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Original Research Article

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

ABSTRACT

Leucaena leucocephala is a species that occurs in semiarid regions capable of developing physiological and biochemical strategies ~~underin~~ 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 effects the abiotic stresses, ~~being necessary with this~~ ~~weto implement have looked for~~ alternatives ~~able to that~~ 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 ~~submitted-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, 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 leaves of Germitest[®], moistened with distilled water 2.5 times the dry weight and incubated in B.O.D. at 25 °C for 10 days. Then, normal seedlings, germination speed index, seedling length and seedling mass were measured. Eleven treatments with four replicates of 50 seeds were used. The design was completely randomized, and the statistical analyzes were done in SAS. The use of potassium nitrate (KNO₃) contributed to the maintenance of the physiological quality of *L. leucocephala* seeds under conditions of saline stress and water restriction.

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Keywords: *Leucena*; water potencial; salinity

1. INTRODUCTION

~~The~~ *Leucaena leucocephala* (Lam.) ~~b~~ belongs to the family Fabaceae (Subfamily Mimosidae), is ~~a~~ fast growing ~~specie~~, ~~is~~ nitrogen fixer and has been highlighted as a promising alternative for the recovery vegetation ~~cover~~ and rehabilitation of 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 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]. ~~H~~ However, sowing ~~in~~ saline soils in ~~those~~ regions with a low frequency of rainfall ~~can restrict soil moisture~~, ~~resultings~~ in slow and irregular emergence, with direct reflexes in the development of seedling [3].

The effects of salinity on germination can be noticed both by interference of salts in the cellular metabolism and by reduction the osmotic potential of seed, causing water stress and making water absorption difficult [4]. Salinity may also cause injury, such as chlorophyll

29 degradation and changes in protein metabolism, important amino acid levels linked to the
30 germination process, and seedling initial development [5].

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

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

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

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

52 53 2. MATERIAL AND METHODS

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

61 After collection, the seeds were manually removed from the pods, selected and mechanically
62 scarified, using N°. 100 sandpaper, on side opposite the thread, only to break the tegument.
63 After scarification, the water content of seeds was determined by greenhouse method at 105
64 ± 3 °C for 24 hours, as recommended by Brazil [15]. And the results were expressed in
65 average percentage of humidity (wet basis).

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

74
75 For germination, four replicates of 50 seeds were used for each treatment, distributed on two
76 sheets of Germitest® type paper towels and covered with a third sheet of the same paper,

77 then rolled into a roller. The substrate was moistened with distilled water (AD) in an amount
78 2.5 times the dry substrate weight, according to Brazil [15].

79 Subsequently, paper rolls with seeds that were not conditioned and conditioned with
80 potassium nitrate were subjected to saline stress and water stress separately and incubated
81 in a type germination chamber (Biochemical Oxygen Demand) at 25 °C for 10 days, when
82 final seedlings were counted. Results were expressed as percentage of germination.

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

90
91 The dry mass of seedlings was performed with total of ten normal seedlings per
92 experimental unit, randomly taken at the end of germination test, dried for 24 hours in a
93 greenhouse at 105 ± 2 °C, weighed in precision scale and the results expressed in grams of
94 dry mass of seedlings.

95 The experiment was carried out in a completely randomized design with four replicates of 50
96 seeds, using the factorial arrangement scheme: 2 (unconditioned and conditioned with
97 KNO₃) x 6 (NaCl concentrations), for salt stress and 2 conditioned and conditioned with
98 KNO₃ x 5 (osmotic potentials), for water stress. The results were submitted to analysis of
99 variance and, when there was significance, by the F test, submitted to regression analysis.
100 Statistical analyzes were performed using SAS® software [19].

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102 3. RESULTS AND DISCUSSION

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

113

114 **Table 1. Summary of variance analysis for the variables germination (G), germination**
115 **velocity index (GVI), seedling length (SL) and seedling dry mass (SDM) from**
116 **unconditioned seeds and conditioned with potassium nitrate subjected to saline and**
117 **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**

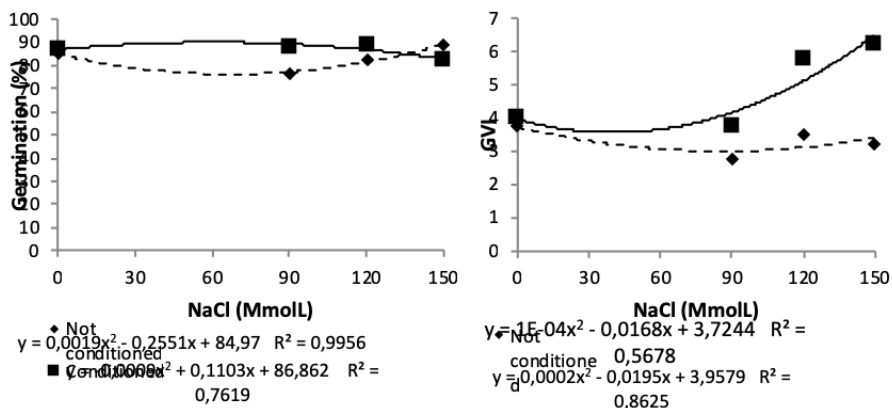
(C) x (S)	74,83 ^{ns}	13,73**	25,97**	0,06**
CV (%)	7,82	14,83	11,83	12,15
F values – Hydrical stress				
Source of variation	G (%)	GVI	SL (cm)	SDM (g)
Conditioning (C)	22,94 ^{ns}	14,88**	14,21**	0,0016**
Osmotic potentials (O)	26,56 ^{ns}	51,37**	9,08**	0,0012**
(C) x (O)	28,02 ^{ns}	55,43**	120,70**	0,0010**
CV (%)	7,53	3,90	1,94	7,45

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

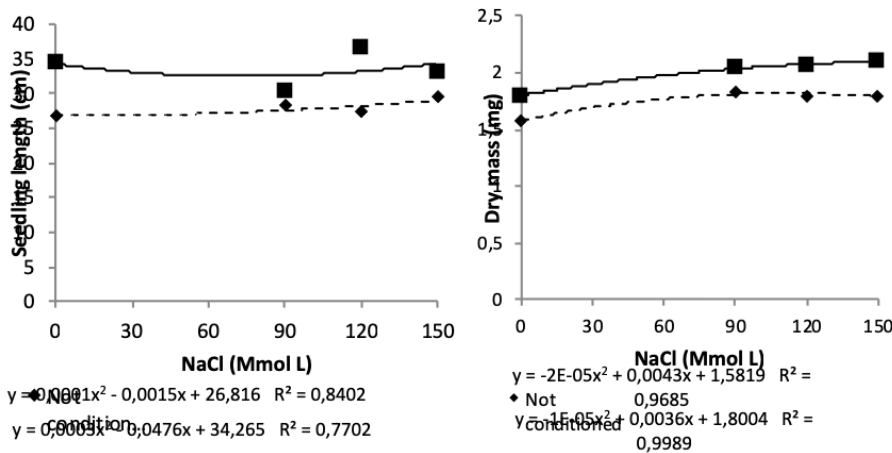
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120 The results of the germination test on conditioned or non-saline seeds (Figure 1 A) show
 121 that, despite salinity, there was a positive effect of conditioning on this variable. Although the
 122 seeds did not present significant variation with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

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135 **Figure 1. (A) Germination, (B) Germination velocity index, (C) Seedling length, and (D)**
 136 **Dry mass of *L. leucocephala* seedlings from not conditioned seeds conditioned with**
 137 **potassium nitrate to saline stress).**

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

146 However, it was verified that potassium nitrate (KNO_3) did not promote seed germination
 147 improvement, since these already present high viability even under conditions of salt stress.

148 It was observed that the rate of germination, length and dry mass of seedlings increased as
 149 the substrate concentrations increased, evidencing that the potassium nitrate (KNO_3) and
 150 salt stress *L. leucocephala* seeds (Figures 1 B, C and D), evidencing that potassium nitrate
 151 (KNO_3) concentrations helped to reverse and/or minimize the negative effects of salinity in
 152 seed germination velocity index and seedling initial development. The plausible explanation
 153 for this phenomenon is the possibility of nitric oxide liberators promoting seedling tolerance
 154 to salinity by reducing Na^+ and Cl^- transport to leaves and by the ability to compartmentalize
 155 these ions in vacuoles to avoid their accumulation in the cytoplasm or cell walls and thus
 156 avoid salt toxicity [35].

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 158 This is because the mechanism of action of potassium nitrate acts on the reception of
 159 electrons, reducing the nitrite form inside the seeds, reoxidizing the NADPH and increasing
 160 the availability of NADP for the reduction of the dehydrogenases of the cycle of pentose
 161 phosphate, helping the overcoming of seed dormancy [3]. In addition to being an excellent
 162 growth promoter of seedlings, potassium nitrate can also act to maintain balance in plant
 163 cells, promoting respiration and metabolism of carbohydrates [23].
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165 Regarding the unconditioned seeds, lower performance was observed in all salinity
166 concentrations when the germination rate, length and dry mass of seedlings were evaluated.
167 As for the variable germination, the vigor of the unconditioned seeds was more affected by
168 the salinity. Evaluating the physiological quality of seeds of *Crambe abyssinica* Hochst. ex R.
169 E. Fr., Nunes et al. [24] found that the increase in saline concentration of the substrate also
170 reduced the rate of germination. This decrease in germination speed was also observed by
171 Sousa Filho [25] in *Erythrina mulungu* Mart ex Benth. (Fabaceae) seeds under saline stress.
172 These results confirm the negative effect of soil salinity on the vigor of seeds of different
173 species, reducing the emergence speed and the growth of the seedlings, generating
174 damages to agricultural production.

175 Salinity probably interferes with the physiological quality of seeds as well, in terms of
176 reservoir energy expenditure to absorb water and subsequently not reserving the reservoir
177 for other processes, inducing changes in the activities of catalase, polyphenoloxidase and
178 peroxidase enzymes [26]. In studies carried out by Marques et. [27], the effect of saline
179 stress on emergence and establishment of seedlings seems to have been responsible for
180 the inhibition of cotyledonary reserve depletion and embryonic axis growth, since in the
181 analyzes the seedlings presented excessive accumulation of Na⁺ and Cl⁻ ions.

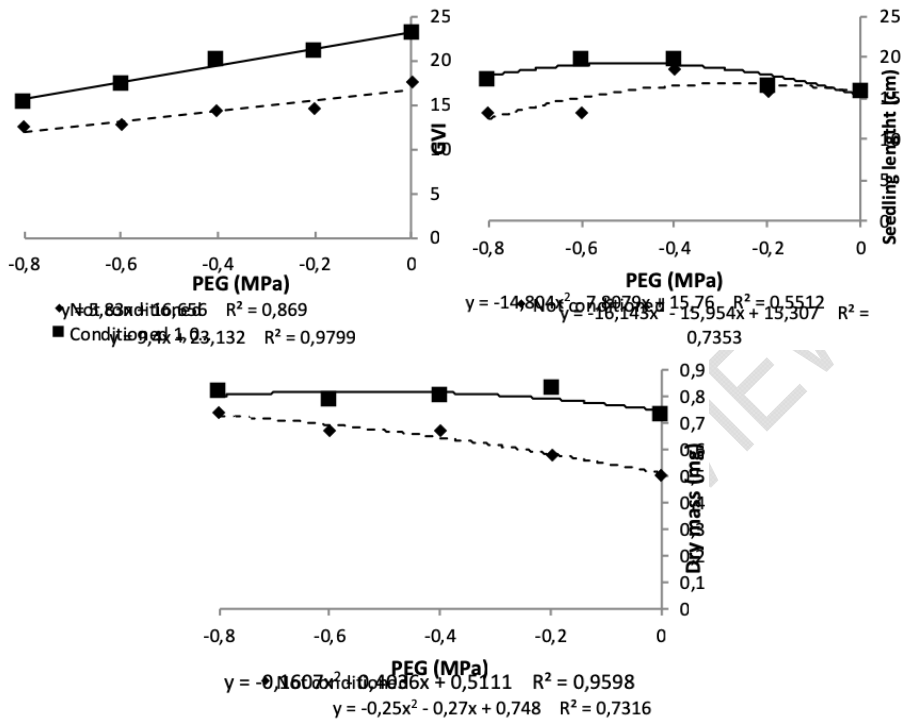
182 The excess Na⁺ and Cl⁻ ions seem to play a role in reducing the physiological quality of
183 seeds, since they tend to cause a decrease in protoplasmic intumescence (the ions in
184 solution initially cause a decrease in intumescence, and only after absorption and
185 accumulation in the vacuoles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

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

196 However, as the water potential became more negative, there was a decrease in the rate of
197 germination of *L. leucocephala* seeds conditioned and not conditioned with potassium nitrate
198 (KNO₃), and conditioned seeds presented higher results in relation to unconditioned seeds
199 (Figure 2 A). KNO₃ under water stress showed a significant interaction and showed a
200 positive influence on the evaluations described in *L. leucocephala* seeds (Figure 2A), since
201 conditioning has the function of repairing damaged macromolecules and cellular structures
202 with the activation of metabolic events in stages I and II of imbibition without, however,
203 occurring root protrusion [36]. The pPotassium nitratee (KNO₃) also helped to reverse and/or
204 minimize the negative effects of seed germination and initial seedling development, in
205 addition to being a very tolerant to negative water potentials. Thus, the conditioning
206 technique establishes favorable conditions to increase seed germinability and vigor of
207 seedlings [37], thus contributing to high-quality seedlings even under stress conditions [38].

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Figure 2. Germination velocity index (A), seedling length (B) and dry mass (C) of *L. leucocephala* from seeds not conditioned and 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 the more negative the potentials, the lower germination speed averages were -0.4 and -0.8 MPa obtained 3.80 and 3.74, respectively. When Felix et al. [34] evaluated the effects of water stress, verified that the germination speed in *L. leucocephala* seeds were reduced when submitted to the most negative water potentials, -0.3 and -0.6 MPa, obtaining a mean of 8.0 and 4.0, respectively.

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

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It is observed that *L. leucocephala* seeds presented high rate of germination 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 semiarid regions, as they acquire biochemical and physiological strategies that can be attributed to membrane repair even in situations of low water potential.

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234 Conditioning with potassium nitrate (KNO₃) has influenced the reversal of harmful changes
235 in cell membranes, caused by inactivation of enzymes and inhibition of protein synthesis
236 [29]. This shows that even under conditions of stress of abiotic origin, *L. leucocephala* seeds
237 had positive effects on the physiological responses involved in the whole germination
238 process, regulating percentage of germination and rate germination, as well as the growth,
239 development and establishment of seedlings.

240 There was a tendency to increase the length and dry mass of *L. leucocephala* seedlings as
241 the water restriction was increased, regardless of the potassium nitrate (KNO₃) or not.
242 However, the seeds presented different results for length and dry mass of seedlings when
243 compared to conditioned and unconditioned seeds. With the reduction of the water potential,
244 the length of the seedlings resulting from conditioned seeds presented results with a
245 tendency to increase the difference in relation to the unconditioned seeds, indicating an
246 increase in the size conditioned seedlings in relation the untreated seedlings under
247 conditions of lack of water (Figure 2 B). On the other hand, the dry matter of seedlings
248 showed a decrease in the water potential (Figure 2 C), in relation to the unconditioned seeds.
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250 The ability to develop roots and aerial parts, even under conditions of stress and water
251 scarcity, varies widely between species and may be related to the hypothesis of adaptation,
252 due to the proliferation and the increase of the roots increase the capacity of water
253 absorption in a limit situation, or may be related to the growth rate of a given plant [32].

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

259

260 4. CONCLUSION

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262 The use of potassium nitrate (KNO₃) showed a positive action on the reversal of salt stress
263 and the conditioning of the seeds under low osmotic potential, influencing the maintenance
264 of the physiological quality of *L. leucocephala*.

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266 COMPETING INTERESTS

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268 Authors have declared that no competing interests exist.

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270 AUTHORS' CONTRIBUTIONS

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272 This work was carried out in collaboration with all authors.

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