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Inducing salt tolerance in castor bean through seed priming

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Abstract

Castor bean (*Ricinus communis L.*) cultivation requires production techniques appropriate for its growth conditions. Thus, the characterization of the deleterious effects of salinity on seed germination and on seedling establishment and the evaluation of the use of priming techniques on seeds could reduce replanting costs and improve emergence uniformity, preparing plant responses to salt stress. The goals of this current research were to characterize the deleterious effects of salinity on seed germination and on seedling establishment and to evaluate the use of the seed priming techniques as a tool to minimize these deleterious effect. In this research, castor bean seeds, cv. BRS-Energia was used to characterize effects of salt stress on germination and seedling establishment. Then, some chemicals such as PEG-6000 and H_2O_2 were evaluated to find the beneficial priming techniques for castor bean seeds against salt stress. Our results indicate that water imbibition by the seed under salinity conditions decelerated the time of exposure to salinity. Salinity also affected the germination of castor bean seeds under an Ψs of -0.4 MPa and seedling growth was already affected under an Ψs of -0.16 MPa. Priming with $CaCl_2$, $NaNO_2$, $NaNO_3$ and PEG-6000 showed promising results under an Ψs of 0.0 MPa, but priming with $NaNO_3$ and PEG-6000 were ones that contributed most to better germination and establishment of seedlings under saline conditions. However, $NaNO_3$ is the most recommended priming agent for castor bean because accelerated seed germination, reducing the time to emergence and planting risks.

Keywords: Germination and vigor tests, imbibition curve, physiological conditioning, *Ricinus communis* L., salinity.
Abbreviations: ABA_abscisic acid; BOD_biochemical oxygen demand; DM_dry mass; EMBRAPA_Brazilian Company of Agricultural Research; GERBOX_plastic boxes to germinate; PEG-6000_polyethylene glycol; Ψs_osmotic potential.

Introduction

Castor bean (*Ricinus communis* L.) is an oilseed with high commercial value for industrial and biofuel applications (Lima et al., 2013). It has specific oil properties that is particularly suited for industurial applications; therefore, the economic growth potential of this crop is immense. In fact, global consumption is driven by industrial and biofuel demands, but insufficient and unreliable feedstock are limiting global consumption (Severino et al., 2013).

The inclusion of oil crops in family farming indicates the consolidation of economic activity, maintains food security, keeps people in non-urban area and indicates bioenergetic self-sufficiency of the country (Marcovith, 2006). Based on these principle, the EMBRAPA released the BRS-Energia castor bean cultivar, characterized by excellent cultivation, allowing farmers to harvest bunches of castor bean in half the estimated time need harvest other varieties available on the market.

The precocity of the BRS-Energia cultivar significantly reduces the time of exposure to biotic and abiotic stresses in the cultivation environment such as soil and irrigation water salinity. However, the degree of injuries caused by salinity depends on the plant species, variety, growth stage, environmental factors and salt conditions, which makes the accurate definition of saline soils difficult (Bray et al., 2000; Zeng et al., 2003; Ali et al., 2014). The growth of castor bean plants is affected 50 days after emergence in saline soils with an electrical conductivity greater than 4 dS m^{-1}, which corresponds to an Ψs of approximately -0.2 MPa (Santos et al., 2010).

Salinity is also a source of environmental stress in the early stages of growth of castor bean plants such as germination and seedling establishment (Severino et al., 2013). The inhibition of germination can be expected as salt stress impairs water absorption and promotes excessive ion entry into the cells. This phenomenon can create an ionic imbalance in the cell cytoplasm, altering the K^+/Na^+ ratio and causing other metabolic disorders (Shabala and Munns, 2012; Khan, et al., 2015). The castor bean seeds naturally have a slow and uneven germination, which extends their expousure to soil pathogens and other environmental stresses, resulting in irregular stands. Therefore, the use of seeds with high germination rates and vigor guarantees producers success regarding proper stand establishment (Dias et al. 2009).

Physiological conditioning techniques can increase seed vigor, and they can potentially reduce the cost of replanting

and can help standardization of both planting and preparation of the seedlings to cope better with abiotic and biotic stresses (Harris et al., 2001; Kubala et al., 2015; Ibrahim, 2016). According to Conrath et al. (2002) and Pastori and Foyer (2002), physiological conditioning improves the ability of plants to perceive biotic and abiotic stresses more quickly and to counteract stresses more efficiently. Seed priming is a simple, low-cost and low-risk technique used to overcome salinity problems in agricultural lands. The physical invigoration treatments can involve chemical and physical methods (Araujo et al., 2016). Several priming techniques are used and their classification relies on the priming agent (e.g., hydropriming, osmopriming, halopriming, hormone priming, hardening, solid matrix priming, humidification, stratification and thermal shock) (Ibrahim, 2016).

No studies on the benefits of seed priming of castor bean under salinity exist in the literature, but the benefits are promising. Based on the information above, the objectives were to characterize the deleterious effects of salinity on seed germination and seedling establishment of castor bean and to evaluate the use of seed priming for minimizing these effect.

Results

Influence of salt stress on seed germination and seedling establishment

The germination of castor bean seeds was inhibited due to the reduction of the Ψs by NaCl (Fig 1a; Supplementary Table 1). Castor bean seeds did not suffer from deleterious effects on germination until the Ψs reached -0.4 MPa, corresponding to a concentration of 80 mM NaCl (Fig 1a). The reduction in germination by salinity (Ψs < -0.4 MPa) was accompanied by an increased percentage of dead seeds and a majority of non-germinated seeds (Fig 1b, c). The Ψs values lower than -0.4 MPa also reduced the percentage of normal seedlings and the germination speed index; thus, extending the average 7-day time of germination under control conditions (Fig 1d, e, f).

Root DM and length decreased when Ψs < -0.31 MPa (Fig 2a, d), whereas hypocotyl DM and length decreased at Ψs values less than -0.23 and -0.16MPa, respectively (Fig 2b, e). Under an Ψs between -0.4 and -0.6 MPa, the reduction in hypocotyl DM was 1.4 times greater than the reduction in root DM (Fig 2a, b, c), and the reduction in hypocotyl length was 1.2 times greater than the reduction in root length (Fig 2d, e, f).

Salinity affected the accumulation of Na^+ and K^+ ions in the roots and hypocotyls of seedlings (Fig 3; Supplementary Table 1). A decrease in Ψs increased the accumulation of Na^+ in the organs of castor bean seedlings (Fig 3a, b). In the hypocotyls, the Na^+ content at an Ψs of -0.6 MPa was 24 times (510 µmol g^{-1} DM) greater than at an Ψs of 0.0 MPa (Fig 3b). On the other hand, in the roots of seedlings, the maximum Na^+ content was 947 µmol g^{-1} DM at an Ψs of -0.46 MPa, which was an increase of 10 times the Na^+ content compared with that at an Ψs of 0.0 MPa (Fig 3a). Under Ψs values of -0.29 and -0.36 MPa, the K^+ content became larger than that in the control conditions in the roots and hypocotyls, respectively (Fig 3c, d). The K^+/Na^+ content ratio in the roots and hypocotyls decreased sharply when the Ψs decreased from 0.0 MPa to -0.2 MPa (Fig 3e, f). At an Ψs less than -0.2 MPa, only slight variations in both K^+/Na^+ relations were observed (Fig 3e, f).

The seeds imibibed water at a constant rate of 0.004487% h^{-1} (1.08 mg water h^{-1}) during germination according to the standard imbibition curve (Fig 4a). However, imbibition curves for Ψs values of -0.2, -0.4 and -0.6 MPa exhibited a uniformly retarded water absorption, whereas seeds soaked at the salt solution with an Ψs of -0.2 MPa exhibited a deceleration of 0.00002% h^{-2} (4.8 µg water h^{-2}) and those under Ψs values of -0.4 and -0.6 MPa exhibited a deceleration of 0.000026% h^{-2} (6.3 µg water h^{-2}) (Fig 4b, c, d; Supplementary Table 2). Despite changes in the rate of absorption of water in the imbibition curves, the seeds in the four analyzed salinity conditions did not differ until 108 h of imbibition and seeds displayed similar water contents (Supplementary Table 2). However, 10 days after germination, the deceleration of water intake caused by salinity resulted in a water inflow reduction of 121 mg under -0.2 MPa and 157 mg under both -0.4 and -0.6 MPa per seed.

Effective seed priming of castor bean

Priming strategies positively affected the establishment of castor bean seedlings (Fig 5; Supplementary Table 3). According to the electrical conductivity tests, seed priming reduced electrolyte loss during emergence (Fig 5a). PEG-6000 and $NaNO_3$ priming treatments led to increased numbers of normal seedlings (Fig 5b). The $CaCl_2$, KCl, $NaNO_2$ and PEG-6000 priming treatments resulted in hypocotyl lengths similar to those of seedlings of unprimed seeds, whereas the other priming treatments negatively affected hypocotyl length (Fig 5c). Roots were longer in seedlings primed with H_2O, $CaCl_2$, KCl, $NaNO_2$, $NaSiO_3$ and H_2O_2 and none of the other priming agents significantly affected root length, compared to the unprimed seeds (control) (Fig 5d). The root/hypocotyl length ratio and hypocotyl length well-maintained in seedlings primed with $CaCl_2$, KCl, $NaNO_2$, $NaNO_3$ and PEG-6000 (Fig 5e, c). Priming agents did not affect DM accumulation in roots (Fig 5g), but the PEG-6000 and $NaNO_3$ priming agents increased hypocotyl DM and reduced the root/ hypocotyl DM ratio (Fig 5f, h). Based on this positive outcome, priming with $CaCl_2$, $NaNO_2$, $NaNO_3$ and PEG-600 were selected for further studies.

Action of the best seed priming agents for castor bean against salt stress

Among the four selected priming agents, only $CaCl_2$, $NaNO_3$ and PEG-6000 increased the percentage of germination and numbers of normal seedlings under salinity (Fig 6a, d). Germination was improved by nearly 90%. The percentage of non-germinated seeds was decreased by 47% and the percent of normal seedlings increased by 50% on average (Fig 6a, b, d). However, no significant improvement in the number of dead seeds was observed (Fig 6c). In particular, $NaNO_3$ priming increased the germination speed index from 3.5 to 11.9 and reduced the average time of germination from 10.4 to 6.2 days (Fig 6e, f).

Only the $NaNO_3$ and PEG-6000 priming treatments increased both root and hypocotyl DM under salinity (Fig 7a, b). The $CaCl_2$, $NaNO_3$ and PEG-6000 priming treatments

increased root length by 56% compared with the control, and seedlings primed with PEG-6000 presented the longest hypocotyls (Fig 7c, d). The $NaNO_2$, $NaNO_3$ and PEG-6000 priming treatments most strongly reduced the root/hypocotyl DM ratio and root/hypocotyl length ratio (Fig 7e, f).

None of the osmopriming agents affected the accumulation of Na^+ in castor bean seedlings (Fig 8a, b; Table 4, supplementary data). However, the $CaCl_2$, $NaNO_3$ and PEG-6000 priming treatments reduced the K^+ content in roots and $NaNO_3$ priming reduced the K^+ content in the hypocotyl (Fig 8c, d). The $CaCl_2$, $NaNO_3$ and PEG-6000 priming treatments reduced the K^+/Na^+ content ratio in the roots, but priming did not alter the K^+/Na^+ content ratio in the hypocotyl (Fig 8e, f; Table 4, supplementary data).

Discussion

Similar to *Apuleia leiocarpa* seeds (Henicka et al. 2006), germinated castor bean were relatively tolerant to salinity and exhibited unaffected by NaCl concentrations up to 80 mM NaCl (Ψs = -0.4 Mpa) (Fig 1). Other species are less tolerant to osmotic effects, such as the African savannah legumes *Combretum apiculatum*, *Colophospermum mopane*, *Acacia karroo* and *Acacia tortilis*, displaying reduced germination at Ψs < -0.3 MPa (Choinsky and Tuohy 1993). There are even more sensitive species such as *Guizotia abyssinica* (Lf) Cass. and *Atriplex halimus*, which exhibit reduced seed germination from a minimum exposure to NaCl solutions with Ψs < -0.1 MPa (Gordin et al. 2012; Shaygan et al. 2017). On the other hand, some plants, such as *Prosopis juliflora*, have an increased tolerance to osmotic effects and can tolerate an Ψs of -1.6 MPa without any reduction in germination (Iqbal et al. 2002).

Moderate salinity stress delays germination and limits the growth and development of plants and crop yield, but high salinity stress greatly reduces germination, causing plant death (Kranner et al., 2010; Sobhanian et al., 2011; Ibrahim, 2016). On this basis, castor bean seedlings began to suffer from the effects of moderate salt stress at an Ψs < -0.16 MPa, at which the harmful effects of salinity on hypocotyl growth began (Fig 2e), and the effects of high salt stress could be observed at an Ψs = -0.8 MPa, in which 98% of the germination castor bean seeds was inhibited (Fig 1b).

The largest reductions in growth occurred between Ψs values of -0.4 and -0.6 MPa, resulting in increased root/hypocotyl DM and root/hypocotyl length ratios of nearly 200% (Fig 2c, f). The most sensitive part of castor bean seedlings to the reduction of Ψs caused by NaCl was the hypocotyl, in which salinity began to reduce hypocotyl length from a NaCl solution with Ψs < -0.16 MPa (Fig 2e), whereas the growth and development of castor bean roots was maintained until Ψs of -0.36 MPa. This maintained growth an develoopment can be advantageous (Fig 2a, d). Plants with increased root growth are prone to increased resistance to the effects of water stress by providing conditions for plant growth and subsequent recovery of shoots, as the regulation of leaf expansion during the exposure to osmotic stress, depending on the amount of water in the roots (Toorchi et al. 2009). Azevedo Neto and Tabosa (2000) and Carvalho et al. (2012) also reported a greater sensitivity to salinity in the growth of shoots of maize and soybean seedlings, respectively, whereas the roots maintained their normal development.

A great reduction in the K^+/Na^+ content ratio in the roots and hypocotyls between Ψs values of 0.0 MPa and -0.2 MPa (Fig 3e, f) was the result of high Na^+ absorption, which was far greater than K^+ translocated from the endosperm (the main source of K^+) to the seedling (Fig 3a-d). In the hypocotyl, the higher the Ψs gradient was, the greater the Na^+ absorption (Fig 3b). However, there was a little resistance to increased Na^+ content in the roots due to a possible adjustment to higher K^+ content in the roots (Fig 3a, c). The compartmentalization of K^+ in the hypocotyl in response to salinity conditions in castor bean seedlings differs from the reduction in K^+ content in the shoots of seedlings of common salt-resistant species such as *Suaeda salsa* and *Triticum turgidum* spp. durum cv. Cappelli (Song et al. 2008; Spanò and Bottega, 2016).

The imbibition curve of castor bean seeds under control conditions (Ψs = 0.0 MPa) (Fig 4a) and the imbibition curves under salinity (Fig 4b, c, d) did not provide a clear distinction among the three stages of the model proposed by Bewley and Black (1978). The similar water absorption in the first 108 h of germination was occurred under Ψs values between 0 and -0.6 MPa (Supplementary Table 2) because the matric potential (Ψm) is largely responsible for the initial water absorption by the seed. Therefore, Ψs becomes responsible for leading the water flow to the seed over time (Nonogaki et al. 2007). According to Hasegawa et al. (2000), plants grown under salt stress absorb less water because they have less osmotic adjustment capacity, which strongly impacts growth and development. After 252 h, the castor bean seeds under an Ψs of -0.6 MPa showed a 2.6% reduction in water content compared with than under an Ψs of 0.0 MPa (Fig 4). Therefore, this reduction in water content seems to reduce growth and development.

Basavarajappa et al. (1991) highlighted the relationship between the physiological quality of seeds and both the organization of membranes and the leakage of substances by membranes. However, it was not possible to distinguish the quality of the seed priming treatments by the electrical conductivity test, as all priming treatments resulted in similar values (Fig 5a). This might imply that the organization of the castor bean seed cell membrane system does not depend on the type of salt treatment or the Ψs used in the conditioning, only the absorption of water (hydropriming). A similar result was reported in conditioned lettuce seeds. According to the authors, the electrical conductivity test can be useful in the evaluation of conditioned seeds, but it only indicated an increase in seed vigor of the conditioned seeds compared with those of the control treatment (Colete et al., 2004; Rodrigues et al., 2012).

Hydropriming and H_2O_2 priming did not produce good results in castor bean seeds but did in other species. Hydropriming is an effective tool for improving the quality of pyrethrum seeds (Lia et al., 2011). Ellouzi et al. (2017) attributed osmotic stress tolerance of *Cakile maritima* to H_2O_2 priming and reported that the H_2O_2 signal enables the plant to memorize and decode early signals rapidly activated, when plants are exposed to subsequent stress.

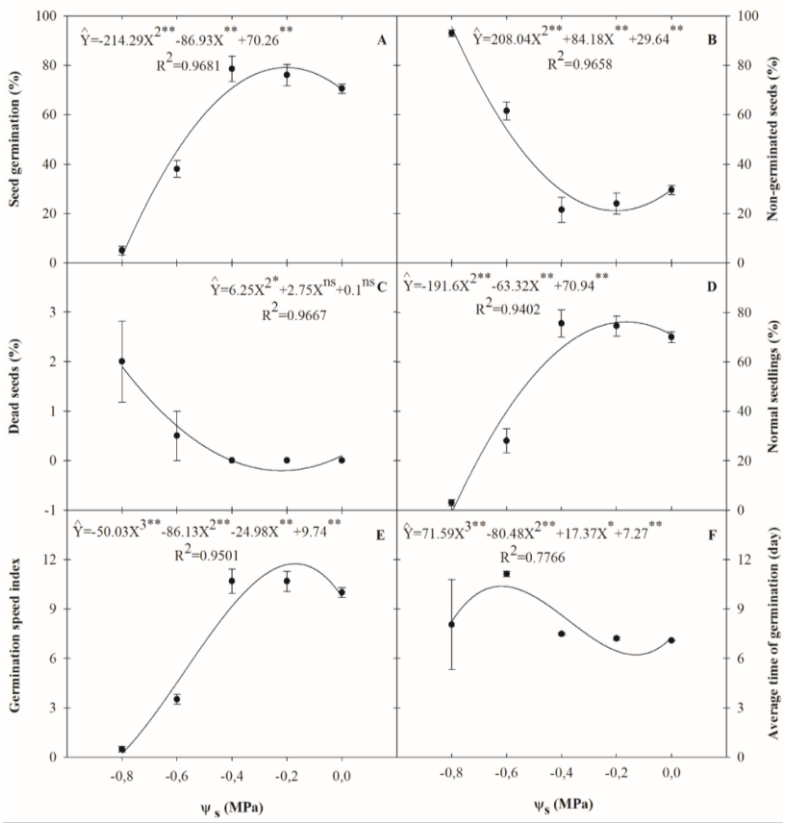

Fig 1. Germination test of castor bean seeds under different values of Ψs obtained with NaCl salt. Seed germination (a), non-germinated seeds (b), dead seeds (c), normal seedlings (d), germination speed index (e) and average time of germination (f). * $p < 0.05$; ** $p < 0.01$; ^{ns} $p > 0.05$.

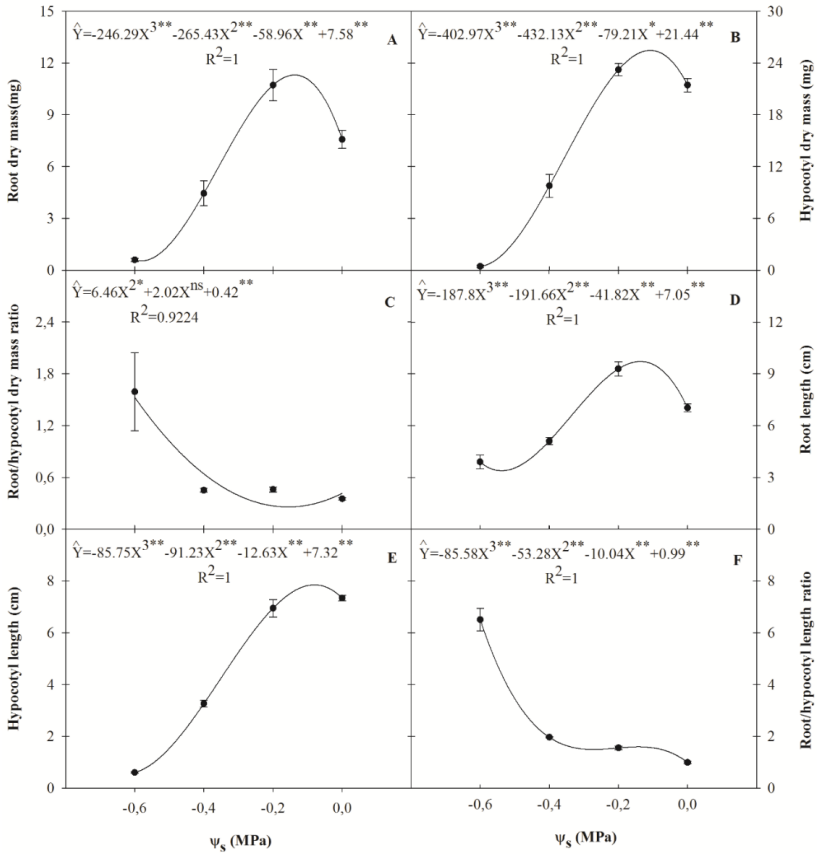

Fig 2. Growth and development of castor bean seedlings under different values of Ψs obtained with NaCl salt. Root DM (a), hypocotyl DM (b), root/hypocotyl DM ratio (c), root length (d), hypocotyl length (e) and root/hypocotyl length ratio (f). * $p < 0.05$; ** $p < 0.01$; ^{ns} $p > 0.05$.

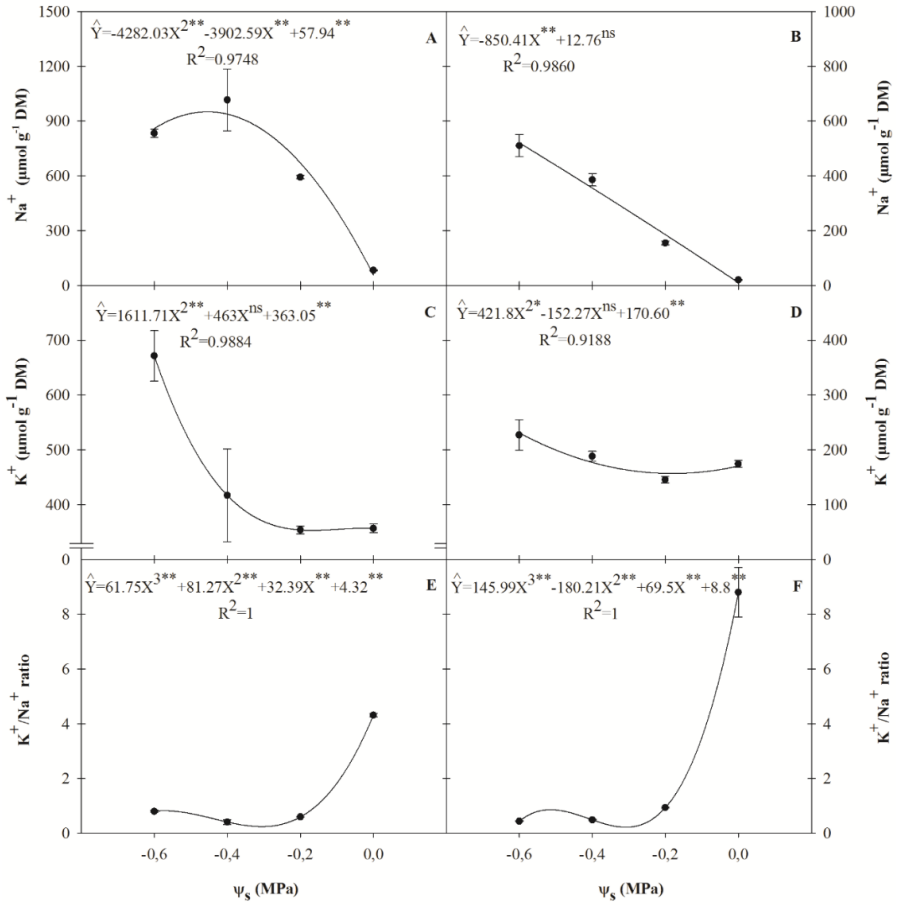

Fig 3. Ionic absorption in castor bean seedlings under different values of Ψs obtained with NaCl salt. Na^+ content in the roots (a) and hypocotyl (b), K^+ content in the roots (c) and hypocotyl (d), and K^+/Na^+ content ratio in the roots (e) and hypocotyl (f). * $p < 0.05$; ** $p < 0.01$; ^{ns} $p > 0.05$.

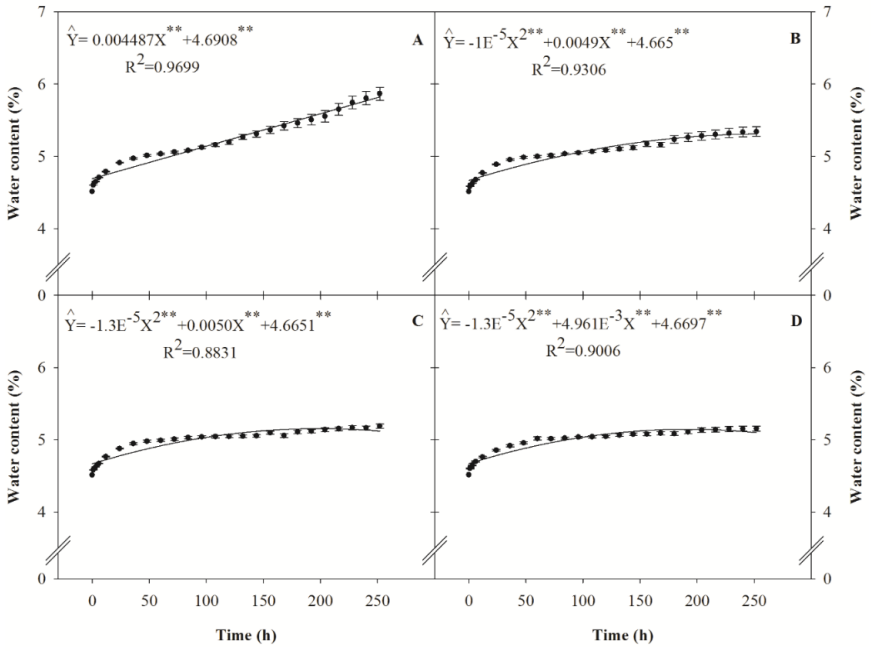

Fig 4. Water content in castor bean seeds during germination under Ψs values of 0.0 MPa (a), -0.2 MPa (b), -0.4 MPa (c) and -0.6 MPa (d) obtained with NaCl salt. * $p < 0.05$; ** $p < 0.01$; ns $p > 0.05$.

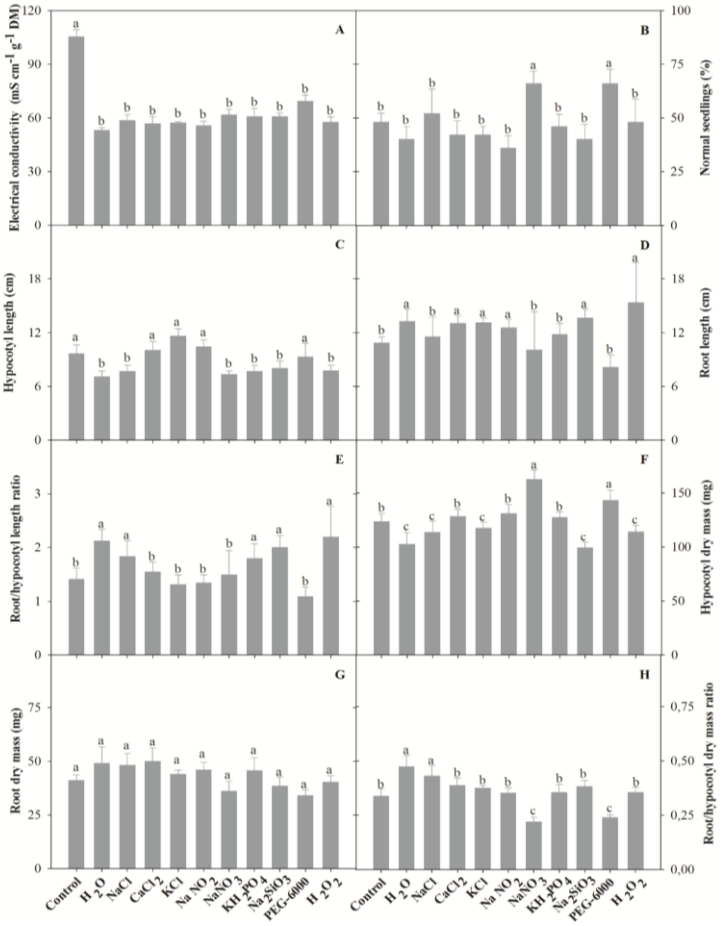

Fig 5. Selection of priming agents for castor bean seeds on non-salt conditions. Electrical conductivity (a), percentage of normal seedlings (b) as well as the hypocotyl length (c), root length (d), root/hypocotyl length ratio (e), hypocotyl dry mass (f), root dry mass (g) and root:hypocotyl dry mass ratio (h) of castor bean seedlings.

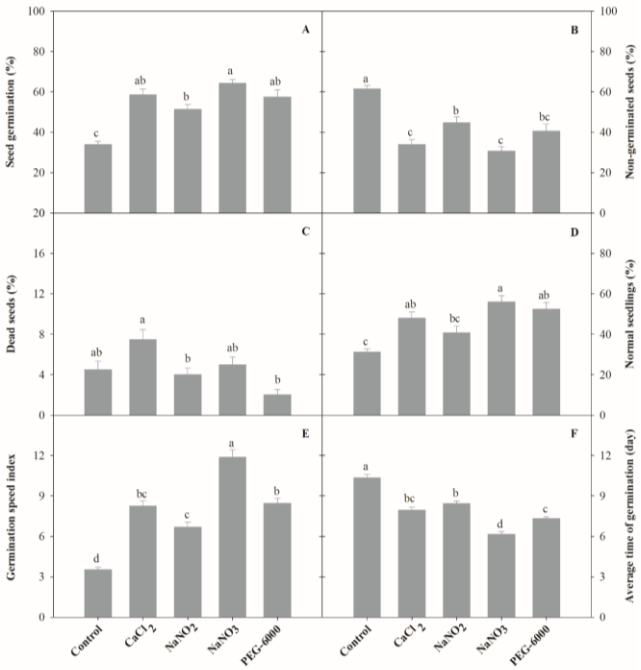

Fig 6. Germination test of unprimed and primed seeds of castor bean under saline conditions (Ψs of -0.6 MPa). Seed germination (a), non-germinated seeds (b), dead seeds (c), normal seedlings (d), germination speed index (e) and average time of germination (f).

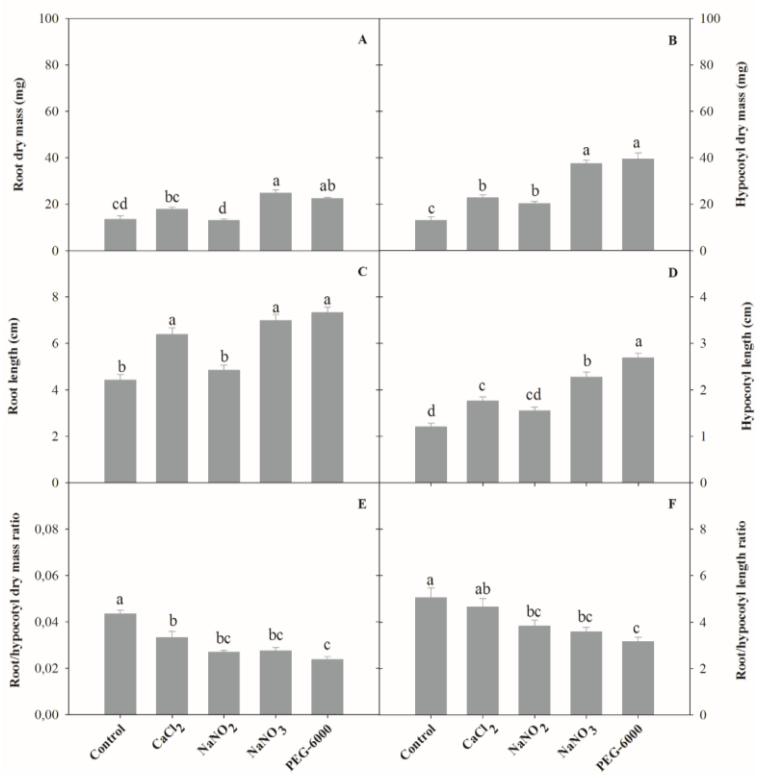

Fig 7. Growth and development seedlings of castor bean seedlings originating from unprimed and primed seeds under saline conditions (Ψs of -0.6 MPa). Root dry mass (a), hypocotyl dry mass (b), root length (c), hypocotyl length (d), root/hypocotyl dry mass ratio (e) and root/hypocotyl length ratio (f).

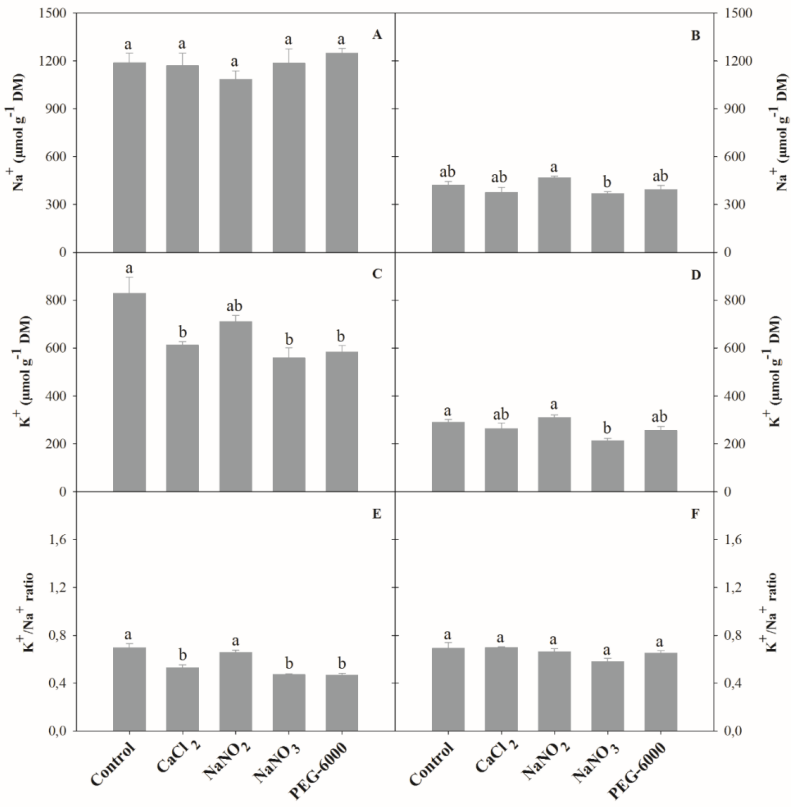

Fig 8. Ionic absorption in castor bean seedlings from unprimed and primed seeds under saline conditions (Ψs of -0.6 MPa). Na^+ content in the roots (a) and hypocotyl (b), K^+ content in the roots (c) and hypocotyl (d) and K^+/Na^+ content ratio in the roots (e) and hypocotyl (f).

stress. Hydropriming and H_2O_2 priming impaired hypocotyl growth and reduced hypocotyl DM (Fig 5c, f).

To select good priming agents for castor bean seeds, four priming agents with two typical responses were selected for evaluation under salinity: $CaCl_2$ and $NaNO_2$, which promoted root and hypocotyl elongation, and $NaNO_3$ and PEG-6000, which promoted normal seedlings and the accumulation of hypocotyl DM (Fig 5). These results indicate the importance of the Ψs during the priming process and the salt used for the Ψs to produce primed seeds with good qualities. Priming with PEG-6000 was more effective than hydropriming and not all salts improved the physiological qualities of the seeds.

Under salinity, $CaCl_2$ maintained the ability to promote seedling elongation, whereas $NaNO_2$ did not promote elongation as expected during the selection phase of good priming agents. All four selected osmopriming agents conferred benefits to seed vigor, although $NaNO_3$ and PEG-6000 priming were most beneficial to seed germination and to the establishment of castor bean plants (Figs 6, 7). In addition, promoting the percentage of normal seedlings and hypocotyl DM, the $NaNO_3$ and PEG-6000 priming treatments also improved seedling elongation. Theese treatments increased the percentage of normal seedlings by 72%; root and hypocotyl DM by 40% and 23%, respectively; and root and hypocotyl lengths by 38% and 25%, respectively (Fig 7). As reported in the conditioned seeds of *Cuminum cyminum* L., as well as in those of castor bean, PEG-6000 greatly improved root length (Rahimi 2013). The Ψs during the priming process is important for high quality seed priming.

Even though benefits can be optimal using the appropriate Ψs for seed priming, the benefits can still be improved by the action of NO_3^- derived from the salt $NaNO_3$. Atia et al. (2009), usied KNO_3 and reported increases of 35% and 330% in the percentage of germination in *Crithmum maritimum* seeded in media containing 100 mM and 200 mM NaCl, respectively. Positive effects on germination with the use of nitrogenous salts have also been documented in halophytes such as *Atriplex griffithii* (Khan et al., 2000), *Zygophyllum simplex* L. (Khan et al., 2002) and *Suaeda salsa* (Li et al., 2005) as well as in tolerant species such as *Lactuca sativa* (Hendricks et al., 1974) and *Avena fatua* (McIntyre et al., 1996).

The high percentage of non-germinated seeds under salinity may be related to the inhibition of germination caused by ABA. The ABA level increases in response to both drought and salt stress in the cell signaling process and involve both the perception and transduction of stress, regulating the gene expression of key enzymes for the biosynthesis and catabolism of ABA (Zhang et al., 2006). According to Atia et al. (2009), the increase in germination percentage caused by priming with KNO_3 or $NaNO_3$ is due to the action of the ion NO_3^-, which alleviates secondary dormancy by decreasing ABA levels in the seed (Rachedi-Ali et al. 2004). According to Copeland and McDonald (1995), this process is involved in breaking dormancy. In addition, NO_3^- can relieve the typical osmotic effect of salinity.

In addition, salinity can affect germination by reducing the N content available for embryo growth in seeds, as it delays the mobilization of protein stores, delaying the development of the new plant (Voigt et al., 2009). Atia et al. (2009) reported that NO_3^- derived from the salts $NaNO_3$ and KNO_3 increased germination and the germination speed index of both control and stressed seeds (100 mM NaCl). Abdelgabir et al. (2012) demonstrated that NO_3^- priming promoted the longitudinal growth of the roots of *Jatropha curcas* L. seedlings. These results were consistent with the $NaNO_3$ action on castor bean seeds by reducing the germination time by 4.2 days under salinity (Fig 1f). Thus, NO_3^- assimilation leads to the production of amino acids and nitrogenous compounds that promote growth by providing basic structural materials (Atia et al., 2009).

The accumulation of Na^+ both in the roots and hypocotyls was not affected by the osmopriming agents evaluated (Fig 8a, b). Therefore, the positive effects of osmopriming are not related to blocking the absorption or excluding Na^+ from plant tissues. Nevertheless, $NaNO_3$ priming alleviated the salinity effect and promoted a reduced K^+/Na^+ content ratio in the roots by reducing the K^+ content as well as salt-resistant species (Fig 8c, e). Therefore, the action of effective priming treatments such as $NaNO_3$ reaffirms that salinity tolerance is related not only to the presence of higher levels of Na^+ in the tissue but also to the tolerance of the tissue itself, as highlighted by Khan et al. (2015) and Kotula et al. (2015).

Materials and Methods

Plant materials and experimental design

Castor bean (*Ricinus communis* L. cv. BRS-Energia) seeds used in this research were provided by EMBRAPA, Campina Grande, Paraíba, Brazil. According to Sá et al. (2016), this cultivar is more salt tolerant than other commercial cultivars. The research was divided into three stages: (i) characterization of the effects of salt stress on seed germination and seedling establishment, (ii) selection of beneficial priming techniques for castor bean seeds, and (iii) evaluation of the action of the best castor bean priming techniques against salt stress. In all steps, the seeds were germinated in BOD chambers at temperature of 25 °C and a photoperiod of 12 h.

During the first stage, seeds were subjected to germination and vigor tests. The concentrations of Na^+ and K^+ in the roots and hypocotyls of seedlings as well as water uptake during germination were evaluated. The salt treatments included NaCl solutions with Ψs values of 0.0, -0.2, -0.4, -0.6 and -0.8 MPa. The Ψs of the NaCl solutions were calculated by the Van't Hoff equation and the concentration was adjusted via the purity of the reagent. The calculated values of Ψs were validated using an osmometer (Wescor Vapor Pressure 5500).

During the second stage, the seed primings treatments included H_2O; H_2O_2 at 10 mM (Gondim et al., 2011); and solutions of NaCl, $CaCl_2$, KCl, $NaNO_2$, $NaNO_3$, KH_2PO_4, $NaSiO_3$ and PEG-6000 with an Ψs of - 0.2 MPa, applied for 24 h. After applications, the seeds were dried in BOD chambers at 25 °C and 50% humidity to restore to the original mass of seeds. The Primed seeds were subjected to vigor tests without salt stress.

During the third stage, the seed priming treatments that showed the best results were subjected to germination and vigor tests, and the concentrations of Na^+ and K^+ in the roots and hypocotyls of seedlings under salt stress were evaluated. An Ψs of a NaCl solution that affected more than

50% of growth and development parameters was used to produce environmental salt stress.

Germination test

The germination test was performed using eight repetitions with 50 seeds each. Seeds were germinated on germitest paper moistened with the appropriate solution for the Ψs treatment. The first germination count was performed on the second day of soaking and additional daily counts were carried out until the 14th day. Seeds were considered dead if they presented signs of necrosis. Moreover, seeds were considered non-germinated seed if they did not germinate and showed no necrosis at the end of the test. Seedlings were considered normal if they did not lack parts and did not have organs. Germination speed index and average germination time were calculated in accordance with formulas defined by Maguire (1962) and Labouriau (1983), respectively.

Seed vigor test

Seedling growth and electrical conductivity tests were used to evaluate seed vigor. For the electrical conductivity test, seeds (five repetitions of 10 seeds per treatment) were weighed and then soaked in10 mL of deionized water in plastic cups under controlled conditions at a constant temperature of 25 ± 1°C for 24 h (Vieira and Krzyzanowski, 1999). Electrical conductivity was measured using a Micronal AJX-515 conductivity meter and the results were expressed in microSiemens per centimeter per gram of seed. Seedling growth was assessed by the length and dry mass of the hypocotyls and roots (Vieira and Krzyzanowski, 1999). Seeds were sown in paper rolls (eight repetitions of 10 seeds per treatment) and randomly arranged with the caruncle facing downward. Evaluations were performed only on the 14th day. Lengths were measured using a digital caliper. To determine the DM, normal seedlings were divided into hypocotyl and roots and dried in an oven with forced air circulation at a temperature of 105 ± 2 ° C for 24 h.

Imbibition curve

The seeds were placed on paper inside GERBOX to soak. For each NaCl solution treatment (0.0, -0.2, -0.4 and -0.6 MPa) 10 repetitions with 30 seeds each were performed. Fresh mass determination was performed prior to the beginning of imbibition (0 h) and at 1, 3, 6 and 12 h, followed by every 12 h until 252 h after the start of imbibition. At the time of each evaluation, fresh mass of each repetition was measured.

Determination of inorganic solutes

Inorganic solutes were extracted by homogenizing 10 mg of lyophilized leaf or root tissue ground with a mortar and pestle with the addition of 1 mL of deionized water. The homogenate was maintained under constant agitation for 1 h at 95°C and then centrifuged at 3,000 g for 15 min. The supernatant was kept at -25 °C until the determination of Na^+ and K^+ concentrations, which was performed using flame photometry according to the methods of Malavolta et al. (1989). Ionic concentrations were expressed as micromoles per gram of DM.

Statistical analysis

The experimental design was completely randomized. The data were subjected the Shapiro-Wilk test before being subjected to the analysis of variance (ANOVA F-test). For the first stage, the mean treatment values subjected to regression analysis ($p \leq 0.05$). For the second stage, the mean treatment values were separate by Scott-Knott's test ($p \leq 0.05$). For the third stage, the mean treatment values were separate by Tukey's test ($p \leq 0.05$). The software program Sisvar was used to analyze the data (Ferreira 2011).

Conclusion

Based on the results obtained in this research, we can conclude the following: (i) Salinity affects the germination of castor bean seeds under Ψs < -0.4 MPa, but the growth of the seedlings is affected by Ψs < -0.16 MPa. Furthermore, the hypocotyl was the most salt-sensitive organ in castor bean seedlings; (ii) The imbibition curve of castor bean seeds did not clearly exhibit the second phase or lag phase of the model proposed by Bewley and Black (1978); (iii) Seed soaking in a saline environment decelerates the water uptake with time of exposure to NaCl solution; (iv) The main effects of salinity on castor bean germination and seedling establishment can be partially reversed by priming under an Ψs -0.2 MPa. These benefits can also be maintained or enhanced depending on the type of salt during osmopriming; and (v) Among the osmopriming agents tested under saline conditions (Ψs = -0.6 MPa), $NaNO_3$ priming showed the best results by improving the physiological qualities of seeds and seedlings of castor bean cv. BRS-Energia.

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