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Water memory in moringa seeds (*Moringa oleifera* Lam.)[1]

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ABSTRACT - Moringa is a plant native to India, with a wide distribution worldwide, ranging from Asia to Africa, with more occurrence in regions with hot and dry climates, such as the Brazilian semiarid region. The aim of this study was to verify whether moringa seeds present water memory in response to discontinuous hydration and dehydration cycles. The study was conducted at the seed laboratory of the State University of Maranhão in the municipality of Balsas, Maranhão. The experimental design was completely randomized, with a 3 × 3 + 1 factorial arrangement, three soaking times (10, 36 and 52 hours), three hydration cycles (1, 2 and 3) and an additional treatment (control), with four replicates of 25 seeds each. After the seeds were subjected to hydration and dehydration cycles, an emergence test was performed, and the following characteristics were analyzed: emergence, first emergence count, emergence speed index, shoot length, root length, shoot mass and dry mass. The data obtained were subjected to analysis of variance and regression with a 5% probability level, and principal component analysis and response surface analysis were performed. It is assumed that (*Moringa oleifera* Lam). Exhibits water memory has a hydration time of up to 10 hours with a maximum of up to two hydration cycles, and dehydration positively influenced the improvement in most agronomic variables compared with those of the control.

Keywords: Discontinuous hydration. Hydration cycles. Semiarid.

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INTRODUCTION

Moringa (*Moringa oleifera* Lam.) is a plant native to India, belongs to the Moringaceae family, and has a wide distribution worldwide, ranging from Asia, South America and Central America to Africa, with adaptations to locations with dry and hot climates. This region is similar to the semiarid region of Brazil, which has low rainfall rates (Nascimento *et al*., 2021).

This species was introduced in Brazil in 1950 through the Secretary of Agriculture of the State of Maranhão, and from then on, its cultivation has increased over the years in Brazil, especially in the semiarid regions of Northeast Brazil, owing to its great adaptability to climatic conditions varied (Oliveira *et al*., 2020; Silva; Souto; Santos, 2019). One of the most common limitations of Northeast and other regions is related to water scarcity, so the use of species that are tolerant to low water availability without low productivity has become a challenge (Costa *et al*., 2019).

The loss of water by seeds during germination is more common in regions with hot and dry climates, such as semiarid regions, as there is water limitation in these places, and the amount of rainfall is low; thus, the amount of water available for these seeds is short, even in the rainy season (Lima; Meiado, 2017). The seeds of species adapted to these regions undergo long periods of drought, and water, in turn, is one of the factors responsible for the reactivation of the metabolism of these seeds, directly influencing germination; thus, when rains occur, water becomes available, allowing germination to resume (Camacam; Omena Messias, 2022).

Through these cycles of discontinuous hydration and dehydration, the seeds may acquire a high survival rate under these conditions of low water availability, inferring to these seeds the possibility of the presence of some form of water memory, which influences the seed, preserving aspects of previous hydration (Dubrovsky, 1996; Rito *et al*., 2009). Thus, hydration memory is the ability of some seeds to respond more effectively to water stress after undergoing cycles of hydration and dehydration, promoting germination and early growth (Lima; Meiado, 2024). This leads to several advantages for the germination process of seeds, such as a considerable increase in the germination percentage, uniformity and speed, as well as in the production of vigorous seedlings (Hora; Santos; Meiado, 2018). Thus, with the hydration and dehydration of the seeds in the soil in these regions due to the environmental conditions, the hydration and dehydration cycles may enable these seeds to acquire greater resistance as desiccation occurs, resulting in water memory caused by the imbibition process. and drying (Santos; Dantas, 2021).

Water memory allows seeds to maintain the biochemical changes that occur when there is no water available and thus are able to withstand periods of drought, and when water becomes available again, the seeds resume the germination process, thus enabling a high survival rate. during times of low water availability (Santos; Santos; Meiado, 2018; Sarmento *et al*., 2020).

Strategies that use the effect of water memory in seeds can be used to improve germination, growth and seedling yield, increasing the degree of permanence of the crop in the field (Santos Junior; Freitas; Silva, 2021). Therefore, the objective of this study was to determine whether moringa seeds (*Moringa oleifera* Lam.) have water memory occurs in response to discontinuous hydration and dehydration cycles.

MATERIAL AND METHODS

Location of the experiment

The study was conducted at the Seed Laboratory of the State University of Maranhão, Campus Balsas, municipality of Balsas, Maranhão. The seeds of Moringa (*Moringa oleifera* Lam.) were acquired through producers and/or sales specialists in the seeds of native and exotic plants.

Imbibition and dehydration curves

To determine the imbibition curve, four replicates of 25 seeds were weighed on an analytical scale to obtain their initial weight. Each replicate was subsequently placed on germitest paper and moistened with 2.5x the weight of the paper by volume of sterile water. Each replicate was weighed at two-hour intervals during the first 24 hours and at four-hour intervals until the radicle protruded into the seeds. After the imbibition curve of the species was established, three points, X, Y and Z, were selected on the curve.

To determine the dehydration curve, four replicates of 25 seeds were weighed on an analytical scale to obtain the initial weight. Each replicate was subsequently placed on germitest paper and moistened with 2.5x the weight of the paper by volume of sterilized water for a period corresponding to the Z time of the imbibition curve, where the seeds absorbed the maximum amount of water before germination. After hydration at time Z, the replicates were removed from contact with water, placed to dry and weighed on an analytical scale at intervals of 2 hours until the weight of the replicates returned to the initial weight.

Hydration and dehydration (HD) cycles

For each time point established through the soaking curve (X, Y and Z), the seeds were subjected to 0 (control), 1, 2 and 3 hydration cycles, with a dehydration time between each cycle corresponding to the drying time of the seeds, which was obtained through the dehydration curve. The experimental design adopted was completely randomized, with a 3 x 3 + 1 (control) factorial arrangement, with three soaking times and three hydration cycles with 12 treatments of 25 seeds each, where 100 seeds were used per cycle, totaling 300 seeds for each hydration time + 100 seeds that composed the control, totaling 1000 seeds. The discontinuous hydration of the seeds was performed in plastic trays containing two layers of germistest paper moistened with 2.5x the weight of the paper by volume of sterilized water. For the dehydration phase, the seeds were transferred to plastic trays with paper towels. Each cycle corresponds to a hydration phase followed by a dehydration phase.

Emergence tests, evaluated parameters and statistical analysis

After the seeds went through the hydration and dehydration cycles, the emergence test was carried out, referring to the 12 treatments, which consisted of 4 replicates of 25 seeds in each treatment, where they were placed in 10 kg pots with sand, totaling 48 pots, and their irrigation was carried out to keep the soil close to its field capacity. The number of emerged seeds was counted daily for a period of 21 days. During the experiment, the following characteristics were analyzed:

Emergence (EME): In the emergence test, the germinated seeds were counted daily until their complete stabilization. Those whose cotyledons were above the soil surface were considered emerged seedlings. First emergence count (FEC): This test was performed together with the seedling emergence test, and normal seedlings were counted four days after the beginning of the test. Those whose cotyledons were above the soil surface were considered. The results are expressed as the percentage of normal seedlings. Emergence velocity index (EVI): calculated according to the formula of Maguire (1962). Shoot length (SL): The shoot part growth assessment was performed only on normal seedlings on the twenty-first day after sowing, and the shoot part length was measured with the aid of a ruler graduating in centimeters from the seedling neck to the top of the primary leaf. Root length (RL): This measurement was performed only on normal seedlings. On the twenty-first day after sowing, the length of the taproot was measured with the aid of a ruler graduating in centimeters from the root tip to the neck of the seedling. Shoot dry mass (SDM): The dry mass was obtained after the shoot was dried. These samples were weighed on a precision analytical balance, and the values were expressed in mg/seedling (Nakagawa *et al.*, 1999). Root dry mass (RDM): The dry mass was obtained after drying the root mixture. The roots were weighed on a precision analytical balance, and the values are expressed in mg/seedling (Nakagawa *et al.*, 1999).

The data obtained were subjected to analysis of variance, and when significant, complementary analyses were applied to identify the presence of water memory. Since the study has two sources of variation with quantitative effects (hydration and dehydration cycles) and the variables analyzed are also quantitative, the presence of water memory in the species, in each variable, will be verified using response surface analysis, in which the existence of an optimum point for a variable outside the initial levels of hydration and dehydration will be an indication of the presence of water memory. Finally, to verify the possible effect of water memory considering all levels of hydration and dehydration and all variables simultaneously, the data were subjected to principal component analysis. The analyses were performed using the Genes program (Cruz, 2016), and principal component analysis was performed using the PAST 13 program.

RESULTS AND DISCUSSION

Hydration and dehydration curves

The water absorption in the first phase was considerably fast (Phase I), followed by a stable phase (Phase II) and then a resumption of water absorption (Phase III), which occurred only when germination was accomplished, as the embryonic axis elongates and breaks through its covering structures, presenting a three-phase model similar to the model proposed by Bewley and Black (1994).

The beginning of phase I in moringa seeds occurred in the first hours, with a high speed of water absorption, which lasted 20 hours (Figure 1A). Coll *et al.* (2001) reported that the amount of soaked water and the absorption rate change according to the composition of the seed coat. Moringa has a smooth and porous tegument, which favors water absorption (Noronha; Medeiros; Pereira, 2019).

In phase I, the beginning of seed hydration, and subsequently, of subcellular proteins, structural changes such as membrane and DNA repair, in addition to the beginning and increase of respiration, occur in the first 12 hours (Turazzi *et al*., 2021). The increase in respiratory activity is proportional to the increase in seed tissue hydration.

The period between 22 and 68 hours was characterized as phase II, called stationary, identified by the large reduction in the hydration rate. In moringa seeds, the amount of water imbibed is reduced, and from this point on, there is a resumption of water absorption and the beginning of the third phase. Phase III lasted longer than did Phase I (48 hours). The imbibition curve of *Moringa oleifera* Lam. showed a three-phase pattern, with germination occurring 128 hours after the beginning of seed hydration. Points X, Y and Z of the soaking process corresponded to 10, 36 and 52 hours, respectively, and the soaked seeds took 30 hours to dehydrate and return to the initial weight.

After the imbibition curve of the crop species was defined, the dehydration curve (Figure 1B) was obtained by imbibing the seeds until time X (10 h) of Phase I, when the seed absorbs the maximum amount of water until the beginning of the phase. II; thus, the seeds were dehydrated from this point, resulting in 30 hours of total dehydration.

Analysis of variance and response surface

Tables 1 and 2 show the mean data of the variables evaluated as a function of hydration, cycles, interaction between hydration and cycles and the contrast between hydrations, cycles and the control.

Figure 1 - Hydration curves (A) and dehydration (B) of *Moringa oleifera* Lam seeds (I, II and III) after hidratation

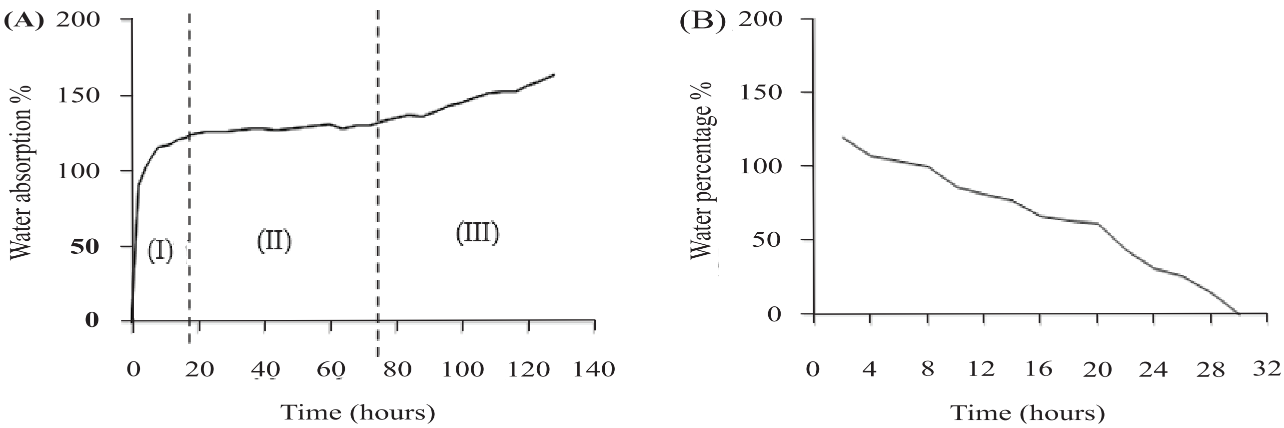


Table 1 - Analysis of variance refers to the evaluated characteristics, first emergence count (FEC), emergence (EME), emergence speed index (ESI) and shoot length (SL) as a function of different hydration times (H), cycles (C), interactions between hydration cycles (H×C), and contrasts between hydrations, cycles and controls (H×C×T)

	Mean Squares			
	FEC	EME	ESI	SL
Hydration (H)	300.44*	940.33**	0.78*	3.25
Cycles (C)	120.44*	4107.0**	8.37**	27.68
H_x×C	52.44*	531.83*	0.75**	6.88
H×C×T	1012.87**	359.76*	0.86*	478.65**
CV (%)	33.39	14.77	18.62	18.77

**and *indicate significance at the 1% probability and 5% probability, respectively, according to the F test. CV - Coefficient of variation

Table 2 - Analysis of variance, referring to the evaluated traits, including root length (RL), shoot dry mass (SDM) and root dry mass (RDM) as a function of the different hydration times (H), cycles (C), interactions between hydration and cycles (H×C), and contrasts between hydrations, cycles and controls (H × C × T)

	Mean Squares		
	RL	SDM	RDM
Hydration (H)	0.22	1.80**	0.001
Cycles (C)	4.89*	11.11**	0.03**
H × C	4.11**	1.95**	0.008
H × C × T	0.03	0.21	0.03**
CV (%)	10.98	24.27	28.46

**and *indicate significance at the 1% probability and 5% probability, respectively, according to the F test. CV - Coefficient of variation

In terms of the first emergence count (FEC), the percentage of seeds belonging to the 36 h group that underwent 3 hydration and dehydration cycles (H36-C3) was not significantly different from that of the control, with values of 21 and 28%, respectively (Figure 2).

In terms of emergence (Figure 3), after 10 hours with up to 2 hydration and dehydration cycles and 36 hours with up to 1 hydration and dehydration cycle, the other treatments differed from H36-C3 and the control, with lower average values for the first emergence count. According to Nakagawa *et al.* (1999), the first count test is based on the principle that the samples with a higher percentage of normal seedlings at the first count are more vigorous because they germinate more quickly. Santos, Santos and Silva (2024) reported that hydration/dehydration treatments can promote, inhibit or have no effect on seeds. In the studies of Contreras-Quiroz *et al*. (2016) of eight tested species, only four species were affected by the treatments (*Echinocactus platyacanthus, Ferocactus pilosus, Lesquerella virginicum* and *Nassella tenuissima*), resulting in higher percentages of germination in one or more of the hydration/dehydration treatments than in the control group, whereas the three cycles and longer cycles promoted seed germination in four and two species, respectively, which is in agreement with Dubrovsky (1996), who reported that repeated hydration/dehydration cycles had a cumulative effect on the seeds.

Figure 2 - First emergence count (FEC) of *Moringa oleifera* Lam. seeds under different hydration and dehydration times and cycles. Different capital letters in columns indicate significant differences according to Tukey's test. C1: one hydration cycle, C2: two hydration cycles, C3: three hydration cycles, H10: hydration for 10 hours, H36: hydration for 36 hours, H52: hydration for 52 hours

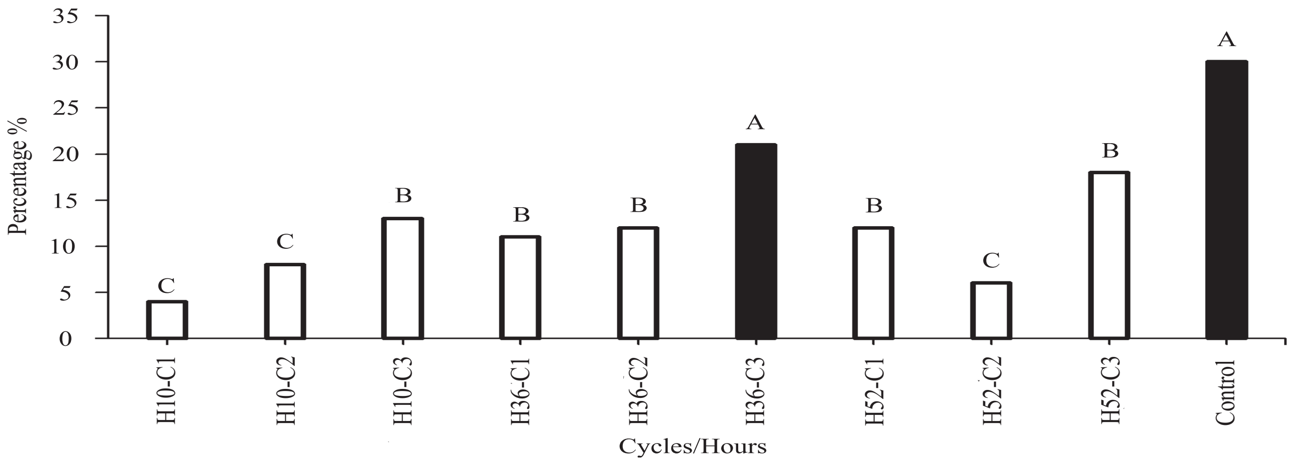
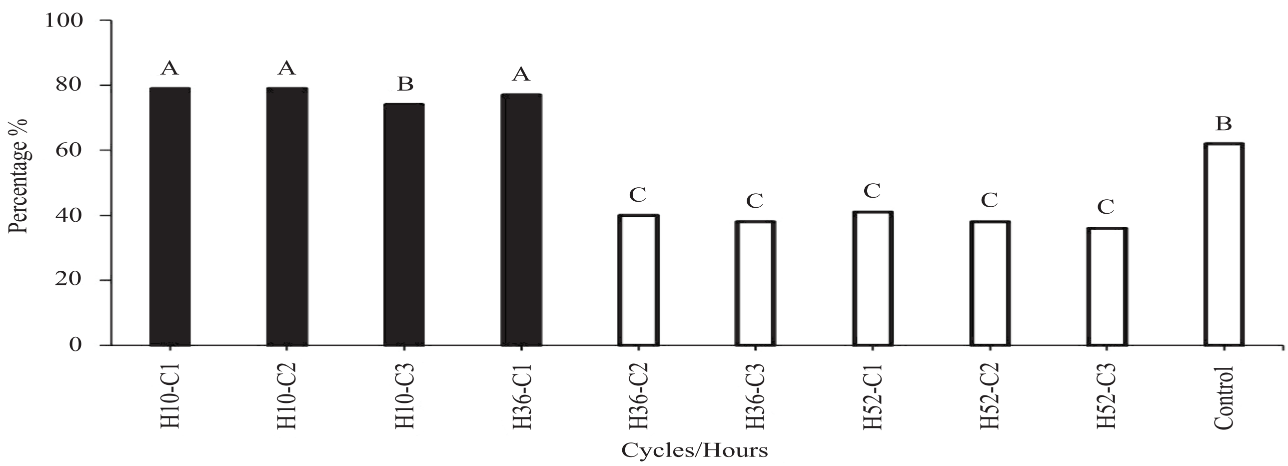


Figure 3 - Emergence of *Moringa oleifera* Lam. seeds under different hydration and dehydration times and cycles. Different capital letters in columns indicate significant differences according to Tukey's test. C1: one hydration cycle, C2: two hydration cycles, C3: three hydration cycles, H10: hydration for 10 hours, H36: hydration for 36 hours, H52: hydration for 52 hours



In terms of the emergence velocity index (EVI), the treatments involving times of 10 with up to 3 hydration and dehydration cycles and 36 with up to 1 cycle yielded better means and significantly different results than did the control, where H10-C1, H10-C2 and HC10-C3 presented IVE values of 3.1, 3.1, and 3.3, respectively, while the control had a value of 1.8 (Figure 4). In the studies by Sarmento *et al*. (2020), the application of three cycles of hydration and dehydration for a period of 13 h in sorghum seeds improved tolerance to water deficiency conditions during seedling development.

Depending on the plant species, successive hydration–dehydration cycles may be a tool capable of improving the germination performance of seeds (Caçula *et al*., 2022). With respect to shoot length, the results revealed that there was no difference between the treatments that passed through for 10, 36 and 52 h, regardless of the number of hydration cycles (0, 1, 2 and 3), for this variable; a difference was observed only in comparison with the control, which presented the worst averages, with plants reaching 13 cm in length. (Figure 5).

For root length, we can observe from the response surface graph (Figure 6) that the maximum point occurred at a hydration time of 52 hours, with 0 dehydration cycles.

Figure 4 - Emergence velocity index (ESI) of *Moringa oleifera* Lam. seeds under different hydration and dehydration times and cycles. C1: one hydration cycle, C2: two hydration cycles, C3: three hydration cycles, H10: hydration for 10 hours, H36: hydration for 36 hours, H52: hydration for 52 hours

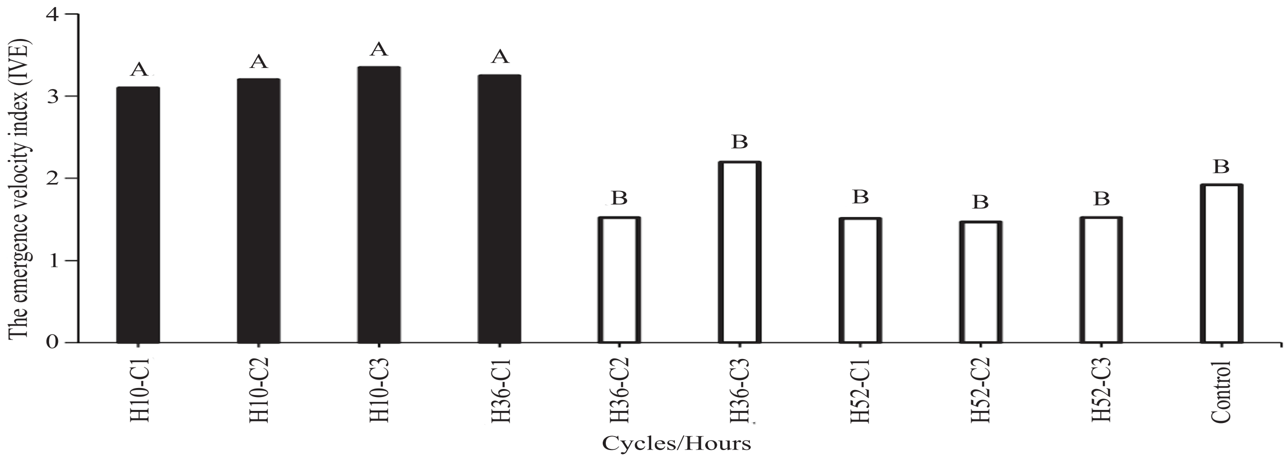
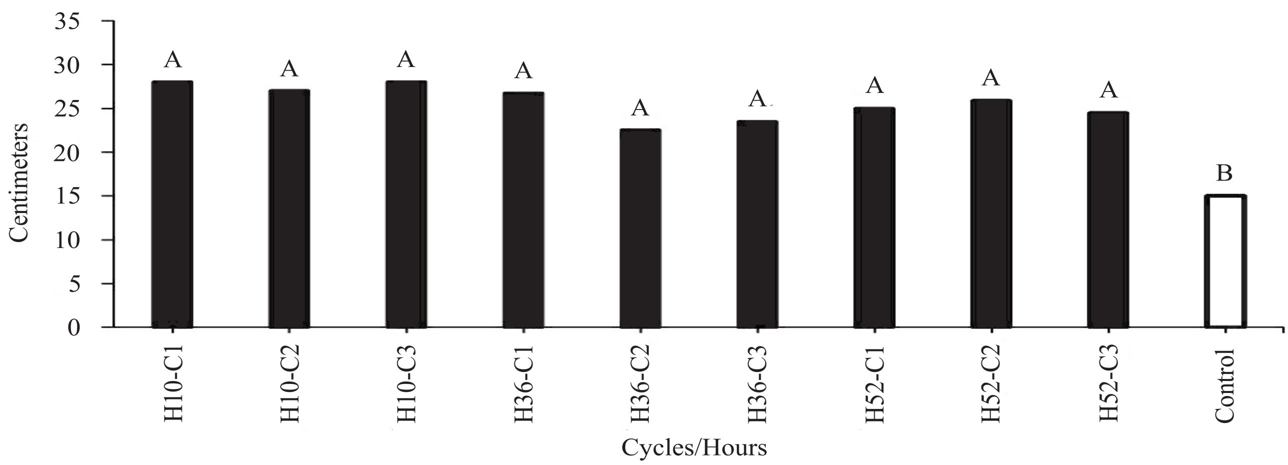
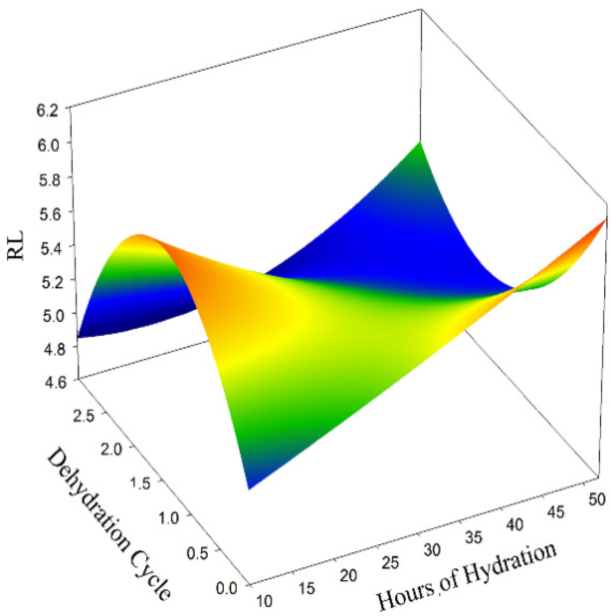


Figure 5 - The shoot length (SL) of *Moringa oleifera* Lam. seeds under different hydration and dehydration times and cycles. C1: one hydration cycle, C2: two hydration cycles, C3: three hydration cycles, H10: hydration for 10 hours, H36: hydration for 36 hours, H52: hydration for 52 hours



These results can be explained by the need for a high water content by the seed for a certain period of time to trigger the germination process, which comprises the mobilization of reserves and the readjustment of the cell membrane, culminating in the protrusion of the root, where this measurement is of paramount importance because plants with greater root depth have better fixation when planted in the field and the ability to obtain water and nutrients from deeper soil layers, thus better resisting environmental variations (Silva; Viégas; Galvão, 2020).

Figure 6 - Root length of *Moringa oleifera* Lam (RL) over different hydration and dehydration times and cycles



For the shoot dry mass (SDM), the best average value of 3.1 grams refers to the set of 10 hours of hydration with 0.7 hydration and dehydration cycles, whereas for the root dry mass (RDM) variable, the best average value refers to the time of 10 hours with up to 1.5 hydration cycles, reaching 0.31 grams (Figures 7A and 7B). Ozden, Ermis and Demir (2017), through experiments with *Antirrhinum* spp. (Plantaginaceae) and *Dahlia* spp. (Asteraceae), reported that the seedlings of these studied species, which were produced from seeds previously subjected to hydration and dehydration cycles, presented greater biomass.

Principal component analysis

The principal component analysis (PCA) graph below (Figure 8) involves the variables evaluated in relation to hydration time (0, 10, 36 and 52 h) and dehydration (30 h) together with the number of cycles (0, 1, 2 and 3) and explains 86.501% of the total variance in the data, with 63.517% explained by component 1 and 22.984% explained by component 2.

According to Rencher and Christensen (2002), the percentage of total variance that must be explained by the first two principal components is at least 70%; thus, PCs 1 and 2 are sufficient to represent the variation in the original data.

Figure 7 - (A) Shoot dry mass (DSM) and (B) root dry mass (RMS) of *Moringa oleifera* Lam under different hydration and dehydration times and cycles

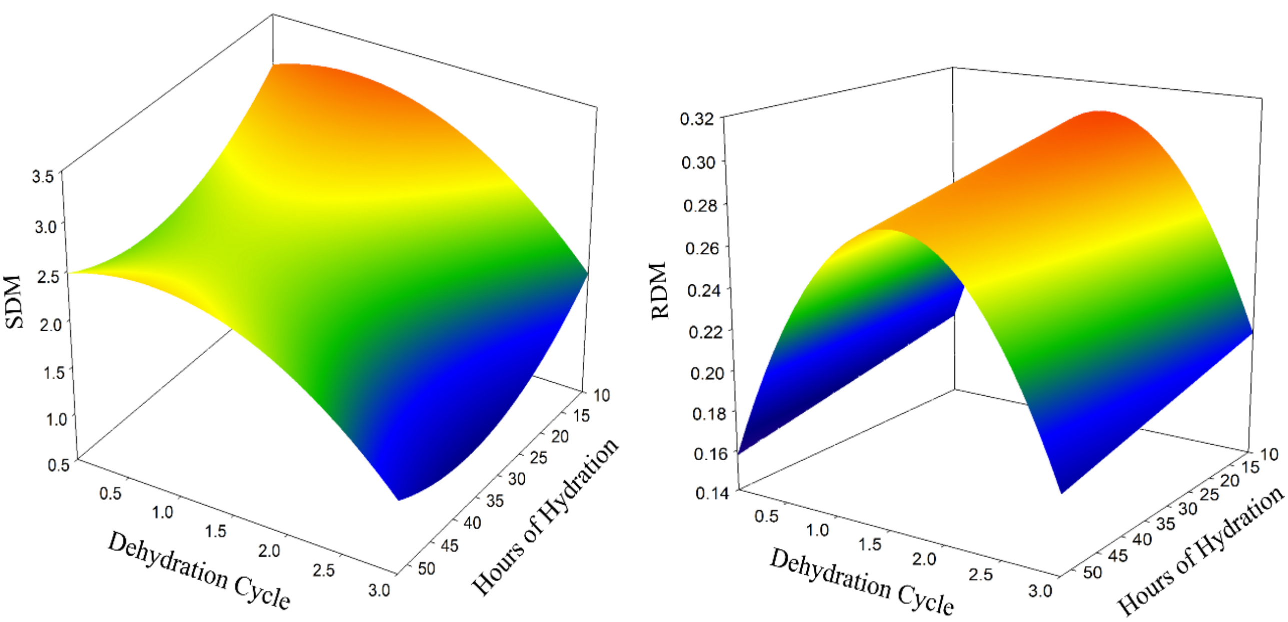
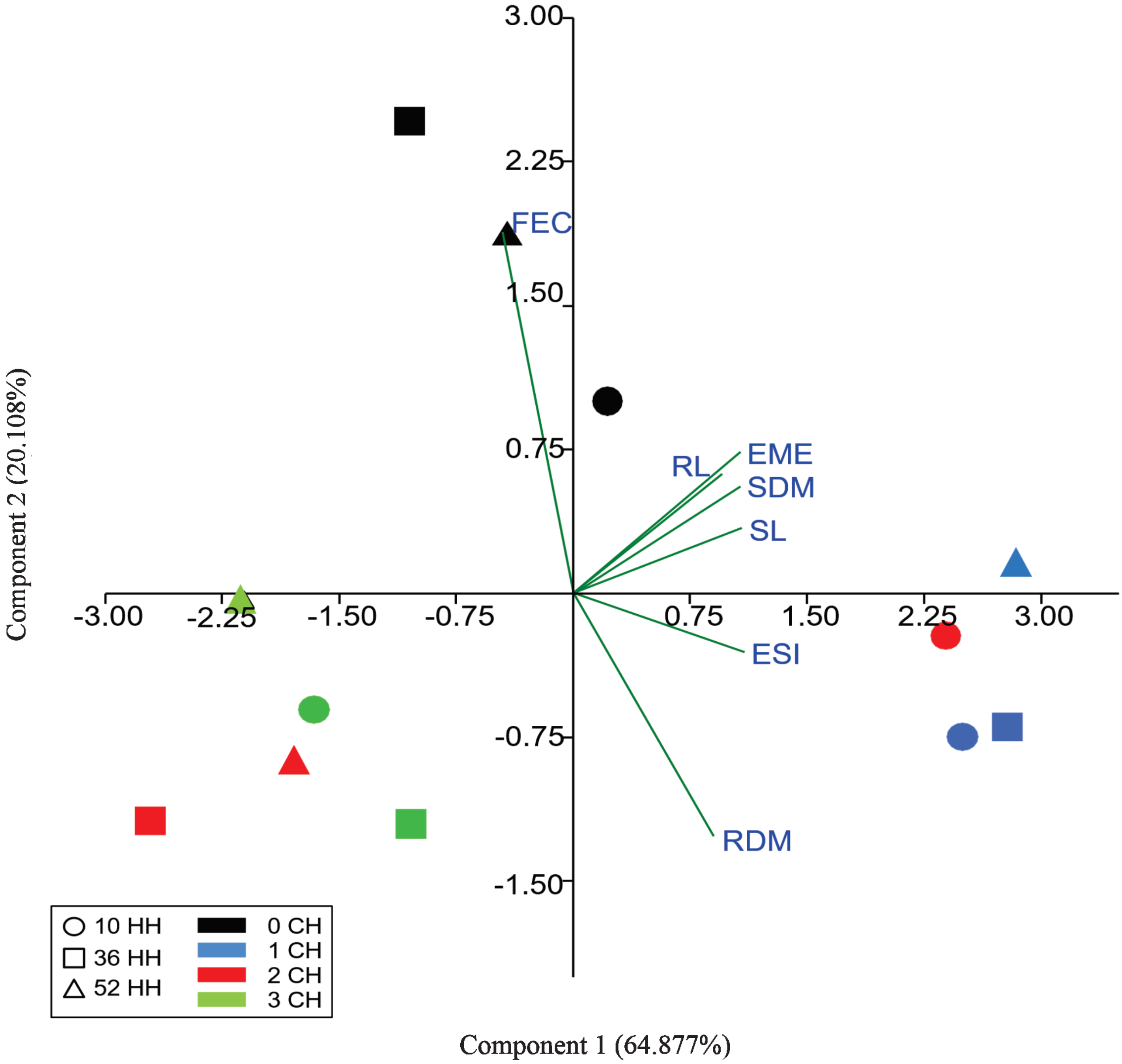


Figure 8 - Grouping of the evaluated variables on the basis of analysis of the principal components. Abbreviations: first emergence count (FEC), emergence speed index (ESI), emergence speed (EME), shoot length (SL), root length (RL), shoot dry mass (SDM), root dry mass (RDM), hours of hydration (HH), dehydration cycle (HC)



Through the grouping of the variables, we can observe that the variables emergence velocity index (EVI), root dry mass (RDM), root length (RL), shoot dry mass (SDM) and emergence (EME) have the largest contributions to principal component 2, since the vectors of these variables point to points that indicate that treatments that underwent 1 hydration cycle, regardless of the number of hours of hydration (10, 36, 52), obtained better results for the characteristics mentioned above. However, the graph shows that *Moringa oleifera* Lam. up to 2 hydration cycles and limited to 10 hours of hydration; however, the average results were the same. The hydration and dehydration cycles in the aroeira-do-sertão seeds promoted better initial seedling development, more significantly influencing germination, which is directly related to other factors of initial development. The treatments used for the production of the seedlings included 2 cycles of hydration and dehydration (Hora; Meiado, 2016). We can see that the vector of the variable First Emergence

Count (ECP) is heading toward the indicator point of the control, indicating that the same presented higher means for the First Emergence Count compared with the treatments that went through the hydration times and cycles and dehydration.

In the studies conducted by Lima and Meiado (2018), *M. tenuiflora* seeds that underwent hydration and dehydration cycles produced more vigorous seedlings with greater shoot and root lengths and consequently greater shoot and root dry mass, corroborating this hypothesis. Seeds that undergo hydration and dehydration cycles produce more vigorous seedlings. In studies by Lima and Meiado (2017) on discontinuous hydration with two populations of cactus *Pilosocereus catingicola* (Gürke) Byles & Rowley subsp. salvadorensis, germination was favored, and the presence of water memory was confirmed. The presence of water memory was also recorded in the seeds of *Senna spectabilis* (DC.) HS Irwin & Barneby var. *excelsa* (Schrad.) HS Irwin & Barneby (Fabaceae) through hydration and dehydration cycles (Lima *et al.*, 2018).

Cycles of hydration and dehydration in nature enable biochemical changes and degrade macromolecules in reserve tissues, such as sugars and proteins, which are entirely related to the effects of passage through cycles (Alvarado-López *et al*., 2018; Dubrovsky, 1998).

Some species have the ability to undergo hydration cycles, mobilizing macromolecules for the embryo and maintaining physiological changes soon after dehydration; this effect is called seed hydration memory (hydric memory), an important mechanism of action that influences the life cycle of plants (Dubrovsky, 1996; Rito *et al*., 2009). The potential to resist hydration and dehydration cycles is an important adaptation to improve seedling establishment in arid and semiarid environments. The presence of water in the seeds of *Moringa oleifera* Lam. This may explain the wide distribution of this species in hot regions with little rainfall from which it originated.

CONCLUSIONS

1. It is assumed that the species *Moringa oleifera* Lam. has water memory has a hydration time of up to 10 hours with a maximum of up to two hydration cycles, and dehydration positively influences the improvement in most agronomic variables compared with those of the control;
2. The hydration and dehydration intervals should be analyzed according to the behavior of each species, as they can be influenced by the different ecosystems where they are located.

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