Revista Ciência Agronômica, v.37, n.1, p.110-112, 2006
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br ISSN 0045-6888

Proline accumulation in sorghum leaves is enhanced by salt-induced tissue dehydration[1]

Aumento da concentração de prolina em folhas de sorgo devido à desidratação pelo estresse salino

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Abstract – Seedlings of two forage sorghum genotypes differing in salt tolerance were subjected to 0 and 100 mM NaCl and inorganic solutes (Na^+ plus Cl^-) and proline contents in leaves were measured. Proline contents increased in the leaves of the seedlings after treatment with 100 mM NaCl, mainly in the salt sensitive one. Ion accumulation under saline conditions was higher in basal leaves, but proline accumulation occurred mainly in the apical leaf zones, which suffered higher dehydration. Proline accumulation did not seem to be a good indicator of plant tolerance to salinity but of tissue water dehydration which may occur during salt treatment.

***Index terms*:** salinity, *Sorghum bicolor*, solute accumulation

Resumo – Plântulas de dois genótipos de sorgo forrageiro com diferentes graus de tolerância ao estresse salino foram submetidas a 0 e 100 mM NaCl e mediram-se os teores de íons (Na^+ e Cl^-) e de prolina nas folhas. A concentração de prolina aumentou nas folhas das plântulas submetidas ao estresse com NaCl 100 mM, principalmente no genótipo sensível. O acúmulo de íons sob condições salinas foi maior na região basal das folhas, enquanto o acúmulo de prolina ocorreu principalmente na região apical, a qual sofreu maior grau de desidratação. O acúmulo de prolina não parece ser um bom indicador de tolerância ao estresse salino, sendo uma resposta ao processo de desidratação dos tecidos o qual pode ocorrer durante o tempo de exposição ao tratamento salino.

***Termos para indexação*:** salinidade, *Sorghum bicolor*, acúmulo de solutos.

[1] Recebido para publicação em 11/05/2005, aprovado em 30/08/2005.
Trabalho financiado pelo CNPq.
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Increase in leaf proline content is a common plant response to salt stress in many species (Hasegawa et al., 2000; Lacerda et al., 2001). This osmolyte may contribute to the osmotic equilibrium in the cell (Hasegawa et al., 2000) and to the stabilization of protein molecules and membranes (Hare et al., 1998) or it may be used as storage of carbon, nitrogen and energy for plant metabolism (Serrano and Gaxiola, 1994). Negative correlation between leaf proline accumulation and salt tolerance, however, has been observed in some species (Lutts et al., 1996; Lacerda et al., 2001). In a recent paper we showed that leaf proline content increased with salt treatment but this accumulation occurred especially in more injured leaf blade zones of the salt sensitive genotype (Lacerda et al., 2003). Since proline accumulation did not seem to be correlated with salt tolerance in sorghum genotypes and since proline accumulation may also be induced by water deficit (Claussen, 2005) the aim of this work was to test the hypothesis that the proline accumulation observed previously (Lacerda et al., 2003) was induced by tissue dehydration during salt treatment.

Seven days old seedlings of two sorghum genotypes (*Sorghum bicolor* (L.) Moench), one salt tolerant (CSF20) and another salt sensitive (CSF18), obtained from the Empresa Pernambucana de Pesquisa (IPA), Brazil, were transplanted into 2.5 liters pots (four seedlings per pot) containing aerated full strength Clark's nutrient solution, in the absence and presence of 100 ml NaCl (Clark, 1975). Salt treatment was obtained by adding NaCl to the nutrient solution at increments of 25 mM NaCl every 12 hr to reach the final concentration of 100 mM. The experiment was carried out in a growth chamber programmed to a temperature of 25 ± 3°C, relative humidity of 70± 10%, photoperiod of 16 hr and PFD of 230 μmol m^{-2} s^{-1} (Lacerda et al., 2003).

The experimental design was a completely randomized 2 x 2 factorial with four replicates per treatment. The data were subjected to analysis of variance, and the means were compared by Tukey's test at 5% probability.

Mature leaves 1 and 2, i.e., 2nd and 3rd leaf from the top, respectively, after 7 days of salt treatment, were collected, the leaf blades divided into three parts of approximately the same size and water, proline and Na^+ *plus* Cl^- contents determined at the basal and apical portions. Proline and inorganic ions were extracted in a 0,2% toluene aqueous solution (v/v), as suggested by Weimberg et al., 1984 and quantified by routine analytical methods (Bates et al., 1973; Gaines et al., 1984)

Regardless of leaf age and sorghum genotype, the basal zone of the leaf blade had higher water and Na^+ *plus* Cl^- but lower proline contents than the apical leaf blade zone (Table 1), similarly to previous results obtained by Lacerda et al. (2003). In the salt tolerant genotype salt stress affected neither water content nor proline accumulation in the basal and apical zones of the leaf 1 and in the basal zone of leaf 2. In the apical zone of leaf 2 of this genotype, however, water content reduced and proline accumulated about 3.6 times relative to control plants. In the salt sensitive genotype, also, there was no change in water content in the basal zones of leaves 1 and 2 but proline content increased about 2 times. In the apical zones of this genotype there was a reduction in water but an increase in proline contents in both leaf types. In this genotype the greatest tissue dehydration was observed in the apical zone of leaf 2 which coincided with the greatest proline accumulation.

Table 1 - Water, proline and Na^+ *plus* Cl^- contents at different parts of leaves of two sorghum genotypes seedlings exposed to NaCl, in nutrient solution.

Leaf type	NaCl	Tolerant		Sensitive	
	(mM)	Basal	Apical	Basal	Apical
		Water Content (%)			
Leaf 1[1]	0	88.2 a[2]	84.1 a	88.7 a	83.0 a
	100	88.1 a	82.5 a	88.6 a	80.9 b
	-	-	-	-	-
Leaf 2	0	85.2 a	82.9 a	85.2 a	81.3 a
	100	84.0 a	78.2 b	85.1 a	72.9 b
		Proline (mmol kg^{-1} dw)			
Leaf 1	0	3.11 a	5.23 a	3.24 b	6.94 b
	100	4.35 a	5.67 a	6.49 a	10.94 a
	-	-	-	-	-
Leaf 2	0	3.53 a	6.99 b	5.70 b	12.32 b
	100	4.24 a	25.04 a	11.69 a	175.51 a
		Na^+ *plus* Cl^- (mmol kg^{-1} dw)			
Leaf 1	0	247.3 b	209.7 b	271.2 b	142.4 b
	100	1987.8 a	572.1 a	2352.0 a	914.9 a
	-	-	-	-	-
Leaf 2	0	189.9 b	290.8 b	179.8 b	284.2 b
	100	1363.3 a	549.8 a	2155.8 a	1194.0 a

[1] Leaf 1 and Leaf 2: 2nd and 3rd leaves from the plant top, respectively.

[2] Means followed by the same letter in the columns, for each leaf type, do not differ statistically at 5% probability, by Tukey's test.

Correlations between tissue water status and leaf proline accumulation have been observed in salt-stressed sorghum seedlings as well as in other species subjected to other stressing factors (Nalini et al., 2002; Souza et al., 2004; Claussen, 2005), suggesting the existence of a common mechanism controlling proline biosynthesis. In this experiment, in the salt sensitive genotype the increase in proline content in the basal zone was much smaller than in the apical zone and occurred without a significant reduction in water content. It was not clear if this was caused by the higher ion accumulation at this leaf zone or to a proline translocation from other portions of leaf blade, as suggested by Weimberg et al. (1984) and Aziz et al. (1999).

Ion accumulation in the sensitive genotype under saline conditions was higher in basal leaves, but proline accumulation occurred mainly in the apical leaves, which suffered higher dehydration. Under saline conditions, both leaf types of the two sorghum genotypes, proline content was negatively correlated to water content (r = - 0,79** in leaf 1 and r = - 0,87** in leaf 2). So, proline accumulation did not seem to be a good indicator of plant tolerance to salinity but of tissue water dehydration which may occur during salt treatment.

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