Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Seed priming with light quality and *Cyperus rotundus* L. extract modulate the germination and initial growth of *Moringa oleifera* Lam. seedlings

Condicionamento de sementes com qualidade de luz e extrato de *Cyperus rotundus* L. modula a germinação e o crescimento inicial de plântulas de *Moringa oleifera*

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Abstract

Improving plant germination is essential to guarantee better quality seedlings. Thus, this research aimed to evaluate whether the seed priming with light quality (LIQ) and the aqueous extract of *Cyperus rotundus* (AEC) tuber could modulate the germination and initial growth of *Moringa oleifera* L. seedlings. The experimental design was a completely randomized in the 4x4 factorial scheme, composed of four LIQ conditions (white, blue, red, and distant red light) and four AEC concentrations (0, 25, 50 and 100%). Seed priming with red light reduced the average emergence time, while blue, red, and extreme red lights associated with 50% of aqueous extract of *C. rotundus* increased shoot initial length and photosynthetic pigment accumulation. Seed priming with blue light resulted in seedlings with a shorter final shoot length. However, application of 100% of aqueous extract of *C. rotundus* reversed this. The white light in combination with concentrations of 50 and 100% of AEC promoted a higher relative shoot growth rate of seedlings. The research revealed that seed priming with light quality and aqueous extracts of *C. rotundus* tubers modulates the germination and initial growth of *M. oleifera* seedlings. More work needs to be done to determine the responsible compounds in AEC that is responsible for priming growth as phytohormones.

Keywords: germination, light spectrum, plant extract, plant hormones.

Resumo

A melhoria da germinação de plantas é fundamental para garantia de mudas de melhor qualidade. Assim, objetivou-se avaliar se o condicionamento de sementes com qualidade de luz (light quality - LIQ) e extrato aquoso de tubérculos de *Cyperus rotundus* (AEC) modula a germinação e o crescimento inicial de plântulas de *Moringa oleifera.* Utilizou-se delineamento inteiramente casualizado, em esquema fatorial 4x4, sendo quatro condições de LIQ (luz branca, azul, vermelha e vermelho distante) e quatro concentrações de AEC (0, 25, 50 e 100%). O condicionamento de sementes com luz vermelha reduziu o tempo médio de emergência, enquanto que as luzes azul, vermelha e vermelho extremo associadas a 50% de extrato aquoso de *C. rotundus* aumentaram o comprimento inicial da parte aérea e o acúmulo de pigmentos fotossintéticos. Condicionamento de sementes com luz azul induziu a formação de plântulas com menor comprimento final da parte aérea, no entanto, a aplicação de 100% de extrato aquoso de *C. rotundus* reverteu o menor crescimento. A luz branca associada às concentrações de 50 e 100% de fitormônios promoveu maior taxa de crescimento relativo da parte aérea de plântulas. Nossa pesquisa mostra que o condicionamento de sementes com radiação espectral de luz e extrato aquoso de tubérculos de *Cyperus rotundus* modula a germinação e o crescimento inicial de plântulas de *Moringa oleifera*. Mais trabalhos precisam ser feitos para determinar os compostos do AEC responsáveis que que atuam como fitormônios e são responsáveis pelo crescimento inicial.

Palavras-chave: germinação, espectro de luz, extrato vegetal, hormônios vegetais.

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Received: August 29, 2021 – Accepted: March 16, 2022

1. Introduction

Improving plant germination is essential to guarantee better quality seedlings, especially under abiotic stress conditions (Silva et al., 2024). However, physiological treatments can improve seed performance under these conditions (Sá et al., 2022). Physiological conditioning has been the most recent and interesting treatment for this purpose. This treatment synchronizes germination as much as possible through the activation of seed metabolism, seeking to reach a uniform level and as close as possible to the stage of primary root protrusion, via controlled hydration (Costa et al., 2022).

The abovementioned situation sets pressure on the agricultural sector to set aside and prepare more arable land and production factors as water, fertilizers and pesticides, whose use which has been intensely by the negative effects of human actions on agro-ecosystems and climate change (Martins et al., 2019). Thus, the use of plant extracts can be a viable alternative, as it has lower cost and greater availability, such as the extract of *Cyperus rotundus* (Cyperaceae) tubers (Cavalcante et al., 2018; Santos et al., 2021). Changes in climate variables such as temperature and light can influence seed germination and plant development, mainly due to variations in light quality and its relationship with plant photoreceptors (Bornman et al., 2019; Weber et al., 2019).

Growing plants with potential for multiple applications (functional foods) is an important strategy to ensure sustainability and food security. Those plants have genotypic and phenotypic resilience to adjust to much more diverse agroecosystems as under an integrated crop-livestock-forest system (Bussoni et al., 2019; Cortner et al., 2019; Melo et al., 2022). In that respect, *Moringa oleifera* Lam. has attained global significance because all its parts can be used for food, medicines, and industrial purposes (Liu et al., 2019; Macário et al., 2020; Parveen et al., 2024).

Moringa oleifera belongs to the Moringaceae family, native to India and Pakistan (Domenico et al., 2019) and has been introduced as a cultivated crop in arid and semi-arid regions. It is an alternative for human and animal nutritional security due to its potential to provide essential amino acids, macronutrients, and micronutrients (Karthickeyan, 2019; Páramo-Calderón et al., 2019). Furthermore, it can be used for water purification and has antifungal, analgesic, anti-inflammatory, antioxidant, antidiabetic, antitumor, and antibacterial activity (Garcia et al., 2019).

Although *M. oleifera* is resilient and can with stand climate changes, however, events such as abiotic stresses that occur during its seed germination may influence seedlings' growth and development, showing a reduction in phytomass accumulation and fruit and seed yield (Hasan et al., 2019). Availability, quality, and time of exposure to light are critical for seed germination. Thus, *M. oleifera* reacts to light variations, chiefly because light influences factors such as soil temperature and humidity, air, and plant metabolism (Ahmed et al., 2014; Silva et al., 2020).

The response of photoreceptors to light plays a crucial role in plants' physiological processes. Light signals transduction involves important biochemical events for biosynthesis and action of phytohormones responsible for photomorphogenic changes and tolerance to abiotic stresses (Matsuo et al., 2019; Polesi et al., 2019; Vaishak et al., 2019). Despite the importance of the interaction between light and phytohormones in plant development, little is known about the combined effect of those constituents on seed germination and initial seedling growth of *M. oleifera*.

Auxins, gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids, and strigolactones are phytohormones that regulate seed germination and plant growth and development (Pawela et al., 2019; Peres et al., 2019). These hormones have been obtained from synthetic and natural sources such as 3-indol butyric acid (auxin), present in *Cyperus rotundus* tubers, which can be utilized as a technology to promote dormancy break and seed germination (Cavalcante et al., 2018; Rifna et al., 2019).

Seed germination is a complex physiological process that starts with water absorption and ends with radicle protrusion (Tuan et al., 2019). Hence, seed pre-treatment consists of controlling the water content of these germinative structures to partially activate the germination processes, however it is observed that the seed prime can prevent the total seed germination and those may return to quiescence phase (Sano and Seo, 2019).

Based on the above considerations, it has been predicted that seed priming, varying the light spectrum radiation conditions and concentration of aqueous extract from *C. rotundus* tubers, can increase seed germination, seedling growth, and the balance of chloroplast pigments. Thus, the objective was to evaluate whether the seed priming with light spectral radiation and aqueous extract from *C. rotundus* tubers modulates seeds' physiological quality and growth and chloroplast pigments accumulation in *M. oleifera* seedlings.

2. Material and Method

2.1. Geographical localization

The research was carried out between October and December 2018, at the Phytopathology Laboratory, in a screen-protected environment at the Center for Agricultural and Environmental Sciences (CCAA) of Paraiba State University (UEPB), located in the municipality of Lagoa Seca - PB, in coordinates Latitude 7º 09 'S, Longitude 35º 52' W, and 634 m of altitude (Soares et al., 2017). The climate of the region according to the Köppen classification is As' (humid tropical), with an average annual temperature of 22 ºC, with minimum and maximum temperatures of 18 ºC and 33 ºC respectively, rainfall of 800 mm, and relative humidity 80% (Silva et al., 2020).

2.2. Experimental design

Seed priming application was performed in a completely randomized experimental design, in a 4x4 factorial scheme, with four replications with 24 seeds (Pereira et al., 2015). The factors consisted of four conditions light quality (LIQ) measured with a digital lux meter model LD-400 (WL = white light with emission of 202 lumens m^{-2}, BL = blue light with emission of 108 lumens m^{-2} and wavelengths

from 400 to 485 nm, RL = red light with emission of 184 lumens m^{-2} and wavelength 600 to 680 nm, and FR = distant red light with emission of 32 lumens m^{-2} and wavelength 680 to 720 nm) plus four concentrations of aqueous extract of *C. rotundus* (AEC0 = control 0%, AEC25 = 25%, AEC50 = 50% and AEC100 = 100%).

2.3. Light quality treatments

Biochemical Oxygen Demand (B.O.D.) germinating chamber equipped with LED strips with white light (emission of 205 lumens m^{-2}) was used as a light source. Transparent gearboxes (11x11x3.5 cm in length, width and height) were used to obtain the light quality. For obtain of white, blue and red lights, the boxes were covered with four layers of clear, blue and red cellophane, respectively. For obtain of distant red light, the boxes were covered with two layers of blue and two red cellophane, for a total of four layers (Yamashita et al., 2011).

2.4. Preparation of aqueous extract from Cyperus rotundus tuber

Cyperus rotundus tubers were obtained at CCAA/UEPB Experimental Field (Latitude 7 ° 09 'S, Longitude 35 ° 52' W, and altitude 634 m) (Silva Filho et al., 2016). Fresh tubers without shot and roots were washed with running water and neutral detergent and dried to a constant weight under shed. The dried plant material was ground into powder. The powder, 10.0 g was extracted with 200 mL of distilled water on a sonication bath for 1hr followed by filtration through a Whatman No. 1 filter paper to obtain a stock solution with 100% of extract concentration (AEC100) (Simões et al., 2003).

Concentrations corresponding to each treatment were obtained by diluting the stock solution (AEC100) with distilled water as follows: control treatment (AEC0 = 0%) just distilled water, (AEC25 = 25%) dilution with 75% distilled water + 25% stock solution, (AEC50 = 50%) dilution with 50% distilled water + 50% stock solution, (AEC100 = 100%) stock solution without dilution with distilled water (Rezende et al., 2013; Scariot et al., 2017).

2.5. Seed priming application

Seeds of *Moringa oleifera* Lam., obtained from a domesticated variety (2018 harvest), lot 00016, were used in the experiment. Initially, 4 samples (100 seeds weighing 30 g) were characterized by length 10.59 ± 1.58 mm, width 9.61 ± 0.90 mm, weighing 0.29 ± 0.06 g, with electrical conductivity of 172 16 ± 41.82 µS cm^{-1} g^{-1}, moisture content of 11.57 ± 0.30%, 96% pureness, 4% inert matter, and 90% germination. Afterward, seeds went through a cleaning process with sodium hypochlorite (1%) for 3 minutes (Carvalho and Carvalho, 2009). The procedure was carried out under a green light, recognized as a safety light because it does not influence phytochromes (Parreira et al., 2011).

Each box received a substrate composed of two layers of 'germitest' paper sheet, sprayed with solutions matching each AEC concentration with added volume corresponding to approximately 2.5 times germitest paper dry mass (Ferreira et al., 2017). The boxes and seeds were stored in a Biochemical Oxygen Demand (B.O.D.) germinating chamber, at a temperature of 30 ± 5 °C and photoperiod of 8 hours (Pereira et al., 2015).

Seed priming was applied for 24 h, the time necessary for soaking the seeds (phase II) without concluding the germination process (Guimarães et al., 2008). Next, seeds were dried on polyethylene trays (30 cm x 20 cm x 5 cm length, width, and height, respectively) for 24 h. The trays were covered with two layers of absorbent paper and covered with cellophane in the colors matching the same lighting conditions applied in the gearboxes.

2.6. Seed sowing

After these priming treatments, 48 hours after drying, seeds were sown at a depth of 0.02 m in polyethylene trays, filled with 3.0 dm^3 of autoclaved sandy substrate, and its humidity maintained between 90 and 100% of field capacity (CC). Trays were kept in shaded conditions with a 15% reduction of natural light. Irrigation management was carried out every day by the weighing method (Silva et al., 2020).

2.7. Variables evaluated

Assessments were done to determine the percentage of emerged seedlings (PES, %), emergence speed index (ESI, dimensionless), average emergence time (AET, days), initial shoot average length (ISL, cm), final shoot average length (FSL, cm), relative shoot growth rate (RSGR, cm cm^{-1} day^{-1}), initial root average length (IRL, cm), final root average length (FRL, cm), relative root growth rate (RRGR, cm cm^{-1} day^{-1}), initial shoot phytomass (ISP, mg), final shoot phytomass (FSP, mg), shoot phytomass relative gain (SPRG, mg mg^{-1} day^{-1}), initial root phytomass (IRP, mg), final root phytomass (FRP, mg), root phytomass relative gain (RPRG, mg mg^{-1} day^{-1}) (Ferraz et al., 2017).

2.8. Seedling emergence

Seedling emergence was evaluated at 24-hour intervals as those that appeared on the substrate surface (epicotyl ≥ 2.0 mm). Percentage of emerged plants was calculated per evaluations done within 24 days (Equation 1):

$$PES = \left(\frac{N_2}{N_1}\right) x100 \quad (1)$$

where: PES = percentage of emergence of seedlings, N_1= total number of seeds sawn, and N_2= number of seedlings emerged.

Emergence speed index (ESI) estimates the average number of seedlings that emerge per day and, the higher the value obtained from the ESI, the greater the emergence speed and, consequently, greater the seedlings' vigor (Maguire, 1962). ESI was calculated by Equation 2

$$ESI = \left(\frac{100}{N}\right) x \sum \left(\frac{n}{j}\right) \quad (2)$$

where: N = number of seeds sowed, n = number of seedlings emerged on day j (j = number of days after sowing) (A-As-Saqui and Corleto, 1978).

The mean emergence time (MET) corresponds to the weighted average of the time required for the emergence of seedlings, that is, the shorter that time, the greater the emergence speed (Edmond and Drapala, 1958). It has used the following relationship to estimate the average emergency (Equation 3):

$$MET = \frac{\sum \frac{n}{t}}{\sum n} \quad (3)$$

where: n = number of emerged seedlings and t = number of days after sowing (Labouriau and Valadares, 1976).

2.9. Seedling growth

Two standardized seedlings were selected per plot after thirteen days from the beginning of the experiment. These seedlings were measured to get the initial shoot (ISL) and root average length (IRL) with a graduated ruler (mm). Afterward, the two seedlings were separated into shoot and root parts. These materials were packed in paper bags and stored in a forced air circulation oven at 70 ºC until stable weight and weighed on an analytical scale to determine the initials shoot (ISP) and root phytomass (IRP) (Ferraz et al., 2017).

Twenty-one days after the beginning of the experiment, two seedlings were selected per plot among the remaining ones and measured to obtain the finals shoot (FSL) and root average lengths (FRL). Afterward, plant material was sectioned, packed in a paper bag, and dry to determine the final shoot (FSP) and root phytomass (FRP) (Ferraz et al., 2017).

Relative shoot (RSGR) and root growth rate (RRGR), relative shoot (SPRG) and root phytomass gain (RPRG) were obtained by the relationship (Equation 4):

$$R = \frac{lnW2 - lnW1}{t2 - t1} \quad (4)$$

where: *R*= growth rate or relative gain; ln = Neperian logarithm; W_1 = initial length or phytomass; W_2 = final length or phytomass; t_1 = initial time, and t_2 = final time (Echer et al., 2010; Ferraz et al., 2017).

2.10. Photosynthetic pigments

Twenty-four days after setting up the experiment, among remaining seedlings, were collected leaflets from two standardized seedlings per plot. The leaflets were wrapped in aluminum film and stored in a refrigerated environment at -20 °C. Pigments extraction was assessed with 0.5 g of leaflet tissue, digested in 6.0 mL of acetone (80%) for 24 hours. Subsequently, the samples have diluted on a 2:1 basis with 2 ml of acetone (80%) for each mL of sample. The extract absorbances were analyzed in a spectrophotometer at the wavelengths of 663, 646, and 470 nm, for chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), total carotenoids (Cart), and total chlorophylls (Chl *t*), respectively. The pigment contents have obtained by Lichtenthaler and Buschmann's equation (Lichtenthaler and Buschmann, 2001) namely (Equations 5-8):

$$Chl\ a\left(\mu g\ g^{-1}\right) = 12.25\ x\ A663 - 2.79x\ A646 \quad (5)$$

$$Chl\ b\left(\mu g\ g^{-1}\right) = 21.5x\ A646 - 5.10x\ A663 \quad (6)$$

$$Car\ t\left(\mu g\ g^{-1}\right) = [1000x\ A470 \div (1.82\ Chl\ a - 85.02x\ Chl\ b)] / 198 \quad (7)$$

$$Chl\ t\ \left(\mu g\ g^{-1}\right) = (12.25x\ A663 - 2.79\ x\ A646) + (21.5x\ A646\ - 5.10\ x\ A663) \quad (8)$$

The values obtained were multiplied by 6.0 mL (volume of the digestion tube) and divided by the sample mass (0.5 g) for conversion of µg mL^{-1} to µg g^{-1}. With these data were calculated Chl a/Chl b and Chl a/Car t relationships.

2.11. Statistical analysis

Original variables data were submitted to the Shapiro-Wilk normality test (Shapiro and Wilk, 1965). When postulates of normality were achieved, each variable data were standardized to obtain the variable Z with null mean ($\overline{X}$ = 0.0) and unit variance (σ^2 = 1.0). Equation 9:

$$Z = \frac{X - \overline{X}}{\sigma^2} \quad (9)$$

Where: X is each observation of the variable's data set, $\overline{X}$ is the mean, and σ^2 is the variance of the data set.

Converted data were subjected to the Principal Component Analysis (PCA) exploratory procedure. The choice of Principal Components (PCs) was based on eigenvalues greater than one (λ> 1.0), which explained a percentage greater than 10% of total variance (Govaerts et al., 2007; Hair Junior et al., 2009). Original data from each PC were submitted to multivariate analysis of variance (MANOVA) by Roy's test. Afterward, seed primings were grouped based on variables from each PC by Ward's minimum variance hierarchical method.

Variables, not associated with any of the PCs, were removed from PCA and subjected to univariate analysis of variance (ANOVA) by the F test with 95% confidence (Barbosa and Maldonado Júnior, 2015). Statistica v. 7.0 (Statsoft Inc., 2004) was used to perform the analyzes.

3. Results

3.1. Principal components and multivariate variance

Four PCs with λ > 1 and σ^2 > 10% were formed from the linear combination of the 18 original variables because of the combination of LIQ and AEC with 78.74% of total σ^2. PC_1 represents 27.91% of σ^2, composed by the combination of ISL, RSGR, IRL, RRGR, ISP, SPRG, and Cart;

PC_2 represents 21.44% of σ^2, formed by FRL, FSP, Chl a, and Chl t; PC_3 represents 16.61% of σ^2 composed by RPRG, Chl b, Chl a/Chl b, and Chl a/Car t; and PC_4 represents 12.79% of σ^2, made by the combination of PES, ESI, and FRP. MET, FSL, and IRP Variables did not match in any PCs and, therefore, were excluded from PCA to be assessed by a univariate analysis. There was a significant interaction between LIQ and AEC combinations in the four PCs (MANOVA results) (Table 1).

In two-dimensional projection of the first two PCs (Figures 1A-1B), it was observed that in PC_1 the seed priming with LIQ – light quality in the BL - blue light, RL - red light, and FR - distant red light region, combined with AEC - aqueous extract of *C. rotundus*, triggered different processes in *M. oleifera* seedlings in contrast to WL - white light. Seeds irradiated with distant red light without the addition of AEC (FR-0) plus the addition of 50% AEC (FR-50) induced seedlings with higher ISL, IRL, and ISP with consequent reduction in RSGR, RRGR, and SPRG that increased in seedlings irradiated with white light and the addition of 50% (WL-50) and 100% (WL-100) of AEC; while RL-25 and RL-100 increased Cart contents.

It was observed that in PC_2 that seeds under irradiation with blue light and the addition of 25% (BL-25) and 50% (BL-50) of AEC combined with RL-50 originated seedlings with the higher levels of Chla and Chlt, whereas, irradiation with BL, without addition of AEC, significantly decreases the level of these pigments. It is possible to note that the seedlings from seeds irradiated with FR-100 and BL-0 had higher FRL and FSP in PC_2.

In the two-dimensional projection of the third and fourth PC (Figures 1C-1D), in PC_3, it was observed that irradiation with RL had a synergistic effect on 50% of AEC concentration, which triggered a process of rising Chla/Chlb and Chla/Cart due to raised Chla contents and the decrease of Chlb and Cart contents. It was observed that in PC_4 the emergence of seedlings occurred in higher percentages (PES) and speed

Table 1. Correlation among original variables and principal components, eigenvalues, explained and accumulated variance, and probability significance of hypothesis test in the first four main interaction components (PCs 1, 2, 3 and 4) among light quality levels and aqueous extract concentrations of *Cyperus rotundus* on *Moringa oleifera* seeds.

EV – Evaluated Variables	Principal Components			
	PC_1	PC_2	PC_3	PC_4
PES - Percentage emerged seedlings	-0.56	-0.12	0.08	-0.57*
ESI - Emergency speed index	-0.53	-0.15	0.04	-0.63*
ISL - Initial shoot length	-0.78*	-0.48	0.03	0.19
RSGR - Relative shoot growth rate	0.70*	0.11	0.09	-0.40
IRL - Initial root length	-0.55*	-0.55	-0.35	0.12
FRL - Final root length	-0.27	-0.62*	0.05	0.44
RRGR - Relative root growth rate	0.59*	-0.03	0.45	0.35
ISP - Initial shoot phytomass	-0.75*	-0.35	-0.19	0.05
FSP - Final shoot phytomass	-0.03	-0.75*	-0.19	0.06
SPRG - Shoot phytomass relative gain	0.91*	-0.11	0.10	-0.14
FRP - Final root phytomass	-0.03	-0.45	0.39	0.55*
RPRG - Root phytomass relative gain	0.32	-0.20	0.62*	0.51
Chla - Chlorophyll a content	-0.52	0.77*	0.05	0.28
Chlb - Clofophyll b content	-0.39	0.16	0.83*	-0.20
Cart - Total carotenoid content	-0.60*	0.55	0.47	0.16
Chlt - Total chlorophyll content	-0.57	0.69*	0.36	0.15
Chla/Chlb - Relationship between chlorophylls a and b	0.06	0.40	-0.71*	0.46
Chla/Cart - Relationship between Chla and Cart	-0.05	0.62	-0.69*	0.26
λ – Eigenvalues	5.02	3.86	2.99	2.30
S^2 (%) – Explained variance	27.91	21.44	16.61	12.79
S^2 (%) – Accumulated variance	27.91	49.34	65.95	78.74
MANOVA	Significance probability (p value)			
Roy's test for LIQ	< 0.01	< 0.01	< 0.01	< 0.01
Roy's test for AEC	0.11	< 0.01	< 0.01	0.09
Roy's test for interaction LIQ x AEC	< 0.01	< 0.01	< 0.01	0.04

*Variables considered in PC formation.

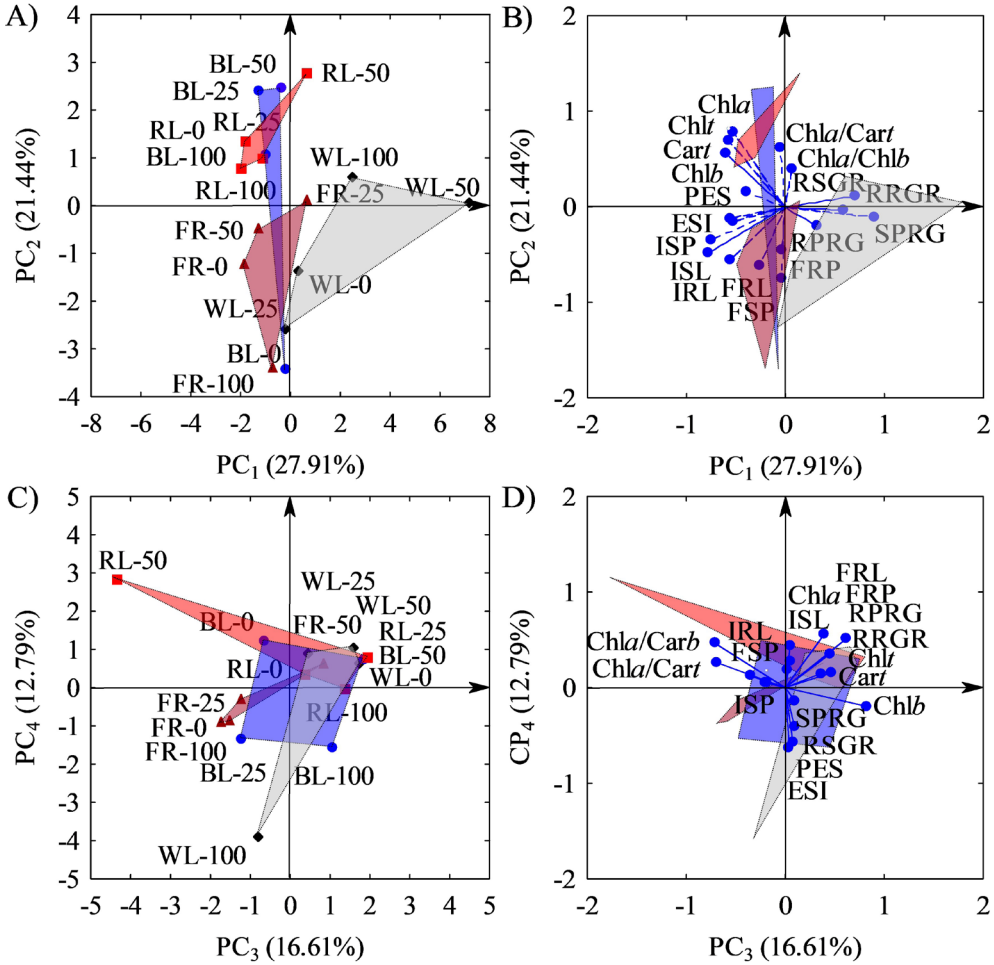

Figure 1. Two-dimensional projection of factorials scores (A and C) and variables (B and D) in the first four main interaction components (CPs 1, 2, 3, and 4) among light quality levels and aqueous extract concentrations of *Cyperus rotundus* (0, 25, 50 and 100%) on *Moringa oleifera* seeds. WL - white light, BL - blue light, RL - red light, FR - distant red light, PES - percentage emerged seedlings, ESI - emergency speed index, ISL - initial shoot length, RSGR - relative shoot growth rate, IRL - initial root length, FRL - final root length, RRGR - relative root growth rate, ISP - initial shoot phytomass, FSP - final shoot phytomass, SPRG - shoot phytomass relative gain, FRP - final root phytomass, RPRG - root phytomass relative gain, Chla - chlorophyll a content, Chlb - chlorophyll b content, Cart - total carotenoid content, Chlt - total chlorophyll content, Chla/Chlb - relationship between chlorophylls a and b, Chla/Cart - relationship between Chla and Cart.

(ESI) under irradiation of seeds, WL potentiated by using 100% of AEC. This could be attributed to the reduction of final root phytomass accumulation (FRP), which showed the partition of a greater amount of energy supply to hypocotyl growth and lesser to root extension.

Applying Euclidean Distance (ED) as a measure of dissimilarity and subjective visual inspection criteria to establish the cutoff point with ED = 4, the seed priming from LIQ and AEC combination was grouped into four groups (1, 2, 3 and 4) of PC_1 (Figure 2A), PC_2 (Figure 2B) and PC_3 (Figure 2C) and in three groups of PC_4 (Figure 2D). Those groups are characterized by having greater homogeneity (similarity) between seed priming of each group and higher heterogeneity (dissimilarity) among groups regarding variables considered because Ward's method (Minimum Variance Method) minimizes the sum of squares within the group.

Moringa oleifera seedlings had a shorter average emergence time (0.086 days) when seeds were submitted to seed priming in red light (Figure 3A). *Moringa oleifera* seeds irradiated with WL and bio induced with 100% of AEC gave rise to seedlings with higher FSL (10.55 cm), followed by those generated from the combination of FR with 50% AEC that had FSL of 10.31 cm (Figure 3B). Seeds not treated with AEC generated seedlings with a greater initial root phytomass (IRP) accumulation when irradiated with RL (50.38 mg) and BL (45.75 mg), respectively. Seedlings originated from seeds treated with 100% AEC showed higher IRP when irradiated by RL and FR with 49.13 mg and 45.38 mg, respectively (Figure 3C).

Original averages data of all individual variables assessed in this study and the F (Fc) test are in Table 2.

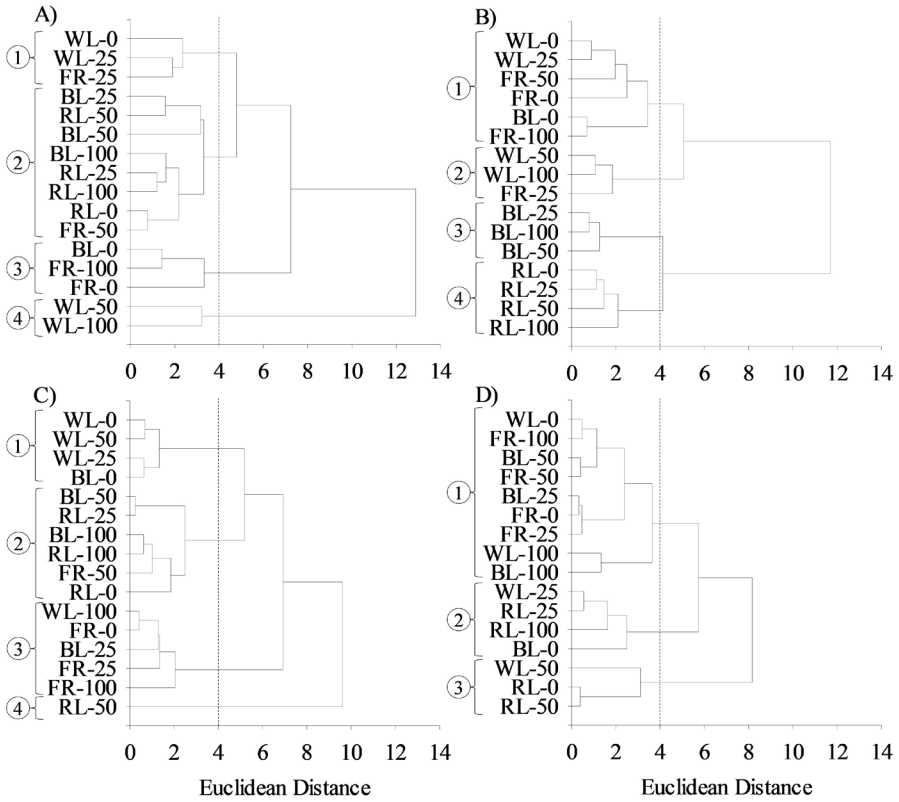

Figure 2. Grouping dendrograms of seed priming of *Moringa oleifera* assembled with PC_1 (A), PC_2 (B), PC_3 (C), and PC_4 (D) variables according to the interaction among light quality levels and aqueous extract concentrations of *Cyperus rotundus* (0, 25, 50 and 100%). WL - white light, BL - blue light, RL - red light, FR - distant red light.

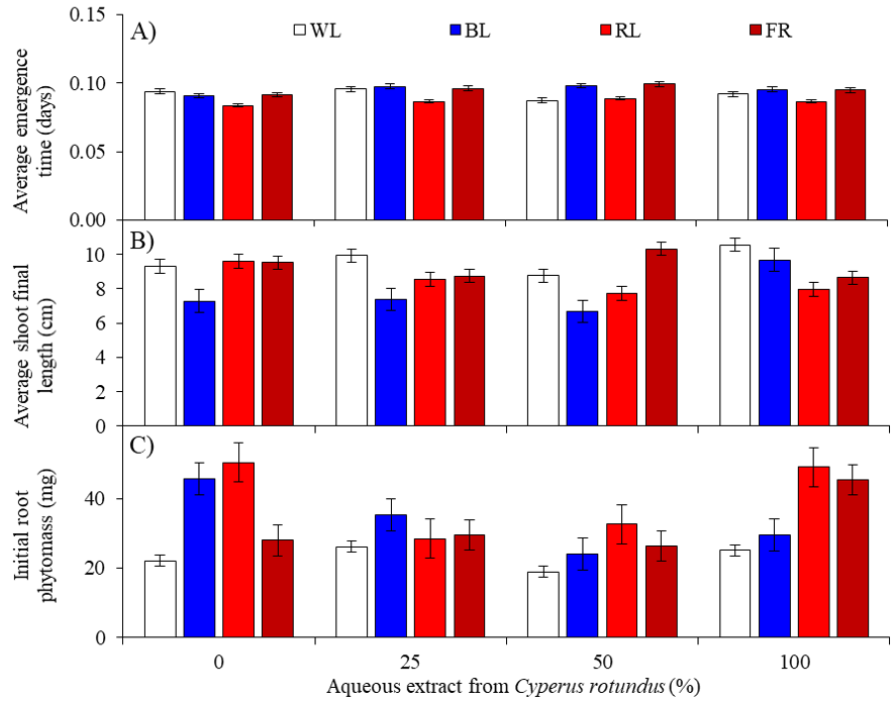

Figure 3. Average emergence time (A), average shoot final length (B), and initial root phytomass (C) of *Moringa oleifera* seedlings as a function of the interaction among light quality levels and aqueous extract concentrations *Cyperus rotundus*.

Table 2. Variables averages evaluated in function of the interaction among light quality levels and aqueous extract concentrations of *Cyperus rotundus*.

Seed priming	Mean ± Standard Deviation						
	PES	**ESI**	**MET**	**ISL**	**FSL**	**RSGR**	**IRL**
WL-0	36.46±10.42	3.41±0.93	0.09±0.01	6.79±0.38	9.3±0.45	0.04±0.01	3.65±0.54
WL-25	36.46±12.44	3.36±0.8	0.1±0.01	6.76±0.54	9.93±0.9	0.04±0.01	4.46±0.64
WL-50	22.92±8.67	2.02±0.8	0.09±0.01	3.93±1.29	8.75±0.94	0.09±0.02	3.25±2.02
WL-100	41.67±14.03	3.82±1.26	0.09±0	4.9±0.25	10.55±0.6	0.09±0	3.63±1
BL-0	34.38±14.97	3.15±1.42	0.09±0	6.54±0.78	7.29±0.93	0.01±0.01	4.81±0.77
BL-25	34.38±6.25	3.35±0.63	0.1±0.01	5.29±0.97	7.39±0.62	0.04±0.03	4.04±0.36
BL-50	32.29±13.77	3.18±1.45	0.1±0.01	6.11±0.48	6.68±1.12	0.01±0.01	3.44±0.95
BL-100	38.54±10.96	3.72±1.27	0.1±0.01	6.09±1.02	9.68±0.65	0.05±0.02	3.88±0.9
RL-0	31.25±8.67	2.60±0.74	0.08±0.01	6.2±1.23	9.58±1.21	0.05±0.03	4.45±0.54
RL-25	36.46±9.24	3.12±0.6	0.09±0.01	5.98±1.02	8.54±1.7	0.04±0.04	4.13±0.09
RL-50	30.21±6.25	2.66±0.48	0.09±0.01	5.58±0.55	7.71±1.59	0.03±0.03	4.21±1.31
RL-100	41.67±17.35	3.51±1.2	0.09±0.01	5.86±1.15	7.95±1.34	0.03±0.02	4.39±1.39
FR-0	35.42±7.22	3.26±0.89	0.09±0.02	6.45±1.02	9.53±1.61	0.04±0.01	4.45±0.38
FR-25	33.33±14.43	3.24±1.51	0.1±0.01	5.96±1.31	8.74±1.43	0.04±0.01	3.73±0.47
FR-50	33.33±4.81	3.31±0.62	0.1±0.01	6.36±0.24	10.31±1.45	0.05±0.02	4.64±0.45
FR-100	35.42±5.38	3.35±0.44	0.09±0	6.49±0.77	8.65±1.05	0.03±0.03	5.18±0.71
F – test	0.67^{ns}	0.78^{ns}	1.37^{ns}	2.82^{**}	3.85^{**}	3.54^{**}	1.34^{ns}
	FRL	RRGR	ISP	FSP	SPRG	IRP	FRP
WL-0	6.24±0.96	0.06±0.03	37±7.54	129.88±16.81	0.14±0.02	22.13±5.95	77.13±25.58
WL-25	6.68±1.11	0.04±0.02	43.38±8.04	131.75±43.74	0.12±0.04	26.13±4.99	94.75±52.42
WL-50	5.06±0.2	0.07±0.07	13.88±6.79	114.38±50.4	0.23±0.07	19±19.14	84.75±110.17
WL-100	4.64±0.9	0.03±0.02	20.5±1.08	120.63±8.1	0.2±0.01	25.13±9.12	34.25±7.26
BL-0	5.81±0.46	0.02±0.01	43.75±9.19	141.63±30.45	0.13±0.04	45.75±21.43	137.25±75.37
BL-25	4.56±0.92	0.01±0.01	33.88±11.16	89.38±28.62	0.11±0.04	35.25±17.1	46.63±11.6
BL-50	4.65±0.73	0.04±0.02	41.38±17.72	90.75±24.66	0.09±0.03	24.13±8.44	71.75±22.56
BL-100	4.89±0.54	0.03±0.03	40.25±16.86	99.63±33.54	0.1±0.04	29.63±14.91	62.38±20.23
RL-0	5.61±0.92	0.03±0.02	41.5±12.55	128.75±38.25	0.13±0.05	50.38±17.5	64±15.17
RL-25	5.4±0.49	0.03±0.01	43±4.45	108.88±61.16	0.09±0.08	28.5±11.25	96.38±70.78
RL-50	5.6±1.05	0.03±0.03	34.13±3.61	110.75±49.53	0.12±0.06	32.63±7.77	56.61±1.79
RL-100	6.3±0.94	0.04±0.03	38.5±9.11	92.63±24.01	0.1±0.05	49.13±15.63	89.25±28.49
FR-0	5.38±0.74	0.02±0.01	72.13±55.06	155.38±92.26	0.09±0.05	28±7.67	43.13±9.67
FR-25	5.59±0.76	0.04±0.02	45.88±23.29	99.38±19.69	0.1±0.04	29.5±13.31	48.75±15.71
FR-50	6.38±1.72	0.03±0.02	45.5±11.55	132.5±9.03	0.12±0.03	26.38±4.13	68.63±13.55
FR-100	6.1±0.92	0.02±0.02	50.88±13.21	135.75±23.53	0.11±0.05	45.38±17.58	67.25±15.84
F – test	2.22^{*}	1.18^{ns}	1.99^{*}	0.97^{ns}	2.98^{**}	2.22^{*}	1.42^{ns}
	RPRG	Chla	Chlb	Cart	Chlt	Chla/Chlb	Chla/Cart
WL-0	0.14±0.06	305.83±58.31	108.16±11.84	188.15±26.12	413.99±69.63	2.81±0.3	1.62±0.15
WL-25	0.13±0.05	287.36±86.98	100.9±34.09	168.62±51.27	388.26±118.58	2.87±0.39	1.7±0.15
WL-50	0.16±0.08	254.22±49.73	99.51±21.58	153.49±28.35	353.72±71.18	2.56±0.09	1.66±0.05
WL-100	0.04±0.03	284.14±23.22	98.32±15.11	160.42±15.86	382.46±38.02	2.91±0.22	1.77±0.04
BL-0	0.11±0.08	263.18±42.35	92.83±11.11	150.95±20.87	356.02±53.4	2.82±0.13	1.74±0.05
BL-25	0.04±0.03	348.32±14.47	103.13±23.07	184.52±23.24	451.44±16.3	3.56±1.07	1.91±0.3
BL-50	0.12±0.06	371.27±42.23	132.13±22.43	209.04±20.62	503.4±64.66	2.84±0.17	1.77±0.03
BL-100	0.09±0.06	333.44±42.52	126.68±11.92	193.99±18.18	460.12±53.63	2.63±0.15	1.72±0.13
RL-0	0.03±0.02	364.43±58.41	131.7±27.55	208.98±36.3	496.14±85.9	2.79±0.14	1.75±0.02
RL-25	0.12±0.08	377.73±96.42	136.79±29.41	212.42±40.31	514.52±125.31	2.74±0.21	1.76±0.16
RL-50	0.06±0.03	389.61±104.93	51.6±37.5	178.24±52.44	441.2±142.02	11.83±9.32	2.2±0.06
RL-100	0.07±0.04	360.67±17.89	125.59±11.07	207.12±10.67	486.26±27.95	2.88±0.16	1.74±0.03
FR-0	0.05±0.02	315.33±60.62	105.92±20.38	177.73±35.17	421.25±81	2.98±0	1.78±0.01
FR-25	0.06±0.06	298.89±1.46	81.7±13.48	165.07±6.05	380.6±12.97	3.73±0.59	1.81±0.07
FR-50	0.11±0.04	351.97±36.79	115.66±19.72	209.15±23.96	467.63±52.51	3.08±0.43	1.68±0.05
FR-100	0.05±0.05	247.38±4.1	91.08±3.63	153.95±4.71	338.46±6.86	2.72±0.09	1.61±0.03
F – test	2.41^{*}	2.82^{**}	4.17^{**}	2.31^{*}	2.36^{*}	3.63^{**}	6.20^{**}

**, * and ns: Probability of the F test significance $p < 0,01$, $p < 0,05$ and $p > 0,05$, $n = 4$.

4. Discussion

Plant plasticity is closely related to environmental conditions and endogenous phytohormones balances (Melo et al., 2022). Interactions between LIQ and AEC, in germination, growth, and accumulation of photosynthetic pigment variables, occur because light is the main environmental factor that influences cell elongation, division, differentiation and induces plant photomorphogenesis. This is mainly because of photoreceptors excitation that trigger the synthesis of phytohormones (Bîlc and Luchian, 2020).

The intrinsic mechanisms by which light interacts with phytohormones in *M. oleifera* are not yet clear, principally when applied seed priming. However, shifts in LIQ levels promote microclimate changes which influence photomorphogenesis and water consumption by *M. oleifera* mini-cuttings when treated with AEC in a vegetative propagation system. This response occurs because of the reduction in air temperature under BL and the increase in air temperature and relative air humidity, and soil temperature under RL and FR (Silva et al., 2020).

Photomorphogenesis is regulated by a set of light signal photoreceptors (Kami et al., 2010; Neff, 2012), thus can be inferred that seed priming with RL and FR influenced the phytohormones activity, for example, phyA, phyB, phyC, phyD, and phyE since these photoreceptors are responsible for light reception and modulation and gene expression by way of signal transduction systems (Oka and Yamamoto, 2019). This explains PES and ESI reduction and ISL, IRL, and ISP increase, which may be related to the enhanced production of phenolic and flavonoid compounds and the resulting photoprotection mentioned in *M. oleifera*.

Possibly, seed priming with BL, RL, and FR plus doses of AEC increased the action of phytochrome interaction factors (PIFs) from rapid phosphorylation, ubiquitination, and proteasome-mediated degradation, which accelerated the transition state from skotomorphogenesis to photomorphogenic development (Liang et al., 2020). The interaction between LIQ and AEC supported the activity of phyA under FR and stimulated the action of phyB under RL, which promoted greater germination and growth (Oh et al., 2020), mainly under seed priming with WL-100, because WL is composed of all colors balance.

This data reported here, on the LIQ and AEC effect, is important for making decisions on seedling management of *M. oleifera* because there may be competition for light in dense crops which can stimulate stem elongation, decrease photoassimilates allocation to leaves, and reduce seedlings growth, a phenomenon that occurs more frequently at a greater FR rate (Kong et al., 2018; Shibuya et al., 2020). FR increases the quantum efficiency of photosystems (PSI and PSII) due to the enhanced electron flow and H^+ in the thylakoid lumen and the synthesis of adenosine triphosphate (ATP) in chloroplasts stroma of plants under light availability fluctuations (Kono et al., 2020).

The knowledge acquired in this research is important to induce abiotic stresses tolerance in *M. oleifera* and allow its cultivation at restrictive environments because the luminous stress, caused by seed priming, can stimulate the synthesis and action of phytohormones as auxin, gibberellic acid, cytokinins, ethylene, and abscisic acid by regulating plant defense mechanisms (Banerjee and Roychoudhury, 2016). Our results propose that the interaction between LIQ and AEC induced further chloroplasts development and describe the rise of chlorophyll biosynthesis as observed on *Arabidopsis thaliana* and *Camellia sinensis* (Liu et al., 2020).

Light induces endogenous phytohormones synthesis. However, their activity can be initiated by natural phytohormones obtained from species as *C. rotundus*. If applied in supra-optimal amounts, these auxin-producing species can provide a herbicide-like effect that reduces plant growth (LV et al., 2019; Bieleszová et al., 2019). This response may explain the lower performance of *M. oleifera* under WL combined with 50 and 100% of AEC, respectively.

Seed priming with WL activated the photo perception and triggered adaptive responses of *M. oleifera* seedlings resulting in a higher relative growth rate. Although wavelengths in the BL (400-500 nm) and RL (600-700 nm) regions from the visible spectrum are more efficient in capturing CO_2 and releasing O_2. Additions seen in RSGR under WL and AEC may be related to the fact that up to 50% of white light spectral composition is between 500 and 600 nm, comprising blue, green, yellow, red, and extreme red lights (Mickens et al., 2018).

5. Conclusion

Seed priming with red light reduced the average emergence time while blue, red, and extreme red lights, associated with 50% of aqueous extract of *C. rotundus*, resulted in an increase in initial shoots length and photosynthetic pigments accumulation. Seed priming with blue light exhibited a shorter final shoot length seedlings, although using 100% of the aqueous extract of *C. rotundus* reversed that lower growth. The association among white light to 50 and 100% of extract promoted a higher rate of seedlings relative shoot growth. Our research shows that seed priming modulates the ecophysiology of *M. oleifera* seedlings when applied in combination with light spectral radiation and the aqueous extract of *C. rotundus* tubers. More work to determine the mechanisms of these actions are needed.

Acknowledgements

Authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for funding this work under Research project No. 160862/2019-1. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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