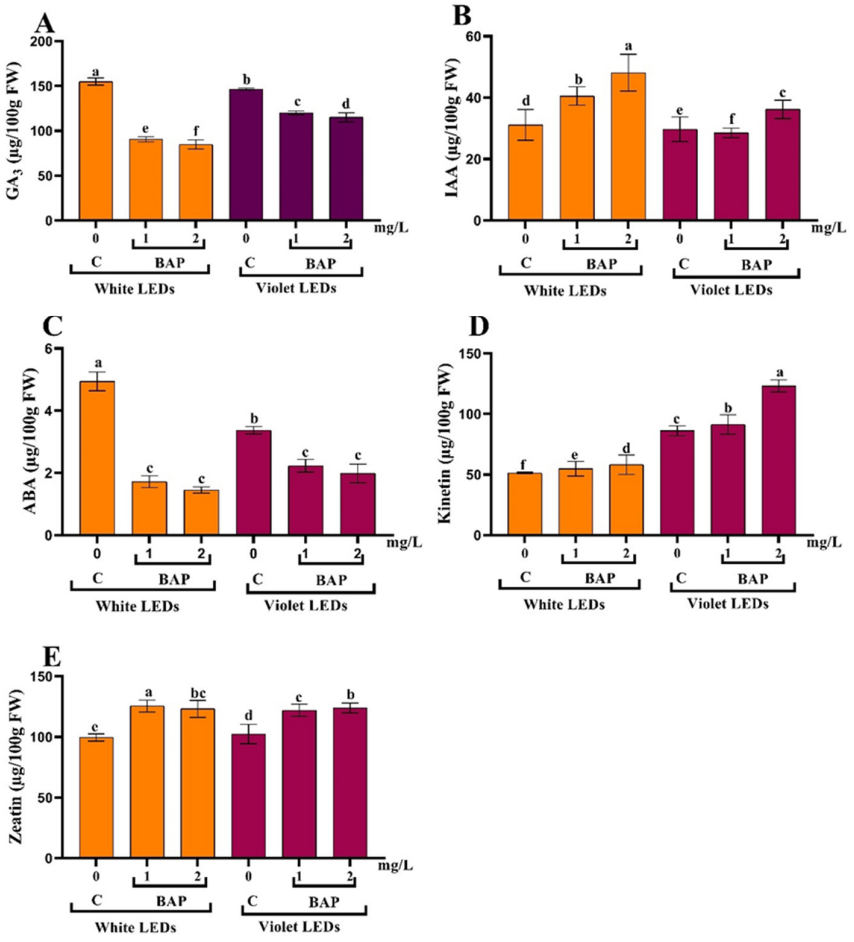

Figure 3. Effect of two different visible light emitting diodes (white& violet) LEDs on phytohormones content

A) gibberellic acid (GA_3), B) Indole3- acetic acid (IAA), C) Abscisic acid (ABA), D) Kinetin and E) Zeatin of micro-propagated *Moringa oleifera* after 30 days from transplanting. BAP**:** 6-benzylaminopurine, TDZ: thidiazuron**,** LEDs: light-emitting diode systems.

Principal component analysis

The Principal Component Analysis (PCA) identified substantial differences in physiological parameters and morphological criteria (Figure 5). The PCA identified two primary components of the measured variables, and PC1 and PC2 accounted for 83.3% and 7.3%, respectively, of the total variance in *Moringa oleifera* plants (Figure 4).

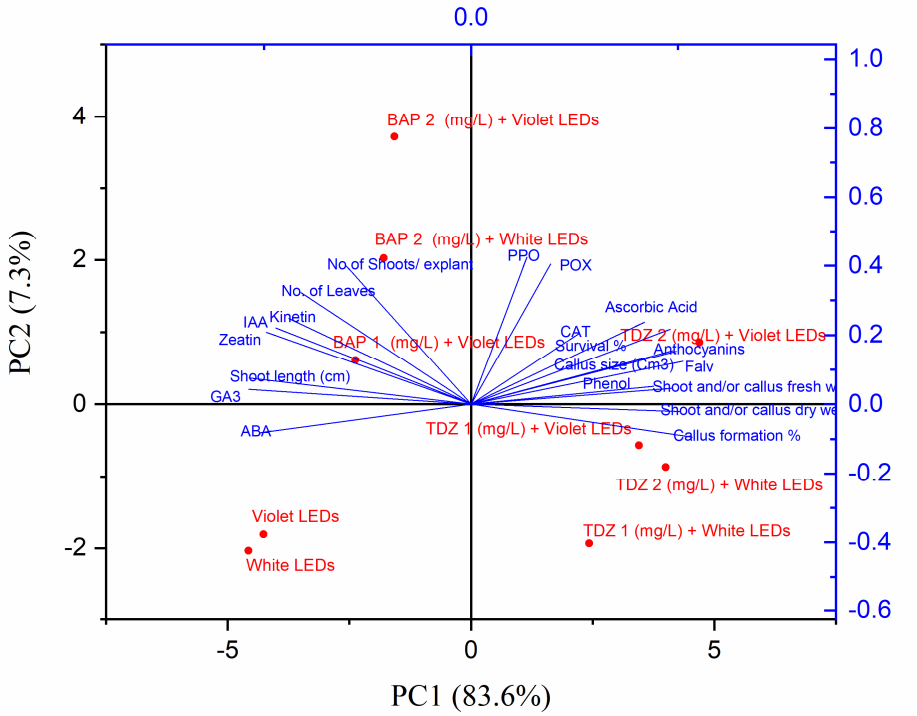

Figure 4. Principal component analysis (PCA) to understand variable treatment relationships in *Moringa oleifera* plants

PCA was used to examine the full dataset. The percentages on biplots represent the proportion of data variability that each component contributes to explaining. The lines emerging from the center point show positive or negative correlations between various factors, and the closer the lines are together, the stronger the association with a certain treatment.

Discussion

In this study, under white and violet LEDs illumination, all the used treatments caused a marked increase in the survival percentage of micro-propagated *Moringa oleifera*. This may be due to the micro-propagation of *Moringa oleifera* by using the seeds. Drumstick is often reproduced through seed in this regard. However, the viability of the seeds soon diminishes (Fotouo *et al*., 2015). *Moringa* is traditionally propagated by means of epigeal seed germination, which typically ranges from 60 to 98 percent for fresh seeds (Nouman *et al*., 2012). While Gupta *et al*. (2020) discovered that the proportion of *Moringa* responsive explants reduced with increased doses of cytokinin's or auxins like BA and IAA, our findings are the exact reverse. The findings obtained in this study demonstrated that, after 30 days of micropropagation, only BAP (1 or 2 mg/L) could promote the number of axillary shoots per explant. The most induced shoots per explant appeared during incubation under white or violet LED light when 2 mg/L BAP was applied. According to Saini *et al*. (2013), MS media containing 2.0 mg/L BAP generated the greatest mean number of shoots per explant, followed by

those treated with 1.0 and 3.0 mg/l of BAP, respectively. According to Azmi *et al*. (2014), roses grown in vitro with LEDs in combination generate more shoots than roses grown traditionally. They concluded that the combined LED light colors (red and blue) were advantageous for growing ornamental plants in general and roses in particular. The current finding is consistent with those of other researchers who have noted the effectiveness of BAP for the induction of shoots in *Moringa oleifera* shoot explants that were cultured on MS medium supplemented with various concentrations of BAP. All the media-induced 100% shoot induction (Pham *et al*. 2020). Sang *et al*. (2018) noted that while the only explants treated with TDZ generated no shoots at all in response to an increase in concentration, this was the sole exception to the general tendency (Grzegorczyk-Karolak *et al*. 2021). Numerous studies have revealed that *M. oleifera* shoot numbers tend to increase when BAP concentrations rise (Ridzuan *et al*., 2020).

Additionally, unlike the findings of Galán-Ávila *et al*. (2020) the control medium without growth regulators failed to produce any multiple shoots. Still, it did produce shoots of a fair length after 30 days of incubation under either white or violet LEDs. The information provided here demonstrated that adding thidiazuron (TDZ) did not cause the shoot to spread. The maximum shoot length was considerably compared to hormone-free media (control) when being incubated under white LEDs compared to all other treatments. These findings concurred with those of (Ridzuan *et al*., 2020). They reported that explants grown on MS media without a growth regulator were lengthened and developed into new drumstick plantlets with strong roots. Compared to the control, kinetin at lower concentrations, zeatin, and TDZ either generated statistically comparable or shorter-length shoots (Kousalya and Bai, 2016). With hormone-free media (control), under two distinct lighting conditions, there was no callus development, according to the tabulated data in the present research (white and violet LEDs). As evidence, Torrizo and Zapata (1986) demonstrated that low ABA concentrations induced the callus to proliferate. The greatest outcome in this study (100%) was found when TDZ was applied and incubated in both white and violet LEDs. These are backed up by Zimmerman and Scorza (1992), who concluded that TDZ is not necessarily preferable to BAP. For instance, TDZ resulted in severe callus and little shoot proliferation in peach, while BAP caused the most shoot multiplication.

According to Sang *et al*. (2018), greater cytokinin concentrations promote the beginning of calluses at the cut ends of shoots. The presence of endogenous hormones in explants as well as those added to the media, may have an impact on callus induction variation. Callus's morphology and weights differed in addition to their size (Mahood *et al*., 2018). Additionally, TDZ encourages callus development more than other regulators do in several jurisdictions (Traore *et al*., 2003). In this research, under violet light, the callus size was enhanced in MS medium containing 2 mg/L TDZ. These outcomes might be explained by the fact that a rise in cell division and cell expansion leads to an increase in the fresh callus weight.

Additionally, the water and carbohydrates in the culture medium impact the callus' fresh weight physiologically (Bajji *et al*., 2000), and the quantity of fresh weight generated is greatly influenced by how quickly these cells divide and proliferate before becoming a callus (Phua *et al*., 2018). To research cell growth, use products from primary and secondary metabolism, and gain cell suspension for propagation, the generation of callus form from pieces of stems, leaves, and roots has recently become more important. It can also make it possible to isolate economically advantageous phytochemicals, preventing the need to harvest plant materials from their natural sources (Ogita *et al*., 2009). To manufacture pharmaceutical and bioactive substances from plants on a large scale, callus and cell culture play a specific significance in the field of plant biotechnology. Pharmaceuticals, flavors, agrochemicals, colors, biopesticides, and food additives are all primarily derived from phytochemicals (Maksoud *et al*., 2022).

The determined antioxidant enzymes (peroxidase, polyphenol oxidase, and catalase) were significantly increased when supplemented with MS media containing BAP and TDZ under white, violet LEDs. Many results have shown that peroxidase (POX) and polyphenol oxidase (PPO) are important defense-related enzymes of plants (El-Sheshtawy *et al*., 2022; Mowafy *et al*., 2022). Al-Mayahi (2016) researched the effects of LED lighting on date palm shoot multiplication, phytochemicals, and changes in the activities of antioxidant

enzymes. He reported that the total antioxidant activity revealed no appreciable differences in the antioxidant power of the biomass grown under various lighting conditions. However, when red + blue (9:1) mixed LED treatment was used instead of fluorescent lighting, *P. dactylifera* in vitro shoot cultures showed increased POX activity as well as better growth (Al-Mayahi, 2016), indicating a close connection between the light environment, antioxidative metabolism, and morphogenesis. According to the findings of this experiment, *M. oleifera* exhibits the highest levels of peroxidase, polyphenol oxidase, and catalase activity when BAP is used under violet light. Kapoor *et al*. (2018) findings on the impact of LED light quality on antioxidant activity lend credence to this. Additionally, they claimed that tests on plant defense enzymes showed that polyphenol oxidase (PPO) activity was lower in vitro samples than it was in samples grown in soil. However, peroxidase (POX) activity was greater in plants (Dawood *et al*., 2022a).

Tissue culture is growing and used to produce secondary metabolites for therapeutic application. Future consumer product development will be based on safe, efficient, high-quality processes incorporating cultural systems (Lemma *et al*., 2020). The results obtained in this study showed that the phenolic content from *M. oleifera* leaves increased with the treatments used. Similarly, *M. oleifera* is well recognized for having a number of health advantages, and they have been connected to its phytochemicals, such as phenolic compounds (Vongsak *et al*., 2013). According to reports, the phenolic content of medicinal herbs influences how effective they are as antioxidants (Dawood *et al*., 2022b). Additionally, it was shown that different quantities of 1-naphthalene acetic acid (NAA) and 6-benzyl aminopurine (BAP) had a substantial impact on phenolic exudation (North *et al*., 2012). Recently, it has been discovered that the leaves of *Moringa oleifera* are full of phenolic compounds with antioxidant properties (Zullaikah *et al*., 2019). In vitro-grown *M. oleifera* samples exhibit a buildup of phenolic chemicals, indicating that the defensive response has been triggered (Ridzuan *et al*., 2020).

When compared to hormone-free media, the treatments utilized in this research significantly increased the micropropagation of *Moringa oleifera* under two distinct lighting conditions (white and violet LEDs) (control). In this regard, the phytochemical and antioxidant studies of the extract from different *Moringa oleifera* plants showed that the leaves had the greatest concentrations of total phenolics (9.58 mg/g), α-carotene (14.10 mg/g), and lycopene (2.60 mg/g). In addition, the greatest concentrations of anthocyanin (52.80 mg/g) and total flavonoids (3.5 mg/g) were found in the bark and flowers, respectively (Vats and Gupta, 2017). The presence of phytochemicals in various morphological sections of *M. oleifera* has long been known. For instance, *M. oleifera's* leaf, root, blossom, and seed coat all contain different flavonoids (Wang *et al*., 2017). The results of this study showed that, in comparison to hormone-free media, all of the applied treatments substantially boosted anthocyanin synthesis in the micropropagated *Moringa olifera* under two distinct lighting conditions (white and violet LEDs) (control). According to some data, anthocyanins may shield photosynthetic tissues from photo-inhibition by absorbing blue-green light and decreasing the quantity of light that reaches the chloroplasts (Merzlyak *et al.,* 2008). This increase in anthocyanin synthesis may be brought on by mild stress in the context of micropropagation. When vegetative tissues are stressed by factors like intense light, low temperatures, nutritional shortages, or pathogen attacks, anthocyanins are often formed (Chalker-Scott, 1999). This induction indicates that anthocyanins operate as a protective agent. However, this might be because of their antioxidant qualities or their visual characteristics (or both).

The production of flavonoids and anthocyanin under the same conditions and the greater growth rate in the micropropagation of *Moringa oleifera* were shown to be positively correlated in this research. Because anthocyanins are powerful antioxidants, flavonoids, in general, may be able to remove reactive oxygen species (ROS) produced during photosynthesis, especially in the presence of photoinhibition (typically high light and low temperature). In addition, although anthocyanins are often contained in vacuoles, colorless cytosolic anthocyanins may function to remove ROS produced by mitochondria and chloroplasts (Neill and Gould, 2003; Sheteiwy *et al*., 2022).

The findings from this investigation showed that the *Moringa oleifera* micropropagation trend responded to the various employed treatments under two distinct light conditions (white and violet LEDs) in a manner that was most comparable to those of the other identified antioxidant compounds. An appropriate plant growth promoter is moringa leaf extract, which is rich in K, Ca, Fe, amino acids, carotenoids, phenols, ascorbate, and growth-regulating hormones (Basra *et al*., 2009; Ragaey *et al*., 2022). Plant defense against oxidative stress depends on antioxidants like ascorbic acid and glutathione, which are abundant in the chloroplasts of the moringa plant and other cellular compartments (Badawy *et al*., 2022; Sofy *et al*., 2021). According to Lee *et al.,* (2008), the combined action of red and blue light may boost the accumulation of bioactive substances such as total polyphenols, anthocyanins, and flavonoids for all the antioxidant components thus tested. Red LED therapy boosts the antioxidant activity of tomato, Chinese cabbage, pea, and Chinese kale during storage and has a greater effect on anthocyanin than blue LED treatment does (Kim *et al*., 2013). In their study of the impact of LEDs on the overall nutritional profile of cabbage, Maksoud *et al*. (2022) noted improvements in the amounts of total phenolics, total chlorophyll, ascorbic acid, and reactive oxygen species.

Additionally, the response of *Moringa olifera* micropropagation to TDZ addition was better than that of BAP addition. As a result, BAP was used as some studies indicated that TDZ might affect endogenous plant growth regulators either directly or indirectly and cause cellular/tissue responses required for cell/tissue division/regeneration. Other options include cell membrane modification, energy levels, nutritional absorption, transport, and assimilation (Guo *et al*., 2011; Younes *et al*., 2021).

Moringa and other species' tissues have been shown to contain a variety of phytohormones (Safi-naz and Rady, 2015). It is important to reiterate that only in the case of BAP treatment were phytohormones determined since the micro propagated *Moringa oleifera* underwent differentiation under the two distinct lighting conditions (white & violet LEDs).

The analysis of the data from the current study showed that in comparison to control values, all the treatments used in the propagated *Moringa oleifera* under two different light conditions (white & violet LEDs) decreased the determined GA_3, IAA, and ABA, while increasing kinetin and zeatine at the same conditions. In this regard, light and its characteristics, such as wavelength or photoperiod, control the metabolism of gibberellin in plants (Gaspar *et al*., 1996; Mourad *et al*., 2021). In agreement, there is a direct correlation between the quantity of GA_3 hormone and the degree of regeneration (Weiss and Ori, 2007; Fouda and Sofy, 2022). Gibberellins, a sizable family of tetracyclic diterpenoid plant hormones, control a variety of aspects of plant growth and development throughout the course of the plant's life cycle, including encouraging cell division and elongation, seed germination, stem and hypocotyl elongation, root growth, and flower induction (Vera-Sirera *et al*., 2016; Mohamed *et al*., 2021).

On the other hand, in comparison to a hormone-free medium, the treated samples with the two levels of BAP had the lowest value (control). Gibberellins influence the pace of shoot proliferation in this situation and may lengthen shoots, particularly if the shoots are short owing to high cytokinin levels (Cioć *et al*., 2022). These authors also claimed that the blue LED light reduced the overall gibberellin content in the final stage of shoot propagation (B and RB). The most widely used auxin class plant hormone, IAA controls a number of aspects of plant growth and development (Fu *et al*., 2015). Auxin levels in the tissues of *Gerbera jamesonii* may be decreased by red light spectrum (Red and Red-Blue LED), culture time, and axillary shoot multiplication, according to Cioć *et al*. (2022). Blue LED or control fluorescent did not cause a decrease in auxin levels. The measured antioxidant enzymes and auxin concentration in Moringa were shown to be positively correlated in this research. This is important because ROS increases the development of root nodes and auxin synthesis (Joo *et al*., 2001). By creating ROS, which induces cell wall loosening and aids in cell elongation and division, auxin plays a crucial part in elongation and cell division (Kawano, 2003). Also, because GA_3 and ABA are included in this theory, hydrogen peroxide (H_2O_2) is a ROS that aids in root regeneration (Li *et al*., 2007). Gibberellic acid

production is decreased by raising the quantity of ROS (Bazin *et al.,* 2011). ABA is one of the elements that boost the generation of extracellular ROS (Marten *et al*., 2007). The ABA works against cytokinin antagonistically (Huang *et al*., 2018).

The current data support the negative relationship between the quantity of abscisic acid (ABA) and the percentage of regeneration (Saeedpour *et al*., 2021). In this regard, LED light did not disturb the endogenous phytohormone balance and more effectively reduced the stress experienced by in vitro grown plants than fluorescent lamps did (Cioć *et al*., 2022). This study's phytohormone data of Moringa clarified the consistency between promotors and inhibiters. According to Torrizo and Zapata (1986), there is an inverse link between certain rice types' ABA content and regeneration. However, it was shown that lower values for shoot height, leaf number, and root number were linked with greater ABA concentrations in the culture medium (Ramírez-Mosqueda *et al*., 2019), in research on the regeneration of *Laelia anceps*. Cytokinin levels in moringa leaves are very high (Anwar *et al*., 2007). And one of them truly captured our attention with its unusual focus. More zeatin than any other plant discovered to date is found in the moringa tree leaves. Several research has been conducted to increase *M. oleifera* by tissue culture, but only with a small range of cytokinin (Leone *et al*., 2015). Hisano *et al*. (2016) demonstrated that cultivars of Golden Promise with high regeneration rates have extremely high amounts of cytokinin, which perfectly matches the present study's findings. Kinetin is a hormone that is recognized to be crucial for plants and these creatures (Muslihatin *et al*., 2018). Zeatin, which is abundant in the moringa plant leaves and can promote plant development, may also be utilized as a natural source of cytokinin. According to Hönig *et al*. (2018), zeatin seems particularly adept in regulating cell proliferation, cell division, and differentiation.

The results of Lappas (2015), who reported that zeatin has potent antioxidant and immunity-enhancing properties and thereby protects cells against oxidation, are consistent with the positive correlation between the determined explant growth, antioxidants (enzymes & compounds), and cytokinins (kinetin & zeatin) by the application of the two levels (1 and 2 mg/L) of BAP in propagated *Moringa olifera*. Patterns of plant regeneration rely on the precise balance of internal hormones (Bidabadi and Jain, 2020). Using pistils as explants, direct interactions between auxin and cytokinin during shoot regeneration have also been discovered (Cheng *et al*., 2013). The current findings confirmed that the tissues of vitrified shoots had more internal cytokinins than normal shoots did in this research. On the one hand, our findings are consistent with those of other writers, whose research showed the value of exogenous cytokinin in vitrification induction and promotion (Leshem *et al*., 1988). On the other hand, the mean total content of all detected cytokinins was lower than in the beginning material at all phases of the cultivation of *Gerbera jamesonii* and for all light quality (Cioć *et al*., 2022).

From a different perspective, micropropagation is more successful when lit by violet LEDs than by white LEDs. According to estimates from Olle and Viršile (2013), LED absorption affects over 90% of plant physiology and growth. In tissue culture investigations, white, red, blue, and a blue-red mixture are the most often utilized LED colors (wavelengths) or color combinations (wavelength combinations). The production of chlorophyll, photosynthesis, the opening of stomata, and the maturity of chloroplasts are all significantly influenced by blue light (Tibbitts *et al*., 1983). Chen *et al.,* (2014) found that red and blue light greatly impacted plant morphology and that red light increased leaves.

Conclusions

Plant cell culture innovations may open up new avenues for the economically viable commercial cultivation of even uncommon or exotic plants. The addition of thidiazuron to the medium did not propagate shoot but recorded 100% callus formation other than benzyl aminopurine. A sparring response was detected in the determined metabolites by thidiazuron and benzyl aminopurine, but in general, the former is the most

superior, and anyhow, violet LEDs are more effective than white. Although protocols for optimizing the use of medical plants have made excellent progress, more study is still required in these areas to optimize and preserve the use of medicinal plants.

Authors' Contributions

LMZ, SAH, MAA, RMG, and HYE Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization: LMZ, SAH, RMG, MAA, and HYE; Writing - original draft; Writing - review and editing. LMZ, SAH, MAA, RMG, HYE and MRS. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abu-Shahba MS, Mansour MM, Mohamed HI, Sofy MR (2022). Effect of biosorptive removal of cadmium ions from hydroponic solution containing indigenous garlic peel and mercerized garlic peel on lettuce productivity. Scientia Horticulturae 293:110727. *https://doi.org/10.1016/j.scienta.2021.110727*

Ahmadi T, Shabani L, Sabzalian MR (2021). LED light sources improved the essential oil components and antioxidant activity of two genotypes of lemon balm (*Melissa officinalis* L.). Botanical Studies 62(1):1-13. *https://doi.org/10.1016/j.scienta.2021.110727*

Al-Mayahi AMW (2016). Effect of red and blue light emitting diodes "CRB-LED" on in vitro organogenesis of date palm (*Phoenix dactylifera* L.) cv. Alshakr. World Journal of Microbiology and Biotechnology 32(10):1-8. *https://doi.org/10.1007/s11274-016-2120-6*

ALHaithloul HAS, Khan MI, Musa A, Ghoneim MM, ALrashidi AA, Khan I, Azab E, Gobouri AA, Sofy MR, El-Sherbiny M (2022). Phytotoxic effects of Acacia saligna dry leachates on germination, seedling growth, photosynthetic performance, and gene expression of economically important crops. PeerJ 10:e13623. *https://doi.org/10.7717/peerj.13623*

Anwar F, Latif S, Ashraf M, Gilani AH (2007). *Moringa oleifera*: a food plant with multiple medicinal uses. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 21(1):17-25. *https://doi.org/10.1002/ptr.2023*

Azmi NS, Ahmad R, Ibrahim R (2014). Effects of red and blue (RB) LED on the *in vitro* growth of *Rosa kordesii* in multiplication phase. In: 2nd International Conference on Agriculture and Biotechnology. Singapore.

Babar H, Rizwan B, Babar A, Nazia H, Noreen S, Naeem N, Raza F, Seed Z, Imran S (2022). Therapeutic effect of *Moringa oleifera:* A review. Pakistan BioMedical Journal 10-13. *https://doi.org/10.54393/pbmj.v5i1.161*

Badawy IH, Hmed AA, Sofy MR, Al-Mokadem AZ (2022). Alleviation of cadmium and nickel toxicity and phyto-stimulation of tomato plant by endophytic *Micrococcus luteus* and E*nterobacter cloacae*. Plants 11(15):2018. *https://doi.org/10.54393/pbmj.v5i1.161*

Bajji M, Lutts S, Kinet J-M (2000). Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus cultures issued from durum wheat (*Triticum durum* Desf.) cultivars differing in drought resistance. Journal of Plant Physiology 156(1):75-83. *https://doi.org/10.1016/S0176-1617(00)80275-8*

Barceló-Muñoz A, Barceló-Muñoz M, Gago-Calderon A (2021). Effect of LED lighting on physical environment and microenvironment on *in vitro* plant growth and morphogenesis: the need to standardize lighting conditions and their description. Plants 11(1):60. *https://doi.org/10.3390/plants11010060*

Basra S, Zahar M, Rehman H, Yasmin A, Munir H (2009). Evaluating the response of sorghum and moringa leaf water extracts on seedling growth in hybrid maize. In: Proceedings of the International Conference on Sustainable Food Grain Production: Challenges and Opportunities. University of Agriculture, Faisalabad, Pakistan, pp 22.

Batista DS, Felipe SHS, Silva TD, de Castro KM, Mamedes-Rodrigues TC, Miranda NA, ... Chagas K (2018). Light quality in plant tissue culture: does it matter? In Vitro Cellular & Developmental Biology-Plant 54(3):195-215. *https://doi.org/10.1007/s11627-018-9902-5*

Bazin J, Langlade N, Vincourt P, Arribat S, Balzergue S, El-Maarouf-Bouteau H, Bailly C (2011). Targeted mRNA oxidation regulates sunflower seed dormancy alleviation during dry after-ripening. The Plant Cell 23(6):2196-2208. *https://doi.org/10.1105/tpc.111.086694*

Bidabadi SS, Jain SM (2020). Cellular, molecular, and physiological aspects of *in vitro* plant regeneration. Plants 9(6):702. *https://doi.org/10.1105/tpc.111.086694*

Cavallaro V, Pellegrino A, Muleo R, Forgione I (2022). Light and plant growth regulators on *in vitro* proliferation. Plants 11(7):844. *https://doi.org/10.1105/tpc.111.086694*

Chalker-Scott L (1999). Environmental significance of anthocyanins in plant stress responses. Photochemistry and Photobiology 70(1):1-9. *https://doi.org/10.1111/j.1751-1097.1999.tb01944.x*

Chen X-l, Guo W-z, Xue X-z, Wang L-c, Qiao X-j (2014). Growth and quality responses of 'Green Oak Leaf'lettuce as affected by monochromic or mixed radiation provided by fluorescent lamp (FL) and light-emitting diode (LED). Scientia Horticulturae 172:168-175. *https://doi.org/10.1016/j.scienta.2014.04.009*

Chen Y, Cao X, Lu Y, Wang X (2000). Effects of rare earth metal ions and their EDTA complexes on antioxidant enzymes of fish liver. Bulletin of Environmental Contamination and Toxicology 65(3):357-365. *https://doi.org/10.1016/j.scienta.2014.04.009*

Cheng ZJ, Wang L, Sun W, Zhang Y, Zhou C, Su YH, Li W, Sun TT, Zhao XY, Li XG (2013). Pattern of auxin and cytokinin responses for shoot meristem induction results from the regulation of cytokinin biosynthesis by AUXIN RESPONSE FACTOR3. Plant Physiology 161(1):240-251. *https://doi.org/10.1016/j.scienta.2014.04.009*

Cioć M, Dziurka M, Pawłowska B (2022). Changes in Endogenous phytohormones of *Gerbera jamesonii* axillary shoots multiplied under different light emitting diodes light quality. Molecules 27(6):1804. *https://doi.org/10.3390/molecules27061804*

Dawood MF, Abu-Elsaoud AM, Sofy MR, Mohamed HI, Soliman MH (2022a). Appraisal of kinetin spraying strategy to alleviate the harmful effects of UVC stress on tomato plants. Environmental Science and Pollution Research:1-21. *https://doi.org/10.1007/s11356-022-19378-6*

Dawood MF, Sofy MR, Mohamed HI, Sofy AR, Abdel-kader HA (2022b). Hydrogen sulfide modulates salinity stress in common bean plants by maintaining osmolytes and regulating nitric oxide levels and antioxidant enzyme expression. Journal of Soil Science and Plant Nutrition 1-19. *https://doi.org/10.1007/s42729-022-00921-w*

Egbuna C, Ifemeje JC, Maduako MC, Tijjani H, Udedi SC, Nwaka AC, Ifemeje MO (2018) Phytochemical test methods: qualitative, quantitative and proximate analysis. Phytochemistry. Apple Academic Press, pp 381-426. https://doi.org/10.1201/9780429426223-15

El-Sheshtawy HS, Mahdy HM, Sofy AR, Sofy MR (2022). Production of biosurfactant by *Bacillus megaterium* and its correlation with lipid peroxidation of *Lactuca sativa*. Egyptian Journal of Petroleum 31(2):1-6. *https://doi.org/10.1201/9780429426223-15*

El-Sheshtawy HS, Sofy MR, Ghareeb DA, Yacout GA, Eldemellawy MA, Ibrahim BM (2021). Eco-friendly polyurethane acrylate (PUA)/natural filler-based composite as an antifouling product for marine coating. Applied Microbiology and Biotechnology 105(18):7023-7034. *https://doi.org/10.1007/s00253-021-11501-w*

Fotouo-M H, du Toit ES, Robbertse PJ (2015). Germination and ultrastructural studies of seeds produced by a fast-growing, drought-resistant tree: implications for its domestication and seed storage. AoB Plants 7. *https://doi.org/10.1093/aobpla/plv016*

Fouda HM, Sofy MR (2022). Effect of biological synthesis of nanoparticles from *Penicillium chrysogenum* as well as traditional salt and chemical nanoparticles of zinc on canola plant oil productivity and metabolic activity. Egyptian Journal of Chemistry 65(3):1-2. *https://doi.org/10.21608/ejchem.2021.95120.4469*

Fu S-F, Wei J-Y, Chen H-W, Liu Y-Y, Lu H-Y, Chou J-Y (2015). Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signaling & Behavior 10(8):e1048052. *https://doi.org/10.1080/15592324.2015.1048052*

Galán-Ávila A, García-Fortea E, Prohens J, Herraiz FJ (2020). Development of a direct in vitro plant regeneration protocol from *Cannabis sativa* L. seedling explants: developmental morphology of shoot regeneration and ploidy level of regenerated plants. Frontiers in Plant Science 11:645. *https://doi.org/10.3389/fpls.2020.00645*

Galicia LN, Rosales E, A Palacios Rojas N (2009) Laboratory protocols 2008: Maize nutrition quality and plant tissue analysis laboratory. Cimmyt.

Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA (1996). Plant hormones and plant growth regulators in plant tissue culture. In vitro Cellular & Developmental Biology-Plant 32(4):272-289. *https://doi.org/10.3389/fpls.2020.00645*

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. John Wiley & Sons

Gopalakrishnan L, Doriya K, Kumar DS (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. Food Science and Human Wellness 5(2):49-56. *https://doi.org/10.1016/j.fshw.2016.04.001*

Grzegorczyk-Karolak I, Hnatuszko-Konka K, Krzemińska M, Olszewska MA, Owczarek A (2021). Cytokinin-based tissue cultures for stable medicinal plant production: regeneration and phytochemical profiling of *Salvia bulleyana* shoots. Biomolecules 11(10):1513. *https://doi.org/10.3390/biom11101513*

Guo B, Abbasi BH, Zeb A, Xu L, Wei Y (2011). Thidiazuron: a multi-dimensional plant growth regulator. African Journal of Biotechnology 10(45):8984-9000. *https://doi.org/10.5897/AJB11.636*

Gupta S, Kachhwaha S, Kothari SL, Jain R (2020). Synergistic effect of cytokinins and auxins enables mass clonal multiplication of drumstick tree (*Moringa oleifera* Lam.): a wonder. In Vitro Cellular & Developmental Biology-Plant 56(4):458-469. *https://doi.org/10.1007/s11627-020-10065-0*

Hisano H, Matsuura T, Mori IC, Yamane M, Sato K (2016). Endogenous hormone levels affect the regeneration ability of callus derived from different organs in barley. Plant Physiology and Biochemistry 99:66-72. *https://doi.org/10.1016/j.plaphy.2015.12.005*

Hönig M, Plíhalová L, Husičková A, Nisler J, Doležal K (2018). Role of cytokinins in senescence, antioxidant defence and photosynthesis. International Journal of Molecular Sciences 19(12):4045. *https://doi.org/10.3390/ijms19124045*

Huang X, Hou L, Meng J, You H, Li Z, Gong Z, Yang S, Shi Y (2018). The antagonistic action of abscisic acid and cytokinin signaling mediates drought stress response in *Arabidopsis*. Molecular Plant 11(7):970-982. *https://doi.org/10.1016/j.molp.2018.05.001*

Joo JH, Bae YS, Lee JS (2001). Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiology 126(3):1055-1060. *https://doi.org/10.1016/j.molp.2018.05.001*

Kapoor S, Raghuvanshi R, Bhardwaj P, Sood H, Saxena S, Chaurasia OP (2018). Influence of light quality on growth, secondary metabolites production and antioxidant activity in callus culture of *Rhodiola imbricata* Edgew. Journal of Photochemistry and Photobiology B: Biology 183:258-265. *https://doi.org/10.1016/j.jphotobiol.2018.04.018*

Kar M, Mishra D (1976). Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiology 57(2):315-319. *https://doi.org/10.1104/pp.57.2.315*

Kawano T (2003). Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. Plant Cell Reports 21(9):829-837. *https://doi.org/10.1007/s00299-003-0591-z*

Khan T, Khan MA, Karam K, Ullah N, Mashwani Z-u-R, Nadhman A (2021). Plant *in vitro* culture technologies; a promise into factories of secondary metabolites against COVID-19. Frontiers in Plant Science 12:356. *https://doi.org/10.1007/s00299-003-0591-z*

Kim K, Kook H, Jang Y, Lee W, Kamala-Kannan S, Chae J, Lee K (2013). The effect of blue-light-emitting diodes on antioxidant properties and resistance to *Botrytis cinerea* in tomato. Journal of Plant Pathology and Microbiology 4(9).

Knegt E, Bruinsma J (1973). A rapid, sensitive and accurate determination of indolyl-3-acetic acid. Phytochemistry 12(4):753-756. *https://doi.org/10.1007/s00299-003-0591-z*

Kousalya L, Bai VN (2016). Effect of growth regulators on rapid micropropagation and antioxidant activity of *Canscora decussata* (Roxb.) Roem. & Schult.–A threatened medicinal plant. Asian Pacific Journal of Reproduction 5(2):161-170. *https://doi.org/10.1007/s00299-003-0591-z*

Lappas CM (2015). The plant hormone zeatin riboside inhibits T lymphocyte activity via adenosine A2A receptor activation. Cellular & Molecular Immunology 12(1):107-112.

Lee B, Martin P, Bangerth F (1989). The effect of sucrose on the levels of abscisic acid, indoleacetic acid and zeatin/zeatin riboside in wheat ears growing in liquid culture. Physiologia Plantarum 77(1):73-80. *https://doi.org/10.1007/s00299-003-0591-z*

Lee C, Lee Y, Kuo H-C, Tsai M, Lu T, Wang S (2008). High brightness GaN-based flip-chip light-emitting diodes by adopting geometric sapphire shaping structure. Semiconductor Science and Technology 23(2):025015. *https://doi.org/10.1088/0268-1242/23/2/025015*

Lemma DT, Banjaw DT, Megersa HG (2020). Micropropagation of medicinal plants. International Journal of Plant Breeding and Crop Science 7:796-802.

Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: An overview. International Journal of Molecular Sciences 16(6):12791-12835. *https://doi.org/10.3390/ijms160612791*

Leshem B, WERKER E, Shalev D (1988). The effect of cytokinins on vitrification in melon and carnation. Annals of Botany 62(3):271-276. *https://doi.org/10.1093/oxfordjournals.aob.a087658*

Li S, Xue L, Xu S, Feng H, An L (2007). Hydrogen peroxide involvement in formation and development of adventitious roots in cucumber. Plant Growth Regulation 52(2):173-180. *https://doi.org/10.1007/s10725-007-9188-9*

Lin M, Zhang J, Chen X (2018). Bioactive flavonoids in *Moringa oleifera* and their health-promoting properties. Journal of Functional Foods 47:469-479. *https://doi.org/10.1016/j.jff.2018.06.011*

Mahood HE, Alwash BM, Ibrahim KM (2018). Improvement of alkaloids yield using phenylalanine as a precursor supplemented to *Moringa oleifera* L. callus cultures. Biochemical and Cellular Archives 18(Suppl. 1):913-919.

Maksoud MA, Bekhit M, El-Sherif DM, Sofy AR, Sofy MR (2022). Gamma radiation-induced synthesis of a novel chitosan/silver/Mn-Mg ferrite nanocomposite and its impact on cadmium accumulation and translocation in brassica plant growth. International Journal of Biological Macromolecules 194:306-316. *https://doi.org/10.1016/j.jff.2018.06.011*

Marrazzo P, O'Leary C (2020). Repositioning natural antioxidants for therapeutic applications in tissue engineering. Bioengineering 7(3):104. *https://doi.org/10.1016/j.jff.2018.06.011*

Mathur M, Yadav S, Katariya PK, Kamal R (2014). *In vitro* propagation and biosynthesis of steroidal sapogenins from various morphogenetic stages of *Moringa oleifera* Lam., and their antioxidant potential. Acta Physiologiae Plantarum 36(7):1749-1762. *https://doi.org/10.1016/j.jff.2018.06.011*

Merzlyak MN, Chivkunova OB, Solovchenko AE, Naqvi KR (2008). Light absorption by anthocyanins in juvenile, stressed, and senescing leaves. Journal of Experimental Botany 59(14):3903-3911. *https://doi.org/10.1093/jxb/ern230*

Mohamed HI, Abd-Elsalam KA, Tmam AM, Sofy MR (2021) Silver-based nanomaterials for plant diseases management: Today and future perspectives. Silver Nanomaterials for Agri-Food Applications. Elsevier, pp 495-526. *https://doi.org/10.1016/B978-0-12-823528-7.00031-7*

Moraes RM, Cerdeira AL, Lourenço MV (2021). Using micropropagation to develop medicinal plants into crops. Molecules 26(6):1752. *https://doi.org/10.3390/molecules26061752*

Mourad AM, Amin AE-EAZ, Dawood MF (2021). Genetic variation in kernel traits under lead and tin stresses in spring wheat diverse collection. Environmental and Experimental Botany 192:104646. *https://doi.org/10.1016/j.envexpbot.2021.104646*

Mowafy A, Agha M, Haroun S, Abbas M, Elbalkini M (2022). Insights in nodule-inhabiting plant growth promoting bacteria and their ability to stimulate *Vicia faba* growth. Egyptian Journal of Basic and Applied Sciences 9(1):51-64. *https://doi.org/10.1080/2314808X.2021.2019418*

Mukherjee S, Choudhuri M (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiologia plantarum 58(2):166-170. *https://doi.org/10.1080/2314808X.2021.2019418*

Muslihatin W, Jadid N, Saputro T, Purwani K, Himayani C, Calandry A Characteristic of synthetic seeds from two medicinal plants (*Moringa oleifera* and *Camellia sinensis*). In: Journal of Physics: Conference Series 1040:012005. *https://doi.org/10.1080/2314808X.2021.2019418*

Naik SK, Chand PK (2006). Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. Scientia Horticulturae 108(3):247-252. *https://doi.org/10.1080/2314808X.2021.2019418*

Neill SO, Gould KS (2003). Anthocyanins in leaves: light attenuators or antioxidants? Functional Plant Biology 30(8):865-873. *https://doi.org/10.1080/2314808X.2021.2019418*

North JJ, Ndakidemi PA, Laubscher CP (2012). Effects of antioxidants, plant growth regulators and wounding on phenolic compound excretion during micropropagation of *Strelitzia reginae*. International Journal of the Physical Sciences. *https://doi.org/10.5897/IJPS11.786*

Nouman W, Siddiqui MT, Basra S, Ahmed M (2012). Moringa oleifera leaf extract: An innovative priming tool for rangeland grasses. Turkish Journal of Agriculture and Forestry 36(1):65-75. *https://doi.org/10.5897/IJPS11.786*

Ogita S, Miyazaki J, Godo T, Kato Y (2009). Possibility for selective accumulation of polyphenolics in tissue cultures of senno (*Lychnis senno* Siebold et Zucc.). Natural Product Communications 4(3):1934578X0900400312. *https://doi.org/10.5897/IJPS11.786*

Olle M, Viršile A (2013). The effects of light-emitting diode lighting on greenhouse plant growth and quality. Agricultural and Food Science 22(2):223-234. *https://doi.org/10.23986/afsci.7897*

Pham TL, Nguyen VH, Hoang TLT, Ha TTT, Tran TK, Vu XD, Cao PB, Nguyen QT (2020). Micropropagation from root segments to improve seedling quality in Chinese foxglove crops. Journal of Plant Biotechnology 47(3):235-241. *https://doi.org/10.23986/afsci.7897*

Phua QY, Subramaniam S, Lim V, Chew BL (2018). The establishment of cell suspension culture of sabah snake grass (*Clinacanthus nutans* (Burm. F.) Lindau). In Vitro Cellular & Developmental Biology-Plant 54(4):413-422. *https://doi.org/10.1007/s11627-018-9885-2*

Ragaey MM, Sadak MS, Dawood MF, Mousa NH, Hanafy RS, Latef AAHA (2022). Role of signaling molecules sodium nitroprusside and arginine in alleviating salt-induced oxidative stress in wheat. Plants 11(14):1786. *https://doi.org/10.3390/plants11141786*

Ramírez-Mosqueda M, Cruz-Cruz C, Atlahua-Temoxtle J, Bello-Bello J (2019). *In vitro* conservation and regeneration of *Laelia anceps* Lindl. South African Journal of Botany 121:219-223. *https://doi.org/10.1016/j.sajb.2018.11.010*

Ridzuan N, Abdullah N, Vun Y, Supramaniam C (2020). Micropropagation and defence enzymes assessment of *Moringa oleifera* L. plantlets using nodal segments as explant. South African Journal of Botany 129:56-61. *https://doi.org/10.1016/j.sajb.2018.12.010*

Saeedpour A, Godehkahriz SJ, Lohrasebi T, Esfahani K, Salmanian AH, Razavi K (2021). The effect of endogenous hormones, total antioxidant and total phenol changes on regeneration of barley cultivars. Iranian Journal of Biotechnology 19(1):e2838.

Safi-naz S, Rady MM (2015). Moringa oleifera leaf extract improves growth, physio-chemical attributes, antioxidant defence system and yields of salt-stressed *Phaseolus vulgaris* L. plants. International Journal of ChemTech Research 8(11):120-134.

Saini R, Saad K, Ravishankar G, Giridhar P, Shetty N (2013). Genetic diversity of commercially grown *Moringa oleifera* Lam. cultivars from India by RAPD, ISSR and cytochrome P450-based markers. Plant Systematics and Evolution 299(7):1205-1213. *https://doi.org/10.1016/j.sajb.2018.12.010*

Saini R, Shetty N, Giridhar P, Ravishankar G (2012). Rapid in vitro regeneration method for *Moringa oleifera* and performance evaluation of field grown nutritionally enriched tissue cultured plants. 3 Biotech 2(3):187-192. *https://doi.org/10.1016/j.sajb.2018.12.010*

Sang YL, Cheng ZJ, Zhang XS (2018). Plant stem cells and de novo organogenesis. New Phytologist 218(4):1334-1339. *https://doi.org/10.1111/nph.15106*

Sheteiwy MS, Ulhassan Z, Qi W, Lu H, AbdElgawad H, Minkina T, ... Dawood M (2022). Association of jasmonic acid priming with multiple defense mechanisms in wheat plants under high salt stress. Frontiers in Plant Science 13: 886862. *https://doi.org/10.3389/fpls.2022.886862*

Sofy M, Mohamed H, Dawood M, Abu-Elsaoud A, Soliman M (2021). Integrated usage of *Trichoderma harzianum* and biochar to ameliorate salt stress on spinach plants. Archives of Agronomy and Soil Science 7:1-22. *https://doi.org/10.1080/03650340.2021.1949709*

Steinitz B, Tabib Y, Gaba V, Gefen T, Vaknin Y (2009). Vegetative micro-cloning to sustain biodiversity of threatened *Moringa* species. In Vitro Cellular & Developmental Biology-Plant 45(1):65-71. *https://doi.org/10.1111/nph.15106*

Stephenson KK, Fahey JW (2004). Development of tissue culture methods for the rescue and propagation of endangered *Moringa* spp. germplasm. Economic Botany 58(1):S116-S124. *https://doi.org/10.1663/0013-0001(2004)58[S116:DOTCMF]2.0.CO;2*

Tibbitts T, Morgan D, Warrington I (1983). Growth of lettuce, spinach, mustard, and wheat plants under four combinations of high-pressure sodium, metal halide, and tungsten halogen lamps at equal PPFD. Journal of American Society of Horticultural Sciences 108(4):622-630. *https://doi.org/10.21273/JASHS.108.4.622*

Torrizo L, Zapata F (1986). Anther culture in rice: IV. The effect of abscisic acid on plant regeneration. Plant Cell Reports 5(2):136-139. *https://doi.org/10.1007/BF00269253*

Touaibia M, Chaouch F (2017). Propriétés antioxydantes et antimicrobiennes des extraits de *Myrtus nivellei* Batt et Trab. obtenus in situ et *in vitro*. Phytothérapie 15(1):16-22. *https://doi.org/10.1007/s10298-015-1011-6*

Traore A, Maximova SN, Guiltinan MJ (2003). Micropropagation of *Theobroma cacao* L. using somatic embryo-derived plants. In Vitro Cellular & Developmental Biology-Plant 39(3):332-337. *https://doi.org/10.1079/IVP2002409*

Vats S, Gupta T (2017). Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. from Rajasthan, India. Physiology and molecular biology of plants 23(1):239-248. *https://doi.org/10.1007/s12298-016-0407-6*

Vendrame WA, Xu J, Beleski D (2022). Evaluation of the effects of culture media and light sources on *in vitro* growth of *Brassavola nodosa* (L.) Lindl. hybrid. Horticulturae 8(5):450. *https://doi.org/10.3390/horticulturae8050450*

Vera-Sirera F, Gomez MD, Perez-Amador MA (2016) DELLA proteins, a group of GRAS transcription regulators that mediate gibberellin signaling Plant transcription factors. Elsevier, pp 313-328. *https://doi.org/10.1016/B978-0-12-800854-6.00020-8*

Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. Industrial Crops and Products 44:566-571. *https://doi.org/10.1016/B978-0-12-800854-6.00020-8*

Wang S, Yang C, Tu H, Zhou J, Liu X, Cheng Y, Luo J, Deng X, Zhang H, Xu J (2017). Characterization and metabolic diversity of flavonoids in citrus species. Scientific Reports 7(1):1-10. *https://doi.org/10.1016/B978-0-12-800854-6.00020-8*

Weiss D, Ori N (2007). Mechanisms of cross talk between gibberellin and other hormones. Plant Physiology 144(3):1240-1246. *https://doi.org/10.1104/pp.107.100370*

Younes NA, Rahman M, Wardany AA, Dawood MF, Mostofa MG, Keya SS, Abdel Latef AAH, Tran L-SP (2021). Antioxidants and bioactive compounds in licorice root extract potentially contribute to improving growth, bulb quality and yield of onion (*Allium cepa*). Molecules 26(9):2633. *https://doi.org/10.3390/molecules26092633*

Yu L, Song CM, Sun LJ, Li LL, Xu ZG, Tang CM (2020). Effects of light-emitting diodes on tissue culture plantlets and seedlings of rice (*Oryza sativa* L.). Journal of Integrative Agriculture 19(7):1743-1754. *https://doi.org/10.1016/S2095-3119(19)62793-0*

Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64(4):555-559. *https://doi.org/10.1016/S0308-8146(98)00102-2*

Zimmerman TW, Scorza R (1992). Shoot growth and proliferation of peach under varying environmental regimes. HortScience 27(6):696a-696. *https://doi.org/10.21273/HORTSCI.27.6.696a*

Zullaikah S, Naulina R, Meinawati P, Fauziyah K, Rachimoellah M, Rachmaniah O, ... Prasetyo E (2019). Enhanced Extraction of phenolic compounds from *Moringa oleifera* leaves using subcritical water ethanol mixture. In: IOP Conference Series: Materials Science and Engineering. 543:012021. *https://doi.org/10.1088/1757-899X/543/1/012021*