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Seed priming as a strategy to increase the performance of drumstick tree

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ABSTRACT

Moringa oleifera Lam. is a multiple purpose tree used as human and animal food, cosmetic production and water purification. Seed priming combined with growth promoters and natural substances has been used to improve plant performance. This study aimed to verify the efficiency of seed priming using growth-promoting substances such as brassinosteroids, ascorbic acid, and moringa leaf extract on seed germination and seedling growth of *Moringa oleifera*. The seeds were primed with water and 24-epibrassinolide solutions (EBL 10^{-10}, 10^{-8} and 10^{-6} M), ascorbic acid (AsA 50, 100 and 150 mg.L^{-1}) and Moringa Leaf Extract (MLE 1:30). Primed seeds with EBL 10^{-8} M improved the speed of seed germination. Seedling length and vigor increased mainly in treatments with AsA 100 mg.L^{-1} and MLE 1:30. The activity of the antioxidant enzyme catalase increased mainly in primed seeds with AsA. Plant height, steam base diameter, number of leaves and gas exchange parameters such as photosynthetic rate, stomatal conductance, and internal CO_2 concentration increased in plants which were from primed seeds with MLE 1:30. Therefore, we recommend seed priming to improve plant growth of *Moringa olefeira*, mainly using MLE, a natural and ecological product.
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1. Introduction

To improve the performance of any crop under field conditions it is necessary to use high-quality seeds. However, seed quality and viability tend to decline over time due to the deteriorative chemical process that happen in a dry seed (Bewley et al., 2013). This reduction in seed quality can cause a decrease in the speed of germination and provoke uneven germination of a seed lot. As a result, the seed priming technique can be an alternative to increase uniformity and reduce the time for seedling emergence, mainly in low-vigor seeds. Therefore, seed priming consists of controlled hydration to induce the pre-germinative metabolism without root protrusion (Marcos-Filho 2015). During the priming process, the seed is submitted to moderate stress as being hydrated and dried later, which confers a "priming memory", being triggered upon the second hydration. This happens because when a seed is hydrated, events such as mechanisms of Deoxyribonucleic Acid (DNA) repair, mobilization of reserves, and antioxidative system are activated. Hence, this information is preserved as a "priming memory"(Chen e Arora, 2013). The seed priming technique has been used in seed industry, in which patents are released for specialized treatments to improve germination and seedling uniformity emergence (Paparella et al., 2015). On the other hand, seed priming approach is recognized for being a traditional and low-cost technique that farmers around the world have applied to ensure improvements for sustainable plant growth (Carrillo-Reche et al., 2018).

Along with priming, growth-promoting substances such as plant hormones can also be used to boost seed germination and plant growth (Jisha et al., 2013). Among them, brassinosteroids (BR) are classified as a new steroidal plant hormone, derived from campesterol and synthesized in young plant tissues. BRs operate in a variety of functions to induce plant growth, especially in cell division and elongation (Kanwar et al., 2013; Vadhini et al., 2010), and confer resistance to a variety of stress, such as drought (Talaat and Shawky 2016), saline (Dong et al., 2017), high and low temperatures (Jin et al., 2015; Gornik and Lahuta 2017), and heavy metals (Soares et al., 2020). The ascorbic acid is considered another promising product to be used in seed priming (Ahmad et al., 2015). Commonly known as vitamin C, ascorbic acid consists of a non-enzymatic antioxidant that reduces or prevents oxidative stress caused by oxygenated free radicals (Hussain et al., 2017). Ascorbic acid is produced in small amounts and act not only to promote the plant growth by itself but also play a role in biosynthesis of some phytohormones such as gibberellin and ethylene (Kasim et al., 2017).

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Similarly, the use of natural products with growth-promoting substances seems to be a relevant and efficient approach to improve plant growth (Bibi et al., 2016; Pervez et al., 2017). For example, Moringa Leaf Extract (MLE) has been used in seed priming as an ecological alternative that farmers can easily adapt (Nouman et al., 2014). MLE is constituted of nutrients, enzymatic and non-enzymatic antioxidants, and plant hormones, such as auxin, cytokinin and gibberellin (Khan et al., 2017; Rady et al., 2013). The effects of MLE on improving germination and plant growth have already been reported in some crops such as maize (Bibi et al., 2016), pea (Iqbal, 2015), wheat (Yasmeen et al., 2013), beans (Howladar, 2014), and grasses (Nouman et al., 2012). Although positive results ensure the efficiency of seed priming application for different agronomic species (Ali, 2017; Li et al., 2017; Nawaz et al., 2016; Yan, 2015), there is a lack of information about seed priming applied to forest species (Missio et al., 2018).

Moringa oleifera Lam., popurlarly known as drumstick, is the most recognized species among the 13 species of the Moringaceae family (Tshabalala et al., 2019). Also known as the "miracle tree", *Moringa oleifera* is native to northern India and cultivated in a wide range of tropical countries (Leone et al., 2015). Historically, this species is considered a multipurpose tree, with different parts that can be used not only in animal and human nutrition but also in traditional medicine (Jaja-Chimedza et al., 2017). In addition, *Moringa oleifera* seeds have high oil content, called Ben oil that can be used in the food industry, being an alternative to olive oil (Foidl et al., 2001). It can be propagated by seeds or cuttings, being preferable seeds due to the availability and lower costs and labor. Therefore, using seeds with a higher germination rate is an important step for planting success (Leone et al., 2015). Seed priming is recognized for improving germination speed due to the action on DNA repair, ROS signaling, antioxidants activation, early reserve mobilization, endosperm weakening, and altering hormonal regulation, culminating in a greater germination process (Chen e Arora, 2013). In the literature, the use of seed priming in *Moringa oleifera* has been already reported by Nouman et al. (2014), however it only focused on improving plant growth under salinity stress. Thus, this study aimed to verify the efficiency of seed priming using plant growth promoting substances such as brassinosteroids, ascorbic acid, and Moringa Leaf Extract on seed germination, seedling and plant growth of *Moringa oleifera* Lam.

2. Material and methods

2.1. Seed priming application

The seeds of *Moringa oleifera* were collected from local matrices trees, located in city of Aracaju, Brazil (−10.944410, −37.075507). Firstly, the seeds were pre-soaked for 12 h (Nouman et al., 2014) in water (hydroconditioning), 24-epibrassinolide (EBL) solutions at concentrations of 10^{-10}, 10^{-8} and 10^{-6} M; ascorbic acid (AsA) at concentrations of 50, 100 and 150 mg.L^{-1}; and Moringa Leaf Extract (MLE 1:30). After pre-soaking, the seeds were dried at room temperature until they reached constant weight for 72 h. The control treatment was composed of seeds without priming.

2.2. Preparation and characterization of MLE

The MLE was prepared using young leaves which were overnight frozen and then grinded with a blender to extract its juice. The extract was filtered by passing through cheesecloth and diluted 30 times with distilled water, according to the method of Basra et al. (2011) adapted.

Both macro and micronutrients of MLE were determined using atomic absorption spectrophotometer, according to Silva (2009). In addition, total phenolic was determined by using the Folin-Ciocalteu methodology Barbosa et al. (2019), in which a mix composed of 0.5 mL of MLE, 2.25 mL of Folin-Ciocalteu (7% v/v), 1.75 mL of sodium carbonate, and 0.5 mL of distilled water. Then, the quantification was determined by using a UV−vis spectrophotometer at 765 nm absorbance and the calibration curve was obtained from the gallic acid standard. Similarly, the determination of flavonoid content was performed according to Barbosa et al. (2019), using an aluminum nitrate colorimetric method. In brief, a mix of 0.5 mL of MLE, 0.1 m of aluminum, 0.1 mL of potassium acetate and 4.3 mL of distilled water was prepared and determined in a spectrophotometer at 425 nm absorbance. The standard for the calibration curve was obtained using rutin. Finally, all assays were performed in triplicate.

2.3. Germination and seedling growth parameters

Twenty moringa seeds were placed to germinate in water-moistened germinating paper rolls in the amount of 2.5 times the weight of the dried paper. The rolls were packed into germinators at a constant temperature of 25 °C for 10 days following Pereira et al. (2015). Each treatment was composed of five repetitions and each roll represented a repetition. The following evaluations were performed with the germination test:

- Root protrusion (RP): percentage of seeds with 1 mm long radicle protruded through the seed coat recorded on 10th day after sowing (DAS). The results were expressed in%.
- Germination (G): percentage of normal seedlings recorded on the 10th DAS. The standard adopted to characterize normal and abnormal seedling is shown in Fig. 1. A normal seedling was considered when all essential structures were well developed, completed, proportional, and health, according to Brasil (2009). The results were expressed in%.
- First germination count (FGC): percentage of normal seedlings on 5th DAS. The results were expressed in%.
- Root Protrusion Speed Index (RPSI) and Germination Speed Index (GSI): obtained by daily counting of the number of seeds with radicle protruded and the number of normal seedlings observed,

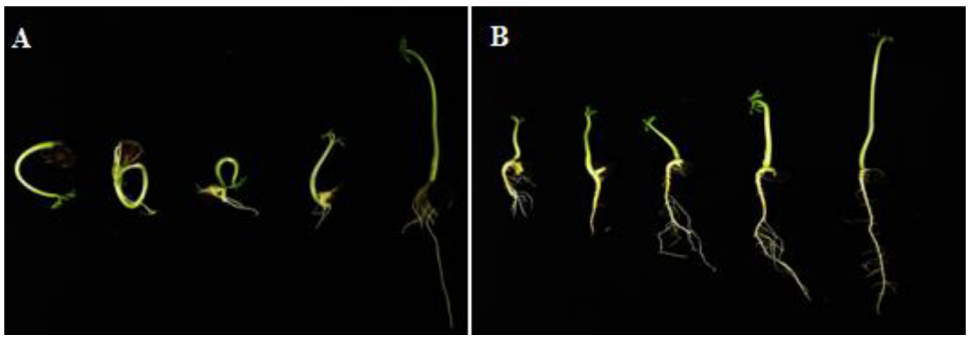

Fig. 1. The standard of abnormal (A) and normal (B) *Moringa oleifera* seedlings from the germination test.

respectively, according to the formula proposed by Maguire (1962).
- Mean Germination Time (MGT): calculated by using the number of normal seedlings per day in the equation proposed by Laboriau (1983).
- Shoot and root length of seedling: on the 5th and 10th DAS, 10 seedlings were randomly selected from each roll and manually measured using a graduated ruler to determine the shoot and root length. The results were expressed in cm.seedling^{-1}.
- Vigor and Uniformity index: obtained using seedling length on the 5th and 10th DAS in the equation proposed by Sako et al. (2001).
- Fresh and dry weight of seedling: after measuring the seedling length on the 10th DAS, the fresh weight was determined by using an analytical balance. Then, the seedlings were placed into paper bags, dried into a forced ventilation even at 65 °C for 48 h and weighed in an analytical balance to determine the dry weight. The results were expressed in g.seedling^{-1}.

2.3. Estimation of antioxidant enzymes

The activity of antioxidant enzymes catalase (CAT) and ascorbate peroxidase (APX) were determined in primed seeds and unprimed seeds (control). The crude enzymatic extracts were obtained by maceration of 0.2 g of seeds after 16 h of soaking in 1.5 mL of 50 mM phosphate buffer (pH 7) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 1% (p/v) polyvinylpolypyrrolidone (PVPP) (Peixoto et al., 1999). The homogenized extract was centrifuged at 14,000 g for 20 min at 4 °C and the supernatants were used to measure the antioxidant enzymatic activity.

The CAT activity was determined by adding 100 μL of the crude enzymatic of seed extract to 2 mL of reaction medium consisting of 50 mM phosphate buffer (pH 7) and 10 mM H_2O_2. The reduction in absorbance was assessed by spectrophotometer at the wavelength of 240 nm for two minutes reaction, recorded at every 15 s (Havir and McHale 1987). The results were expressed in μmol min^{-1}. g^{-1} FW.

The APX activity was determined by adding 100 μL of the crude enzymatic seed extract to 2 mL of reaction medium consisting of 50 mM phosphate buffer (pH 7), 0.1 mM EDTA, 0.25 mM sodium ascorbate and 1 mM H_2O_2. The reduction in absorbance was assessed by spectrophotometer at wavelength 290 nm for 5 min, recorded at every 30 s (Nakano and Asada 1981). The results were expressed in μmol.min^{-1}.g^{-1} FW.

2.4. Plant growth and gas exchange parameters

Hydroprimed seeds and primed seeds with EBL 10^{-8} M, AsA 100 mg.L^{-1}, and MLE 1:30 were sown in 1000 ml polyethylene bags. Non-primed seeds were taken as the control. Three seeds were sown in each bag at a depth of 2 cm using an agricultural substrate. One week later, the more vigorous seedling was left. Each treatment had four repetitions, each one composed of three plants. The plants were kept in a greenhouse for 45 days and the following morphologic parameters were mesured, thus plant height using a graduated ruler from the stem base to the last pair of leaves; the stem diameter determined with a digital calliper and; the number of leaves by counting fully expanded leaves.

To evaluate gas exchange parameters was used an infrared gas analyzer, IRGA equipment (model LI-6400 xt, Li-color Nebraska, USA). Measurements were taken from mature and fully expanded Moringa leaves from 8:00 to 10:00 AM after 45 DAS. The variables evaluated were the photosynthetic rate (μmolCO_2.m^{-2}.s^{-1}), stomatal conductance (molH_2O.m^{-2}.s^{-1}), transpiration rate (mmolH_2O.m^{-2}.s^{-1}) and internal CO_2 concentration (mol.CO_2.mol.ar^{-1}).

2.5. Experimental design and statistical analysis

The experiment was conducted in a completely randomized design (CRD). Germination, seedling growth, and vigor variables were calculated using the Germcalc and PlantCalc functions of the R software (Silva et al., 2019). Data were submitted to normality tests and analysis of variance. The means were compared by the Tukey test at 5% probability using the software R version 4. 2. 1. (R Development Core, 2016).

3. Results

3.1. Moringa leaf extract characterization

The characterization of MLE was performed and the analysis of its constitutes is described as follows: Na (202.50 mg.L^{-1}), K (461 mg.L^{-1}), Ca (214.50 mg.L^{-1}) Mg (101.40 mg.L^{-1}), Fe (0.09 mg.L^{-1}), Mn (0.12 mg.L^{-1}), Zn (0.10 mg.L^{-1}), total phenols (42.10 mg. g^{-1}) and flavonoids (38.17 mg.g^{-1}).

3.2. Germination, seedling growth and antioxidant enzymes

Even though the seed germination percentage of *Moringa oleifera* was not affected by seed priming on the 10th DAS, all primed seeds showed an increase in the FGC (5th DAS) (Table 1). The EBL treatment at 10^{-8} M concentration was the most efficient by increasing germination from 33 to 57% on the 5th DAS. Similarly, the EBL 10^{-8} M provided the highest value of GSI. All primed seeds had a reduction in approximately one day of MGT, compared to the control. Contrary to germination percentage, lower values of MGT are positively related to germination in a shorter time. Although none of the seed priming treatments affected the root protrusion percentage, its speed of occurrence increased significantly in primed seeds with EBL 10^{-10} M and 10^{-8} M compared to the control.

The shoot length of seedlings on the 5th DAS increased in all primed treatments, except for hydroprimed seeds and primed seeds with AsA 50 mg.L^{-1} (Table 2). The highest shoot length was observed

Table 1
Effect of seed priming on Germination (G), First Germination Count (FCG), Germination Speed Index (GSI), Mean Germination Time (MGT), Root Protrusion (RP) and Root Protrusion Speed Index (RPSI) of *M. oleifera*. Values are means ± SE (n = 5) and differences between means were compared by Tukey test (P = 0.05). Means followed by different letters are significantly different.

Priming	Dose	G (%)	FGC (%)	GSI	MGT (days)	RP (%)	RPSI
Non-Primed(Control)	–	47 ± 4.90^{a}	33 ± 7.18^{b}	1.85 ± 0.20^{b}	5.26 ± 0.20^{a}	65 ± 7.58^{a}	5.37 ± 0.62^{c}
Hydro	–	53 ± 6.82^{a}	46 ± 6.96^{ab}	2.49 ± 0.35^{ab}	4.39 ± 0.09^{b}	70 ± 4.18^{a}	7.77 ± 0.77^{abc}
24-EBL	10^{-10} M	57 ± 6.44^{a}	51 ± 6.40^{ab}	2.75 ± 0.30^{ab}	4.29 ± 0.08^{b}	74 ± 3.67^{a}	8.53 ± 0.88^{a}
	10^{-8} M	65 ± 3.54^{a}	57 ± 5.39^{a}	3.11 ± 0.20^{a}	4.37 ± 0.11^{b}	72 ± 3.39^{a}	8.22 ± 0.19^{ab}
	10^{-6} M	45 ± 4.06^{a}	35 ± 2.92^{ab}	2.14 ± 0.23^{ab}	4.42 ± 0.18^{b}	70 ± 8.72^{a}	6.36 ± 0.79^{abc}
AsA	50 mg.L^{-1}	50 ± 4.74^{a}	39 ± 5.57^{ab}	2.18 ± 0.24^{ab}	4.78 ± 0.13^{ab}	76 ± 5.34^{a}	6.38 ± 0.49^{abc}
	100 mg.L^{-1}	60 ± 2.74^{a}	50 ± 2.74^{ab}	2.73 ± 0.12^{ab}	4.51 ± 0.06^{b}	77 ± 3.00^{a}	7.07 ± 0.26^{abc}
	150 mg.L^{-1}	49 ± 2.92^{a}	42 ± 2.00^{ab}	2.35 ± 0.09^{ab}	4.34 ± 0.24^{b}	62 ± 4.64^{a}	6.25 ± 0.32^{abc}
MLE	1:30	57 ± 4.06^{a}	45 ± 3.54^{ab}	2.53 ± 0.17^{ab}	4.67 ± 0.17^{ab}	70 ± 4.47^{a}	5.67 ± 0.34^{bc}

Table 2
Effect of seed priming on shoot and root length of *M. oleifera* seedlings on 5th and 10th day after sowing. Values are means ± SE (n = 5) and differences between means were compared by Tukey's test (P = 0.05). Mean followed by different letters are significantly different.

Priming	Dose	Shoot Length (cm.seedling^{-1})		Root Length (cm.seedling^{-1})	
		5th day	10th day	5th day	10th day
Non-Primed (Control)	–	1.45 ± 0.08^{c}	4.62 ± 0.27^{b}	4.53 ± 0.63^{b}	6.59 ± 0.63^{b}
Hydro	–	1.96 ± 0.11^{bc}	4.59 ± 0.19^{b}	5.64 ± 0.56^{ab}	6.78 ± 0.56^{b}
24-EBL	10^{-10} M	2.23 ± 0.06^{ab}	4.80 ± 0.26^{ab}	7.34 ± 1.10^{a}	8.57 ± 1.10^{ab}
	10^{-8} M	2.43 ± 0.08^{ab}	5.06 ± 0.11^{b}	7.57 ± 0.70^{a}	8.10 ± 0.70^{ab}
	10^{-6} M	2.14 ± 0.14^{ab}	4.36 ± 0.26^{b}	5.70 ± 0.73^{ab}	7.49 ± 0.73^{b}
AsA	50 mg.L^{-1}	1.91 ± 0.16^{bc}	4.90 ± 0.12^{b}	6.65 ± 0.81^{ab}	9.39 ± 0.81^{ab}
	100 mg.L^{-1}	2.18 ± 0.07^{ab}	4.80 ± 0.17^{b}	7.29 ± 0.80^{a}	9.21 ± 0.80^{ab}
	150 mg.L^{-1}	2.75 ± 0.26^{a}	4.79 ± 0.19^{b}	6.82 ± 0.87^{a}	7.46 ± 0.87^{ab}
MLE	1:30	2.32 ± 0.12^{ab}	6.03 ± 0.28^{a}	7.20 ± 0.98^{a}	10.98 ± 0.98^{a}

Table 3
Effect of seed priming on Uniformity and Vigor Index of *M. oleifera* seedlings on 5th and 10th day after sowing. Values are means ± SE (n = 5) and differences between means were compared by Tukey test (P = 0.05). Mean followed by different letters are significantly different.

Priming	Dose	Uniformity Index		Vigor Index	
		5th day	10th day	5th day	10th day
Non-Primed (Control)	–	649 ± 29^{b}	710 ± 17^{a}	490 ± 17^{c}	660 ± 35^{b}
Hydro	–	683 ± 52^{ab}	712 ± 28^{a}	574 ± 28^{bc}	673 ± 32^{b}
24-EBL	10^{-10} M	780 ± 30^{ab}	735 ± 44^{a}	712 ± 44^{ab}	794 ± 71^{ab}
	10^{-8} M	817 ± 15^{a}	798 ± 22^{a}	739 ± 22^{a}	785 ± 43^{ab}
	10^{-6} M	697 ± 41^{ab}	691 ± 38^{a}	583 ± 38^{bc}	723 ± 52^{b}
AsA	50 mg.L^{-1}	728 ± 45^{ab}	758 ± 47^{a}	650 ± 47^{abc}	853 ± 55^{ab}
	100 mg.L^{-1}	816 ± 29^{a}	819 ± 31^{a}	719 ± 31^{ab}	859 ± 44^{ab}
	150 mg.L^{-1}	721 ± 28^{ab}	771 ± 33^{a}	665 ± 33^{ab}	734 ± 51^{ab}
MLE	1:30	783 ± 25^{ab}	735 ± 37^{a}	704 ± 37^{ab}	954 ± 72^{a}

on primed seeds with AsA 150 mg. L^{-1}. However, only primed seeds with MLE 1:30 performed better than the control evaluated on the 10th DAS, increasing by approximately 1.4 cm of shoot length. In addition, all primed seeds improved the root length of seedlings on the 5th DAS, except for hydroprimed seeds, seeds primed with EBL 10^{-6} M and AsA 50 mg.L^{-11}. These observations of our study suggest that there is a narrow concentration range in which 24-epibrassinolide promotes better seedling growth. Evaluating the root length of seedlings on the 10th DAS, MLE 1:30 performed better than any other seed priming studied, increasing approximately 4.39 cm over the control.

Seedling uniformity and vigor were also affected by the priming substances applied (Table 3). EBL 10^{-8} M and AsA 100 mg. L^{-1} provided the highest uniformity index on the 5th DAS, although these differences were not significant on the 10th DAS. The vigor index reached the maximum on EBL 10^{-8} M on the 5th DAS. However, MLE 1:30 stood out for being the greater one to improve this parameter on the 10th DAS. The highest value of fresh and dry seedling weight was observed in MLE 1:30 treatment (Table 4).

The antioxidant system was evaluated by the activity of CAT and APX enzymes in unprimed and primed seeds (Table 4). There was no significant difference among priming treatments for APX activity. However, the maximum activity of CAT was under the treatments of AsA 100 and 150 mg.L^{-1}. The lowest values of CAT activity were observed in AsA 50 mg.L^{-1} and EBL 10^{-6} M treatment. Thus, both concentrations were not efficient in improving the effects of seed priming by increasing the antioxidant system activity, which were reflected in the seedling growth, especially the root length of seedlings on the 5th DAS (Table 2).

Table 4
Effect of seed priming on fresh and dry weight of *Moringa oleifera* seedlings and the activity of CAT and APX enzymes in *M. oleifera* seeds. Values are means ± SE (n = 5) and differences between means were compared by Tukey test (P = 0.05). Mean followed by different letters are significantly different.

Priming	Dose	Fresh Weight (g.seedling^{-1})	Dry Weight	CAT activity Mmol. min^{-1} g FW	APX activity
Non-Primed (Control)	–	0.274 ± 0.017^{b}	0.022 ± 0.001^{b}	0.06 ± 0.01^{ab}	0.22 ± 0.06^{a}
Hydro	–	0.259 ± 0.017^{b}	0.020 ± 0.002^{b}	0.14 ± 0.05^{ab}	0.27 ± 0.09^{a}
24-EBL	10^{-10} M	0.241 ± 0.012^{b}	0.020 ± 0.002^{b}	0.13 ± 0.06^{ab}	0.47 ± 0.21^{a}
	10^{-8} M	0.263 ± 0.011^{b}	0.023 ± 0.002^{ab}	0.05 ± 0.03^{ab}	0.82 ± 0.24^{a}
	10^{-6} M	0.240 ± 0.007^{b}	0.017 ± 0.001^{b}	0.02 ± 0.01^{b}	0.53 ± 0.31^{a}
AsA	50 mg.L^{-1}	0.265 ± 0.016^{b}	0.021 ± 0.001^{b}	0.02 ± 0.01^{b}	0.05 ± 0.02^{a}
	100 mg.L^{-1}	0.282 ± 0.008^{b}	0.022 ± 0.003^{b}	0.20 ± 0.02^{a}	0.05 ± 0.01^{a}
	150 mg.L^{-1}	0.250 ± 0.007^{b}	0.019 ± 0.001^{b}	0.19 ± 0.03^{a}	0.22 ± 0.05^{a}
MLE	1:30	0.391 ± 0.026^{a}	0.033 ± 0.005^{a}	0.11 ± 0.04^{ab}	0.40 ± 0.18^{a}

3.2. Plant growth and gas exchange parameters

Primed seeds with MLE 1:30 was the most efficient to improve the plant height of *Moringa oleifera*, which increased vyapproximately 4.1 cm compared to the control (Fig. 2A). Although the priming products did not show any effect on the stem diameter, the number of leaves was significantly higher in primed seeds with EBL at 10^{-8} M and MLE 1:30 (Fig. 2B and 2C).

The positive effects of seed priming were also observed in the photosynthetic rate of *Moringa oleifera* plants, in which the treatment with MLE 1:30 showed the highest value for this parameter (Fig. 3A). Moreover, treatments with MLE 1:30 and AsA 100 mg.L^{-1} increased significantly the stomatal conductance, compared to the control (Fig. 3B). Similarly, the concentration of internal CO_2 increased in all products primed seeds (Fig. 3C), although there was no significant difference in the transpiration rate (Fig. 3D).

4. Discussion

The positive effects of seed priming on plants are recognized for hastening the germination process and improving plant growth under optimal and stressful conditions (Lutts et al., 2016). Seed priming can reduce imbibition time during germination and also plays an essential role in increasing the speed of germination. Therefore, more vigorous seeds usually take less time to start germination when all external conditions are supplied with water, temperature, and light (Iqbal, 2015). That could be explained by the priming process be consisted of two phases, the first one is partial imbibition followed by re-drying back to the same moisture content before hydration. In fact, this first imbibition provides a "head-start" that accelerate germination (Chen e Arora, 2013; Kubala et al., 2015b). In the present study, all primed seeds increased germination speed, although the most prominent effects were observed in primed seeds with EBL at the concentration of 10^{-10} and 10^{-8} M.

Brassinosteroids are recognized for acting on different processes of plant growth, especially cellular expansion and division (Taiz et al., 2017). Despite being positively regulated by gibberellins during the germination stage, brassinosteroids also contribute to this process but using different metabolic routes (Leubner-metzger, 2001). All plant hormones are characterized by being naturally produced in small quantities. Therefore, BRs are typically known for promoting plant growth in an extreme low amount (Mandava, 1988). Thus, our findings suggested that the 10^{-6} M dose may have interfered in the beneficial effects of BRs to improve germination speed. To maximize the positive effects of BRs, its necessary to adjust its concentration following experimental procedures. For this reason, using exogenous brassinosteroids, such as 24-epibrassinolide seems to be appropriated to improve growth at a certain concentration. Consequently, when the threshold level is reached, the plant may have injurious effects (Wu et al., 2019). In addition, the effects caused by exogenous application of brassinosteroids can vary among different plant species or plant growth stages (Gomes, 2011).

Similarly, the EBL at 10^{-10} and 10^{-8} M improved seedling growth, especially root length at 5 DAS. Reduction in root growth could be associated to one of the first symptoms of seed deterioration during the germination. Even though root protrusion occurs, any failure in this process can affect the root growth for a plant's sensitive part (Bewley et al., 2013). The positive effect of EBL in increasing root growth was reported in sunflower seedlings, in which these both concentrations (10^{-10} and 10^{-8} M) reduced the inhibition of radicle elongation caused by chilling at a rate of 20.6 and 23.9%, respectively (Gornik and Lahuta 2017). The application of brassinosteroids enhanced the root length under stress conditions such as drought (Fariduddin et al., 2009), lead toxicity (Soares et al., 2020), and saline stress (Larré et al., 2015). In addition, BRs seem to be a nontoxic product and environmentally friendly plant hormone, which has gotten the attention of the scientific community and industry market as a promising technology for enhancing yield and quality of different crops (Kang and Guo 2011).

Overall the treatment with MLE 1:30 stood out for being the best priming product to improve seedling growth, mainly root and shoot length, vigor index at 10th DAS, and fresh and dry weight. The

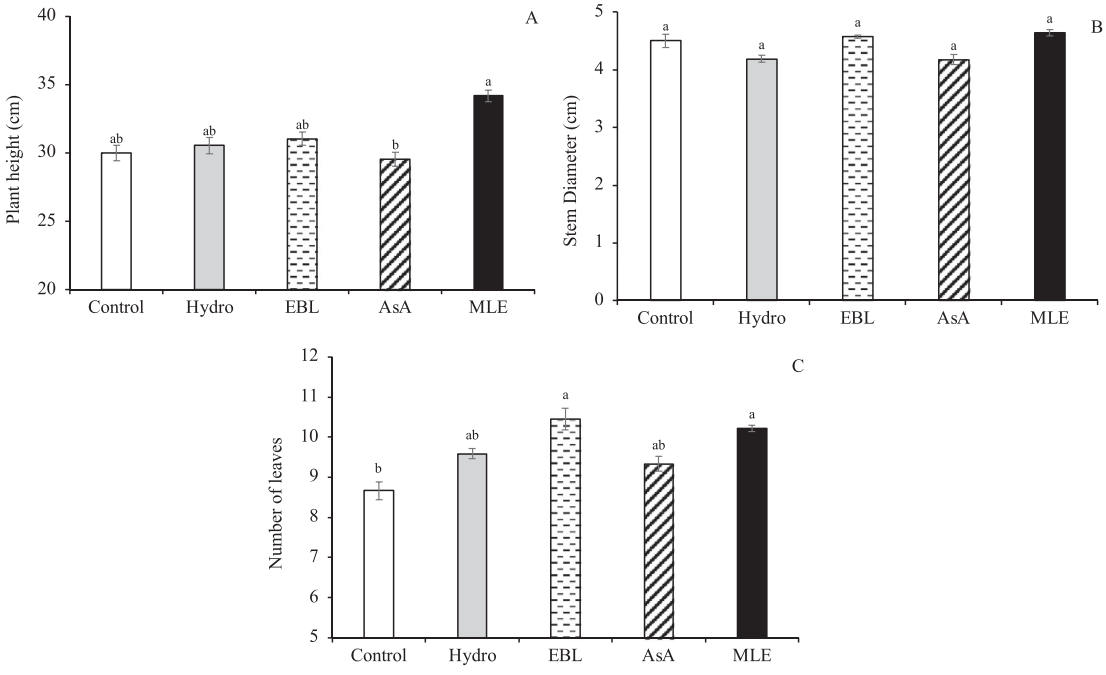

Fig. 2. Effect of seed priming on plant height (A), stem base diameter (B) and number of leaves (C) of *Moringa oleifera* seedling grown in greenhouse after 45 days of sowing. Values are means ± SE (n = 4). Control: no priming; Hydro: primed seeds with water; EBL: primed seeds with 24-Epibrassinolide at 10^{-8} M; AsA: primed seeds with AsA at 100 mg.L^{-1}; MLE: primed seeds with Moringa Leaf Extract at 1:30.

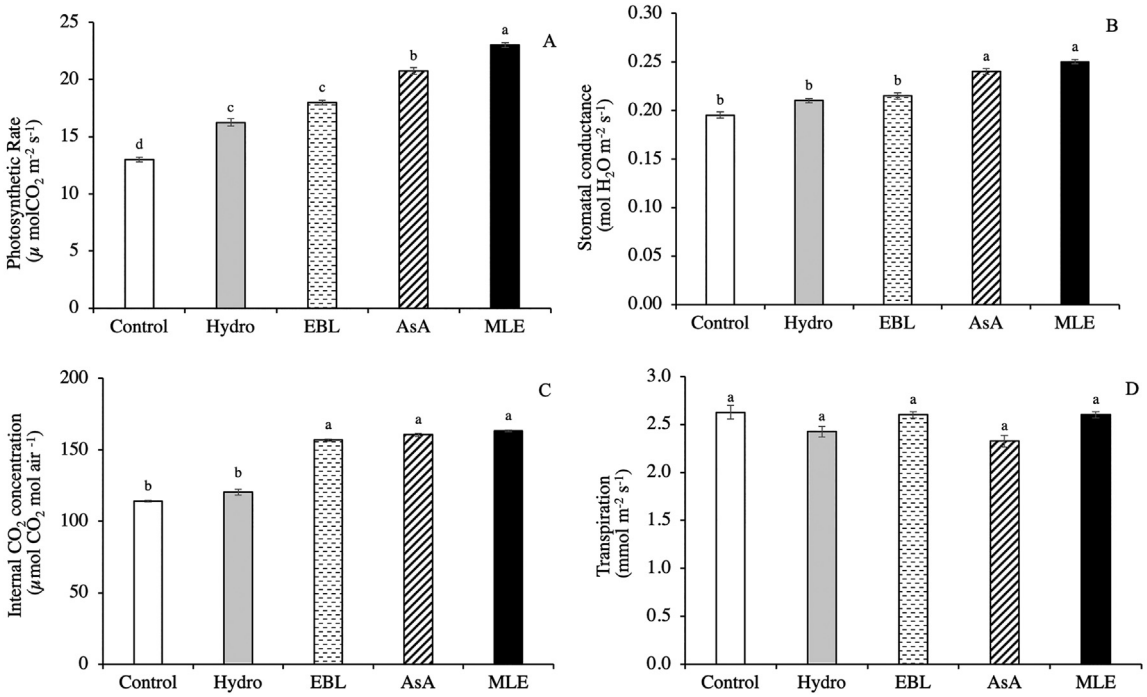

Fig. 3. Effect of seed priming on Photosynthetic rate (A), Stomatal conductance (B), Internal CO_2 concentration (C), and Transpiration (D) of *M. oleifera* seedling grown in greenhouse after 45 days of sowing. Values are means ± SE (n = 5) and differences between means were compared by Tukey test (P = 0.05). Means followed by different letters are significantly different. Control: no priming; Hydro: primed seeds with water; EBL: primed seeds with 24-Epibrassinolide at 10^{-8} M; AsA: primed seeds with AsA at 100 mg.L^{-1}; MLE: primed seeds with Moringa Leaf Extract at 1:30.

benefits provided by MLE seems to be linked with the presence of micro and micronutrients, such as Na, K, Ca, Mg, Fe, Zn and Mn and non-enzymatic compounds, such as flavonoids and phenolic acids as were described in our study. In addition, *Moringa oleifera* leaves are characterized by the presence of plant hormones such as auxin, gibberellins, cytokinin, salicylic acid, jasmonic acid, and abscisic acid, which could also explain its positive effects on plant growth (Ali et al., 2018; Rehman et al., 2017). The MLE utilization in agriculture is recognized for being practical, nontoxic, and improving agronomic cultures' performance (H ur Rehman et al., 2015; Yasmeen et al., al.,2013). MLE can be stored for one month at ambient temperature without decreasing its potential effects (Khan et al., 2017). Seed priming treatment with MLE improved seedling length and dry weight of cowpea (Iqbal, 2015) and wheat (Khan et al., 2017). Moreover, MLE applied in the seed priming is also recognized not only for improving plant growth under optimal conditions but also for ameliorating the adverse effects of a variety of stress, such as $HgCl_2$ (Bibi et al., 2016), saline (Nouman et al., 2014; Rady et al., 2013) and drought (Pervez et al., 2017).

Priming technique is based on hydrating seeds until they reach a point where its metabolism is ready for germination germinate. During this process, many biochemical changes occurs, such as DNA repair mechanisms, degradation and mobilization of reserves, biosynthesis of proteins, and restoration of membranes. In addition, the enzymatic system is active to deal with the oxidative stress by preventing the generation of reactive oxygen species (ROS) (Marcos-Filho, 2015). It is suggested that seed priming responds to proteins that regulate the oxidative stress on post-priming germination (Kubala et al., 2015a). Some genes, such as CAT2 and PER21, were identified as regulators of antioxidative enzymes, e.g., catalase and peroxidase, respectively, and both operate after the application of seed priming techniques (Kubala et al., 2015a). Under optimal conditions, the cell produces a low amount of free radicals (Mittler, 2002). Although, during the germination process, the antioxidative activity is usually increased to deal with ROS generation to develop the embryonic axis (Garnczarska e Wojtyla, 2008). In the present study, primed seeds with AsA 100 and 150 mg.L^{-1} reached the highest values of CAT activity. It is suggested that improvements caused by AsA treatment applied in seed priming are related to the increase of phenolic activity and the activity of antioxidant enzymes that reflects on the enhancement of seed vigor response (Burguieres et al., 2007). Similarly, in our study we noted the increase of seedling growth (Table 2) and seed vigor parameters (Table 3) in the primed seed with AsA 100 and 150 mg.L^{-1}, and probably it consequently reflected on gas exchange parameters such as photosynthetic rate, stomatal conductance, and internal CO_2 concentration of *Moringa oleifera* plants (Fig. 3).

Ascorbic acid improves plant growth by enhancing antioxidant capacity, cell division, and cell enlargement and seems to protect against oxidative damage by controlling cellular redox state (Athar et al., 2008). Normally the increase of ROS implies the increase of antioxidative enzymes to minimize the negative effects of oxidative damage in the cell. In okra seeds treated with AsA 100 mg.L^{-1}, under conditions of lead stress, the activity of CAT increased significantly (Hussain et al., 2017). Besides ascorbic acid be known as an important part of seed antioxidant defense, its function also includes being a co-substrate of enzyme required to the synthesis of other hormones such as ethylene, gibberellins and abscisic acid. Ascorbic acid plays a role during the whole life span of seeds, from embryogenesis to seed filling and dehydration. It is believed that the seed germinability could be assured by the ability to quickly restore the biosynthesis of enzymes related to the ascorbic acid (De Tullio e Arrigoni, 2003).

Primed seeds often generate plants with good growth behavior compared to unprimed seeds. However, there is no agreement if that increase in plant growth is provided by a reduction in time to establish the seedling stage or as a result of physiological adjustments induced by seed priming memory (Lutts et al., 2016). In the present study, plants from primed seeds had a higher rate photosynthetic, stomatal conductance, and internal CO_2 concentration (Fig. 3), which may have lead to improvements in morphological parameters such

as plant height, steam base diameter, and number of leaves (Fig. 2). The transpiration rate (Fig. 3D) had no significant differences among treatments. Transpiration is important to evaluate the water loss in the plant and correlate with leaf water status. To maintain the plant growth, the transpiration process should not surpass the amount of water absorbed by plant. Otherwise, high values of transpiration rate can indicate that the plant metabolism is under a stressful condition (Mir et al., 2020).

In this study, the MLE was the best product to maximize the results of seed priming technique, mainly related to gas exchange parameters (Fig. 3). Similarly, a previous study with the application of MLE in rocket plants showed to increase not only the photosynthetic rate and stomatal conductance, but also photosynthetic pigments, being correlated to the increase of antioxidative enzymes (Abdalla, 2013). Changes in plant metabolism can increase the photosynthetic CO_2 assimilation, such as improvements in photosynthetic machinery by electron transport chain and Rubisco activity; higher stomatal efficiency, which can improve water status; or increases in nutritional content i.e. nitrogen uptake (Mohammadi et al., 2017). Seed priming increases the performance of the antioxidative system that reacts against the ROS formation and represents the second line to control the photoinhibition. The detoxification of ROS by antioxidants is an important protection of photosynthetic apparatus (Logan et al., 2006).

Priming benefits are most usually related to plants in non-optimal conditions due to its impact on plant growth be more evident for increasing stress resistance (Lutts et al., 2016). Despite the idea of using seed priming to improve plant growth under optimal conditions is still not well explored, there are some studies focus on agronomical species such as maize (H ur Rehman et al., 2015), wheat (H ur Rehman et al., 2015), and okra (Sharma et al., 2014). However, there is still a lack of information about this subject related to other forest species, especially using plant growth regulators or natural products such as MLE. To sum up, although brassinosteroids have been considered the best product to increase the speed germination of *Moringa oleifera* seeds, MLE at 1:30 stood out as a better one for the general growth of Moringa seedlings and plants.

5. Conclusions

The present study showed the beneficial role of seed priming treatment to increase seed germination, seedling and plant growth of *Moringa oleifera*. Although MLE did not show any positive effect on seed germination, it was the best product used to improve seedling and plant growth for this species, especially for being a natural eco-friendly substance with a low cost.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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