

Water stress on germination and vigor of ‘mofumbo’ (*Combretum leprosum* Mart.) seeds at different temperatures¹

Estresse hídrico na germinação e vigor de sementes de mofumbo (*Combretum leprosum* Mart.) em diferentes temperaturas

Caio César Pereira Leal^{2*}, Salvador Barros Torres², Nadjamara Bandeira de Lima Dantas², Gutierrez Silva Medeiros Aquino² and Tatianne Raianne Costa Alves²

ABSTRACT - *Combretum leprosum* Mart., commonly known as ‘mofumbo’, is used for medicinal purposes, recovery of degraded areas and apiculture. The objective of this study was to evaluate the effect of water stress on the germination and vigor of *C. leprosum* seeds as function of different temperatures. To compose the treatments, the substrate used was paper roll moistened with polyethylene glycol solutions (PEG 6000), at the following levels of osmotic potential: 0.0; -0.1; -0.2; -0.2; -0.4; and -0.5 MPa, and placed in chamber at constant temperatures of 25, 30 and 35 and alternating temperature of 20-30 °C, forming a 6 x 4 factorial. The seeds were submitted to the following evaluations: germination percentage, germination speed index, root length, shoot length, root dry matter, shoot dry matter. There was a significant interaction for all variables, with a negative effect as the osmotic potentials decreased. Thus, *C. leprosum* showed high sensitivity to low osmotic potentials, showing a steeper decrease from the potential of -0.2 MPa for all evaluated temperatures. Initial germination and development of *C. leprosum* seedlings was negatively affected by low osmotic potentials, with a tolerance limit of -0.4 MPa. Temperatures of 25 and 35 °C accentuate the negative effect of the low osmotic potential of water on the germination and early development of *C. leprosum* seedlings.

Key words: Combretaceae. Osmotic potential. Abiotic stress.

RESUMO - *Combretum leprosum* Mart., conhecida popularmente como mofumbo, é utilizada para fins medicinais, melífera, forrageira e recuperação de áreas degradadas. Objetivou-se avaliar o efeito do estresse hídrico sobre a germinação e vigor de sementes de *C. leprosum* em diferentes temperaturas. Para isso, as sementes foram semeadas em substrato rolo de papel, umedecido com soluções de polietileno glicol (PEG 6000), nos potenciais osmóticos de 0,0; -0,1; -0,2; -0,3; -0,4; e -0,5 MPa; os testes de germinação foram conduzidos em germinadores sob as temperaturas de 25, 30, 35 e 20-30 °C, formando o fatorial 6 x 4. As avaliações foram as seguintes: germinação, índice de velocidade de germinação, comprimento da raiz, comprimento da parte aérea, massa seca da raiz e massa seca da parte aérea. Houve interação significativa para todas as variáveis, verificando-se efeito negativo com a diminuição dos potenciais osmóticos. A *C. leprosum* apresentou alta sensibilidade a baixos potenciais osmóticos, com decréscimo mais acentuado a partir do potencial de -0,2 MPa para todas as temperaturas avaliadas. A germinação e o desenvolvimento inicial de plântulas de *C. leprosum* foram afetados de forma negativa pelos baixos potenciais osmóticos, com limite de tolerância máximo partir de -0,4 MPa. As temperaturas de 25 e 35 °C acentuam o efeito negativo do baixo potencial osmótico da água sobre a germinação e o desenvolvimento inicial de plântulas de *C. leprosum*.

Palavras-chave: Combretaceae. Potencial osmótico. Estresse abiótico.

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*Author for correspondence

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²Departamento de Ciências Agronômicas e Florestais, Centro de Ciências Agrárias, Universidade Federal Rural do Semi-Árido/UFERSA, Mossoró-RN, Brasil, caioleal3@hotmail.com (ORCID ID 0000-0001-7745-1836), sbtorres@ufersa.edu.br (ORCID ID 0000-0003-0668-3327), nadybandeira@hotmail.com (ORCID ID 0000-0002-1261-2181), gutierrezsilva@hotmail.com (ORCID ID 0000-0003-4492-5550), tatianne_ralves@hotmail.com (ORCID ID 0000-0002-5729-3268)

INTRODUCTION

Combretum leprosum Mart., Combretaceae family, is a species known as 'mofumbo' and used in medicine, honey production, as forage and in the recovery of degraded areas. Because of these characteristics, this species is pointed by the project 'Plantas para o Futuro' as priority for research (MAIA, 2012).

The environmental, chemical and pharmacological importance of some native species leads to more-advanced studies on the production techniques. However, the number of species for which the effective means are known, on this topic, is still scarce (PRAVUSCHI *et al.*, 2010). Hence, seedling production becomes one of the biggest obstacles when the goal is to establish rational cultivation (MOREIRA *et al.*, 2007).

Among the limiting factors for seed germination and, consequently, for the success of the seedlings at field, water availability is one of the most common, especially in semi-arid regions. The influence of this factor has been widely studied, particularly on germination and seedling development, to identify the tolerance of each species to this abiotic stress (MOURA *et al.*, 2011).

Temperature variation also differently influences the characteristics of germination, leading to higher values of germination percentage and speed at higher temperatures (MARTINS; MACHADO; NAKAGAWA, 2008), which mainly occur because water absorption becomes faster. Despite this initial benefit, higher temperatures tend to accentuate the effect of water deficit according to the decrease in the osmotic potential (NOGUEIRA *et al.*, 2018). In the literature there are works whose results show the influence of water stress under different temperature regimes on several species, such as *Dimorphandra mollis* Benth. (MASETTO; SCALON; REZENDE, 2014), *Amburana cearensis* (Allemão) A. C. Smith. (ALMEIDA *et al.*, 2014), *Piptadenia moniliformis* Benth. (AZEREDO; PAULA; VALERI, 2016) and *Chorisia glaziovii* O. Kuntze. (SILVA *et al.*, 2016). Nogueira *et al.* (2018) also inform that, for the Brazilian subtropical and tropical species, the optimum temperature of germination is between 20 and 30 °C.

Studies have been conducted using solutions with different osmotic potentials to moisten the substrate, simulating the conditions of low water availability in the soil (MORAES; MENEZES, 2003). Among these solutions, the utilization of PEG 6000 stands out because it is an osmotic agent, chemically inert, atoxic and of easy absorption, thus simulating the drought (GUEDES *et al.*, 2013).

Studies on water stress involving species of the Combretaceae family are scarce. However, others

with different native and exotic species, adapted to the semi-arid region, stand out: Pelegrini *et al.* (2013) with 'corticeira-da-serra' (*Erythrina falcata* Benth.), Mota, Scalon and Mussury (2013) with 'angico' (*Anadenanthera falcata* Benth. Speg.), Guedes *et al.* (2013) with 'pente-de-macaco' (*Apeiba tibourbou* Aubl.), Azerêdo, Paula and Valeri (2016) with 'angico-de-bezerra' (*Piptadenia moniliformis* Benth.), Silva *et al.* (2016) with 'paineira branca' (*Chorisia glaziovii* O. Kuntze), Silva *et al.* (2017) with coconut (*Cocos nucifera* L.), and Santos *et al.* (2018) with ipê-roxo (*Handroanthus impetiginosus* (Mart. ex DC)).

Thus, the objective was to evaluate the effect of water stress on germination and vigor of *C. leprosum* seeds under different temperatures.

MATERIAL AND METHODS

C. leprosum seeds were obtained from ripe fruits harvested from different mother plants in the municipality of Mossoró, RN (5°12'14" S, 37°19'26" W and approximate altitude of 23 m). According to Köppen, the climate of the area is BSwh', dry and very hot, with two climate seasons: a dry one, which usually comprehends the period from June to January and a rainy one, between the months of February and May (CARMO FILHO; ESPÍNOLA SOBRINHO; MAIA NETO, 1991).

After harvest, the fruits were dried in the shade (± 30 °C and 60% of RH), for 72 h, and then manually opened to remove the seeds. The seeds were placed in glass packages and stored in cold chamber (10 ± 2 °C and 50% RH) during the entire experimental period.

The experimental design was completely randomized in 4 x 6 factorial scheme (four temperatures x six osmotic potentials), with four replicates of 25 seeds each.

Germination tests were conducted using the substrate paper roll (Germitest®), which was moistened with polyethylene glycol (PEG 6000) solutions, at the following osmotic potentials: 0.0 (distilled water); -0.1; -0.2; -0.3; -0.4; and -0.5 MPa according to Villela, Doni-Filho and Sequeira (1991). The tests were conducted in Biochemical Oxygen Demand (B.O.D.) chambers at constant temperatures of 25, 30 and 35 °C, and alternated temperatures of 20-30 °C, with the following evaluations:

Germination percentage: performed at the end of the 19th day (PACHECO *et al.*, 2014), using the criterion of normal seedlings established by the Rules for Seed Analysis (RAS) (BRASIL, 2009).

Germination speed index: determined through daily count of the number of germinated seeds, at the

same time, from the first day until to stabilization of germination, and calculated using the formula proposed by Maguire (1962).

Lengths of main root and shoot of the seedlings: at the end of the germination test, the primary root and hypocotyl of the normal seedlings of each replicate were measured with a ruler graduated in millimeters, with results expressed in cm seedling⁻¹.

Dry matter of root and shoot of the seedlings: at the end of the germination test, roots and hypocotyls of normal seedlings were fractioned, placed in Kraft paper bags, dried in forced-air oven at 65 °C until constant weight and then weighed on analytical scale (0.001 g), with results expressed in mg seedling⁻¹.

The results were subjected to analysis of variance by F test at 0.05 probability level. In case of significance, qualitative data were subjected to Tukey test at 0.05 probability level using the statistical program SISVAR (FERREIRA, 2011). Quantitative data were subjected to regressions generated using the software Sigmaplot® 11.0. The model was selected based on the biological explanation and significance of the mean square of the regression.

RESULTS AND DISCUSSION

The germination and vigor of *C. leprosum* seeds showed sensitivity to the decrease in the osmotic potential of the water, as well as to the studied temperatures, with significant interaction between both factors at 0.01 probability levels for all analyzed variables (Table 1).

Germination percentage decreased with the reduction in the osmotic potential of the solution, regardless of the evaluated temperature, indicating the negative effects that the low water availability can cause to the seed. However, the species showed higher tolerance at the alternated temperature of 20-30 °C, at which the

germination, despite the reduction, was on average 80% at the lowest osmotic potential studied (-0.5 MPa) (Figure 1A).

At the temperature of 30 °C, the effects were similar to those at the alternated temperature of 20-30 °C, but with lower mean values, in which the germination percentage of 85% in the control decreased to 70% at the osmotic potential of -0.5 MPa. At the temperatures of 25 and 35 °C, there was a sharper reduction in the germination percentage, reaching 25% and zero, respectively, at the lowest osmotic potential.

This negative effect on germination is related to the low water availability and consequent decrease in water absorption, due to the lower osmotic potential of the solution, which is accentuated at temperatures higher than 30 °C. Besides the observed effects, high temperatures may lead to thermal dormancy or even loss of seed viability due to the thermal stress (VIDAVER; HSIAO, 1975). The result observed for temperature of 25 °C may be linked to a mild condition, but not within the optimal range required by the species, so there is a sharp drop from -0.3 MPa.

The same effect was observed by Moura *et al.* (2011), who worked with *M. caesalpinifolia* seeds and found out reduction of germination percentage due to the osmotic potential of the solution. It should be pointed out that, for this species, the authors found null germination at the potential of -0.5 MPa and temperature of 30 °C, which differs from the present study, in which the germination at this temperature was around 70% for the same potential. This demonstrates the need to study each species individually and their responses to different temperatures, in order to find an optimum range of tolerance for each one, since the intensity of the germination response to water stress varies between the seeds of different species (MARCOS-FILHO, 2015).

Similarly, Pelegrini *et al.* (2013) found inhibitory effect of the PEG 6000 solutions from -0.4 MPa in seeds of *E. falcata*, also observing that the species has

Table 1 - Summary of the analysis of variance for the variables evaluated in the germination and seed vigor of *Combretum leprosum* Mart., as a function of osmotic potential and different temperatures

SV	DF	MS					
		G	GSI	SDM	RDM	RL	SL
Potentials	5	10630.4	16.38	0.00002	0.00001	5.58	8.99
Temperatures	3	1634.00**	3.39**	0.00005**	0.00001**	20.98**	11.36**
Pot. x Temp.	15	999.20**	1.61**	0.00005**	0.00002**	43.55**	29.41**
CV (%)	-	13.46	14.60	30.32	39.71	35.26	29.31

SV - Sources of variation; DF - Degrees of freedom; CV- Coefficient of variation; G - Germination; GSI - germination speed index; SDM - Shoot dry matter; RDM - Root dry matter; RL - root length; SL - Shoot length; MS - Mean square; ** - Significant at 1% by F test

maximum tolerance of up to -0.2 MPa. Braga *et al.* (2008) found reduction in the germination percentage of seeds of *Schizolobium amazonicum* (Huber) ex. Ducke, at potentials from -0.1 to -0.5 MPa of PEG 6000, differing from the control. Guedes *et al.* (2013), with seeds of *A. tiburou*, observed that the tolerance range of this species is between -0.4 and -0.6 MPa of osmotic potential in the irrigation water, at temperatures of 25 and 30 °C. Similarly, Silva *et al.* (2016) observed that the reduction in osmotic potentials from -0.2 MPa negatively affects the germination and vigor of *Chorisia glaziovii* seeds, especially at temperature of 20 °C.

The behavior of the germination speed index (GSI) was similar to germination percentage, with reduction from the potential of -0.1 MPa, for all evaluated temperatures, and highest values for the alternated temperature of 20-30 °C and constant temperature of 30 °C (Figure 1B). The reduction in the osmotic potential of the substrate substantially reduced the germination speed of *Jatropha curcas* L. seeds, as well as the development of the seedlings (PEREIRA; LOPES, 2011). More negative water potentials reduce water imbibition by the seeds and can prevent the sequence of events of the germination process, acting in the reduction of the germination speed and percentage, and each species requires a value of water potential in which if below, germination does not occur (STEFANELLO *et al.*, 2008).

The reduction in GSI is related to the delay in the phase III of germination, which requires greater amount

of water for the radicle protrusion to occur. Therefore, with the reduction of water absorption, due to the decrease in osmotic potential, physiological processes are compromised, reducing the germination speed of the seeds (MOURA *et al.*, 2011). Similarly, Azerêdo, Paula and Valeri (2016) emphasized that the occurrence of the germination requires energy from respiration; if it is low, there will not be adequate conditions to trigger the germination process, which becomes more visible at high temperatures. In most cases, the thermal stress delays the development of the germination process and can suppress it in quiescent seeds or in those that had already started the germination (POLLOCK; ROSS, 1972).

Similar results with *Gliricidia sepium* (Jacq.) Steud were found by Farias *et al.* (2009), who also used PEG 6000 as osmotic agent and observed reduction of 50% in GSI when the osmotic potential changed from 0 MPa to -0.5 MPa. Many other species showed reductions in germination percentage and speed, at different levels, as in the case of *Ateleia glazioviana* Baill. (ROSA *et al.*, 2005) and *Plantago ovata* Forsk (SOUSA *et al.*, 2008). In addition, it was also observed that the reduction of water potential and the PEG 6000 concentrations from -0.8 MPa on prevented water absorption by the seeds of *Anadenanthera colubrina* (Veloso) ex. Brenan (REGO *et al.*, 2007).

For seedling length (roots and shoots), there was a reduction as the osmotic potential of the solution decreased (Figures 2A and 2B). However, these

Figure 1 - Germination (A) and germination speed index (B) of *Combretum leprosum* Mart. seeds submitted to different osmotic potentials at different temperatures

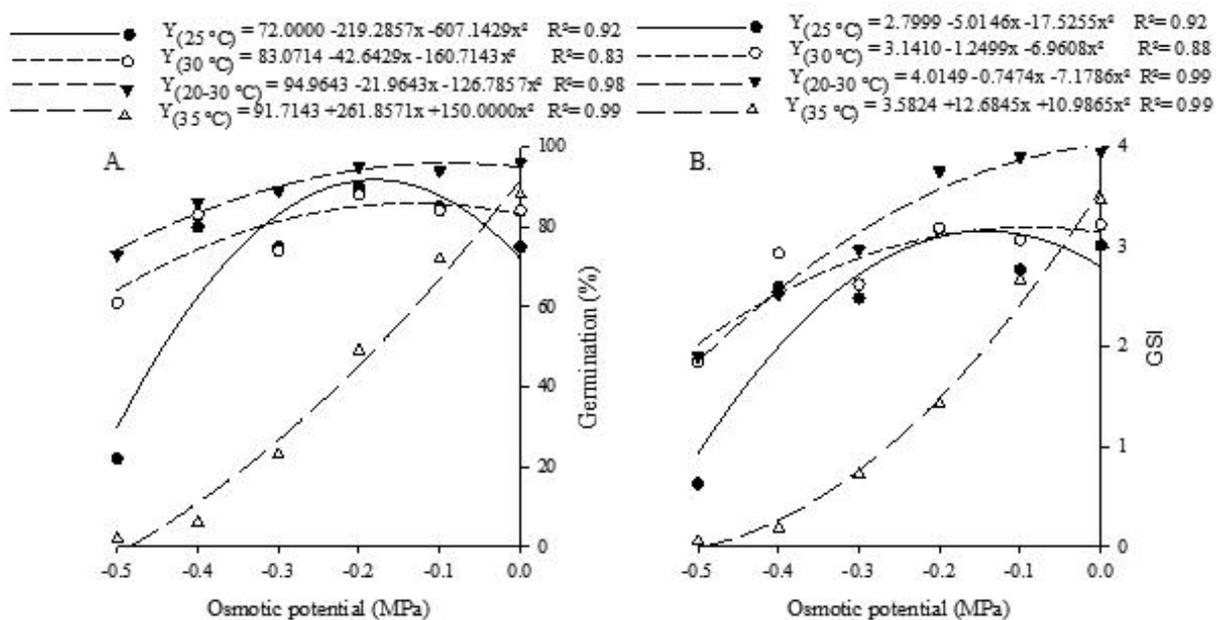
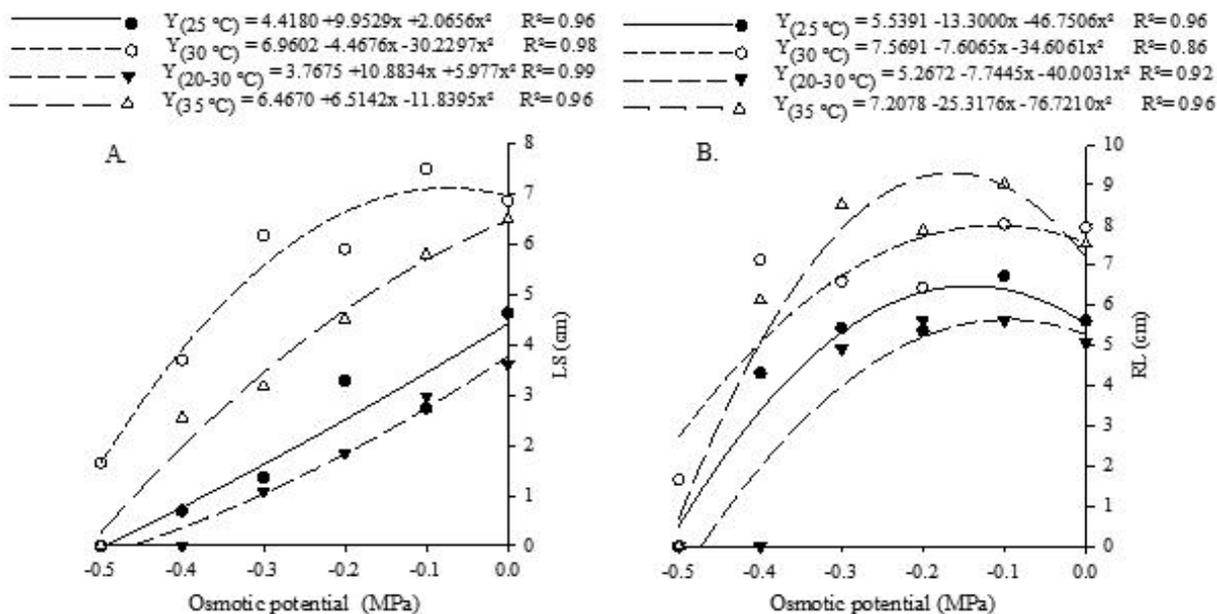


Figure 2 - Length of shoot (A) and root length (B) of *Combretum leprosum* Mart. seedlings submitted to different osmotic potentials at different temperatures



variables showed different responses to the various temperatures, with higher means at the constant temperature of 30 °C. Similar result was also observed in *C. glaziovii* seedlings at the same temperature and there was a reduction in seedling length as the water availability was restricted at all tested temperatures (SILVA *et al.*, 2016). These results corroborate those reported by Guedes *et al.* (2013), with seedlings of *A. tibourbou*, in which the greatest length (3.28 cm) was observed at temperature of 30 °C, while the temperature of 25 °C led to length of 2.28 cm.

Considering root length individually, a peculiar effect was observed, its slight increase until the potential of -0.2 MPa. This is a response of the seedling to the environment with low water availability, where there is higher investment in root length in the search for water, i.e., there was an adaptation of the species to the water stress condition, also showing its maximum limit of tolerance at this potential (AL-KARAKI; AL-AJMI; OTHMAN, 2007). According to these authors, seedlings growing under water stress conditions have the ability to develop an efficient root system as soon as germination occurs, in order to ensure continuous water supply to meet transpiration and growth. This fact was not observed by Silva *et al.* (2016) in *C. glaziovii*, who reported reduction in root length as the osmotic potential became more negative, for all temperatures tested.

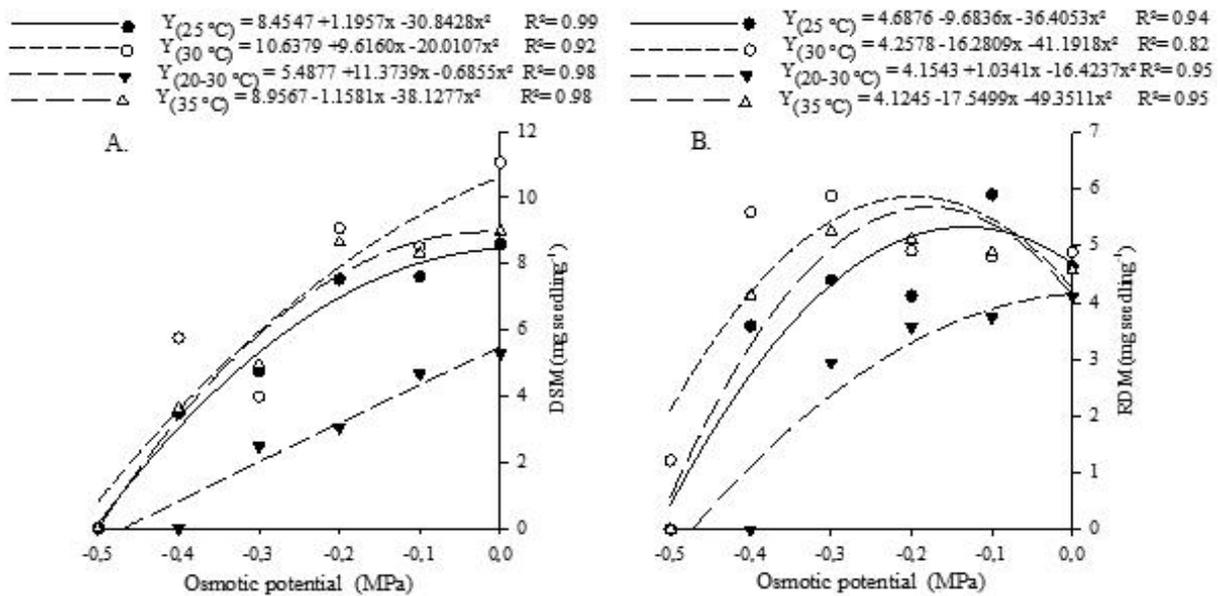
The curve of dry matter accumulation (roots and shoots) followed the behavior of the curve of length, with

higher values at the constant temperature of 30 °C and lower values for the other temperatures. A more severe effect occurred on shoot dry matter (Figure 3A), in which the reduction occurs from 0.0 MPa on for all temperatures. For root dry matter (Figure 3B), there was an increase up to the potential of -0.2 MPa, demonstrating the effect of the stress on the seedling and its adaptation to the stress, giving greater importance to the root and leading to better water absorption.

In seeds of *Foeniculum vulgare* Mill., the reduction of the osmotic potential also led to significant reductions in the dry matter of the seedlings (STEFANELLO *et al.*, 2006). Probably, this reduction occurs due to the demand of the physiological and biological processes or to the difficulty of hydrolysis and mobilization of the reserves stored in the seeds. In seedlings of *C. glaziovii*, Silva *et al.* (2016) observed that the results obtained for temperatures of 20 and 30 °C evidence that the thermal stress produced by low or high temperature negatively affects the root dry matter content in the initial stage of its development.

Similarly, Oliveira *et al.* (2017), worked with 'pereiro-vermelho' (*Simira gardneriana* M.R. Barbosa and Peixoto) and observed that the tolerance of germination of seeds of this species to the water stress varies among the temperatures, limiting the formation of normal seedlings from -0.5 MPa at the temperatures of 25 and 30 °C and of -0.4 and -0.2 MPa for temperatures of 20-30 and 35 °C, respectively.

Figure 3 - Dry shoot matter (A) and root dry matter (B) of *Combretum leprosum* Mart. seedlings submitted to different osmotic potentials at different temperatures



The results found in the present research emphasize the increasing need of studies on the effects of water and thermal stresses on seed germination, highlighting the importance of the individual study, in order to determine the osmotic potential and the temperature from which germination is inhibited because, for each species, there is a value of water potential in the soil or in the water in which if below the germination does not occur (AZERÊDO; PAULA; VALERI, 2016), and this value varies according to the temperature.

CONCLUSIONS

1. The germination and initial development of *C. leprosum* seedlings are negatively affected by the low osmotic potentials, with maximum tolerance limit from -0.4 MPa;
2. The temperatures of 25 and 35 °C accentuate the negative effect of the low osmotic potential of the water on the germination and initial development of *C. leprosum* seedlings.

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