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ORIGINAL PAPER

Physiological response of *Luffa aegyptiaca* seeds subjected to water deficit and heat

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Abstract: Sponge gourd (*Luffa aegyptiaca*) is a plant widely used in folk medicine, mainly in Northeast Brazil. It is propagated by seeds and can be influenced by biotic factors such as temperature and water deficit. Thus, the objective was to evaluate the effect of water deficit and heat stress on the seed quality of *L. aegyptiaca*. The experiment was performed at the Seed Analysis Laboratory of the Federal University of Paraíba, Areia, Paraíba, Brazil. The statistical design was completely randomized in a 5 × 4 factorial scheme, with five water potentials (0.0, -0.2, -0.4, - 0.8, and -1.2 MPa) and four temperatures (20, 30, 35, and alternating between 20-35°C), with four replications of 50 seeds. *L. aegyptiaca* was tolerant to high temperatures and water deficit during the germination phase and initial growth of seedlings. The highest germination percentage of *L. aegyptiaca* seeds was observed at a temperature of 35°C and water potential of -0.4 MPa.

Keywords: Sponge gourd, germination, osmotic potential, vigor.

Introduction

Luffa aegyptiaca Mill. is a species belonging to the Cucurbitaceae family that is widespread in several parts of the world, such as South America, Africa, and Asia (Mavi et al., 2018). In Brazil, it is found mainly in the North and Northeast regions, and it is popularly known as sponge gourd, "esfregão", vegetal sponge, and "bucha dos paulistas". It has several uses, such as handicrafts, packaging, filters for swimming pools, lining benches, and removing pollutants from water (Kesraoui et al., 2016). The extract of this plant is used in folk medicine as a purgative, deblocking agent, vermifuge and wound healing (Ani et al., 2018).

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Sponge gourd is propagated sexually. Its seeds are distributed variably in fruits, being necessary ideal conditions for the seeds to germinate. However, several environmental factors, such as temperature and water availability, have a direct influence on seed germination and quality (Ribeiro et al., 2019).

Water availability is one essential factor for seed germination since it is, directly and indirectly, involved in all germination metabolism stages (Nóbrega et al., 2021). Thus, the water acts as a stimulating and controlling agent, regulating the rehydration of seed tissues, promoting an increase in respiratory intensity, and stimulating other metabolic processes, favoring the degradation of reserves and promoting the embryonic axis growth (Lechowska et al., 2019).

Temperature is another factor that directly influences germination, acting on speed, uniformity, and the total percentage of germinated seeds. Temperature interferes both in the dynamics of water absorption, as well as in biochemical reactions, in addition to the physiological processes that determine the entire germination process (Lima et al., 2019; Leal et al., 2020; Nóbrega et al., 2022).

Thus, the present work was developed with the objective was to evaluate the effect of water deficit and heat stress on the seed quality of *L. aegyptiaca*.

Materials and Methods

The experiment was performed at the Seed Analysis Laboratory, Department of Plant Science and Environmental Sciences, of the Agricultural Sciences Center, Federal University of Paraíba, Campus II, Areia (6° 57’ 42” S and 37° 41’ 42” W), Paraíba, Brazil.

Seeds were collected from the fruits of 60 plants of *L. aegyptiaca*, originating from the second multiplication of propagating material (seeds) in an experimental field belonging to the Department of Plant Science of the Federal University of Santa Maria (UFSM), Santa Maria (29º 42’ S, 53º 49’ W, at an elevation of 95 m), Rio Grande do Sul, Brazil.

Seeds were manually extracted and placed at room temperature for 48 h to remove the mucilage. Samples were taken for analysis and the other seeds were packed in kraft paper and stored in a cold chamber at 15ºC temperature and 60% relative humidity.

The statistical design was completely randomized in a 5 × 4 factorial scheme, with five water potentials (0.0, -0.2, -0.4, -0.8, and -1.2 MPa) and four temperatures (20, 30, 35, and alternating between 20-35°C), with four replications of 50 seeds. Polyethylene glycol (PEG 6000) was used for the water deficit simulation, and its concentrations were formulated according to the specifications of Villela et al. (1991). The temperatures were based on a study by Nóbrega et al. (2022) with the species *Crotalaria spectabilis*.

Germination was performed in a BOD (Biochemical Oxygen Demand) germination chamber, set at pre-established temperatures and a photoperiod of 8 h, using sheets of Germitest® paper moistened with 2.5 times the weight of dry paper with the PEG 6000 solutions at the aforementioned potentials. Germination (G%) was evaluated from the 4th to the 15th day after the test began, considering the germinated seeds that emitted root protrusion and leaf primordia, and the results were expressed in the percentage of normal seedlings (BRASIL, 2025).

The first germination count (FCG) was obtained by counting the number of seeds germinated on the 4th day of the germination test, and the results were determined as the percentage of germinated seeds. The germination speed index (GSI) was determined by daily counts of the number of germinated seeds, calculating the results according to the Equation 1 proposed by Maguire (1962).

$$\mathrm{GSI} = \frac{G1}{N1} + \frac{G2}{N2} \ldots + \frac{Gn}{Nn} \qquad (1)$$

Where: G1, G2 ... Gn – number of seeds germinated in each count; N1, N2 ... Nn – number of days elapsed from sowing to the 1st, 2nd... nth count.

The average germination time (AGT) was established from daily counts of the number of germinated seeds, with results determined according to the Equation 2 proposed by Labouriau (1983).

$$\text{AGT}=\frac{\sum (\text{ni} \times \text{ti})}{\sum \text{ni}} \qquad (2)$$

Where: ni – number of seeds germinated in the given time interval; ti – time (days or hours) elapsed from the start of the test until interval i; ∑ni – total number of seeds germinated at the end of the test.

Abnormal seedlings (AS%) were measured from the seedling count on the 15th day of the germination test, with the results expressed as a percentage (BRASIL, 2025). The average germination speed (AGS) was established according to the methodology of Labouriau and Valadares (1976), using Equation 3.

$$\text{AGS} = \frac{\sum \text{ni}}{\sum (\text{ni} \times \text{ti})} \qquad (3)$$

Where: ni – number of seeds germinated in the given time interval; ti – time (days or hours) elapsed from the start of the test until interval i; ∑ni – total number of seeds germinated at the end of the test.

Root (RL), shoot (ShL), and seedlings lengths (SL) were evaluated 15 days after installation of the germination test using a graduated ruler, and the results were expressed in cm. At the end of the experiment, root (RDM), shoot (ShDM), and total dry mass (TDM) were quantified. The fresh material was placed in Kraft paper bags and placed to dry in a forced air circulation oven at 65°C until they had constant weight. A precision analytical scale (0.0001 g) was used to weigh the material, with the results expressed in g per seedling.

Data normality was evaluated using the Shapiro-Wilk test ($p \leq 0.05$). Subsequently, analysis of variance was performed by the F-test ($p \leq 0.05$). Data on water potentials were subjected to polynomial regression analysis, while temperature means were separated by Tukey's test ($p \leq 0.05$). All statistical analyses were performed using the statistical program Sisvar (Ferreira, 2019). A principal component analysis with clusters was conducted to study the interrelationship between treatments and variables. The statistical program R (R Core Team, 2023) was used for this analysis.

Results

According to the summary of the analysis of variance (Table 1), significant interactions ($p \leq 0.01$) between water potentials and temperatures were observed for germination percentage (G%), germination speed index (GSI), average germination time (AGT), average germination speed (AGS), and percentage of abnormal seedlings (AS%). For the first germination count (FGC), there was an isolated effect on temperature.

For the G% (Figure 1A), the data obtained at 30, 35, and the alternating temperatures between 20-35ºC were well fitted by a quadratic model. Maximum G% values of 77.4, 87.1, and 79.3% were estimated at water potentials of -0.7, -0.6, and -0.2 MPa, respectively. At 20ºC, G% increased progressively across the water potentials levels, ranging from 69.5% at -0.2 MPa to 78.6% at -1.2 MPa.

For the GSI (Figure 1B), it can be observed that for all water potentials, the temperature of 35ºC showed the highest values, while 20ºC presented the lowest values at potentials of 0.0, -0.2, -0.4, and -0.8 MPa. When observing the effect of water potentials, it is verified that under temperatures of 20 and 35ºC, the highest values (6.06 and 7.03, respectively) were obtained at a potential of -0.7 MPa, while at 30ºC and alternating between 20-35ºC, the highest estimated values occurred at a potential of -0.5 MPa.

Table 1. Summary of analysis of variance for germination percentage (G%), first germination count (FGC), germination speed index (GSI), average germination time (AGT), average germination speed (AGS), and percentage of abnormal seedlings (AS%) of *Luffa aegyptiaca* Mill seeds subjected to different water potentials and temperatures.

Sources of variation	DF	Mean squares					
		G%	FGC	GSI	AGT	AGS	AS%
Water potentials (Ψ_s)	3	251.78^{**}	166.80^{ns}	3.41^{**}	0.26^{ns}	0.012^{ns}	124.87^{**}
Linear regression	1	176.18^{*}	0.0017^{ns}	0.35^{ns}	0.093^{ns}	0.0016^{ns}	14.29^{ns}
Quadratic regression	1	557.73^{**}	50.95^{ns}	2.48^{**}	0.39^{ns}	0.0006^{ns}	81.69^{*}
Temperatures (T)	4	1599.41^{**}	1016.73^{**}	7.39^{**}	8.99^{**}	0.040^{**}	900.72^{**}
Interaction $\Psi_s \times$ T	12	187.68^{**}	89.06^{ns}	1.11^{**}	0.38^{**}	0.0019^{**}	123.91^{**}
Residue	60	37.00	95.93	0.19	0.14	0.00066	11.58
CV (%)		8.17	13.58	7.25	9.96	9.50	22.59

DF – degree of freedom; CV – coefficient of variation; ns, * and ** – not significant, significant at $p < 0.05$ and significant at $p < 0.01$, respectively, by F-test.

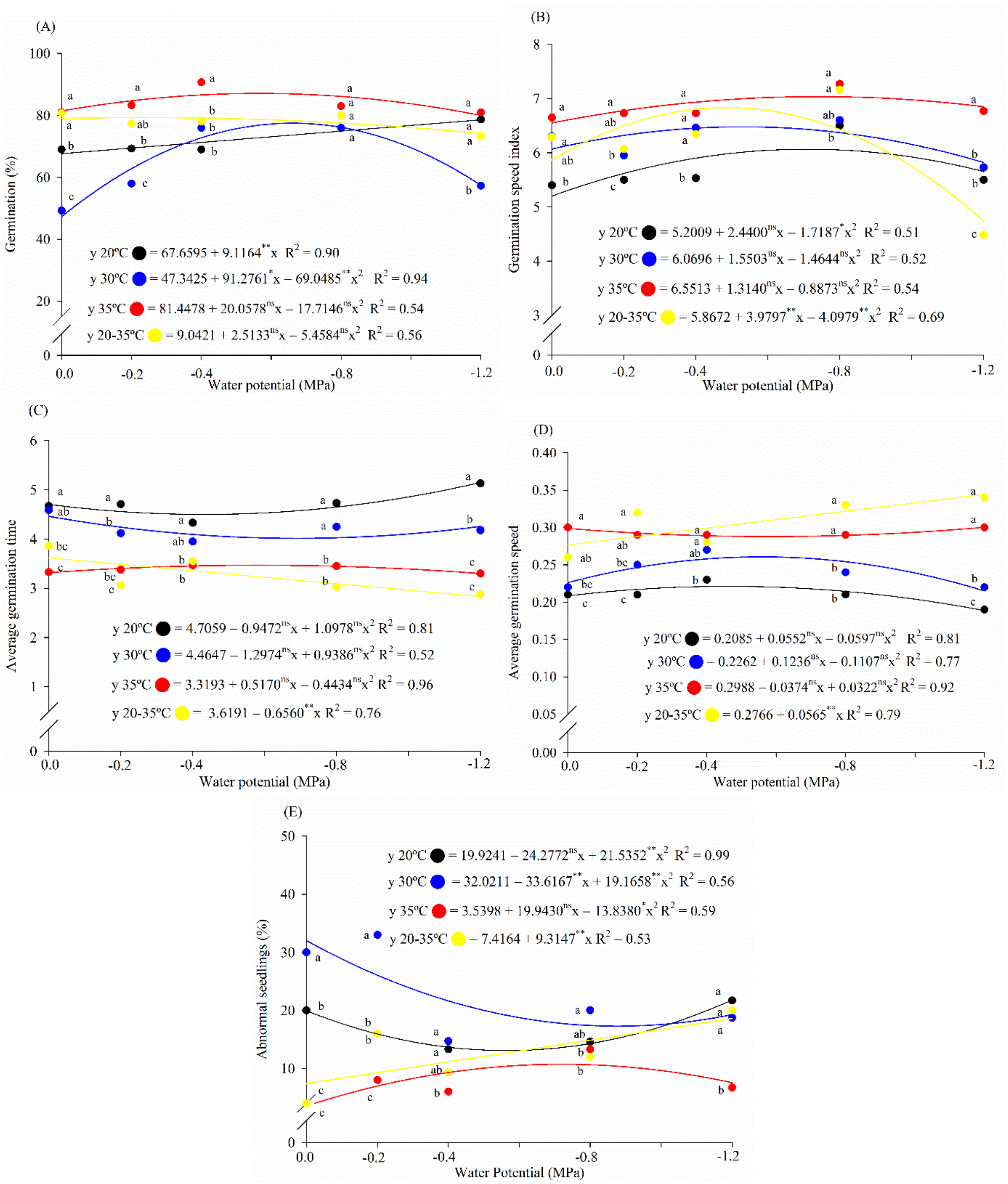
The AGT was shorter (2.89 and 3.30) in seeds subjected to alternating temperatures between 20-35 and 35ºC under a potential of -1.2 MPa (Figure 1C). At a temperature of 30°C, the lowest AGT was under a potential of -0.7 MPa. The temperature of 20°C promoted an increase (5.15) in germination time at a potential of -1.2 MPa. When comparing the effect of temperatures on AGT, it is possible to verify that 20°C delayed the germination process of the seeds, presenting the highest values in relation to the other temperatures.

The AGS was higher (0.30) in seeds subjected to a temperature of 35ºC and under a water potential of -1.2 MPa (Figure 1D). At temperatures of 20 and 30ºC and alternating temperatures of 20-35ºC, the highest values (0.21, 0.26, and 0.27) occurred at water potentials of -0.1, -0.6, and 0.0 MPa, respectively. Regarding the effect of temperature, it was observed that the AGS was higher at temperatures of 35ºC and alternating temperatures of 20-35ºC, exceeding the other temperatures.

For the AS% (Figure 1E), the highest estimated value (32%) occurred at a temperature of 30ºC and under a water potential of 0.0 MPa. At temperatures of 20ºC and alternating 20-35ºC, the highest values (21.8 and 18.6%) occurred at the highest water potential of -1.2 MPa, while at 35ºC the highest value (10.7%) was observed at -0.7 MPa. Regarding the effect of temperatures, it was possible to verify that the temperature of 35ºC produced the lowest values regardless of the water potential, proving to be more efficient for the seed germination process.

L. aegyptiaca seeds do not tolerate low temperatures, as observed in the FGC (Table 2). When the seeds were incubated at 20°C, they presented 63.6% of normal seedlings. This value did not differ significantly from the FGC observed under the alternating temperature regime of 20-35°C (69.4%). Maximum FGC of 75.8 and 79.8% were observed at temperatures of 30 and 35°C, respectively. However, the value recorded at 30°C did not differ significantly from that observed under the alternating temperature regime of 20-35°C. This may explain the species' wide adaptation to the conditions of the Northeastern semiarid region.

There was a significant effect of the interaction between water potentials and temperatures for shoot length (ShL), shoot dry mass (ShDM), and total dry mass (TDM) of seedlings. On the other hand, the variables root length (RL), seedling length (SL), and root dry mass (RDM) showed a significant effect ($p \leq 0.01$) for the temperature factor (Table 3).



ns, * and ** – not significant, significant at $p < 0.05$ and significant at $p < 0.01$, respectively, by F-test. Within each water potential, means followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$). The equations were calculated using the absolute values of the water potentials. However, for the purpose of graphical representation, the values on the X-axis are presented on a negative scale.

Figure 1. Germination (A), germination speed index (B), average germination time (C), average germination speed (D), and abnormal seedlings (E) of *Luffa aegyptiaca* Mill., subjected to different water potentials and temperatures.

Table 2. First germination count (FGC) of *Luffa aegyptiaca* Mill seeds subjected to different temperatures.

Temperatures (ºC)	FGC (%)
20	63.6c
30	75.8ab
35	79.8a
20-35	69.4bc

Means followed by the same letter in the column do not differ from each other by the by Tukey's test ($p \leq 0.05$).

Table 3. Summary of analysis of variance for root length (RL), shoot length (ShL), seedling length (SL), root dry mass (RDM), shoot dry mass (ShDM), and total dry mass (TDM) of seedling of *Luffa aegyptiaca* Mill seeds subjected to different water potentials and temperatures.

Sources of variation	DF	Mean squares					
		RL	ShL	SL	RDM	ShDM	TDM
Water potentials (Ψ_s)	3	2.34^{ns}	9.24**	14.47^{ns}	0.000005^{ns}	0.00006**	0.00009**
Linear regression	1	0.011^{ns}	20.30**	29.15*	0.000006^{ns}	0.00018**	0.00016**
Quadratic regression	1	1.54^{ns}	2.88^{ns}	8.64^{ns}	0.000006^{ns}	0.00009*	0.00014**
Temperatures (T)	4	15.80**	59.67**	27.65**	0.00003**	0.00053**	0.00079**
Interaction $\Psi_s \times$ T	12	1.99^{ns}	2.92*	5.97^{ns}	0.000008^{ns}	0.00005**	0.00006**
Residue	60	2.63	1.48	6.30	0.000005	0.000014	0.000017
CV (%)		24.50	19.86	19.69	56.48	12.10	12.02

DF – degree of freedom; CV – coefficient of variation; ns, * and ** – not significant, significant at $p < 0.05$ and significant at $p < 0.01$, respectively, by F-test.

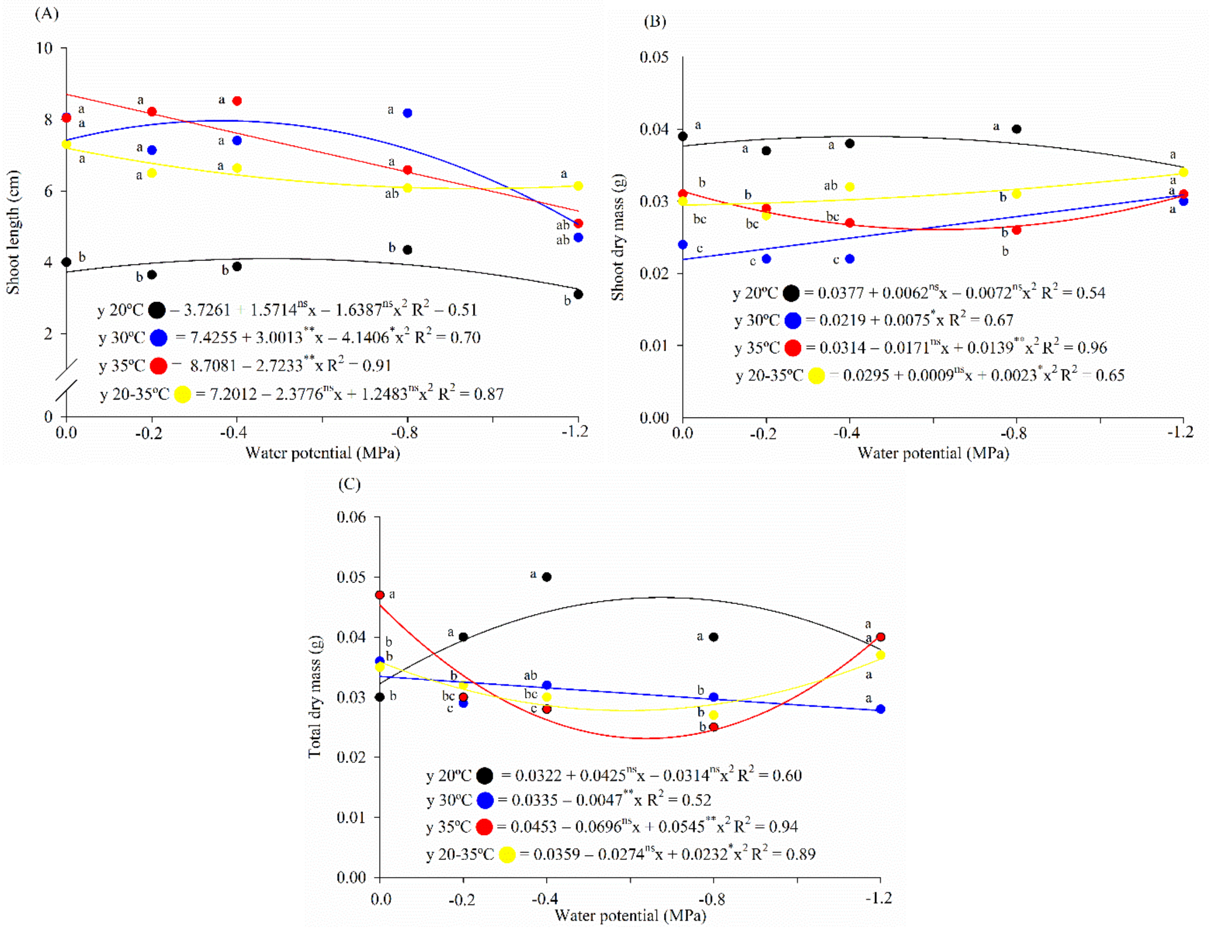
ShL was greater (8.71 cm) in seedlings originating from a temperature of 35ºC and under a water potential of 0.0 MPa (Figure 2A). For temperatures of 20, 30, and alternating 20-35ºC, the highest values (4.10, 7.96, and 7.20 cm) were obtained at water potentials of -0.5, -0.4, and 0.0 MPa, respectively. Regarding the effect of temperatures, it was found that seedlings originating from a temperature of 20ºC presented lower values than the other temperatures evaluated at all water potentials.

For ShDM (Figure 2B), the highest values were observed at a water potential of -1.2 MPa across different temperature regimes, of 0.0309, 0.0308, and 0.0338 g at 30, 35, and alternating 20-35ºC, respectively. The highest value (0.039 g) at 20ºC was obtained at a potential of -0.4 MPa. Regarding the effect of the different temperatures, it was observed that the temperature at 20ºC was higher than the others at all water potentials, except for -1.2 MPa, where there was no statistical difference.

The TDM of seedlings was higher (0.046 g) at a temperature of 20ºC and under a potential of -0.7 MPa (Figure 2C). At temperatures of 35ºC and alternating 20-35ºC, the highest values (0.045 and 0.036 g, respectively) occurred at a potential of -1.2 MPa. At a temperature of 30ºC, the highest value was obtained at the lowest water potential (0.0 MPa). Regarding the effect of temperatures, it was found that at the lowest osmotic potential (0.0 MPa), the temperature of 35ºC was higher than the others. At water potentials of -0.2, -0.4, and -0.8 MPa, the temperature of 20ºC was statistically higher than the other temperatures, while at a potential of -1.2 MPa there was no statistical difference.

Temperature is fundamental for the development of the initial structures of the seedling, such as the primary root, and plays a crucial role in the process of seedling establishment. Under stress conditions, the plant reacts by translocating assimilates to the roots for rapid development. This behavior was observed for RL, at low temperature (20°C) the roots were longer

(7.55 cm), followed by alternating temperature (25-35°C), with 7.06 cm. The highest accumulation of RDM (0.0057 g) was observed at 20°C. The SL had a difference between the temperatures, the increase in temperature increased the seedling length, increasing from 11.14 cm (20°C) to 13.65 cm (35°C) (Table 4).



ns, * and ** – not significant, significant at $p < 0.05$ and significant at $p < 0.01$, respectively, by F-test. Within each water potential, means followed by the same letter are not significantly different according to Tukey's test ($p \leq 0.05$). The equations were calculated using the absolute values of the water potentials. However, for the purpose of graphical representation, the values on the X-axis are presented on a negative scale.

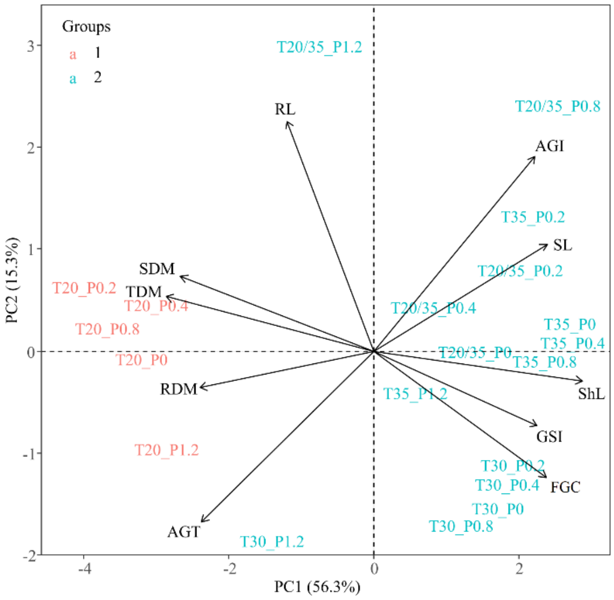
Figure 2. Shoot length (A), shoot dry mass (B), and total dry mass (C) of the seedlings of *Luffa aegyptiaca* Mill., subjected to different water potentials and temperatures.

Table 4. Root length (RL), seedling length (SL), and root dry mass (RDM) of *Luffa aegyptiaca* Mill., subjected to different temperatures.

Temperatures (°C)	RL (cm)	SL (cm)	RDM (g)
20	7.55a	11.14b	0.0057a
30	5.51b	12.61ab	0.0036b
35	6.36ab	13.65a	0.0028b
20-35	7.06a	13.59a	0.0030b

Means followed by the same letter in the column do not differ from each other by the by Tukey's test ($p \leq 0.05$).

The first two principal components explained 71.6% of the variance. Two groups were formed based on the combination of the studied factors. Group 1 consisted of all water potentials at a temperature of 20°C, while group 2 included all other treatments. Group 1 showed a greater association with the variables RL, SDM, TDM, and AGT. Meanwhile, group 2 showed a strong affinity with AGS, SL, ShL, GSI, and FGC (Figure 3).



T – temperature; P – osmotic potential; RL – root length; SDM – shoot dry mass; TDM – total dry mass; RDM – root dry mass; AGT – average germination time; AGI – average germination speed; SL – seedling length; ShL – shoot length; GSI – germination speed index; FGC – first germination count.

Figure 3. Principal component analysis with clusters for the combination *of Luffa aegyptiaca* Mill., subjected to different temperatures and water potentials.

Discussion

A temperature of 35°C stimulates the germination of *L. aegyptiaca* seeds (Figure 1A). According to Ataíde et al. (2016), the ideal temperature for germination is the one that allows the maximum number of seeds to germinate in the shortest period of time, thus characterizing it as the optimal temperature for this species. However, it was observed that germination decreases at all temperatures due to the reduction in the osmotic potential and the water absorption capacity of the seed. Furthermore, PEG 6000 has a high viscosity, which reduces the availability of oxygen to the seed (Santos et al., 2018).

The decrease in the germination capacity of the seeds due to the reduction of the water potential was observed in other species, such as in *Handroanthus impetiginosus*, which had a germination percentage of 64% at a temperature of 30ºC and water potential of -0.4 MPa and 0% at the potential of -1.2 MPa (Santos et al., 2018). The temperature of 35ºC and water potential of -0.5 MPa totally inhibited the germination of *Combretum leprosum* seeds (Leal et al., 2020).

As the percentage of germination (Figure 1A), the speed and time of germination (Figures 1B and 1C, respectively), for the seeds to germinate are affected by the low availability of water in the substrate, due to the absorption of water being slow at lower temperatures, resulting in the prolongation of the germination process. According to Antala et al. (2025), water deficit can cause changes in plant behavior, the intensity depends on the genotype, duration, severity and stage of plant development. The initial seed soaking period is the most critical for germination, where negative water potentials can prevent water absorption, making the sequence of events in the germination process unfeasible, as observed by Matias et al. (2015) in *Cucumis sativus* L., and by Nóbrega et al. (2024) in *Cenostigma pyramidale*.

In order for germination to occur, an increase in the respiratory activity must occur, with higher temperatures favoring the activation of germination (Azerêdo et al., 2016). This vigor test is a good index to assess the occupation of a species in a given environment since rapid germination is characteristic of species whose strategy is to establish themselves in the environment as quickly as possible because they take advantage of favorable conditions (Melo et al., 2018). The physiological quality of the seeds can be reduced as a function of the osmotic conditioning and the exposure time of these seeds in the solution, as observed by Pereira et al. (2019) in *Draucus carota* L., by Nóbrega et al. (2022) in *Crotalaria spectabilis*, and by Nóbrega et al. (2024) in *Cenostigma pyramidale.*

Water deficit promoted alterations in germination metabolism, reducing the growth and vigor of *L. aegyptiaca* seedlings. Thus, metabolic activity and the synthesis of new tissues are reduced by cellular dehydration promoted by low water absorption, resulting in seedlings with low vigor (Nascimento et al., 2019).

Similar results were observed by Silva et al. (2016) who studying the germination of *Chorisia glaziovii* seeds subjected to water deficit at different temperatures, noticed a reduction in the shoot length at temperatures of 20, 25, and 30°C in water potentials -0.1, -0.2, and -0.3 MPa. The process of cell wall elongation and synthesis is very sensitive to water deficit, due to the reduction in growth caused mainly by the decrease in cell turgidity (Hellal et al., 2018).

Water deficit also induced changes in the seedling dry mass, inducing the seed to translocate its reserves to shoot development (Figures 2A and 2B), as a mechanism to survive. This result differs from those observed by Santos et al. (2018), who observed a reduction in the root dry mass of *Handroanthus impetiginosus*, with a decrease in the osmotic potential, reaching null values from the potential of -0.8 MPa at temperatures of 25 and 30°C.

This reduction in seedling dry mass (Figure 2C), caused by water restriction, may occur due to the demand of biological and physiological processes or to the difficulty in hydrolysis and mobilization of reserves stored in the seeds (Bewley et al., 2013). The dry mass of *Piptadenia moniliformis* seedlings was reduced by -0.6 MPa at temperatures of 25 and 30°C (Azerêdo et al., 2016), results similar to these in this study, indicating that water deficit compromises the initial growth of *L. aegyptiaca*.

The temperature alone influenced the vigor evaluated by the first count test (Table 2), root and seedlings growth (Table 4), indicating that the increase in temperature up to 35ºC stimulates germination and initial growth of *L. aegyptiaca*. As observed by Ribeiro et al. (2019) in *Erythroxylum pauferrense*, temperature can interfere with germination, influencing the speed of water absorption and affecting biochemical reactions.

Other species also have variation in vigor when evaluated at different temperatures, according to results observed by Gomes et al. (2016), evaluating germination in *Eugenia involucrata* seeds under different substrates and temperatures, noted that the first germination count was higher (24%) at alternating temperatures of 20-30°C and 0% at 15°C. Oliveira et al. (2014) found that the temperature of 25ºC improved the performance of seeds and seedlings of *Miconia albicans*.

Conclusions

Temperature of 35°C provides the best conditions for seed germination and initial seedling growth, down to a water potential of -1.2 MPa.

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