BIOLOGIA PLANTARUM 49 (2): 285-288, 2005

BRIEF COMMUNICATION

Growth and ion uptake in *Annona muricata* and *A. squamosa* subjected to salt stress

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Abstract

The effects of treatment with NaCl (3, 100 and 300 mM) for 1, 2, 3 and 7 d on plant growth and ion accumulation were analyzed in 2-week and 8-week-old *Annona muricata* and *A. squamosa* plants. Fresh mass and root growth inhibition were directly related to the increase in salinity, particularly for *A. squamosa*. Two-weeks old seedlings were sensitive to 100 and 300 mM NaCl particularly after 7 d, whereas 8-week-old plants were shown to be more resistant to NaCl even at 300 mM NaCl. Na^+ and Cl^- mostly accumulated in young leaves. Our results suggest that *A. squamosa* is more sensitive than *A. muricata* to salt stress and that older seedlings of both species are more tolerant than younger seedlings.

Additional key words: abiotic stress, ion translocation, NaCl.

Plants may tolerate salt concentrations of approximately 100 mM NaCl without any damage (Serrano 1996). Salt stress involves two main components, the first is related to the decrease in the osmotic potential in the soil solution (ψ_s), and an ionic component, which is related to the accumulation of toxic ions and to the decrease of essential elements, such as K^+ and Ca^{2+}. An excess of salt can cause various modifications of plant metabolism, such as changes in phosphorylation state, inhibition of enzyme activity, or production of reactive oxygen species (Allakhverdiev *et al*. 2000, Blumwald *et al*. 2000).

The identification of salt tolerant species that can be cultivated under high salinity is particularly important (Latha *et al*. 2004). *Annonaceae* plant species are particularly important in semi-arid regions in terms of agro-economic aspects and food alternative and certain aspects related to salt stress have been studied (Marler and Zozor 1996). Salt stress in *A. squamosa* has been shown to decrease gas exchange, chlorophyll *a* fluorescence, and the uptake of water and nutrients, suggesting that *A. squamosa* is sensitive to salt stress (Marler and Zozor 1996). Ebert (1998) also observed a significant growth reduction of *A. cherimola* by NaCl, with both ions being accumulated in all tissues,

Received 19 April 2004, *accepted* 27 August 2004.
Acknowledgements: We wish to thank Prof. Peter Lea (Lancaster University, U.K.) for critical reading of the manuscript. The authors gratefully acknowledge the financial support of this work provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Grant no. 99/06429-0) and the Universidade Estadual de Feira de Santana (BA-Brazil). R.A.A. and F.C.G. received a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil).
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particularly in the leaves and shoots.

Although a significant amount of information is available concerning the effects of NaCl on higher plants (Fedina *et al.* 2003, Qasim *et al.* 2003, Smýkalová and Zámečníková 2003, Stoynova-Bakalova and Toncheva-Panova 2003, Azevedo-Neto *et al.* 2004, Viégas *et al.* 2004), a comparative study investigating tolerance/ sensitivity during the growth of Brazilian cultivated *Annona* species has not been carried out. We have investigated the responses of the two main *Annona* species to salt stress, at two different stages of development.

A. squamosa and *A. muricata* plants were grown hydroponically (Hoagland and Arnon 1950) in a glass-house for 2 and 8-weeks, at 38 % relative humidity, a temperature varying from 20 to 26 °C and a 12-h photoperiod, with the maximum irradiance reaching 2000 μmol m^{-2} s^{-1}. Two-week-old plants were subjected to 3, 100 and 300 mM NaCl, whereas 8-weeks-old plants were subjected to 100 and 300 mM NaCl for 1, 2, 3 and 7 d. The growth rate of the shoot and root [cm d^{-1}], and fresh and dry mass accumulation [g d^{-1}] were determined. The experimental design employed three to five replicates in a complete randomized block design and appropriate statistical analyses of variance and means were applied (Tukey test).

The Na^+ and Cl^- concentrations in the roots and shoots of control and NaCl treated plants, were determined after 7d of growth. The plants were extensively washed and the shoots and roots separated and dried at 60 °C for 4 d. The Na^+ content of dried homogenous powder was determined by perchloric nitric acid digestion and the Cl^- concentration was determined through the titration of silver nitrate and the results expressed as percentage of Na^+ and Cl^- of the dry mass.

The exposure to NaCl reduced plant growth in most of the parameters evaluated. However, these growth reductions varied depending upon the species, plant age and treatment. For both species, the 7 d treatment period for 2-week-old plants at 100 mM and 300 mM NaCl was lethal, causing wilt, followed by chlorosis and defoliation. In 2-week-old plants of *A. squamosa* (Table 1), a decrease in the shoot growth rate was observed at all NaCl concentrations, whereas the shoots of 2-week-old *A. muricata* plants and the shoots of the 8-week-old plants of both species, were not significantly affected (Table 1). The root growth rates of both the 2-week-old *A. squamosa* and *A. muricata* plants were affected by all NaCl concentrations. However, in 8-week-old plants only the roots of *A. muricata* exhibited a decreased growth rate and only at 300 mM. The rate of increase of the fresh mass of the *A. squamosa* plants was more sensitive to NaCl than *A. muricata*, particularly for the 2-week-old plants (Table 1). Although there was a salt induced decrease in the fresh mass of *A. squamosa* plants, the same was not verified for the dry mass of the 8-weeks-old plants after the 7 d of treatment (data not shown), thus suggesting that the reduction of growth rate observed in the plants occurred due to a reduction in water uptake.

An increase in the concentrations of both Na^+ and Cl^- was observed in the roots and young leaves of *A. squamosa* and *A. muricata*, after NaCl treatment (Table 2). In mature leaves, there was accumulation of Cl^- in both plants but little of Na^+. *A. muricata* and *A. squamosa* exhibited more significant accumulation in young leaves when treated with 100 mM NaCl for 7 d (Table 2). Cl^- was present in higher concentration in all tissues of 100 mM NaCl treated plants when compared to control plants. Considering all the tissues together, there was a greater accumulation of Na^+ and Cl^- in *A. muricata* than in *A. squamosa*.

Salt stress has been shown to cause a reduction in the water potential of the soil-plant system, thus making difficult for the plant to take up water. Induced water deficit is responsible for the reduction in plant growth (Soares *et al.* 2001) and leaf area (Bernstein *et al.* 1993). In a previous report by Ebert (1998), the growth of 2-year-old *A. muricata* plants subjected to 30 and 60 mM NaCl for 8 weeks was not affected by the NaCl treatment as was observed with other *Annona* species. In a similar way in this study, *A. muricata* was more resistant to the action of NaCl, when compared to *A. squamosa*. Based upon Munns (2002), when plants are subjected to salt stress, there is a fast and transient reduction in growth, followed by a gradual recovery at a new growth rate. This temporary effect is due to variations in the water relations.

Salt stress and other abiotic stresses can also promote a series of metabolic and anatomic alterations in the leaf and root systems, which may lead to the inhibition of growth (Sam *et al.* 2003, Sousa *et al.* 2003, Vitória *et al.* 2003). Hossain *et al.* (2004) reported that the root formation of *Chrysanthemun morifolium*, submitted to several NaCl concentrations was inhibited due to a decrease in mitotic activity. Alterations in the activity of antioxidant enzymes have also been observed as a result of the oxidative stress induced by NaCl (Khan 2003, Panda and Upadhyay 2004). The oxidative stress has also been shown to contribute to the reduction of growth (Azevedo *et al.* 1998, Vitória *et al.* 2001, Sairam *et al.* 2003, Medici *et al.* 2004).

The susceptibility of plants to high concentrations of NaCl is demonstrated by leaf necrosis, growth reduction, and loss of fresh mass. These effects are probably due to an excessive increase and translocation of Na^+ and Cl^- ions to the leaf tissue (Munns and Termaat 1986), which causes alterations in the osmotic potential (Ho and Adams 1989). Studies conducted by Lorenzo *et al.* (2000) reported the effects of Na^+ ions on the mineral nutrition of hybrid roses. Their results showed that the elongation of the rose stems was inversely proportional to the presence of sodium ions. The excess of an element can

Table 1. The growth rate of shoots and roots, and increase in fresh mass of 2-week-old and 8-week-old plants of *A. squamosa* and *A. muricata* subjected to 3, 100 and 300 mM NaCl for 1, 2, 3 and 7 d. Data were analyzed using the Tukey´s test and means with same letters in the same column were not significantly different at 5 %, $n = 5$.

Parameter	Age [weeks]	NaCl [mM]	*A. squamosa* 1 d	2 d	3 d	7 d	*A. muricata* 1 d	2 d	3 d	7 d
Shoot growth rate [cm d^{-1}]	2	0	10.50a	12.00a	12.46a	13.10a	13.83a	11.83a	11.50a	13.70a
		3	10.00b	9.60b	9.60b	10.30b	13.37a	12.16a	11.60a	13.20a
		100	9.00b	8.75b	9.33b	-	13.27a	13.16a	10.60a	-
		300	8.32b	7.75b	8.50b	-	12.44a	12.16a	10.16a	-
	8	0	12.40a	12.70a	12.80a	11.30a	16.30a	16.25a	16.40a	16.40a
		100	13.10a	12.80a	13.40a	11.80a	15.70a	15.40a	15.20a	15.70a
		300	12.40a	11.90a	11.60a	10.50a	14.90a	14.50a	14.90a	14.30a
Root growth rate [cm d^{-1}]	2	0	15.66a	16.10a	17.33a	19.20a	14.12a	15.16a	15.45a	15.07a
		3	16.33a	15.80a	14.75b	15.80b	13.55a	13.16b	13.50b	13.30b
		100	13.66a	15.25a	12.00b	-	13.55a	13.00b	13.66b	-
		300	14.50a	15.16a	14.00b	-	12.77a	12.83b	12.66b	-
	8	0	11.70a	10.80a	11.30a	10.60a	13.70a	13.90a	13.90a	13.70a
		100	12.70a	12.30a	13.00a	12.90a	12.80ab	12.80ab	12.75ab	12.70ab
		300	13.10a	12.20a	11.90a	11.10a	12.20b	11.40b	11.35b	11.70b
Fresh mass accumulation [g d^{-1}]	2	0	0.98a	1.03a	1.02a	-	1.26a	1.13a	1.35a	-
		3	0.88ab	0.84ab	0.90ab	-	1.21a	1.25a	1.14b	-
		100	0.79ab	0.73b	0.77ab	-	1.18a	1.24a	1.14b	-
		300	0.70b	0.65b	0.63b	-	1.10a	1.25a	1.04b	-
	8	0	1.30a	1.20a	1.20a	1.10a	1.42a	1.36a	1.42a	1.42a
		100	1.30a	1.30a	1.20a	1.10a	1.48a	1.35a	1.45a	1.42a
		300	1.10a	1.10a	0.90b	0.60b	1.48a	1.45a	1.38a	1.45a

Table 2. Percentage of Na^+ and Cl^- in the dry mass of roots and young and mature leaves from 8-week-old plants of *A. squamosa* and *A. muricata* subjected to 100 mM NaCl for 7 d. Data were analyzed using the Tukey´s test and means with same letters for the same ion, were not significantly different at 5 %, $n = 3$.

Species	Tissue	Na^+ [%] control	100 mM	Cl^- [%] control	100 mM
A. squamosa	root	0.21a	1.34c	1.20a	2.47b
	young leaves	0.26a	2.56c	1.90ab	3.68c
	mature leaves	0.74b	1.06c	0.94a	2.74b
A. muricata	root	0.39b	3.75c	1.50a	2.92b
	young leaves	0.08a	2.56c	1.09a	3.68c
	mature leaves	0.51b	0.66b	1.90ab	4.88c

damage plants in several different ways. The toxicity can result in a low water potential as seen in saline soils. Moreover, each element can cause specific effects. Plant growth occurs within certain salt concentration limits in the medium, while outside these limits, the plant is not able to survive (Asch *et al*. 1999).

Mature leaves of both species and in particular *A. muricata*, accumulated a considerable absolute amount of Cl^-, however, the relative Na^+ increases, particularly in young leaves, were much higher when compared to Cl^- (Table 2). Evidence for the restriction of the translocation of Na^+ to the leaves has been provided by Ramoliya *et al.* (2004). Ebert (1998) reported a greater amount of Cl^- in all parts of *A. muricata* with the exception of the roots, while in *A. cherimola* less Cl^- was present in young leaves. The control values of Na^+ and Cl^- concentrations observed in this study are very close to those reported by Ebert (1998) when analyzing *A. muricata*. A greater accumulation of Cl^- was observed in our study which is likely to be due to the higher NaCl concentrations used in our experiments. Furthermore, the accumulation trends were also very similar in both studies, indicating a direct relationship between higher accumulations of the ions with increase NaCl concentration in the growth solution. Storey and Walker (1999), who analyzed *Citrus*, stated that the injuries caused by NaCl were mainly burning and defoliation, which are directly associated with the accumulation of Na^+ and Cl^- in the leaves. This could explain the defoliation of the 2-weeks-old plants when subjected to a 7-d treatment period (data not shown). Furthermore, although *A. muricata* grew better than *A. squamosa*, it appeared to accumulate more Na^+ and Cl^-.

The salt concentrations and the treatment periods tested for young plants showed a more significant decrease in the fresh mass in *A. squamosa* plants, whereas mature plants of both species did not show major alterations due to the presence of NaCl. In conclusion, the results suggest that *A. squamosa* is more sensitive than *A. muricata* to NaCl, but only in the early stages of plant development.

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