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Physiological Response of Seeds of *Crotalaria spectabilis* under Drought and Heat Stress

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HIGHLIGHTS

- Water stress compromises seed germination.
- *C. spectabilis* tolerates up to an osmotic potential of -0.2 MPa.
- The temperature range between 25 and 30 ºC favors germination.

Abstract: This study evaluate the effect of drought and heat stress on the quality of *C. spectabilis* seeds. A completely randomized design in a 4×4 factorial scheme, referring to four osmotic potentials based on Polyethylene glycol (PEG 6000 = 0.0, -0.2, -0.4 and -0.8 MPa) and four temperatures (25, 30 and 35 ºC constant and alternating between 25-35 ºC), with four replications of 50 seeds per plot was used. The germination percentage, first count germination, germination speed index, average germination time, average germination speed, length and dry mass of the root, shoot and seedlings were evaluated. *C. spectabilis* is sensitive to drought stress during germination and seedling establishment, tolerating up to -0.2 MPa. The physiological quality of seeds is reduced with the decrease of osmotic potentials. The temperature range between 25 and 30 ºC are the one that provide better conditions for the germination of the seeds of this species.

Keywords: Legume; seed physiology; germination temperature; vigor.

INTRODUCTION

Crotalaria spectabilis is a species of the legume family, originally from the Indo-Malay region, with an annual cycle and distributed around the world [1]. It is a plant widely used in the agricultural production system, whether in the succession of crops or intercropped with other crops [2]. The production of biomass is the main purpose of production, with excellent accumulation and high nutrient cycling, with emphasis on N and Ca, in addition to reducing the incidence of weeds and nematodes in the soil [3].

However, its exploration in semi-arid regions can be compromised by low rainfall, water scarcity and high temperatures, especially in the germination and initial seedling establishment phases [4]. Drought stress promotes a series of changes in germination, affecting the imbibition of embryonic tissue, respiratory activity, degradation and mobilization of reserves, losses in membrane functionality and cellular activity [5].

Some substances are used to simulate water stress conditions in germination under controlled conditions, such as mannitol and polyethylene glycol (PEG 6000). During conditioning, the seeds absorb water in quantities necessary to activate the germination metabolism, however, the imbibition of the seed is stopped before the protrusion of the radicle [6].

In semi-arid regions, in addition to water stress, high temperatures can affect the germination capacity of seeds. Temperature has a direct influence on germination metabolism, affecting germination percentage, speed and uniformity [7, 8]. The kinetics of water uptake by the seed can be altered by temperature, with the higher kinetics tending to accentuate the effects of water scarcity as the osmotic potential is reduced [9, 10].

Information about the germination and quality of *C. spectabilis* seeds under conditions similar to those found in semi-arid regions, as occurs, in a large part of Northeast Brazil and where drought deficit and high temperatures occur are scarce. Thus, the objective of this work was to evaluate the effect of drought and heat stress on the quality of *C. spectabilis* seeds.

MATERIAL AND METHODS

The research was carried out at the Seed Analysis Laboratory, Agricultural Sciences Center, Universidade Federal da Paraíba (CCA/UFPB), Campus II, Areia, Paraíba, Brazil.

A completely randomized design in a 4 × 4 factorial scheme, referring to four osmotic potentials based on Polyethylene glycol (PEG 6000 = 0.0, -0.2, -0.4 and -0.8 MPa) and four temperatures (25, 30 and 35 ºC constant and alternating between 25-35 ºC), with four replications of 50 seeds per plot was used.

The seeds used were previously disinfected with 5% sodium hypochlorite solution for five minutes. Then, the seeds were sown on sheets of germitest® paper and placed in B.O.D. type chamber (Biological Oxygen Demand), at the established temperatures and under a photoperiod of 8h.

Polyethylene glycol 6000 (PEG 6000) was used to obtain osmotic potentials, with the quantity established according to Villela [11]. The solutions were prepared by diluting the PEG 6000 in distilled water equivalent to 2.5 times the weight of the dry sheet, making rolls of germitest® paper moistened with the appropriate osmotic solutions.

The germination percentage was measured by counting the number of seeds germinated on the 10th day after sowing, considering germinated the seeds that had the radicle protrusion and the emission of leaf primordia. The first count germination was established on the first counting day (3rd day) of the germination test. The germination speed index was measured by the count of germinated seeds in each day and calculated according to Maguire [12]. The average germination time was determined according to the methodology proposed by Labouriau [13] and the average germination speed was established according to the methodology proposed by Labouriau [14].

The root length, shoot length and seedling length, expressed in centimeters, were obtained with the aid of a graduated ruler. Subsequently, each part was placed in Kraft paper bags and placed to dry in an oven with forced air circulation at 65 ºC until it had a constant weight, being weighed in a precision analytical balance (0.001g), with the results expressed in g per seedling.

The data were subjected to analysis of variance at 1% and 5% probability and in cases of significant effect, a regression analysis was performed. The Sisvar software version 6.5 was used for statistical analysis [15].

RESULTS

The interaction between osmotic potential and temperature promoted a significant effect on all variables analyzed at 1% probability (P = <0.01). The germination percentage decreased with the reduction of the osmotic potential (Figure 1A). All seeds of *Crotalaria spectabilis* germinated when subjected to a temperature of 30 ºC and potential of -0.2 Mpa. The highest germination percentage at temperatures of 25, 35 ºC and alternating between 25-35 ºC were obtained at the osmotic potentials of -0.3, 0.0 and -0.2 MPa, with values of 96.9, 95.9, and 92.1%, respectively. The seeds submitted to -0.8 MPa at temperatures of 35 ºC alternating between 25-35 ºC did not germinate.

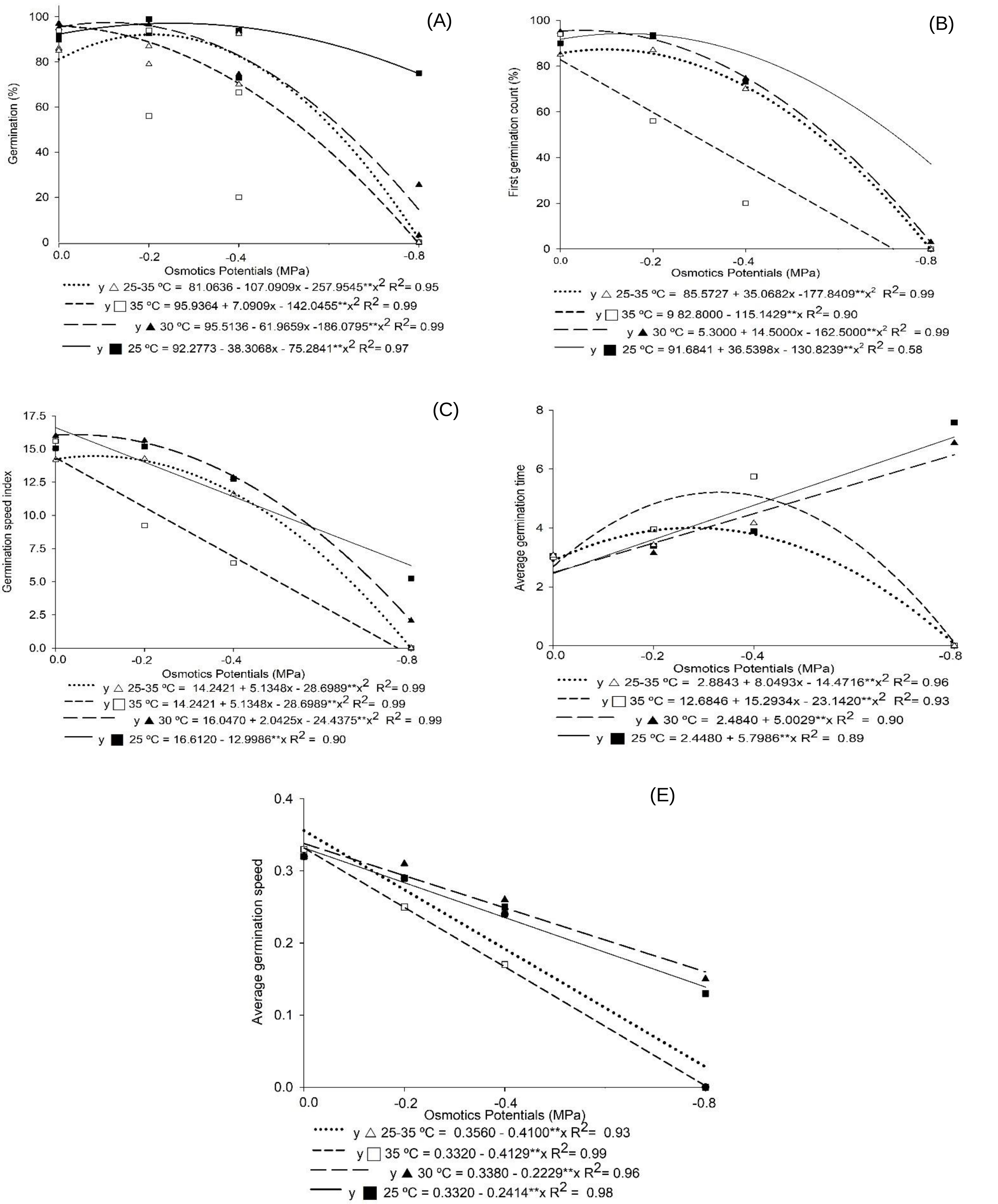

Figure 1. Germination percentage (A), first germination count (B), germination speed index (C), average germination time (D) and average germination speed (E) of *Crotalaria spectabilis* seeds under drought and heat stress. ** significant at 1% respectively.

Seed vigor, represented by the first count and the germination speed index, was affected by drought, with the highest values in the control treatment, regardless of the temperature (Figure 1B and 1C). The highest values (95.3%) for the first germination count were obtained at a constant temperature of 30 ºC and the germination speed index (16.6) at a constant temperature of 25 ºC.

The average germination time increased with the reduction of the osmotic potential, with the shortest time observed in the seeds submitted to a temperature of 25 ºC and 0.0 MPa (Figure 1D). It is noteworthy that at temperatures of 35 ºC and alternating between 25-35 ºC, there was a quadratic effect due to the germination in the potential of -0.8 MPa to have been null, resulting in the opposite effect to the other temperatures. The average germination speed decreased as the osmotic potential was more negative, regardless of the temperature imposed on the seeds, with the highest values (0.35) occurring in the seeds submitted to an alternating temperature between 25-35 ºC (Figure 1E).

The growth of *C. spectabilis* seedlings was negatively affected due to the reduction of osmotic potentials at the imposed temperatures (Figure 2). The longest root length was observed in plants without water stress (0.0 MPa) and at temperatures ranging from 25-35 °C (3.14 cm), 25 °C (2.75 cm) and 35 °C (2.68 cm). The longest root length (2.73 cm) in seedlings submitted to a temperature of 35 ºC occurred in the seeds under an osmotic potential of -0.2 MPa (Figure 2A).

The shoot length (SL) decreased with the reduction of the osmotic potential, mainly in the alternating temperature of 25 - 35 ° C. The highest SL (7.46 cm) was observed in seedlings without drought stress and at a temperature of 35 °C, which represents an increase of 98.2% in the shoot length under higher drought stress (Figure 2B).

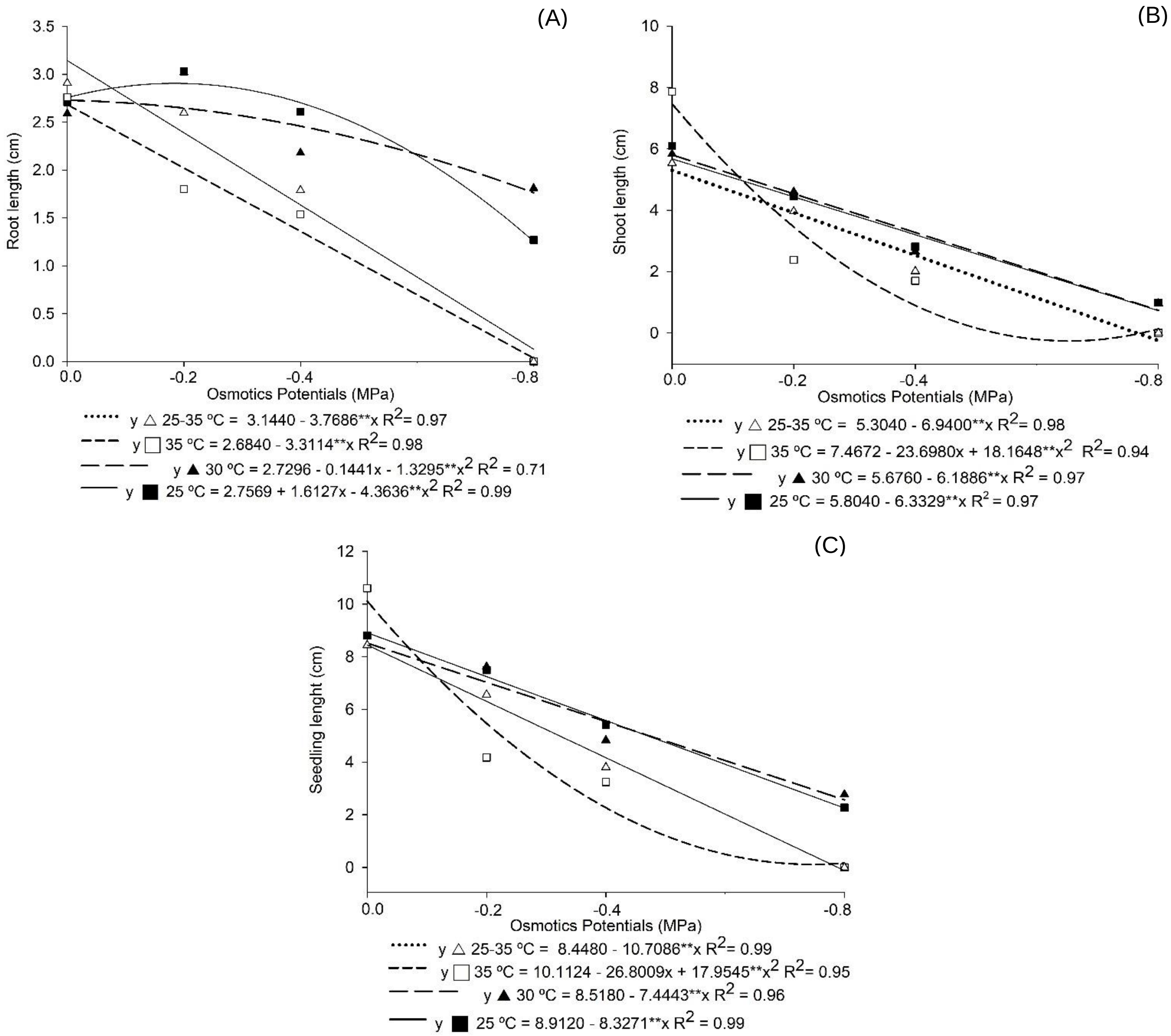

Figure 2. Root length (A), Shoot length (B) and seedling length (C) of *Crotalaria spectabilis* under drought and heat stress. ** significant at 1% respectively.

The losses in the shoot length were 87.3% when submitted to temperatures of 25 and 30 ºC and 100% in the alternate between 25-35 ºC. Similarly, it occurred in the total seedlings length, with decreases as the drought stress increased, with the highest values (10.1 cm) in the seedlings submitted to a temperature of

35 ºC (Figure 2C). The reduction of potentials down to -0.8 MPa promoted losses of 74.7, 69.9, 98.45, and 100% at temperatures of 25, 30, 35 ºC and alternating temperatures between 25-35 ºC, respectively.

Drought stress induced a greater accumulation of root biomass in seedlings that were submitted to a temperature of 30 °C, with increments of 0.0023 g per seedling between seedlings without stress and with greater water stress (-0.8 MPa) (Figure 3A). The temperatures of 25, 35, and alternating between 25-35 ºC had the values of 0.00066, 0.00063, and 0.0012 g per seedling, in the potentials of -0.4, -0.2, and -0.3 MPa, respectively.

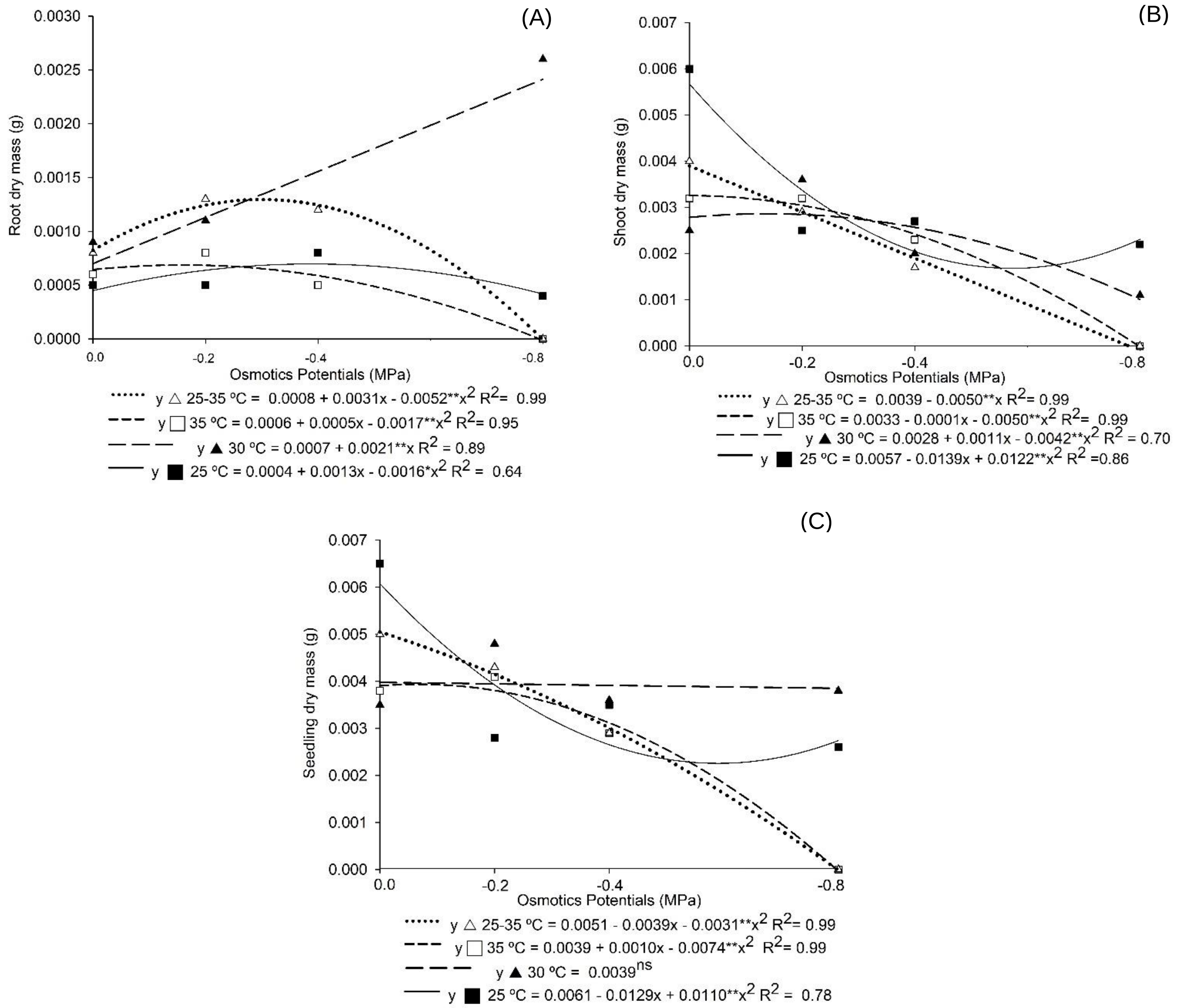

Figure 3. Root dry mass (A), shoot dry mass (B) seedlings dry mass (C) of *Crotalaria spectabilis* under drought and heat stress. significant at 1$^{(**)}$ and 5%$^{(*)}$, respectively. ^{ns} not significant.

The shoot dry mass was higher in seedlings without water stress, regardless of the temperature applied (Figure 3B). Drought stress reduces the shoot dry mass, with the highest values (0.0057 g) observed at a temperature of 25 ºC, while temperatures of 30, 35 and alternating temperature between 25-35 ºC had the maximum increments of 0.0028, 0.0033, and 0.0039 g seedling, respectively. The seedlings dry mass was reduced with the reduction of osmotic potentials, providing low biomass accumulation, with the largest increments (0.0061 g) at a temperature of 25 ºC (Figure 3C). The seedlings dry mass at temperatures of 30 ºC, 35 ºC and alternating temperature between 25-35 ºC was 0.0039g, 0.0039 and 0.0051g, respectively.

DISCUSSION

The germination of *C. spectabilis* decrease to zero levels with the reduction of the osmotic potential at temperatures equal to or higher than 30 ºC is due to the low capacity of water absorption by the seed, affecting the biochemical reactions during germination. Water is essential for germination to occur, from the hydration of tissues and increased respiratory activity, causing the breakdown of reserves and the resumption of the embryonic axis development, resulting in the embryo expansion and integument rupture until the radicle protrusion [16].

The variation in thermal amplitude between 25 and 30 ºC is the most efficient range for the germination of *C. spectabilis* seeds under drought conditions. Temperature is a crucial factor for biochemical reactions to occur in an orderly manner during germination, regulating the percentage and establishment of seedlings [17, 9] with temperatures or temperature ranges that the germinative process occurs more efficiently.

The reduction of the osmotic potential induces low water absorption by the seed, negatively affecting the metabolic activity and reduces the degradation and translocation of reserves to the embryonic axis [18], combined with low respiratory activity due to the decrease in the input of oxygen due to the viscosity of PEG 6000 [19], which have directly affected the vigor presented by the first count and the germination speed index of *C. spectabilis* seeds. The low water absorption reduced the reactions during the germination metabolism, promoting the germination speed reduction and prolonging the seed germination process. Such effects can be attributed to changes in water absorption kinetics, affecting the imbibition of the seed's internal tissues and compromising cell expansion and division, which can cause the embryo's death [20].

The reduction in germination and seed vigor was reported by other authors, such as Leal [4] that who observed that *Combretum leprosum* Mart. seeds did not germinate when subjected to the osmotic potential of -0.5 MPa at a temperature of 35 ºC. A similar trend was observed by Felix [7] when verifying that *Leucaena leucocephala* (Lam.) Wit seeds did not germinate at temperatures above 35 ºC and the osmotic potential of -0.3 MPa.

The vigor presented by the growth of *C. spectabilis* seedlings indicates that the nitric stress reduces the capacity of cell multiplication and elongation, compromising the initial establishment of the seedlings. Drought stress can interrupt or cease cell stretching, this is due to the decrease in turgor pressure in plant tissues [21]. Growth reduction was also observed by Sahay [22] in mustard seedlings (*Brassica Juncea* L.) and also by Jain [23] in milk thistle (*Silybum marianum* (L.) Gaertn) under water stress.

Accumulation of root biomass of *C. spectabilis* was possibly stimulated as a mechanism to survive drought stress. This increase is associated with the plant's antioxidant defense system, stimulated by the action of phytohormones in response to the oxidative stress imposed by the drought deficit, enabling greater root development to favor greater water uptake and absorption [24].

Drought stress can promote changes in the metabolism of degradation and mobilization of reserves for the embryonic axis, affecting the establishment of the seedling [25]. Among the effects promoted by drought deficit, oxidative stress can alter the activity of enzymes proteases and amylases, which reduces the capacity for degradation of reserves and accumulation of seedling biomass [26].

The reduction in seedling vigor, represented by the accumulation of biomass, induced by drought stress was observed by other authors, such as Silva [27] in crambe (*Crambe abyssinica* Hochst), where PEG-induced low water absorption decreased the seedling dry mass accumulation. The biomass of quinoa seedlings (*Chenopodium quinoa* Willd.) was reduced with exposure to drought stress [18].

CONCLUSION

The temperature range between 25 and 30 ºC are the one that provide better conditions for the germination of the seeds of *Crotalaria spectabilis* under drought stress conditions. *C. spectabilis* is a species that is poorly tolerant of water stress during the germination phases and the establishment of seedlings tolerating up to -0.2 MPa.

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Conflicts of Interest: The authors declare no conflict of interest.

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