COMMUNICATIONS IN PLANT SCIENCES

RESEARCH ARTICLE

Effect of hydration and dehydration cycles on *Macroptilium atropurpureum* seeds germination under water deficit conditions

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The imbibition process, which is essential for seed germination, can be interrupted due to lack of water in the environment. Once this occurs, seeds have its germination process interrupted and loses the water absorbed to the environment. The objective of this study was to evaluate the effects of hydration and dehydration cycles (HD cycles) on seed germination of *Macroptilium atropurpureum* under water deficit conditions. Seeds of *M. atropurpureum* were submitted to 0 (control), 1, 2 and 3 HD cycles at different hydration times. In addition, the seeds were also submitted to osmotic potentials of 0.0, -0.1, -0.3, -0.6 and -0.9 MPa for water deficit simulation. Although the seeds germinated in all of the osmotic potentials used, the seeds presented a reduction in the percentage of germination, independently of the number of HD cycles and the hydration time between each cycle. In addition, HD cycles also negatively affected the mean germination rate, promoting a reduction of this parameter as seeds were submitted to HD cycles and osmotic potentials. Thus, the results demonstrated that HD cycles did not provide an increase in water stress tolerance in *M. atropurpureum* seeds during germination.

Highlighted Conclusions

1) *Macroptilium atropurpureum* seeds can germinate even in low osmotic potentials conditions.

2) Discontinuous hydration confers a negative effect on *M. atropurpureum* germination and does not provide a greater tolerance to water deficit.

Seed imbibition is a key process in the plant life cycle determining whether seed germination and individual growth will be successful or not (Ribeiro et al. 2015). The imbibition process, which is essential for seed germination, can be interrupted due to lack of water in the environment. Once this occurs, the seed has its germination process interrupted and loses the water absorbed to the environment (Fenner and Thompson 2005). These interruptions during germination process occur in arid and semi-arid environments, where water presents a limit of space and time in its availability, subjecting seeds to hydration and dehydration cycles (HD cycles) (Lima and Meiado 2017). Dubrovsky (1998) pointed that discontinuous hydration ensures a high survival rate during desiccation, demonstrating that seeds may present seed hydration memory, caused by the discontinuous imbibition process.

In addition to the high survival rate during desiccation highlighted by Dubrovsky (1998), HD cycles may provide other advantages during the germination process of the seeds of some species. Rito et al. (2009) demonstrated that seeds of *Cereus jamacaru* DC. subsp. *jamacaru* (Cactaceae) present seed hydration memory, since, after being submitted to HD cycles, seeds had their percentage of seed germination increased. Pedrero-López et al. (2016), in experiments with seeds of *Dodonaea viscosa* Jacq. (Sapindaceae) also observed a significant increase of germinability after HD cycles from 22.5% to 63.3%. Discontinuous hydration may also provide greater tolerance to abiotic stress conditions, as observed by Li et al. (2017) in *Medicago sativa* L. (Fabaceae) seeds, which showed an increase in germinability under water and saline stress conditions after being submitted to discontinuous hydration. Lima and Meiado (2018) also observed a reduction of the time for seed germination in *Mimosa tenuiflora* (Willd.) Poir. (Fabaceae) after 1, 2 and 3 HD cycles, however, seed germinability did not differ statistically after HD cycles.

In studies with cactus species, Contreras-Queiroz et al. (2016) observed that the presence of seed hydration memory, resulting of HD cycles, depends on the climate and the microenvironment where individuals occur, being then an indication that seed germination of some species is influenced by environmental conditions imposed to parental plants. Lima and Meiado (2017) demonstrated in experiments with seeds of *Pilosocereus catingicola* (Gürke) Byles & G.D. Rowley subsp. *salvadorensis* (Werderm.) Zappi (Cactaceae) from populations of different ecosystems in Northeast region of Brazil that the ecosystem influences the effects that HD cycles promote in seed germination. On the other hand, discontinuous hydration may also have negative effects on germination of some species. Santini et al. (2017) demonstrated that HD cycles reduced the percentage of seed germination and increased the mean germination time of *Echinocereus engelmannii* (Parry ex Engelm.) Lem. and *Ferocactus hamatacanthus* (Muehlenpf.) Britton & Rose, both species of Cactaceae.

Fabaceae Lindl. is among the angiosperm families present in semiarid regions of Brazil that are subject to water deficit conditions. This family presents a great diversity of seed shape, size, color, structure and characteristics, and many species present economic value for the Brazilian Northeast (Queiroz 2009). *Macroptilium atropurpureum* (Sessé & Moc. ex DC.) Urb. known as Siratro is a species of Fabaceae family that is distributed throughout the Brazilian territory, being present in different ecosystems and submitted to diverse environmental conditions, including climatic conditions of the Brazilian semi-arid (Snak and Salinas 2017). Moreover, this species is a tropical pasture legume used for permanent and short-term pastures (Rusdy 2016). Furthermore, a little is known about the ecophysiological behavior of this species. Thus, the objective of this study was to evaluate the effects of HD cycles on seed germination of *M. atropurpureum* under water deficit conditions.

MATERIAL AND METHODS

General information. The study was carried out at the Laboratory of Seed Physiology, at the Federal University of Sergipe, Campus Professor Alberto Carvalho, in Itabaiana, Sergipe. Seeds of *M. atropurpureum* were collected on September, 2017, in Caatinga areas of the municipality of Nossa Senhora da Glória, Sergipe. According to Köppen and Geiger, the weather rating is Aw and the average annual temperature is 23,4 °C. The average annual rainfall is 763 mm (Climate Data 2017). Caatinga is a semi-arid ecosystem located in the Northeast region of Brazil and characterized by a deficiency in water availability during a large part of the year and a temporal irregularity in the distribution of rainfall (Queiroz 2009, Santana and Souto 2011).

Treatment to overcome seed dormancy. All of the seeds of *M. atropurpureum* used in this study to the determination of the imbibition curve and germination tests were previously scarified with sulfuric acid (Sigma-Aldrich® P.A., 95-97%) in glass beakers for 5 minutes to overcome the physical dormancy presented by the seeds of the species. After the period immersed in the sulfuric acid, seeds were washed in running water for 10 minutes and dried on paper.

Imbibition curve and hydration times. To determine the imbibition curve, four replicates of 25 seeds were weighed on analytical balance to obtain the seeds initial weight. After this, each replicate was placed in 9 cm diameter Petri dishes containing two layers of filter paper moistened with 8 mL of distilled water at a temperature of 25°C (McDonald 2002). The Petri dishes were maintained in a growth chamber. No water was added to the Petri dishes during determination of the imbibition curve. Thereafter, each repetition was weighed at 60-minute intervals, after being placed to imbibition, until they completed the germination process with radicle protrusion. After establishing the imbibition curve of *M. atropurpureum* seeds, three points in the curve were selected, which were denominated as times X, Y and Z, corresponding to ½ of phase I, ¼ of phase II and ¾ of phase II of the imbibition process, respectively (Lima et al. 2018).

Hydration and dehydration cycles (HD cycles). HD cycles corresponded to pre-germination treatments to evaluate the influence of discontinuous hydration on seed tolerance to water deficit. For each time established through the imbibition curve (times X, Y and Z), seeds of *M. atropurpureum* were submitted to 0 (control), 1, 2 and 3 cycles of hydration with times of dehydration of 48 h for all cycles. Discontinuous hydration of the seeds was carried out in 15 cm diameter Petri dishes containing two layers of filter paper moistened with 20 mL of distilled water. For the dehydration phase, seeds were transferred to 15 cm diameter Petri dishes with dry paper and kept at a temperature of 25°C. Each cycle corresponded to a hydration phase followed by a dehydration phase (Lima et al. 2018).

Germination tests and parameters evaluated. Seed germination was evaluated using distilled water as control and under the osmotic potentials of -0.1; -0.3; -0.6 and -0.9 MPa obtained with polyethylene glycol 6000 solution (PEG 6000) (Villela et al. 1991) in the water deficit simulation. For each treatment in this study, four replicates with 25 seeds were used, which were placed to germinate in 5 cm diameter Petri dishes containing two layers of filter paper moistened with 3 mL of PEG 6000 solution. The Petri dishes were sealed with parafilm plastic and maintained under white light (12 h photoperiod) and 25°C (Lima and Meiado 2017). The experiment took place in a growth chamber. The number of germinated seeds was counted daily during a period of 15 days and radicle protrusion was considered as the criterion for seed germination. A 15 days germination test were used once *M. atropurpureum* is a fast germination species (McDonald 2002).

Processing data and statistics. After the evaluation, we calculated, using the GerminaQuant software (Marques et al. 2015), the germination percentage (G = %) and the mean germination rate: MGR = 1/t, where $t = \sum n_i.t_i / \sum n_i$, where t_i is the period between the beginning of the experiment and the nth observation (days) and n_i is the number of seeds germinated in the time *i* (number corresponding to the nth observation) (Labouriau 1983). Before statistical analysis, the germinability data obtained underwent an angular transformation (arc sine √%). The Shapiro-Wilk and Levene tests were used to verify the data normality and the homogeneity of the variances. The results were submitted to factorial variance analysis with three factors (hydration time, number of HD cycles and osmotic potential) and the means were compared by Tukey test (Ranal and Santana 2006). Analyzes were performed in STATISTICA 13 software with $\alpha = 5\%$ (StatSoft 2016).

RESULTS

The seeds of *M. atropurpureum* presented a three-phase pattern in their imbibition curve (Figure 1). The root protrusion was observed after 12 hours of the beginning of seed hydration. Thus, the hydration times X, Y and Z corresponded to 1:30, 5:15, and 9:45 hours, respectively. The osmotic potentials presented significant influence on germination of *M. atropurpureum* seeds that did not undergo HD cycles (Table 1). Although seeds germinated in all osmotic potentials, demonstrating a great tolerance to water deficit, there was a reduction in the seeds germinability as the osmotic potential increased. The seeds reduced their germination percentage from 87% in 0.0 MPa (control) to 27% in -0.9 MPa (Figure 2). In addition, MGR was also affected by water deficit conditions (Table 1). A reduction of the MGR was observed as the osmotic potential became more negative (Figure 3).

HD cycles and the hydration time during the cycles showed significant effects on *M. atropurpureum* germinability (Table 1). However, these effects were negative, since HD cycles at all hydration times reduced the percentage of germination in all osmotic potentials (Figure 2). This result also demonstrated that HD cycles did not provide the seeds of *M. atropurpureum* an increase in tolerance to water deficit conditions to which the seeds were submitted. Although not influenced by hydration times, MGR was influenced by HD cycles (Table 1). It was observed a reduction of the MGR as the seeds were submitted to HD cycles and water deficit conditions (Figure 3).

DISCUSSION

In a condition of water availability and permeability of the seed coatings, the imbibition process usually presents three phases (Rajjou et al. 2012). This three-phase pattern has already been observed in seeds of several species, such as *Schinopsis brasiliensis* Engl. (Anacardiaceae) (Dantas et al. 2008); *Jatropha curcas* L. (Euphorbiaceae) (Araujo et al. 2014) and *Senna spectabilis* (DC.) H.S. Irwin & Barneby var. *excelsa* (Schrad.) H.S. Irwin & Barneby (Fabaceae) (Lima et al. 2018).

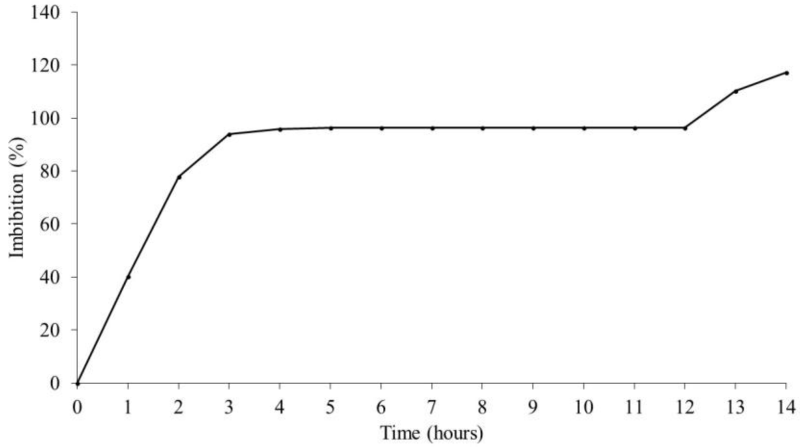

Figure 1. Imbibition curve of *Macroptilium atropurpureum* (Sessé & Moc. ex DC.) Urb. (Fabaceae) seeds.

Table 1. Results of the factorial ANOVA of germinability and the mean germination rate of *Macroptilium atropurpureum* (Sessé & Moc. ex DC.) Urb. (Fabaceae) seeds that were submitted to hydration and dehydration cycles (HD cycles) and were submitted to water deficit at different osmotic potentials.

Factors	*F*	d.f.	*p*
Germinability (%)			
Hydration times	17.06	2	< 0.0001
Number of HD cycles	211.37	3	< 0.0001
Osmotic potential	629.43	4	< 0.0001
Mean germination rate (days $^{-1}$)			
Hydration times	2.64	2	0.0744
Number of HD cycles	76.22	3	< 0.0001
Osmotic potential	300.32	4	< 0.0001

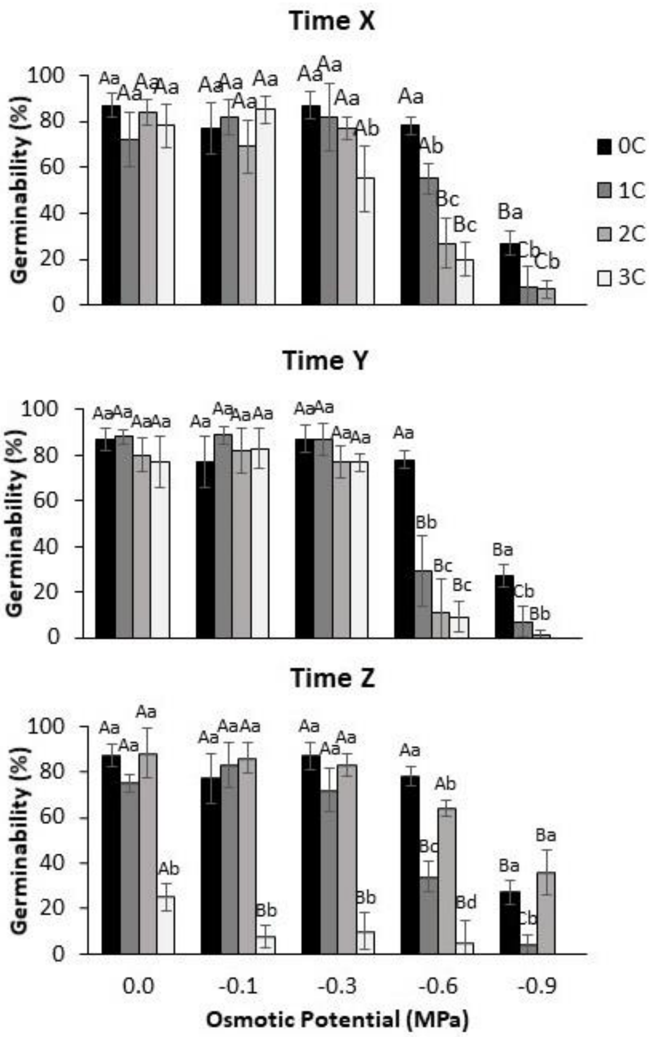

Figure 2. Germinability (%) of *Macroptilium atropurpureum* (Sessé & Moc. ex DC.) Urb. (Fabaceae) seeds that passed through 0, 1, 2 and 3 hydration and dehydration cycles (0C, 1C, 2C and 3C, respectively) in different hydration times (Time X: 1:30 hours, Time Y: 5:15 hours, Time Z: 9:45 hours) and were submitted to water deficit at different osmotic potentials. Data were expressed as mean ± standard deviation. Uppercase letters compare different cycles at the same osmotic potential. Lowercase letters compare the same cycle in different osmotic potentials.

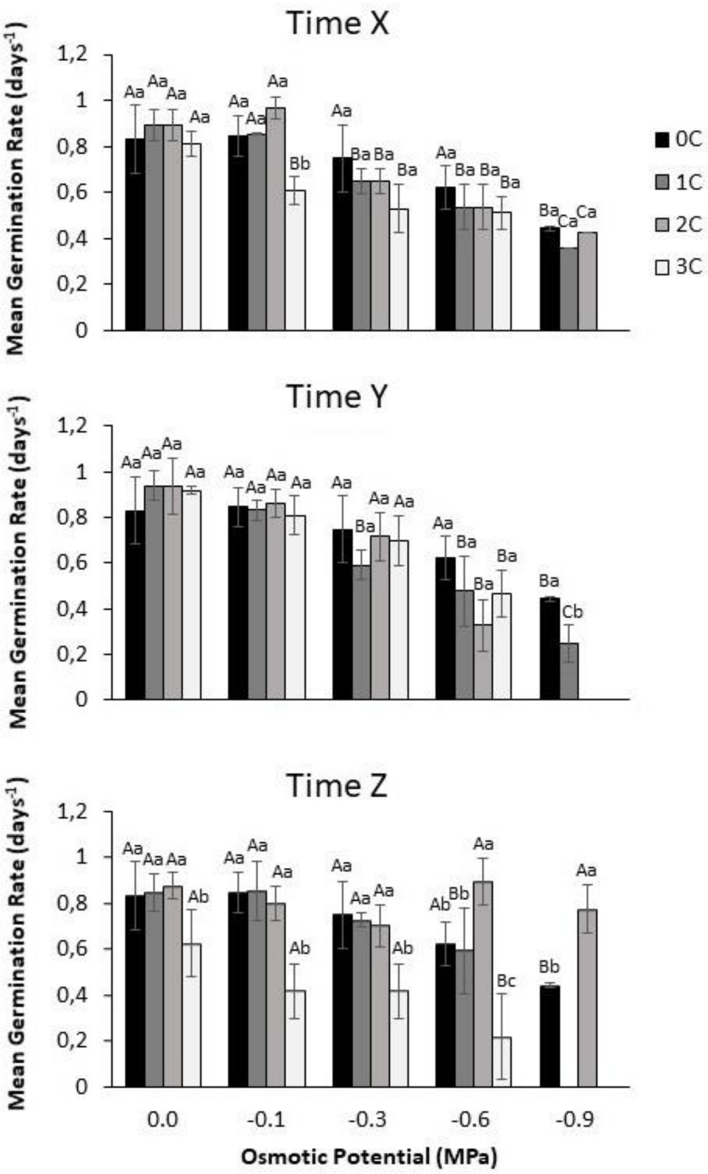

Figure 3. Mean germination rate (days $^{-1}$) of *Macroptilium atropurpureum* (Sessé & Moc. ex DC.) Urb. (Fabaceae) seeds that passed through 0, 1, 2 and 3 hydration and dehydration cycles (0C, 1C, 2C and 3C, respectively) in different hydration times (Time X: 1:30 hours, Time Y: 5:15 hours, Time Z: 9:45 hours) and were submitted to water deficit at different osmotic potentials. Data were expressed as mean ± standard deviation. Uppercase letters compare different cycles at the same osmotic potential. Lowercase letters compare the same cycle in different osmotic potentials.

Each species germinates at a specific critical osmotic potential (Oliveira et al. 2017). In addition, the environmental conditions from which the seeds were produced may influence the physiological responses to the abiotic conditions present during the germination of these seeds (Lima and Meiado 2017). Sevik and Cetin (2015), in germination experiments with *Cupressus sempervirens* L. (Cupressaceae), *Koelreuteria paniculata* Laxm. (Sapindaceae) and *Pinus nigra* J.F.Arnold (Pinaceae) showed that these species, as well as *M. atropurpureum*, also germinate at low osmotic potentials (-0.8 MPa), even though in a reduced percentage compared to the control. Santos et al. (2018), evaluating *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (Bignoniaceae) seeds germination under water stress conditions, observed 0% of germinability at the osmotic potential -0.8 MPa. Unlike *M. atropurpureum*, as demonstrated in this study, *H. impetiginosus* is an example of a species with greater sensitivity to water stress conditions.

As observed, *M. atropurpureum* has the capacity to germinate at low osmotic potentials (-0.9 MPa), although it reduces the mean germination rate and the final percentage of germination. This inhibition or reduction of the germinability and the mean germination rate of seeds under water deficit conditions is related to the reduction of enzymatic activities due to the low availability of water and, consequently, to the reduction of seed metabolism, which needs to be activated for the reserve mobilization process, fundamental for seeds germination (Bewley and Black 1994).

Yan (2017) emphasized that the benefits of HD cycles are related to the improvement of the germinative metabolism. Many studies pointed positive responses regarding the HD cycles effects on germination of different species, increasing the percentage of germination, mean germination rate and tolerance to abiotic stresses. Alvarado-López et al. (2014) observed positive effects of HD cycles on seeds of *Tecoma stans* (L.) Juss. ex Kunth (Bignoniaceae) and *Cordia megalantha* S.F.Blake (Boraginaceae). HD cycles also favored the germination of *Parkia nitida* Miq. (Fabaceae) (Moraes et al. 2015) and *Tanacetum cinerariifolium* (Trev.) Sch. Bip. (Asteraceae) (Li et al. 2011).

Although discontinuous hydration favors the germination of many species, HD cycles did not provide positive effects on *M. atropurpureum* germination. Santini et al. (2017) demonstrated that discontinuous hydration also did not favor seed germination of *Echinocereus engelmannii* (Parry ex Engelm.) Lem. (Cactaceae). According to Sliwinska and Jendrzejczak (2002), this response may be related to loss of desiccation tolerance after the seeds are submitted to HD cycles, causing an increase in seed mortality. In *M. atropurpureum* seeds, HD cycles may not have provided the germinative metabolism, inhibiting the positive effects that the HD cycles could confer during the seeds germination. Lima and Meiado (2017) demonstrated that the numbers and the hydration times had direct effects on seeds response to HD cycles. In this present study, hydration times and numbers of HD cycles may had interfered negatively on *M. atropurpureum* seed germination. Contreras-Queiroz et al. (2016) observed that the effects of HD cycles are related to the climate and the microenvironment where the parental individuals occur. Lima and Meiado (2017) demonstrated that seeds produced in different ecosystems present differentiated germinative responses after being submitted to the cycles of HD.

In conclusion, the present study suggests that *M. atropurpureum* seeds is highly tolerant to water deficit conditions due to the ability to germinate at low osmotic potentials (-0,9 MPa). HD cycles did not improve seed tolerance to water deficit and had a negative effect on final germination percentage and mean germination rate. This could be attributed to climate conditions where seeds were produced. Therefore, future studies can evaluate the effects of HD cycles on *M. atroporpureum* seeds from different populations.

References

Alvarado-López S et al. 2014. Priming effects on seed germination in *Tecoma stans* (Bignoniaceae) and *Cordia megalantha* (Boraginaceae), two tropical deciduous tree species. Acta Oecologica 61:65-70.

Araujo RF et al. 2014. Curva de absorção de água em sementes de pinhão-manso (*Jatropha curcas* L.). Idesia (Arica) 32:5-10.

Bewley JD, Black M. 1994. Seed: Physiology of Development and Germination. Plenum: New York.

Climate Data. 2017. Dados Climáticos Para Cidades Mundiais. Available at https://pt.climate-data.org. Accessed on Dec. 10, 2017.

Contreras-Queiroz M et al. 2016. Is seed hydration memory dependent on climate? Testing this hypothesis with Mexican and Argentinian cacti species. Journal of Arid Environments 130:94-97.

Dantas BF et al. 2008. Alterações bioquímicas durante a embebição de sementes de baraúna (*Schinopsis brasiliensis* Engl.). Revista Brasileira de Sementes 30:214-219.

Dubrovsky JG. 1998. Discontinuous hydration as a facultative requirement for seed germination in two cactus species of the Sonoran Desert. Journal of the Torrey Botanical Society 125:33-39.

Fenner M, Thompson K. 2005. The Ecology of Seeds. Cambridge University Press: Cambridge.

Labouriau LG. 1983. A germinação das sementes. Secretaria da OEA: Washington.

Li et al. 2011. Effects of light, hydropriming and abiotic stress on seed germination, and shoot and root growth of pyrethrum (*Tanacetum cinerariifolium*). Industrial Crops and Products 34:1543-1549.

Li R et al. 2017. Hydropriming accelerates seed germination of *Medicago sativa* under stressful conditions: A thermal and hydrotime model approach. Legume Research 40:741-747.

Lima AT, Meiado MV. 2017. Discontinuous hydration alters seed germination under stress of two populations of cactus that occur in different ecosystems in Northeast Brazil. Seed Science Research 27:292-302.

Lima AT, Meiado MV. 2018. Effect of hydration and dehydration cycles *on Mimosa tenuiflora* seeds during germination and initial development. South African Journal of Botany 116:164-167.

Lima AT et al. 2018. Does discontinuous hydration of *Senna spectabilis* (DC.) H.S. Irwin & Barneby var. *excelsa* (Schrad.) H.S. Irwin & Barneby (Fabaceae) seeds confer tolerance to water stress during seed germination? Journal of Seed Science 40:36-43.

Marques FRF et al. 2015. GerminaQuant: A new tool for germination measurements. Journal of Seed Science 37:248-255.

McDonald CK (2002) Germination response to temperature in tropica and subtropical pasture legumes. 1. Constant temperature. Australian Journal of Experimental Agriculture 42:407-419.

Moraes GJP et al. 2015. Physiological immaturity and hydropriming of *Parkia nitida* Miq. seeds with physical dormancy. Ciência Florestal 25:1053-1059.

Oliveira DM et al. 2017. O Aumento da Temperatura Reduz a Tolerância ao Estresse Hídrico na Germinação de Sementes de *Pereskia grandifolia* Haw. subsp. grandifolia (Cactaceae)? Gaia Scientia 11:26-36.

Pedrero-López LV et al. 2016. Effects of Hydropriming Treatments on the Invigoration of Aged *Dodonaea viscosa* Seeds and Water-Holding Polymer on the Improvement of Seedling Growth in a Lava Field. Restoration Ecology 24:61-70.
Queiroz LP. 2009. Leguminosas da Caatinga. Universidade Estadual de Feira de Santana: Feira de Santana.
Rajjou L et al. 2012. Seed Germination and Vigor. Annual Review of Plant Biology 63:507-533.
Ranal MA and Santana DG. 2006. How and why to measure the germination process? Revista Brasileira de Botânica 29:1-11.
Ribeiro PR et al. 2015. Metabolite profiling of the oilseed crop *Ricinus communis* during early seed imbibition reveals a specific metabolic signature in response to temperature. Industrial Crops and Products 67: 305-309.
Rito KF et al. 2009. As sementes de mandacaru têm memória hídrica? Boletín de la Sociedad Latinoamericana y del Caribe de Cactáceas y otras Suculentas 6:26-31.
Rusdy M. 2016. Enhancement of Seed Germination and Seedling Growth of Siratro (*Macroptilium atropurpureum*). International Journal of Science and Research 5: 820-823.
Santana JAS, Souto JS. 2011. Produção de serapilheira na Caatinga da região semi-árida do Rio Grande do Norte, Brasil. Idesia 29:87-94.
Santini BA et al. 2017. Priming effect on seed germination: Is it always positive for cacti species? Journal of Arid Environments 147:155-158.
Santos PCS et al. 2018. Water stress and temperature on germination and vigor of *Handroanthus impetiginosus* (Mart. ex DC). Revista Brasileira de Engenharia Agrícola e Ambiental 22:349-354.
Sevik H, Ceting M. 2015. Effects of water stress on seed germination for select landscape plants. Polish Journal of Environmental Studies 24: 689-693.
Sliwinska E, Jendrzejczak E. 2002. Sugar-beet seed quality and DNA synthesis in the embryo in relation to hydration-dehydration cycles. Seed Science and Technology 30:597-608.
Snak C, Salinas AOD. 2017. *Macroptilium*. In: Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro. Available at http://reflora.jbrj.gov.br/reflora/floradobrasil/FB29786 Accessed on Dec. 10, 2017.
Statsoft. 2016. STATISTICA 13. StatSoft South America.
Villela FA et al. 1991. Tabela de potencial osmótico em função da concentração de polietileno glicol 6000 e da temperatura. Pesquisa Agropecuária Brasileira 26:1957-1968.
Yan M. 2017. Prolonged storage reduced the positive effect of hydropriming in Chinese cabbage seeds stored at different temperatures. South African Journal of Botany 111:313-315.