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Seed germination in *Tephrosia egregia* Sandwith (Fabaceae), a species native to the brazilian Caatinga ecoregion with potential for recovery of degraded areas

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ABSTRACT – (Seed Germination in *Tephrosia egregia* Sandwith (Fabaceae), a species native to the Brazilian Caatinga Ecoregion with Potential for Recovery of Degraded Areas). *Tephrosia egregia* Sandwith is a species that develops in some States of the Northeast region of Brazil. It is propagated by seeds and has considerable potential for restoration. Thus, studies related to seed germination are important for the species. The aims were to characterize the post-seminal development and evaluate the germination response of the seeds under different temperatures and water stress and salt stress. Germination tests were performed at 20, 25, 30 and 20-30 °C and under PEG 6000 and NaCl concentrations (-0.2, -0.4, -0.6 and -0.8 MPa). The germination test should be conducted in a paper roll at 25 ºC for 14 days. Seed germination declines from the water potential of -0.2 MPa on; the species is sensitive to water stress. Under salt stress, there is more accentuated decline in germination as from -0.4 MPa, and this is an indication have a certain tolerance to salinity.
Keywords: anil-bravo, effect of salinity on germination, germination test, water stress

RESUMO – (Germinação de sementes de *Tephrosia egregia* Sandwith (Fabaceae), uma espécie nativa da Caatinga brasileira com potencial para recuperação de áreas degradadas). *Tephrosia egrégia* Sandwith é uma espécie que se desenvolve em alguns Estados do Nordeste. Sua propagação é realizada por meio de sementes, sendo uma espécie potencial para restauração. Os objetivos foram caracterizar o desenvolvimento pós-seminal e avaliar a germinação das sementes sob diferentes temperaturas e sob estresse hídrico e salino. Testes de germinação foram conduzidos a 20, 25, 30 e 20-30 °C e sob concentrações de PEG6000 e NaCl (-0.2, -0.4, -0.6 e -0.8 MPa). O teste de germinação deve ser conduzido em rolo de papel sob temperatura constante de 25 ºC durante 14 dias. A germinação das sementes é reduzida a partir do potencial hídrico de -0.2 MPa, sendo a espécie sensível ao estresse hídrico. Sob estresse salino, a redução mais acentuada na germinação, a partir de -0.4 MPa, sendo isso um indicativo de uma certa tolerância à salinidade.
Palavras-chave: anil-bravo, efeito da salinidade na germinação, estresse hídrico, teste de germinação

Introduction

The Caatinga is a biome endemic to Brazil in the semi-arid region of the country, a region with extensive periods of little rainfall (Silva *et al.* 2017). Its flora is abundant and diverse, with many endemic species (Queiroz *et al.* 2019). However, it has sustained intense human activities that have required the removal of natural vegetation (principally by the establishment of crops) and soil displacement (principally by the construction industry) (Carvalho *et al.* 2012, Schulz *et al*. 2016, Queiroz *et al.* 2020).

In addition, the seasonal climate is another factor that affects the native vegetation of these regions (Ferreira *et al.* 2016). The low rainfall and high temperatures in the

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region result in low fertility soils with high salinity (Santos *et al.* 2018), which can hurt or inhibit the establishment and development of species sensitive to these conditions.

The native species found in degraded and anthropized areas are potential sources for recovery of these areas (Carvalho *et al.* 2020). In addition, another important element for success in a rehabilitation plan is the use of soil recovery species, such as leguminous plants (Fabaceae). These species promote soil cover, have considerable biomass production, and assist in nutrient cycling and incorporation of atmospheric nitrogen, which contributes to soil recovery (Pereira *et al.* 2013).

Tephrosia egregia Sandwith (Fabaceae) is a species endemic to Brazil, popularly known as *anil-bravo*, which occurs in the Caatinga and Atlantic Forest phytogeographic domains (BFG 2018). It is mainly in regions where the soils are considered sub-saline (Bolland 1947). Seeds are the means of its propagation (Moreira & Bragança 2011), and it is therefore a species of considerable potential for recovery of degraded areas of the Caatinga.

In this context, the use of good quality seeds is fundamental, and germination capacity is a determining characteristic for the seed to be valuable for sowing. Thus, studies regarding seed germination are fundamental for sustainable production of seeds and seedlings of species native to the Caatinga, both for preventing loss of biodiversity (Dantas *et al.* 2014, Silva *et al.* 2014) and for successful propagation.

The seed germination process is directly affected by environmental factors, such as humidity, temperature, and light (Oliveira *et al.* 2015). Temperature acts on the speed at which water is absorbed by seeds and also on biochemical reactions that act in the process, and this is directly reflected in the percentage, speed, and uniformity of germination (Carvalho & Nakagawa 2012, Marcos Filho 2015). Seeds have variable responses to this factor, depending on the species. Cardinal temperatures for germination of a given species are defined as base, optimum, and maximum (Carvalho & Nakagawa 2012). The maximum germination percentage and germination speed occur under optimum temperature, whereas the maximum and base temperatures are defined as the temperature limits at which germination still occurs, that is, the temperature is not considered lethal for the seed (Dürr *et al.* 2015). The range from 20 to 30 °C proves to be adequate for the germination of a large number of subtropical and tropical species (Piña-Rodrigues *et al.* 2015).

Seed germination capacity is evaluated by the germination test, conducted in a laboratory under ideal conditions for the species, so as to indicate the maximum germination potential of a seed lot (Brasil 2009) to know its value for sowing.

For *T. egregia*, which is a species of the herbaceous stratum of the native flora of the Caatinga biome, with potential for plant cover in degraded areas, there is no information regarding the most suitable conditions for germination. Thus, the aims of this study were to characterize the post-seminal development of *T. egregia* and evaluate the germination response of the seeds under different temperatures to define the most suitable conditions for germination and the effects of water and salt stress on germination of *T. egregia*.

Materials and methods

The study was conducted in the Seed Analysis Laboratory of the Department of Agronomy of the Universidade Federal de Viçosa in Viçosa, MG, Brazil.

We used *Tephrosia egregia* Sandwith seeds obtained from pods collected from the municipalities of Mata Grande, AL, more specifically at the following coordinates and on the corresponding date: 9°06'07.2"S and 37°39'32.7"W, 9°03'15.1"S and 37°45'44.9"W, and 9°04'04.6"S and 37°44'58.0"W in November 2018. The pods were collected when they were straw-colored and beginning to open. The experiments were installed after the harvest.

This material was taken to the Seed Analysis Laboratory of the Department of Agronomy of the Universidade Federal de Viçosa. The seeds were manually removed from the pods.

Effect of temperature on germination of *T. egregia* seeds

Trial 1 – The seeds were distributed on two sheets of germitest (germination testing) paper moistened with water in the amount of 2.5 times the weight of the dry paper and covered with one more sheet, creating rolls that were kept in seed germinators regulated to temperatures of 20 ºC, 25 ºC, 30 ºC, and 20-30 ºC (16 hours at the temperature of 20 ºC and 8 hours at the temperature of 30 ºC).

Daily counts were made of the number of normal seedlings up to stabilization of the counts so as to obtain the cumulative germination curve of the seeds under different temperatures. For that purpose, the mean of the germination percentage (% of normal seedlings) was calculated daily, based on these data.

Trial 2 – Four replications of 50 seeds were used in this trial, under two forms of sowing: 1. on paper – the seeds were distributed on two sheets of germitest paper moistened with water in the amount of 2.5 times the weight of the dry paper in gerboxes (plastic germination boxes) and 2. in rolls – the seeds were distributed on two sheets of germitest paper moistened as described above and covered with one more sheet, creating rolls of paper. After that, the two forms of sown seeds were placed in seed germinators regulated to the following temperatures: 20 ºC, 25 ºC, 30 ºC, and 20-30 ºC (16 h at 20 ºC and 8 h at 30 ºC every 24 h), with 8 h (light) photoperiod.

Daily counts were made up to stabilization of the count so as to define the period necessary for seed germination. The number of normal seedlings were calculated, determining the germination percentage (% of normal

seedlings), the germination speed index (GSI), and the germination speed (GS).

Effect of water stress and salt stress on germination of *T. egregia* seeds

Trial 3 – in this third trial, the osmotic agents polyethylene glycol (PEG 6000) and sodium chloride (NaCl) were used to simulate water stress and salt stress, respectively, at the potentials of 0.0 (distilled water), -0.2, -0.4, -0.6, and -0.8 MPa.

The PEG solutions were prepared according to Villela *et al.* (1991). The salt concentration to prepare the saline solution was calculated through the van Hoff equation (Hillel 1971):

$$\Psi_s = - R \times T \times C \times i$$

where Ψ_s = osmotic potential, R = ideal gas constant: 8.314 J/mol, C = salt concentration in mol/L, and i = isotonic constant of NaCl: 2.

The seeds were distributed on two sheets of germitest paper moistened with the PEG 6000 and NaCl solutions at the respective osmotic potentials that were established, and distilled water for the control, in the amount of 2.5 times the weight of the dry paper. The seeds, covered with one more sheet of paper, were formed into rolls. They were then placed in seed germinators at the constant temperature of 25 ºC.

Daily counts were made for 15 days of number of normal seedlings, calculating the germination percentage (% of normal seedlings), the germination speed index (GSI), and the seed germination speed (GS).

The germination speed index (GSI) was calculated according to the following formula (Maguire 1962).

$$GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \cdots + \frac{G_n}{N_n}$$

where G_1, G_2, and G_n are the number of germinated seeds on the day of observation; and N_1, N_2, and N_n are the number of days after sowing.

The germination speed (GS) in days was calculated according to the following formula (Souza & Varela 1989);

$$GS = \frac{(N_1 \, x \, G_1) + (N_2 \, x \, G_2) + \cdots + (N_n \, x \, G_n)}{G_1 \; + \; G_2 + \cdots + \; G_n}$$

where GS is germination speed (days); G_1, G_2, and G_n are number of normal seedlings counted in the first, second, and last count; and N_1, N_2, and N_n are the number of days from sowing to the first, second, and last count.

Post-seminal development was monitored daily during the seed germination process, characterizing the different seedling phases. The criteria for classification of seedlings as normal and abnormal was according to Brasil (2009).

The experiments were installed in a completely randomized design, with four replications of 50 seeds. Analysis of variance was performed on the data on germination percentages, GSI, and GS under different temperatures; and the mean values were compared by Tukey's test at 5% probability (Zar 1996). The data of the different stresses (water and salt) were interpreted by regression analysis, the model for which was chosen based on the significance of the regression coefficients through use of the "t" test at 1% probability, on the coefficients of determination, and on the biological phenomenon using the SYSTAT 8.0 program for Windows®.

Results and discussion

The *Tephrosia egregia* Sandwith seeds (figure 1 a) rapidly soak up water from the substrate (figure 1 b); thus, radicle emergence begins from three to six days after sowing (figure 1 c). The hypocotyl-radicle axis is sinuous, and at this stage, the root collar and root are not clearly defined (figure 1 d). The greenish cotyledons emerge from the seminal remains, exhibiting the cotyledonary auricular sinus (figure 1 f, g). In the normal seedling, the primary root is observed, with or without secondary roots; the hypocotyl is cylindrical, green, slender, and has hirsute-pilose indumentum from the middle third up to the point of connection with the cotyledons. When the cotyledons are fully expanded, the convex surface is exposed or they undergo rapid abscission from the hypocotyl, which clearly shows phanero-epigeal germination. The epicotyl raises the eophyll, which comprises a pair of leaves, with multiple leaflets, paripinnation, and well-defined central venation (figure 1 h).

The normal seedling should have all its essential structures present and healthy, according to Brasil (2009). When this does not occur, we have abnormal seedlings, which exhibit some deformity or anomaly that impedes their complete development until giving rise to plants. The most frequent types of abnormalities found during the *T. egregia* germination process are necroses in the hypocotyl and/or primary root (figure 1 i, j, k), as well as absence or atrophy of the primary root, and poorly developed hypocotyl, which is short and thick.

Trial 1 and 2 – Effect of temperature on germination of *Tephrosia egregia* seeds

Detailed study on the germination of *Tephrosia egregia* seeds under different temperatures is shown in Figure 2. At the temperature of 20 ºC, the germination of the seeds began at approximately 11 days after sowing, with an increase in germination from that point, reaching the maximum value of 4% at 13 days after sowing, and stabilizing from then on.

At 25 ºC, germination began on the sixth day after sowing and increased progressively over time, reaching the value of

Figure 1. Initial phases of the post-seminal development of *Tephrosia egregia* Sandwith. a. Dry seed. b. Soaked seed. c-h. Beginning of germination up to formation of a normal seedling. i-k. Abnormal seedlings.

80% at 12 days after sowing, stabilizing by the thirteenth day of evaluation, with an increase of 8%, thus achieving 88% germination on the fourteenth day after sowing (figure 2). In the same Figure, we noted that at the temperature of 30 ºC, the seeds began the germination process on the third day after sowing, that is, the process was faster than at 20 ºC and at 25 ºC, reaching the value of 65% at 7 days and 69% at 14 days.

At the alternating temperature of 20-30 ºC, the seeds began their germination on the sixth day after sowing (figure 2), similar to that observed at the temperature of 25 ºC, and achieved maximum value at 13 days with 89%, with stabilization of the process on the fourteenth day of evaluation (figure 2).

There was stabilization in the germination process as of the thirteenth day after sowing for the temperatures of 20 ºC, 30 ºC, and 20-30 ºC, with a small increase for 25 ºC, which stabilized as of the fourteenth day of observation. This shows that the germination test of *T. egregia* seeds can be ended at 14 days after sowing without compromising the results (figure 2).

In summary, the germination process was slower at the temperature of 20 ºC, whereas at the temperature of 30 °C, the germination speed was greater. The best response at this temperature may be related to the adaptation of *T. egregia* to the environmental conditions of Caatinga and Atlantic Forest (BFG 2018), indicating tolerance to high temperature. In many Caatinga species, seed germination occurs under high temperatures (30 to 35 °C) (Dantas *et al.* 2020).

Temperature directly affects the speed and percentage of seed germination, because it alters the speed of water absorption and of the biochemical and enzymatic system reactions, which require specific thermal conditions to occur (Bewley *et al.* 2013). At lower temperatures, imbibition, enzymatic activity, and metabolism of the seeds are reduced, leading to decreases in the speed and percentage of seed germination (Marcos-Filho 2015). At higher temperatures, the biochemical/physiological processes occur at greater speed, contributing to greater germination speed. However, the high temperatures result in denaturation of enzymes that are important for the metabolic process and that of the antioxidant systems, adding to the number of abnormal seedlings (Flores *et al.* 2014, Matos *et al.* 2014, Ataíde *et al.* 2016).

Maximum germination of the seeds was obtained at 14 days after sowing at the temperature of 25 ºC and of 20-30 ºC (figure 2). The alternating temperature of 20-30 ºC

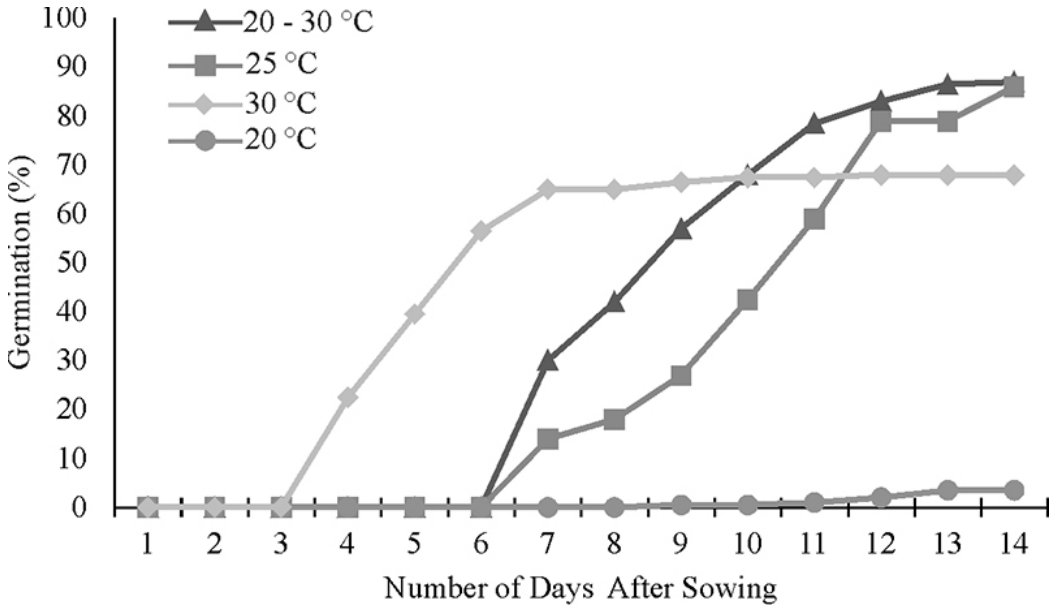

Figure 2. Cumulative germination percentages of *Tephrosia egregia* Sandwith under different temperatures.

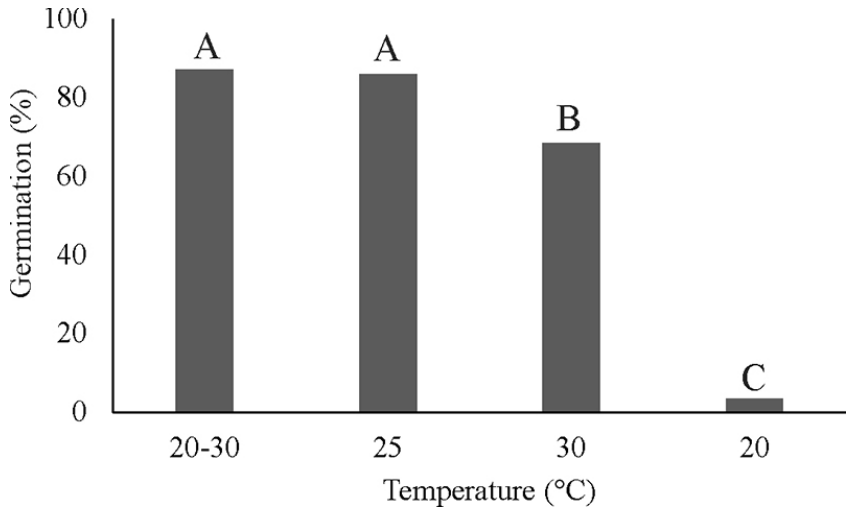

Figure 3. Germination of *Tephrosia egregia* Sandwith under different temperatures.

was also recommended for seed germination of *Caesalpinia pyramidalis* Tul. (Lima *et al.* 2011), *Schizolobium parahyba* (Vell.) Blake (Souza *et al.* 2012), and *Senna uniflora* (Mill.) HSIrwin & Barneby (Carvalho *et al.* 2020).

As sowing on paper led to considerable tangling of the roots, creating difficulties for evaluation, the choice was made to present the data only from the experiment conducted in rolls of germitest paper, and this manner of sowing was defined as most adequate for conducting germination tests with *T. egregia* seeds.

Evaluation of the effect of temperature on seed germination shows that the highest germination values were obtained at the constant temperature of 25 ºC and alternating temperature of 20-30 ºC, which did not differ from each other and were superior to the values obtained at 30ºC and 20 ºC (figure 3). At the temperature of 20 ºC, germination was very low (3%), which indicates great sensitivity of the *T. egregia* seeds to temperatures below 25 ºC, since at that temperature (25 ºC), the seeds achieved 86% germination. At 30 ºC, germination was significantly superior to that observed at 20 ºC. Comparing the absolute values, the seeds of this species are much more tolerant to higher temperature (above 25 ºC) than to lower temperature (below 25 ºC, that is, 20 ºC).

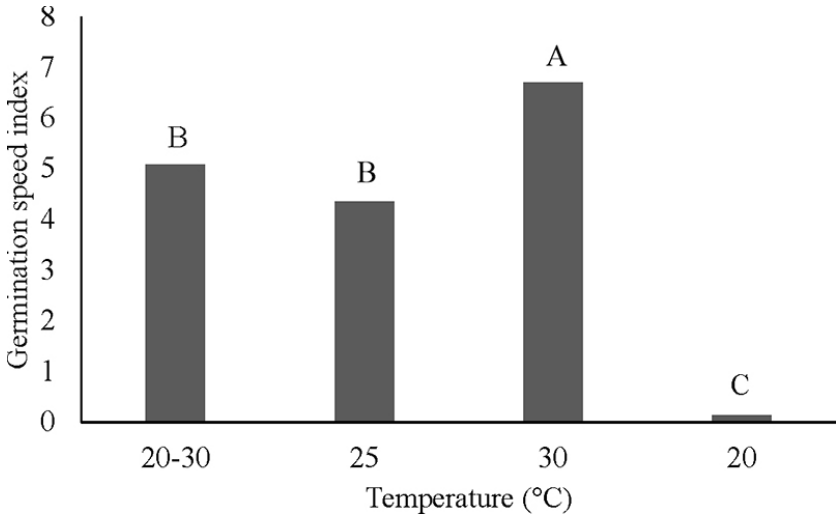

Figure 4. Germination speed index (GSI) of *Tephrosia egregia* Sandwith under different temperatures.

For Brazilian native species, the optimum germination temperature is between 15 °C and 30 °C, which is normally related to the temperatures of the region of origin of the species in the season favorable for germination (Andrade *et al.* 2000). Thus, there are species whose germination process is favored by constant temperature (Guedes *et al.* 2010, Socolowski *et al.* 2010, Benedito *et al.* 2017, Guo *et al.* 2020), by alternating temperature (Souza *et al.* 2012, Carvalho *et al.* 2020), and by a wide interval of temperature (Ferraz *et al.* 2012, Lemes & Lopes 2012). In the case of *T. egregia* seeds, the temperatures of 25 ºC or 20-30 ºC were suitable for germination.

In the Rules for Seed Analysis (Brasil 2009) for *T. candida*, the use of alternating temperature of 20-30 ºC or constant temperature of 30 ºC is recommended, partially corroborating that observed in our study for *T. egregia*. Whereas the constant temperature recommended for *T. candida* is 30 ºC, the ideal observed for *T. egregia* is 25 ºC.

Nevertheless, it should be emphasized that for the germination test conducted in the laboratory, when possible, the use of constant temperatures is preferrable to alternating temperatures, to more easily carry out the test. Alternating temperatures should be used when the species shows problems for germination under constant temperature, especially due to dormancy. Alternating temperatures are an important factor for breaking dormancy in various species (Bewley *et al.* 2013). The high germination values observed for *T. egregia* seeds in the germination test indicate that the species does not have post-harvest dormancy. In addition, hard seeds due to impermeability to water were not observed during evaluations. This dormancy mechanism is common in Fabaceae (Marcos-Filho 2015).

Unlike this study on *T. egregia*, studies performed on seeds of *T. bracteolata* Guill. & Perr., *T. candida* DC., and *T. linearis* (Willd.) Pers (Babayemi *et al.* 2003), and on seeds of *T. apollinea* (Delile) DC. (Al-Ansari & Ksiksi 2016), showed varied levels of dormancy, requiring the use of treatments such as immersion of seeds in hot water for 30 seconds (Babayemi *et al.* 2003) and/or use of sulfuric acid for 40-60 minutes (Al-Ansari & Ksiksi 2016) to break dormancy.

The Germination Speed Index (GSI) presented in figure 4 showed better seed performance at the temperature of 30 ºC, with a mean value of 6.7. This value was significantly higher than that observed for the other temperatures tested, which were alternating temperature of 20-30 ºC, with a mean of 5.09; constant temperature of 25ºC with a mean of 4.35; and constant temperature of 20 ºC, with a mean of 0.14. The alternating temperature of 20-30 ºC and the constant temperature of 25 ºC showed no significant difference for GSI, indicating that the germination speed was similar at the two temperatures, just as occurred for germination percentage (figure 3).

The greatest number of seedlings that germinated per day was found at the constant temperature of 30ºC, with approximately seven seedlings germinated per day. Under higher temperatures, germination speed is greater, due to more rapid absorption of water by seeds and an increase in metabolic activity. However, the final germination percentage is generally lower, due to inactivation of proteins and enzymes, which does not occur under optimum temperature (Marcos Filho 2015).

According to Baskin & Baskin (2014), temperatures lower or higher than the optimum tend to reduce the speed of the germination process, exposing seeds to adverse factors for a longer period, leading to reduction in total germination, which occurred at the temperature of 35 °C, inadequate for the species under the conditions tested. Marcos Filho (2015) explains this lower germination due to the fact that high temperatures cause damage to the seeds of determined species, leading, for example, to enzymatic alterations, reducing the amount of free amino acids, and modifying

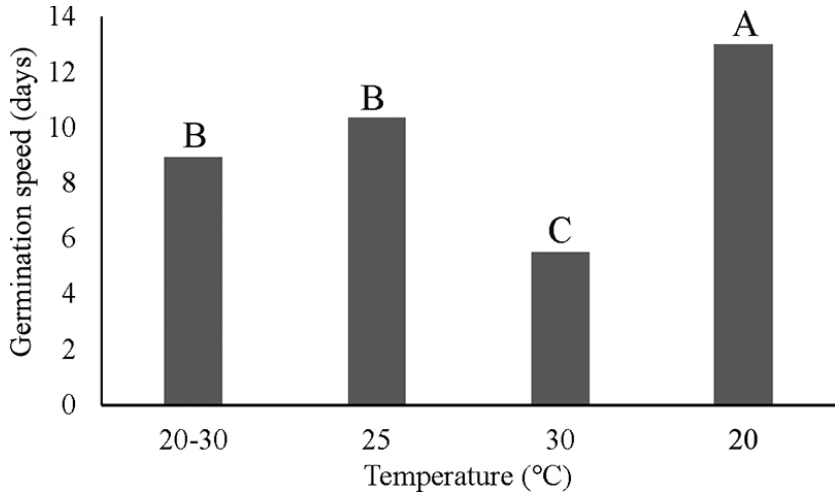

Figure 5. Germination speed (days) of *Tephrosia egregia* Sandwith under different temperatures.

the speed of metabolic reactions. In a study on enzymatic activity in *braúna* (*Melanoxylon brauna* Schott.) seeds at 40 °C, a temperature considered to be above the optimal range for germination of the species, there was reduction in activities of the enzymes α-amylase, β-amylase, and G6PdH; and lower germination values were observed compared to those obtained at 25 °C and 30 °C (Ataíde *et al.* 2016).

Regarding the number of days necessary for germination (GS) (figure 5), the results are in agreement with those of the GSI (figure 4), showing slower germination speed at the temperature of 20 ºC and faster at 30 ºC (figure 5). The germination speed at 25 ºC and at 20-30 ºC was similar, but significantly different from the other treatments (figure 5), as also observed for GSI (figure 4).

Trial 3 – Effect of water stress and salt stress on germination of *Tephrosia egregia* seeds

The *Tephrosia egregia* seeds under water stress and salt stress at more negative osmotic potentials, from -0.2 MPa on, affected all the variables studied.

The regression for the germination variable of *T. egregia* seeds under water deficit using PEG 6000 and under salt stress from NaCl (figure 6) shows a quadratic curve response. For water stress (figure 6), the highest germination percentage occurred in the control treatment (0 MPa), whereas for salt stress (figure 6), the highest germination percentages occurred at the potentials of 0 and -0.2 MPa, with a slight increase in the potential of -0.2 MPa in relation to the control (0 MPa).

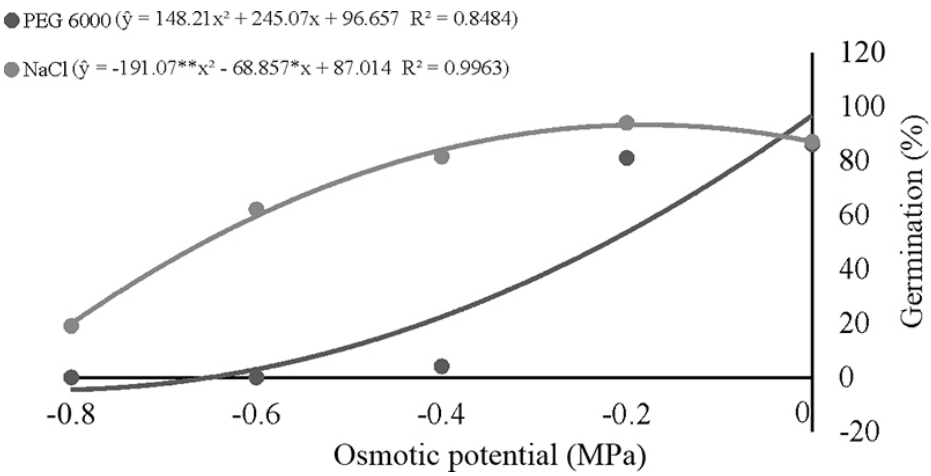

Figure 6. Germination percentage of *Tephrosia egregia* Sandwith seeds under different osmotic potentials of PEG 6000 and NaCl.

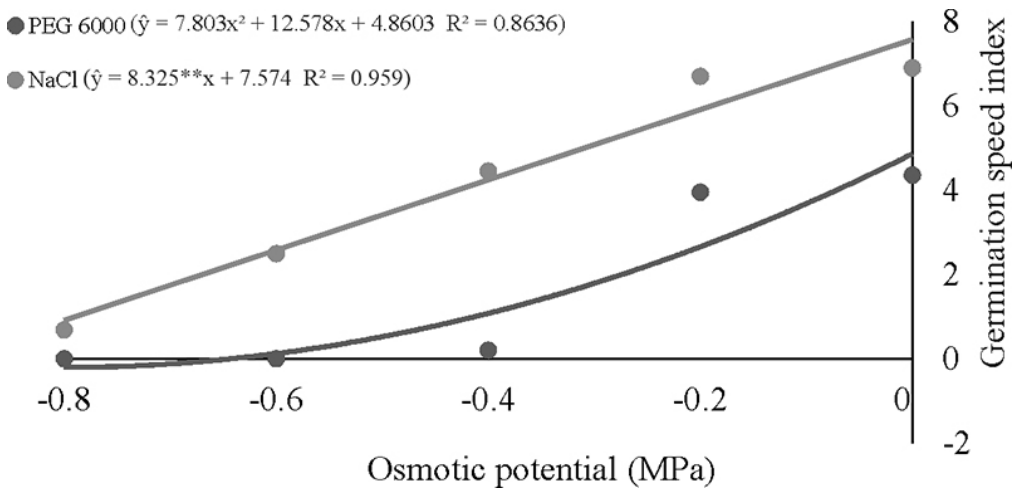

Figure 7. Germination speed index of *Tephrosia egregia* Sandwith seeds under different osmotic potentials of PEG 6000 and NaCl.

Furthermore, for water stress (figure 6), a sharp reduction in germination is observed from the potential -0.2 MPa to -0.4 MPa, in which germination is considered nearly null; and at the potentials -0.6 and -0.8 MPa, germination is null (figure 6). A similar response was observed for the germination speed index (figure 7). For salt stress (figure 6), a gradual reduction in germination percentage is observed from the potential -0.4 MPa on, coming to obtain a germination percentage of 19% at the potential of -0.8 MPa (figure 6).

The reductions observed can be explained by reduction in enzymatic activity, which hurts seed metabolism. This occurs because of the low availability of water present to carry out digestion and transport of seed reserve substances (Bewley *et al.* 2013). In addition, salinity can cause ionic toxicity, which results in inhibition of growth and cell division (Morais *et al.* 2012).

Santos *et al.* (2016) studied the germination of two forest species from the Caatinga, *Poincianela pyramidalis* (Tul.) L. P. Queiroz and *Anadenanthera colubrina* (Vell.) Brenan, under water deficit and salinity and observed that when PEG 6000 was used, the lowest germination percentages occurred at the potentials of -0.8 and -1.2 MPa, while under NaCl, lower germination occurred only at the potential of -1.2 MPa.

Almeida *et al.* (2014) studied *cumaru* [*Amburana cearensis* (Allemão) A.C. Smith] seeds under water stress imposed by PEG 6000 and observed higher germination percentages at the potentials of 0 and -0.2 MPa, with reduction from the potential of -0.6 MPa on, and null germination at the potentials of -0.8 and -1.0 MPa. Pelegrini *et al.* (2013) found that the germination of Brazilian coral tree (*Erythrina falcata* Benth) seeds was affected from -0.5 MPa on, while Fanti & Perez (2004), evaluating the effect of the potentials from 0 to -0.7 MPa on silk floss tree (*Chorisia speciosa* St. Hil.) seeds, observed reduction in germination percentage from the potential of -0.5 MPa on, and null germination at the potential of -0.7 MPa.

As observed in our study, in a study on *Erythrina falcata* seeds, Pelegrini *et al.* (2013) observed a slight increase in p.p. in germination at the potential of -0.2 MPa compared to the control (0 MPa) for both osmotic solutions studied (PEG, Mannitol, and NaCl).

In salt stress (figure 7), a linear fit of the GSI was observed, with reduction in speed associated with decline in osmotic potential of the NaCl solution, showing the effect of salinity on delay in germination of *T. egregia* (figure 7). Reduction in speed of seed germination is cited as one of the main effects caused by salinity (Andréo-Souza *et al.* 2010, Gordin *et al.* 2012, Pelegrini *et al.* 2013, Santos *et al.* 2016).

Germination speed (GS) also shows a linear fit for both stresses (figure 8), however, in a manner opposite to that observed for the germination speed index (figure 7); that is, the greater the number of days to germination, the slower the process. Thus, as osmotic potential becomes more negative, the lower the speed of seed germination of *T. egregia*, that is, the seeds require a longer time to germinate. Lower GS (in number of days) is observed for the control (0 MPa), and an increase in GS (number of days) is observed as osmotic potential becomes more negative (figure 8). The potential of -0.8 MPa required the longest time for germination – normal seedlings were obtained only on the tenth day after sowing for water stress and on the fourteenth day after sowing for salt stress (figure 8).

In niger (*Guizotia abyssinica* (L.f.) Cass.) seeds, there was reduction in germination percentage and in germination speed index of the seeds as of the potential of -0.3 MPa in NaCl solution (Gordin *et al.* 2012). In physic nut (*Jatropha curcas* L.) seeds, germination speed is directly affected by reduction in water availability, due to salinity, because a longer time is required to conclude the germination process (Andréo-Souza *et al.* 2010).

In *angico* (*Anadenanthera colubrina*) seeds, Santos *et al.* (2016) found that the lowest values of GSI were found

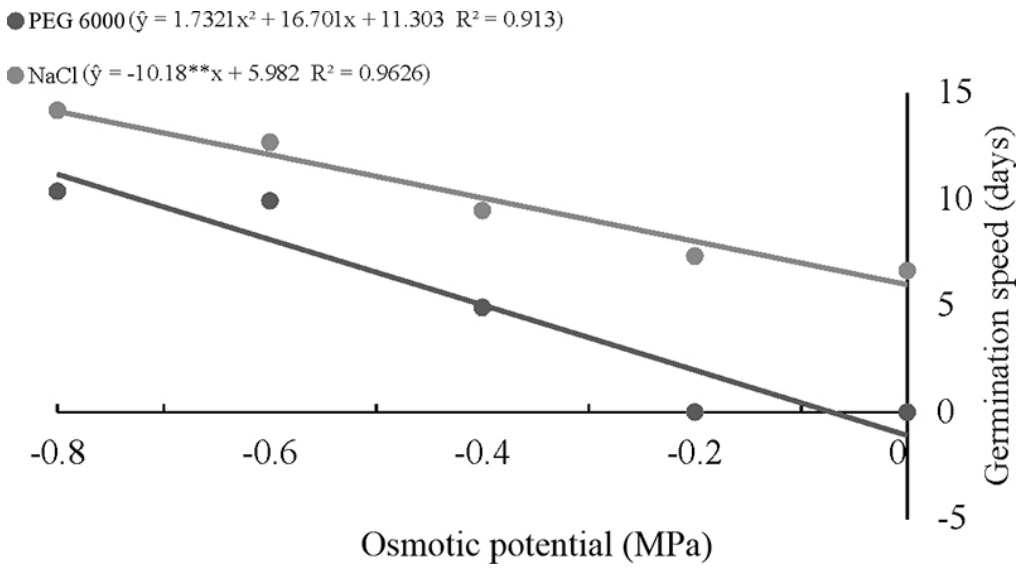

Figure 8. Germination speed (days) of *Tephrosia egregia* Sandwith seeds under different osmotic potentials of PEG 6000 and NaCl.

at the most negative osmotic potentials (-1.2 MPa of NaCl and -0.8 MPa of PEG 6000).

Comparison of the data from the germination variables (figure 6) and the germination speed index (figure 7) shows that the *T. egregia* seeds are more susceptible to water stress with the use of PEG 6000 than to salt stress with NaCl. With the use of PEG 6000, from the potential of 0.4 MPa on, germination and the GSI were practically null. This response was not found under salt stress with NaCl, because even at the most negative osmotic potentials, the germination values obtained were not null.

Greater sensitivity to water stress (PEG 6000) than salt stress (NaCl) is also observed in *Senna uniflora* (Mill.) H.S.Irwin & Barneby (Carvalho *et al.* 2020). According to these authors, germination and the germination speed index of *S. uniflora* seeds were significantly less when placed under PEG 6000 at the potentials of -0.2 and -0.4 MPa.

Santos *et al.* (2016) observed that *Poncianella pyramidalis* was more tolerant to salt stress, whereas *Anadenanthera colubrina* to water stress.

Rego *et al.* (2011) evaluated water stress and salt stress in germination of *Anadenathera colubrina* (Veloso) Brenan seeds and found reduction in germination and in the germination speed index as of the potential of -0.6 MPa when PEG 6000 was used for simulation of water stress. For salt stress, these authors used KCl and observed that reduction in germination occurred only as of the potential of -1.0 MPa; nevertheless, for GSI, a decrease was observed as of the potential of -0.6 MPa.

Studying the effect of salt stress and water stress on germination of *juazeiro* (*Zizyphus joazeiro* Mart.), Lima & Torres (2009) found a significant reduction in germination percentage as of the potential of -0.3 MPa in NaCl solutions.

Conclusions

The most suitable temperatures for germination of *Tephrosia egregia* are 25 ºC and 20-30 ºC, at which greater values of germination were obtained.

For this species, the germination test should be conducted in a roll of paper under constant temperature of 25 ºC or alternating temperature of 20-30 ºC for 14 days.

Seed germination in *T. egregia* is reduced as of the water potential of -0.2 MPa; we can therefore classify the species studied as sensitive to water stress.

Under salt stress with NaCl solution, the sharpest reduction in germination was as of 0.4 MPa, and this indicates that *T. egregia* may have a certain tolerance to salinity. This behavior combined with tolerance to high temperatures during germination indicate that it is the species of considerable potential for recovery of degraded areas.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author contributions

Paulo José de Moraes: conceptualization, collect seeds, carried out the experiments, writing original draft.

João Paulo Oliveira Ribeiro: writing, prepare the manuscript and illutrations, editing.

Mariana Miranda Silva: carried out the experiments in laboratory.

Denise Cunha Fernandes dos Santos Dias: resources, supervision the study and the experiments in laboratory, review the manuscript and editing.

Paulo Roberto Cecon: statistical analysis of data.

Jéssica Vieira dos Santos: carried out the experiments in laboratory.

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