



Effects of salinity on growth, water content and distribution of Na⁺ and K⁺ in the organs of *Avicennia germinans* L. seedlings

Victor D. TAFFOUO^{1*}, Théophile FONKOU², Martin KENNE³, Oscar W. FOTSOP¹ and A. AMOUGOU⁴

¹ Department of Botany, Faculty of Science, University of Douala, P.O. Box 24157 Douala-Cameroon

² Department of Botany, Faculty of Science, University of Dschang, PO Box 377 Dschang – Cameroon. tfonkou@yahoo.fr

³ Department of Biology and Animal Physiology, Faculty of Science, University of Douala, PO Box 24157 Douala – Cameroon, E-mail: medoum@camail.com

⁴ Department of plant Biology and Physiology, Faculty of Science, University of Yaoundé I, PO Box 812 Yaoundé – Cameroon.

*Author for correspondence: vtaffouo@yahoo.fr

ABSTRACT

Effects of 4 different concentrations of NaCl on plant height, on water content and on the distribution of monovalent cations (Na⁺ and K⁺) in organs of *Avicennia germinans* seedlings in semi-controlled conditions were investigated. After 4 weeks of cultivation, results showed that 200 mmoles sodium chloride reduced the height of leaves, but roots and stems growth was stimulated at 100 mmoles of NaCl. A high retention rate of sodium was noted in seedling epicotyl axes, contributing to delay the invasion of young leaves, thereby preventing toxic effects of the ion. Adaptation of mature leaves to the salt stress was found to be associated with succulence, which was achieved by the absorption of large quantities of water and K⁺. In leaves, uptake of K⁺ was not affected by the NaCl concentration in the medium. As a result, absorption of Na⁺ and K⁺ reduces the water potential, and consequently increases the water content in the studied organs. The high concentrations of Na⁺ and K⁺ in the leaves suggested that these ions might be the principal mineral elements responsible for the osmotic adjustment in the resistance of *A. germinans* to salinity stress.

Key words: mineral nutrition salinity, ionic transport, *Avicennia germinans*

RESUME

L'effet des différentes concentrations du milieu en NaCl sur la taille, l'état hydrique et la distribution des cations monovalents (Na⁺ et K⁺) au niveau des organes de *Avicennia germinans* en conditions semi-contrôlées a été étudié. Après 4 semaines de pépinière, les résultats montrent que le chlorure de sodium affecte beaucoup plus la croissance des feuilles que celle des racines et des tiges. Pour ces derniers organes végétaux, une concentration de 100 mmoles de NaCl entraîne l'augmentation de la croissance. Une forte rétention du sodium a été notée au niveau des axes épicotylés des plantules, ce qui contribue à retarder l'envahissement des jeunes feuilles et par conséquent, éviter les effets toxiques de cet ion. Les feuilles âgées s'adaptent aux conditions de salinité en augmentant leur succulence par absorption massive d'eau et de potassium. L'approvisionnement en K⁺ des feuilles n'est pas affecté par la présence du NaCl dans le milieu. Par conséquent, l'absorption des cations monovalents Na⁺ et K⁺ accroît la teneur en eau des organes étudiés. Les fortes accumulations de Na⁺ et K⁺ au niveau des feuilles suggèrent que ces ions sont les principaux minéraux responsables de l'ajustement osmotique chez *A. germinans*.

Mots clés: nutrition minérale, salinité, transport ionique, *Avicennia germinans*

INTRODUCTION

Mangrove vegetation is generally dominated by mangrophytic woody plant species, among which *Avicennia* spp are the most tolerant to both high and fluctuating salinity, ranging from low values in estuarine habitats to hypersaline conditions in sites regularly fed by seawater [1,2,3]. As the mangrove environmental conditions affect the survival and the productivity of the colonising plants species, plant structures and physiological features explain their ecological success under harsh conditions [4,5]. For example, several studies revealed that photosynthesis activity and growth of mangrove plant species are reduced as the medium salinity increases [6,7,8,9,10]. Moreover, studies in Australian mangrove sites showed that the height of mangrove species and their diameter at breast height are related to soil-water salinity, soil-water content and distance from the mouth of the estuary. Consequently, characteristics of those mangroves are not a simple

response to salinity gradients in diverse systems, and other variables such as nutrient availability may also be important [11].

Although studies have demonstrated that mangroves are able to tolerate large rates of soil salinities and water potentials, the physiological mechanisms involving several processes are poorly elucidated. Regulation of ion uptake and ion transport mechanism allow plants to adapt easily to high salinity rates, thus maintaining a favourable water and carbon balance [4]. In mangroves, water deficits are generally tolerated due to the uptake of ions and an increase of intracellular solute concentration. Consequently, the resulting decrease in osmotic potential allows mangroves to lower their water content below that of seawater, in order to maintain a positive water uptake at their roots level [12,13].

In *Avicennia germinans* (which is the common species of old mangrove vegetation where the effect of

nutrient availability is overwhelmed by the tolerance of individual species to salt stress) leaves adapt easily to hypersaline soils by increasing their solute concentration and cell elasticity [3,14]. Although *A. germinans* plays an important ecological role in coastal structures by trapping sediments and stabilising the delta plain [15], little is known about the salinity effects at the levels of its organs, except few studies concerning carbon assimilation in the leaves [8,9,10]. In this study, we hypothesised that differences in cations concentrations from roots to leaves of *A. germinans* seedlings may be modified in response to salinity conditions to maintain a favourable water balance and the positive turgor required for growth. Therefore, the aim of the study was to assess the cations distribution in different organs of *A. germinans* seedlings subjected to different NaCl concentrations under controlled conditions.

MATERIALS AND METHODS

Seedlings

Seeds collected from the mangrove of the Cameroonian estuary were disinfected using a 10% sodium hypochlorite solution for 1 hr, rinsed with distilled water and kept under germinating conditions. After three days, seedlings obtained were separated into four groups of 15 individuals each. Five seedlings were then randomly selected from each group and planted together in one pot containing sand. This sand was previously washed using HCl 0.1 N and rinsed several times with distilled water. Pots were placed in the laboratory at $26 \pm 3^\circ\text{C}$; 5000 lux light 12h/day and 51-70 % relative humidity [16].

Treatments and analysis of K^+ and Na^+ contents

The control group of seedlings was watered at three-day intervals with nutrient solution (composition: 0.4 mmole of KNO_3 , 0.2 mmole of KH_2PO_4 , 1.0 mmole of Ca_2NO_3 and 0.4 mmole of MgSO_4 , pH 6, with 0 mmoles of NaCl) while for the three experimental groups, 50, 100 and 200 mmoles of NaCl were added to the nutritive solution. One week onwards after cultivation, two growth parameters were monitored in each group of seedlings: (1) the height of each plant was measured at two-day intervals, and (2) the dry weight was obtained weekly on randomly sampled plants. Four weeks after, water, Na^+ and K^+ contents of leaves, stems and roots were determined on 5 randomly sampled plants as described previously [17,18]. Na^+ and K^+ contents in dried pulverized plant samples were determined using Corning 410™ Flame Photometer, after concentrated acid digestion.

Data analysis

Data are presented in the form of mean \pm standard deviation. Correlation coefficients between studied parameters as well as coefficient of determination (square of correlation coefficients) and regression equations are given. Correlation coefficients and linear regression slopes (\pm standard deviation) were compared using the Student t-test. Multiple comparisons of several means were made using the ANOVA method followed by all pairwise analysis using the Student-Newman-Keuls procedure when the normality and equal variance conditions passed. When conditions were not matched, the Kruskal-Wallis non-parametric multiple tests were used and analytic comparisons performed using the *Dunn's* method. Multiple comparisons of data noted in experimental groups versus those recorded in the single control group were performed using the Dunnett's procedure in the SigmaStat™ software.

RESULTS

Seedling's growth

Seedlings height varied positively with the duration of culture, apparently without any relation with the degree of salinity of the medium. Growth inhibition effect of the salt was significantly noted for 200 mmoles of NaCl (Table 1). Moreover, the dry weight was higher in stems and roots than in leaves. Comparisons between the control and treated plants revealed that the presence of salt in the nutritive solution had no effect on dry weight of roots and stems except with 100 mmoles of NaCl (Fig. 1).

Ionic distribution

Potassium ions were more concentrated in leaves than in the roots and the stems. Pairwise comparisons between the control and the three experimental groups of plants showed that differences in ionic distribution recorded in leaves and roots were in all cases significant (Dunnett's test: $P < 0.001$ for leaves and roots respectively), whereas in epicotyl and hypocotyl axis, a significant difference was noted only between the control plants and those treated with 200 mmoles of NaCl (Fig. 2A).

Contrary to K^+ , Na^+ accumulated more in organs of the treated plants than in those of the control. In the treated plants, Na^+ was more concentrated in epicotyl axis than in other organs (Fig. 2B). Moreover, the ionic ratio for all the plants was more important in epicotyl and hypocotyl axis than in leaves and roots (Fig. 2C). Results presented in Table 2 show that contrary to stems, treatment with NaCl induced a significant increase of water content in roots and leaves.

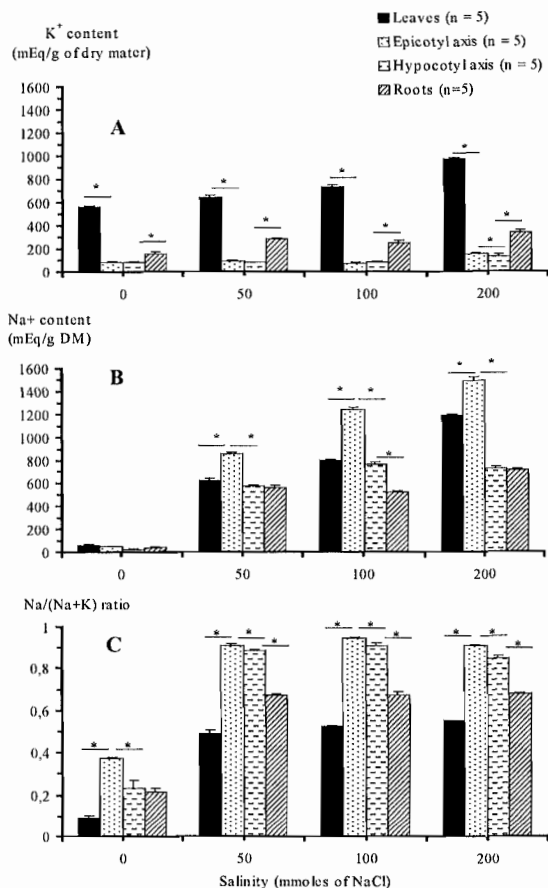
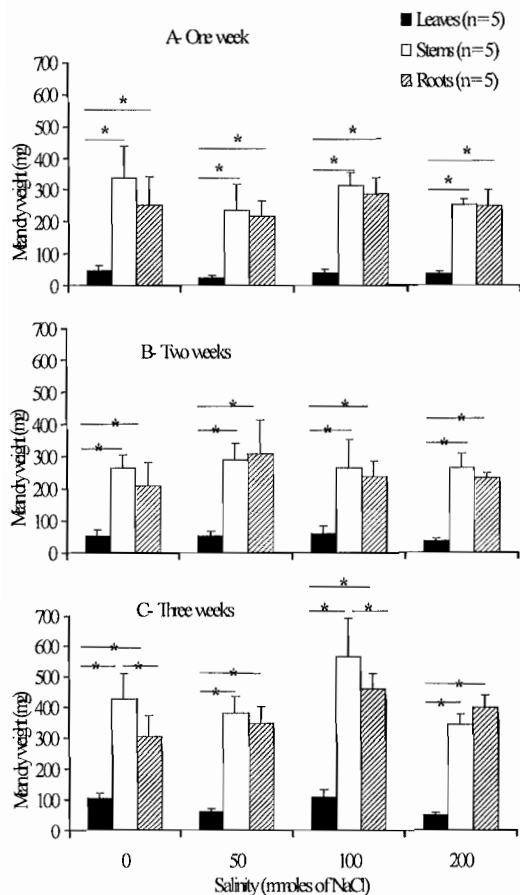


Figure 1. Effects of salinity (mmole of NaCl) and age on the dry weight (mg) of different seedlings' organs. Vertical bars represent standard deviation. Statistical analyses were set up using the one way ANOVA method followed by the Student-Newman-Keuls pairwise multiple comparisons. * = significant difference.

Figure 2. Effects of salinity (mmole of NaCl) on the distribution of potassium and sodium ions in seedling's organs. Vertical bars represent standard deviation. Statistical analysis were set up using the one way ANOVA method and the Student-Newman-Keuls pairwise multiple comparisons. * = significant difference.

Table 1: Effect of salinity on the height of the seedlings and Pearson correlation coefficients between plant height variation and duration of culture.

Salinity (mmoles of NaCl)	Height of plants (cm)			Pearson correlation	
	Min.	Max.	Mean ± SD	r	p-value
A : 0	4.5	10.2	7.8 ± 1.4	0.752	4.5x10 ⁻¹²
B : 50	4.5	8.5	7.9 ± 1.1	0.615	1.8x10 ⁻⁷
C : 100	4.5	10.5	8.4 ± 1.6	0.793	1.5x10 ⁻¹³
D. 200	4.3	7.5	5.9 ± 0.9	0.454	2.5x10 ⁻⁴

n = 60

Multiple comparison: normality test failed (P<0.001); *Kruskal-Wallis* One Way ANOVA: H = 88.64; df = 3; P <0.001.

Pairwise multiple comparison procedures using *Dunn's* method (d = difference of ranks; Q = *Dunn's* index):

A vs. B: d = 4.28; Q = 0.34; ns B vs. C: d = 19.12; Q = 1.51; ns

A vs. C: d = 23.40; Q = 1.85; ns B vs. D: d = 90.10; Q = 7.11; *

A vs. D: d = 85.82; Q = 6.77; * C vs. D : d = 109.22; Q = 8.62; *

= P<0.05; ns = no significant difference

Table 2. Effect of Salinity on the water content of seedling's organs. Multiple comparisons between treated and control groups (0 mmoles of NaCl) using Dunnett's method: n=5, * = P<0,05.

Salinity (mmoles of NaCl)	Roots			Stems			Leaves		
	Min.	Max.	Mean±SD	Min.	Max	Mean±SD	Min.	Max.	Mean ± SD
A : 0	76.8	78.1	77.4±0.6	77.6	79.2	78.5±0.7	66.1	68.2	67.3±0.8
B : 50	84.8	86.2	85.4±0.5 *	76.8	78.2	77.5±0.6	63.6	65.8	64.7±0.8 *
C : 100	86.9	88.5	87.5±0.7 *	81.8	83.1	82.4±0.6	78.1	80.0	79.1±0.9 *
D : 200	85.2	86.8	85.6±0.7 *	79.5	81.0	80.2±0.8	73.8	75.0	74.2±0.5 *

DISCUSSION

Growth inhibition in the presence of high concentrations of NaCl has been documented in glycophytes and several halophytes [10,19,20,]. The same phenomenon is observed in *A. germinans* whose growth is slowed by 200 mmoles of NaCl (Table 1). In *A. germinans*, the negative effect of salt on biomass production is important at the edge of leaves. Three weeks after cultivation, elongation of stems and roots was stimulated by 100 mmoles of NaCl (Figure 1). Similar results have been obtained on *Helianthus annuus* seedlings [21].

In this study, the NaCl concentration in the substrate was not correlated with the concentration of accumulated K⁺, which was higher in leaves than in stems and roots (Fig. 2A). Na⁺ even at high concentrations did not affect the transfer of K⁺ from the mature leaves (power supply) to the youngest ones (the well). This phenomenon is not restricted to mangrove plant species, as it has also been reported in *Phaseolus vulgaris* and *Canavalia obtusifolia* [19,22]. The reduction of leaves biomass was not correlated with the K⁺ uptake deficiency. High accumulation rate of K⁺ observed in the leaves may confirm the conclusions that potassium corresponds to about 25 to 50 % of the plants mineral constitution especially in young organs where it is suggested to intervene during cell division [23].

In high salinity sites, anions and cations are highly concentrated while water content per unit dry mass is low. Consequently, leaves adapt easily to hypersaline soils by increasing their solute concentration and cell elasticity [3,10,24,25]. *A. germinans* seedlings cultivated on soil supplied with NaCl maintained high water content as compared to the control (Table 2). The flow of water from roots to leaves may be facilitated by the increase of the ionic ratio Na/(Na+K) (Figure 2C). In some halophytes, especially mangroves, the principal mechanism of salt tolerance is associated with the presence of specific glands responsible of the active excretion of salts at the level of leaves surface [26]. The present investigation has shown that the succulence of mature leaves is due to water retention, which may be facilitated by Na⁺ and K⁺ accumulation. The consequence is the high intracellular dilution and the avoidance of toxicity. These results

corroborate with reports on *Medicago sativa* and *Hedysarum carnosum* [19,27]. *A. germinans* appeared as a facultative halophyte whose seedlings adapt easily to high salinity conditions. Young seedlings are able to accumulate important amounts of Na⁺ in epicotyl's axis, increasing the succulence of the mature leaves through absorption of high quantities of water. The high concentrations of Na⁺ and K⁺ in the leaves suggested that these ions might be the principal mineral elements responsible for the osmotic adjustment in the resistance of *A. germinans* to salinity stress.

ACKNOWLEDGEMENT: This work is part of an ongoing project supported by the International Foundation for Science (IFS) through a grant to Dr Taffouo Victor Désiré. The authors are also grateful to Dr. Mvondo Ze of the Soil Science Laboratory of the Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon, for his help during the chemical analysis of samples.

REFERENCES

- Mallery C.H. and Teas H.J. 1984. The mineral ion relations of mangroves. 1. Root cell compartments in a salt excluder and a salt excreter species at low salinities. *Plant Cell Physiology* **25**: 1123-1131.
- Ball M.C. 1996. Comparative ecophysiology of mangrove forest and Tropical lowland moist rainforest. In: S.S. MULKEY, R.L. CHAZDON and A.P. SMITH (Eds.). *Tropical forest plant ecophysiology*. Chapman and Hall, New York, 461-496.
- Suárez N., Sobrado M.A. and Medina E. 1998. Salinity effects on the leaf water relations' components and ion accumulation patterns in *Avicennia germinans* L. seedlings. *Oecologia* **114**(3): 299-309.
- Clough B.F., Andrew T.J. and Cowan I.R. 1982. Physiological processes in mangroves. In : B.F. Clough (ed.). *Mangrove ecosystem in Australia. Structure, Function and Managment*. Australia National Press, Camberra, 193-210.
- Smith J.A.C., Poop M., Lütge U., Cram W.J., Diaz M., Griffith H., Lee H.S.J., Medina E., Schaefer C.,

- Stimmel K.H. and Thonke B. 1989. Ecophysiology of xerophytic and allophytic vegetation of a costal alluvial plain in northern Venezuela. VI. Water relations and gas exchange of mangroves. *New Phytol.* **11**: 293-307
6. Ball M.C. and Farquhar C.D. 1984. Photosynthetic and stomatal responses of the grey mangrove, *Avicennia marina*, to transient salinity conditions. *Plant Physiol.* **74**: 7-11.
 7. Azócar A., Rada F. and Orozco A. 1992. Water relations and gas exchange in two mangrove species with contrasting mechanisms of salt vegetation. *Ecotropicos* **5**: 11-19.
 8. Sobrado M.A., 1999a. Drought effects on photosynthesis of the mangrove, *Avicennia germinans*, under contrasting salinities. *Trees* **13**: 125-130.
 9. Sobrado M.A., 1999b. Leaf photosynthesis of the mangrove *Avicennia germinans* as affected by NaCl. *Photosynthetica* **36**(4): 547-555.
 10. Sobrado M.A. and Ball M.C. 1999. Light use in relation to carbon gain in the mangrove, *Avicennia marina*, under hypersaline conditions. *Austrian Journal of Plant Physiology* **26**: 245-251.
 11. Saintilan N. 1998. Relationships between height and girth of mangroves and soil-water conditions in the Mary and Hawkesbury River estuaries, eastern Australia. *Austrian Journal of Plant Physiology* **23**(4): 322-328.
 12. Scholander, P.F. 1964. Hydrostatic pressure and osmotic potential in leaves of mangroves and some other plants. *Proceeding National Academy of Science* **52**: 119-125.
 13. Scholander P.F. 1968. How mangroves desalinate seawater. *Physiologia. Plantarum* **21**: 251-261.
 14. Chen R. and Twilley R.R. 1998. A gap dynamic model of mangrove forest development along gradients of soil salinity and nutrient resources. *Journal of Ecology* **86**(1): 37-51.
 15. Froimard F. 1998. L'écosystème mangrove. Biodiversité, fonctionnement, restauration et gestion. *Bois et Forêt des Tropiques* **256**: 80-86.
 16. Wacquant J.P. 1974. *Recherche sur les propriétés d'absorption cationique des racines. Rôle physiologique et importance écologique.* Thèse de doctorat, Université de Montpellier. 155 p.
 17. Savoure J.C. 1980. *Manipulations pratiques en physiologie végétale.* Paris, Masson. 258 p.
 18. Tsoata E. & Taffouo V.D. 1997. Absorption du sodium par les parties aériennes de quatre espèces de Légumineuses. *Agronomie Africaine* **9** (1): 37-43.
 19. Hamza M. 1977. *Action de différents régimes d'apport du chlorure de sodium sur la physiologie de deux Légumineuses: Phaseolus vulgaris (sensible) et Hedysarum carnosum (tolérante).* Relations hydriques et relations ioniques. Thèse de Doctorat, Université Paris VII. 252 p.
 20. Brun A. 1987. Effets de NaCl sur les teneurs en Na⁺ et K⁺ des feuilles de *Canavalia obtusifolia* DC: Comparaison du tissu du limbe et du pétiole. *Compte Rendu de l'Académie des Sciences de Paris* **304**(8): 181-184
 21. Gharsalli M. & Cherif A. 1979. Action du chlorure de sodium sur la croissance et la teneur en lipide des plants de tournesol (*Helianthus annuus* L.). *Physiologie Végétale.* **17** (2): 215-229.
 22. Brun A. 1988. Effets de NaCl sur une halophyte tropicale (*Canavalia obtusifolia* DC): Croissance, distribution de Na⁺ et K⁺, état hydrique. *Oecologia Plantarum* **9**(2): 173-186.
 23. Grillot G. 1957. *Les problèmes biologiques relatifs aux plantes tolérant l'eau salée ou saumâtre et à l'utilisation d'une telle eau pour l'irrigation.* Recherche sur la zone aride. Tome IV: *Utilisation des eaux salines.* Unesco, Paris, 152 p.
 24. Tattini M. R., Gucci M.A., Coradeshi C., Pangio and Everard J.D. 1995. Growth gaz exchange and ion content in *Olea europaea* plants during salinity stress and subsequent relief. *Physiologia Plantarum* **95**(2): 203-210.
 25. Rajesh A., Arumugam R. and Venkatesalu V. 1998. Growth and photosynthetic characteristics of *Ceriops roxburghiana* under NaCl stress. *Photosynthetica* **35**(2): 285-287.
 26. Albert R. 1975. Salt regulation in halophytes. *Oecologia* **21**(1): 57-71.
 27. Lessani H. 1969. *Recherches sur le comportement physiologique de la luzerne en présence de chlorure de sodium. Etude de quelques aspects de la nutrition minérale et du métabolisme respiratoire.* Thèse de Doctorat ès-Science, Université de Paris VI, 152 p.