

Blue light and ethylene modulate the quality of *Moringa oleifera* seedlings under abiotic stresses

Luz azul e etileno modulam a qualidade de mudas de *Moringa oleifera* sob estresses abióticos

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ABSTRACT - *Moringa oleifera* Lam. is a strategic crop in the Brazilian semi-arid region due to its moderate tolerance to abiotic stresses and nutritional value. Therefore, the objective was to evaluate the effect of seed priming with ethylene under monochromatic blue light on mitigating water and heat stress in *M. oleifera* seedlings. The experiment was conducted in a completely randomized design, in a 2 x 2 x 3 factorial scheme, with two levels of water replacement (50% and 100%), two temperatures (30/25 °C and 40/35 °C day/night), and three seed priming treatments (NP - non-primed; PB - blue light; and PBE - ethylene + blue light). Isolated heat stress (40/35 °C) reduced growth but increased chlorophyll and carotenoid content. Under water stress (30 °C), the PBE treatment resulted in seedlings 6.55% taller than the control. Under combined stress conditions (50% and 40/35 °C), PBE priming resulted in higher levels of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, with increases of 26.78%, 21.72%, 24.56%, and 14.13%, respectively, compared to non-primed seedlings. Furthermore, seedlings subjected to PBE showed the highest Dickson Quality Index (1.98) under combined stress, surpassing those under PB. Seed priming with monochromatic blue light combined with ethylene (PBE) is an efficient strategy to mitigate the deleterious effects of combined water and heat stress, favoring the photosynthetic apparatus and the quality of *M. oleifera* seedlings.

RESUMO - A *Moringa oleifera* Lam. é uma cultura estratégica para o semiárido brasileiro devido à sua tolerância moderada a estresses abióticos e à sua importância nutricional. Portanto, objetivou-se avaliar o efeito do condicionamento de sementes com etileno sob luz monocromática azul na mitigação dos estresses hídrico e térmico em mudas de *Moringa oleifera*. O experimento foi conduzido em delineamento inteiramente casualizado, em esquema fatorial 2 x 2 x 3, com dois níveis de reposição hídrica (50% e 100%), duas temperaturas (30/25 °C e 40/35 °C dia/noite) e três condicionamentos de sementes (NP - não condicionadas; PB - luz azul; e PBE - etileno + luz azul). O estresse térmico isolado (40/35 °C) reduziu o crescimento, mas aumentou os teores de clorofilas e de carotenoides. Sob estresse hídrico (30 °C), o tratamento PBE proporcionou mudas 6,55% maiores do que as do controle. Nas condições de estresse combinado (50% ETc e 40/35 °C), o condicionamento PBE resultou em maiores teores de clorofila *a*, *b*, total e de carotenoides, com incrementos de 26,78%, 21,72%, 24,56% e 14,13%, respectivamente, em relação às mudas não condicionadas. Além disso, o PBE apresentou o maior Índice de Qualidade de Dickson (1,98) sob estresse combinado, superando o do PB. O condicionamento de sementes com luz monocromática azul associada ao etileno (PBE) é uma estratégia eficiente para mitigar os efeitos deletérios dos estresses hídrico e térmico combinados, favorecendo o aparato fotossintético e a qualidade das mudas de *M. oleifera*.

Keywords: Water Stress. Irradiation. Chloroplast pigments.

Palavras-chave: Estresse hídrico. Irradiação. Pigmentos cloroplásticos.

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INTRODUCTION

Global warming, coupled with population growth, intensifies climate change and affects livelihood systems, causing imbalances and vulnerabilities in agroecosystems worldwide (ELSHAMLY; ABAZA, 2024). This factor poses significant challenges to agricultural production, particularly in semi-arid regions, where abiotic stresses, especially water and thermal stresses, limit plant growth and development (MELO et al., 2024). In this context, there is a need for studies on crops that exhibit functionality, adaptability, and tolerance to drought and high temperatures, aiming to increase agricultural production (BONOU et al., 2025).

Among the promising species in the region, *Moringa oleifera* Lam. stands out as a plant widely cultivated for human and animal nutrition (DIVYA et al., 2024) and used as a raw material in the pharmaceutical industry (PAREEK et al., 2023). Although *M. oleifera* exhibits edaphoclimatic adaptability in semi-arid environments, its initial establishment and seedling development stages are sensitive to water restrictions and heat stress, which can severely compromise plant survival and early growth in the Brazilian semi-arid region (HAJAJI et al., 2024).

In *M. oleifera* seedlings, prolonged exposure to heat and drought alters

metabolic balance, inducing photo-oxidative stress, which directly affects chloroplast stability and biomass allocation (MASHAMAITE; RAMATSITSI; MANYEVERE, 2024). This stress condition triggers the degradation of photosynthetic pigments (chlorophylls and carotenoids) and impairs leaf expansion, compromising light interception and energy conversion. Consequently, phytomass accumulation in shoot and root tissues is severely reduced, altering growth kinetics and key morphophysiological indices, such as leaf area duration and the Dickson Quality Index (BONOU et al., 2025).

Therefore, the search for efficient strategies to mitigate the adverse effects of abiotic stress is essential. Among the promising alternatives to mitigate the adverse effects of drought and high temperature on plants is the association between blue light and ethylene in seed priming (COSTA et al., 2022). Among the promising alternatives for mitigating abiotic stresses, seed priming combined with blue light and ethylene emerges as an innovative strategy (COSTA et al., 2022). Rather than relying on direct foliar irradiation, seed priming with monochromatic blue light activates photoreception via cryptochromes during imbibition, while exogenous ethylene accelerates the seed's metabolic reactivation, stimulates reserve mobilization, and modulates early stress-signaling networks. Acting synergistically during imbibition, blue light and ethylene establish a persistent priming memory within embryonic tissues. This memory induces the sustained activation of antioxidant enzymes, preserves the integrity of chloroplast membranes, and optimizes osmotic regulation, thereby protecting seedlings against heat- and drought-induced oxidative stress during early growth (CHIBANI; GHERLI; FAN, 2025; ZHANG et al., 2025).

Despite growing scientific interest in *M. oleifera*, the synergistic potential of blue light and ethylene in seed priming remains unexplored, particularly regarding the physiological memory of seedlings under multiple stresses. It is hypothesized that seed priming under monochromatic blue irradiation, combined with ethylene, promotes morphological adjustments and increased chloroplast pigment supply, resulting in higher-quality seedlings with greater biomass accumulation, even under conditions of water deficit and heat stress.

In this context, the objective was to evaluate the effect of priming seeds with ethylene under monochromatic blue light on mitigating water and heat stress in *M. oleifera* seedlings.

MATERIAL AND METHODS

Seed priming was carried out at the Plant Physiology Laboratory (LFV) of the Academic Unit of Agricultural Engineering (UAEA), part of the Center for Technology and Natural Resources (CTRN) of the Federal University of Campina Grande (UFCG), located in Campina Grande, Paraíba, Brazil (7° 12' 51.9" S, 35° 54' 24.1" W; 551 m altitude). The local climate is classified as equatorial semi-arid (AS) tropical, with a dry season, an average temperature of 25 °C, and relative humidity ranging from 72% to 91% (ALVARES et al., 2013).

The experiment was conducted in a completely

randomized design, in a 2 × 2 × 3 factorial scheme. The first factor consisted of two levels of soil water replenishment, based on crop evapotranspiration (ET_c): 50% and 100%. The second factor included two thermal conditions: one with thermal stress (40 °C daytime / 35 °C nighttime) and another without thermal stress (30 °C daytime / 25 °C nighttime). The third factor comprised three seed treatments: a control (NP – non-primed seeds), seed priming with light (PB – priming in distilled water under monochromatic blue light irradiation [380–500 nm]), and seed priming with light and ethylene (PBE – priming in an ethylene solution [500 mg L⁻¹ of Ethrel, i.e., ethephon (2-chloroethylphosphonic acid)] under monochromatic blue light irradiation). Each treatment was replicated 5 times, with one plant per plot, for a total of 60 experimental units.

Seeds of *M. oleifera* were collected from mother plants cultivated by a rural producer in the municipality of Catolé do Rocha, Paraíba, Brazil (6° 20' 38" S, 37° 44' 49" W; 272 m altitude). Initially, the seeds were subjected to surface sterilization with 1% sodium hypochlorite for 3 minutes (CARVALHO; CARVALHO, 2009), carried out under green light (500–565 nm), which is considered "safe light" because it does not influence phytochrome activity (BONOU et al., 2025), thus avoiding the interference of monochromatic blue light in the priming effects.

Monochromatic blue light was installed in a germination chamber with biochemical oxygen demand (BOD) (model MA 402, Marconi, Brazil). For this purpose, LED strips emitting white light (205 lumens m²) were installed. To obtain the blue light, Gerbox-type plastic boxes were lined with four layers of blue cellophane, as described by Costa et al. (2022).

The ethylene solution (500 mg L⁻¹ of Ethrel) was prepared by diluting 2-chloroethylphosphonic acid (C₂H₄ClO₃P), the active ingredient in commercial Ethrel (24% a.i., molar mass 144.5 g mol⁻¹, density 1.41 g cm³), in deionized water (VASCONCELOS et al., 2015). The conditioning temperature of 25 °C was maintained using thermostatic programming of the BOD chambers, following the procedures of Tesfay, Modi, and Mohammed (2016), with 100% relative humidity and a 24-hour photoperiod.

After surface sterilization, 100 seeds were placed in transparent acrylic plastic boxes (11 × 11 × 3.5 cm; length × width × height). The substrate consisted of two sheets of moistened germination paper towel, each containing the respective treatment solution (control or ethylene), with a volume equivalent to approximately 2.5 times the dry weight of the substrate (FERREIRA et al., 2017).

The boxes containing the seeds were placed in climate-controlled germination chambers equipped with LED panels to ensure monochromatic blue light. The priming period was set at 24 hours, sufficient time for the seeds to soak without inducing germination (BONOU et al., 2025). After this period, the seeds were transferred to lidless transparent acrylic plastic boxes containing two dry sheets of germination paper towel and dried under the same temperature and light conditions used during priming.

Primed seeds were sown in 300 cm³ polyethylene pots filled with a substrate composed of sandy soil and humus in a 3:1 (w/w) ratio (Table 1), with moisture adjusted to field capacity (100%).

Table 1. Chemical analysis of the substrate used in the production of *Moringa oleifera* seedlings.

pH	OM	P	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺	H ⁺	SB	CEC	V	ESP
H ₂ O	g kg ⁻¹	cmol _c dm ⁻³									%	
7.6	32.3	1.91	2.49	0.70	5.8	2.1	0.0	0.74	11.09	11.3	98.1	6.2

OM: Organic matter content by the Walkley-Black method; P, K⁺, Na⁺: extracted by Mehlich 1; Ca²⁺ and Mg²⁺: extracted by 1 M KCl; SB: Sum of exchangeable bases; CEC: Cation exchange capacity; V: Base saturation; ESP: Exchangeable sodium percentage.

The pots were then transferred to a growth chamber (Fitotron, Weissttechnik® SGC 120), located at the Experimental Station of the State University of Paraíba (UEPB), in Campina Grande, Paraíba, Brazil (07° 12' 42.99" S, 35° 54' 36.27" W; 521 m altitude).

Environmental conditions inside the growth chamber (Fitotron, Weissttechnik® SGC 120) were strictly controlled and continuously monitored throughout the experimental period. To simulate heat stress conditions, the chamber was programmed with daytime/nighttime temperatures of 40 °C/35 °C, relative air humidity of 40% during the day and 50% at night, a 12-hour photoperiod, and an ambient CO₂ concentration of 400 ppm. Due to the availability of only one Fitotron chamber, the experiment under thermally stress-free conditions (30 °C daytime / 25 °C nighttime; relative humidity of 50% during the day and 60% at night) was carried out in a subsequent experimental cycle.

Substrate moisture was controlled through daily irrigation using the gravimetric method adapted from Silva et al. (2020). Initially, the dry mass of each polyethylene pot filled with substrate (VS, kg) was recorded. To allow drainage, a smaller 80 cm³ pot was attached to the bottom of each main pot. The substrate was saturated with water to an initial water volume (IV, L) equal to 75% (v/w) of its dry mass. The pots were then placed in polyethylene trays, covered with black plastic, and stored in a dark, climate-controlled environment at 20 °C to prevent evaporation. After 24 h, the volume of water drained (VWD, L) was quantified, and the pot mass at field capacity (MFC, kg) was recorded. Daily irrigation was performed at 09:00 h, with each pot weighed on a digital balance (0.01 g precision). Daily crop evapotranspiration (ET_c) was determined by the difference between MFC and the current pot mass before irrigation (MC). For the 100% ET_c treatment (control), water was added to restore the pot mass to MFC. For the water-deficit treatment (50% ET_c), initiated 18 days after sowing (DAS), only 50% of the daily ET_c volume was replaced ($V = 0.5 \times (MFC - MC)$).

At 35 DAS (days after sowing), corresponding to 17 days of exposure to water restriction and heat stress treatments, sampling was performed. This evaluation date was selected because *M. oleifera* seedlings had reached the commercial standard and optimal physiological vigor for field transplanting, enabling a precise assessment of seed-priming effects at the end of the nursery production stage.

After 35 days (DAS), morphometric evaluations were performed at 9-day intervals to measure the growth of *M. oleifera* seedlings. Plant height (PH, in cm), stem diameter (SD, in mm), and number of leaflets (NL) were measured. From these data, the relative growth rate (RGR) was obtained using the relationship described by Benincasa (2003), through Equation 1:

$$RGR = \frac{\ln M_2 - \ln M_1}{T_2 - T_1} \quad (1)$$

where:

RGR - relative growth rate, cm cm⁻¹ day⁻¹;

Ln - natural logarithm of the height measurement at time 1 (T₁) and of the measurements taken at 9-day intervals until the last evaluation (T₂).

After performing the morphometric measurements, the length (L) and width (W) of the leaflets were measured and used in the model proposed by Macário et al. (2020) to estimate the leaf area (LA) using Equation 2.

$$LA = 0.035 + 0.720 \cdot LW \quad (2)$$

Where:

LA - leaf area, cm²;

L - length, cm; and

W - width, cm

The leaf area ratio (LAR), also known as the leaf area quotient, was obtained using Equation 3, defined as the ratio of leaf area to total plant dry mass.

$$LAR = \frac{LA - 35}{TDM} \quad (3)$$

Where:

LAR - leaf area ratio, cm² g⁻¹;

LA - leaf area, cm²; and

TDM - total dry mass, g⁻¹

The ratio between leaf dry mass and the total dry mass accumulated in the plant was obtained using Equation 4:

$$LMR = \frac{LDM}{TDM} \quad (4)$$

Where:

LMR - leaf mass ratio, g g⁻¹;

LDM - leaf dry mass, g⁻¹; and

TDM - total plant dry mass, g⁻¹

Leaf area duration (LAD) was determined by Equation 5:

$$LAD = \frac{(LAI_1 + LAI_2) \cdot (t_2 - t_1)}{2} \quad (5)$$

Where:

LAD - leaf area duration, cm² day⁻¹;

LAI₁ and LAI₂ - minimum and maximum leaf area index,

respectively, cm²; and

t₁ and t₂ - times, in days, when the LAI is at its maximum and minimum values.

Subsequently, the plants were sectioned into leaves, stems, branches, and roots. The plant material was placed in correctly labeled paper bags and taken to a forced-air circulation oven at 65 °C until it reached a constant mass. Afterward, these plant parts were weighed on an analytical balance to determine root dry mass (RDM, g), shoot dry mass (SDM, g), and total dry mass (TDM, g), obtained by summing the other parts, were determined. Shoot length (SL, cm) and stem diameter (SD, mm) were also measured. From these values, the Dickson Quality Index (DQI) was determined using a balanced formula that includes the relationships of the morphological variables: TDM, SDM, RDM, SL, and SD (DICKSON; LEAF; HOSNER, 1960), described below in Equation 6:

$$DQI = \frac{TDM}{\frac{SL}{SD} + \frac{SDM}{RDM}} \quad (6)$$

where:

DQI - Dickson Quality Index

TDM - Total Dry Mass, g;

SDM - Shoot Dry Mass, g;

RDM - Root Dry Mass, g;

SL - Shoot Length, cm; and

SD - Stem Diameter, mm

At 35 DAS (days after sowing), fully expanded leaflets were identified and collected. 1.0 g of leaf tissue was macerated in 7.0 mL of acetone (80%), and the volume was made up to 25 mL. The absorbances of the extract were determined using a spectrophotometer (Nova 2000 UV) at

wavelengths of 663, 646, and 470 nm for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total carotenoids (Car *t*) determined by Equations 7, 8 and 9 respectively, while total chlorophyll (Chl *t*) was obtained by the sum of Chl *a* + Chl *b*. The pigment contents were obtained using the equations according to Lichtenthaler and Buschmann (2001), as follows:

$$\text{Chl } a \text{ (}\mu\text{g g}^{-1}\text{)} = 12.25 * A_{663} - 2.79 * A_{646} \quad (7)$$

$$\text{Chl } b \text{ (}\mu\text{g g}^{-1}\text{)} = 21.5 * A_{646} - 5.10 * A_{663} \quad (8)$$

$$\text{Car } t \text{ (}\mu\text{g g}^{-1}\text{)} = \frac{[100 * A_{470} - (1.82 * \text{Chl } a - 85.02 * \text{Chl } b)]}{198} \quad (9)$$

To convert the values in $\mu\text{g mL}^{-1}$ to $\mu\text{g g}^{-1}$, the results were multiplied by the flask volume (25 mL) and divided by the sample weight (1.0 g).

The data obtained were subjected to the Shapiro-Wilk normality test (SHAPIRO; WILK, 1965). Once the normality assumptions were met, the data were analyzed using analysis of variance (ANOVA) with the F test at a significance level of 5%. The means were compared by Tukey's test ($p \leq 0.05$) in the SISVAR 6.5 software (FERREIRA, 2019).

RESULTS AND DISCUSSION

The triple interaction between seed priming and the two water and heat stress conditions studied significantly influenced plant height (PH) ($P \leq 0.01$), number of leaflets (NL) ($P \leq 0.01$), leaf dry mass (LDM) ($P \leq 0.01$), stem dry mass (SDM) ($P \leq 0.01$), root dry mass (RDM) ($P \leq 0.01$), leaf dry mass ratio (LDMR) ($P \leq 0.01$), and Dickson quality index (DQI) ($P \leq 0.01$) (Table 2).

Table 2. Summary of analysis of variance for plant height (PH), number of leaflets (NL), leaf dry mass (LDM), stem dry mass (SDM), leaf dry mass ratio (LDMR), root dry mass (RDM), and Dickson Quality Index (DQI) in *M. oleifera* seedlings derived from seeds primed in water under blue light irradiation (PB) and in an ethylene solution (500 mg L⁻¹ Ethrel) under blue light irradiation (PBE) under water and thermal stress conditions (WWS 40 °C) at 35 days after sowing.

SV	DF	Mean squares						
		PH	NL	LDM	SDM	RDM	LDMR	DQI
SP	2	1.99 ^{ns}	242.61 ^{**}	0.0009 [*]	0.0002 ^{ns}	0.00006 ^{ns}	0.004 ^{**}	0.89 ^{**}
WS	1	896.29 ^{**}	10428.02 ^{**}	0.29 ^{**}	0.309 ^{**}	0.04 ^{**}	0.009 ^{**}	5.75 ^{**}
TS	1	382.54 ^{**}	0.15 ^{ns}	0.43 ^{**}	0.33 ^{**}	0.14 ^{**}	0.007 ^{**}	1.48 ^{**}
SP x WS	2	3.36 ^{ns}	26.52 ^{ns}	0.0009 ^{**}	0.0006 ^{ns}	0.0006 ^{**}	0.003 ^{**}	0.90 ^{**}
SP x TS	2	45.21 ^{**}	126.95 ^{**}	0.0001 ^{ns}	0.009 ^{**}	0.0011 ^{**}	0.01 ^{**}	1.23 ^{**}
WS x TS	1	7.28 [*]	453.75 ^{**}	0.045 ^{**}	0.06 ^{**}	0.03 ^{**}	0.01 ^{**}	4.95 ^{**}
SP x WS x TS	2	12.34 ^{**}	161.45 ^{**}	0.0011 ^{**}	0.001 [*]	0.0027 ^{**}	0.004 ^{**}	2.06 ^{**}
Residual	48	1.43	10.86	0.00018	0.0002	0.00004	0.0002	0.021
Average		36.74	85.28	0.24	0.26	0.11	0.38	2.25
CV (%)		3.26	3.86	5.72	5.88	5.89	3.96	6.42

SV – Sources of variation; SP – Seed priming; WS – Water stress; TS – Thermal stress; DF – Degrees of freedom; CV – Coefficient of variation; ^{ns} – not significant; * – significant at $P \leq 0.05$; ** – significant at $P \leq 0.01$.

M. oleifera seedlings grown from seeds primed with an ethylene solution (500 mg L⁻¹ of Ethrel) under blue light irradiation (PBE), under water stress conditions (WWS 30 °C), showed the highest average plant height (PH) of 31.74 cm. In contrast, unprepared seedlings (NP) showed the lowest

average height, 29.66 cm, which corresponds to a 6.55% decrease compared with prepared seedlings (NP). Under combined stress conditions (WWS 40 °C), seed priming did not differ statistically (Figure 1A).

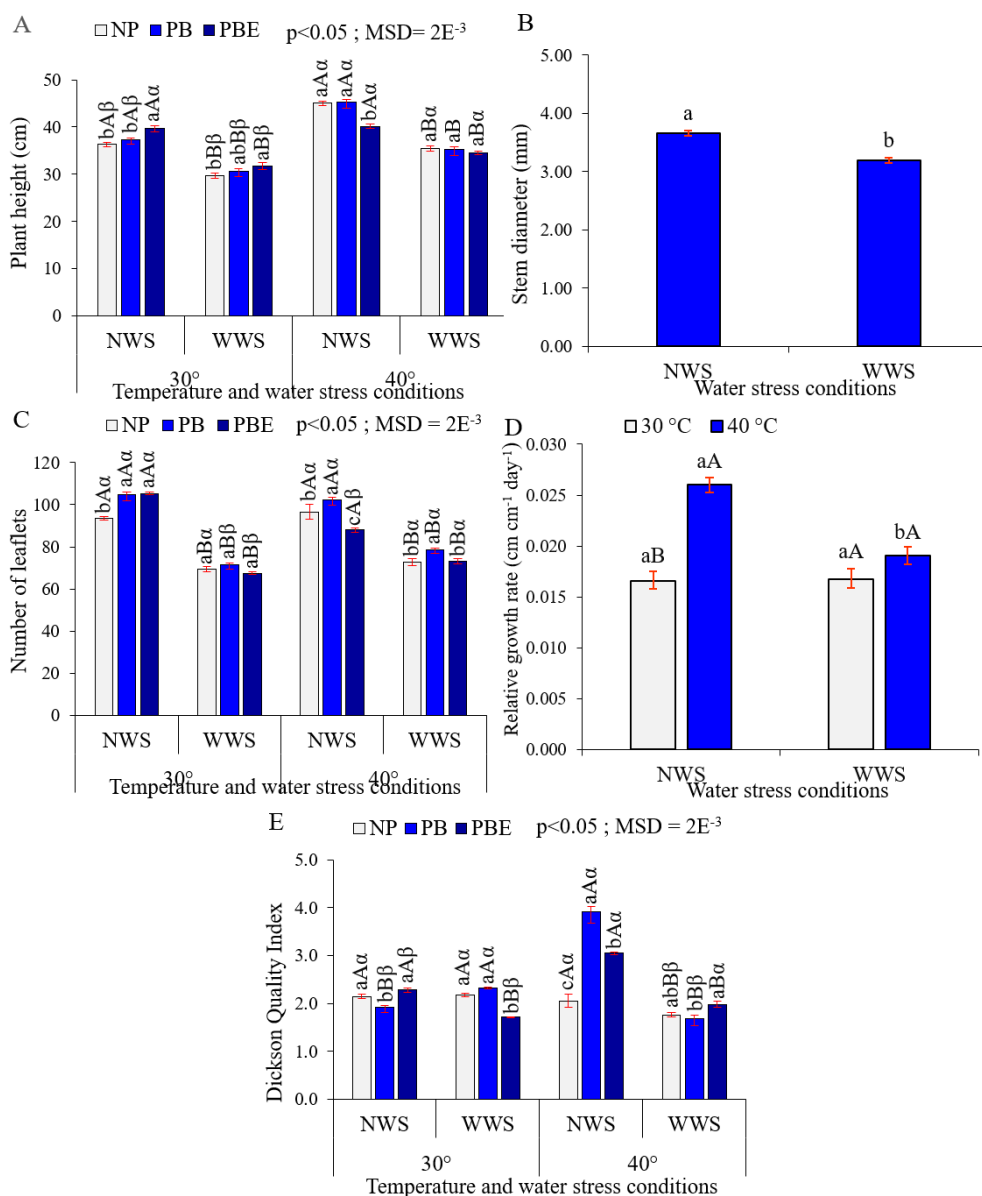


Figure 1. Plant height (PH) (A), stem diameter (SD) (B), number of leaflets (NL) (C), relative growth rate (RGR) (D), and Dickson quality index (DQI) (E) in *Moringa oleifera* seedlings grown from seeds primed with water under blue light irradiation (PB) and in ethylene solution (500 mg L⁻¹ Ethrel) under blue light irradiation (PBE) under water and heat stress conditions (WWS 40 °C) 35 days after sowing. Treatments: NP = unprimed seeds; PB = seeds primed with blue light; PBE = seeds primed with blue light and ethylene. Bars represent means ± SE (n = 5). Different letters indicate significant differences according to Tukey's test ($P \leq 0.01$): lowercase letters = differences between seed priming treatments within each stress condition; uppercase letters = differences between water stress levels within each priming treatment; Greek letters = differences between temperature levels within each combination of priming and water stress. MSD = minimum significant difference.

This 6.55% increase suggests that priming with PBE induced a 'physiological memory' in the seeds, optimizing elongation and cell division processes, even under water stress. Blue light is a known signaling molecule that regulates early development and phytohormone synthesis, while ethylene, at low concentrations, can modulate cell expansion

under adverse conditions (BONOU et al., 2025).

The largest stem diameters were obtained in seedlings under water stress conditions (3.66 cm) compared to those under normal irrigation conditions (3.19 cm) (Figure 1B). Increased stem diameter is a common mechanism in forest species under water deficit, aimed at increasing the capacity

for water and reserve compound storage in the stem parenchyma tissues and reducing resistance to sap flow in the xylem (HACKMANN et al., 2024). Therefore, *M. oleifera* demonstrated phenotypic plasticity, prioritizing stem robustness to ensure survival under conditions of reduced water replenishment.

The number of leaflets (NL) did not differ statistically between seed priming treatments under water stress (WWS 30 °C) (Figure 1C). However, under combined stress conditions (WWS 40 °C), the highest number of leaflets was observed in seedlings primed with PB (78.60), and the lowest values were found in seedlings grown from unprimed seeds - NP (72.80) and seeds primed with PBE (73.00), which were statistically equal (Figure 1C). This suggests that priming the seeds to isolated blue light may have conferred greater morphological stability to the plant, minimizing leaflet loss under extreme conditions (PECH et al., 2024).

Blue light acts on cryptochrome signaling, which influences tissue differentiation and the maintenance of photosynthetic area under thermal stress. However, the addition of ethylene (PBE) did not increase the number of leaflets under combined stress (WWS 40 °C), indicating that, in situations of simultaneous water and thermal stress, ethylene signaling may have prioritized other survival mechanisms, such as stomatal closure or osmotic adjustment, to the detriment of expanding the number of leaflets (BONOU et al., 2025).

The relative growth rate (RGR) of *M. oleifera* seedlings was higher under normal irrigation conditions and under heat stress (NWS at 40 °C), with 0.02599 cm cm⁻¹ day⁻¹, which corresponds to a 36.51% increase in RGR compared to the stress-free condition (NWS at 30 °C) (Figure 1D), reflecting the thermophilic characteristic of *M. oleifera*. This behavior demonstrates that elevated temperature, in isolation, can serve as a stimulus for the development of *M.*

oleifera, provided that the plant can maintain its transpiration rate and leaf cooling through adequate soil water availability (COSTA et al., 2022).

The Dickson quality index (DQI) is considered one of the best indicators of seedling quality, as it integrates the robustness and balance of biomass distribution (KOLLN et al., 2025). The highest DQI under water stress (WWS 30 °C) was observed in seedlings primed with PB, 2.33, statistically equal to that of unprepared seedlings (NP), 2.18, representing a 26.61% increase compared to the lowest value (Figure 1E). This indicates that even under water-stress conditions with moderate temperature (WWS 30 °C), *M. oleifera* possesses intrinsic tolerance mechanisms that maintain seedling quality, regardless of priming (COSTA et al., 2022).

Under combined stress (WWS 40 °C), the DQI was higher in seedlings from seeds primed with PBE, being equal to 1.98; however, the lowest DQI (1.69) was observed in seedlings primed with PB (Figure 1E). This result demonstrates the synergistic effect of ethylene, as a stress signaling molecule, when associated with blue light. Ethylene may have induced the synthesis of heat shock proteins (HSPs) and modulated the antioxidant system, conferring greater stability on membranes and allowing a more balanced accumulation of phytomass, even under combined stress conditions (WWS 40 °C) (COSTA et al., 2022).

The interaction between seed priming and water stress significantly influenced leaf area (LA; $P \leq 0.05$). The interaction between seed priming and heat stress significantly influenced leaf area (LA) ($P \leq 0.05$), leaf area ratio (LAR) ($P \leq 0.01$), and leaf area duration (LAD) ($P \leq 0.05$). The interaction between water stress and heat stress significantly influenced the relative growth rate (RGR; $P \leq 0.01$). Water stress, in isolation, significantly influenced stem diameter (SD) ($P \leq 0.01$) (Table 3).

Table 3. Summary of analysis of variance for leaf area (LA), leaf area ratio (LAR), leaf area duration (LAD), relative growth rate (RGR) and stem diameter (SD) in *M. oleifera* seedlings derived from seeds primed in water under blue light irradiation (PB) and in an ethylene solution (500 mg L⁻¹ Ethrel) under blue light irradiation (PBE) under water and thermal stress conditions (WWS 40 °C) at 35 days after sowing.

SV	DF	Mean squares				
		LA	LAR	LAD	RGR	SD
SP	2	0.48 ^{ns}	1.12 ^{ns}	0.009 ^{ns}	0.000006 ^{ns}	0.15 ^{ns}
WS	1	2.75 ^{**}	21.75 ^{**}	0.05 [*]	0.0001 ^{**}	3.29 ^{**}
TS	1	26.20 ^{**}	0.33 ^{ns}	0.68 ^{**}	0.0005 ^{**}	0.017 ^{ns}
SP x WS	2	1.26 [*]	3.12 ^{ns}	0.02 ^{ns}	0.000001 ^{ns}	0.03 ^{ns}
SP x TS	2	1.14 [*]	5.53 ^{**}	0.03 [*]	0.000007 ^{ns}	0.09 ^{ns}
WS x TS	1	0.008 ^{ns}	4.70 [*]	0.0002 ^{ns}	0.0002 ^{**}	0.11 ^{ns}
SP x WS x TS	2	0.33 ^{ns}	0.21 ^{ns}	0.008 ^{ns}	0.00004 ^{ns}	0.06 ^{ns}
Residual	48	0.33	1.03	0.008	0.000012	0.064
Average		2.22	3.88	0.37	0.019	3.42
CV (%)		25.78	26.14	23.50	17.89	7.38

SV – Sources of variation; SP – Seed priming; WS – Water stress; TS – Thermal stress; DF – Degrees of freedom; CV – Coefficient of variation; ^{ns} – not significant; * – significant at $P \leq 0.05$; ** – significant at $P \leq 0.01$.

The largest leaf area (LA) was observed in *M. oleifera* seedlings under normal irrigation conditions (NWS) and from unprepared seeds (NP), with 2.76 cm²; the other treatments did not differ statistically (Figure 2A). Under thermal stress

conditions (NWS 40 °C), the largest leaf area was observed in seedlings from unprepared seeds (NP), 3.16 cm², statistically equal to that observed in seedlings primed with PB, 3.02 cm², at 30 °C (Figure 2B).

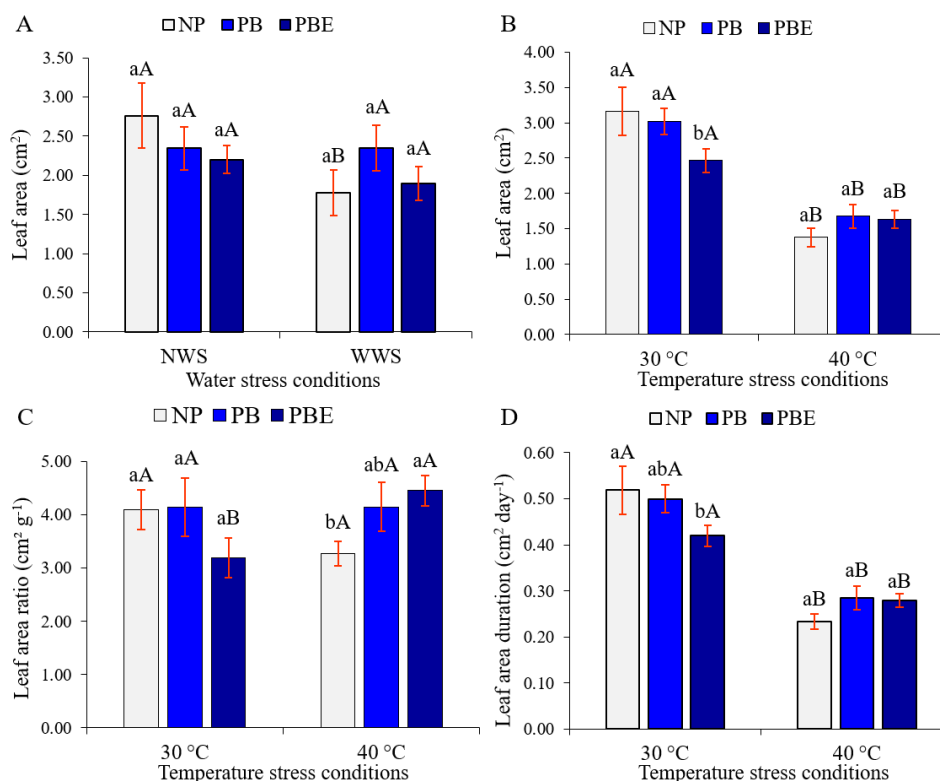


Figure 2. Leaf area (LA) (A and B), leaf area ratio (LAR) (C) and leaf area duration (LAD) (D) of *Moringa oleifera* seedlings grown from seeds primed with water under blue light irradiation (PB) and in ethylene solution (500 mg L⁻¹ Ethrel) under blue light irradiation (PBE) under water and heat stress conditions (WWS 40 °C) 35 days after sowing. Treatments: NP = unprimed seeds; PB = seeds primed with blue light; PBE = seeds primed with blue light and ethylene. Bars represent means $\pm$ SE (n = 5). Different letters indicate significant differences according to Tukey's test ($P \leq 0.01$): lowercase letters = differences between seed priming treatments within each stress condition; uppercase letters = differences between water stress levels within each priming treatment; Greek letters = differences between temperature levels within each combination of priming and water stress. MSD = minimum significant difference.

The increase in leaf area corroborates the RGR results, indicating that elevated temperature under full irrigation accelerates metabolism and leaf expansion to maximize light interception and transpiration rates, thereby cooling the leaves (KOLLN et al., 2025). Furthermore, it is noteworthy that a reduction in leaf area is considered an adaptive response in plants subjected to abiotic stresses, such as water and thermal stresses, aimed at preventing water loss through transpiration and reducing heat absorption (MELO et al., 2024). Seed priming partially attenuated the effects of stress on *M. oleifera* seedlings, indicating that monochromatic blue light can induce tolerance to the stress conditions studied.

The highest leaf area ratio (LAR) under water stress (WWS 30 °C) was obtained in seedlings primed with PB (5.16 cm² g⁻¹), and the lowest value was observed in NP seedlings (3.90 cm² g⁻¹), which corresponds to a 24.42% increase in the LAR of seedlings treated with PB compared to NP under water stress (Figure 2C). However, under heat stress (NWS 40 °C), the highest LAR was observed in seedlings with PBE, 4.45 cm² g⁻¹, and the lowest value was observed in NP seedlings, 3.27 cm² g⁻¹, which corresponds to a 26.51% increase compared to unprepared seedlings (Figure 2D). The most extended leaf area duration (LAD) was obtained in unprepared seedlings, under conditions without heat stress (30 °C), of 0.52 cm² day⁻¹, statistically equal to the value found in seedlings primed with PB, 0.499 cm² day⁻¹. The lowest value was observed in seedlings with PBE, 0.42 cm² day⁻¹ (Figure 2D).

These results suggest that priming with PBE favored the maintenance of more efficient or morphologically finer leaf tissues, allowing the seedling to maintain a functional leaf structure and for the plant to maintain light uptake, including for thermoregulation (via transpiration) and photosynthesis, without compromising metabolic stability, and consequently plasticity under water or thermal restrictions (BONOU et al., 2025).

The triple interaction between seed priming and the two water and heat stress conditions significantly influenced chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents, as well as total carotenoids ($P \leq 0.01$) (Table 4).

The levels of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *t*), and total carotenoids (Car *t*) under water stress conditions (WWS at 30 °C) did not differ statistically between the conditions studied (Figures 3A, 3B, 3C, and 3D). However, under combined stress conditions (WWS at 40 °C), the content of photosynthetic pigments showed similar behavior, in which the highest levels of Chl *a*, Chl *b*, Chl *t*, and Car *t* were obtained in *M. oleifera* seedlings from seeds primed with PBE, with values of 3099.72 $\mu\text{g g}^{-1}$, 2417.62 $\mu\text{g g}^{-1}$, 5517.35 $\mu\text{g g}^{-1}$, and 481.11 $\mu\text{g g}^{-1}$, respectively. The lowest levels were observed in unprepared seedlings (NP), of 2269.73 $\mu\text{g g}^{-1}$, 1892.59 $\mu\text{g g}^{-1}$, 4162.33 $\mu\text{g g}^{-1}$, and 413.12 $\mu\text{g g}^{-1}$, respectively, providing increases of 26.78%, 21.72%, 24.56% and 14.13%, respectively, compared to NP (Figures 3A, 3B, 3C, and 3D).

Table 4. Summary of analysis of variance for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *t*), and total carotenoids (Car *t*) in *M. oleifera* seedlings derived from seeds primed in water under blue light irradiation (PB) and in an ethylene solution (500 mg L⁻¹ Ethrel) under blue light irradiation (PBE) under water and thermal stress conditions (WWS 40 °C) at 35 days after sowing.

SV	DF	Mean squares			
		Chl <i>a</i>	Chl <i>b</i>	Chl <i>t</i>	Car <i>t</i>
SP	2	343968.96**	137641.93**	916787.55**	2308.12**
WS	1	772.52 ^{ns}	309.14 ^{ns}	2059.03 ^{ns}	5.18 ^{ns}
TS	1	880382.29**	352292.33**	2346499.53**	5907.56**
SP x WS	2	322386.77**	129005.53**	859262.93**	2163.27**
SP x TS	2	294267.97**	117753.72**	784317.81**	1974.61**
WS x TS	1	809910.53**	324092.37**	2158672.59**	5434.62**
SP x WS x TS	2	144113.06**	57668.10**	384107.78**	967.06**
Residual	48	18922.26	7571.89	50433.88	126.97
Average		2688.31	2157.38	4845.69	447.41
CV (%)		5.12	4.03	4.63	2.52

SV – Sources of variation; SP – Seed priming; WS – Water stress; TS – Thermal stress; DF – Degrees of freedom; CV – Coefficient of variation; ^{ns} – not significant; * – significant at $P \leq 0.05$; ** – significant at $P \leq 0.01$.

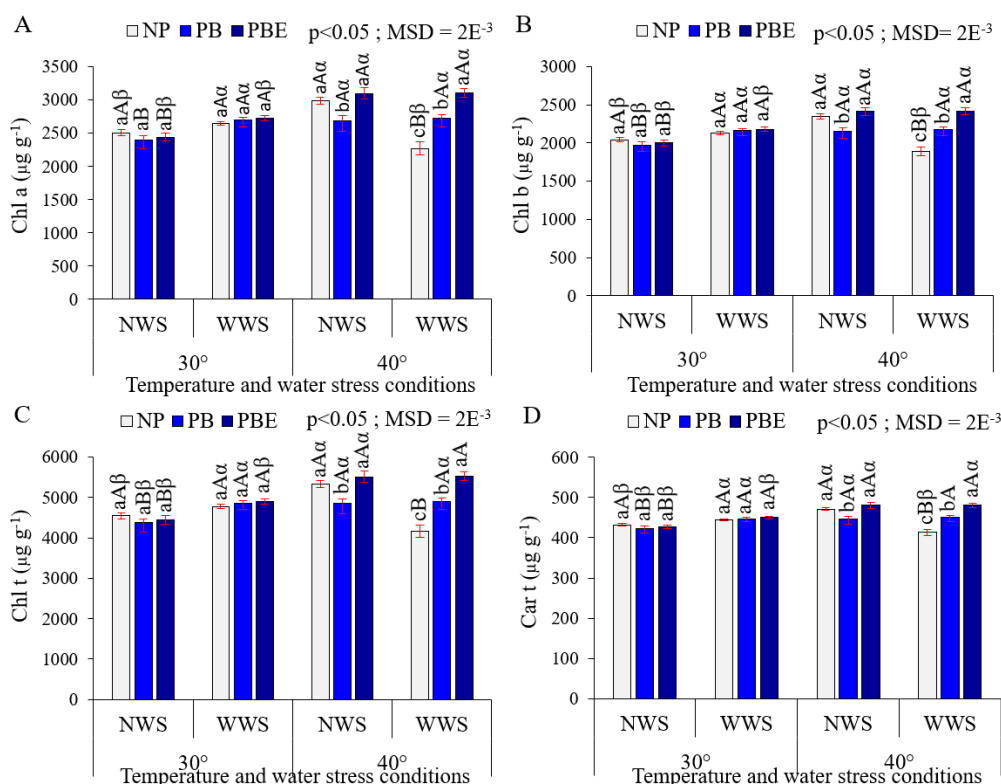


Figure 3. Chlorophyll *a* (Chl *a*) (A), chlorophyll *b* (Chl *b*) (B), total chlorophyll (Chl *t*) (C), and total carotenoids (Car *t*) (D) in *Moringa oleifera* seedlings from seeds primed with water under blue light irradiation (PB) and in ethylene solution (500 mg L⁻¹ Ethrel) under blue light irradiation (PBE) under water and heat stress conditions (WWS 40 °C), 35 days after sowing. Treatments: NP = unprimed seeds; PB = seeds primed with blue light; PBE = seeds primed with blue light and ethylene. Bars represent means $\pm$ SE ($n = 5$). Different letters indicate significant differences according to Tukey's test ($P \leq 0.01$): lowercase letters = differences between seed priming treatments within each stress condition; uppercase letters = differences between water stress levels within each priming treatment; Greek letters = differences between temperature levels within each combination of priming and water stress. MSD = minimum significant difference.

The maintenance of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid levels in seedlings grown from seeds subjected to PBE priming, even under combined stress conditions (WWS 40 °C), suggests a protective effect of blue light combined with ethylene (PBE) on the photosynthetic

apparatus. Blue light is known to stimulate chlorophyll synthesis and stomatal development, while ethylene, at appropriate concentrations, can act as a signaling molecule in modulating antioxidant defenses (COSTA et al., 2022; BONOU et al., 2025).

Similar results were reported by Costa et al. (2022), who evaluated seed priming with light and observed increases in the accumulation of photosynthetic pigments in *M. oleifera* seedlings. These results indicate that priming with light and phytohormones, alone or in combination, can favor the accumulation of these pigments (COSTA et al., 2022).

The dry mass of all parts of the seedlings was reduced as a function of the stresses studied. The reduction in dry mass of different plant parts under water and heat stress conditions is a typical acclimation response aimed at conserving energy and reducing transpiration demand (IJAZ; KHAN; HAIDAR, 2025). However, the analysis of the partitioning of this phytomass indicated distinct strategies induced by the studied conditions.

The highest leaf dry mass (LDM) under water stress (WWS 30 °C) was observed in seedlings primed with PB (0.24 g) and non-primed (NP) (0.23 g), which were statistically equal (Figure 4A). Under combined stress (WWS

40 °C), there was no significant difference in the priming (Figure 4A).

Similarly to LDM, the highest leaf dry mass ratios (LDMR) under water stress (WWS 30 °C) were observed in seedlings primed with PB (0.43 g) and non-primed (NP) (0.41 g), which were statistically equal (Figure 4B). However, under combined stress (WWS 40 °C), the highest LDMR was observed in seedlings primed with PBE (0.37 g); the other seed priming treatments showed lower values that were not statistically different (Figure 4B). This indicates that priming with PBE maintained functional leaf tissues even under combined stress.

The highest stem dry mass (SDM) under water stress (WWS 30 °C) was observed in seedlings primed with PBE (0.26 g); the other priming treatments showed lower, statistically equal values (Figure 4B). Under combined stress (WWS 40 °C), there was no significant difference between the priming treatments (Figure 4B).

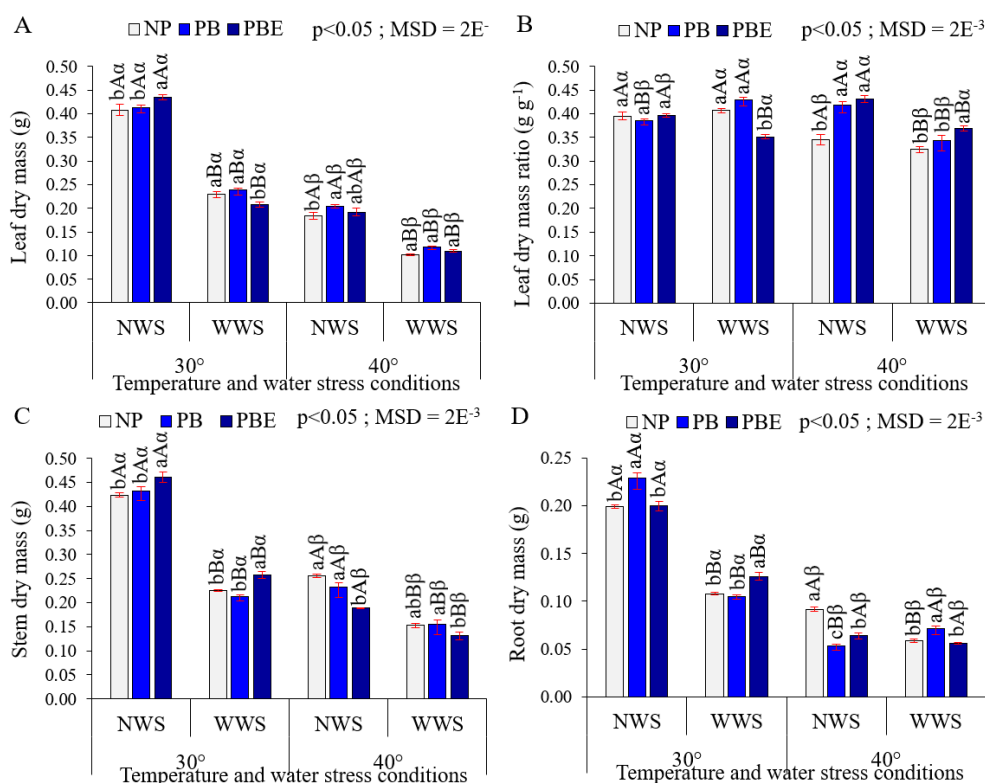


Figure 4. Leaf dry mass (LDM) (A), leaf dry mass ratio (LDMR) (B), stem dry mass (SDM) (C), and root dry mass (RDM) (D) in *Moringa oleifera* seedlings from seeds primed with water under blue light irradiation (PB) and in ethylene solution (500 mg L⁻¹ Ethrel) under blue light irradiation (PBE) under water and heat stress conditions (WWS 40 °C), 35 days after sowing. Treatments: NP = unprimed seeds; PB = seeds primed with blue light; PBE = seeds primed with blue light and ethylene. Bars represent means $\pm$ SE (n = 5). Different letters indicate significant differences according to Tukey's test ($P \leq 0.01$): lowercase letters = differences between seed priming treatments within each stress condition; uppercase letters = differences between water stress levels within each priming treatment; Greek letters = differences between temperature levels within each combination of priming and water stress. MSD = minimum significant difference.

The highest root dry mass (RDM) under water stress (WWS 30 °C) was observed in seedlings primed with PBE (0.13 g); the other priming conditions showed lower, statistically equal values (Figure 4C). However, under combined stress (WWS 40 °C), the highest RDM was observed in seedlings primed with PB (0.071 g); the other

priming conditions showed lower, statistically equal values (Figure 4C). This pattern of resource allocation to the root system is an important adaptive response, as it enables exploration of larger soil volumes and optimizes water absorption, thereby giving seedlings greater resilience to water deficits (HUANG; HONG; LIANG, 2024).

This protection of the biochemical apparatus, through osmotic adjustment and mitigation of oxidative damage to chloroplast membranes (Figures 3A, 3B, 3C, and 3D), allowed for the continuous flow of photoassimilates. The channeling of these resources to the root system and the maintenance of stem robustness explain the higher Dickson quality index (DQI = 1.98) value in the PBE treatment under combined stress (WWS 40 °C). Therefore, priming with PBE acted as an 'early warning' signal, preparing *M. oleifera* to reorganize its metabolism and morphology, prioritizing supporting and absorption organs, which is essential for the successful establishment of seedlings in semi-arid regions subject to thermal and hydrological fluctuations.

CONCLUSIONS

Priming seeds with monochromatic blue light and ethylene (PBE) is an efficient strategy to mitigate the deleterious effects of combined water and heat stress in *M. oleifera* seedlings.

M. oleifera seedlings produced from seeds primed in an ethylene solution under irradiation with blue light (PBE), when grown in the absence of heat and water stress, showed an increase in biomass accumulation.

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REFERENCES

ÁLVARES, C. A. et al. Köppen's climate classification map for Brazil. **Meteorologische Zeitschrift**, 22: 711-728, 2013.

BENINCASA, M. M. P. **Análise de crescimento de plantas: noções básicas**. 2. ed. Jaboticabal, SP: FUNEP, 2003. 41 p.

BONOU, S. I. et al. Seed priming with blue light and ethylene modulates responses to drought and heat stress in *Moringa oleifera*. **BMC Plant Biology**, 26: 1-17: 2025.

CARVALHO, D. B.; CARVALHO, R. I. N. Qualidade fisiológica de sementes de guanxuma sob influência do envelhecimento acelerado e da luz. **Acta Scientiarum. Agronomy**, 31: 489-494, 2009.

CHIBANI, K.; GHERLI, H.; FAN, M. The role of blue light in plant stress responses: modulation through photoreceptors and antioxidant mechanisms. **Frontiers in Plant Science**, 16:

e1554281, 2025.

COSTA, P. S. et al. Seed priming with light quality and *Cyperus rotundus* L. extract modulate the germination and initial growth of *Moringa oleifera* Lam. seedlings. **Brazilian Journal of Biology**, 84: e255836, 2022.

DICKSON, A.; LEAF, A.; HOSNER, J. F. Quality appraisal of white spruce and white pine seedling stock in nurseries. **The Forest Chronicle**, 36: 10-13, 1960.

DIVYA, S. et al. Exploring the phytochemical, pharmacological, and nutritional properties of *Moringa oleifera*: a comprehensive review. **Nutrients**, 16: e3423, 2024.

ELSHAMLY, A. M. S.; ABAZA, A. S. Precise partial root-zone irrigation technique and potassium-zinc fertigation management improve maize physio-biochemical responses, yield, and water use in an arid climate. **BMC Plant Biology**, 24: 775, 2024.

FERREIRA, D. T. R. G. et al. Germinação de três Euphorbiaceae influenciada pela luz e níveis de palhada. **Revista Agro@mbiente On-line**, 11: 215-222, 2017.

FERREIRA, D. F. Sisvar: A computer analysis system to fixed effects split-plot type designs. **Revista Brasileira de Biometria**, 37: 529-535, 2019.

HACKMANN, C. A. et al. Local neighborhood affects stem rehydration under drought: evidence from mixtures of European beech with two different conifers. **Tree Physiology**, 44: tpae114, 2024.

HAJAJI, A. N. et al. Multivariate investigation of *Moringa oleifera* morpho-physiological and biochemical traits under various water regimes. **BMC Plant Biol**, 24: e505, 2024.

HUANG, Y.; HONG, Y.; LIANG, K. Impact of tree root structure on soil water dynamics: drought adaptation mechanisms and ecological significance. **Molecular Soil Biology**, 15: 2024.

IJAZ, A.; KHAN, M. K. R.; HAIDAR, S. Early-stage identification of heat-tolerant cotton genotypes under simulated episodic heat stress in controlled conditions. **BMC Plant Biology**, 25: 1194, 2025.

KOLLN, A. M. et al. Initial vegetative vigor of *Coffea canephora* genotypes grown in the western Amazon. **Revista Caatinga**, 38: e12955, 2025.

LICHTENTHALER, H. K.; BUSCHMANN, C. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. **Current Protocols in Food Analytical Chemistry**, 173: F4.3.1-F4.3.8, 2001.

MACÁRIO, A. P. S. et al. Allometric models for estimating *Moringa oleifera* leaflets area. **Ciência e Agrotecnologia**, 44: e005220, 2020.

MASHAMAITE, C. V.; RAMATSITSI, M. N.;

MANYEVERE, A. *Moringa oleifera* Lam.: A versatile climate-smart plant for nutritional security and therapeutic usage in semi-arid regions. **Journal of Agriculture and Food Research**, 16: e101217, 2024.

MELO, A. S. D. et al. Modulation of grought-induced stress in cowpea genotypes using exogenous salicylic acid. **Plants**, 13: 634, 2024.

PAREEK, A. et al. *Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. **International Journal of Molecular Sciences**, 24, e2098, 2023.

PECH, R. et al. Blue light as an important factor increasing plant tolerance to acute photooxidative stress. **Environmental and Experimental Botany**, 226: 105923, 2024.

SHAPIRO, S. S.; WILK, M. B. An analysis of variance test for normality (complete samples). **Biometrika**, 52: 591-609, 1965.

SILVA, A. E. et al. Microclimate changes, photomorphogenesis, and water consumption of *Moringa oleifera* cuttings under different light spectrums and exogenous phytohormone concentrations. **Australian Journal of Crop Science**, 14: 751-760, 2020.

TESFAY, S. Z.; MODI, A. T.; MOHAMMED, F. The effect of temperature in moringa seed phytochemical compounds and carbohydrate mobilization. **South African Journal of Botany**, 102: 190-196, 2016.

VASCONCELOS, L. H. C. et al. Utilização de métodos físicos e de fitorreguladores para superação de dormência em sementes de pinha. **Revista de Agricultura Neotropical**, 2: 20-24, 2015.

ZHANG, Z. et al. Ethylene-mediated drought tolerance in the critically endangered *Artocarpus nanchuanensis*: insights from physiological and transcriptomic analyses. **Plants**, 14: e2636, 2025.