Accumulation of organic and inorganic solutes in NaCl-stressed sorghum seedlings from aged and primed seeds

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ABSTRACT: Although it has been known that the seed's physiological potential affects its response to osmoconditioning and abiotic stresses, researches involving seed aging and priming associated to abiotic stresses are scarce. The aim of this work was to evaluate the role of seed priming on salt tolerance in sorghum seedlings from seeds with two vigor levels (aged or non-aged) and to verify the organic and inorganic solute contributions as osmoregulators in NaCl-stressed seedlings from aged and primed seeds. The combinations of two seed vigor levels (aged or not), two seed types (primed or not) and two salinity levels (exposed to NaCl at 100 mM or not) were evaluated. In low physiological quality seeds (aged seeds), priming provided an attenuation of salinity's negative effects (0 or NaCl at 100 mM) on seedling growth. The accumulation of Na^+ and Cl^- ions in NaCl-stressed sorghum seedling shoot from primed seeds indicate a plant osmotic adjustment induced by seed priming, which was efficient in reducing the osmotic stress caused by salinity. Proline was the main organic solute that contributed to osmoregulation in NaCl-stressed sorghum seedling shoot and its levels increased due to seed priming. Changes in inorganic and organic solute contents, in both shoot and roots, could have been induced by seed priming and as a function of salt stress tolerance, although the changes in these organs were poorly related to each other.
Keywords: *Sorghum bicolor*, accelerated aging, priming, salinity, osmoregulation

Introduction

Seed priming has been recognized as one of the most promising seed vigor treatments, consisting of seed-controlled hydration to facilitate initial germination process without radicle protrusion (Heydecker et al., 1973). Although the seed's physiological potential affects its response to osmoconditioning and abiotic stresses, researches involving seed aging and priming associated to abiotic stresses are scarce (Oliveira et al., 2010).

Salinity is one of the most severe abiotic factors affecting crop growth and productivity (Munns and Tester, 2008). Salt's negative effects on plant growth have initially been associated with the osmotic stress component caused by decreases in soil water potential and, consequently, restriction of water uptake by roots. Thereafter, plant roots take up and accumulate salt ions (usually Na^+ and Cl^-) that trigger toxic effects upon several biochemical and physiological processes of cell metabolism and can also cause a nutritional imbalance, affecting seedling growth (Zhu, 2002; Munns, 2002).

Plants usually develop mechanisms that allow the maintenance of growth under salt-stress conditions; these mechanisms tend to limit salt absorption into the plant in order to avoid toxic ion concentrations in the cytosol (Witzel et al., 2010). In general, salt-tolerant plants perform osmotic adjustments through the accumulation of osmotically active solutes, such as ions in the vacuole and organic solutes, which allow water uptake, cell enlargement and plant growth under salinity (Yamaguchi and Blumwald, 2005). The osmolytes that usually participate in osmotic adjustment vary between species and plant developmental stage and consist of sugars (glucose and fructose), alcohols (glycerol) and amino quaternary compounds (glycinebetaine and proline) (Ashraf and Harris, 2004).

Sorghum is distinguished by its moderate tolerance to salt stress (Lacerda et al., 2003, 2005) and could be considered as an alternative food crop production in soils exposed to salt stress. Thus, we aimed to evaluate the role of seed priming on salt tolerance in sorghum seedlings from seeds with two vigor levels (aged or non-aged) and to verify the organic and inorganic solute contributions as osmoregulators in NaCl-stressed seedlings from aged and primed seeds.

Materials and Methods

Plant material, artificial aging and seed priming: The initial seed lot of sorghum seeds [*Sorghum bicolor* (L.) Moench], cultivar IPA 1011 was divided into two sub-lots to create a difference in seed physiological potential. The first seed sub-lot was subjected to accelerated aging by distributing them in a single and uniform layer on an aluminum screen attached to a covered, transparent plastic box (11 × 11 × 3.5 cm) containing 40 mL of distilled water and maintained in a seed germination chamber at 43 °C with approximately 100 % relative humidity for 72 h (ISTA, 1999), and thereafter the seeds were dried on filter paper for 72 h at room temperature (25 ± 2 °C). The other sub-lot was not subjected to accelerated aging (non-aged seeds) and was used as control. After this procedure, 50 % of the seeds from each sub-lot were subjected to priming. For this treatment, seeds were placed in 400 mL beakers containing 200 mL of a 250 g L^{-1}

PEG-6000 solution with a -0.86 MPa osmotic potential at 15 ºC for 48 h, as reported previously (Foti et al., 2002; Patanè et al., 2009). Priming was performed in a seed germination chamber under continuous darkness and constant aeration to guarantee normal seed respiration. A narrow hose was attached to the bottom of each beaker; a porous stone attached at the end of the hose was connected to a small aquarium pump, which was responsible for pumping air through the solution (Bujalski and Nienow, 1991). After extensive rinsing with distilled water, seeds were dried on filter paper for 72 h at room temperature (25 ± 2 ºC). Thereafter, seeds were surface-sterilized for 5 min in a 1 % sodium hypochlorite solution and used in the greenhouse experiment. The moisture contents and germination percentage of seeds submitted or not to artificially aging and osmoconditioning are shown in Table 1.

Seed germination and seedling development conditions: The experiment was carried out in a greenhouse under natural conditions, where the mean air temperature varied between 24 ºC (minimum) and 36 ºC (maximum) with a mean temperature of 29 ºC and a 65 % mean air relative humidity. First, 200 seeds per treatment, divided into four replicates of 50 seeds, were sown in three rolls of neutral pH Germitest® paper moistened with distilled water or NaCl solution (100 mM) equivalent to 2.5 times the substrate dry mass, which were kept in seed germination chamber at 25 ºC and continuous darkness. Twelve 10-day-old seedlings of each replicate, selected for uniformity in size and form, were transferred to 10-L plastic pots that contained aerated, half-strength Hoagland's nutrient solution (control) or NaCl at 100 or 200 mM (salt-stress treatments). The nutrient solutions were replaced every five days, and the pH was adjusted to 5.5 with 0.1 M NaOH or 0.1 M HCl. The amount of water lost was replaced daily with distilled water.

Growth parameter determinations: Seedlings grown hydroponically for 14 days were harvested, the shoot and roots separated and then frozen and stored at -25 ºC; subsequent lyophilization resulted in the shoot (SDM) and root (RDM) dry mass. In addition, full dry mass (FDM) was obtained from sum of SDM and RDM, whereas SDM/RDM ratio was obtained from division of SDM by RDM. Growth parameters were evaluated for all treatments.

Table 1 – Moisture contents and germination (%) of seeds exposed or not to artificially aging and osmoconditioning.

Treatment	Non-aged	Artificially aged
	Moisture content	
Non-osmoconditionated	11.11 aA	11.92 aA
Osmoconditionated	11.93 aA	11.88 aA
	Germination percentage	
Non-osmoconditionated	89 aA	65 aB
Osmoconditionated	87 aA	67 aB

Means followed by the same capital letter in the lines and lowercase letter in the columns do not differ (Tukey, $p < 0.05$).

Organic and inorganic solute determinations: Lyophilized material (shoot and root) was extracted with 15 mL of deionized water and placed in a 100 ºC bath for 1 h. The extracts were then filtered and stored at -20 ºC for later analyses. The analyzed organic solutes were proline (Bates et al., 1973), free amino acids (Yemm and Cocking, 1955), soluble carbohydrates (Dubois et al., 1956) and proteins (Bradford, 1976). Na^+ and K^+ contents were determined by flame photometry, $Cl^–$ content was determined by visible spectrophotometry (Gaines et al., 1984) and $NO_3^–$ content was evaluated according to Cataldo et al. (1975). Organic and inorganic solutes were evaluated in shoot and roots of seedlings grown in nutrient solution without NaCl and with 100 mM NaCl. However, inorganic solutes were analyzed only in shoot, due to limited availability of root material.

Experimental design and statistical analysis: The experimental design was completely randomized, with four replicates per treatment, in a 2 × 2 × 2 factorial arrangement composed by interaction of two seed vigor levels (aged or not), two seed types (primed or not) and two salinity levels (exposed or not to NaCl at 100 mM). The data were subjected to analysis of variance, and the means were compared by Tukey's test at 5 % (Snedecor and Cochran, 1971).

Results and Discussion

Salinity negatively affected SDM ($p < 0.05$) (Table 2), with the most severe effects being observed in seedlings from the aged seed lot, in which the highest significant decreases related to salt increases were found (Figures 1A-B). In contrast, seedlings from the most vigorous seeds (non-aged seed group) did not show significant differences between 100 and 200 mM NaCl ($p < 0.05$). However, priming was responsible for the

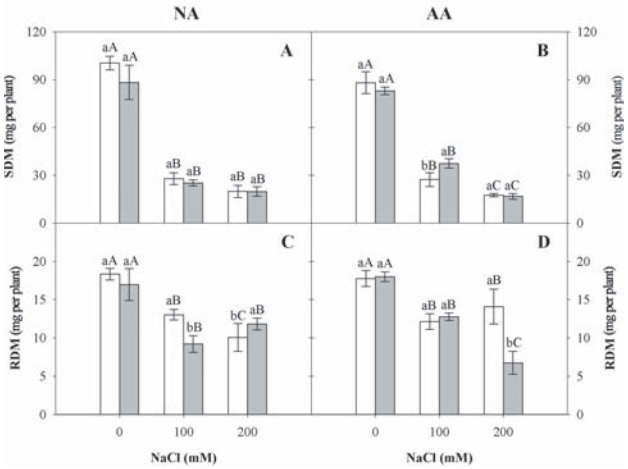

Figure 1 – Shoot (SDM, A and B) and root (RDM, C and D) dry mass of sorghum seedlings from non-aged (NA) or artificially aged (AA) seeds and subjected (■) or not (□) to seed priming under different salt levels.

The columns with distinct colors and equal lowercase let for seedlings under same NaCl levels, or columns with same capital letters for seedlings under NaCl levels, did not differ between treatments (Tukey, $p < 0.05$). The standard error is represented by vertical bars.

Table 2 – Three-way variance analysis (F values) of the effect of artificial aging (AA), seed priming (SP) and salt stress (SS) on growth parameters and inorganic and organic solutes in shoot and roots.

Traits	AA	SP	SS	AA × SP	AA × SS	SP × SS	AA × SP × SS
SDM	292.7**	98.1**	17.3**	46.8**	13.9**	8.5**	7.4**
RDM	34.9**	8.4**	10.9**	4.9*	1.5 n.s.	2.6 n.s.	1.7 n.s.
FDM	268.5**	87.6**	19.3**	42.3**	12.3**	8.0**	7.2**
SDM/RDM	107.9**	41.1**	1.7 n.s.	15.7**	6.7**	3.4*	2.4 n.s.
K^+_{shoot}	373.0**	1.4 n.s.	0.6 n.s.	1.2 n.s.	26.6**	0.3 n.s.	5.0 *
Na^+_{shoot}	187.8**	0.2 n.s.	2.2 n.s.	0.07 n.s.	2.5 n.s.	2.1 n.s.	2.1 n.s.
$NO^-_{3\ shoot}$	75.4**	0.4 n.s.	0.8 n.s.	1.35 n.s.	0.07 n.s.	0.3 n.s.	1.9 n.s.
Cl^-_{shoot}	155.8**	0.5 n.s.	18.9**	3.4 n.s.	0.03 n.s.	1.5 n.s.	0.58 n.s.
$Prol_{shoot}$	82.2**	5.3*	12.2**	9.2**	25.8**	1.3 n.s.	0.64 n.s.
$Prol_{root}$	19.9**	0.1 n.s.	4.1 n.s.	13.7**	0.31 n.s.	1.6 n.s.	1.7 n.s.
$N\text{-}amin_{shoot}$	22.3**	5.7*	4.3*	26.8**	6.3*	16.9**	2.5 n.s.
$N\text{-}amin_{root}$	16.8**	0.4 n.s.	0.3 n.s.	9.7**	6.7*	4.7*	0.2 n.s.
$Prot_{shoot}$	53.8**	0.1 n.s.	1.6 n.s.	7.2*	0.77 n.s.	0.0 n.s.	7.2*
$Prot_{root}$	0.6 n.s.	4.4*	0.2 n.s.	0.02 n.s.	5.2*	0.4 n.s.	0.47 n.s.
$Carb_{shoot}$	0.0 n.s.	0.0 n.s.	4.8*	1.9 n.s.	0.001 *	0.3 n.s.	9.5**
$Carb_{root}$	0.5 n.s.	10.8**	0.0 n.s.	19.5**	15.6**	5.6*	29.1**

SDM: shoot dry mass, RDM: root dry mass, FDM: Full dry mass, SDM/RDM: shoot and root dry mass ratio, Prol: proline, N-amin: soluble N-amino, Prot: soluble proteins, Carb: soluble carbohydrates. (n.s., *, ** non-significant or significant at $p \leq 0.05$ or 0.01, respectively).

SDM increase in seedlings grown at 100 mM NaCl. Indeed, the seedling performance improvement under abiotic stress has been reported to be a direct response of seed priming. Fanti and Perez (2003) reported that seed priming was responsible for an increased tolerance to water stress. Similarly, Patanè et al. (2009) demonstrated that osmoconditioning of sweet sorghum seeds attenuated the negative effects of salt and cold stresses during germination and primary root growth.

The RDM values varied as a function of the evaluated factors, and there was a significant interaction among them ($p < 0.05$) (Table 2). The highest decreases in RDM were observed for non-aged and unprimed seeds (Figure 1C) and for aged seeds that were subjected to priming (Figure 1D). This observation confirms previously published results that reported the variable effects of seed priming between species, cultivars and seed lots (Costa and Villela, 2006).

The priming of the most vigorous seeds mitigated the salt-stress effects in seedlings that were grown under excessive salinity (200 mM), which showed similar values to those seedlings subjected to FDM at 100 mM NaCl (Figure 2A). In aged seeds, we found beneficial effects of priming only at 100 mM NaCl (Figure 2B). These results confirm that priming is efficient in promoting the adaptation of sorghum seedlings to salt stress, probably through osmotic adjustment mechanisms. Similarly, Oliveira et al. (2010) reported in their study with seedlings from sorghum seeds that priming increases shoot growth.

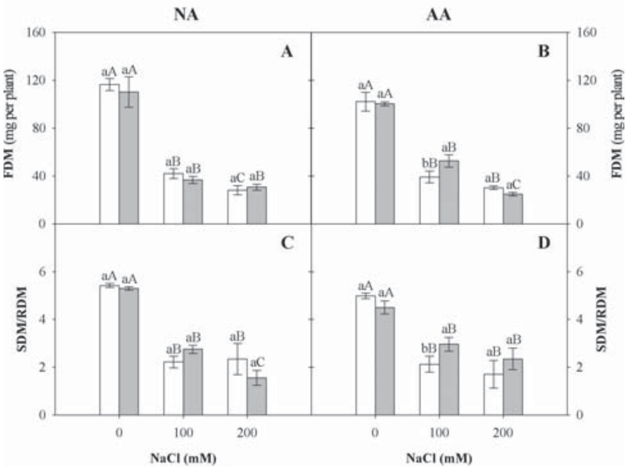

Figure 2 – Full dry mass (FDM, A and B) and shoot and root dry mass ratio (SDM/ RDM, C and D) of sorghum seedlings from non-aged (NA) or artificially aged (AA) seeds and subjected (▨) or not (☐) to seed priming under different salt levels.
The columns with distinct colors and equal lowercase letters for seedlings under same NaCl levels, or columns with same capital letters for seedlings under NaCl levels, did not differ between treatments (Tukey, $p < 0.05$). The standard error is represented by vertical bars.

Smaller SDM/RDM ratio decreases in seedlings from aged and primed seeds subjected to salt stress may have been one of the factors responsible for low sensitivity to salt stress (Figures 2C-D). A small decrease in root growth may result in surface area increase for nutrient and water uptake, thus minimizing the negative effects of the salt (Lacerda et al., 2003). Salinity affects biomass production and could modify the carbon partitioning among plant tissues because these responses are related to osmotic, toxic and nutritional effects (Munns and Tester, 2008).

Seedlings subjected to salt stress did not show increases in K^+ content as a function of PEG-6000 seed treatment (Figures 3A-B). This observation contrasts with the observed results in melon (*Cucumis melo* L.) seedlings from primed seeds with NaCl, which displayed high salinity tolerance due to increases in K^+ and Ca^{2+} content that induced osmoregulation by organic solute accumulation (Sivritepe et al., 2003). In addition, the differences between these studies may be related to osmotic adjustment of sorghum seedlings subjected to salt stress with NaCl, which was not verified in seeds treated with PEG-6000, as reported by Oliveira and Gomes-Filho (2009).

Na^+ content was highly increased by salinity in both aged and non-aged seeds subjected to osmoconditioning or not (Figures 3C-D). Na^+ content in shoot (Figures 3C-D) showed a trend that was inversely proportional to the K^+ content (Figures 3A-B). In this paper, it is suggested that a decrease in K^+ content in leaves must result from reduced uptake due to Na^+ competing for the same sites in root cell membrane transport systems (Marschner, 1995). Therefore, the highest K^+ decrease and Na^+ increase in seedlings from non-aged and primed seeds could be related to lower uptake efficiency, likely as a result of reduced root development (Figure 1C), enabling a lower K^+ absorption in relation to Na^+. In this context, the maintenance of more appropriate K^+ levels in photosynthetic tissues of sorghum seedlings from aged and primed seeds could be an important characteristic of salt-stress adaptation (Lacerda et al., 2005).

Seed priming did not change NO_3^- content in seedlings grown in nutrient solution without NaCl (Table 2). However, under salt-stress conditions, NO_3^- contents were significantly reduced in shoot, mainly in seedlings from non-aged and primed seeds (Figure 4A). These results suggest that salt stress negatively affects shoot NO_3^- accumulation, which corroborates the results by Meloni et al. (2004), who reported that salinity treatments in *Prosopis alba* also leads to shoot NO_3^- decreases.

The Cl^- content increased in seedling shoot from non-aged and artificially aged seeds subjected to salt stress, and these contents were highest as a function of seed priming (Figures 4C-D). As noted by Oliveira and Gomes-Filho (2009), the present study suggests that primed seeds could have shown higher ion accumulation than unprimed seeds, allowing an osmotic adjustment mainly in seedlings from high vigor seeds. This condition was verified by growth parameter improvements of seedlings cultivated under salt stress at 100 mM (Figures 2B and 5B). In this way, inorganic solute compartmentalization in the vacuole is an osmotic adjustment component necessary for salt stress acclimation and adaptation (Munns and Tester, 2008).

Seed priming caused an increase in proline content in shoot of salt-stressed seedlings, and there were no significant differences for this solute (Table 2 and Figures 5A-B). In contrast, proline content decreased in seedling roots from the most vigorous seeds (non-aged), which was attributed to osmoconditioning because under these conditions, there was a less pronounced proline decrease as function of seed priming in NaCl-stressed root (Figure 5C). In a study with *Jatropha curcas* L., Silva et al. (2009) observed a significant increase in proline concentration in the leaves of seedlings subjected to 100 mM NaCl, which was similar to what was observed in the present study for seedlings from aged seeds. Another important finding in our study was that priming treatment in low vigor seeds resulted in the highest proline concentration in seedling shoot grown at 100 mM NaCl (Figure 5B), which may represent an important mechanism for osmotic adjustment of these seedlings to salt stress.

In general, soluble amino-N contents increased in shoot due to NaCl, except in seedlings from non-aged seeds subjected to priming with PEG-6000, which showed a reduction of these compounds (Figures 6A-B). In contrast, there was no difference in the soluble amino-N contents in roots as a function of salt stress (Table 2). Praxedes et al. (2010) observed a similar result in cowpea subjected to salt stress. Thus, we suggest that these compounds do not participate effectively in the osmotic adjustment because we did not observe any correlation between

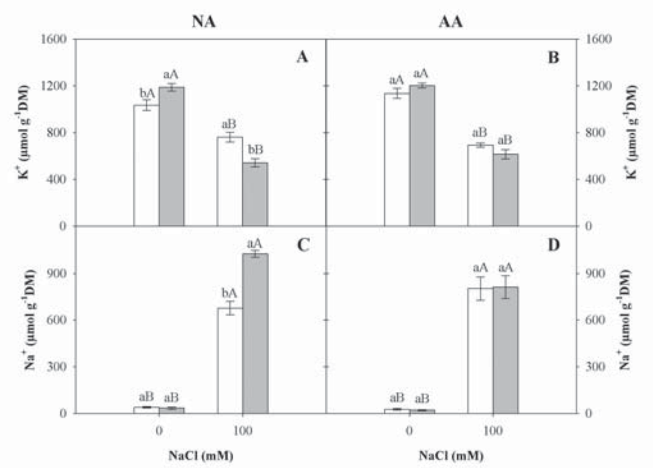

Figure 3 – Potassium (K^+, A and B) and sodium (Na^+, C and D) contents in sorghum seedling shoot from non-aged (NA) or artificially aged (AA) seeds and subjected (▨) or not (☐) to seed priming under salt levels.

The columns with distinct colors and equal lowercase letters for seedlings under same NaCl levels, or columns with same capital letters for seedlings under NaCl levels, did not differ between treatments (Tukey, $p < 0.05$). The standard error is represented by vertical bars.

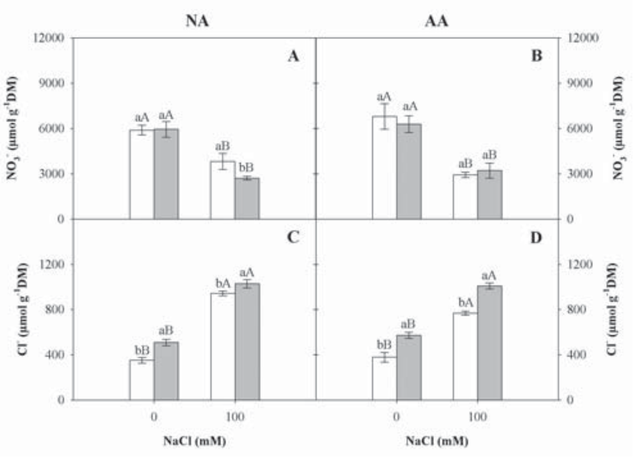

Figure 4 – Nitrate (NO_3^-, A and B) and chloride (Cl^-, C and D) contents in sorghum seedling shoot from non-aged (NA) or artificially aged (AA) seeds and subjected (▨) or not (☐) to seed priming under salt levels.

The columns with distinct colors and equal lowercase letters for seedlings under same NaCl levels, or columns with same capital letters for seedlings under NaCl levels, did not differ between treatments (Tukey, $p < 0.05$). The standard error is represented by vertical bars.

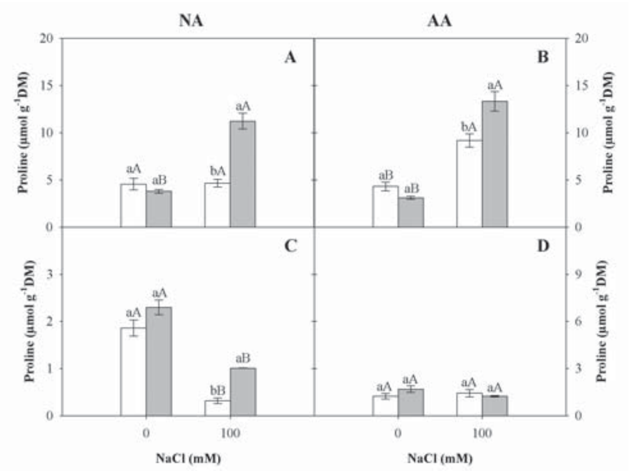

Figure 5 – Proline contents in shoot (A and B) and roots (C and D) of sorghum seedlings from non-aged (NA) or artificially aged (AA) seeds and subjected (■) or not (□) to seed priming under salt levels.

The columns with distinct colors and equal lowercase letters for seedlings under same NaCl levels, or columns with same capital letters for seedlings under NaCl levels, did not differ between treatments (Tukey, $p < 0.05$). The standard error is represented by vertical bars.

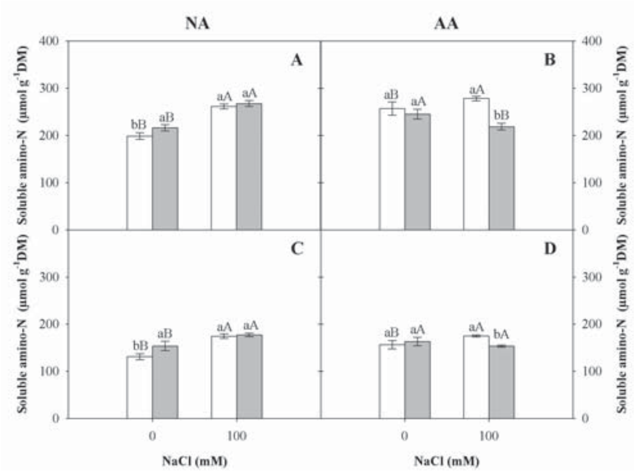

Figure 6 – Amino soluble-N contents in shoot (A and B) and roots (C and D) of sorghum seedlings from non-aged (NA) or artificially aged (AA) seeds and subjected (■) or not (□) to seed priming under salt levels.

The columns with distinct colors and equal lowercase letters for seedlings under same NaCl levels, or columns with same capital letters for seedlings under NaCl levels, did not differ between treatments (Tukey, $p < 0.05$). The standard error is represented by vertical bars.

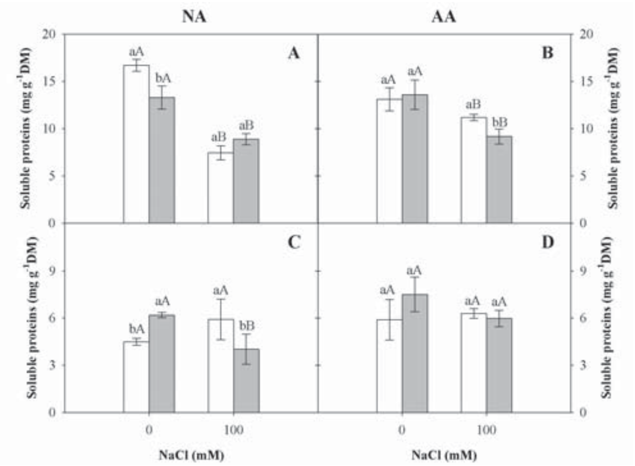

Figure 7 – Soluble protein contents in shoot (A and B) and roots (C and D) of sorghum seedlings from non-aged (NA) or artificially aged (AA) seeds and subjected (■) or not (□) to seed priming under salt levels.

The columns with distinct colors and equal lowercase letters for seedlings under same NaCl levels, or columns with same capital letters for seedlings under NaCl levels, did not differ between treatments (Tukey, $p < 0.05$). The standard error is represented by vertical bars.

the concentration of soluble nitrogen compounds in leaves and growth maintenance (Figures 2 and 6).

The soluble protein concentration was reduced in shoot subjected to NaCl, with the highest reductions observed in non-aged and unprimed seeds (Figure 7A). These results explain soluble amino-N increase in response to salinity in shoot of seedlings originated from non-aged seed sub-lot (Figure 6A). This behavior may be associated with a decrease in total protein in this treatment (Figure 7A) that normally would have been caused by degradation and/or reduction in protein synthesis. Generally, in seedlings subjected to salt stress, there is a decrease in total protein (Parida et al., 2004), although some proteins can have their synthesis stimulated (Mansour, 2000). In seedling roots originating from the non-aged seed sub-lot, priming provided an increase or decrease in total protein content due to NaCl absence or presence in the cultivation medium, respectively (Figure 7C), whereas in seedling roots from aged seeds, there were no significant influences by any of the analyzed treatments (Table 2 and Figure 7D).

Soluble carbohydrates were not affected in seedling shoot as a response to analyzed treatments (Table 2 and Figures 8A-B). This result agrees with those observed by Costa et al. (2003), in which no effect on soluble carbohydrates in leaves due to salt stress was found for seven cowpea cultivars with different tolerances to salinity. In roots, there was a variation in carbohydrate concentration, except in the treatment involving aged and primed seeds; these seedlings did not show changes in this variable as a function of salinity (Figures 8C-D).

In conclusion, priming of low physiological quality seeds (non-aged seeds) provided an attenuation of salinity negative effects (100 mM NaCl) on seedling growth. Na^+ and Cl^- accumulation in shoot of NaCl-stressed sorghum seedlings from primed seeds indicated an osmotic adjustment induced by seed priming, which was efficient in reducing the osmotic stress caused by salinity. Proline was the main contributing organic solute to osmoregulation in seedling shoot subjected to salinity, and its levels increased due to seed priming. Finally, the observed results suggest that changes in inorganic and organic solute concentration in both shoot and roots could be induced by seed priming as a function of salt stress tolerance caused by this treatment, although the changes in these organs are poorly related to each other.

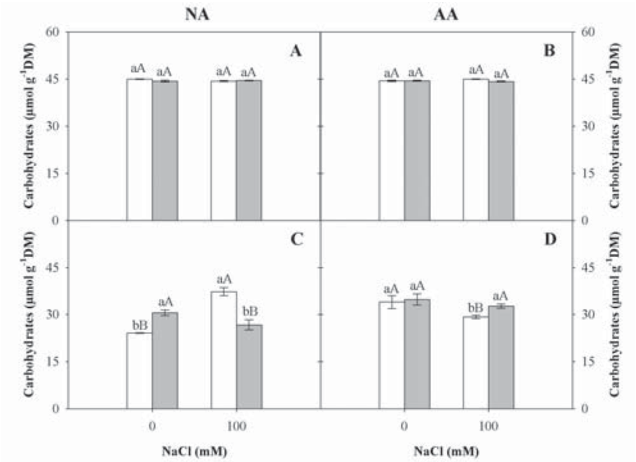

Figure 8 – Soluble carbohydrate contents in shoot (A and B) and roots (C and D) of sorghum seedlings from non-aged (NA) or artificially aged (AA) seeds and subjected (■) or not (☐) to seed priming under salt levels.

The columns with distinct colors and equal lowercase letters for seedlings under same NaCl levels, or columns with same capital letters for seedlings under NaCl levels, did not differ between treatments (Tukey, $p < 0.05$). The standard error is represented by vertical bars.

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