



# Seed discontinuous hydration does not benefit germination, but improves drought tolerance of *Triplaris gardneriana* seedlings

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**ABSTRACT.** The discontinuous seed hydration enables the acquisition of tolerance to environmental stresses, causing a stress imprint. It may modify metabolic patterns and lead to improved stress responses. This study aims to evaluate the effects of discontinuous hydration on germination and on seedling growth of *Triplaris gardneriana* Wedd. under intermittent drought. The seeds have gone through cycles (0, 1, 2 and 3) of hydration and dehydration (HD). The seedlings produced were subjected to water deficit (daily watering and intervals of seven and fourteen days between watering). Seed germinability parameters and, relative growth rate (RGR) of seedling, leaf area, dry matter yield and leaf relative water content (RWC) were evaluated. The HD cycles did not benefit germination, but two HD cycles induced a better biomass accumulation and increased leaf area in seedlings under moderate water deficit, while three HD cycles promoted an increase in RGR and influenced the RWC values. Severe stress affects seedling growth, but subjection to HD cycles minimizes the deleterious effects of drought, suggesting discontinuous hydration acts leading stress imprint in plants.

**Keyword:** Growth rate; HD cycles; riparian tree; water deficit; drought stress; Caatinga.

Received on September 26, 2020.

Accepted on May 6, 2021.

## Introduction

Plant organisms do not always find favorable conditions for growth and development during their life cycle. Plant growth and development are closely associated with a positive balance of available soil water, nutrients, light, and carbon dioxide (Zandalinas, Mittler, Balfagón, Arbona, & Gómez-Cadenas, 2017; Lamers, Meer, & Testerink, 2020). Plants respond to the scarcity of these resources in complex ways, involving adaptive changes or deleterious responses. In field conditions, these responses may be synergistically or antagonistically modified due to multiple pressures, such as the environmental stresses (Chaves et al., 2002). These pressures may cause disturbances in the appropriate levels of these resources and may promote negative effects on plant growth and establishment (Zlatev & Lidon 2012; Brunner, Herzog, Dawes, Arend, & Sperisen, 2015; Zandalinas et al., 2017).

Currently, some approaches have been used to elucidate the process of acquiring tolerance to environmental stresses in plants (Ali, Bano, & Fazal, 2017; Ashraf et al., 2018; Kumar & Verma 2018), focusing on understanding how these processes affect plant growth and yield. Recently, the use of priming stimuli in seeds has been discussed as a strategy of induced tolerance in plants (Hossain, Liu, Burritt, Fujita, & Huang, 2020) and it is generally considered a primary germinative metabolism that allows the activation of repair mechanisms in the seeds, thus enabling germination and seedling development (Thomas, Dinakar, & Puthur, 2020).

Seed priming is a physiological technique of hydration in a solution of any priming agent followed by drying of seeds that promotes improvements in aspects and pre-germinative processes, seedling growth and yield under normal and stress conditions (Lima & Meiado 2018a). Seeds that go through this process exhibit a faster and more uniform germination (Ghassemi-Golezani, Chadordooz-Jeddi, Nasrollahzadeh, & Moghaddam, 2010) due to the activation of different enzymes, metabolic activities, biochemical and cellular

repair processes (Paparella et al., 2015), protein synthesis (Varier, Vari, & Dadlani, 2010), and increase of the antioxidant system, in relation to non-primed seeds (Hasanuzzaman & Fotopoulos 2019).

There is a variety of priming treatments (e.g. hydropriming, osmopriming, holopriming, biopriming, UV-B priming) and their use has been associated with the improvement of seeds and seedlings in normal and stressful conditions (Ghassemi-Golezani et al., 2010; Paparella et al., 2015; Lima & Meiado 2018a; Hasanuzzaman & Fotopoulos 2019; Thomas et al., 2020). Between priming treatments, the discontinuous hydration of “hydropriming” seeds, which occurs naturally in arid and semiarid environments (Lima, Cunha, Dantas, & Meiado, 2018), has been referred to as a cheaper, accessible and eco-friendly technique, because there is no additional use of chemical compounds when compared to seed priming that activates seed improvement (Singh et al., 2020).

Otherwise, seed priming does not always give a better germinative performance to the seeds, especially when comparing different kinds of priming agents (Esper-Neto et al., 2020). Aluko, Ayodele, Salami, & Olaleye (2020) did not find differences in the  $T_{50}$  (described as the time required for 50% germination) for seeds treated with NaCl (osmopriming) and aqueous extract of *Moringa oleifera* leaves (phyto-priming) which were placed to germinate in ideal conditions when compared to the control treatment, as well as no difference was found for the mean emergence time (MET) when the seeds were treated with water and NaCl. Likewise, Silva et al. (2019) found no differences in the percentage of germination and  $T_{50}$  of *Handroanthus serratifolius* (Vahl) S. Grose (Bignoniaceae) seeds comparing hydro and osmopriming treatments at various times, including, with a reduction in the percentage of germination when the seeds were treated with hydropriming at 5 °C in relation to the control, showing that despite the benefits of the technique, the responsibility of the seeds does not always occur.

However, some studies with plants from Caatinga, a Brazilian Tropical Dry Forest, have demonstrated that, sometimes, discontinuous hydration do not favor germination in adequate water availability, but it does benefit the process in drought or salt stress condition (Lima & Meiado 2017; Lima & Meiado 2018b). On the other hand, some observation has showed that, for some species, the benefit of discontinuous hydration has been recognized during plant initial growth (Lima & Meiado 2018a) or during stress environmental conditions conferring to drought or salt-tolerance (Lima & Meiado 2018b).

On this study, we have tested the hypothesis that hydration and dehydration cycles (HD cycles) of seeds act as a priming stimulus, conferring benefits on seed germination and acquisition of water deficit tolerance during the early development phase of seedlings, maintaining growth rhythms under stress. For this, we analyzed the species *Triplaris gardneriana* Wedd. (Polygonaceae), a riparian plant, with a wide geographical distribution that grows in semiarid areas in northeastern Brazil and has been of profound importance in projects of ecological restoration in riparian environments in Caatinga. In order to verify our hypothesis, we addressed some ecophysiological aspects to observe the real benefits of hydropriming for this species, as maintenance of high relative water content in leaves and growth rates, even under stress, characteristics which are important for the ecology and permanence of the species studied in a stressful condition. Thus, this study aims to evaluate the germination and initial growth responses of *T. gardneriana* seedlings subjected to soil water deficiency from seeds that have been undergone HD cycles, a process adopted as a pre-germination priming stimulus. If our hypothesis is corroborated, discontinuous hydration should promote benefits to seed germination and initial development of seedlings generated from previously treated seeds.

## Material and methods

We conducted the experiments in the Laboratory of Plant Physiology and Ecophysiology and in the agricultural greenhouse belonging to the Department of Biology of the *Universidade Federal de Sergipe* (UFS) in the municipality of São Cristóvão, Sergipe, Northeast Brazil (10°92' S and 37°10' W).

### Hydration and dehydration cycles (HD cycles) and seed germination assay

Seeds were provided by the Ecology and Environmental Monitoring Center of the *Universidade Federal do Vale do São Francisco*. They were submitted to four discontinuous hydration treatments (0, 1, 2 and 3 HD cycles), whose hydration and dehydration time was determined after soaking and dehydration curves.

To plot the soaking curve, we weighed the seeds ( $n = 100$  seeds divided into four replications of 25) and placed to soak in glass containers containing approximately 100 mL of distilled water. The containers were kept in the laboratory at room temperature (approximately 25°C) constantly in the dark. At one-hour

intervals, we dried the seeds using absorbent paper and weighed on an analytical balance (Shimadzu AUY220). We estimated imbibition by seed biomass variation in the different intervals evaluated until the completion of the three phases. On the other hand, we estimated the dehydration in a laboratory environment (25°C), by weighing at intervals of 1 hour, and it was determined as the time necessary for the seeds hydrated in time X to return to the initial weight (pre-soaking).

The hydration time chosen for the HD cycles was determined, being  $\frac{1}{2}$  of the time the greatest water absorption in phase I of the three-phase pattern of water absorption by the seeds, called time X (Lima et al. 2018). In turn, the seed dehydration time corresponded to the time required for the seeds to return to their initial fresh biomass before the seed hydration phase (Lima et al., 2018). The hydration and dehydration times of the studied species were 14 and 18 hours, respectively.

After determining hydration and dehydration times, we subjected the seeds ( $n = 75$  seeds per treatment, divided into three replications of 25) to the HD cycles (0 cycle as control and 1, 2 and 3 cycles of 14 hours of hydration followed by 18 hours of dehydration before soaking). Then, we placed the seeds to germinate in 57 x 38 cm plastic trays containing 20 kg of washed sand as substrate. The maintenance of the water supply of the trays was performed daily, and we accounted for the germination in 24-hour intervals. The seedling hypocotyl emergence was the criterion used to consider a seed as germinated. From these data, we calculated each discontinuous hydration treatment germinability [ $\%G = (\sum n_i \cdot N^{-1}) \cdot 100$ , where  $\sum n_i$  is the total sum of germinated seeds in relation to the number of seeds ready to germinate in the tray ( $N$ ); data being expressed as percentage], mean germination time ( $MGT = \sum n_i \cdot t_i / \sum n_i$ , where  $n_i$  is the number of germinated seeds within a given time range  $t_{i-1}$  and  $t_i$ ; data expressed in days), emergence rate index ( $ERI = E_1/N_1 + E_2/N_2 + \dots E_n/N_n$ , where  $E_1, E_2, \dots E_n$  correspond to the number of seedlings that emerged in the first, second and up to the ninth observation, and  $N_1, N_2, \dots N_n$  correspond to the number of days after sowing), and Synchrony ( $Z = \sum C_{ni,2} / N$ , where  $C_{ni,2} = n_i(n_i - 1)/2$  and  $N = \sum n_i(\sum n_i - 1)/2$ , where  $C_{ni,2}$  is the combination of seeds germinated during the time  $i$ , by pairs, and  $n_i$  is the number of seeds germinated in the time  $i$ ) of each cycle (Ranal & Santana, 2006).

### Design and experimental conditions

After emergence, we selected 15 homogeneous seedlings from each treatment, taking account sanity and same stage of development, totaling 60 seedlings. The seedlings were transferred to pots with a capacity of 4 kg containing vegetable soil, washed sand and cattle manure at a ratio of 3:1:1 (v/v) as substrate. We watered seedlings close to field capacity (approximately 20% of the volumetric capacity of the soil) during the period prior to differentiation of water treatments (15 days for acclimatization). After this period, we subjected the seedlings to irrigation suspension cycles at intervals of seven and fourteen days between irrigation times, and daily irrigation was used as a control treatment, maintaining them close to field capacity as mentioned above.

The experiment was completely randomized in a 4x3x5 factorial design corresponding to four hydration and dehydration (HD) treatments before germination (0, 1, 2 and 3 cycles of HD) and three water treatments [daily watering as control, and water suspension intervals of seven (S7) (moderate water stress) and fourteen (S14) (severe water stress) days between watering], with five replications per treatment, totaling 60 seedlings.

### Growth analysis and relative water content

We evaluated seedling growth weekly by measuring height, leaf count and basal stem diameter, with aid of a ruler and a caliper. From these data, we calculated the relative growth rate (RGR) under the conditions studied at 28 and 56 days of seedling development according to the formula below (Benincasa, 2003):

$$RGR = \frac{(\ln A2 - \ln A1)}{(t2 - t1)}$$

whereupon A2 and A1 refer to seedling height, stem diameter or number of leaves obtained at the end and the beginning of the analyzed period, evaluating the actual growth of individuals against a pre-existing value, for each respective attribute. T2 and T1 are the relative analysis times, final and initial. RGR values in plant height were expressed as  $\text{cm cm}^{-1} \text{ day}^{-1}$ , stem diameter as  $\text{mm mm}^{-1} \text{ day}^{-1}$ , and number of leaves as  $\text{un. un.}^{-1} \text{ day}^{-1}$ .

At the end of the experimental period (56 days), we also determined the leaf area using the leaf contour method (Benincasa, 2003; Freitas & Silva, 2018), the dry matter production for leaves (LDM), stem (SDM) root (RDM), total dry matter (TDM), and the biomass partition for the different organs. With this data, we calculated the leaf area (LA), leaf area ratio (LAR), specific leaf area (SLA), root/shoot ratio (R/S), and the biomass partition (Benincasa, 2003).

We also evaluated the relative water content (RWC) from the leaves every fourteen days, according to the methodology described by Weatherley (1950). For this analysis, we removed five 1 -cm discs from the leaf limb of mature and fully expanded leaves collected between 11 a.m. and 12 p.m. By varying the weight of fresh, turgid and dry discs, we calculated the RWC based on the following equation, expressed as percentage:

$$RWC = \frac{(WFD - DWD)}{TWD - DWD} * 100 \text{ (Weatherley, 1950).}$$

RWC: relative water content.

WFD: fresh weight.

WTD: turgid weight.

WDD: dry weight.

### Statistical analyses

We calculated the germination parameters using the GerminaQuant 1.0 software (Marques et al., 2015), and the HD cycle treatments were compared using analysis of variance with a *posteriori* Tukey test. In turn, we subjected the data obtained during the initial seedling development analysis to factor variance analysis (Two-Factors ANOVA). The two factors corresponded to HD cycles in seed and water treatments in seedlings. Means were compared using the Tukey test ( $p < 0.05$ ). Prior to the analyses, we transformed the germinability data into arcsine $\sqrt{\%}$  and the normality of data residues and the homogeneity of variances were verified using Leneve test. We performed all statistical analyses using the STATISTICA 13.0 and SigmaPlot 11.0 software at 5% significance and the graphs were plotted using the SigmaPlot 11.0 software.

### Results

According to the germination aspects analyzed for *Triplaris gardneriana*, the discontinuous seed hydration does not influence the parameters evaluated when seeds are under ideal germination conditions. We did not observe significant differences in germinability (%G), emergence rate index (ERI), mean germination time (MGT, days), and synchrony (Z). Seeds were undergone HD cycles and were put to germinate under optimal germination conditions (Table 1).

**Table 1.** Germinability (%G), mean germination time (MGT – days), emergence rate index (ERI), and seed synchrony of germination (Z) from germination of *Triplaris gardneriana* Wedd. (Polygonaceae) seeds submitted to discontinuous seed hydration (0, 1, 2 and 3 HD cycles). Equal letters do not differ by Tukey's test at 5% probability.

HD CYCLES	%G	MGT	ERI	Z
0	48.0 ± 14.4 a	9.92 ± 1.04 a	1.37 ± 0.29 a	0.16 ± 0.10 a
1	60.0 ± 14.4 a	8.65 ± 0.96 a	2.07 ± 0.22 a	0.20 ± 0.09 a
2	62.7 ± 10.1 a	8.02 ± 0.18 a	2.28 ± 0.15 a	0.25 ± 0.04 a
3	49.3 ± 22.0 a	8.32 ± 0.45 a	1.37 ± 0.92 a	0.22 ± 0.13 a
F	0.6578	3.758	1.6935	0.4293
df	3	3	3	3
p	0.6005	0.0596	0.2449	0.7376

F: F-statistics, df: degrees of freedom, and p: p-value.

On the other hand, initial growth of *T. gardneriana* reduced as the stress intensified. The unavailability of water reduced RGR in height and stem diameter in these plants (Tab. 2). In addition, seedlings from seeds that underwent discontinuous hydration of three cycles (3C) cultivated under greater water restriction (S14) showed a higher stem diameter RGR at 28 days when compared to other HD cycles, being 87% higher in relation to S14-0C (Table 2).

An increase in stem diameter RGR was also observed at 56 days, when we compared seedlings of the control treatment and S7, being the seedlings of 3C those that presented a greater RGR in stem diameter, showing values 77 and 34% larger in relation to the 0C in moderate stress S7 and control, respectively (Table 2). Similar behaviors to this can also be observed for RGR in number of leaves at 28 days, with the seedlings of the treatments S7 and S14 showed higher values in these attributes when coming from 3C, exhibiting values 98 and 5% higher in relation to 0C, for S7 and S14, respectively. We did not identify highly expressive values for RGR in height and number of leaves at 56 days comparing the performance of HD cycles (Table 2).

**Table 2.** Relative growth rate in height (RGR), stem diameter and leaf number in *Triplaris gardneriana* Wedd. (Polygonaceae) seedlings produced by seeds that underwent discontinuous hydration (0, 1, 2 and 3 cycles of HD). C, S7 and S14 correspond to the control water treatments with daily watering and intervals of seven (moderate stress) and fourteen days (severe stress) between waterings, respectively. Lower case letters compare the same treatment in different cycles, upper case letters compare the water treatments within each cycle. Equal letters do not differ by Tukey's test at 5% probability.

		Relative growth rate in height (cm cm <sup>-1</sup> day <sup>-1</sup> ) AGRH		Relative growth rate of stem diameter (mm mm <sup>-1</sup> day <sup>-1</sup> ) AGRSD		Relative growth rate of leaf number (un. un. <sup>-1</sup> day <sup>-1</sup> ) AGRLN	
HD Cycles	Water treatments	0DS-28DS	28DS-56DS	0DS-28DS	28DS-56DS	0DS-28DS	28DS-56DS
0	C	0.0179 aA	0.0085 aA	0.0214 aAB	0.0046 bA	0.0070 aA	-0.0005 aA
	S7	0.0201 aA	0.0065 aA	0.0251 aA	0.0035 bAB	0.0057 bA	-0.0034 abA
	S14	0.0129 aA	0.0031 aB	0.0143 bB	0.0025 aB	0.0091 bA	-0.0045 aA
1	C	0.0174 aA	0.0072 aA	0.0198 aA	0.0054 bA	0.0079 aA	-0.0077 bA
	S7	0.0203 aA	0.0050 aA	0.0214 aA	0.0037 bA	0.0043 bA	-0.0089 bA
	S14	0.0155 aA	0.0023 aB	0.0150 bA	0.0012 aB	0.0015 bA	-0.0093 aA
2	C	0.0201 aAB	0.0072 aA	0.0257 aA	0.0048 bA	0.0092 aA	-0.0046 bA
	S7	0.0232 aA	0.0045 aB	0.0251 aA	0.0035 bA	0.0047 bA	-0.0069 bA
	S14	0.0129 aB	0.0011 aC	0.0129 bB	0.0006 aB	0.0061 bA	-0.0035 bA
3	C	0.0194 aA	0.0070 aA	0.0123 bB	0.0079 aA	0.0095 aB	-0.0027 aA
	S7	0.0216 aA	0.0059 aA	0.0263 aA	0.0062 aA	0.0113 aA	-0.0013 aA
	S14	0.0166 aA	0.0027 aB	0.0268 aA	0.0016 aB	0.0096 aA	-0.0134 aA

The critical water restriction has reduced leaf area in plants that have undergone discontinuous hydration or not. In contrast, the seedlings from two cycles of HD 2C showed higher LA when cultivated with moderate watering intervals S7, being 33% higher when compared to 0C seedlings and 78% higher when compared to seedlings that underwent three cycles of HD.

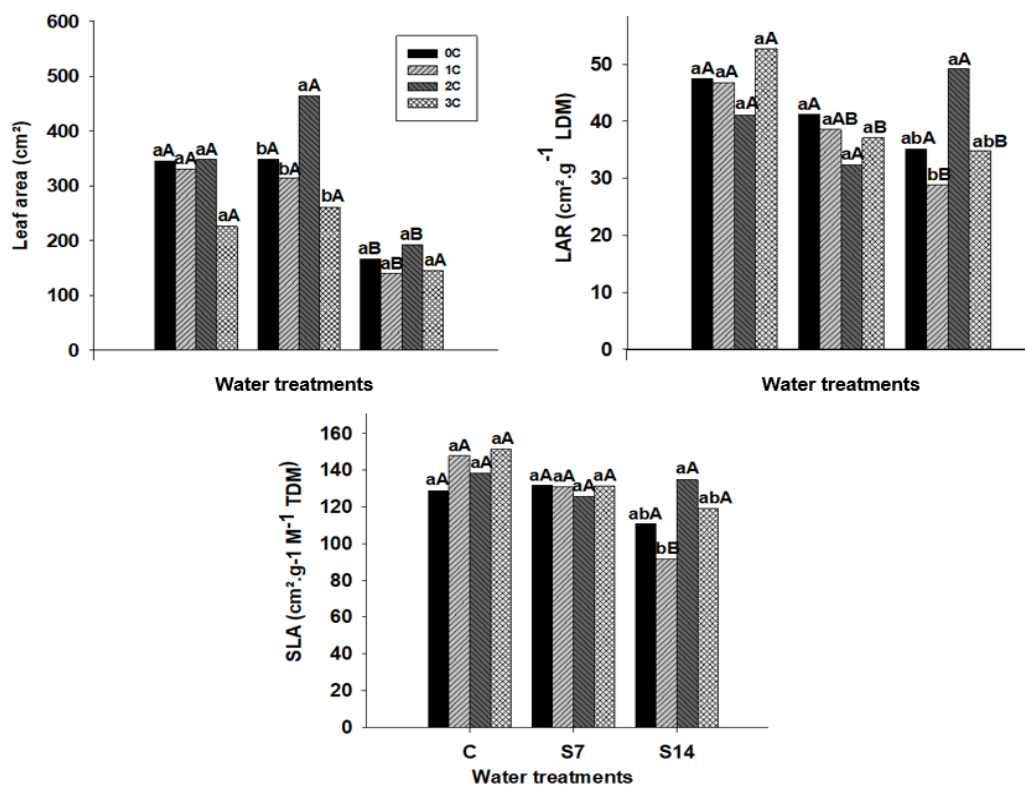
The leaf area ratio (LAR) was significantly reduced in severe stressed plants S14 that underwent one cycle of HD in seeds (Figure 1). The seedlings from seeds that underwent two cycles of HD had reduced LAR in moderate and increased in severe water stress treatments. As for specific leaf area (SLA), we only observed reductions in seedlings that underwent one cycle of HD cultivated in severe water stress. The other treatments did not differ from each other.

Given the differences caused by HD cycles and water treatments on RGR, LA and LAR of *T. gardneriana* seedlings (Table 2, Figure 1), we proposed to investigate whether these changes also express themselves at different levels of leaf water content. We found out marked reductions in the RWC of leaves of stressed plants. During the evaluation period, leaf RWC values decreased in all seedlings regardless of the passage through discontinuous hydration cycles under severe water stress when compared to control plants for each respective HD cycle (Figure 2). We observed higher percentages of RWC in leaves of seedlings cultivated for three HD cycles in severe water stress at 28 days after treatment differentiation, with values of RWC 60% higher when compared to the other (0C) HD cycle under the same conditions. However, after 56 days, RWC values in these plants also dropped sharply to below 40%, tending to show a subtle increase of 24% in RWC at 42 days compared to 0C but which does not present a statistical difference between 0C and other HD cycles in this water treatment S14 (Figure 2).

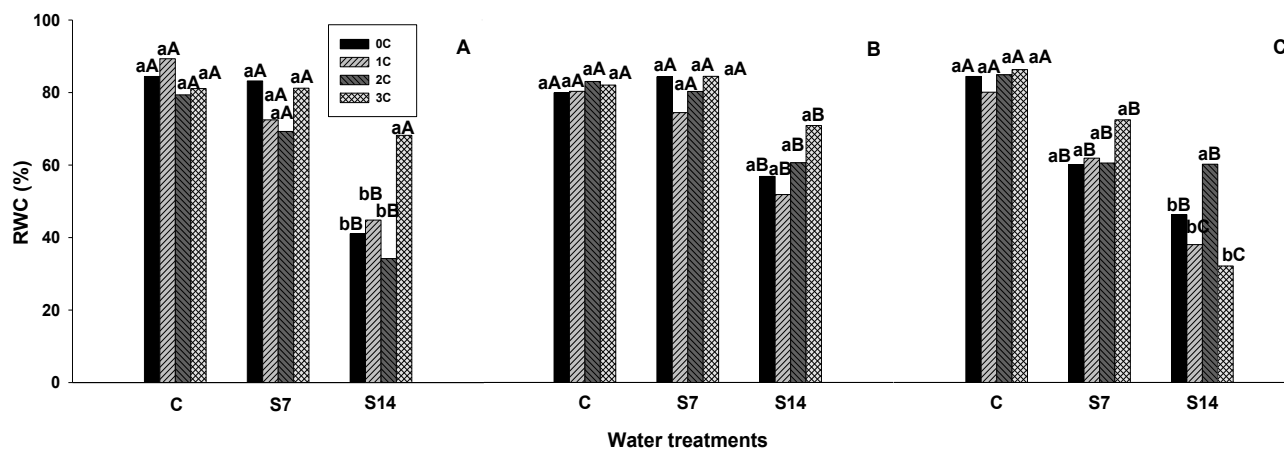
Finally, while evaluating the dry matter production of these plants, we observed a positive effect of HD cycles on the production of leaf dry matter (LDM), root dry matter (RDM) and total dry matter (TDM). We also noticed a higher biomass accumulation in two-cycle 2C plants cultivated in the moderate water stress S7 with increases of 46, 44 and 32% in LDM, RDM and TDM, respectively, compared to 0C seedlings in the same water condition (Figure 3). However, regardless of discontinuous hydration treatments, plants from the severe water stress treatment evidenced a sensitivity to this critical condition, producing less dry matter in the various organs analyzed (Figure 3).

The plants of 0C and those submitted to one cycle of HD obtained similar values in dry matter production for the majority of organs we analyzed independently of water treatment exposure. In turn, seedlings from three cycles of HD showed a behavior to accumulate the lowest values of dry matter in the

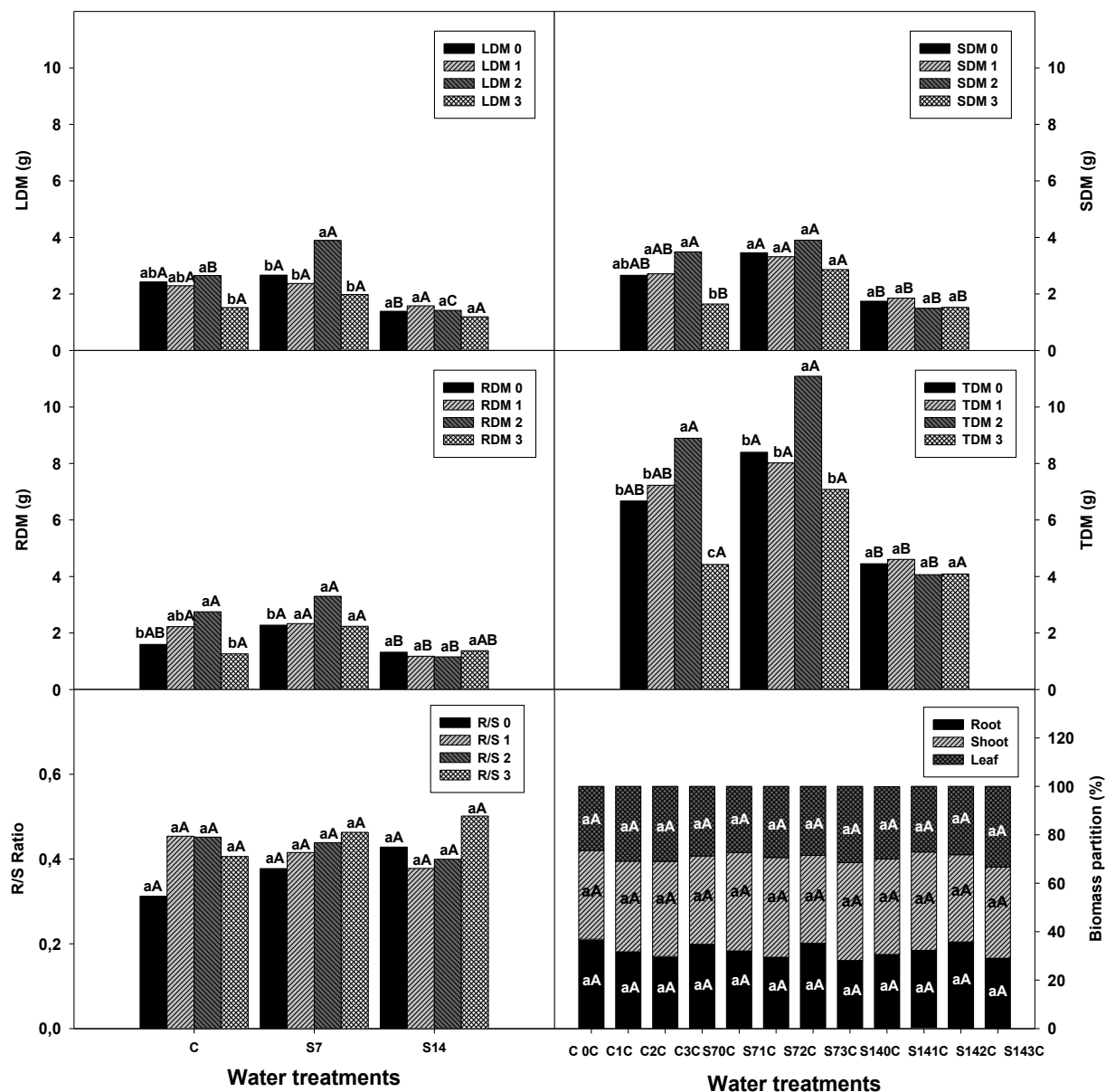
organs analyzed. For TDM, this reduction was 66% when compared to seedlings that do not undergo discontinuous hydration, 61, and 49% when compared to plants for one and two HD cycles, respectively, when grown in the control treatment. These reductions demonstrate impairment in the capture and/or assimilation of carbon dioxide under these conditions, even in a good water condition regime, which ultimately reflect in a lower output of dry matter. We did not observe significant differences for root/shoot ratio (R/S) and biomass partition (Figure 3).



**Figure 1.** Leaf area (LA), leaf area ratio (LAR) and specific leaf area (SLA) in *Triplaris gardneriana* Wedd. (Polygonaceae) seedlings produced by seeds that underwent discontinuous hydration (0, 1, 2 and 3 cycles of HD). C, S7 and S14 correspond to the control water treatments with daily watering and intervals of seven (moderate stress) and fourteen days (severe stress) between waterings, respectively. Lower case letters compare the same treatment in different cycles, upper case letters compare the water treatments within each cycle. Equal letters do not differ by Tukey's test at 5% probability.



**Figure 2.** Relative water content (RWC) in leaves of *Triplaris gardneriana* Wedd. (Polygonaceae) seedlings produced by seeds that underwent discontinuous hydration (0, 1, 2 and 3 cycles of HD). C, S7 and S14 correspond to the control water treatments with daily watering and intervals of seven (moderate stress) and fourteen days (severe stress) between waterings, respectively. Measurements performed at 28 (A), 42 (B) and 56 (C) days after differentiation of water treatments. Lower case letters compare the same treatment in different cycles, upper case letters compare the water treatments within each cycle. Equal letters do not differ by Tukey's test at 5% probability.



**Figure 3.** Leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM), total dry matter (TDM), root/shoot ratio (R/S) and biomass partition in *Triplaris gardneriana* Wedd. (Polygonaceae) seedlings produced by seeds that underwent discontinuous hydration (0, 1, 2 and 3 cycles of HD). C, S7 and S14 correspond to the control water treatments with daily watering and intervals of seven (moderate stress) and fourteen days (severe stress) between waterings, respectively. Lower case letters compare the same treatment in different cycles, upper case letters compare the water treatments within each cycle. Equal letters do not differ by Tukey's test at 5% probability.

## Discussion

Changes in seed germination indicate interferences in germinative processes, which may be synergistic or antagonistic in nature (Yan, 2015; Yigit, Sevik, Cetin, & Kaya, 2016). Although discontinuous hydration has ecophysiological implications for the germination process of tree species from semiarid environments (Dubrovsky, 1996), our results indicated that HD cycles do not benefit the germination parameters evaluated under favorable conditions for seed germination.

Although HD cycles favored germination parameters in some Caatinga tree species, such as *Mimosa tenuiflora* (Willd.) Poir. (Fabaceae) (Lima & Meiado 2018a) and *Myracrodruon urundeuva* Allemão (Anacardiaceae) (Hora & Meiado 2016), which showed a faster and synchronized germination, as well as *Handroanthus chrysotrichus* (Mart. Ex DC.) Mattos (Bignoniaceae) (Hora, Santos, & Meiado, 2018), which had a greater than 30% increase in seed germinability after discontinuous hydration, other species are not favored in germination process after experience HD cycles, if these seeds are under ideal conditions of water and temperature (as we did in this study) (Santos & Meiado (2017). That way, the benefits of discontinuous

hydration of seeds for the germination process can be profoundly better evaluated if these seeds are put to germinate in stressful conditions, which, in our personal opinion, reflects the real environmental conditions that these seeds have to deal with.

However, discontinuous hydration can confer tolerance to environmental stress, as drought and salinity, during the germination process, as observed by Lima et al. (2018), Santos, Santos, and Meiado (2018), and Lima and Meiado (2017). We believe that the benefits of discontinuous hydration to the germination phase are directly related to the different requirements needed to promote seed germination of tree species from semiarid environments, and that species producing seeds more tolerant to environmental factors are less favored by discontinuous hydration, especially when seeds of these species are placed to germinate under favorable conditions for germination.

Although in our study none of the germination aspects did not benefit from HD cycles under optimal germination conditions, the growth of *T. gardneriana* seedlings was the result of the interaction between water conditions and HD cycles. Intermittent drought caused reductions in seedling growth in relative values. On the other hand, HD cycles acted to minimize the detrimental effects of drought.

Drought has been regarded as one of the environmental phenomena that causes most changes in plant communities and that has a large potential for negative impacts on plant growth and development (Anderegg et al., 2015; Adams et al., 2017). In our study, the most severe water restriction promoted a series of disturbances to plants, such as reduced growth rates and leaf water content, especially as the experimental period progressed. On the other hand, the discontinuous hydration in seeds allowed the adoption of protective measures against the imposed condition, such as a better regulation of water content at moderate and severe stress levels, conferring acquisition of water deficit tolerance during the early development phase of seedlings, maintaining growth rates under stress.

Water stress caused by drought is a process that occurs gradually and can be noticed by a series of sequential processes (Martins, Nogueira, Azevedo Neto, & Santos, 2010), such as the reduction of water content, leaf area, wilting and decrease in water activity, and cell stretching and division (Manivannan et al., 2007; Jaleel et al., 2009). The interruption of growth rates due to water unavailability is a reflection of the absence of this preceptor, which is intimately involved with the processes of growth and differentiation (Shao, Chu, Jaleel, & Zhao, 2008; Osakabe, Osakabe, Shinozaki, & Tran, 2014), and it is a response that depends on the volume of water lost, dehydration rate, and duration of stress (Bray, 1997). Reductions in growth rates in response to water deficits have been observed in some species occurring in Caatinga, such as *Erythrina velutina* Willd. (Fabaceae) (Silva, Silva, Nogueira, & Albuquerque, 2010; Oliveira, Dombroski, Medeiros, & Medeiros, 2016), *Poincianella bracteosa* (Tul.) L.P. Queiroz (Fabaceae), and *Libidibia ferrea* (Mart. Ex Tul.) L.P. Queiroz (Fabaceae) (Ferreira, Lacerda, Costa, & Medeiros Filho, 2015). Conversely, for *Aspidosperma pyrifolium* Mart. (Apocynaceae) (Freitas & Silva 2018), intermittent drought benefited plant growth, showing that responses to water deficiency tend to vary among species that are already adapted to this condition, favoring individuals that have a xeromorphic or a rustication trait during the stress period.

On the other hand, at the same stress level in the present work, we noticed that seedlings from seed that underwent discontinuous hydration were less sensitive to stress when compared to seedlings from seeds that have not undergone HD cycles, especially when observing both RGR in stem diameter and RGR in number of leaves at 30 days for seedlings 3C. This suggests that, even with the reduction of soil moisture, seedlings of *T. gardneriana* can maintain growth rates higher when compared to those that have not undergone this treatment and obtain higher values in comparison with the other HD cycles. These results give us insight into the real benefits to seedlings from the exposure to seed hydropriming.

The adverse effects of drought and responsive seedling improvement due to seed HD cycles are well evidenced by leaf area and leaf area ratio of plants, with seedlings cultivated in moderate stress that underwent two HD cycles showing greater and lower values in these traits, respectively. In this study, we demonstrated that these attributes tend to decrease vigorously under water deficiency conditions, reflecting the inefficiency of CO<sub>2</sub> stretching and assimilation processes under the conditions we evaluated, thus affecting the reduction of these traits (Shao et al., 2008; Osakabe et al., 2014).

The highest values of SLA that we observed in seedlings from seeds that underwent 2C when grown in a critical water regime cannot be seen as a vantage, because this characteristic signalizes as an impairment in carbon uptake by stomata, being necessary a higher leaf area to maintain photosynthesis in adequate levels. This is an unfavorable trait because it will expose these plants to the risk of desiccation, once those plants generally tend to deal with the stomata paradox (Lawson & Viallet-Chabrand, 2019; Chaves et al., 2002).



In the same manner, as we observed for SLA the highest values of LAR in the same seedlings can be an evidence of impairment in the capacity of the fountain organs (leaves) to export the photoassimilates. Since LAR can be interpreted as the thickness of the leaf, this important anatomical trait serves to represent the retention of sugars from photosynthesis in the tissues of the leaf itself (Benincasa, 2003). Although that occurs, analyze of the real concentration of soluble leaf sugars would be necessary to inflict that.

Similar results were found by Silva-Pinheiro et al. (2016) for seedlings of *Bauhinia monandra* Kurz (Fabaceae) and *Tabebuia aurea* (Bignoniaceae), both species found in the Caatinga. The authors observed decreases in the leaf area of these species, resulting in a lower biomass accumulation. In addition, the authors also observed the behavior of *Hymenaea courbaril* L. (Fabaceae) seedlings under the same conditions. However, they did not decrease leaf area and dry matter production, suggesting that drought affects vegetative growth differently among the three species. The justification for this behavior is the loss of turgor pressure, enzymatic disorder and decreased energy supply for photosynthesis, resulting in a loss of cellular homeostasis and interruption of growth rates (Bhargava & Sawant 2013; Ding, Tao, & Zhu, 2013; Osakabe et al., 2014). In this case, the reduction in the rate of expansion (Mwale, Azam-Ali, & Massawe, 2007) and loss of photosynthetically active leaf area has profound implications for the final carbon balance, ultimately resulting in a marked decrease in growth (Mediavilla, García-Cunchillos, Andrés-Rivera, & Escudero, 2018).

On the other hand, exposure to HD cycles promoted a substantial increase in leaf area under the conditions of two HD cycles, with a drastic decrease after undergoing three consecutive cycles. These results, along with the relative growth analysis, show us that there should probably be a discontinuous hydration threshold that, once exceeded, no longer acts as a modular growth agent for the species, and that may impair the initial development of seedlings originating from these seeds hydro-conditioned by many HD cycles.

The reduction in growth rates under water deficiency is related to the low values of RWC when plants are subjected to water stress. In our study, we showed that *T. gardneriana* seedlings showed a reduced RWC only when grown at severe water stress conditions. We also observed that plants that underwent longer HD cycles were able to accumulate higher RWC values even under stress. Although we observed reductions in RWC in seedlings of *T. gardneriana* under water deprivation, this behavior varies among Caatinga species, especially among the most adapted ones, as observed by Costa, Freire, Bakke, and Pereira (2015) for *Myracrodruon urundeuva* Allemão (Anacardiaceae), whose RWC did not reduce due to a higher regulation of stomatal activity under these conditions, controlling the levels of leaf transpiration, which is one of the strategies used by more adapted plants to prevent dehydration of their tissues (Albuquerque et al., 2013).

Successive exposure of plants to a stressful condition may enable them to improve their response to a stressor. However, this adaptive response was only observed when exposure to the conditioning priming agent in seeds occurred moderately, with no positive response to a long-term condition (Singh et al., 2014; Thomas et al., 2020). Our results are partially in line with this statement, demonstrating that seedlings from seeds that have undergone two cycles of HD (moderate exposure), associated with moderate stress exposures, seem to accumulate greater acclimatizing stress responses, such as increased biomass production and leaf area, a behavior also observed by Lima and Meiado (2018b) in Cactaceae. In its turn, the benefits of exposure to the three HD cycles (long-term condition) manifest into greater regulation of RGR in stem diameter and leaf number, and maintenance of RWC even under the most severe stress. Similar results were found by (Thomas et al., 2020) in their work, using UV-B priming. The authors demonstrate that moderate exposure to the priming promoted benefits to seedlings generated from primed seeds, with no positive response observed at high exposures. We believe that although the exposure of seeds to a priming agent promotes stress imprint in seedlings, this benefit is only observed in moderate doses and that when exceeded acts as a stressor, and that stress imprint are expressed in some attributes to the detriment of others, as it was observed in the present study.

Finally, we conclude that the effects of water deficiency for the species studied are very clear, showing a high stress sensitivity, as well as the benefit of the exposure to HD cycles in seeds during the early phase of their development at least in some aspects. These stress responses reflect only physiological and biochemical delays in the severe conditions imposed (Ribeiro, Dantas, Matias, & Pelacani, 2017). Although it occurs in Caatinga, the vulnerability of *T. gardneriana* to low soil water levels can be explained by the fact that this species is considered a riparian species (Souza & Rodal, 2010). In its natural environment, characterized by well-marked rainfalls and scarce soil water availability, the species tends to occur in places with greater water availability.

## Conclusion

Seeds from *T. gardneriana* were not primed by the HD cycles, showing no improvements in any germination trait when grown in ideal conditions. However, the discontinuous hydration of seeds benefits seedling development under water deficit, leaving a stress imprint in seedlings. The benefits of exposure to hydropriming translate into increased growth rates, during a more severe water deprivation, and dry matter yields when grown under a moderate water restriction regime. On the other hand, exposure to several HD cycles coupled with a more severe water regime affects seedling growth, showing that there may be a discontinuous hydration threshold for the real benefits of prior exposure to be expressed. That is specifically an important trait of the technique, requiring a deeper explanation for further research.

## Acknowledgements

We thank the Ecology and Environmental Monitoring Center of the *Universidade Federal do Vale do São Francisco* (UNIVASF) for the seeds used in this study.

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