Original Research Article

Physiological quality in *Leucaena leucocephala* seeds conditioned with potassium nitrate submitted to saline and water stresses

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ABSTRACT

Leucaena leucocephala is a species that occurs in semi-arid regions capable of developing physiological and biochemical strategies under adverse environmental situations, such as saline soils and water deficiency. Even so, there are still losses in germination and initial development of plants, mainly due to abiotic stress effects, making it necessary to implement alternatives which are able to diminish the impact on the seeds with the use of potassium nitrate. The objective of this work was to evaluate the physiological quality in *L. leucocephala* seeds conditioned in potassium nitrate solution and subjected to saline and water stresses. After scarification, the unconditioned leucine seeds were separated and identified, and the conditioners were immersed in the 1.0 Mmol/L solution of potassium nitrate for 24 hours. The treatments were NaCl: 0; 30; 60; 90; 120 and 150 Mmol/L and the water potential: 0,0; -0.2; -0.4; -0.6, and -0.8 MPa. The seeds were distributed over two Germitest® sheets, moistened with distilled water 2.5 times the dry weight and incubated in a B.O.D. at 25 °C for 10 days. Then, the germination speed index, seedling length and seedling mass were measured for the normal seedlings. Eleven treatments with four replicates of 50 seeds were used. The design was completely randomized, and the statistical analyzes were performed in SAS. The use of potassium nitrate (KNO_3) contributed to maintaining the physiological quality of *L. leucochephala seeds* under conditions of saline stress and water restriction.

Keywords: Leucena; water potential; salinity.

1. INTRODUCTION

Leucaena leucocephala (Lam.) belongs to the Fabaceae family (Subfamily Mimosidae), is a fast growing specie, nitrogen fixer and has been highlighted as a promising alternative for recovering vegetation and rehabilitating degraded areas, since it has symbiosis with nitrogen-fixing bacteria, improving soil fertility [1].

Its use has met a wide variety of demands, including reforestation in disturbed areas for erosion control, animal feed, green manuring, fence posts, poles and cellulose. Sowing can be done either by seedlings or directly on the site [2]. However, sowing in saline soils in regions with a low frequency of rainfall results in slow and irregular emergence, with direct reflexes on the seedling development [3].

The effects of salinity on germination can be noticed by both interference of salts in the cellular metabolism and by the reduced osmotic potential of seeds, causing water stress and making water absorption difficult [4]. Salinity may also cause injury, such as chlorophyll degradation and changes in protein metabolism, important amino acid levels linked to the germination process, and initial seedling development [5].

The presence of water is essential, and its scarcity is one of the adversities encountered by species of plants that develop in saline or dry environments, since water availability to the seed, as well the speed of absorption, is directly influenced by the water potential difference between seed and soil [6]. Therefore, Fabaceae species seeds, even in arid and semi-arid regions, may develop physiological and biochemical strategies capable of developing in adverse environmental situations, such as in soils with high saline concentration or affected by water deficiency [7,8].

Due to the severity of losses caused by the effects of saline and water stress on germination and during the initial plant development, alternatives and treatments have been sought to reduce the impact on seeds. Among the substances able to induce a resistance to these types of stress in seeds, nitric oxide (NO), inorganic free radical and an extremely versatile biological messenger can be supplied exogenously to the seeds through their conditioning in potassium nitrate solutions [9].

Research has demonstrated the involvement of nitric oxide in hormonal signaling [10] and in numerous physiological plant processes, including: protective function against oxidative stress [11]. In seeds, nitric oxide stimulates germination, both under normal conditions and under stress, in addition to favoring seed dormancy of some species [12] and promoting the elongation and formation of adventitious roots [13].

The objective of this work was to evaluate physiological quality in *Leucaena leucocephala* seeds conditioned in potassium nitrate solution and submitted to saline and water stresses.

2. MATERIAL AND METHODS

L. leucocephala seeds were collected from mature fruits with brown staining from ten matrices, spaced at a distance of 6 meters, located in the vicinity of the municipality of Macaíba - RN (Latitude: -5.86, Longitude: -35.33) in the period from July to August 2017. The region presents an Aw tropical rainy climate, with an average temperature of 26 ^{o}C, relative air humidity of 74 % and precipitation of 1,134 mm annually, according to Köppen and Geiger [14].

After collection, the seeds were manually removed from the pods, selected and mechanically scarified using No. 100 sandpaper, on the side opposite to the hilum, only to break the tegument. After scarification, the water content of seeds was determined by the greenhouse method at 105 ± 3 °C for 24 hours, as recommended by Brazil [15], and the results were expressed in average percentage of humidity (wet basis).

Next, the unconditioned seeds were separated and deposited in identified glass containers. The conditioned seeds were immersed in glass containers containing the 1.0 Mmol/L solution of potassium nitrate for 24 hours. The following concentrations of NaCl were used to simulate the stress conditions: 0; 30; 60; 90; 120 and 150 Mmol/L [16]; and also the water potentials: 0; -0.2; -0.4; -0.6 and -0.8 MPa, calculated according to the equation proposed by Michel and Kaufmann [17]. The seeds had their physiological potential evaluated after conditioning and under saline and water stress conditions through the tests described below.

For germination, four replicates of 50 seeds were used for each treatment, distributed on two Germitest® paper towel sheets and covered with a third sheet of the same paper, then rolled into a roller. The substrate was moistened with distilled water (DW) in an amount 2.5 times the dry substrate weight, according to Brazil [15].

Paper rolls with seeds that were not conditioned and conditioned with potassium nitrate were subsequently subjected to saline stress and water stress separately and incubated in a germination chamber (Biochemical Oxygen Demand) at 25 °C for 10 days, when final seedlings were counted. The results were expressed as a percentage of germination.

The germination speed was carried out in conjunction with the germination test, distributed on two Germitest® paper towel sheets and covered with a third sheet of the same paper, then rolled into a roller. The substrate was moistened with distilled water (DW) in an amount 2.5 times the dry substrate weight, according to Brazil [15], with daily counts of seeds that emitted a primary root of at least 2 mm from the first to the tenth day. The germination speed was determined from the germination velocity index (GVI), calculated according to the formula proposed by Maguire [18].

The dry mass of seedlings was performed with a total of ten normal seedlings per experimental unit, randomly taken at the end of germination test, dried for 24 hours in a greenhouse at 105 ± 2 ºC, weighed on a precision scale and the results were expressed in grams of dry mass of seedlings.

The experiment was carried out in a completely randomized design with four replicates of 50 seeds using a factorial arrangement scheme: 2 (unconditioned and conditioned with KNO_3) x 6 (NaCl concentrations) for salt stress, and 2 conditioned and conditioned with KNO_3) x 5 (osmotic potentials) for water stress. The results were submitted to analysis of variance, and when there was significance by the F-test, they were submitted to regression analysis. Statistical analyzes were performed using SAS® software [19].

3. RESULTS AND DISCUSSION

According to the analysis of variance, when applying the F-test, statistical differences were verified for the variables in which *L. leucocephala* seeds were submitted, both in relation to the conditioning and the saline stress (NaCl) concentrations and different osmotic potentials (PEG). Significance was also observed for the interaction between the factors, observing different behaviors for physiological analyzes, except for the germination variable, when subjected to saline stress conditioning and interaction, for which there was no significant effect on the evaluated treatments. No difference was found between the means of the evaluated treatments when the germination of seeds submitted to water stress was evaluated (Table 1).

Table 1. Summary of variance analysis for the variables of germination (G), germination velocity index (GVI), seedling length (SL) and seedling dry mass (SDM) from unconditioned seeds and conditioned with potassium nitrate subjected to saline and water stresses.

F values – Saline stress				
Source of variation	G (%)	GVI	SL (cm)	SDM (g)
Conditioning (C)	175.91**	334.67**	387.11**	0.16**
Saline concentrations (S)	21.10^{ns}	34.08**	4.01**	0.08**
(C) x (S)	74.83^{ns}	13.73**	25.97**	0.06**
CV (%)	7.82	14.83	11.83	12.15
F values – Hydrical stress				
Source of variation	G (%)	GVI	SL (cm)	SDM (g)
Conditioning (C)	22.94^{ns}	14.88**	14.21**	0.0016**
Osmotic potentials (O)	26.56^{ns}	51.37**	9.08**	0.0012**
(C) x (O)	28.02^{ns}	55.43**	120.70**	0.0010**
CV (%)	7.53	3.90	1.94	7.45

^{ns} not significant, ** significant at 1% by the F test.

The results of the germination test on conditioned or non-saline seeds (Figure 1 A) show that, there was a positive effect of conditioning on this variable despite salinity. Although the seeds did not present significant variation with the progressive increase of the salinity of the substrate, a conditioning effect on the germination of seeds was observed until the concentration of 90 Mmol/L of NaCl, which presented higher germination than the unconditioned seeds. In general, the seeds presented high germination, even when not conditioned. A similar result was verified by Amaro [20], who evaluated *Copaifera langsdorffii* Desf. (Fabaceae) seeds on a substrate moistened with NaCl solution, with a high germination percentage of around 95 %. For *Leucaena leucocephala* (Lam.) De Wit seeds, Cavalcante and Perez [21] added sodium chloride (NaCl) to a substrate and observed high germination percentages of the seeds, which reaffirms the ability of the seeds of this species to present high germinative performance even under saline stress conditions.

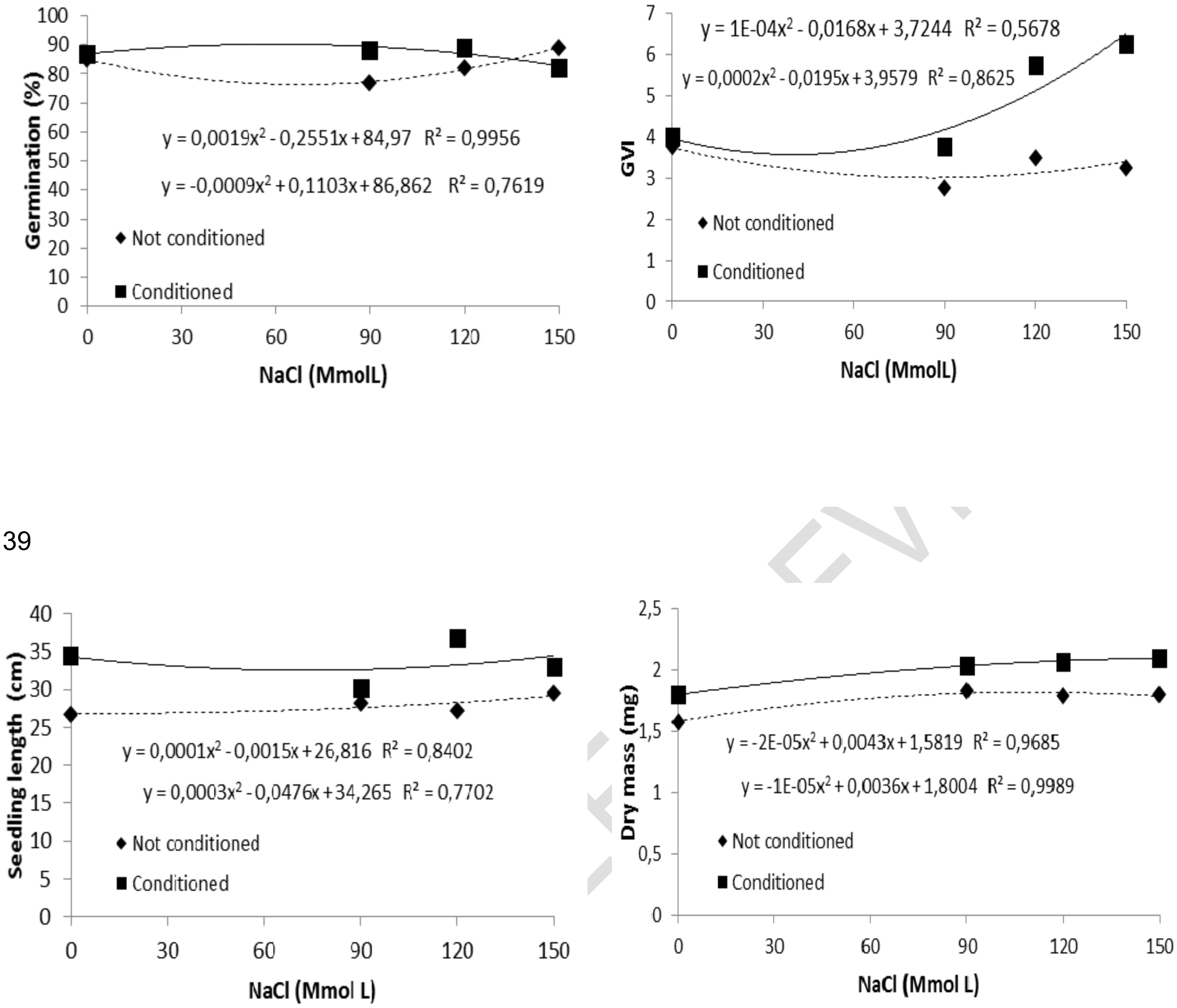

Figure 1. (A) Germination, (B) Germination velocity index, (C) Seedling length, and (D) Dry mass of *L. leucocephala* seedlings from unconditioned seeds and those conditioned with potassium nitrate (submitted to saline stress).

Thus, *L. leucocephala* seeds, as well as those of several other species of the Fabaceae family, show resistance or some kind of defense system which makes them, capable of synthesizing and accumulating different compounds of small molecular mass such as sugar alcohols, proline and glycine betaine, which are referred to as compatible osmopolymers, osmoprotectants or solutes. These compounds have an exact function in the plants, being able to protect the plants from abiotic stresses, functioning as a tool for the cellular osmotic adjustment, and protection against oxidative damage under adverse conditions, such as in drought and salinity tolerance [22].

However, it was verified that potassium nitrate did not promote seed germination improvement, since these seeds already present high viability even under salt stress conditions.

It was observed that the germination rate, length and dry mass of seedlings increased as the substrate concentrations increased, evidencing that the potassium nitrate and salt stress in *L. leucocephala* seeds (Figures 1 B, C and D) and the potassium nitrate concentrations helped to reverse and/or minimize the negative effects of salinity in seed germination velocity index and initial seedling development. The plausible explanation for this phenomenon is the possibility of nitric oxide liberators promoting seedling tolerance to salinity by reducing Na^+ and Cl^- transport to leaves and by the ability to compartmentalize these ions in vacuoles to avoid their accumulation in the cytoplasm or cell walls and thus avoid salt toxicity [35].

This is because the mechanism of action of potassium nitrate acts on the reception of electrons, reducing the nitrite form inside the seeds, reoxidizing the NADPH and increasing the availability of NADP which reduces the dehydrogenases in the pentose phosphate cycle, helping to overcome seed dormancy [3]. In addition to being an excellent growth promoter of seedlings, potassium nitrate can also act to maintain balance in plant cells, promoting respiration and metabolism of carbohydrates [23].

Regarding the unconditioned seeds, lower performance was observed in all salinity concentrations when the germination rate, length and dry mass of seedlings were evaluated. Regarding the variable germination, the vigor of the unconditioned seeds was more affected by the salinity. In evaluating the physiological quality of *Crambe abyssinica* Hochst. ex R. E. Fr. seeds, Nunes et al. [24] found that the increase in saline concentration of the substrate also reduced the germination rate. This decrease in germination speed was also observed by Sousa Filho [25] in *Erythrina mulungu* Mart ex Benth. (Fabaceae) seeds under saline stress. These results confirm the negative effect of soil salinity on the vigor of seeds of different species, reducing the emergence speed and the growth of the seedlings, generating damage to agricultural production.

Salinity probably interferes with the physiological quality of seeds, and also in terms of reservoir energy expenditure to absorb water and subsequently does not reserve the reservoir for other processes, inducing changes in the activities of catalase, polyphenoloxidase and peroxidase enzymes [26]. In studies carried out by Marques et al. [27], the effect of saline stress on emergence and establishment of seedlings seems to have been responsible for inhibiting cotyledonary reserve depletion and embryonic axis growth, since the seedlings in these analyzes presented excessive accumulation of Na^+ and Cl^- ions.

The excess Na^+ and Cl^- ions seem to play a role in reducing the physiological quality of seeds, since they tend to cause a decrease in protoplasmic intumescence (the ions in solution initially cause a decrease in intumescence, and only after absorption and accumulation in the vacuoles and apoplast is the rate of water absorption normalized), affecting the enzymatic activity, mainly resulting in the inadequate production of energy by disturbances in the respiratory chain [28]. In addition, it is necessary to emphasize its toxic effect, resulting from the concentration of ions in the protoplasm. As the saline concentration increases in the soil solution, there is an increase in the osmotic pressure, and therefore the plant does not absorb the soil water, causing physiological and morphological disturbances that hinder the plant’s survival in this stress condition [29].

No changes in seed germination were observed along the reduction of water availability when seeds were conditioned or not subjected to water stress, and no differences between the two treatments for any of the potentials were tested.

However, as the water potential became more negative, there was a decrease in the germination rate of *L. leucocephala* seeds conditioned and not conditioned with potassium nitrate (KNO_3), and conditioned seeds presented higher results in relation to unconditioned seeds (Figure 2 A). KNO_3 under water stress showed a significant interaction and showed a positive influence on the evaluations described in *L. leucocephala* seeds (Figure 2A). Potassium nitrate also helped to reverse and/or minimize the negative effects of seed germination and initial seedling development, in addition to being very tolerant to negative water potentials. Thus, the conditioning technique establishes favorable conditions to increase seed germinability and vigor of seedlings [36], contributing to high-quality seedlings even under stress conditions [37].

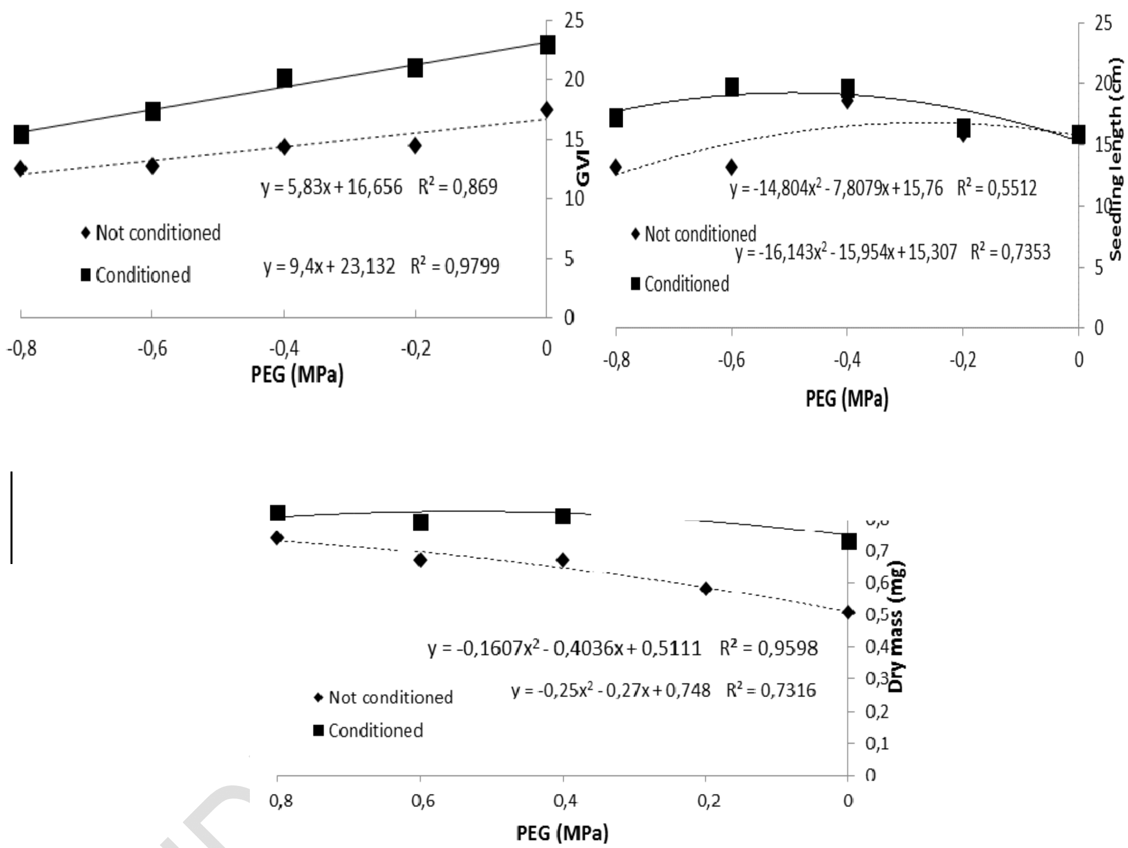

Figure 2. Germination velocity index (A), seedling length (B) and dry mass (C) of *L. leucocephala* from unconditioned seeds and seeds conditioned with potassium nitrate (and submitted to water stress).

When evaluating the viability of *Apuleia leiocarpa* (Vogel) J.F.Macbr. (Fabaceae) in different water potentials, Spadeto et al. [31] also observed that more negative potentials of -0.4 and -0.8 MPa obtained lower germination speed averages of 3.80 and 3.74, respectively. When Felix et al. [34] evaluated the effects of water stress, they verified that the germination speed in *L. leucocephala* seeds were reduced when submitted to the most negative water potentials of -0.3 and -0.6 MPa, obtaining a mean of 8.0 and 4.0, respectively.

Therefore, the results of these researchers corroborate with those obtained in this research, indicating the negative effect of reducing the water availability in substrate on the germination of seeds for this species. This fact is probably due to a decrease in water availability, which limits imbibition and reduces seed water intake, reducing the metabolic activities expected during germination phases, slowing down the process of mobilization and utilization reserves, leading to a reduction in the germination rate [5].

It is observed that *L. leucocephala* seeds presented a high germination rate in the conditions in which the lowest water restrictions were observed. According to Bewley et al. [5], seeds such as *L. leucocephala* are tolerant to water stress due to their natural occurrence in semi-arid regions, as they acquire biochemical and physiological strategies that can be attributed to membrane repair even in situations of low water potential.

Conditioning with potassium nitrate (KNO_3) has influenced the reversal of harmful changes in cell membranes caused by inactivation of enzymes and inhibition of protein synthesis [29]. This shows that even under stress conditions of abiotic origin, *L. leucocephala* seeds had positive effects on the physiological responses involved in the whole germination process, regulating germination percentage and germination rate, as well as the growth, development and establishment of seedlings.

There was a tendency to increase the length and dry mass of *L. leucocephala* seedlings as the water restriction was increased, regardless of the potassium nitrate (KNO_3) or not. However, the seeds presented different results for length and dry mass of seedlings when compared to conditioned and unconditioned seeds. With the reduction of the water potential, the length of the seedlings resulting from conditioned seeds presented results with a tendency to increase the difference in relation to the unconditioned seeds, indicating an increase in the size of conditioned seedlings in relation to the untreated seedlings under water stress conditions (Figure 2 B). On the other hand, the dry matter of seedlings showed a decrease in the water potential (Figure 2 C) in relation to the unconditioned seeds.

The ability to develop roots and shoots even under conditions of stress and water scarcity, varies widely between species and may be related to the adaptation hypothesis, due to the proliferation and increase of the roots which increase the capacity of water absorption in a limiting situation, or may be related to the growth rate of a given plant [32].

Many proteins that are induced in the early stages of water stress are also involved in root morphogenesis and carbon/nitrogen metabolism, which may contribute to the nullification of stress by increasing root growth [33]. In the case of *L. leucocephala*, the literature reports that it is a plant which can adapt to inhospitable conditions, and such rusticity can favor the tolerance of its seeds under different conditions.

4. CONCLUSION

The use of potassium nitrate (KNO_3) showed a positive action on the reversal of salt stress and the conditioning of the seeds under low osmotic potential, influencing the maintenance of the physiological quality of *L. leucocephala.*

COMPETING INTERESTS

The authors declare they have no competing interests.

AUTHOR CONTRIBUTIONS

This work was carried out in collaboration with all of the authors.

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