

# Physiological potential of sorghum seeds under discontinuous hydration and water deficiency conditions<sup>1</sup>

## Potencial fisiológico de sementes de sorgo sob condições de hidratação descontinuada e déficit hídrico

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**ABSTRACT** - Hydration and dehydration cycles during imbibition of seeds may influence their physiological performance, making them more vigorous and resistant to abiotic factors such as water stress. The aim was to investigate whether the times and discontinued hydration cycles condition higher tolerance to water deficiency conditions in germination and seed vigor and accumulation of organic compounds in sorghum seedlings. The experiment was carried out in a randomized experimental design with four replicates of 50 seeds. The treatments were arranged in a  $3 \times 4 \times 6$  factorial scheme (hydration times, hydration and dehydration cycles, osmotic potentials). The imbibition curve of the species was determined based on three times (X, Y and Z); and the dehydration curve was constructed. With these times, the seeds were submitted to 0, 1, 2 and 3 cycles of hydration and dehydration (HD). Then, they were submitted to germination test on paper moistened with polyethylene glycol 6000 solution at the osmotic potentials of 0.0 (distilled water), -0.2, -0.4, -0.6, -0.8 and -1.0 MPa. Germination and vigor of sorghum seeds are severely impaired in water potentials equal to or greater than -0.4 MPa. Application of three cycles of hydration and dehydration for a period of 13h in sorghum seeds improves tolerance to water deficiency conditions during germination and seedling development, and provides greater contents of soluble N-amino content and total soluble carbohydrates.

**Key words:** Germination. *Sorghum Bicolor*. Water Memory. Water Stress. Vigor.

**RESUMO** - Ciclos de hidratação e desidratação durante a embebição das sementes podem influenciar o seu desempenho fisiológico, tornando-as mais vigorosas e resistentes a fatores abióticos como o estresse hídrico. Objetivou-se investigar se os tempos e ciclos de hidratação descontinuada condicionam maior tolerância ao déficit hídrico na germinação e vigor das sementes e acúmulo de compostos orgânicos em plântulas de sorgo. Foi desenvolvido estudo em delineamento experimental casualizado, com quatro repetições de 50 sementes. Os tratamentos foram arranjados em fatorial  $3 \times 4 \times 6$  (tempos de hidratação; ciclos de hidratação e desidratação; potenciais osmóticos). Determinou-se a curva de embebição da espécie, em três tempos X, Y e Z; e a curva de desidratação. Com esses tempos, as sementes foram submetidas a 0, 1, 2 e 3 ciclos de hidratação e desidratação (HD). Após, foram submetidas ao teste de germinação sob papel umedecido, com solução de polietileno glicol 6000 nos potenciais osmóticos 0,0 (água destilada), -0,2; -0,4; -0,6; -0,8 e -1,0 MPa. A germinação e vigor de sementes de sorgo são severamente prejudicados em potenciais hídricos iguais ou superiores a -0,4 MPa. A aplicação de três ciclos de hidratação e desidratação por um período de 13h em sementes de sorgo melhora a tolerância ao déficit hídrico durante a germinação e desenvolvimento de plântulas, e proporciona maiores conteúdos de N-aminossolúveis e carboidratos solúveis totais.

**Palavras-chave:** *Sorghum Bicolor*. Memória Hídrica. Germinação. Estresse Hídrico. Vigor.

DOI: 10.5935/1806-6690.20200069

Editor do artigo: Professor Alek Sandro Dutra - alekdutra@ufc.br

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Received for publication in 24/02/2020; approved in 18/06/2020

<sup>1</sup>Estudo realizado pela primeira autora durante a disciplina Pesquisa Orientada em Tecnologia de Sementes, no Programa de Pós-Graduação em Agronomia/Fitotecnia, Universidade Federal do Ceará/UFC

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## INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench.] is the fifth most cultivated cereal in the world. The species is capable of producing economic yields in arid and semiarid regions, with low water availability and high temperatures, conditions under which other cereal crops are not normally able to grow (PEREIRA *et al.*, 2014; SUTKA *et al.*, 2016). The crop has been cultivated in these regions under dry conditions mainly for grain and forage production (SILVA *et al.*, 2014; WANI; ALBRIZIO; VAJJA, 2012).

Studies show that in arid and semiarid regions water deficiency occurs in the soil in certain periods (summer) due to the lack and irregularity of rainfall (AZERÊDO; PAULA; VALERI, 2016; SOUSA *et al.*, 2018), resulting in a limitation on agricultural production (JADOSKI; SUCHORONCZEK; SANTOS, 2017).

The first stage of the plant cycle affected by these dry spells is germination, since water is responsible for activating all the metabolic processes that culminate in germination (MARCOS-FILHO, 2015). Thus, the lack or even the loss of water by the seed to the external environment can cause cycles of hydration and dehydration (HD) during imbibition (LIMA; MEIADO, 2017; LIMA; OLIVEIRA; MEIADO, 2018b), causing the germination process to stop and the accumulation or scarcity of organic compounds such as total soluble carbohydrates and soluble N-amino, which has an important function in maintaining cell turgor and physiological processes of growth and development when plants are exposed to various forms of stress, such as water, salt or temperature stresses (AHANGER *et al.*, 2018).

Some species have the capacity to suspend imbibition when there is water restriction in the soil, resuming the process when it is ceased, without damage to the internal tissues (LIMA; MEIADO, 2018), because they reactivate their metabolic processes instead of starting from the beginning (DUBROVSKY, 1996). Thus, these seeds have a water memory, undergoing cycles of HD, and possibly can have a high survival rate during water deficit periods (LIMA *et al.*, 2018a).

Therefore, the hypothesis was raised that sorghum capacity for tolerance to low water availability conditions may be related to the water storage mechanism of its seeds, since the species originates in dry environments and, for being a plant with C4 photosynthetic mechanism, it has the capacity to grow under conditions of high temperatures and low or erratic rainfall. Understanding the performance of this species at the germination stage under these environmental conditions and the specific characteristics of the plant that increase its tolerance to water deficits can be an important tool for productivity

improvement programs, especially in near future scenarios of climate change, which predict increased frequency and duration of water deficit periods.

In view of the above, this study aimed to investigate whether the times and discontinued hydration cycles condition higher tolerance to water deficiency conditions in germination and seed vigor and accumulation of organic compounds in sorghum seedlings.

## MATERIAL AND METHODS

### Conducting the experiment

The experiment was conducted at the Laboratory of Seed Analysis, Department of Plant Science, Center of Agricultural Sciences, Federal University of Ceará, Pici *Campus*, Fortaleza-CE, Brazil. Sorghum seeds of the cultivar EA-955 were used. The seeds were manually harvested, stored in plastic bottles and stored in a controlled environment (temperature of  $10\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and relative humidity of 40%) during the whole experimental period. In order to do so, the curves of seed imbibition and dehydration and HD cycles were initially determined.

### Imbibition and dehydration curve, and HD cycles

For the imbibition curve, four replicates of 50 seeds were used. The seeds were weighed to obtain the initial weight and then placed to soak in transparent acrylic boxes ( $11 \times 11 \times 3.5$  cm), on two sheets of blotting paper, moistened with distilled water in quantity equivalent to 2.5 times the dry paper weight. After, the boxes were kept in Biochemical Oxygen Demand (BOD) germination chambers at  $25\text{ }^{\circ}\text{C}$  with 12-h photoperiod.

The weight of the seeds was measured every 60 minutes until the first germinated seed (root protrusion) appeared, which indicated the end of the second imbibition phase. The curve was estimated based on the variation of seed biomass at the intervals evaluated. Three points were selected on the curve, being called X, Y and Z, where X corresponds to  $\frac{1}{2}$  of the time of phase I of the imbibition, Y to  $\frac{1}{4}$  of the time of phase II and Z to  $\frac{3}{4}$  of the phase II. These intervals represented periods of hydration (LIMA *et al.*, 2018a).

For the dehydration curve, the same methodology of the imbibition curve was followed; however, the seeds were hydrated until the periods X, Y and Z determined in the imbibition curve, then placed to dry on aluminum screen, fixed in transparent acrylic box, containing silica at the bottom, kept at  $25\text{ }^{\circ}\text{C}$ . Every 60 minutes, the seeds were weighed until they returned to the initial weight. The time required for the seeds to return to their initial biomass represented the periods of seed dehydration.

The information of the X, Y and Z times of imbibition and the times required for drying the seeds determined the periods of hydration and dehydration. From these, four replicates of 50 seeds were used, which were submitted to 0, 1, 2 and 3 cycles of HD, following the same methodology adopted for the previous imbibition and dehydration curves. The seeds of cycle 0 did not undergo any cycle of HD, while the others underwent the X, Y and Z times of hydration and their respective times of dehydration (LIMA *et al.*, 2018a).

### Germination and vigor under water deficiency

Seeds that underwent the HD cycles were submitted to water deficiency simulated by Polyethylene glycol (PEG 6000). Seeding was carried out on paper towel rolls, moistened with commercial PEG 6000 solution (equivalent to 2.5 times the dry paper weight) at the osmotic potentials of 0.0 (distilled water), -0.2, -0.4, -0.6, -0.8 and -1.0 MPa. Then, the seeds were conditioned in BOD germinators at 25 °C, with photoperiod of 12 h. The osmotic potentials were prepared according to the table of Villela, Doni-Filho and Sequeira (1991).

Water content, germination, germination speed index (GSI), length and dry mass of seedling shoots and roots, and contents of soluble carbohydrates and soluble N-amino were evaluated. The water content in the seeds was determined with two subsamples of 5 g of seeds, using the oven method, at  $105 \pm 3$  °C for 24 hours (BRASIL, 2009). Germination test evaluations were carried out daily after sowing, for a period of ten days, using root protrusion as the criterion for germinated seeds, the results being expressed as a mean percentage and transformed to  $\arcsin \sqrt{x}/100$ . The germination speed index was determined in conjunction with the germination test, being evaluated based on the sum of normal seedlings germinated daily and divided by the number of days corresponding to the count (MAGUIRE, 1962).

The lengths of seedling shoots and roots were evaluated at the end of the germination test, using 10 normal seedlings per replicate. The seedlings were transferred to blue-colored A3 paper for the capture of digital images, performed on an HP Scanjet G2410 scanner, positioned upside down inside an aluminum box. The captured images were saved with digital resolution of 300 dpi in JPEG format and analyzed using ImageJ® software (Image Processing and Analysis in Java) (FERREIRA; RASBAND, 2012). The results obtained were expressed in cm seedling<sup>-1</sup>.

Shoot and root dry masses were obtained at the end of the germination test. Normal seedlings were collected, placed in Kraft paper bags, and dried in a forced air circulation oven at  $65 \pm 3$  °C for 72 hours. Afterwards,

they were weighed on a precision analytical balance (0.0001) and the results were expressed in g seedling<sup>-1</sup> (NAKAGAWA, 1999).

### Determination of organic compounds

For the determination of soluble carbohydrates, extracts were prepared containing 50 mg of the lyophilized sample with 5.0 mL of 80% ethanol in test tubes. The tubes were kept in a water bath at 75 °C for 1h and were shaken every 15 minutes. The samples were then centrifuged at  $3.000 \times g$  for 10 minutes, and the supernatant was collected, filtered on filter paper and completed with 80% ethanol to 25 mL in a volumetric flask. The vials containing the extracts were stored at -20 °C. The reaction mixture was formed by 0.5 mL of the conveniently diluted extract, 0.5 mL of 5% phenol and 2.5 mL of concentrated sulfuric acid. The mixture was stirred and allowed to stand at room temperature. Carbohydrate quantification was performed by absorbance readings at 490 nm and using a standard curve made with D(+) anhydrous glucose. The soluble carbohydrate contents were expressed in  $\mu\text{mol g}^{-1}$  DM and represent the mean of four replicates, each extract being dosed in duplicate (DUBOIS *et al.*, 1956).

The extracts used for the determination of soluble N-amino were the same used for the determination of soluble carbohydrates. The soluble N-amino contents were determined according to the method of Yemm and Cocking (1955). The reaction mixture was formed by 0.5 mL of the conveniently diluted extract, 0.25 mL of 0.2 M sodium citrate buffer (pH 5.0), 0.5 mL of 0.2 mM potassium cyanide (KCN) in 100% methyl cellosolve and 0.1 mL of 5% ninhydrin in 100% methyl cellosolve. The tubes were closed, vortexed and kept in water bath at 95 °C for 15 minutes. The reaction was stopped in an ice bath. Then, 0.65 mL of 60% ethanol was added to each tube. The soluble N-amino contents were estimated by absorbance readings at 570 nm, based on a standard curve with increasing concentrations of glycine. The results of the soluble N-amino contents were expressed in  $\mu\text{mol g}^{-1}$  DM and the results represent the average of four replicates, each extract being dosed in duplicate.

### Statistical analysis

Initially, normality and homogeneity of the variances were evaluated according to the Shapiro-Wilk and Levene tests. The data were submitted to analysis of variance by the F test ( $p \leq 0.05$ ) with three factors (hydration times, HD cycles and osmotic potentials). In cases of significance, means were compared by the Scott-Knott test at 5%. The analyses were performed with the aid of the statistical program SISVAR (FERREIRA, 2011).

## RESULTS AND DISCUSSION

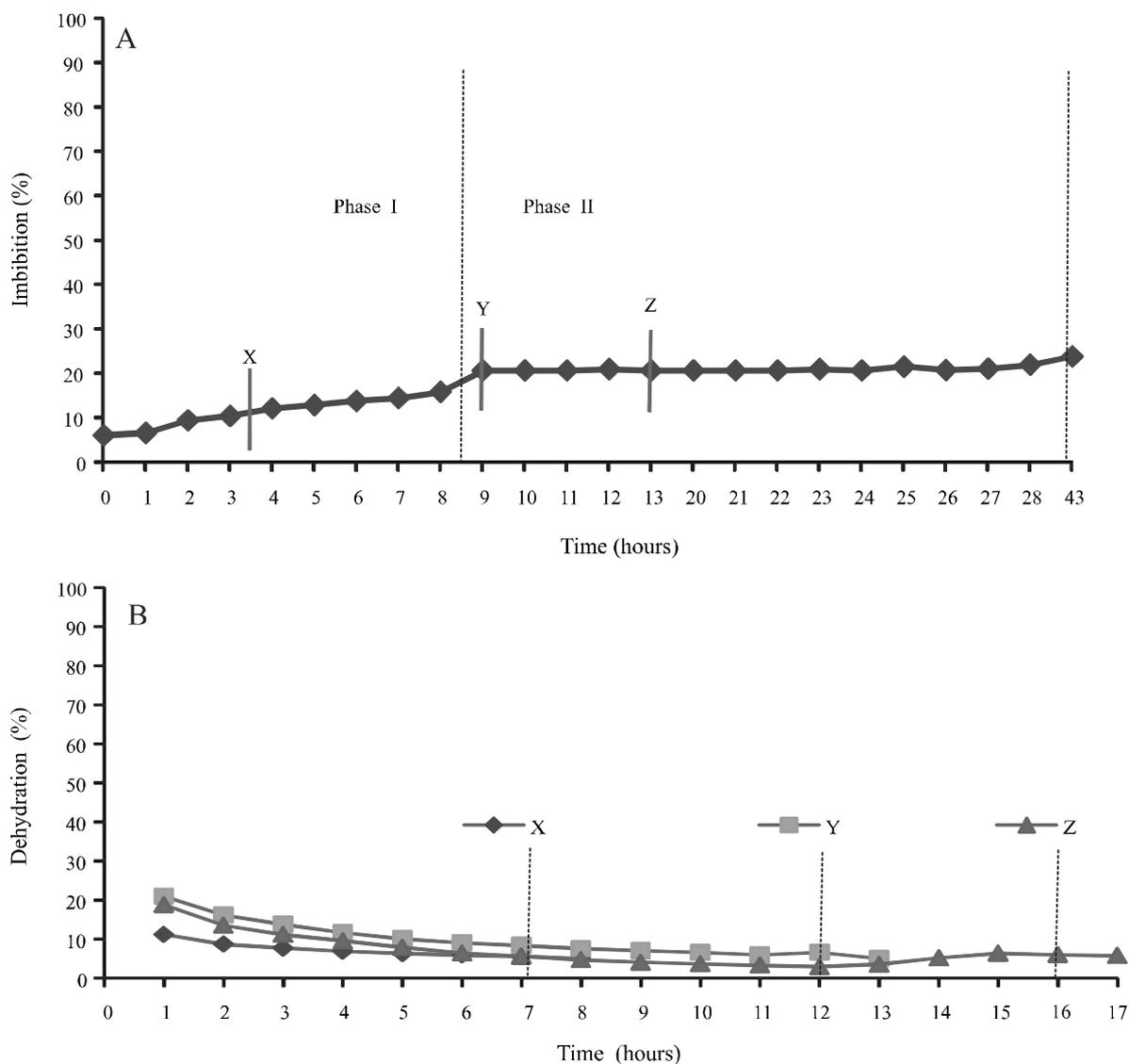
The initial water content of the seeds was 9.3%. The sorghum imbibition curve was described by the three-phase model, with the appearance of the first germinated seed at 13 h and 50% germination at 43 h after the beginning of imbibition. The hydration times X, Y and Z corresponded to 3 h 30, 9 h and 13 h, respectively (Figure 1A). The dehydration curve to return to the initial weight lasted on average 7 h, 12 h and 16 h for the times X, Y and Z, respectively (Figure 1B).

Germination process of sorghum seeds was not influenced by HD times and cycles when submitted to 0.0 and -0.2 MPa potentials (Table 1). On the other hand, as the osmotic potential became more negative, there was a

reduction of germination in seeds submitted to potentials lower than -0.4 MPa, regardless of the hydration times and HD cycles, reaching zero at the potential of -0.6 MPa (Table 1). These negative effects have been reported by other researchers (FERREIRA; MEIADO; SIQUEIRA FILHO, 2017; OLIVEIRA; GOMES-FILHO, 2009), who demonstrated that as water availability decreases, a decrease in seed germination occurs.

The reduction in germination due to the decrease in osmotic potential was also observed in other species that underwent different times and cycles of HD, such as *Senna spectabilis* (DC.) var. *Excelsa* (LIMA *et al.*, 2018a). In spite of the reduced germination at the potential -0.4 MPa, the seeds that underwent more than 1 hydration cycle had higher germination percentages compared to the control

**Figure 1** - Imbibition (A) and dehydration curve (B) of sorghum seeds cv. EA-955



**Table 1** - Germination (%) of sorghum seeds cv. EA-955, submitted to different hydration-dehydration cycles and water deficiency conditions at times X (3 h 30), Y (9 h) and Z (13 h)

Time X (3 h 30)	0.0 MPa	-0.2 MPa	-0.4 MPa
0 cycles	97 Aaa	96 Aaa	21 Aab
1 cycle	96 Aaa	93 Aaa	20 Cbb
2 cycles	93 Aaa	51 Bbb	-
3 cycles	92 Aaa	92 Aaa	30 Cab
Time Y (9 h)			
0 cycles	97 Aaa	96 Aaa	21 Acb
1 cycle	95 Aaa	95 Aaa	55 Abb
2 cycles	97 Aaa	93 Aaa	70 Aab
3 cycles	93 Aaa	92 Aaa	54 Bbb
Time Z (13 h)			
0 cycles	97 Aaa	96 Aaa	21 Adb
1 cycle	94 Aaa	93 Aaa	35 Bcb
2 cycles	93 Aaa	87 Aaa	53 Bbb
3 cycles	96 Aaa	94 Aaa	71 Aab

Means followed by equal uppercase letters do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the times, in each cycle and at each osmotic potential. Means followed by equal lowercase letters do not differ by Scott-Knott's test ( $p < 0.05\%$ ), for the cycles, at each time and at each osmotic potential. Means followed by equal the 2<sup>nd</sup> lowercase letter do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the osmotic potentials, at each time and in each cycle

cycle, at all hydration times. According to Bewley *et al.* (2013) and Oliveira and Gomes-Filho (2010), prolonged hydration, particularly under low water potentials, has a significant influence on the speed, synchrony and percentage of seed germination.

The lowest results at the most negative potentials of PEG are due to the characteristics of this osmotic agent, which due to its high viscosity causes a low rate of water and oxygen diffusion into the seeds (SOUSA *et al.*, 2018). Thus, it is expected that the more negative the osmotic potential, the longer the time of phase II, and therefore, the longer the water absorption time and the greater the delay or impediment of the start of phase III (BEWLEY *et al.*, 2013).

In general, the increase in the number of cycles, at all times and osmotic potentials, led to a higher germination speed, compared to seeds that did not undergo HD cycles, which had the lowest results between the HD cycles, times and potentials for GSI (Table 2).

A similar result was found by Lima *et al.* (2018a), in seeds of *Senna spectabilis*, when submitted to HD cycles and water deficiency, in the mean germination time, which decreased from 1 cycle of HD. The authors explain that this decrease is due to the improvement of the physiological and biochemical events that occur during the germination process of these seeds, which may be

related to the accumulation of LEA proteins during the HD cycles, which are responsible for increasing seed tolerance to desiccation.

Oliveira and Gomes-Filho (2010), testing the effect of osmotic conditioning with PEG in 48h of imbibition and 72 h of drying on the germination and vigor of sorghum seeds with different levels of physiological quality, verified that the hydration time did not influence the germination, but promoted benefits for vigor of seeds of both superior and inferior physiological quality. Hence, seeds that undergo a discontinuous hydration process have a protoplasm with lower viscosity and greater water permeability (LIMA; OLIVEIRA; MEIADO, 2018b), which influence the faster development of the seedlings, in addition, differences in the physiological quality of the seeds lead to variation in the imbibition pattern. This can be closely related to these results.

The shoot and root development had similar behavior, with no influence of the HD cycles at the 0.0 MPa potential (Table 3 and 4). It was also observed that the length values decreased when the seeds were submitted to more negative potentials. At these potentials, in cycle 3, the increase of the HD times improved the results, mainly in the shoot length of the seedlings. An increase in seedling length was also observed by Li *et al.* (2017), when evaluating the effect of thermal, water and salt stresses on

**Table 2** - Germination speed index (GSI) of sorghum seeds cv. EA-955, submitted to different hydration-dehydration cycles and water deficiency conditions at times X (3 h 30), Y (9 h) and Z (13 h)

Time X (3 h 30)	0.0 MPa	-0.2 MPa	-0.4 MPa
0 cycles	16.0 Ada	8.7 Abb	0.17 Aac
1 cycle	20.54 Aba	7.57 Ccb	0.59 Aac
2 cycles	22.27 Aaa	0.84 Bdb	-
3 cycles	17.18 Bca	11.73 Bab	0.57 Cac
Time Y (9 h)			
0 cycles	16 Aca	8.7 Acb	0.17 Abc
1 cycle	21.37 Aba	9.27 Bcb	1.57 Aac
2 cycles	22.73 Aaa	11.78 Abb	2.34 Aac
3 cycles	22.54 Aaa	15.23 Aab	2.51 Bac
Time Z (13 h)			
0 cycles	16 Aca	8.7 Acb	0.17 Acc
1 cycle	19.21 Bba	11.23 Abb	1.04 Acc
2 cycles	21.51 Aaa	12.54 Aab	2.43 Abc
3 cycles	22.36 Aaa	10.73 Bbb	4.86 Aac

Means followed by equal uppercase letters do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the times, in each cycle and at each osmotic potential. Means followed by equal lowercase letters do not differ by Scott-Knott's test ( $p < 0.05\%$ ), for the cycles, at each time and at each osmotic potential. Means followed by equal the 2<sup>nd</sup> lowercase letter do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the osmotic potentials, at each time and in each cycle

**Table 3** - Shoot length of seedlings (cm) of sorghum cv. EA-955, submitted to different hydration-dehydration cycles and water deficiency conditions at times X (3 h 30), Y (9 h) and Z (13 h)

Time X (3 h 30)	0.0 MPa	-0.2 MPa	-0.4 MPa
0 cycles	16.25 Aaa	7.25 Aab	1.0 Aac
1 cycle	15.25 Aaa	6.50 Aab	2.50 Aac
2 cycles	15.75 Aaa	1.25 Ccb	-
3 cycles	15.50 Aaa	4.75 Bbb	1.25 Cac
Time Y (9 h)			
0 cycles	16.25 Aaa	7.25 Aab	1.0 Aac
1 cycle	16.25 Aaa	5.75 Abb	2.50 Aac
2 cycles	15.0 Aaa	5.25 Bbb	2.50 Aac
3 cycles	14.50 Aaa	8.25 Aab	3.0 Bac
Time Z (13 h)			
0 cycles	16.25 Aaa	7.25 Aab	1.0 Abc
1 cycle	15.75 Aaa	5.0 Abb	2.25 Aac
2 cycles	14.50 Aba	7.50 Aab	3.50 Abc
3 cycles	14.0 Aba	8.0 Aab	4.75 Aac

Means followed by equal uppercase letters do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the times, in each cycle and at each osmotic potential. Means followed by equal lowercase letters do not differ by Scott-Knott's test ( $p < 0.05\%$ ), for the cycles, at each time and at each osmotic potential. Means followed by equal the 2<sup>nd</sup> lowercase letter do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the osmotic potentials, at each time and in each cycle

**Table 4** - Root length of seedlings (cm) of sorghum cv. EA-955, submitted to different hydration-dehydration cycles and water deficiency conditions at times X (3 h 30), Y (9 h) and Z (13 h)

Time X (3 h 30)	0.0 MPa	-0.2 MPa	-0.4 MPa
0 cycles	15.50 Aaa	5.25 Abb	3.50 Aab
1 cycle	17.25 Aaa	12.0 Aab	3.50 Aac
2 cycles	17.25 Aaa	5.0 Bbb	-
3 cycles	14.25 Aaa	6.75 Bbb	6.25 Aab
Time Y (9 h)			
0 cycles	15.50 Aaa	5.25 Abb	3.50 Abb
1 cycle	18.25 Aaa	13.0 Aab	6.75 Aac
2 cycles	15.75 Aaa	5.25 Bbb	8.25 Aab
3 cycles	16.25 Aaa	12.25 Aab	7.25 Aac
Time Z (13 h)			
0 cycles	15.50 Aaa	5.25 Abb	3.50 Aab
1 cycle	18.75 Aaa	7.0 Bbb	7.0 Aab
2 cycles	16.25 Aaa	8.75 Abb	7.75 Aab
3 cycles	15.50 Aaa	12.75 Aaa	5.25 Aab

Means followed by equal uppercase letters do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the times, in each cycle and at each osmotic potential. Means followed by equal lowercase letters do not differ by Scott-Knott's test ( $p < 0.05\%$ ), for the cycles, at each time and at each osmotic potential. Means followed by equal the 2<sup>nd</sup> lowercase letter do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the osmotic potentials, at each time and in each cycle

*Medicago sativa* (alfalfa) seeds, which underwent cycles of hydration and dehydration.

Probably this result can be explained by the higher GSI found in at these potentials, leading to a shorter germination time and greater seedling development. The reduction in the total size of the seedlings between the potentials, cycles and times of HD is due to the changes in the cell turgor due to the decrease in protein synthesis under water deficiency conditions (COELHO *et al.*, 2014), since it is the first and more sensitive response to the reduction of the osmotic potential of the imbibition medium, consequently of the growth, since cell division, elongation and differentiation are affected at the various physiological stages (SOUSA *et al.*, 2018).

The seeds that underwent 9 h of hydration led to greater dry mass accumulation in the shoots, compared to the other times, at the control potential of 0.0 MPa (Table 5). Differently from what was observed for root dry mass, in which the seeds that underwent more than 2 cycles at all times at the potential 0.0MPa had less influence on dry mass accumulation and those that underwent 3 cycles at all other times and potentials resulted in greater accumulation of biomass (Table 6).

These data suggest that the species needs more cycles of HD to activate seed metabolism for the digestion of the reserves and translocation of metabolized products,

fundamental for seed germination (BEWLEY *et al.*, 2013), and accumulation of dry mass, which were reduced under low water availability, and that HD cycles do not lead to the same germination responses for seedling vigor when seeds are subjected to different osmotic potentials. Thus, the possible benefits that HD cycles provide for seeds vary between species, cultivars and even between lots of seeds.

Several studies have shown the benefits of HD cycle on the germination of different species, most of them native and forest, in which HD cycles increased germination percentage, germination rate and water deficit tolerance (LIMA *et al.*, 2018a; LIMA; MEIADO, 2017). Different results were reported by Santos and Meiado (2017), who did not observe a positive influence of discontinuous hydration on the germination and initial development of 'umburana-de-cheiro' (*Amburana cearensis*) seeds, as in the present study, in which the cycles did not influence germination, only in isolated cases of variables related to vigor.

The contents of total soluble carbohydrates and soluble N-amino behaved similarly (Table 7 and 8). Seeds that underwent more than one cycle at 0.0 MPa potential showed lower levels than those found in seeds subjected zero cycles at all times; on the other hand, when submitted to 3 cycles of HD at the potential -0.2 MPa, they obtained higher contents of both osmolytes compared to all the other cycles. These results show that sorghum seedlings do not accumulate osmolytes at potential of 0.0 MPa

under different times and cycles, since they are consumed for the development of the seedling and regulation of

energy metabolism after the seedling phase, such as photosynthesis and respiration.

**Table 5** - Shoot dry mass of seedlings (g) of sorghum cv. EA-955, submitted to different hydration-dehydration cycles and water deficiency conditions at times X (3 h 30), Y (9 h) and Z (13 h)

Time X (3 h 30)	0.0 MPa	-0.2 MPa	-0.4 MPa
0 cycles	0.39 Aaa	0.10 Aab	0.01 Aac
1 cycle	0.34 Aba	0.05 Bbb	0.01 Aac
2 cycles	0.36 Aaa	0.01 Bcb	-
3 cycles	0.33 Aba	0.11 Bab	0.01 Bac
Time Y (9 h)			
0 cycles	0.39 Aaa	0.10 Abb	0.01 Aac
1 cycle	0.36 Aaa	0.12 Abb	0.01 Aac
2 cycles	0.37 Aaa	0.12 Abb	0.02 Aac
3 cycles	0.35 Aaa	0.16 Aab	0.03 Aac
Time Z (13 h)			
0 cycles	0.39 Aaa	0.10 Acb	0.01 Abc
1 cycle	0.34 Aba	0.08 Bcb	0.01 Abc
2 cycles	0.36 Aba	0.12 Abb	0.03 Abc
3 cycles	0.34 Aba	0.16 Aab	0.06 Aac

Means followed by equal uppercase letters do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the times, in each cycle and at each osmotic potential. Means followed by equal lowercase letters do not differ by Scott-Knott's test ( $p < 0.05\%$ ), for the cycles, at each time and at each osmotic potential. Means followed by equal the 2<sup>nd</sup> lowercase letter do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the osmotic potentials, at each time and in each cycle

**Table 6** - Root dry mass of seedlings (g) of sorghum cv. EA-955, submitted to different hydration-dehydration cycles and water deficiency conditions at times X (3 h 30), Y (9 h) and Z (13 h)

Time X(3 h 30)	0.0 MPa	-0.2 MPa	-0.4 MPa
0 cycles	0.14 Aaa	0.05 Abb	0.01 Aac
1 cycle	0.13 Aaa	0.03 Bcb	0.01 Aac
2 cycles	0.14 Aaa	0.01 Bdb	-
3 cycles	0.11 Aba	0.07 Bab	0.01 Bac
Time Y (9 h)			
0 cycles	0.14 Aaa	0.05 Adb	0.01 Abc
1 cycle	0.14 Aaa	0.08 Abb	0.02 Aac
2 cycles	0.13 Aba	0.06 Acb	0.02 Aac
3 cycles	0.12 Aba	0.10 Aab	0.02 Aac
Time Z (13 h)			
0 cycles	0.14 Aaa	0.05 Acb	0.01 Acc
1 cycle	0.14 Aaa	0.07 Abb	0.01 Acc
2 cycles	0.12 Aba	0.07 Abb	0.02 Abc
3 cycles	0.12 Aba	0.09 Aab	0.04 Aac

Means followed by equal uppercase letters do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the times, in each cycle and at each osmotic potential. Means followed by equal lowercase letters do not differ by Scott-Knott's test ( $p < 0.05\%$ ), for the cycles, at each time and at each osmotic potential. Means followed by equal the 2<sup>nd</sup> lowercase letter do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the osmotic potentials, at each time and in each cycle

**Table 7** - Content of total soluble carbohydrates ( $\mu\text{mol g}^{-1}$ ) of sorghum seedlings cv. EA-955, submitted to different cycles of hydration-dehydration and water deficiency conditions at times X (3 h 30), Y (9 h) and Z (13 h)

Time X (3 h 30)	0.0 MPa	-0.2 MPa
0 cycles	0.270375 Aaa	0.077500 Aab
1 cycle	0.243625 Aba	0.048625 Cbb
2 cycles	0.255000 Aaa	0.008875 Bcb
3 cycles	0.227250 Aba	0.094500 Bab
Time Y (9 h)		
0 cycles	0.270375 Aaa	0.077500 Acb
1 cycle	0.250250 Aba	0.106125 Abb
2 cycles	0.253750 Aba	0.097875 Abb
3 cycles	0.241125 Aba	0.131500 Aab
Time Z (13 h)		
0 cycles	0.270375 Aaa	0.077500 Acb
1 cycle	0.244875 Bba	0.078500 Bcb
2 cycles	0.243625 Aba	0.103125 Abb
3 cycles	0.234875 Aba	0.129625 Aab

Means followed by equal uppercase letters do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the times, in each cycle and at each osmotic potential. Means followed by equal lowercase letters do not differ by Scott-Knott's test ( $p < 0.05\%$ ), for the cycles, at each time and at each osmotic potential. Means followed by equal the 2<sup>nd</sup> lowercase letter do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the osmotic potentials, at each time and in each cycle

**Table 8** - Soluble N-amino content ( $\mu\text{mol g}^{-1}$ ) of sorghum seedlings cv. EA-955, submitted to different cycles of hydration-dehydration and water deficiency conditions at times X (3 h 30), Y (9h) and Z (13 h)

Time X (3 h 30)	0.0 MPa	-0.2 MPa
0 cycles	0.209725 Aaa	0.065800 Abb
1 cycle	0.191475 Aaa	0.043450 Ccb
2 cycles	0.198775 Aaa	0.009325 Bdb
3 cycles	0.171375 Aba	0.086275 Bab
Time Y (9 h)		
0 cycles	0.209725 Aaa	0.065800 Acb
1 cycle	0.195150 Aba	0.094350 Abb
2 cycles	0.198775 Aba	0.083600 Abb
3 cycles	0.182975 Aba	0.117150 Aab
Time Z (13 h)		
0 cycles	0.209725 Aaa	0.065800 Acb
1 cycle	0.193200 Aba	0.075650 Bcb
2 cycles	0.184100 Aba	0.090600 Abb
3 cycles	0.177600 Aba	0.111450 Aab

Means followed by equal uppercase letters do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the times, in each cycle and at each osmotic potential. Means followed by equal lowercase letters do not differ by Scott-Knott's test ( $p < 0.05\%$ ), for the cycles, at each time and at each osmotic potential. Means followed by equal the 2<sup>nd</sup> lowercase letter do not differ from each other, by the Scott-Knott test ( $p < 0.05\%$ ), for the osmotic potentials, at each time and in each cycle

On the other hand, the time and number of exposures to water stress led to increments in the content of each one of the organic solutes at more negative potentials, especially in the last cycles of HD. This is due to their lower use as a form of protection against drought damage, resulting in an osmotic adjustment, and only some plants have the ability to adjust osmotically under adverse conditions (MATIAS *et al.*, 2018).

This type of physiological response can be observed in different species under stress, but its significance depends on the type of stress (salt stress, water stress, heavy metals, cold, heat), time and number of stress exposure, phenological stage of the plant, as well as other factors (PRISCO; GOMES-FILHO, 2010; SHINOZAKY *et al.*, 2015).

## CONCLUSIONS

1. Germination and vigor of sorghum seeds are severely impaired in water potentials equal to or greater than -0.4 MPa;
2. Application of three cycles of hydration and dehydration for a period of 13h in sorghum seeds improves tolerance to water deficiency conditions during germination and seedling development, and provides greater contents of soluble N-amino content and total soluble carbohydrates.

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