

Abstract

Differential Tolerance of Cowpea Cultivars to Osmotic Stress in Germinative Phase: A Multivariate Approach [†]

Leticia Oliveira ^{1,*}, Maria Silva ¹, Alexandre Oliveira ¹, Aiala Amorim ², Carlos Dias ¹ and Rosilene Mesquita ¹

¹ Departamento de Fitotecnia, Universidade Federal do Ceará, Fortaleza, Ceará, Fortaleza CEP 60430-170, CE, Brazil; lilian.ufc@hotmail.com (M.S.); aleufc@gmail.com (A.O.); ctsdias@ufc.br (C.D.); rosilenemesquita@ufc.br (R.M.)

² Institute Of Rural Development, University of the International Integration of the Afro-Brazilian Lusophony, São Francisco do Conde 62790-000, Bahia, Brazil; aialaamorim@unilab.edu.br

* Correspondence: leticia.kbo7@gmail.com; Tel.: +55-85-997088966

[†] Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1-15 December 2021; Available online: <https://iecps2021.sciforum.net/>.

Abstract: This study aimed to characterize the behavior of different cultivars of *Vigna unguiculata* L. Walp. to osmotic stress, from germination and vigor parameters. The experimental design used was completely randomized, in a 14 × 4 factorial arrangement, with fourteen cultivars and four levels of osmotic potential (0, -0.1, -0.2, and -0.4 MPa) of the germination solution. BRS-Novaera and BRS-Pajeú cultivars were characterized with possible tolerance at both the -0.1 and -0.2 MPa levels. The study pointed to the BRS-Pujante cultivar as the most sensitive to the -0.4 MPa level. The multivariate technique used allowed for a satisfactory characterization of the treatments adopted.

Keywords: *Vigna unguiculata* L. Walp; abiotic stress; germination; vigor; exploratory analysis.

Citation: Oliveira, L.; Silva, M.; Oliveira, A.; Amorim, A.; Dias, C.; Mesquita, R. Differential Tolerance of Cowpea Cultivars to Osmotic Stress in Germinative Phase: A Multivariate Approach. *2021*, *1*, x. <https://doi.org/10.3390/xxxxx>

Academic Editor: Iker Aranjuelo

Published: 30 November 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp.), also known as Macassar and white beans, is one of the most cultivated and consumed legumes in the world, being one of the main sources of vegetable protein for populations, especially for those with lower purchasing power [1]. In Brazil, above all, it is cultivated in the North and Northeast, presenting important socio-economic functions for family farming in these regions [2], considering that it is configured as a source of employment and income for numerous families.

Although cowpea is well adapted to different edaphoclimatic conditions and is considered tolerant to situations of low soil water availability, some studies have shown that osmotic stress is one of the abiotic stresses that most affect this crop, which can cause changes in seed germination and early development processes, among other things, which are the phases considered most vulnerable to water scarcity [3,4].

The osmotic stress influences practically all aspects related to plant development, which ends up culminating in the reduction of crop productivity [3]. This type of stress tends to compromise the initial establishment of the seedling stand, as water limitation ends up reducing the speed of germination and may even impede it [5]. This is because water is the starting factor for germination and is, directly and indirectly, involved in all other stages of germination metabolism [6].

Studies with seeds of different species have been conducted to assess germination and vigor under low humidity conditions [7]. However, studies related to tolerance of different cowpea cultivars to abiotic stresses at these stages of development are still scarce in the literature, especially when referring to data interpreted from multivariate analysis, which consists of a set of statistical methods that allows the simultaneous evaluation of several variables of a sample or population, whose purpose is to simplify and facilitate

the interpretation of certain studied phenomena [8,9]. In this sense, the objective of this work was to characterize, through multivariate data analysis, the behavior of different cowpea bean cultivars to osmotic stress, based on germination and vigor variables.

2. Material and Methods

The experiment was carried out at the Seed Analysis Laboratory of the Federal University of Ceará (UFC), in Fortaleza, from September to October 2018. Cowpea seeds of the cultivars: BRS-Pujante, BRS were used -Guariba, BRS-Potengi, BR 17-Gurgueia, BRS-Tumucumaque, BRS-Pajeú, BRS-Rouxinol, BRS-Novaera, BRS-Xiquexique, BRS-Milênio, BRS-Acauã, Patativa, BR 3-Tracuateua and BRS-Aracê. They were subjected to germination at different osmotic potentials, simulating situations of osmotic stress: 0 (distilled water), -0.1, -0.2, and -0.4 MPa, induced by polyethylene glycol 6000 (PEG) solutions, prepared according to [10].

Initially, we selected uniform seeds with intact integument of each cultivar. Then, the seeds were disinfected in a 1% sodium hypochlorite solution for 1 min and then washed with distilled water. For each treatment, 200 seeds were used, divided into four replicates of 50 seeds, each group being distributed among three sheets of blotting paper moistened with distilled water or PEG 6000 solution in the proportion of 2.5 times the weight of the dry paper [11], obeying the different osmotic potentials previously established. Then, the three sheets were rolled and the resulting rolls were placed inside polyethylene pots covered with transparent plastic, which were kept in a BOD-type germination chamber at a constant temperature of 25 °C [11] and under 12 h light/12 h dark photoperiod.

The count of germinated seeds was performed daily for nine days, considering as germination criterion the emission of a radicle with at least 2.0 mm [12]. The germination variables evaluated were: final germination percentage [13], germination speed index [14], and mean germination time [13].

For vigor evaluation, we experimented with the same conditions and treatments mentioned for the germination test. However, the four repetitions of each treatment had twenty seeds each [15]. The evaluations were carried out nine days after sowing, measuring, with a ruler, the lengths of the aerial part (LAP) and root (LR) of the normal seedlings of each repetition.

Subsequently, the seedlings of each repetition had shoots and roots sectioned, conditioned separately in properly identified paper bags, and taken to an oven with forced air circulation at 65 °C for 48 h. After this period, with the aid of a precision scale, the aerial part dry mass (APDM) and root dry mass (RDM) data were obtained, and then the RDM/APDM ratio was calculated.

The experimental design used was completely randomized, in a 14 × 4 factorial arrangement, referring to fourteen cowpea cultivars and four levels of osmotic potential, with four replications. Statistical analysis of data was performed using the multivariate method of Principal Component Analysis (PCA), an exploratory technique that aims to reduce the number of variables to a smaller number of indices (principal components), which are combinations of linear variables of the original variables [9]. This analysis was performed using the R statistical package v. 4.0.2 [16].

This methodology is widely used when one wants to analyze several characteristics simultaneously and its efficiency is based on a greater correlation between the original variables, whether positive or negative [8]. Therefore, the correlation matrix used in this study was calculated using Spearman's correlation coefficient, due to the abnormality of the data found through the Shapiro-Wilk test, in which a p -value < 0.05 was obtained. for all analyzed variables.

The data set consisting of the means of the variables for each treatment was standardized ($\mu = 0$; $\sigma^2 = 1$), removing the influence of the different measurement units of the variables on the final result. To determine the number of main components, the Kaiser criterion was used, which consists of selecting the components that presented eigenvalues greater than 1 ($\lambda_i > 1$) [9,17].

3. Results and Discussion

Table 1 shows the matrix of correlations between the variables and their respective *p*-values. We observed that the variables were significantly correlated ($p < 0.05$), both positively and negatively, except for the correlations between RDM/APDM and GSI ($\rho = 0.25$), RDM/APDM and LAP ($\rho = 0.23$) and RDM/APDM and APDM ($\rho = 0.22$), which were not significant. Thus, we can conclude that there is evidence of a correlation between the characteristics, indicating that the use of multivariate analysis techniques is adequate.

Table 1. Correlation matrix and their respective significance values (*p*-value) between the studied variables.

Variable	Variable							
	G	GSI	MGT	LR	LAP	RDM	APDM	RDM/APDM
G	1.00							
GSI	0.93 **	1.00						
<i>p</i> -value	(<0.0001)							
MGT	-0.83 **	-0.93 **	1.00					
<i>p</i> -value	(<0.0001)	(<0.0001)						
LR	0.83 **	0.88 **	-0.89 **	1.00				
<i>p</i> -value	(<0.0001)	(<0.0001)	(<0.0001)					
LAP	0.87 **	0.94 **	-0.87 **	0.86 **	1.00			
<i>p</i> -value	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)				
RDM	0.82 **	0.82 **	-0.80 **	0.86 **	0.85 **	1.00		
<i>p</i> -value	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)			
APDM	0.88 **	0.92 **	-0.86 **	0.88 **	0.95 **	0.91 **	1.00	
<i>p</i> -value	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)		
RDM/APDM	0.33 *	0.25	-0.28 *	0.35 **	0.23	0.42 **	0.22	1.00
<i>p</i> -value	(0.013)	(0.062)	(0.039)	(0.008)	(0.090)	(0.001)	(0.108)	

G = Final Germination Percentage; GSI = Germination Speed Index; MGT = Mean Germination Time; LR = length of the root; LAP = Length of the Aerial Part; RDM = Root dry mass; APDM = Aerial Part Dry Mass; RDM/APDM = Root Dry Mass/Aerial Dry Mass Ratio. ** Significant correlation at the 0.01 level; * Significant correlation at the 0.05 level.

The results obtained with PCA reveal that the first two components generated presented eigenvalues greater than 1 ($\lambda_i > 1$), meeting the criterion established by [17] and therefore, selected for the interpretation of the results. (Table 2). The PCA showed that the two selected components explained 91.50% of the total variance of the data, with 74.77% explained by component 1 (CP1) and 16.73% by component 2 (CP2), as shown in Table 2, the first two main components effectively summarize a large part of the total sample variance and can be used to study the dataset.

Table 2. Weight coefficients (eigenvectors), eigenvalues, explained variance (EV), and accumulated explained variance (AEV) for each principal component, based on the studied variables.

Variable	CP1	CP2	CP3	CP4	CP5	CP6	CP7	CP8
G	0.366	0.270	-0.012	0.688	-0.212	-0.085	-0.466	-0.225
GSI	0.397	-0.042	-0.157	0.401	-0.060	0.105	0.735	0.321
MGT	-0.359	0.050	0.829	0.358	0.164	0.011	0.149	0.065
LR	0.394	0.050	0.100	-0.110	0.610	-0.666	-0.012	0.061
LAP	0.340	-0.444	0.108	0.028	0.460	0.570	-0.090	-0.361
RDM	0.382	0.145	0.395	-0.374	-0.437	-0.094	0.284	-0.507
APDM	0.362	-0.342	0.330	-0.179	-0.308	0.050	-0.356	0.622
RDM/APDM	0.177	0.765	0.070	-0.230	0.234	0.449	-0.068	0.254
eigenvalues	5.98	1.34	0.29	0.19	0.10	0.05	0.03	0.02

EV (%)	74.77	16.73	3.61	2.36	1.31	0.62	0.38	0.22
AEV (%)	74.77	91.50	95.11	97.47	98.77	99.39	99.78	100

In the degree of importance of the variables in each of the main components, those with weight coefficients greater than 0.3 in the module were considered relevant (Table 2). Thus, the variables that most contributed to CP1 were G, GSI, MGT, LR, RDM, and APDM, with the variable MGT showing low values; for CP2, the variables that most influenced it were LAP (characterized by low values) and RDM/APDM ratio (characterized by high values). Thus, CP1 indicates germination performance and root growth, while CP2 indicates differences in seedling size.

When analyzing Figure 1, in general, the treatments distributed in the positive region of CP1 and CP2 are characterized by presenting higher values of the variables G, RDM, LR, and RDM/APDM. However, with increasing stress intensity (from -0.1 MPa to -0.2 MPa) there are reductions in the values of these variables according to each cultivar evaluated. However, even with the stress of -0.2 MPa applied, some cultivars managed to practically maintain the germination potential presented at the level of -0.1 MPa and also to increase their root growth at the expense of the aerial part as a way of tolerance to water scarcity, which was the case of cultivars BRS-Novaera and BRS-Pajeú.

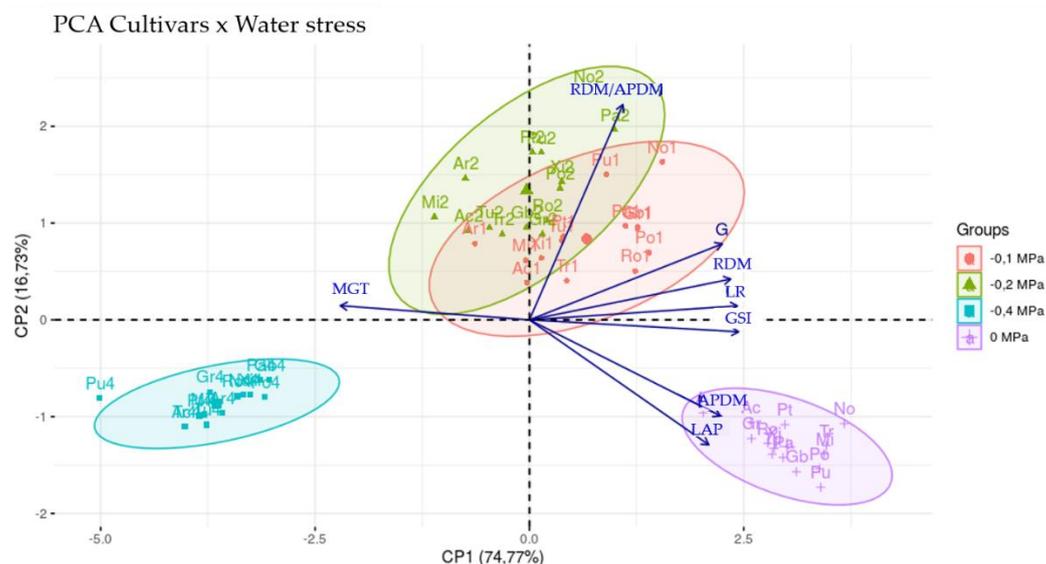


Figure 1. Biplot showing the relationship between variables and treatments for the first two main components (CP1 e CP2). Pu = BRS-Pujante; Gb = BRS-Guariba; Po = BRS-Potengi; Gr = BR 17-Gurgueia; Tu = BRS-Tumucumaque; Pa = BRS-Pajeú; Ro = BRS-Rouxinol; No = BRS-Novaera; Xi = BRS-Xiquexique; Mi = BRS-Milênio; Ac = BRS-Acauã; Pt = Patativa; Tr = BRS-Tracuateua; Ar = BRS-Aracê.

Therefore, most of the reserves metabolized by the seeds of these two treatments were destined for root development (with an increase in lateral roots) and not for shoot growth, which was reflected in the increase in the RDM/APDM ratio. According to [18], the osmotic stress condition suggests a prioritization of root growth, an important feature in the escape from this type of stress, where it can favor water absorption precisely by increasing the surface of contact with the substrate. This continuity in root growth depends on maintaining a minimum turgor pressure in the cells, which is sufficient to allow elongation and cell growth [19,20].

When analyzing the treatments in the negative region of CP1 and positive region of CP2 (Figure 1), they have higher values of the MGT variable and low values of GSI, LAP, and APDM. That is, the levels of osmotic stress (-0.1 and -0.2 MPa) imposed on these cultivars led to a delay in the seed germination process and impaired shoot growth, possibly due to a lower translocation of reserves to the axis. embryonic (growth region) due to the low availability of water. It is important to emphasize the responses of the cultivars BRS-Milênio, BRS-Acauã, and BRS-Aracê that, when submitted to the level of -0.1 MPa,

already presented difficulties to germinate and to develop, intensified, at the level of -0.2 MPa, mainly for BRS-Milênio.

Reductions in seedling LAP values can be explained by the decrease in seed metabolism since there is less water availability for the digestion of reserves and translocation of metabolized products [20]. Similar results were obtained by [21], who, working with the viability of sunflower seeds after water stress, found a reduction in seedling growth as the concentrations of PEG 6000 increased, with the lowest values in the osmotic potential of -0.8 MPa. In *Triticum aestivum* L., a significant reduction was also found for all genotypes evaluated with increasing levels of osmotic stress [22].

When evaluating the mean germination time in seeds of *Poincianella pyramidalis* and *Anadenanthera colubrina* under water stress induced by polyethylene glycol, [23] found that at the levels of more negative osmotic potentials it took a greater number of days for the seeds to germinate. These data corroborate the results obtained in this research. Therefore, we suggest that as the osmotic potential increases, the seed needs more time to germinate [24].

These decreases in the GSI and increases in the MGT, due to the increase in the expressiveness of stress, are widely reported in the literature [25,26]. When exposed to unfavorable conditions of water availability, the time for seed germination to occur tends to increase both due to the reduction in the absorption of water necessary for the activation of the metabolism, which ends up making difficult the digestion of reserves and the translocation of the metabolized products, as well as this time, can increase until the seed can activate tolerance mechanisms to this situation; or, if this does not happen, the germination process may even be completely inhibited [20,24,27], as happened with most of the cultivar seeds which were subjected to the level of -0.4 MPa.

The results showed that, regardless of the studied cultivars, all evaluated variables (except MGT) were severely affected when the seeds were subjected to the highest stress level (-0.4 MPa), where seedlings could not develop to the point of being accounted for the variables LR, LAP, RDM, APDM, and RDM/APDM. In Figure 1, this behavior can be seen and the low values for the germinative performance variables (G and GSI) can also be seen, especially for the BRS-Pujante cultivar, which was very sensitive. According to [28], when seeds are subjected to water deficiency by osmotic solutions, vigor is more affected than germination.

As in the present experiment, the osmotic potential of -0.4 MPa promoted decreases in the germination percentage of *Apuleia leiocarpa* seeds [29,30], working with the simulation of water stress in cowpea genotypes, also found that there was a reduction in the percentage of seed germination with a reduction in the osmotic potential. This same response was found in soybean seeds subjected to osmotic stress conditions [31].

By analyzing the responses of cultivars at the 0 MPa level, we observed that they presented high values of the LAP and APDM variables and low values of MGT. This answer evidences the adequate water availability since the seed has the necessary amount of water for the entire germination process, activation of the metabolism, and translocation of reserves to the embryo's growth region, which culminates in greater development of the aerial part, without the need for greater investments of its reserves for the growth of the root system [20,27].

In general, the LAP demonstrated that the cowpea cultivars presented similar responses and performances when compared within each of the stress levels, which can be observed through the 95% confidence ellipses, except the cultivar BRS-Pujante in the level -0.4 MPa, which was characterized as a discrepant point. The superposition of the ellipses of the treatments with levels -0.1 and -0.2 MPa indicates that the groups share similar characteristics.

4. Conclusions

The Principal Component Analysis allowed the characterization of the treatments, pointing the cultivar BRS-Pujante as the most sensitive at the -0.4 MPa level. The technique showed that the cultivars BRS-Milênio, BRS-Acauã, and BRS-Aracê had difficulties in tolerating the stresses of -0.1 and -0.2 MPa imposed, with emphasis on the cultivar BRS-Milênio at the level -0.2 MPa. The cultivars BRS-Novaera and BRS-Pajeú were characterized with possible tolerance at both the -0.1 and -0.2 MPa levels.

Author Contributions: Conceptualization, L.O.; methodology, A.O.; software, T.D.; validation, R.M.; formal analysis, L.O. and T.D.; investigation, M.S.; resources, L.O.; data curation, R.O.; writing—original draft preparation, L.O.; writing—review and editing, A.A. and R.M.; visualization, L.O.; supervision, A.O., A.A. and R.M.; project administration, A.O. All authors have read and agreed to the published version of the manuscript.

Funding This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Acknowledgments: The authors thank CNPq for granting the scholarship and the Federal University of Ceara for laboratory support.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

- Vijaykumar, A.; Saini, A.; Jawali, N. Phylogenetic analysis of subgenus vigna species using nuclear ribosomal RNA ITS: evidence of hybridization among *Vigna unguiculata* subspecies. *J. Hered.* **2010**, *101*, 177–188. <https://doi.org/10.1093/jhered/esp084>.
- Vale, J.C.; Bertini, C.; Borém, A. *Feijão-Caupi do Plantio à Colheita*, 1st ed.; Editora UFV: Brasil, 2017.
- Nascimento, S.P.; Bastos, E.A.; Araújo, E.C.E.; Freire Filho, F.R.; Silva, E.M. Tolerância ao déficit hídrico em genótipos de feijão-caupi. *Rev. Bras. Eng. Agrícola E Ambient.* **2011**, *15*, 853–861. <https://doi.org/10.1590/S1415-43662011000800013>.
- Freitas, R.M.O.; Dombroski, J.L.D.; Freitas, F.C.L.; Nogueira, N.W.; Pinto, J.R.S. Physiological responses of cowpea under water stress and rewatering in no-tillage and conventional tillage systems. *Rev. Caatinga* **2017**, *30*, 559–567. <https://doi.org/10.1590/1983-21252017v30n303rc>.
- Możdżeń, K.; Bojarski, B.; Rut, G.; Migdalek, G.; Repka, P.; Rzepka, A. Effect of drought stress induced by mannitol on physiological parameters of maize (*Zea mays*) seedlings and plants. *J. Microbiol. Bio-Technol. Food Sci.* **2015**, *4*, 86–91. <https://doi.org/10.15414/jmbfs.2015.4.special2.86-91>.
- Oliveira, A.B.; Gomes Filho, E.; Enéas Filho, J. O problema da salinidade na agricultura e as adaptações das plantas ao estresse salino. *Enciclopédia Biosf.* **2010**, *6*, 1–16.
- Colman, B.A.; Nunes, C.M.; Masson, G.L.; Barbosa, R.H.; Nunes, A.S. Indução de tolerância ao estresse hídrico na germinação de sementes de feijão-caupi. *Comun. Sci.* **2014**, *5*, 449–455. <https://doi.org/10.14295/cs.v5i4.276>.
- Vicini, L.; Souza, A.M.; Morales, F.E.C.; Souza, F.M. *Técnicas Multivariadas Exploratórias: Teorias e Aplicações No Software Statistica*; UFSM: 2018; 240p.
- Manly, B.F.J.; Alberto, J.A.N. *Métodos estatísticos multivariados: uma introdução*. 4nd ed.; Bookman: Brasil, 2019; p.254.
- Villela, F.A.; Filho, L.D.; Sequeira, E.L. Tabela de potencial osmótico em função da concentração de polietileno-glicol 6.000 e da temperatura. *Pesqui. Agropecuária Bras.* **1991**, *26*, 1957–1968.
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. *Regras para análise de sementes*. Brasília: Secretaria de Defesa Agropecuária/Mapa/ACS, 2009.
- Rosa, L.S.; Felippi, M.; Nogueira, A.C.; Grossi, F. Avaliação da germinação sob diferentes potenciais osmóticos e caracterização morfológica da semente e plântula de *Ateleia glazioviana* Baill (timbó). *Cerne* **2005**, *11*, 306–314.
- Labouriau, L.G. *A germinação das Sementes*, 24nd ed.; OEA: USA, 1983; p. 174.
- Maguire, J.D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Sci.* **1962**, *2*, 176–177. <https://doi.org/10.2135/cropsci1962.0011183X0002000200033x>.
- Nakagawa, J. Testes de vigor baseados no desempenho das plântulas. In: *Vigor de sementes: conceitos e testes*; Krzyzanoski, F.C., Vieira, R.D., França Neto, J.B., Eds.; Londrina: ABRATES, 1999; pp. 20-31.
- R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
- Kaiser, H.F. The varimax criterion for analytic rotation in factor analysis. *Psychometrika* **1958**, *23*, 187–200. <https://doi.org/10.1007/BF02289233>.
- Scalon, S.P.Q.; Mussury, R.M.; Euzébio, V.L.D.M.; Kodama, F.M.; Kissmann, C. Estresse hídrico no metabolismo e crescimento inicial de mudas de mutambo (*Guazuma ulmifolia* Lam.). *Ciência Florest.* **2011**, *21*, 655–662. <https://doi.org/10.5902/198050984510>.

19. Marcos Filho, J. *Fisiologia de Sementes de Plantas Cultivadas*. 5nd ed.; Abrates: Brasil, 2015. p. 659.
20. Taiz, L.; Zeiger, E.; Moller, I.M.; Murphy, A. *Fisiologia e Desenvolvimento Vegetal*. 6nd ed.; Artmed, Brasil, 2017.
21. Carneiro, M.M.L.C.; Deuner, S.; Oliveira, P.V.; Teixeira, S.B.; Sousa, C.P.; Bacarin, M.A.; Moraes, D.M. Atividade antioxidante e viabilidade de sementes de girassol após estresse hídrico e salino. *Rev. Bras. De Sementes* **2011**, *33*, 752–761. <https://doi.org/10.1590/S0101-31222011000400017>.
22. Giroto, L.; Alves, J.D.; Deuner, S.; Albuquerque, A.C.S.; Tomazoni, A.P. Tolerância à seca de genótipos de trigo utilizando agentes indutores de estresse no processo de seleção. *Rev. Ceres* **2012**, *59*, 192–199. <https://doi.org/10.1590/S0034-737X2012000200007>.
23. Santos, C.A.; Silva, N.V.; Walter, L.S.; Silva, E.C.A.; Nogueira, R.J.M.C. Germinação de duas espécies da caatinga sob déficit hídrico e salinidade. *Pesqui. Florest. Bras.* **2016**, *36*, 219–224. <https://doi.org/10.4336/2016.pfb.36.87.1017>.
24. Pelegrini, L.L.; Borcioni, E.; Nogueira, A.C.; Koehler, H.S.; Quoirin, M.G.G. Efeito do estresse hídrico simulado com NaCl, manitol e PEG (6000) na germinação de sementes de *Erythrina falcata* Benth. *Ciência Florest.* **2013**, *23*, 511–519. <https://doi.org/10.5902/198050989295>.
25. Oliveira, A.B.; Gomes Filho, E. Efeito do condicionamento osmótico na germinação e vigor de sementes de sorgo com diferentes qualidades fisiológicas. *Rev. Bras. Sementes* **2010**, *32*, 25–34. <https://doi.org/10.1590/S0101-31222010000300003>.
26. Agostini, E.A.T.; Machado-neto, N.B.; Custódio, C.C. Induction of water deficit tolerance by cold shock and salicylic acid during germination in the common bean. *Acta Scientiarum. Agron.* **2013**, *35*, 209–219. <https://doi.org/10.4025/actasciagron.v35i2.15967>.
27. Bewley, J.D.; Bradford, K.J.; Hilhorst, H.W.M.; Nonogaki, H. *Seeds: Physiology of Development, Germination and Dormancy*, 3rd ed.; Springer: USA, 2013; p. 392.
28. Machado Neto, N.B.; Saturnino, S.M.; Bomfim, D.C.; Custódio, C.C. Water stress induced by mannitol and sodium chloride in soybean cultivars. *Braz. Arch. Biol. Technol.* **2004**, *47*, 521–529. <https://doi.org/10.1590/S1516-89132004000400004>.
29. Spadeto, C.; Lopes, J.C.; Mengarda, L.H.G.; Matheus, M.T.; Bernardes, P.M. Estresse salino e hídrico na germinação de sementes de garapa (*Apuleia leiocarpa* (VOGEL.) JF Macbr.). *Enciclopédia Biosf.* **2012**, *8*, 539–551.
30. Ferreira, D.F. Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. *Ciência E Agrotecnologia* **2014**, *38*, 109–112. <https://doi.org/10.1590/S1413-70542014000200001>.
31. Soares, M.M.; Santos Junior, H.C.; Simões, M.G.; Pazzin, D.; Silva, L.J. Estresse hídrico e salino em sementes de soja classificadas em diferentes tamanhos. *Pesqui. Agropecuária Trop.* **2015**, *45*, 370–378. <https://doi.org/10.1590/1983-40632015v4535357>.