

# Germination of *Amaranthus deflexus* L. seeds subjected to different temperature and salt-stress conditions

## Germinação de sementes de *Amaranthus deflexus* L. submetidas a diferentes condições de temperatura e estresse salino

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### Highlights

Osmotic potential affects the germination of *Amaranthus deflexus* seeds.

Light is necessary for *Amaranthus deflexus* to germinate.

*Amaranthus deflexus* seeds have a restricted temperature range.

Seeds resume normal germination once recovered from salt stress.

### Abstract

Weeds compete with crops for limiting factors in the environment in which they live; therefore, studies that demonstrate the germination response of seeds subjected to artificial stress are important to understand the survival and adaptation capacities of these species under natural stress conditions. This study proposes to evaluate the effect of salinity and thermal conditions on the germination and vigor of seeds of *Amaranthus deflexus* L. Two experiments were conducted with sowing in Petri dishes containing two sheets of filter paper moistened with distilled water (control) or NaCl solutions. In experiment 1, a completely randomized experimental design was adopted with a 2 × 6 factorial arrangement consisting of two photoperiods (12 h light and 24 h dark) and six levels of salt stress (0, -0.3, -0.6, -0.9, -1.2, and -1.5 MPa), totaling twelve

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treatments, with four replications. At the end of the test, the seeds that did not germinate were washed and placed on paper towel substrate, which was moistened with water for hydration and to stimulate the germination process. Experiment 2 was also laid out in a completely randomized design and involved eight treatments (germination at 20, 25, 30, 35, 40, 20/30, 25/35, and 30/40 °C) and four replicates each. Salt stress negatively affected the seed vigor of *A. deflexus* from the stress level of -0.3 MPa. The absence of light compromised seed vigor, regardless of the stress level applied. The final germination percentage and the germination speed index of *A. deflexus* seeds were superior at the constant temperature of 25 °C and in the alternating regime of 25-35 °C. The multivariate procedure discriminated treatments 1 and 2 as superior in dendrogram A, as well as 4, 5, and 6 in dendrogram B, making it a robust method for inference in factorial experiments on germination behavior.

**Key words:** Caruru rasteiro. Osmotic potential. Salinity. Vigor. Weed.

## Resumo

As plantas daninhas competem com as culturas por fatores limitantes do meio em que vivem, portanto, estudos que apontem a resposta germinativa de sementes submetidas a estresses artificiais são importantes para entender a capacidade de sobrevivência e adaptação destas espécies sob condições de estresse naturais. Objetivou-se neste trabalho avaliar o efeito das condições salinas e térmicas sobre a germinação e vigor de sementes de *Amaranthus deflexus* L. Foram instalados dois experimentos, com semeadura realizada em placas de petri contendo duas folhas de papel de filtro umedecidas com água destilada (controle) ou com soluções de NaCl. No experimento 1 utilizou-se o delineamento experimental inteiramente casualizado (DIC), em esquema fatorial 2 x 6, constituído de dois fotoperíodos (12h luz e 24h escuro) e seis níveis de estresse salino (0; -0,3; -0,6; -0,9; -1,2 e -1,5 MPa), totalizando doze tratamentos, com quatro repetições. Ao final do teste, as sementes que não germinaram foram lavadas e colocadas em substrato papel toalha, umedecido com água para hidratação e estimular o processo germinativo. No experimento 2 utilizou-se um DIC com oito tratamentos (germinação sob 20; 25; 30; 35; 40°C, 20/30; 25/35 e 30/40°C) e quatro repetições cada. O estresse salino afetou negativamente o vigor das sementes de *A. deflexus* desde o nível de estresse de -0,3 MPa. O vigor das sementes foi comprometido pela ausência de luz, independentemente dos níveis de estresse aplicados. Houve superioridade na percentagem final de germinação e o índice de velocidade de germinação das sementes de *A. deflexus* na temperatura de 25°C constante e no regime alternado de 25-35°C. O procedimento multivariado discriminou os tratamentos 1 e 2 como superiores no dendograma A, bem como 4, 5 e 6 no dendograma B, configurando-se como método robusto para inferência em experimentos fatoriais sobre o comportamento germinativo.

**Palavras-chave:** Caruru rasteiro. Planta daninha. Potencial osmótico. Salinidade. Vigor.

## Introduction

The species *Amaranthus deflexus* L., popularly known as "caruru rasteiro", is an important weed found virtually throughout

the Brazilian territory. Species of this genus greatly reduce agricultural yields when uncontrolled and increase production costs when in high population densities (M. L. M. D. Silva et al., 2016).

Weeds are characterized by competing with crops for the limiting environmental factors found in different soil-climatic conditions. Studies on the germination response of seeds subjected to artificial stress conditions constitute tools for a better understanding of the survival capacity and adaptation of these species under natural stress conditions (Pereira et al., 2012).

One of the main environmental factors that limit plant growth and development is salinity. The effects of excessive accumulation of soluble salts on plants can lead to physiological drought, caused by the decrease in osmotic potential; nutritional imbalance, due to a high concentration of ions, especially sodium, which inhibits the absorption of other nutrients; increased toxicity of specific ions; and interference with different physiological processes (Sousa et al., 2018). Responses are variable and directly related to the species, types of salts present in the water, as well as the intensity and duration of the salt stress to which the seed is subjected (Nóbrega et al., 2018).

Other environmental factors of fundamental importance for seedling emergence under field conditions are temperature ranges and light (E. M. Silva et al., 2019). Phytochromes are responsible for activating or inhibiting seed germination, and the sensitivity to light of a seed depends on the form of phytochrome present in the dry seed (Takaki, 2001). As regards temperature, there is an optimal range that allows for seed germination, and when the maximum temperature limit is exceeded, all processes involved in germination are interrupted. However, some species exhibit better germination parameters when subjected to

alternating temperatures, which correspond to the environmental conditions found in the natural environment (Ribeiro et al., 2016). Fontes et al. (2019) evaluated the influence of light and water stress on the germination behavior and vigor of *Amaranthus deflexus* and found that water stress negatively affects seed performance, reducing germination and vigor, whereas the absence of light completely compromised germination, regardless of the stress level applied. M. L. M. D. Silva et al. (2016) also reported that water stress was harmful at any level, regardless of temperature, for the germination of *Chorisia glaziovii*. In the species *Stigmaphyllon blanchetii*, the constant temperature of 30 °C and the alternating temperatures of 20-30 °C provided better germination and seedling development (E. M. Silva et al., 2019).

Due to the frequent variation observed in the salinity, thermal amplitude, and light factors, which cause seed germination responses to vary, predicting infestations of this species in different agricultural environments is a complex task. In view of this scenario, the present study was undertaken to examine the germination behavior of *Amaranthus deflexus* L. seeds under different salt stress and temperature conditions.

## Material and Methods

The experiments (E) were carried out at the Seed Analysis Laboratory of the Department of Plant Science at the Federal University of Ceará (UFC), in Fortaleza - CE, Brazil. *Amaranthus deflexus* L. seeds collected at the Rafael Fernandes experimental farm at the Federal Rural University of the Semi-Arid (UFERSA), in Mossoró - RN, were used.

In the first experiment (E1) for the germination test, sowing was carried out in Petri dishes lined with two sheets of paper towel (Germitest type) moistened with distilled water (control) or sodium chloride (NaCl) solutions so as to provide the water potentials of -0.3, -0.6, -0.9, -1.2, and -1.5 MPa, in a proportion equivalent to 2.5 times the weight of the dry substrate. The NaCl concentrations for each potential were obtained following Villela et al. (1991). The seeds were kept in Petri dishes with lids, which were sealed with Parafilm® (BRAND, Germany) to reduce moisture loss, and placed in a BOD germination chamber at 25 °C with a 12-h photoperiod of daily light and constant darkness for 14 days.

At the end of E1, the non-germinated seeds in each treatment were washed and placed to germinate in Petri dishes with paper towel (Germitest type) moistened with distilled water to stimulate the continuity of the germination process. The seeds were evaluated for germination and vigor, the latter of which is represented by the first germination count, by analyzing the following variables:

First germination count (FC) - conducted together with the germination test, by computing the percentage of normal seedlings on the fourth day after implementation of the test, as recommended by Ministério da Agricultura, Pecuária e Abastecimento [MAPA] (2009).

Germination test (GT) - carried out on the tenth day after sowing, at the end of the experiment, considering germinated seeds as those that produced primary roots. Results were expressed as a mean percentage based on the number of normal seedlings (MAPA, 2009).

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

$$GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{G_n}{N_n}, \quad \text{where } G_1, G_2, \dots,$$

$G_n$  correspond to the number of seedlings computed in the first, second, and last counts, respectively; and  $N_1, N_2, \dots, N_n$  correspond to the number of days from sowing to the first, second, and last count, respectively.

Mean germination time (MGT) - obtained from daily counts of germinated seeds up to the tenth day after sowing, and calculated using the formula described below, proposed by Labouriau (1983), with results expressed in days.

$$MGT = \frac{\sum(n_i t_i)}{\sum(n_i)},$$

in which MGT = mean germination time (days);  $n_i$  = number of germinated seeds in the interval between each count;  $t_i$  = time elapsed between the beginning of germination and the  $i$ -th count.

A completely randomized experimental design was adopted with four replicates of 50 seeds in a  $2 \times 6$  factorial arrangement. The first factor consisted of two photoperiods (12 h of light and no light) and the second consisted of five levels of salt stress (0, -0.3, -0.6, -0.9, -1.2, and -1.5 MPa).

In the second experiment (E2), the seeds were maintained at the constant temperatures of 20, 25, 30, 35, and 40 °C and alternating temperatures of 20-30, 25-35, and 30-40 °C. The evaluations were carried out daily after the implementation of the test, for a period of 14 days, when

the experiment ended. The counts were performed considering germinated seeds as those that produced a radicle longer than 2.0 mm (Steckel et al., 2004).

The experiment was laid out in a completely randomized design consisting of eight treatments (constant temperatures of 20, 25, 30, 35, and 40 °C and alternating temperatures of 20-30; 25-35 and 30-40 °C), and the same number of replicates in E1 was used.

For E1 and E2, analysis of variance (ANOVA) was performed using Snedecor's F test ( $p < 0.05$ ) to compare treatment variances. The post-ANOVA procedures applied were Tukey's test of multiple comparisons of means ( $p < 0.05$ ) for the first factor and fitting of curves of autoregressive functions for the second factor.

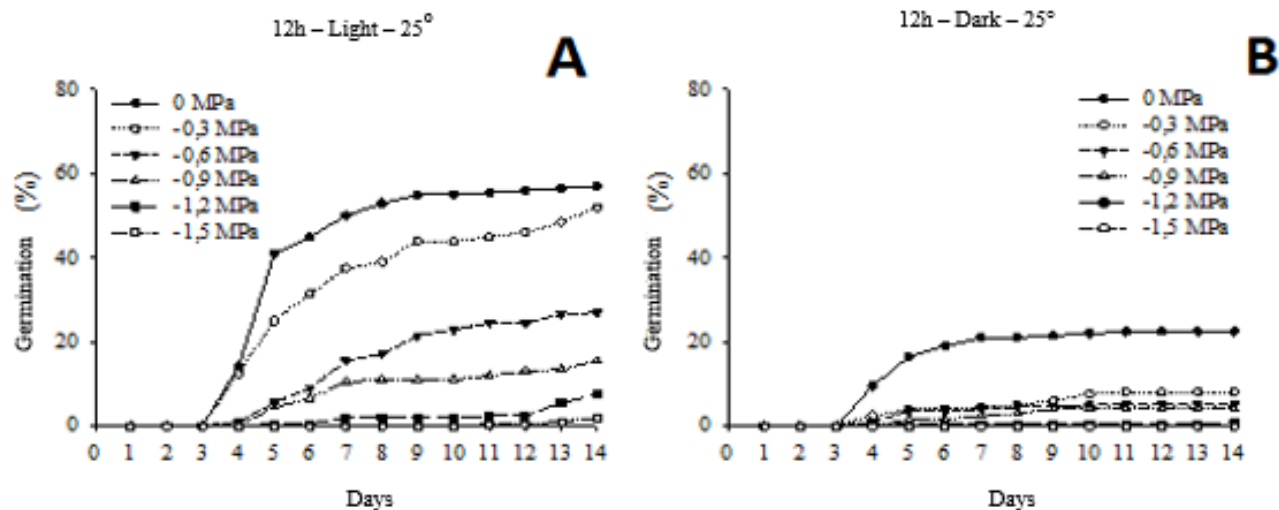
Using the estimated means of the variables, cluster analysis was performed by applying Ward's (1963) hierarchical method. Data were processed using R software version 3.2.0. (R Core Team [R], 2016).

## Results and Discussion

### First experiment

The germination rates of *A. deflexus* seeds over the 14 days in the 12-h-light photoperiod were low: 57 and 52% at the osmotic potentials of 0 and -0.3 MPa, respectively (Figure 1A). In the absence of light, the germination indices were even lower, with the highest percentage of 22.5% observed at the potential of 0 MPa. These results suggest that this species has a positive photoblastic nature, according to Labouriau (1983). The other osmotic potentials associated with the absence of light negatively affected germination (Figure 1B).

Pereira et al. (2012) found similar results in a study with salt stress in *Urochloa decumbens* and *Urochloa ruziziensis*. The authors reported a decrease in *U. ruziziensis* seed germination, with a 22.1% reduction at the potential of -0.2 MPa relative to the control. This difference became even greater: a 61% reduction in osmotic potential at -0.4 MPa. *Vigna unguiculata* seeds showed a germination percentage of less than 80% when cultivated under osmotic potentials lower than -0.35 MPa (Gomes et al., 2019). Water stress negatively affects the performance of *Amaranthus deflexus* seeds, reducing germination and vigor, just as the absence of light compromised germination for seeds of this species regardless of the stress level applied (Fontes et al., 2019).



**Figure 1.** Cumulative germination over time of *Amaranthus deflexus* L. seeds subjected to salt stress induced by NaCl under different osmotic potentials in the photoperiod 12h light/dark (A) and 24 h dark (B).

This reduction in germination parameters may be associated with the fact that when there is an increase in the concentration of ions in the substrate and, consequently, a reduction in osmotic potential, water absorption by the seeds is reduced and the germination phases are delayed (Santos et al., 2016). Salt stress can also trigger secondary effects, such as an accumulation of ions in the cytosol, which can have a cytotoxic action that leads to protein denaturation and membrane

destabilization, causing cell death (Taiz et al., 2017). Furthermore, the increase in the concentration of osmotic solutions in the substrate is responsible for inhibiting the synthesis or activity of hydrolytic enzymes that are essential in the germination process (Campos & Assunção, 1990).

Analysis of variance revealed significant effects ( $p < 0.01$ ) of the interaction between salt stress and photoperiods on the *A. deflexus* seeds in the FC, GT, GSI, and MGT tests (Table 1).

Table 1

Summarized values of analysis of variance for germination test (GT), first germination count (FC), germination speed index (GSI), and mean germination time (MGT) of *Amaranthus deflexus* L. seeds as a function of salt (I) and thermal (II) stress

SV	DF	<sup>1</sup> MS Experiment I				MS Recovery Experiment I			
		FC	GT	GSI	MGT	FC	GT	GSI	MGT
E	5	203.15**	1853.48**	18.35**	73.99 <sup>ns</sup>	414.73**	2011.08**	7.39**	41.31**
F	1	70.08*	4840.08**	30.78**	175.43**	1281.33**	9690.08**	41.02**	1.79 <sup>ns</sup>
E × F	5	34.08*	547.48**	4.80**	24.55*	335.73**	1043.08**	3.34**	5.29 <sup>ns</sup>
Error	36	11.36	58.25	0.49	9.17	31.94	71.53	0.57	6.02

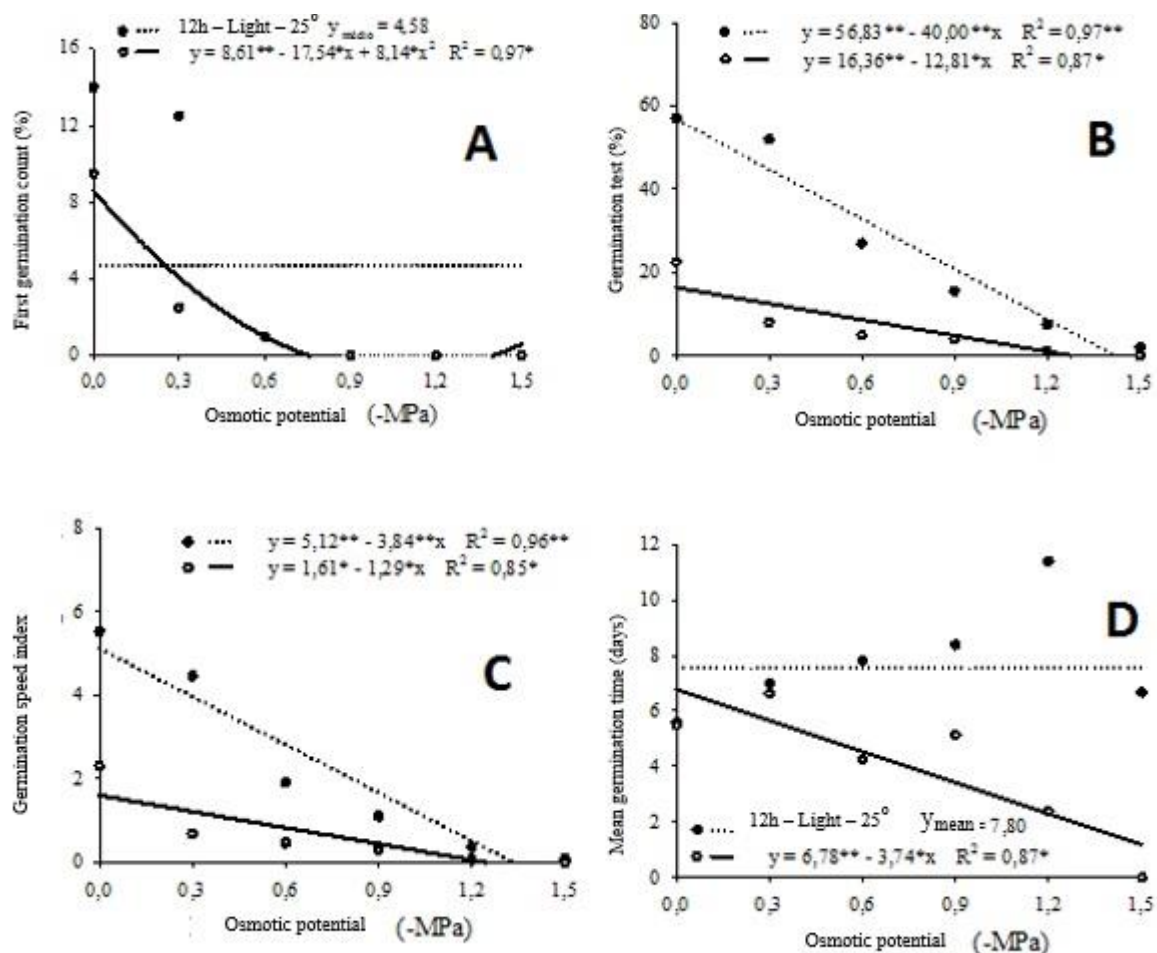
  

SV	DF	<sup>2</sup> MS Experiment II			
		FC	GT	GSI	MGT
Temp.	7	70.55**	2857.26**	12.98**	5.95 <sup>ns</sup>
Residual	24	5.04	123.12	0.67	6.24
Total	31	-	-	-	-
CV (%)	-	32.58	70.44	37.81	30.57

\*\* Significant by Snedecor's F test at 1% probability; <sup>ns</sup> Not significant. <sup>1</sup>Mean squares estimated in Experiment I; <sup>2</sup>Mean squares estimated in Experiment II.

A response function was fitted for FC, GT, GSI, and MGT in the absence of light, reaching 0% in the FC from the level of -0.9 MPa (Figure 2A). For GT, GSI, and MGT, there

was a linear decrease, indicating impairment in the performance of seeds subjected to salt stress from the level of -1.2 MPa (Figures 2B, C, and D).



**Figure 2.** Germination test (A), first count (B), germination speed index (C), and mean germination time (D) of *Amaranthus deflexus* L. seeds in the absence (● —) and presence (● ....) of 12 h of light per day, under different osmotic potentials obtained with NaCl.

In the photoperiod of 12 h of light, a response function was fitted for the variables of GT and GSI, which decreased up to 2% and 0.08, respectively, at the potential of -1.5 MPa (Figures 2B and C). These results corroborate those observed by Bandeira et al. (2018), who reported that salt stress negatively affected seed germination in *Senna obtusifolia*. This finding was already expected, since an increase in the concentration of salts in the substrate induces a reduction in water potential, resulting in a lower capacity

for water absorption by the seeds, which generally influences their germination capacity and seedling development (Oliveira et al., 2012).

For the variables of FC and MGT, there was no consistent fitting of the sampling distribution to any theoretical distribution. However, the increase in water deficit induced by the increase in NaCl concentrations in the solution was responsible for the trend towards a reduction in mean FC values in the 12-h photoperiod (Figure 2A).

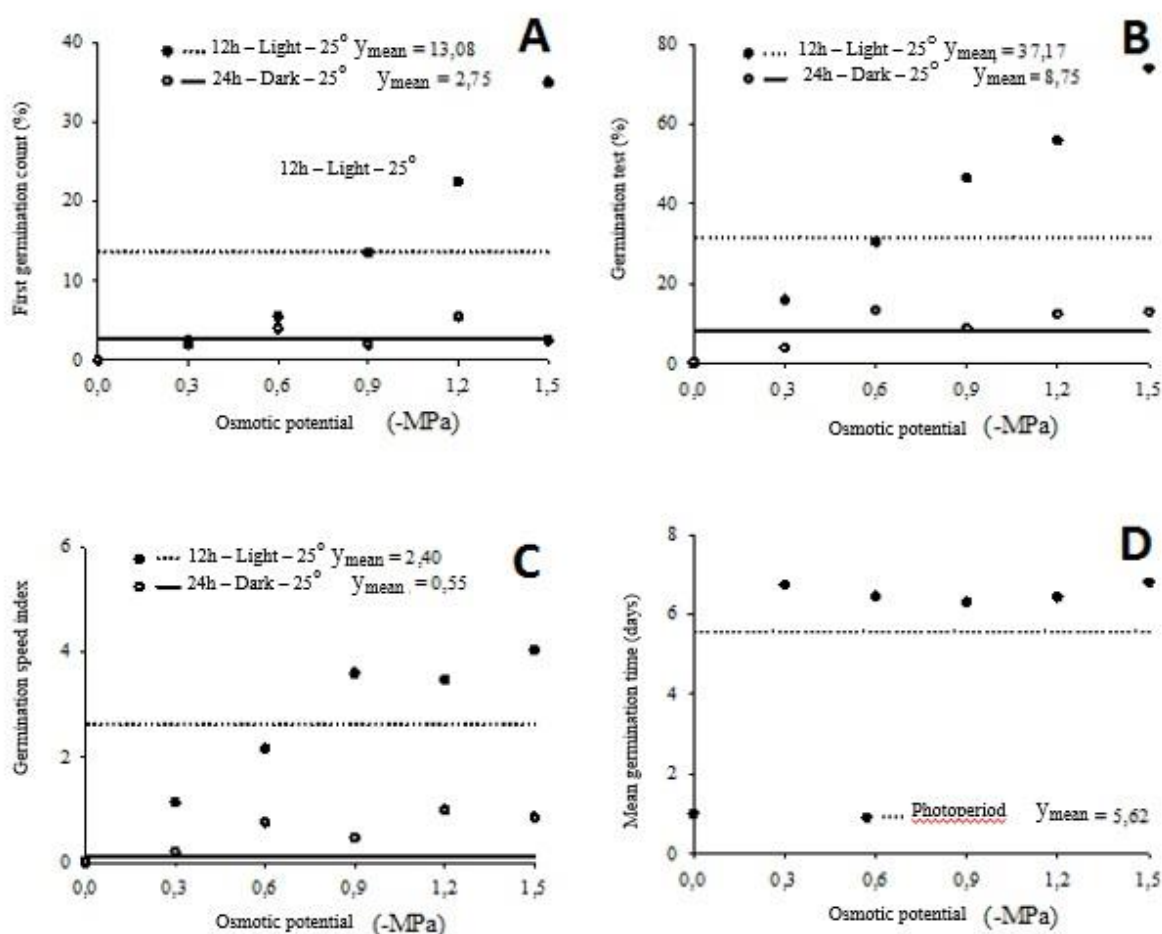


Inhibition of primary root emergence due to less water availability is often related to reductions in the activity of some enzymes, which impairs the general metabolism of seeds (Bewley & Black, 1994). In addition, when seeds are subjected to stress conditions, they direct their metabolism to circumvent these conditions. As a result, energy expenditure is greater for adaptation to this stress than germination itself (Bosco de Oliveira et al., 2018).

At the end of each germination test, the seeds that had not germinated in all

treatments were washed and set to germinate on a paper towel moistened with distilled water.

The sample distribution did not fit any theoretical distribution for the variables FC, GT, GSI, or MGT. However, the increase in water deficit provided by the increase in NaCl concentrations in the substrate solution was responsible for increasing the means of FC, GT, and GSI, which reached maximum values of 35, 74%, and 4.04 at the level of -1.5 MPa in the 12-h photoperiod, respectively (Figures 3A, B, and C).



**Figure 3.** Germination test (A), first count (B), germination speed index (C), and mean germination time (D) in the recovery of *Amaranthus deflexus* L. seeds in the absence (○ —) and presence (● ···) of 12 h of light per day, under different osmotic potentials obtained with NaCl.

These results indicate that the seeds returned to normal germination, suggesting that the inhibition of germination in the treatments was only due to salt stress. In the MGT test (Figure 3D), there was an 86% increase from the osmotic potential of 0 to -0.3 MPa, with germination time stabilizing soon afterwards at an average of 5.62 days.

In the absence of light, sampling distribution did not consistently fit any theoretical distribution for any of the studied variables. Nonetheless, there was a significant trend towards an increase in the mean values of FC, GSI, and GT, which reached the maximum values of 5.5% and 1 (FC and GSI, respectively) at the osmotic potential of -1.2 MPa and 13.5% (GT) at the potential of -0.6 MPa. These results are lower than those observed in the 12-h-light treatment, reinforcing the fact that this species has a positive photoblastic nature, according to Labouriau (1983). This character reveals only the quantitative response, since the seeds germinated both in the presence and absence of light. Carvalho and Christoffoleti (2007) examined five *Amaranthus* species and obtained lower germination rates in *Amaranthus deflexus* when it was subjected to four germination conditions: a photoperiod of 8 h of light and 16 h of dark with alternating temperatures (8 h at 30 °C/16 h at 20 °C); a photoperiod with constant temperature at 25 °C; dark with alternating temperatures; and dark with constant temperature.

Several authors, such as Buhler et al. (1995), Vidal and Bauman (1996), Pereira et al. (2012), and Zandoná et al. (2018), showed how the survival of species considered weeds may be related to non-germination of seeds in unfavorable environmental conditions,

characterizing ecological significance, as it prevents the development of seedlings in soils without sufficient resources to support subsequent growth. Thus, species whose seeds do not have this control mechanism could all germinate at the same time, after a short period of soil wetting, compromising the development of formed individuals and future generations (Van Den Berg & Zeng, 2006; Pereira et al., 2012).

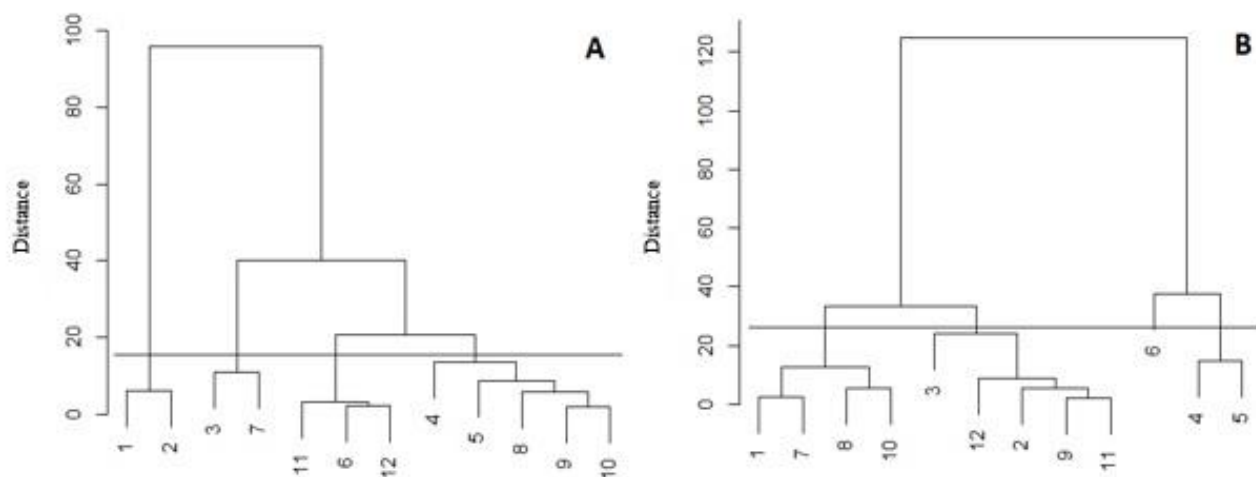
We evaluated the applicability of Ward's multivariate hierarchical clustering method (Figure 4) based on the mean values of the combinatorial effect of salt stress levels in the 12-h-light and 24-h-dark photoperiods (Table 2) to present the best combinations of treatments to respond to the set of variables that qualify the germination and perpetuation of the species, to the detriment of prioritization.

Among the four groups formed in dendrogram A, 1 and 2 (0 and -0.3 MPa) were the most similar and had the highest means of FC, GT, GSI, and MGT resulting from the combined effect of salt stress × photoperiods, defining group I (Table 2). The other groups formed were composed of treatments 3 and 7 (-0.6 and 0 MPa) (group II); 11 and 12 (-1.2 and -1.5 MPa) (group III); and 4, 5, 8, 9, and 10 (-0.9; -1.2; -0.3; -0.6 and -0.9) (group 4) (Figure 4A).

In the recovery experiment, four groups were also formed. Treatments 1, 7, 8, and 10 (0, 0, -0.3, and -0.9 MPa) defined group I and had the lowest germination recovery means (Table 2). The other groups formed were composed of treatments 3, 12, 2, 9, and 11 (-0.6, -1.5, -0.3, -0.6, and -1.2 MPa) (group II); 6 (-1.5 MPa) (group III); and 4 and 5 (-0.9 and -1.2 MPa) (group 4) (Figure 4B). The latter two

were responsible for the highest means of recovery of *A. deflexus* seeds regarding the variables of FC, GT, GSI, and TMG, reaffirming

what is illustrated in Figure 3 and suggesting the use of this multivariate technique in subsequent studies.



**Figure 4.** Ward's hierarchical clustering dendrograms for dissimilarity between treatments in the experiment with salt stress (A) and recovery (B).

**Table 2**

**Ward's hierarchical cluster analysis for first germination count (FC), germination test (GT), germination speed index (GSI), and mean germination time (MGT) for *Amaranthus deflexus* L. seeds as a function of salt stress in the photoperiods of 12 h of light and 24 h of dark**

Treat.	MPa	Experiment Mean				Recovery Experiment Mean			
		12 of light (Dendrogram A)				12 h of light (Dendrogram B)			
		FC	GT	GSI	MGT	FC	GT	GSI	MGT
1	0.0	14.00	57.00	5.52	5.56	0.00	0.00	0.00	0.00
2	0.3	12.50	52.00	4.46	6.98	2.00	16.00	1.64	6.51
3	0.6	1.00	27.00	1.91	7.81	5.50	30.50	3.21	5.63
4	0.9	0.00	15.50	1.11	8.38	13.50	46.50	4.81	5.66
5	1.2	0.00	7.50	0.37	11.39	22.50	56.00	7.05	4.88
6	1.5	0.00	2.00	0.08	0.00	35.00	74.00	9.91	0.00
		24 h of dark				24 h of dark			
7	0.0	9.50	22.50	2.31	5.47	0.00	0.50	0.03	2.00
8	0.3	2.50	8.00	0.68	6.62	2.50	4.00	0.49	5.63
9	0.6	1.00	5.00	0.47	4.25	4.00	13.50	1.34	6.13
10	0.9	0.00	4.00	0.31	5.13	2.00	9.00	0.84	5.48
11	1.2	0.00	1.00	0.07	2.38	5.50	12.50	1.21	6.50
12	1.5	0.00	0.00	0.00	0.00	2.50	13.00	1.15	0.00

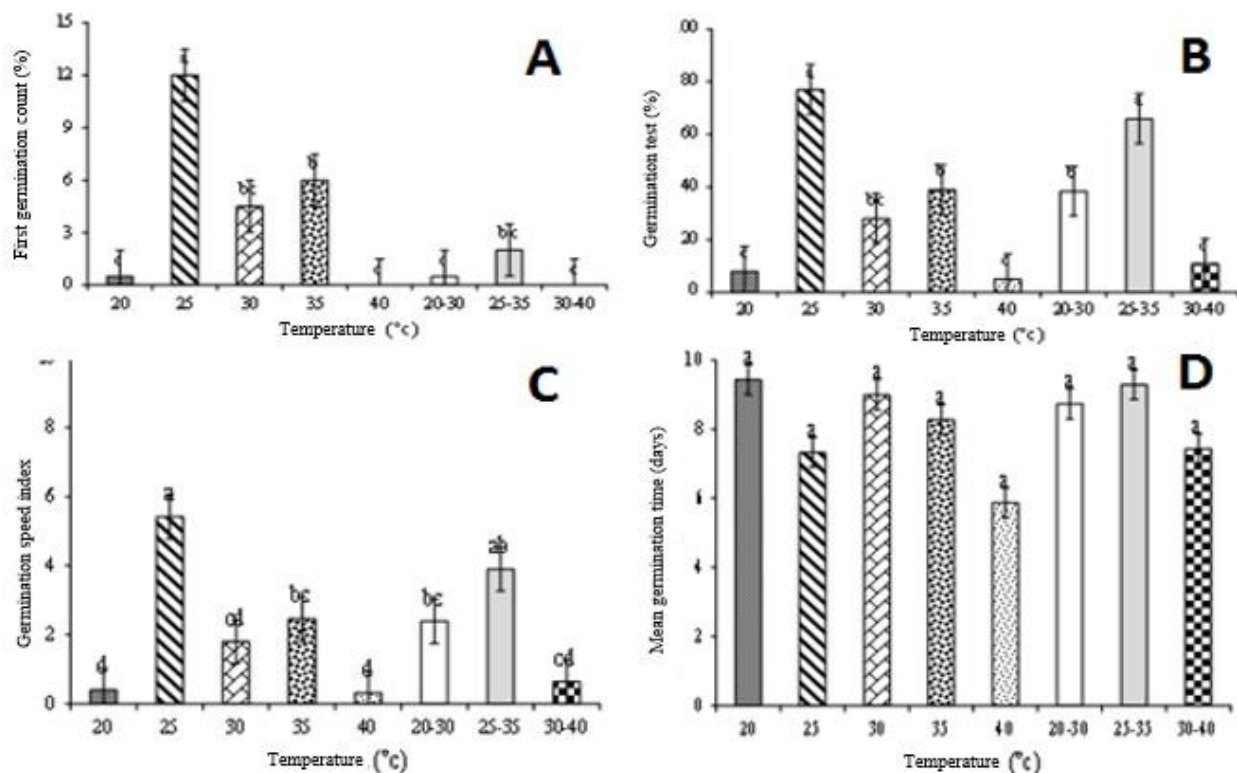
The feasibility of applying multivariate clustering methods is frequently investigated in agronomic experimentation (Cargnelutti et al., 2008). This test also made it possible to observe the response effect of the set of variables that qualify germination. Hierarchical methods are often used to discriminate groups of individuals with similar agronomic attributes. In this study, it was applied to discriminate combinations with similar mean scores and showed coherent and consistent results.

### Second experiment

In the FC, FT, and GSI tests, analysis of variance revealed significant effects ( $p < 0.01$ )

of the temperatures on the physiological performance of *A. deflexus* seeds (Table 1).

There was a significant difference between the tested treatments for FC (Figure 5A), with a highlight on the constant temperature of 25 °C, which provided the highest germination mean, with a 100% increase compared with the second best treatment (35 °C). This information allows us to understand the high levels of infestation of these species in Brazilian agricultural areas, especially in the northeast region, where the average temperature ranges from 23 to 27 °C, with high radiation rates (Instituto Nacional de Meteorologia [INMET], 2014). At a constant temperature of 40 °C and in the alternated regime of 30-40 °C, there was no variation in the germination of *A. deflexus* seeds.



**Figure 5.** First germination count (A), germination test (B), germination speed index (C), and mean germination time (D) of *Amaranthus deflexus* L. seeds under different thermal conditions.

For GT, the treatments also differed significantly (Figure 5B), with the constant temperature of 25 and alternating regime of 25-35 °C providing superior germination, which was 83.3% higher, on average, than that achieved with the treatment of 35 °C. Bandeira et al. (2018) worked with an important weed, *Senna obtusifolia*, and found that the germination percentage of the species is maximized at the temperature of 25 °C. For the species *Senna multijuga*, the temperatures of 30 °C (constant) and 20-30 °C (alternating) resulted in the highest germination percentage (Ribeiro et al., 2016). Wang et al. (2017) reported that the best temperatures for germination in the species *Digitaria sanguinalis* were between 25 and 35 °C. Wenneck et al. (2021) subjected *Amaranthus viridis* seeds to accelerated aging at a constant temperature of 42 °C and obtained a reduced germination potential of the seeds compared with those that were not subjected to this treatment.

The final germination of *A. deflexus* seeds was lower in the treatments of 20, 40, and 30-40 °C. For many species, as seen by Norsworthy and Oliveira (2007) in *Xanthium strumarium*, there is a relationship between thermal amplitude and light. For this species, that constant day and night temperatures were found to reduce the need for light for germination. On the other hand, when seeds are subjected to environments with varied thermal amplitude, e.g., 25/30 or 20/30 °C, the need for high light intensity is confirmed, which constitutes an indispensable condition for their germination to occur. In this sense, these adaptive characteristics provide greater temporal and spatial distribution in the germination of different species, allowing their survival in environments less conducive to their development (Zhou et al., 2005).

In the GSI test, there was a significant difference between the evaluated treatments (Figure 5C), with the temperatures of 25 and 25-35 °C standing out over the others with the indices of 5.41 and 3.89, respectively. The lowest GSI were again observed at the temperatures of 20, 40, and 30-40 °C (Figure 5C). Liu et al. (2017) evaluated the influence of temperature and light on the germination and emergence of *Geranium carolinianum* and found that the alternating temperatures of 15/20 °C and 20/25 °C (day/night) was the most appropriate to express the maximum potential germination of this species, regardless of light. Ataíde et al. (2016) found higher GSI in *Melanoxylon brauna* when grown at 30 °C. For the species *Stigmaphyllon blanchetii*, the constant temperature of 30 °C and alternating temperatures of 20-30 °C provided an increase in GSI and germination percentage (E. M. Silva et al., 2019).

There was no significant difference between the tested treatments for MGT according to analysis of variance (Table 1). However, the comfort zone for germination in *A. deflexus* seeds was clearly around the range of 25 to 35 °C, where it showed good results despite the lack of statistical difference, with a MGT of around eight days (Figure 5D). These results may explain the high competitive capacity of this species over tropical crops, which are usually cultivated in this temperature range throughout the cycle. In this respect, M. Silva et al. (2020) evaluated the effects of light and temperature on the germination of *Macroptilium lathyroides* seeds and described that they completed the germination process more quickly and uniformly when cultivated at constant temperatures between 20 and 30 °C and alternating temperatures of 15/25, 20/30,

and 25/35 °C, and that the temperature of 40 °C delayed this process.

Thermal stress has an influence on plant metabolism, as it has a differential effect on protein stability and enzymatic reactions. Heat stress increases membrane fluidity while cold stress reduces it, causing uncoupling of different multiprotein complexes. Heat or cold can also destabilize and disintegrate secondary DNA and RNA structures (Taiz et al., 2017). These factors may explain the low values found for the parameters evaluated at the temperature extremes in this experiment (20 and 40 °C).

Overall, possibly due to the high adaptation of weeds to hot and dry climates, high temperatures can favor the germination of certain species, as in cold and humid climates, as observed in natural conditions. In the case of the species *A. deflexus*, thermal amplitude was restricted, with no adaptations to the studied extremes.

## Conclusions

Salt stress negatively affected seed vigor in *A. deflexus* from the stress level of -0.3 MPa.

Total darkness compromised the vigor of *A. deflexus* seeds, regardless of the stress level applied.

The final germination percentage and the germination speed index of *A. deflexus* seeds were superior at a constant temperature of 25 °C and in the alternating regime of 25-35 °C.

The multivariate procedure discriminated the combinations referring to treatments 1 and 2 as superior in dendrogram

A, as well as 4, 5, and 6 in dendrogram B, making it a robust method for inference in factorial experiments on the germination behavior of weed species.

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