Stomatic behaviour and leaf water potential in young plants of *Annona squamosa* submitted to saline stress

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Abstract — Introduction. In saline soils, the water absorption process of the plants is negatively affected, due to the permeability of the roots, leading to hydric stress. Plants under saline stress close their stomas earlier than plants not in these conditions; this causes an increase in stomatal resistance due to the decrease in water potential. The aim of the present research was to detect alterations in the stomatic behaviour and leaf water potential in young plants of *Annona squamosa* submitted to saline stress. **Materials and methods**. Sixty-day-old seedlings of *A. squamosa* were acclimated for 15 days in a greenhouse, before being submitted to different saline treatments. Five treatments (T_0 = 0 mM; T_1 = 25 mM; T_2 = 50 mM; T_3 = 75 mM and T_4 = 100 mM of NaCl in a nutritive solution) were applied with six replications. The evaluations of the stomatic response occurred on the 20th, 30th, 40th, 60th, 80th and 90th days after the beginning of the saline treatments. The transpiration measures, diffusive resistance and leaf temperature were registered in the first mature leaf. The photosynthetically active radiation, relative humidity and air temperature within the greenhouse were simultaneously assessed 60 and 90 days after the beginning of treatments; then the leaf water potential (R_{leaf}) was also measured. **Results**. During the time of the experiment the leaf temperature and its difference from the air temperature was not affected by NaCl levels, but only 90 days after the beginning of the treatments the values observed in the control plants were lower than those observed in the 25 mM treatment. The diffusive resistance after 40 days was increased in all NaCl treatments. The transpiration, independent of treatment, was reduced by 44.1% (at 60 days) and 13.4% (at 90 days) with the 100-mM treatment compared with the control treatment. The increment in NaCl induced a decrease in R_{leaf} in all treatments, but an equal reduction was observed for each treatment after 60 and 90 days. **Conclusion**. The NaCl levels affected the transpiration and leaf water potential; however, the leaf water potential showed an equal reduction in all treatments on the 60th and 90th days.

Brazil / *Annona squamosa* / soil salinity / osmotic stress / transpiration / stomata / water potential

Résistance stomatique et potentiel hydrique foliaire chez de jeunes plants d'*Annona squamosa* soumis à des stress salins.

Résumé — Introduction. Dans les sols salins, le processus d'absorption de l'eau par les plants est gêné, du fait de la perméabilité des racines, ce qui entraîne un stress hydrique. Les plants soumis à un stress salin ferment leurs stomates plus tôt que les plants en conditions normales ; cela augmente la résistance stomatique du fait de la diminution du potentiel hydrique. L'objectif de nos recherches a été de détecter des changements du comportement stomatique et du potentiel hydrique chez de jeunes plants d'*Annona squamosa* soumis à un stress salin. **Matériel et méthodes**. Des jeunes plants d'*A. squamosa* âgés de 60 jours ont été acclimatés en serre pendant 15 jours, avant d'être soumis à différents traitements salins. Cinq traitements [(0, 25, 50, 75 et 100) mM de NaCl ajoutés à une solution nutritive] ont été appliqués avec six répétitions. La réponse des stomates a été évaluée (20, 30, 40, 60, 80 et 90) jours après le début des traitements salins. Les mesures de transpiration, la résistance à la diffusion et la température des feuilles ont été enregistrées dans la première feuille adulte. Le rayonnement photosynthétiquement actif, l'humidité relative, la température de l'air dans la serre et le potentiel hydrique dans la feuille ($R_{feuille}$) ont été simultanément évalués (60 et 90) jours après le commencement des traitements. **Résultats**. Durant le temps de l'expérience, la température de la feuille et sa différence avec la température de l'air n'ont pas été affectées par des niveaux de NaCl ; ce n'est que 90 jours après le début des traitements salins que les valeurs observées dans les plants témoins se sont révélées inférieures à celles relevées dans le traitement à 25 mM de NaCl. Après 40 jours d'expérimentation, la résistance à la diffusion a été augmentée dans tous les traitements. La transpiration, indépendante du traitement, a été réduite de 44,1 % (à 60 jours) et de 13,4 % (à 90 jours) dans les plants du traitement à 100 mM de NaCl par rapport à ceux du traitement témoin. L'augmentation du taux de NaCl a induit une diminution de $R_{feuille}$ dans tous les traitements, mais, après (60 et 90) jours, une réduction égale a été observée pour chacun des traitements. **Conclusion**. Les différentes concentrations de NaCl ont affecté la transpiration et le potentiel hydrique de la feuille, toutefois la réduction de $R_{feuille}$ observée après 60 et 90 jours d'expérimentation a été comparable d'un traitement à l'autre.

Brésil / *Annona squamosa* / salinité du sol / stress osmotique / transpiration / stomate / potentiel hydrique

1. Introduction

The sweet apple (*Annona squamosa* L.), also known in Brazil as "*fruta do conde*", belongs to the Annonaceae family. It originates from the West Indies and was introduced into Brazil in 1626 [1]. It is a rustic plant which grows and produces in different types of soils, presenting an excellent adaptation to the climatic conditions of the northeastern, semi-arid region of Brazil [2].

Many species of fruit tree are being introduced into the northeastern region of Brazil, mainly to increase the exports, but this growth is being conditioned by irrigation because the fruit tree needs a good supply to develop and produce satisfactorily [3].

According to Melloni *et al.* [4], the effects of salinity on plant growth are considered important by researchers in countries where arid and semi-arid areas can be found. When the soil shows a high concentration of salts, the water absorption process of the plants is negatively affected, due to the permeability of the roots, leading to hydric stress. Plants under salinity stress close their stomas early in comparison with plants not in these conditions; this causes an increase in stomatal resistance due to the decrease in water potential [5].

Although it saves water, such a mechanism also promotes the reduction of the photosynthesis level and, therefore, affects the growth of the tree [6, 7].

For these reasons, the study of tolerant species and varieties to salt stress is decisive for successful agricultural planning in areas where the reduction of salinity in the soil is not economically viable [8]. Azevedo Neto [9] also states that the use of plants that tolerate saline soils is interesting because it provides a low cost and an immediate recuperation of investment.

The aim of the present research was to detect alterations in the stomatic behaviour and leaf water potential in young plants of *Annona squamosa* submitted to saline stress.

2. Materials and methods

The research took place between December 2001 and June 2002 in a greenhouse of the Laboratory of Plant Physiology of the Biology Department of the Rural Federal University of Pernambuco (Brazil).

The plantlets were obtained from seeds given by the *Empresa Pernambucana de Pesquisa Agropecuária*. These plantlets were submitted to a pre-germinative treatment, immersed in water for 24 h, then planted in polyethylene trays (3-L cleansed sand), approximately 3 cm deep. After 60 days, 30 plantlets, which underwent a selection of height and health, were transplanted into 1.4-L polyethylene trays, containing the same substrate. After an acclimatisation period of 15 days in a greenhouse, receiving a daily Hoagland and Arnon [10] nutrient solution, the differentiation of the saline treatments began. The various steps of the experiment were defined as follows: five treatments (T_0 = 0 mM; T_1 = 25 mM; T_2 = 50 mM; T_3 = 75 mM and T_4 = 100 mM of NaCl in a nutritive solution) with six replications. The salinisation was obtained by the daily addition of 50 mL of nutrient solution with a mix of the respective NaCl levels. The evaluations of the stomatic response occurred on the 20th, 30th, 40th, 60th, 80th and 90th days after the beginning of the saline treatments. To do this, a steady state porometer was used (LI 1600 model, Licor Inc., Nebraska, USA) between 12:00 h and 13:00 h.

The transpiration measurements (E), diffusive resistance (Rs) and leaf temperature (T_{leaf}) were registered in the first mature leaf. The photosynthetically active radiation (PAR), relative humidity (RH) and air temperature within the greenhouse (T_{air}) were simultaneously assessed 60 and 90 days after differentiation; the leaf water potential (R_f) was measured from the leaves which were used for the porometric measures, with the help of a Scholander pressure chamber [11].

After collecting the data, ANOVA statistical analysis was undertaken and the averages compared using the Tukey´s test with a 5% probability level.

3. Results and discussion

The climatic conditions within the greenhouse varied from (362 to 450) $\mu mol \cdot m^{-2} \cdot s^{-1}$

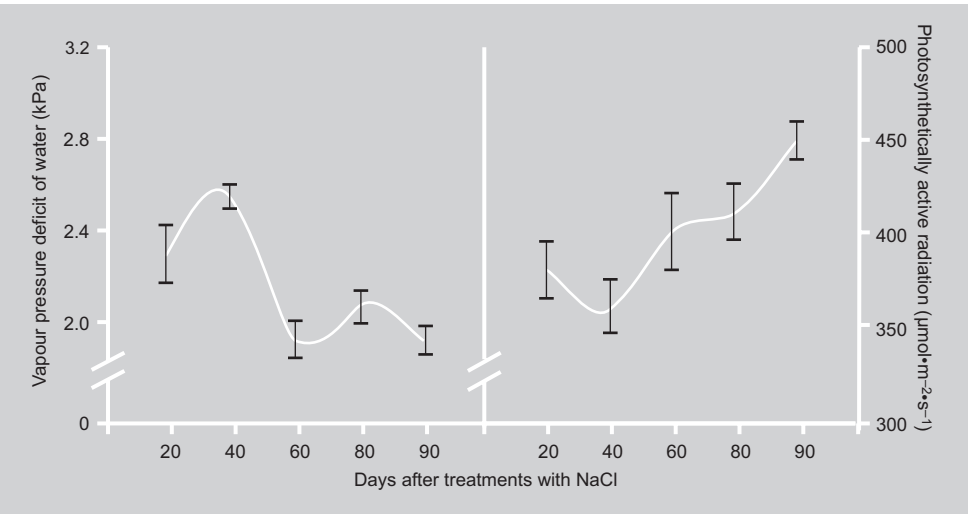

Figure 1.
Climatic conditions within the greenhouse used to assess the effect of different NaCl levels on the stomatic behaviour and leaf water potential of seedlings of *Annona squamosa* (Brazil).

for the photosynthetically active radiation and from (2,56 to 1,94) kPa for the vapour pressure deficit of water (*figure 1*).

The temperature difference between the leaf and the air ($T_{leaf} - T_{air}$) during the experiment did not show a definite pattern (*figure 2*). Nevertheless, on the 90th day it showed lower rates in the control plants and 25-mM treatment plants (0.50 °C and 0.58 °C, respectively) than in the other NaCl treatments. When observing the effects of various NaCl levels on gaseous exchanges in mangaba (*Hancornia speciosa*) plantlets (a tropical fruit tree), Albuquerque *et al.* [12] did not register any significant differences in the ($T_{leaf} - T_{air}$) measurement after a 4-week treatment.

The same behaviour was registered for leaf temperature, which, after the 90th day of differentiation, presented the highest values when submitted to the strongest treatments (31.58 °C, 31.82 °C and 32.02 °C for the 50-mM, 75-mM and 100-mM treatments, respectively) (*figure 2*). Such behaviour is similar to that described by Nogueira [3] in her studies of NaCl effects on acerola (*Malpighia emarginata* DC) plantlets, when cultivated in a hydroponic medium. This author verified an elevation of the temperature level as the stress was increased.

For the diffusive resistance, all the saline treatments showed an increase after the 40th day of differentiation (*figure 2*). In all treatments, the highest levels were obtained on the 80th day (6.28 $s \cdot cm^{-1}$ to 11.82 $s \cdot cm^{-1}$ for the control and 100-mM NaCl treatments, respectively), showing a slight decrease afterwards. The decrease was possibly due to a small diminution of the vapour pressure deficit of water on the 90th day after stress. Nogueira [3], working with acerola plantlets with a 100-mM NaCl treatment, assured that plants undergoing such treatment reached maximum diffusive resistance levels (25.68 $s \cdot cm^{-1}$) after 45 days of differentiation.

Regardless of the treatment, the transpiration suffered reductions of 44.1% (60 days) and 13.4% (90 days) with the 100-mM treatment compared with the control treatment (*figure 2*). Such behaviour may be linked to R_{leaf} reduction, which occurred at the 60-day and 90-day evaluation stages for all treatments (*table I*). Meneses Jr. *et al.* [13], working with cashew clones under saline stress (200 mM NaCl), obtained transpiration values of 1.132 $mmol \cdot m^{-2} \cdot s^{-1}$ for control, and (0.350 and 0.255) $mmol \cdot m^{-2} \cdot s^{-1}$ after (4 and 8) days of exposition, respectively, for the CCP 76 cashew clone.

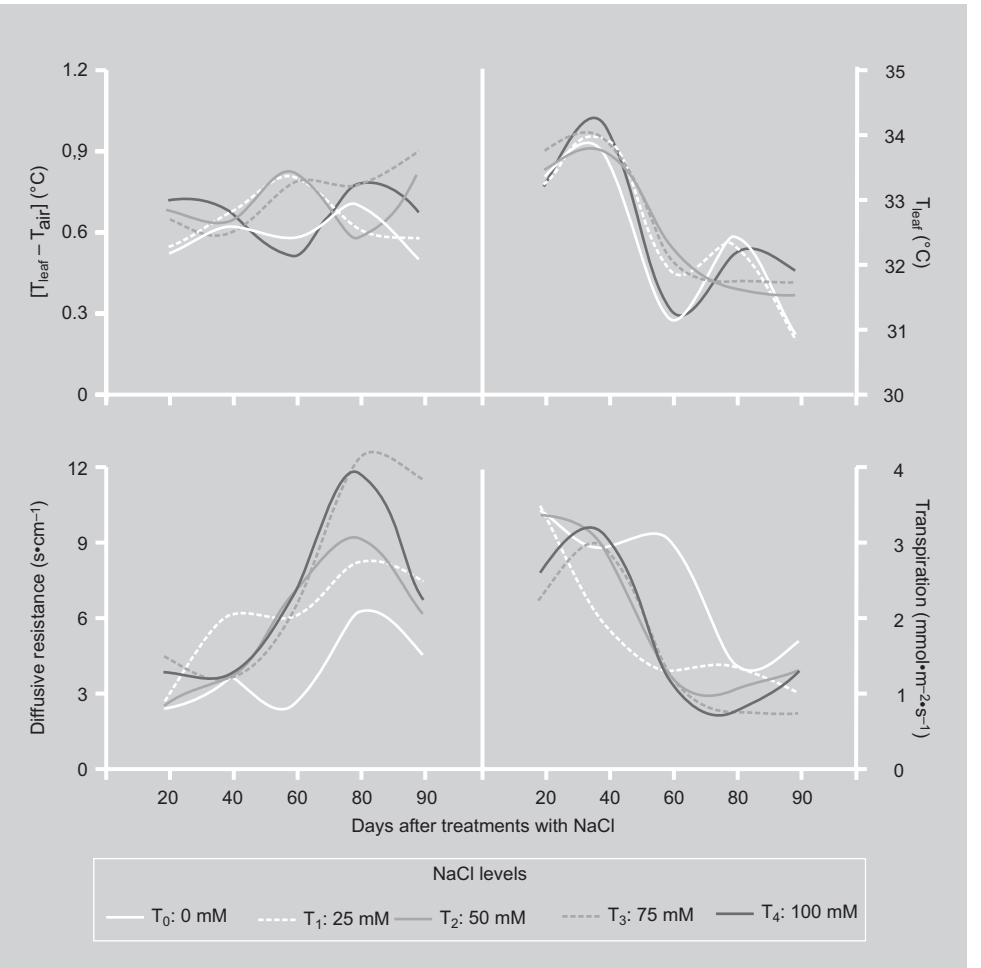

Figure 2.
Evolution of different parameters measured for 90 days of the experiment to assess the effect of five different NaCl levels applied to young plants of *Annona squamosa* (Brazil).

Table I.
Means of leaf water potential (R_{leaf}) in young plants of *Annona squamosa* submitted to different levels of NaCl.

Treatments (mM of NaCl)	R_{leaf} after 60 days (MPa)	R_{leaf} after 90 days (MPa)	Reduction of R_{leaf} (MPa)
0	– 0.66 a A	– 1.10 a B	– 0.44 a
25	– 0.97 b A	– 1.39 b B	– 0.42 a
50	– 1.07 b A	– 1.49 b B	– 0.42 a
75	– 0.96 b A	– 1.35 b B	– 0.39 a
100	– 1.29 c A	– 1.71 c B	– 0.42 a

Values followed by different letters, capital letters between columns and small letters between lines, differ significantly according to Tukey's test at $P < 0.05$.

A reduction in the average values of R_{leaf} related to the different NaCl level applications was verified (60 and 90) days after the beginning of the treatments (*table I*). The decrease in those potential values between the 60th and the 90th day was similar for all treatments.Távora *et al.* [6] observed that the potential reduction can be a reflex of ion accumulation in the cell tissue, in order for the plant to maintain a gradient of water potential with the external solution.

In the most severe treatments, burning of the oldest leaves from the 15th day of differentiation was also verified. With the evolution of the experiment, this effect grew stronger still, also with other NaCl levels, even provoking leaf abscission. Albuquerque *et al.* [12] verified similar behaviour in young plants of mangaba submitted to saline stress.

4. Conclusions

The NaCl levels used in the experiment undertaken affected both the transpiration and leaf water potential. Nonetheless, the leaf water potential presented equal reductions in all treatments resulting from the severity of the salt stress.

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Resistencia estomática y potencial hídrico foliar en plantas jóvenes de *Annona squamosa* sometidas a estrés salinos.

Resumen — Introducción. En los suelos salinos, el proceso de absorción del agua por las plantas se ve obstaculizado, debido a la permeabilidad de las raíces, lo que provoca un estrés hídrico. Las plantas sometidas a un estrés salino cierran sus estomas antes que las plantas en condiciones normales; esto aumenta la resistencia estomática a causa de la disminución del potencial hídrico. El objetivo de nuestras investigaciones consistió en detectar cambios del comportamiento estomático y del potencial hídrico en plantas jóvenes de *Annona squamosa* en condiciones de estrés salino. **Material y métodos**. Durante 15 días, se aclimataron en invernadero plantas jóvenes de *A. squamosa* de 60 días de edad, antes de someterlas a diferentas tratamientos salinos. Se aplicaron cinco tratamientos [(0, 25, 50, 75 y 100) mM de NaCl añadidos a una solución nutritiva] con seis repeticiones. Se evaluó la respuesta de los estomas 20, 30, 40, 60, 80 y 90 días después del inicio de los tratamientos salinos. En la primera hoja adulta, se registraron las medidas de transpiración, la resistencia a la difusión y la temperatura de las hojas. La radiación fotosintéticamente activa, la humedad relativa, la temperatura del aire en el invernadero y el potencial hídrico en la hoja (R_{hoja}) se evaluaron simultáneamente 60 y 90 días después del inicio de los tratamientos. **Resultados**. Mientras duró el experimento, la temperatura de la hoja y su diferencia con la temperatura del aire no se vieron afectadas por los niveles de NaCl; sólo 90 días después del inicio de los tratamientos salinos, los valores observados en las plantas testigo se revelaron inferiores a los obtenidos con el tratamiento con 25 mM de NaCl. Tras 40 días de experimentación, la resistencia a la difusión aumentó en todos los tratamientos. La transpiración, independiente del tratamiento, se redujo un 44,1 % (a los 60 días) y un 13,4 % (a los 90 días) en las plantas con tratamiento de 100-mM de NaCl respecto de los valores del tratamiento testigo. El aumento de los niveles de NaCl indujo una disminución del R_{hoja} en todos los tratamientos pero, tras 60 y 90 días, se observó la misma reducción en todos los tratamientos. **Conclusión**. Las distintas concentraciones de NaCl afectaron a la transpiración y al potencial hídrico de la hoja. No obstante, la reducción del R_{hoja} observada tras 60 y 90 días de experimentación fue semejante entre los distintos tratamientos.

Brasil / *Annona squamosa* / salinidad del suelo / estrés osmótico / transpiración / estoma / tensión de absorción