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Pre-germination treatments in pitaya (*Hylocereus* spp.) seeds for water stress mitigation

Tratamentos pré-germinativos em sementes de pitaya (*Hylocereus* spp.) para atenuação do estresse hídrico

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ABSTRACT - The use of mitigating agents to minimize the deleterious effects of water stress is a promising alternative for plant species, especially during germination and initial seedling development. Thus, the objective was to evaluate the effect of different pre-germination treatments as mitigating agents of water stress during germination and initial development of pitaya seedlings of the species *Hylocereus undatus* and *H. costaricensis*. The experiment was carried out in a completely randomized design, in a 2 x 6 factorial scheme, corresponding to two pitaya species and six pre-germination treatments (T1 = 0.0 MPa (control), T2 = -0.2 MPa (water stress); T3 = hydropriming + water stress; T4 = gibberellic acid + water stress; T5 = salicylic acid + water stress and T6 = thiamethoxan + water stress) with four replicates of 50 seeds. The variables analyzed were germination, germination speed index, shoot and primary root lengths, total dry mass, total soluble sugars and total free amino acids. The water potential of -0.2 MPa is limiting for germination and initial growth of *H. costaricensis* and *H. undatus*, with *H. undatus* being more tolerant to water stress in the germination phase. Pre-germination treatments with hydropriming, gibberellic acid, salicylic acid and thiamethoxan improve the physiological performance of *H. costaricensis* seeds, with gibberellic acid being the best attenuator of water stress. Gibberellic acid improves the physiological performance of *H. undatus* seeds under water deficit conditions.

Keywords: Cactaceae. Mitigation. Gibberellic acid. Hydropriming. Salicylic acid.

RESUMO - A utilização de agentes mitigadores visando minimizar os efeitos deletérios do estresse hídrico constitui alternativas promissoras para as espécies vegetais, sobretudo durante a germinação e o desenvolvimento inicial de plântula. Dessa forma, objetivou-se avaliar o efeito de diferentes tratamentos pré-germinativos como agentes atenuadores do estresse hídrico durante a germinação e desenvolvimento inicial de plântulas de pitaya das espécies *Hylocereus undatus* e *H. costaricensis*. Para isso, realizou-se o experimento em delineamento inteiramente casualizado, em esquema fatorial 2 x 6, correspondente a duas espécies de pitayas e seis tratamentos pré-germinativos (T1 = 0,0 MPa (controle), T2 = -0,2 MPa (estresse hídrico); T3 = hidrocondicionamento + estresse hídrico; T4 = ácido giberélico + estresse hídrico; T5 = ácido salicílico + estresse hídrico e T6 = tiametoxan + estresse hídrico) com quatro repetições de 50 sementes. As variáveis analisadas foram germinação, índice de velocidade de germinação, comprimento da parte aérea e raiz primária, massa seca total, açúcares solúveis totais e aminoácidos livres totais. O potencial hídrico de -0,2 MPa foi limitante para germinação e crescimento inicial da *H. costaricensis* e *H. undatus*, sendo esta última mais tolerante ao estresse hídrico na fase de germinação. Os tratamentos pré-germinativos com hidrocondicionamento, ácido giberélico, ácido salicílico e tiametoxan melhoraram o desempenho fisiológico das sementes de *H. costaricensis*, destacando-se o ácido giberélico como atenuante mais eficiente do estresse hídrico. O ácido giberélico proporcionou melhor desempenho fisiológico das sementes de *H. undatus* em condições de déficit hídrico.

Palavras-chave: Cactaceae. Mitigação. Ácido giberélico. Hidrocondicionamento. Ácido salicílico.

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INTRODUCTION

Brazil stands out among the largest producers of fruits, including those from the unconventional species cultivated. Pitayas are part of this group, which contains *Hylocereus undatus* (white-fleshed, red-skinned pitaya) and *H. costaricensis* (red-fleshed, red-skinned pitaya) with several plantations in Brazil. Although pitaya plants adapt to various edaphoclimatic conditions and are considered rustic (JUNQUEIRA et al., 2002), as any other species they require balanced water supply for germination of their seeds. When analyzing the germination of pitaya genotypes under water stress, Ortiz et al. (2014) observed that from the potential -0.2 MPa there is damage to germination and at the potential -0.6 MPa this process does not occur. Thus, it is necessary to assess the germination behavior of these species under adverse conditions, such as low water availability, and at the same time to seek alternatives to mitigate the effect of this stress during the germination process.

Research has been conducted in an attempt to find techniques that attenuate the deleterious effects during germination. Among these techniques, hydropriming stands out, considered to be promising in the mitigation of damage caused by water stress during the germination of seeds of plant species, such as *Chenopodium quinoa* Willd. (NADALI et al., 2021), *Helianthus annuus* L. (OCVIRK et al., 2021) and *Cymbopogon olivieri* (Boiss) Bor. (PICHAND; TILAKI; SADATI, 2021). In addition, hormones and organic and synthetic compounds, when applied exogenously, can act as germination promoters and embryo vegetative growth activators under water deficit conditions. In this context, thiamethoxan and salicylic and gibberellic acids stand out for promoting higher tolerance to water deficit during germination of maize (*Zea mays* L.) (CATANEO et al., 2011), soybean (*Glycine max* L. Merr.) (SILVA et al., 2019), onion (*Allium cepa* L.) (GNAWALI; SUBEDI, 2021) and common bean (*Phaseolus vulgaris* L.) (SCHMIT et al., 2021). Thiamethoxam is a bioactivating insecticide that can positively contribute to the vigor of plants and protect them from abiotic stresses (HOUSE; SWANTON; LUKENS, 2020). With regard to salicylic acid, it is an organic compound that acts in the regulation of physiological processes, being a signaling agent of great importance in plant stress mitigation (RHAMAN et al., 2021). In the case of gibberellic acid, it has an effective action as a promoter of germination and activator of embryo vegetative growth (TAIZ et al., 2017).

In view of the above, the objective was to evaluate the germination and vigor of pitaya (*H. undatus* and *H. costaricensis*) seeds under different pre-germination treatments as water stress mitigators.

MATERIAL AND METHODS

The experiment was carried out at the Seed Analysis Laboratory of the Federal Rural University of the Semi-Arid Region - UFERSA, Mossoró, Rio Grande do Norte, Brazil (23°17’34” South latitude, 37°20’39” West longitude and average altitude of 20 m). Ripe fruits of red-fleshed (*H. costaricensis*) and white-fleshed (*H. undatus*) pitayas were acquired in the cities of Mossoró (RN) and Londrina (PR) (23° 17’ 34” S, 51° 10’ 24” W and 550 m altitude), respectively.

The seeds were manually extracted in a sieve with running water and washed several times to remove the mucilage. Subsequently, they were arranged on newspaper to dry in a shaded environment for 24 hours. After that, the seeds were homogenized to determine the moisture content, using two replicates of 50 seeds, by the oven method at 105 ± 3 °C, for 24 hours (BRASIL, 2009), and the results were expressed in percentage (wet basis). Then, the seeds were placed in hermetically sealed plastic packages and stored in a refrigerator (4 to 6 °C and 60% RH) for 30 days until the experimental phase.

The experiment was carried out in a completely randomized design (CRD), in a 2 x 6 factorial scheme, corresponding to two pitaya species and six pre-germination treatments (T1 = 0.0 MPa - control), T2 = -0.2 MPa (water stress); T3 = hydropriming + water stress; T4 = gibberellic acid + water stress; T5 = salicylic acid + water stress and T6 = thiamethoxan + water stress) (ORTIZ et al., 2014), with four replicates of 50 seeds. The seeds of treatments T3, T4, T5 and T6 were subjected to pre-imbibition for four hours in hydropriming (distilled water), gibberellic acid (150 mg L^{-1}), salicylic acid (1 µM L^{-1}) and thiamethoxan (1 mL kg^{-1} seed), respectively. After four hours of imbibition, the solution was drained and the seeds from each treatment were sown on two sheets of blotter paper placed inside transparent acrylic boxes and moistened with water volume equivalent to 2.5 times the mass of dry paper, under the osmotic potentials of 0.0 MPa for T1 and -0.2 MPa (PEG 6000 solution), simulating water stress for the other five treatments (VILLELA; DONI FILHO; SIQUEIRA, 1991).

The concentrations of gibberellic and salicylic acids, as well as thiamethoxan, were determined based on pre-tests, from the time required for water gain by the seeds during the construction of the imbibition curve. The potential of -0.2 MPa was also chosen based on pre-tests performed for both species.

At 20 days after sowing, the following physiological evaluations were performed:

a) germination – conducted in a germination chamber at 25 ºC (LONE et al., 2014), with a photoperiod of 12 hours of light, whose results were expressed as an average percentage of normal seedlings for each treatment (BRASIL, 2009);

b) germination speed index - performed simultaneously with the germination test, with daily counts of normal seedlings from the second day after sowing and ended at 20 days. The index was calculated by summing the ratios between the number of seedlings germinated in the period and the number of days from sowing to germination, as recommended by Maguire (1962);

c) Lengths of shoot and primary root of normal seedlings – shoot was measured from the collar the primary root to the apex of the aerial part, while the primary root was measured from the apical part to the basal point, and the results were expressed in centimeters (cm);

d) Dry mass of seedlings – after measurements of shoot and root, the seedlings were placed in paper bags separated by treatment and dried in a forced air circulation oven at 60 °C for 72 hours. Then, the material was weighed on a precision scale and the results were expressed in milligrams per seedling (mg $seedling^{-1}$).

Biochemical determinations were obtained from the fresh mass of fifteen seedlings per replicate, collected at 20 days after sowing.

Initially, the samples were extracted, by weighing 0.2 g of fresh mass, putting it in tubes and adding 3 mL of 80% ethyl alcohol. Then, the material was automatically macerated for two minutes, placed in a water bath at 60 ºC for 20 minutes and centrifuged for eight minutes. The supernatant was collected and this extraction process was repeated two more times. At the end, the resulting supernatant was collected to quantify the contents of total soluble sugars and total free amino acids.

Total soluble sugars were determined based on the measurement of absorbance at 620 nm by applying the anthrone method (YEMM; WILLIS, 1954), using glucose as the standard substance. The results were expressed in GLU.g^{-1} µmol of fresh mass. The total free amino acid content was determined based on the measurement of absorbance at 570 nm, with application of the acid ninhydrin method (YEMM; COCCKING, 1955), with glycine as the standard substance, and the results were expressed in µmol GLY.g^{-1} of fresh mass.

The results were subjected to analysis of variance ($p < 0.05$) and, in case of significance, Tukey test ($p < 0.05$) was applied for interaction and pre-germination treatments, and t-test ($p < 0.05$) was applied for the species, using the statistical program SISVAR® (FERREIRA, 2011).

RESULTS AND DISCUSSION

The results of the analysis of variance indicate interaction between the two pitaya species and the pre-germination treatments (Table 1), with significant values ($p < 0.01$) for the variables germination, germination speed index, shoot length (SL), root length (RL), total dry mass (TDM), total soluble sugars (TSS) and total free amino acids (TFAA).

Table 1. Analysis of variance of germination (G), germination speed index (GSI), shoot length (SL), root length (RL), total dry mass (TDM), total soluble sugars (TSS) and total free amino acids (TFAA) of pitaya (*Hylocereus costaricensis* and *H. undatus*) seedlings from seeds pre-treated and subjected to water stress.

Source of variation	DF	Mean square			
		G	GSI	SL	RL
Species (S)	1	19683.00**	740.26**	0.31**	0.94**
Treatments (T)	5	1454.53**	21.62**	0.79**	0.06**
T x S	5	544.00**	10.38**	0.18**	0.07**
Error	36	25.44	0.38	0.02	0.00
CV (%)		12.64	9.05	10.64	9.27
Overall mean		40.00	6.85	1.32	0.46

Source of variation	DF	Mean square		
		TDM	TSS	TFAA
Species (S)	1	0.01 ns	7.10**	2291.21**
Treatments (T)	5	0.46**	50.03**	2174.08**
T x S	5	0.47**	33.49**	910.84**
Error	36	0.03	0.06	0.90
CV (%)		12.44	2.90	2.54
Overall mean		1.35	9.07	37.45

NS and ** = not significant and significant at 1% probability level ($p < 0.01$).

The germination of *H. undatus* was reduced by half under water stress compared to the control (Table 2). The pre-germination treatments of *H. undatus* seeds with gibberellic acid, salicylic acid and thiamethoxan, under water stress conditions, increased germination by 27, 32 and 19 percentage points, respectively. Hydropriming of seeds promoted results similar to that obtained with water stress (T2 = -0.2 MPa). Although hydropriming did not reduce the effects of stress, on the other hand, the use of salicylic and gibberellic acids and thiamethoxan mitigated the deleterious effects of water stress during the germination of seeds of this species.

The germination of *H. costaricensis* seeds was reduced by 27 percentage points between the control and water stress. The treatments with hydropriming, gibberellic acid, salicylic acid and thiamethoxan promoted increments of 18, 14, 9 and 3 percentage points, respectively, in the germination of seeds of this species under water stress conditions. Seeds under water stress when treated with hydropriming showed germination similar to that obtained in the control.

The species *H. undatus* had higher germination than *H. costaricensis* in all treatments (Table 2). This result occurred because *H. costaricensis* seeds have physiological dormancy attributed to a low ratio between cytokinin and auxin (SHENG et al., 2016).

Table 2. Germination and germination speed index (GSI) of pitaya (*Hylocereus costaricensis* and *H. undatus*) seeds from seeds pre-treated and subjected to water stress.

Treatments	Germination (%)		GSI	
	H. undatus	*H. costaricensis*	*H. undatus*	*H. costaricensis*
T1	90 Aa	35 Ba	14.4 Aa	4.9 Ba
T2	39 Ad	8 Bc	7.7 Ae	1.6 Bc
T3	38 Ad	26 Bab	9.0 Ad	3.1 Bb
T4	66 Abc	22 Bb	11.4 Ac	2.8 Bbc
T5	71 Ab	17 Bbc	9.5 Ad	3.7 Bab
T6	58 Ac	11 Bc	12.7 Ab	1.5 Bc

[1]Means followed by the same letter do not differ statistically from each other, uppercase in the row (species) and lowercase in the column (pre-germination treatments), by Tukey test at 5% probability level. Control (T1); water stress: -0.2 MPa (T2); hydropriming + water stress (T3); gibberellic acid + water stress (T4.); salicylic acid + water stress (T5); thiamethoxan + water stress (T6).

The germination speed index (GSI) of *H. undatus* seeds showed a reduction of 6.7 units under water stress. However, all pre-germination treatments promoted an increase in germination speed index compared to the treatment without stress (T2). When comparing the results of pre-germination treatments with those of T2, there were increments in GSI of 17% for hydropriming, 63% for gibberellic acid, 23% for salicylic acid and 65% for thiamethoxan. On the other hand, a reduction of 3.3 units was observed when comparing T2 with T1 for *H. costaricensis* seeds. However, except for thiamethoxan, the other treatments promoted increments in GSI, which were equal to 1.5, 1.2 and 2.1 units for seeds subjected to hydropriming, gibberellic acid and salicylic acid, respectively.

When analyzing each species, it was observed that for *H. undatus* the highest means of germination and germination speed index were obtained under the treatments with salicylic acid and thiamethoxan, respectively. Conversely, for *H. costaricensis* seeds with the same variables, the highest means were obtained with the use of hydropriming and salicylic acid, respectively.

Although research with gibberellic acid involving abiotic stresses is still incipient, some studies indicate the beneficial effects of its use, for instance in rapeseed (*Brassica napus* L.) (KHAN et al., 2020) and maize (GNAWALI; SUBEDI, 2021), which were subjected to water stress and showed better physiological performance when compared to plants that did not receive the same treatment. As for the results of the present study, this regulator mitigated the damage caused by water stress for both pitaya species, promoting increments for both germination and germination speed index (Table 2). This hormone is considered an endogenous enzymatic activator, promoter of germination, and its exogenous application influences protein metabolism and can double the protein synthesis rate of seeds (BRAUN et al., 2010). For this reason, when used at appropriate concentration, it promotes an increase in germination, mainly due to its performance in cell elongation, causing the primary root to break the tissues that restrict its growth (TAIZ et al., 2017).

As mentioned above, hydropriming increased the germination and germination speed index of *H. costaricensis* seeds. This is because, even when the seeds are under water deficit, a smaller amount of water is required for the resumption of embryo growth, since most reserves have already been mobilized (MARCOS-FILHO, 2015). For some species, hydropriming has been pointed as a viable alternative to mitigate the effects of abiotic stresses. Hydropriming increased salt tolerance of sorghum [*Sorghum bicolor* (L.) Moench] seeds, improving germination and growth of seedlings under salt stress (CHEN et al., 2021). The efficacy of this technique was also verified in sunflower (*Helianthus annuus* L.) seeds, promoting greater expression of vigor, with higher number of normal seedlings and faster and more uniform germination (MATIAS et al., 2019).

The shoot length (SL) of pitaya seedlings was reduced under water stress (T2) by 0.6 cm and 1.1 cm for the seedlings of *H. undatus* and *H. costaricensis*, respectively, when compared to the control (T1) (Table 3). *H. undatus* seedlings, whose seeds were treated with gibberellic acid (T4), showed higher SL compared to the T2 treatment and similar SL compared to the control (T1). This response is due to the action of gibberellin, which is a plant phytohormone that induces cell division and elongation, promoting greater efficiency in reserve mobilization (TAIZ et al., 2017). On the other hand, *H. costaricensis* seeds responded better to the treatment with thiamethoxan (T6), with an increase of 0.7 cm in SL compared to those subjected to water stress (T2). This response may be associated with the fact that thiamethoxan can induce a greater development of the embryonic axis, minimizing the negative effects in water deficit situations, as also occurred for soybean seeds (CATANEO et al., 2011). The other pre-germination treatments did not promote increases in the SL of *H. undatus* and *H. costaricensis* (Table 3).

Table 3. Shoot length (SL), primary root length (RL) and total dry mass (TDM) of pitaya (*Hylocereus costaricensis* and *H. undatus*) seedlings from seeds pre-treated and subjected to water stress.

Treatments	SL (cm)		RL (cm)		TDM (mg)	
	H. undatus	*H. costaricensis*	*H. undatus*	*H. costaricensis*	*H. undatus*	*H. costaricensis*
T1	1.6 Ba	2.1 Aa	0.3 Ba	1.0 Aa	1.6 Aa	1.4 Abc
T2	1.1 Ab	1.0 Ac	0.3 Ba	0.6 Ab	1.3 Aab	1.0 Bde
T3	1.1 Bb	1.3 Ac	0.4 Ba	0.5 Ab	1.3 Aab	0.8 Be
T4	1.5 Aa	1.3 Bc	0.3 Ba	0.5 Ab	1.3 Aab	1.3 Acd
T5	1.0 Ab	1.1 Ac	0.3 Ba	0.5 Ab	1.2 Bb	2.0 Aa
T6	1.2 Bb	1.7 Ab	0.3 Ba	0.6 Ab	1.4 Bab	1.7 Aab

[1]Means followed by the same letter do not differ statistically from each other, uppercase in the row (species) and lowercase in the column (pre-germination treatments), by Tukey test at 5% probability level. Control (T1); water stress: -0.2 MPa (T2); hydropriming + water stress (T3); gibberellic acid + water stress (T4.); salicylic acid + water stress (T5); thiamethoxan + water stress (T6).

Water stress did not influence the root length and total dry mass of *H. undatus* seedlings. Therefore, there was no difference between plants with and without pre-germination treatment, indicating tolerance of *H. undatus* to water potential of -0.2 MPa (Table 3). In *H. costaricensis* seedlings, root length was reduced by water stress, and seedlings originated from seeds with pre-germination treatments also showed reduction (Table 3). Water stress reduces seed hydration, affecting the degradation of reserves and consequently the availability of energy and nutrients for radicle growth (PAIVA et al., 2018; COSTA et al., 2022).

The total dry mass of *H. costaricensis* seedlings was reduced by 29% by water stress (T2) compared to the control (T1). Pre-germination treatment with thiamethoxan (T6) promoted results similar to those found in the control, and treatment with salicylic acid (T5) resulted in 43% higher results compared to those of the control (Table 3). There was a positive effect of these attenuators on the total dry mass of *H. costaricensis* seedlings under water stress was observed, but this same effect was not verified in *H. undatus*. This may be due to the fact that *H. costaricensis* is more adapted to regions of higher temperature and lower relative humidity, responding faster to these defense inducers, which demonstrates once again the differences in the performance of the treatments for both species.

When comparing the total soluble sugars of *H. undatus* seedlings of the control (T1) with the value of seedlings of the other treatments, it was verified that there was a reduction in the content of these carbohydrates. This reduction was even more significant for the results related to pre-germination treatments with hydropriming, gibberellic acid, salicylic acid and thiamethoxan. Regarding the species *H. costaricensis*, it showed a different behavior from that obtained with *H. undatus*. When comparing the control (T1) with the other treatments, there was an increase in sugar levels for seedlings from T2 and also for seedlings from pre-germination treatments with gibberellic and salicylic acids (Table 4).

Table 4. Total soluble sugar contents (TSS) and total free amino acids (TFAA) of pitaya (*H. costaricensis* and *H. undatus*) seedlings from seeds with different pre-germination treatments and germinated under water stress.

Treatments	TSS (μmol GLU /g FM)		TFAA (μmol GLY/g FM)	
	H. undatus	*H.costaricensis*	*H. undatus*	*H. costaricensis*
T1	14.2 Aa	8.6 Bd	26.3 Ae	15.0 Bd
T2	12.1 Ab	9.8 Bc	94.6 Aa	42.5 Ba
T3	4.6 Bf	6.1 Af	36.8 Ac	31.1 Bb
T4	8.1 Bc	13.9 Aa	36.6 Bc	43.4 Aa
T5	6.9 Bd	10.5 Ab	29.7 Bd	31.5 Ab
T6	6.1 Be	7.8 Ae	42.2 Ab	19.8 Bc

[1]Means followed by the same letter do not differ statistically from each other, uppercase in the row (species) and lowercase in the column (pre-germination treatments), by Tukey test at 5% probability level. Control (T1); water stress: -0.2 MPa (T2); hydropriming + water stress (T3); gibberellic acid + water stress (T4.); salicylic acid + water stress (T5); thiamethoxan + water stress (T6).

Among the pre-germination treatments, gibberellic acid stood out for *H. costaricensis* seedlings, promoting the highest level of sugars, compared to the control (without stress - T1) and to the other treatments (Table 4). In seeds, gibberellins act in the production of the enzyme α-amylase, whose function is the breakdown of reserve starch into smaller sugar molecules (TAIZ et al., 2017). Thus, exogenous application of this hormone may have stimulated starch digestion for osmotic adjustment, which is the mechanism of accumulation of osmotically active solutes in the cell (BLUM, 2017). This can be confirmed by the fact that sugar was not used for growth of either shoots or root, being certainly used for osmotic adjustment. This indicates, as mentioned above, that this species is more adapted to the climatic conditions of northeastern Brazil, characterizing a more investment-prone species, capable of accumulating solutes and reducing water potential in response to water stress. On the other hand, this influence of gibberellic acid was not verified for *H. undatus*, from the city of Londrina, Paraná, which showed a lower mean compared to the treatment without attenuator (T2).

Water stress increased the content of total free amino acids for both species (Table 4). This fact may be related to the chemical composition of pitaya seeds, which have a large protein reserve. While the seeds of *Opuntia* sp., belonging to the same family as pitayas, contain 50 g of protein kg^{-1} of seeds (ENNOURI et al., 2005), pitaya seeds have 206 g kg^{-1} of seeds (VILLALOBOS-GUTIÉRREZ et al., 2012). For this reason, during germination under water stress conditions, there was probably a higher hydrolysis of proteins into amino acids than hydrolysis of starch into sugars.

Thus, it can be noticed that there was an attempt to minimize the effects of abiotic stress, through the increase of amino acids for osmoregulation. However, this biochemical behavior was not effective in mitigating physiological damage, because seedlings originated from the situation of water stress at -0.2 MPa (T2) showed low values of germination and vigor, even with a significant increase in amino acids compared to the other treatments (Tables 2 and 3).

The pre-germination treatment of *H. undatus* and *H. costaricensis* seeds with the different techniques used in the present study positively influences germination and vigor. As for organic solutes, this influence is observed mainly in the levels of soluble sugars involved in the osmotic adjustment process.

CONCLUSIONS

Water potential of -0.2 MPa is limiting for germination and initial growth of *H. costaricensis* and *H. undatus*, the latter being more tolerant to water stress in the germination phase.

Pre-germination treatments with hydropriming, gibberellic acid, salicylic acid and thiamethoxan promote better physiological performance of *H. costaricensis* seeds, with gibberellic acid standing out as the best water stress attenuator.

Gibberellic acid improves the physiological performance of *H. undatus* seeds under water deficit conditions.

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