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

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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 100 mmole sodium chloride reduced the height of leaves, but roots and stems growth was stimulated at 100 mmole 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 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 la chlore 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 mmole de NaCl entraîne l’augmentation de la croissance. Une forte rétention du sodium a été notée au niveau des axes epicotyles 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’appréciation de K\(^+\) des feuilles n’est pas affectée 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 ionic, Avicennia germinans

INTRODUCTION
Mangrove vegetation is generally dominated by halophytic 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 ± 3°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 K NO₃, 0.2 mmole of KH₂PO₄, 1.0 mmole of Ca(NO₃)₂ and 0.4 mmole of MgSO₄. 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 ± 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 (± 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 Kruskall-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 mmol 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 mmol 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 mmol 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 (mM 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.

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

<table>
<thead>
<tr>
<th>Salinity (mM NaCl)</th>
<th>Height of plants (cm)</th>
<th>Pearson correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>A: 0</td>
<td>4.5</td>
<td>10.2</td>
</tr>
<tr>
<td>B: 50</td>
<td>4.5</td>
<td>8.5</td>
</tr>
<tr>
<td>C: 100</td>
<td>4.5</td>
<td>10.5</td>
</tr>
<tr>
<td>D: 200</td>
<td>4.3</td>
<td>7.5</td>
</tr>
</tbody>
</table>

n = 60
Multiple comparison: normality test failed (P<0.001); Kuskall-Wallis One Way ANOVA; H = 80.64; 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 = 3.34; ns
A vs. C: d = 20.40; Q = 1.85; ns
A vs. D: d = 85.82; Q = 6.77; *
B vs. C: d = 19.12; Q = 7.11; ns
B vs. D: d = 90.10; Q = 7.11; *
C vs. D: d = 109.22; C = 8.52; *
* = 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 mmole of NaCl) using Dunnett's method; n=5, * = P<0.05.

<table>
<thead>
<tr>
<th>Salinity (mmoles of NaCl)</th>
<th>Roots</th>
<th></th>
<th></th>
<th></th>
<th>Stems</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Leaves</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
<td>Mean ± SD</td>
<td>Min.</td>
<td>Max.</td>
<td>Mean ± SD</td>
<td>Min.</td>
<td>Max.</td>
<td>Mean ± SD</td>
<td>Min.</td>
<td>Max.</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A : 0</td>
<td>76.8</td>
<td>79.1</td>
<td>77.4 ± 0.6</td>
<td>77.6</td>
<td>79.2</td>
<td>78.5 ± 0.7</td>
<td>68.1</td>
<td>68.2</td>
<td>67.3 ± 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B : 50</td>
<td>84.8</td>
<td>86.2</td>
<td>85.4 ± 0.5 *</td>
<td>76.8</td>
<td>78.4</td>
<td>77.5 ± 0.6</td>
<td>63.6</td>
<td>65.8</td>
<td>64.7 ± 0.8 *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C : 100</td>
<td>86.9</td>
<td>88.5</td>
<td>87.5 ± 0.7 *</td>
<td>81.8</td>
<td>83.1</td>
<td>82.4 ± 0.6</td>
<td>78.1</td>
<td>80.0</td>
<td>79.1 ± 0.9 *</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D : 200</td>
<td>85.2</td>
<td>86.8</td>
<td>85.6 ± 0.7 *</td>
<td>79.5</td>
<td>81.0</td>
<td>80.2 ± 0.6</td>
<td>73.8</td>
<td>75.0</td>
<td>74.2 ± 0.5 *</td>
<td></td>
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</table>

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 obliquifolia [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 for 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 Hedyserum catalonum [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 somatic 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. Taffou Victor Désiré. The authors are also grateful to Dr. Myndo Zo 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.

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