

Recovery and germinative response of *Amaranthus deflexus* L. seeds under different levels of water stress and luminosities

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Abstract

The objective of this work was to evaluate, in two experiments, the effect of water stress and luminosity on the germinative behavior and vigor of *Amaranthus deflexus* L seeds. The seeds were sown in Petri dishes containing two sheets of filter paper moistened with distilled water, in the control treatment. Using this treatment-control configuration, as a standard of comparison, two assays were performed using subsamples from the same *A. deflexus* seed population. In the first experiment (experiment 1), solutions of polyethylene glycol 6000 (PEG 6000) were used, providing osmotic potentials of -0.3; -0.6; -0.9; -1.2 and -1.5 MPa; however, in the second trial (experiment 2) osmotic potentials of -0.1; -0.2; -0.3; -0.4 and -0.5 MPa were used. At the end of the test, the ungerminated seeds for each treatment were washed and germinated on a filter paper moistened with water. The germination and vigor of the seeds were evaluated through the first germination count, germination test, germination speed index and average germination time. The experimental design was completely randomized, in a 2 x 6 factorial scheme, totaling twelve treatments, with four replicates of 50 seeds each, in both trials. Water stress negatively affects the performance of *A. deflexus* seeds, reducing germination and vigor from -0.1 MPa. Seed germination was compromised by the absence of light, regardless of the applied stress levels. There was an increase in seed germination after application of the same at higher levels of water stress.

Keywords: pigweed, osmotic stress, photoperiod

Introduction

In recent years, the need to know the main factors governing the germination of weed seeds has acquired greater importance. This is mainly due to the acquisition of the knowledge needed for an efficient management and appropriate to each agricultural situation. In this context, germination is a key process in the dynamics and organization of plant species (Filetiet al., 2011).

Water deficit plays a fundamental role, especially in arid and semi-arid regions (), since it negatively affects germination, the stand of plants and vegetative development (Oliveira & Gomes-Filho, 2009; Nunes et al., 2014). In addition,

this type of abiotic stress is capable of increasing competition between weeds and crops (Souza et al., 2016).

According to Marcos Filho (2005), water is one of the main factors influencing germination, since it is involved directly and indirectly in all stages, besides reactivating metabolism. Very negative osmotic potentials delay and decrease germination, with a minimum level of humidity that the seed must reach to germinate, which depends on the chemical composition and permeability tests (Verslueset al., 2006; Oliveira & Gomes-Filho, 2016). Therefore, it is extremely important to maintain an adequate level of hydration that allows the reactivation of

Received: 23 December 2016
Accepted: 20 July 2017

metabolic processes, culminating in the growth of the embryonic axis (Marcos Filho, 2005).

Other factors that may have a direct influence on the germination process are light and temperature, especially in the emergence of seedlings under field conditions. This situation determines, in some plant species, not only the seed fraction that germinates, but also the speed of germination, even when adequate conditions of humidity and temperature are provided (Vidal et al., 2007).

The species *A. deflexus* L., popularly known as pigweed, occurs spontaneously in tropical and subtropical regions, being one of the important weeds in South America. This annual herbaceous species is found in practically the entire Brazilian territory and is characterized by high seed production, the only means of propagation, causing great reductions of productivity in the agricultural areas when present and uncontrolled (Moreira & Bragança, 2010).

It is known that seeds of the genus *Amaranthus* are present in high quantities in seed banks, and certain species of large pigweed can produce in a single plant, quantities greater than 200,000 seeds (Lorenzi, 2008). Once emerged, it severely infests areas of agricultural production, raising the degree of control difficulty of the weed community and the production costs (Lessa et al., 2017).

To understand the process by which this species infests cultivated areas with or without water deficit, the study of seed germination has become indispensable. In this context, the objective of this work was to evaluate the effects of water stress and luminosity on the germination and vigor of *A. deflexus* seeds.

Materials and Methods

Two experiments were conducted at the Seed Analysis Laboratory of the Phytotechnology Department of the Federal University of Ceará (UFC), in Fortaleza-CE. The first test was called "experiment I" and the second "experiment II". For both, *A. deflexus* seeds were collected at the experimental farm of Rafael Fernandes, Federal Rural University of the Semi-Arid region (UFERSA), Mossoró-RN.

To execute experiment I, a representative subsample was collected from 30 physiologically mature panicles of a population of *A. deflexus* plants. The panicles were placed in paper bags and dried in a greenhouse with forced air circulation at 27-28°C, until dehiscence of the fruits. The processing of seeds (treatment) was carried out by friction of the fruits in a plastic tray, followed by agitation for the separation of seeds from dried fruits.

For the germination test, sowing was done in Petri dishes, containing two sheets of filter paper moistened with distilled water, in the control treatment. Using this treatment-control configuration, as a standard of comparison, two assays were performed using subsamples from the same *A. deflexus* seed population. In the first experiment (experiment 1), solutions of polyethylene glycol 6000 (PEG 6000) were used, providing osmotic potentials of -0.3; -0.6; -0.9; -1.2 and -1.5 MPa; but in the second experiment (experiment 2) it was -0.1; -0.2; -0.3; -0.4 and -0.5 MPa.

The results observed in experiment I necessitated the evaluation of lower levels of stress, aiming to observe better understanding about the point of maximum influence of salt stress levels on seed germination. For that, experiment II was carried out with osmotic potential of -0.1; -0.2; -0.3; -0.4 and -0.5 MPa.

The concentrations of PEG 6000, for each potential, were obtained according to Villela et al. (1991). The seeds were kept in covered Petri dishes, sealed with Parafilm® (BRAND, Germany) in order to reduce moisture loss, and stored in a Biochemical Oxygen Demand (BOD) type germinator at 25°C with a 12-hour photoperiod of daily light and simulating the conditions of germination; and in the absence of a light simulating environment under soil cover, under no-tillage conservation management conditions, for fourteen days.

Seed recovery was evaluated at the end of each experiment, the ungerminated seeds in each treatment were washed and placed to germinate in Petri dishes with Germitest type filter paper, moistened with distilled water to observe the continuity of the germination process. The seeds were evaluated for germination and

vigor, and these were represented by the first germination count, analyzing the following variables:

First germination count (PC) - conducted along with the germination test, counting the percentage of normal seedlings on the fourth day after the test, as recommended by Brasil (2009).

Germination test (TG) - performed on the 14th day after sowing, at the end of the experiment, considering the germination of the seeds that emitted the primary root. The results were expressed as a mean percentage, based on the number of normal seedlings (Brasil, 2009).

Germination speed index (IV)-calculated by the ratio of the sum of the number of germinated seeds each day, and the number of days elapsed between sowing and germination (Maguire, 1962):

$$IV = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{G_n}{N_n}$$

where IV is germination speed index; G_1, G_2, \dots, G_n correspond to the numbers of seedlings computed at the first, second, third and final count; $N_1, N_2, N_3, \dots, N_n$ correspond to the number of days from sowing to the first, second, third and last count.

Mean germination time (TM) - obtained through daily counts of germinated seeds until the 14th day after sowing and calculated using the formula below, proposed by Labouriau (1983), the results are expressed in days.

$$TM = \sum (n_i t_i) / \sum n_i, \text{ where:}$$

TM = mean germination time (days);

n_i = number of seeds germinated in the interval between each count;

t_i = time elapsed between the onset of the germination and the i th count.

In both trials, the experimental design was completely randomized, with four replicates of 50 seeds, in a 2 x 6 factorial scheme, the first factor being two light conditions (F1 = 12 light hours and F2 = absence of light) and the second factor constituted by six levels of water stress (0; -0.3; -0.6; -0.9; -1.2 and -1.5 MPa in experiment 1 and 0; -0.1; -0.2; -0.3; -0.4; and -0.5 MPa in experiment 2).

Variance analysis was performed using the Snedecor F test ($p < 0.05$) for comparison of treatments variances and the Tukey averages ($p < 0.05$) multiple comparisons test was applied to the first and adjustment of regression functions was performed for the second factor.

Results and Discussion

In experiment 1, a low seed germination index of *A. deflexus* was observed during 14 days in the photoperiod of 12 light hours, obtaining 3 and 1.5% in the interval from the seventh to the twelfth day after sowing (DAS), in treatments -0.3 and -0.6 MPa, respectively. A maximum of 20% germination was observed in the treatment of 0 MPa (Figure 1A). In the absence of light (Figure 1B), germination rates were even lower, suggesting that this species has a positive photoblastic nature, according to Labouriau (1983).

In general, weed species are sensitive to the absence of light, inhibiting the metabolic processes of lysis and cell division and, consequently, germination. However, it is not possible to generalize this response, considering that the seeds used in this type of experiment are derived from plants that probably present

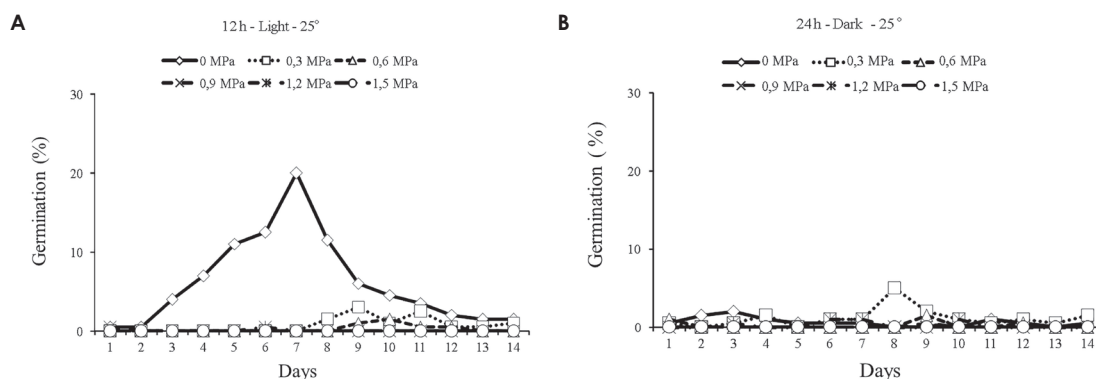


Figure 1. Evolution of the germination of *A. deflexus* seeds at different levels of water stress in a germination chamber under two light conditions.

variable genotypes for this characteristic.

In the first germination count, germination test and germination speed index, analysis of variance showed a significant effect ($p < 0.01$) for the combined effect between water stress and photoperiod (Table 1).

There was an adjustment of the response function at the first germination count in the total absence of light, with a linear decrease reaching 0% TG from the concentration of -1.2 MPa. In the photoperiod of 12 h of light, there was no adjustment of the regression model, as well as for the TG, IV and TM variables. However, the increase in water deficit induced by the increase in PEG

6000 concentrations in the substrate solution was responsible for a tendency of reduction in the mean values of TG and IV in the photoperiod of 12 light hours (Figure 2).

Rizzardì et al. (2009), studying the germination of *Ipomoea triloba* L., an extremely aggressive weed species, observed a reduction of metabolic processes during germination and reported that this is due not only to seed sensitivity and water potential variation, but also to the nature of the inducing substance, which often presents more toxic osmotic effects on germination.

A significant difference was observed

Table 1. Synthesis of variance analysis for the first germination count (PC), germination test (TG), germination speed index (IV) and mean germination time in days (TM), as a function of water stress effects (E), photoperiods (F) and the combined effect (E x F) in the respective stress experiments and physiological recovery with *Amaranthus* seedlings in a germination chamber under two light conditions.

FV	G.L.	¹ QM I				² QM II			
		PC	TG	IV	TM	PC	TG	IV	TM
E	5	8.97**	92.70 ^{ns}	21.77**	119.75**	8.50**	0.17**	11.23**	55.28**
F	1	0.02 ^{ns}	0.26**	6.99**	0.84 ^{ns}	1.40 ^{ns}	0.48**	19.93**	78.87**
E x F	5	1.78**	0.31**	12.49**	0.93 ^{ns}	2.44**	0.13**	6.46**	11.27 ^{ns}
Error	36	0.26	0.01	0.41	7.87	0.66	0.001	0.30	6.89
Recovery Experiment I									
E	5	18.79**	0.26**	37.19**	44.53**	5.38**	0.03**	5.01**	66.79**
F	1	27.72**	1.44**	126.62**	8.14 ^{ns}	69.07**	0.75**	89.03**	37.58**
E x F	5	1.31 ^{ns}	0.32**	15.83 ^{ns}	1.99 ^{ns}	5.38**	0.04**	5.59**	4.95 ^{ns}
Error	36	1.71	0.06	6.56	6.67	0.28	0.01	0.90	5.09

**Significant by Snedecor's F test at 1% probability; ^{ns}Not significant. ¹Average squares estimated in experiment I; ²Average squares estimated in experiment II.

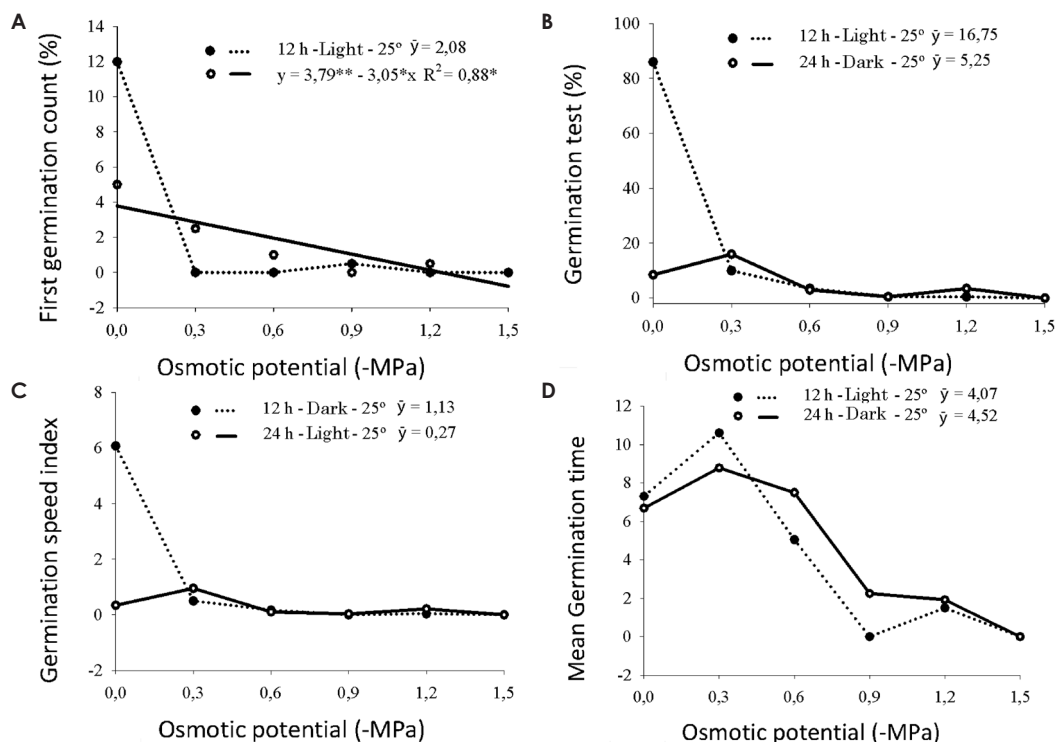


Figure 2. First germination count (A), germination test (B), germination speed index (C) and mean germination time (D) of *A. deflexus* seeds under different levels of osmotic stress in the germination chamber under two light conditions.

between photoperiods in the control (no stress) treatment in PC, TG, IV and TG recovery. In this case, photoperiod 1 (F1), that is, light for 12 hours per day, stood out in all evaluations, except for

TG recovery, where photoperiod 2 (F2), with the seeds in the dark, showed 43% germination (Table 2).

Table 2. First germination count (PC), germination test (TG), germination speed index (IV) and germination test recovery (RECTG) submitted to water stress (E) and light conditioning (F1 = 12 hours of light and F2 = absence of light) in experiment I with *A. deflexus* seeds tested in the germination chamber, determined in experiment I, under two conditions of luminosity.

Water stress (MPa)	PC		TG		IV		RECTG	
	F1	F2	F1	F2	F1	F2	F1	F2
0.0	12 a	5 b	86 a	8.5 b	6.08 a	0.34b	4 b	43 a
-0.3	0a	2.5 a	10 a	16 a	0.5 a	0.95a	58 a	28 b
-0.6	0 a	1 a	3 a	3.5 a	0.17 a	0.10a	66 a	19.5 b
-0.9	0 a	0 a	0.5 a	0.5 a	0 a	0.03a	82 a	37.5 b
-1.2	0 a	0 a	0.5 a	3.5 a	0.4 a	0.20a	80.5 a	22.5 b
-1.5	0 a	0 a	0 a	0 a	0 a	0 a	76 a	51 a
Standard error	0.07		0.01		0.09		0.01	

Means followed by the same letter in the line do not differ by the Tukey test at 5% probability.

This result may be related to the state of physiological dormancy of these seeds, since wild species generally present mechanisms of dormancy and light is linked to the activation of the phytochrome system, in turn, related to the functioning of cell membranes. This situation can cause a change in the flow of numerous substances into cells and membrane

permeability, contributing not only to the break of dormancy and promoting the germination of other species, but also causes inhibition in some of them (Hilhorst & Karszen, 1988).

In the absence of a significant effect, E x F interaction analyzed the simple effect of photoperiods on TM, but also no significant difference was verified (Table 3).

Table 3. Comparison of the averages of first germination count (PC), germination speed index (IV) and mean germination time in days (TM) in experiments I and II and recovery with *A. deflexus* seeds tested in germination chamber under two light conditions (F1 = 12 h of light and F2 = absence of light).

PC I		PC I		IV I	
F1	F2	F1	F2	F1	F2
2.08a	1.50a	24.92a	12.58b	4.61a	2.25b
Standard error		0.19		0.37	
TM I		TM II		TM II	
2.76a	3.66a	3.84a	1.28b	3.35a	1.58b
Standard error		0.40		0.33	

recovery experiment where the variables PC I, IV I, TM I and TM II were determined. Means followed by the same letter in the line do not differ by Tukey test at 5% probability.

In recovery experiment II, significant differences between averages were observed in PC, IV and TM, with photoperiod 1 standing out with 24.92%, 4.61 and 3.35 days, respectively (Table 3). There was no significant difference in the recovery of TM and PC in experiment I, while the mean germination time in experiment II, with photoperiod 1, stood out at 3.84 days.

At the end of each germination test, the seeds that had not germinated in all treatments were washed and placed to germinate on filter paper moistened with distilled water. In fact, there was an increasing response in the germination test up to the maximum value of 82%

at the osmotic potential of -0.9 MPa (Figure 3).

These results suggest that the low values of germination previously observed were observed due to the damage caused to the seeds after the application of severe levels of water stress, showing great sensitivity of the seeds to the levels tested. A similar result was found by Silva Belloet al. (2008), the only work performed using this experimental methodology, but evaluating the germination performance of *Amburanaaccreana* seeds under different temperature and water stress conditions.

The results observed in the present experiment created the demand of the

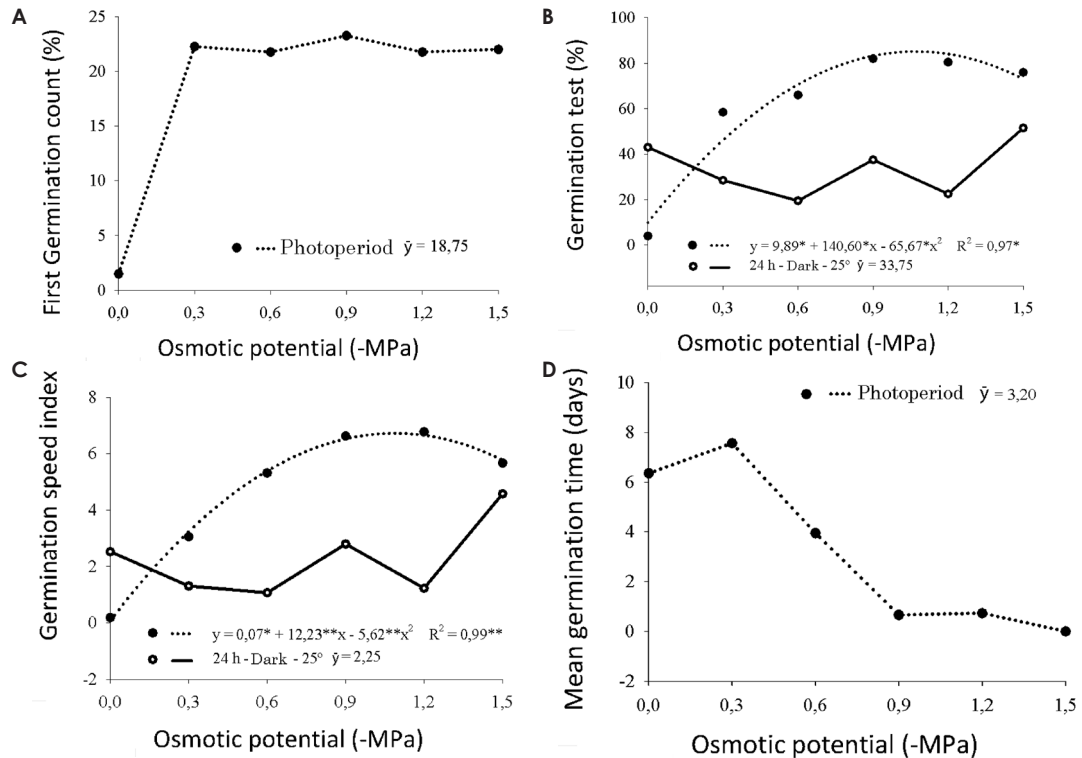


Figure 3. First germination count (A), germination test (B), germination speed index (C) and mean germination time in days (D) of *A. deflexus* seed recovery under different levels of water stress in germination chamber under two light conditions.

adoption of lower levels of stress, aiming to observe a better configuration of the curve and understanding about the extent to which the seeds are influenced by the levels of stress. These responses were obtained in experiment 2.

In experiment 2, even using lower levels of

stress, low seed germination rates were observed during the 14 days in the photoperiod of 12 light hours, reaching a maximum of 13.5 and 7.5% from the third to the sixth DAS, in the treatments of 0 and -0.1 MPa, respectively (Figure 4A).

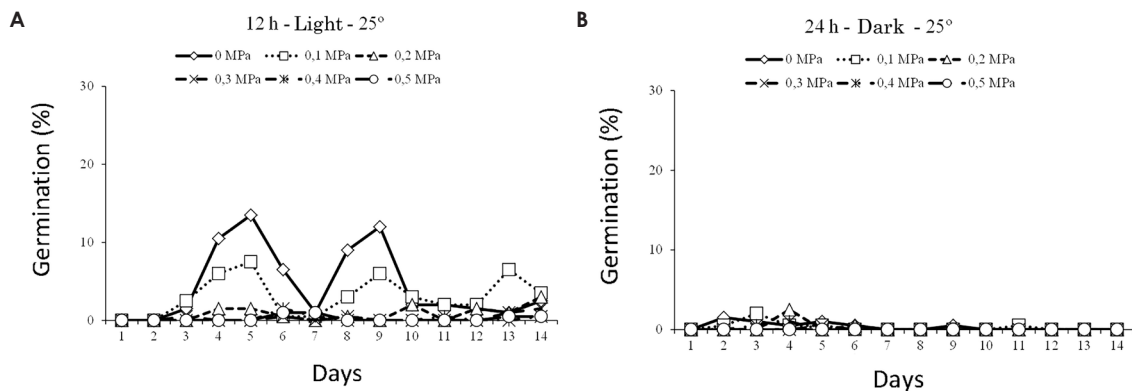


Figure 4. Evolution of germination of *A. deflexus* seeds at different levels of water stress in germination chamber under two light conditions.

Teixeira et al. (2011) observed that osmotic potentials equal to or lower than -0.2 MPa were detrimental to seed germination of *Crambe abyssinica* Hochst, with no normal seedling development at potentials lower than -0.6 MPa. Campos & Assunção (1990) reported that increasing the concentration of osmotic solutions is responsible for inhibition of the synthesis and /

or activity of the hydrolytic enzymes necessary for seed germination. The results of the application of this methodology in populations of *A. deflexus* were not verified in technical-scientific literature; however, the expression of the characteristic in this work corroborated that observed by Teixeira et al. (2011).

In experiment 2, similarity was observed

to what was verified in experiment 1, that is, in PC, TG and IV the analysis of variance showed significant effects ($p < 0.01$) in relation to the combined effect between water stresses and photoperiods in the seeds of *A. deflexus*, with no significant effect of the interaction between water stress and photoperiod in the mean

germination time (Table 1).

In PC, a significant difference was verified only for the potentials -0.1 and -0.2 MPa of water stress. In PC and IV without water stress, the photoperiod 12 h of light per day was highlighted, with 12% and 4.88, respectively (Table 4).

Table 4. First germination count (PC), germination test (TG), germination speed index (IV) and their respective recoveries, submitted to water stress and light conditioning (F1 = 12 light hours and F2 = absence of light) in Experiment II with *A. deflexus* seeds tested in a germination chamber under two light conditions.

Water stress (MPa)	PC II		TG II		IV II	
	F1	F2	F1	F2	F1	F2
0	12a*	3a	5a	3.5b	4.88a	0.23b
-0.1	8.5a	3b	43a	4b	2.80a	0.13b
-0.2	1.5a	2.5b	11a	3b	0.72a	0.35b
-0.3	0a	0.5a	4a	0.5b	4.41a	0b
-0.4	0a	1.5a	3a	1.5b	3.38a	0b
-0.5	0a	0a	3a	0b	3.54a	0.0b
Standard error	0.12		0.004		0.08	
Recovery Experiment II						
0	1a	0a	8a	1.5a	0.61a	0.10 ^a
-0.1	0a	0a	12a	1.5a	0.82a	0.09 ^a
-0.2	11.5a	0b	25.5a	0b	1.74a	0b
-0.3	17a	0b	38a	0b	3.04a	0b
-0.4	11a	0b	30a	0b	3.38a	0b
-0.5	10a	0b	36.5a	0b	3.54a	0b
Standard error	0.08		0.01		0.14	

* Averages followed by the same letter in the row do not differ by the Tukey test at 5% probability.

In recovery experiment II, there was no significant difference between the mean PC, TG and IV between photoperiods in the treatments of 0 and -0.1 MPa. Photoperiod 1 (F1), that is, 12 hours of daily light, was highlighted in PC and TG with 17% and 38% in the potential of -0.3 MPa; and the IV in F1 (3.54) at the potential of -0.5 MPa, respectively.

Even after reducing the levels of water stress, PC, TG and IV (Figure 5), in both treatments (12h and total darkness) were affected. By adjusting the response function for the 12-hour photoperiod, a decreasing behavior was observed up to values of 0.5%; 4% and 0.17 in the osmotic potential of -0.3 MPa, being noticed soon after a stabilization of the behavior until the last level tested. Germination is one of the most critical stages during the plant life cycle, and this water stress condition causes a reduction in physiological quality.

This occurs not only in the seeds of *A. deflexus*, but also in other important weeds, such as: *Conyza canadensis* L. and *Conyza bonariensis* L. (Yamashita & Guimarães, 2010),

Urochloa ruziziensis (Macetto et al., 2013), *Raphanus raphanistrum* L. and *Senna obtusifolia* L. Irwin &. Barneby (Pereira et al., 2014) and *Urochloa brizantha* (Stapf) Webster (Christovam et al., 2015).

In the total dark, a linear decreasing PC and TG adjustment was observed. There was no adjustment of function-response in the total absence of light of the variables IV and TM, respectively.

Comparing the results of vigor at the first count (Figure 5A) with those of germination at the final count of the test (Figure 5B), it was observed through the trend curves and based on the coefficient of determination (R^2) that the seed vigor of *A. deflexus* was more affected than its germination and these results were maintained as the concentrations of the PEG solutions 6000, until reaching 0% germination at -0.5 MPa. This shows the need to determine a suitable level of hydration during the seed imbibition stage, so that it will allow the reactivation of metabolic processes, culminating in the growth of the embryonic axis (Marcos Filho, 2005).

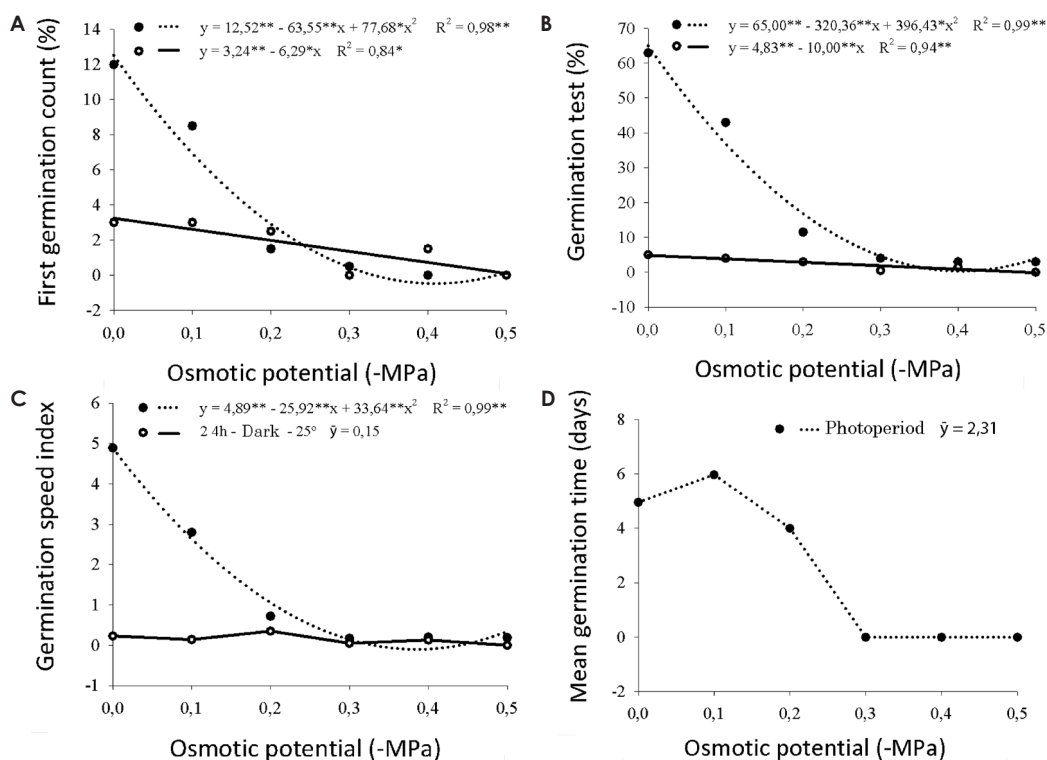


Figure 5. First germination count (A), germination test (B), germination speed index (C) and mean germination time (D) of *A. deflexus* seeds under different water stress levels in two germination conditions.

The increase in water deficit through the increase in PEG 6000 concentrations in the substrate solution was responsible for significant decreases in the mean values of the germination speed index (Figure 5C). Carvalho and Christoffoleti (2007), working with five species of *Amaranthus*, concluded that *A. deflexus* was the species with the lowest rates of germination in four germination conditions: photoperiod (8 hours light / 16 hours dark) with temperature alternation (8 hours at 30°C / 16 hours at 20°C), photoperiod with constant temperature (25°C), dark with alternating temperature and dark with constant temperature.

Therefore, the reduction of the osmotic potential of the substrate solution also influenced the germination, making it slower, especially in the photoperiod with 12h of light. Similar results were found by Rizzardi et al. (2009), who, working with PEG 8000 in the substrate solution in *Ipomoea triloba* L., observed that the germination was reduced with decreasing levels of water potential of the solutions, mainly from -0.1 MPa.

The germination behavior of *A. deflexus* seeds, observed in the present research, can be related to the reduction of the metabolic processes of the seeds, due to the lower

availability of water for the digestion of the reserves and translocation of the products; these processes were characterized by Bewley & Black (1994) by a three-phase germination pattern. According to these authors, water stress can reduce both the germination percentage and germination speed, with a great variation of responses between species, from very sensitive to the most resistant. Thus, resistant seeds have the ecological advantage of establishing seedlings in areas where seeds susceptible to drought cannot.

As in experiment 1, at the end of each germination test of experiment 2, the seeds that had not germinated, in all treatments, were washed and placed to germinate, in a filter paper moistened with distilled water. It was verified that they also re-germinated, corroborating the results of experiment 1 (Figure 6). According to Black & Bewley (1994), the reduction in germination can be attributed to lower mobilization of reserves, reduced synthesis and enzymatic activity or changes in cell turgor. In addition, when the seeds are subjected to stress conditions, they direct their metabolism to circumvent these conditions. Thus, the energy expenditure is greater in relation to the adaptation to this stress than the actual germination (Vaz-de-Melo et al., 2012; Oliveira et

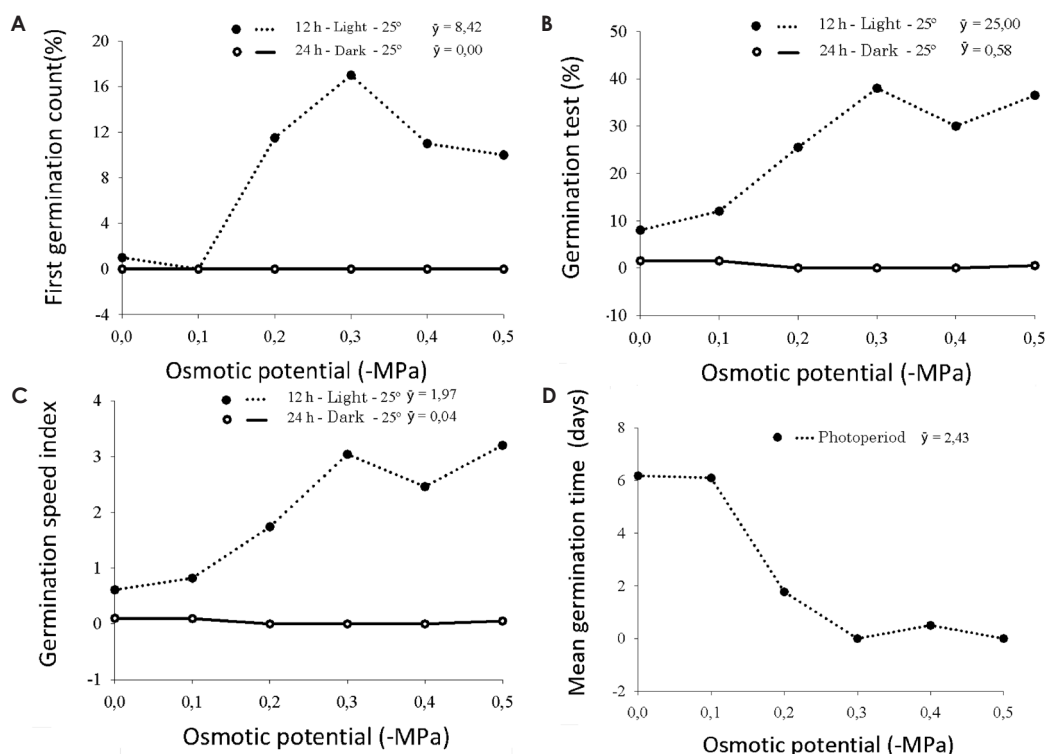


Figure 6. First germination count (A), germination test (B), germination speed index (C) and mean germination time (D) of *A. deflexus* seed recovery after different levels of water stress in a germination chamber under two light conditions.

al., 2018).

There was no function-response adjustment in any of the variables in the seed recovery (Figure 6), obtaining the mean values of PC, TG and IV in the absence of light of 0.00; 0.58% and 0.04 and in the 12-hour photoperiod of 8.42%; 25% and 1.97, respectively. The TM in the photoperiod was 2.43 days.

Conclusion

Water stress negatively affects the seed performance of *A. deflexus*, reducing germination and vigor from -0.1 MPa.

The germination of *A. deflexus* seeds was compromised by lack of light, regardless of the stress levels applied.

There was an increase in seed germination of *A. deflexus* after the recovery of the seeds at the highest levels of water stress.

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