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Responses of *Cymbopogon schoenanthus* to salt stress

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***Cymbopogon schoenanthus* is an aromatic and medicinal plant rich in essential oil. The physiological behavior of this species, related with growth production, the photosynthetic pigments, the nutritional status and the osmotic adjustment were studied. Saline treatments varied from 0 to 150 mM NaCl. The results show that the growth of *C. schoenanthus* was affected by the salinity and the effect increased with more stress at 100 mM of NaCl. Besides the response of *C. schoenanthus* to the salt stress, this study has allowed us to conclude that this plant is excluder glycophyte and less tolerant to salt stress.**

Key words: *Cymbopogon schoenanthus*, NaCl, growth, mineral nutrition, osmotic adjustment.

INTRODUCTION

In arid and semi-arid lands, which cover a third of the globe surface, soil and irrigation water salinity is considered as one of the major factors affecting plant growth and crop yield (Zid and Grignon, 1991). In fact, this can be resulted from a low osmotic potential of the soil solution (osmotic effect), specific ion effects (salt stress), nutritional disequilibrium (nutritional stress), or a combination of the three effects (Ashraf, 1994; Zhu, 2002). Moreover, Munns (2005) separated salt impact on plants into two phases: first, a salt-treated plant faces an osmotic stress that reduces its growth rate, second, when salt reaches toxic levels in leaves, photosynthetic activity decreases in sensitive plants due to the death of some of their leaves which decreases in this way shoot growth rate. Therefore, osmotic adjustment is among the most important mechanisms evolved by salt-tolerant plants at cell level (Munns, 2002) as this enables cells to maintain their turgor in spite of the low water potential in the medium (Garg et al., 2002; Moinuddin et al., 2005), and measures their abilities to accumulate organic (amino acids, sugars, proline, glycinebetaine, etc) or inorganic

(Na⁺, K⁺, Cl⁻ etc) osmotic in their symplasts (Messedi et al., 2004; Morant-Manceau et al., 2004; Ottow et al., 2005; Parida and Das, 2005; Munns et al., 2006; Navarro, 2006; Teakle et al., 2007; Yousfi et al., 2010). As a result, it maintains numerous physiological functions in plants such as water and nutrient uptake, photosynthesis, and growth (Grennan, 2006; Martinez, 2007) at every development stage (Malasses, 1996).

On the other hand, Greenway and Munns (1980) attribute the main differences between dicotyledonous and monocotyledonous halophytes to their cell water contents and consequently their vacuolar volume. Hence, they postulated that dicotyledonous halophytes can maintain higher sodium contents and Na⁺/K⁺ ratios since they can accumulate the major part of sodium in vacuoles and require relatively low potassium amounts for metabolism (Parida and Das, 2005). Although halophytes represent only 1% of the world flora, they can be widely and economically used in arid and semi-arid regions, particularly in the light of the progressive shortage of fresh water resources and soil salinization (Koyro et al., 2008).

Nevertheless, their domestication needs an evaluation of their potential and an understanding of their nutritional behavior and photosynthetic responses. In this context, we investigated salt tolerance in the aromatic and medicinal plant, *Cymbopogon schoenanthus*. The study was

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Table 1. Shoot and root water contents (H₂O) and growth parameters in *C. schoenanthus* plants grown under greenhouse conditions at 0, 50, 100 and 150 mM NaCl over 30 days.

NaCl (mM)	0	50	100	150
Shoots				
DW (g.plant ⁻¹)	1.00 ^b	1.26 ^a	0.83 ^c	0.49 ^d
RGR (g.g ⁻¹ DW d ⁻¹)	0.12 ^a	0.13 ^a	0.11 ^{ab}	0.09 ^b
H ₂ O (mg.g ⁻¹ DW)	12.83 ^a	10.67 ^b	7.93 ^c	6.63 ^d
Roots				
DW (g. plant ⁻¹)	0.18 ^a	0.13 ^c	0.12 ^c	0.16 ^b
RGR (g.g ⁻¹ DW d ⁻¹)	0.05 ^a	0.03 ^b	0.04 ^a	0.03 ^b
H ₂ O (mg.g ⁻¹ DW)	9.82 ^c	17.23 ^a	14.63 ^b	9.37 ^c

Vales are means of eight replicates. Those with different letters are significantly different according to Duncan's test at $p \leq 0.05$. DW, Dry weight; RGR, relative growth rate.

based on biomass production, tissue hydration, nutrient status, chlorophyll concentration and osmotic concentrations at several salinity levels.

MATERIALS AND METHODS

Seeds of *C. schoenanthus* were collected from South Tunisia. After germination, obtained seedlings were hydroponically grown for two months using aerated Hewitt's (1966) nutrient medium. After that, plants were separated into four lots of eight and each subjected to a different treatment (0, 50, 100 and 150 mM NaCl). Plant culture was carried out under growth chamber conditions (a light intensity of 100 $\mu\text{mol photons. m}^{-2}\text{s}^{-1}$, a photoperiod of 16 h, and a day/night temperature regime of 25 /19°C) and salt was added progressively to avoid osmotic shock.

On the 30th day of treatment, a leaf sample was taken from each plant for chlorophyll analysis according to Torrecillas et al. (1984). Then, all plants were harvested and separated into shoots and roots, weighed, oven-dried and weighed again. Thereafter, dry matter was ground and the obtained powder was used for potassium, sodium, chloride, proline, and soluble sugar analyses. Additionally, ions were extracted from the powder using a nitric acid (HNO₃) solution (0.1 N). K⁺ and Na⁺ were titrated with a flame spectrophotometer (Corning), Ca²⁺ with an atomic absorption spectrophotometer (Shimadzu Corporation AA 6800 KYOTO Japon) and Cl⁻ with a chloridometer (Buchler Cotlove) and the determination of soluble sugar and proline contents was performed according to Nelson (1944) and Bates et al. (1973), respectively.

Statistical analysis

The data were subjected to statistical analyses. Analysis of variance was used (ANOVA) and $P < 0.05$ was used to define statistical significance.

RESULTS

Weight growth

Table 1 shows the evolution of the mass of dry matter of shoots and roots of *C. schoenanthus* depending on the

concentration of NaCl. Indeed, the production of dry biomass of shoot showed a stimulation of 22% to 50 mM. Beyond 100 mM, we noted a reduction that reached 50% at 150 mM compared to the control. Moreover, in the treated plants, the production of root dry biomass remained very close to that of the control and the salt treatment did not have effect on these roots organs. However, it had a depressive effect on the production of dry biomass at the shoots.

Tissue hydration

The hydration of different plant organs (roots and shoots), after 30 days of treatment was estimated by water content (Table 1). Examination of this table shows that only in 50 mM was the hydration of shoot reduced by 16.8% compared to the control. It further decreased to 48% at 150 mM. However, the water content of roots was stimulated by salinity primarily at doses 50 and 100 mM, which represents respectively 1.75 and 1.5 times that of the control.

The relative growth rate (RGR)

The relative growth rate (RGR) is defined as the activity of growth per unit biomass and per unit of time. Table 1 shows the evolution of the RGR as a function of salinity. Its examination revealed that the RGR of shoot was not significantly altered at 50 mM dose. However, it decreased at 100 and 150 mM NaCl. This decrease was respectively 12.4 and 27.3% of the control. In addition, the roots decreased by 40% at 50 mM and remained stable at all tested doses of salt.

Chlorophylls pigments

The chlorophyll content of *C. schoenanthus* subjected at varying NaCl-enriched nutrient solutions is showed in

Table 2. Mean chlorophyll content ($\mu\text{g g}^{-1}$ FW shoot tissue) of two-months old *Cymbopogon schoenanthus* subjected for 30 days to salinity at varying NaCl concentrations (0 to 150 mM) in nutrient solution.

NaCl (mM)	Chl a	Chl b	Total Chl	Chl a/b
0	63.55 ^b	28.99 ^b	92.54 ^c	2.46 ^b
50	116.92 ^a	23.7 ^c	140.62 ^a	3.15 ^a
100	65.65 ^b	29.67 ^b	95.32 ^b	2.31 ^b
150	57.32 ^c	40.31 ^a	97.63 ^b	1.84 ^c

Data represent mean \pm 95% confidence limits, n = 8. Different letters indicate significant differences between treatments at $P \leq 0.05$ according to Duncan's test.

Table 3. Mean effect of NaCl stress on Na^+ , K^+ , Ca^{2+} , Cl^- , proline and soluble sugars in the studied *Cymbopogon schoenanthus*.

NaCl (mM)	0	50	100	150
Na shoot (meq.g^{-1} DW)	1358.3 ^c	4889.1 ^b	7015.2 ^a	7197.8 ^a
Na Root (meq.g^{-1} DW)	434.3 ^c	977.8 ^b	1495.0 ^a	1496.5 ^a
K shoot (meq.g^{-1} DW)	937.1 ^a	423.1 ^c	519.6 ^b	413.6 ^c
K Root (meq.g^{-1} DW)	251.0 ^c	372.7 ^b	117.9 ^d	613.9 ^a
Ca shoot (meq.g^{-1} DW)	2.0 ^a	0.9 ^c	1.4 ^b	1.3 ^b
Ca root (meq.g^{-1} DW)	0.5 ^a	0.6 ^a	0.5 ^a	0.5 ^a
Cl shoot (meq.g^{-1} DW)	2.1 ^b	19.7 ^a	19.6 ^a	19.8 ^a
Cl Root (meq.g^{-1} DW)	0.4 ^c	4.2 ^b	5.2 ^a	5.7 ^a
Proline shoot ($\mu\text{mol.g}^{-1}$ DW)	85.7 ^c	148.3 ^b	191.4 ^a	242.3 ^a
Reducer soluble sugar shoot (mg.g^{-1} DW)	8.8 ^b	17.1 ^a	19.0 ^a	22.3 ^a
Reducer soluble sugar root (mg.g^{-1} DW)	4.2 ^b	3.3 ^b	6.2 ^a	6.3 ^a
Total sugar shoot (mg.g^{-1} DW)	16.6 ^b	30.3 ^a	31.8 ^a	35.2 ^a
Total sugar root (mg.g^{-1} DW)	8.9 ^b	10.3 ^a	11.4 ^a	11.8 ^a

Data represent mean \pm 95% confidence limits, n = 8. Different letters indicate significant differences between treatments at $P \leq 0.05$ according to the Duncan's test.

Table 2. Indeed, the chlorophyll content increased in the plants treated with 50 mM NaCl. This increase was 84% compared to the control. However, beyond this dose, we recorded a decrease in content which stabilized at 100 and 150 mM and became equal to that of the control plants. However, chlorophyll b was not changed significantly by salt stress. The levels of total chlorophyll (a + b) follow the same evolution with increasing doses of NaCl than those of chlorophyll a. Thus, we also recorded an increase in the total chlorophyll content at 50 mM, which was 52% compared to the control.

The examination of Table 2 also show that variations in the ratio chlorophyll a to chlorophyll b as a function of different concentrations of NaCl following the same trend as chlorophyll a and total chlorophyll. We found that this ratio increased only at 50 mM NaCl. This result suggests that the photosynthetic system of *C. schoenanthus* grown on 50 mM NaCl is not affected by this dose of NaCl.

Effects of salinity on mineral nutrition of *C. schoenanthus*

It is known that Na^+ disrupts the absorption of major

cations (K^+ , Ca^{2+}), while the excessive accumulation of chlorides reduces the anions required for growth and development of plants. To better analyze the depressive effect of salt on the growth of *C. schoenanthus*, we studied the following distribution of major ions (Na^+ , K^+ , Ca^{2+} and Cl^-) in the shoots and roots of this species.

Sodium

Table 3 shows the variation in sodium content in shoot and root among *C. schoenanthus* depending on the dose of salinity. Examination of this table shows that sodium levels are the lowest recorded in the absence of salt. In terms of shoot we recorded, a considerable enrichment of leaf tissue sodium when we added the lowest dose of NaCl (50 mM). Indeed, the concentration increased by 72% compared to the control at 50 mM NaCl. This continued to increase and then stabilized at 100 and 150 mM, where it was respectively 80.6 and 81% of the control.

At the root, the evolution of the sodium content as a function of doses of NaCl in the culture medium was practically the same pace as that of the contents of this element in shoots. As already mentioned, lower level was

reported in the roots of the control plants. Therefore, this content was twice higher in roots of plants grown on 50 mM NaCl. It continued to grow at 100 mM, became 1.5 times higher than the last. However, it stabilized at 150 mM where we noticed a value very close to the previous (1.495 meq.g⁻¹ DW at 100 mM and 1.497 meq.g⁻¹ DW at 150 mM).

Potassium

Table 3 illustrates the evolution of the levels of K⁺ in roots and shoots of *C. schoenanthus* depending on the concentration of NaCl. In fact, the cultivation of plants in the presence of salt leads to a loss of members in K⁺ and this effect was expressed more in the shoots. On the other hand, in the control plants, the potassium content was higher for the shoots and roots. Indeed, we witnessed a fall of 55% compared to the control in the presence of 50 mM NaCl. However this level, although reduced, remained stable even at high doses of NaCl. The K⁺ content of control roots remained relatively low compared to shoots. Moreover, the presence of NaCl interferes with the content of this element, essential to growth, in root tissues. Hence, our results suggest that salinity has a depressive effect on the levels of potassium in shoots than in roots.

Calcium

The examination of Table 3 shows that the salt treatment induced a considerable variation in the calcium content mainly in the shoots of *C. schoenanthus*. Under the effect of salt, we recorded a significant decrease in the content of Ca²⁺ in shoots of plants after 50 mM NaCl. This decrease was 53% compared to the control and at high doses (100 and 150 mM), calcium content was slightly improved, but was still lower than the control, whereas the roots were not significantly affected by salinity.

Chloride

Table 3 shows the Cl⁻ content of roots and shoots of *C. schoenanthus* under saline conditions. Examination of this table shows that this accumulation is even more important than the culture medium rich in salt. We found that the shoots are richer in Cl than the roots. These levels in the shoots increased significantly at a concentration of 50 mM NaCl to maintain stable doses up to 150 mM NaCl. Generally, changes in the accumulation of sodium chloride reminiscent. However, the rates achieved in the shoots were larger than those of Na⁺ for the same organs and the same concentrations of NaCl medium.

Osmotic adjustment

The accumulation of some osmoregulatory allows plants

to compare the salt stress and to make their adjustment to tolerate osmotic stress. In this context, we followed the evolution of proline and soluble sugars according to the salt treatments.

Proline content

Proline plays an important role in osmotic adjustment (Cayuela, 1996). This is a nitrogenous compound synthesized mainly in case of stress such as salt stress or water stress. We determined its content in shoots of *C. schoenanthus* under salt stress. Table 3 illustrates the changes in proline content of shoots of *C. schoenanthus* in different treatments of NaCl. It was observed that the proline content increased steadily and significantly with the salinity up to 242.3 μmol.g⁻¹ DW to 150 mM NaCl, which was almost three times higher than in control.

Reducer soluble sugars

The examination of the Table 3 shows that the levels of reducer soluble sugars were the lowest recorded in the absence of salt. Besides, the presence of NaCl, even at low doses (50 mM) caused an increase in these levels. This increase was 51.4, 53.6 and 60.6% respectively at 50, 100 and 150 mM NaCl. At the roots, the content of reducer soluble sugars was less than that of shoots both in the controls and in the treaties. Hence, the application of salt causes a slight change not significant at the dose 50 mM.

Total soluble sugars

Examination of Table 3 reveals that the level of shoots and roots of total soluble sugars increased under salt stress. This rate increases of shoots, 45.1; 47.6 and 52.7%, respectively compared with the control for 50, 100 and 150 mM NaCl. The same trend was observed in the roots, with increase of 14.4, 22.4 and 25.1% respectively for 50, 100 and 150 mM NaCl.

DISCUSSION

The study of the growth of *C. schoenanthus* under the effect of different concentrations of NaCl demonstrate its behavior toward salt stress. The results obtained showed that the 50 mM treatment did not disturb the growth of *C. schoenanthus*, hence we saw a stimulation of biomass production of shoots. However, from 100 mM NaCl, we noted a significant decrease in biomass production of shoots, as well as a disruption of water supply to these organs. These results are consistent with those obtained in triticale (Bizid et al., 1988; Haddad and Coudret, 1991) and in durum wheat (Bouaouina, 2000). For instance, at

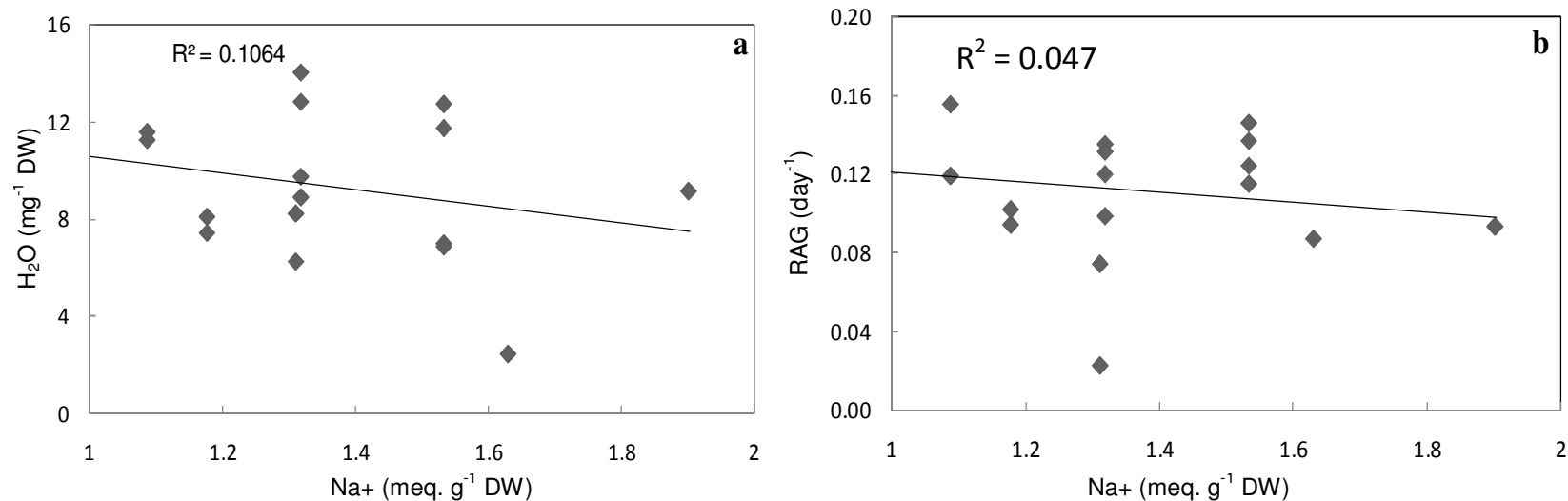


Figure 1. Correlation between water content and the Na content (a), and the RAG and the Na content (b) of the shoots of *Cymbopogon schoenanthus* cultivated under salt stress. The duration of the treatment was 30 days.

high doses (100 and 150 mM), the reduced growth of shoots was greater than that of roots. This result is in agreement with those obtained in maize (Mühling and Läuchli, 2001) and in triticale (Mühling and Läuchli, 2002).

The response of chlorophyll pigments of this constraint depends on its severity. Indeed, the low salinities increase their content while decreasing the high salinity (Winicov and Button, 1991). The effects of NaCl on growth of *C. schoenanthus* are associated with an accumulation of Na⁺ and Cl⁻ in its various organs, particularly in shoots, and disruption of their diet potassium and calcium. It is known that the reduced growth and productivity of plants under salt stress is determined mainly by Na⁺. The rise of salinity induced nutritional stress resulting from an inhibition of nutrient uptake by roots. This inhibition was due to interactions between Na⁺. Indeed, sodium competes with K⁺ at

the absorption sites due to the similarity of the absorption systems of the two cations (Schroeder et al., 1994). In light of these, the results suggest that salt sensitivity of *C. schoenanthus*, is not a very dependent or specific effects of Na⁺, or disturbance of nutrition that cationic induce in plant tissues. It is possible that it is due to the anion accompanying the cation which is Cl⁻. Especially, it is highly accumulated mainly in shoots.

In order to know whether the accumulation of sodium in shoots, exerts a toxic effect or is osmotic, we established a relationship between the change in the RGR on the one hand and that of the water content of leaves with their sodium content on the other hand (Figure 1). Our results show no clear correlation between water content and sodium content. This proved that *C. schoenanthus* is capable of maintaining stable water content in the presence of 150 mM NaCl, thanks to its ability to

achieve osmotic adjustment allowing it to protect cells against dehydration. In addition, the RGR had no correlation with the sodium content, suggesting that Na⁺ does not have a toxic effect when it is accumulated in the shoots of *C. schoenanthus*. These results suggest that Poaceae arid is an excluder type. This suggestion is supported by the fact that Na⁺ is accumulated less than Cl⁻. It would then exclude to the outside. This phenomenon is well known in the Poaceae (Igartua et al., 1995). Moreover, maintaining adequate potassium nutrition to support growth of different plant organs requires a good selectivity of absorption, accumulation and transport of K⁺ versus Na⁺ in the shoots. Numerous studies revealed a relationship between tolerances to NaCl on the one hand and selectivity K⁺/Na⁺ on the other hand that provides information on more or less healthy diet in the presence of potassium salt (Gorham and Wyn

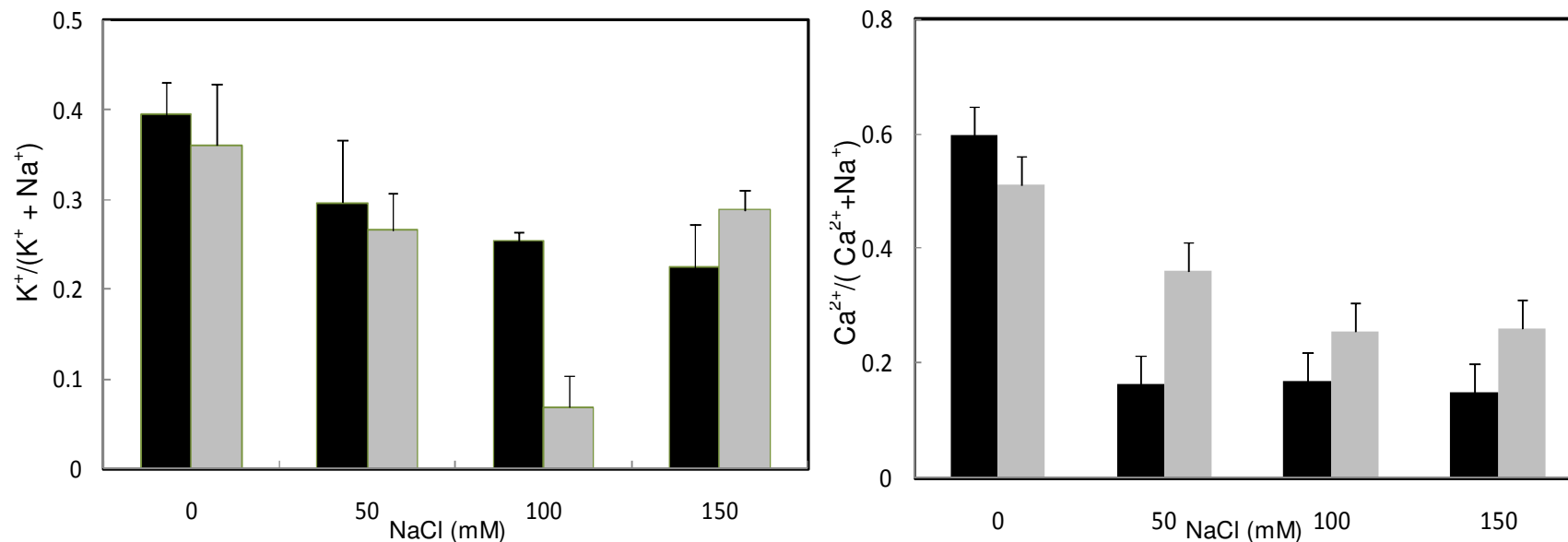


Figure 2. Ratios of $K^+/(K^+ + Na^+)$ and $Ca^{2+}/(Ca^{2+} + Na^+)$ in the roots and shoots of *Cymbopogon schoenanthus* according to different concentrations of NaCl. Treatment duration was 30 days.

Jones, 1990). Figure 2 shows the ratio of ion fractions equivalent $K^+/(Na^+ + K^+)$ in the shoots and roots under different concentrations of NaCl. This figure shows that the ratio $K^+/(Na^+ + K^+)$ of shoots was higher than that of roots. However, this ratio decreases in shoots, according to the different doses of NaCl. This decrease was 25; 35.6 and 43.1%, respectively compared with the control for 50, 100 and 150 mM NaCl. The ratio $K^+/(Na^+ + K^+)$ in roots decreased slightly to 50 mM. This decrease was 26.1% compared to the control. When the culture medium was supplemented with 100 mM, we recorded a sharp decrease of 81% compared to the control. Paradoxically, we noticed an increase to 150 mM. It was 20% compared to the control.

Calcium status was less affected than the potassium status in *C. schoenanthus*. Indeed,

reports in $Ca^{2+}/(Ca^{2+} + Na^+)$ tissue, including roots, and much higher, show a selective absorption of Ca^{2+} vis-à-vis the Na^+ (Figure 2). The ability of a plant to survive under restrictive environmental conditions requires adjustments at various levels. To better appreciate this adaptive nature, we established a relationship between the production of dry matter and concentrations of Na^+ in shoots (Figure 3). Our results suggest that the significant correlation between biomass production and content of Na^+ shoots is of the ionic order in *C. schoenanthus*. Figure 4 shows the variation of the selectivity of absorption K^+/Na^+ and Ca^{2+}/Na^+ . Here, we noticed that the increase of Na^+ is associated with a decrease of K^+ . Therefore, salt stress greatly reduces the salt content of K^+ in tissues of *C. schoenanthus* (Figure 4). This shows a selective absorption and accumulation of Na^+ at

the expense of K^+ . According to Bizid and et al. (1988), the selectivity coefficient K/Na transport in the organs could be used as criteria for the selection of salinity. Increasing the sodium content is translated, also by a decrease in calcium content in shoots. This results in a selective absorption and accumulation of Na^+ depends on Ca^{2+} (Figure 4). The osmotic adjustment results according to several authors, is due to proline accumulation in shoots under the effect of salinity. Indeed, this accumulation is known as an adaptive trait developed by the plant to cope with salt stress (Jin and Ray, 2004). In this regard, our results show that *C. schoenanthus* strongly accumulate proline when grown on salt stress. The increase in proline content could be attributed to its need for nitrogen compounds, amino acid for example, which is counted among the most easily

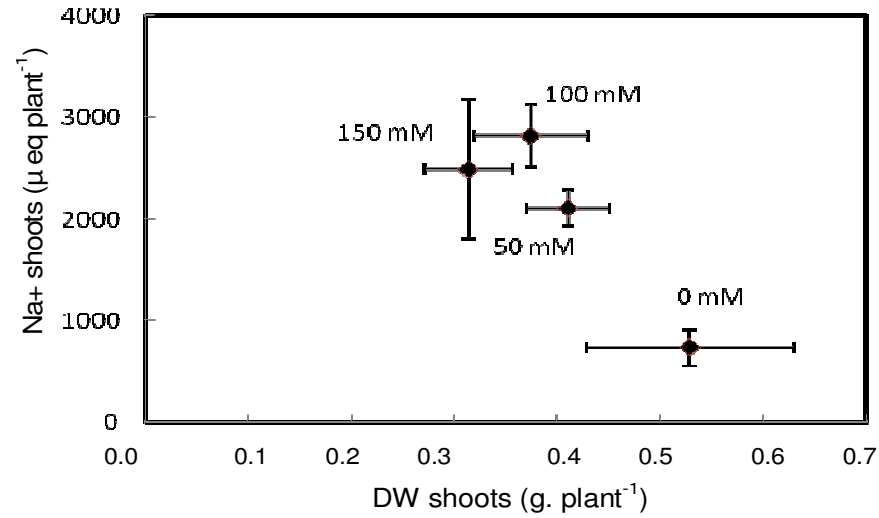


Figure 3. Correlation between Na content and the shoots biomass of *Cymbopogon schoenanthus* cultivated under salt stress. The duration of the treatment was 30 days and the plants were cultivated in the presence of NaCl (from 0 to 150 mM).

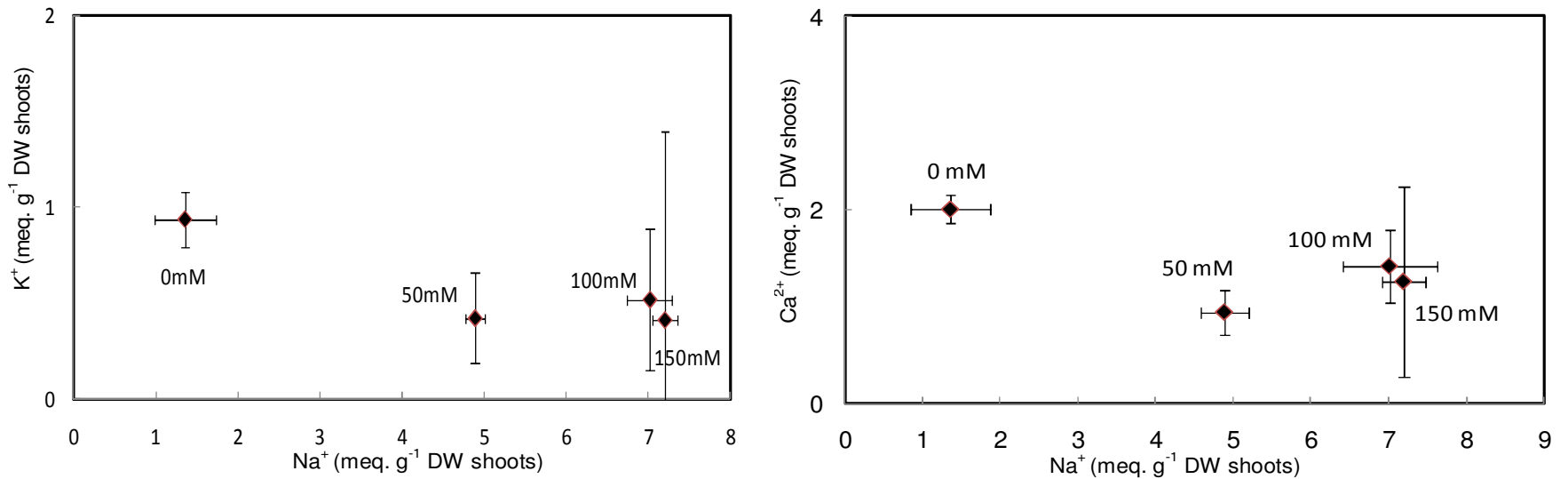


Figure 4. Absorption selectivity of K^+/Na^+ and Ca^{2+}/Na^+ in the shoots of *Cymbopogon schoenanthus* according to different NaCl concentrations.

mobilized. In the Poaceae that do not accumulate salt, soluble sugar content can reach 200 mM (Hammer, 1986). Our results reveal that at the shoots, the rate of total soluble sugar for treated plant was more than that for the control. However, the largest amount of soluble sugars was synthesized in plants grown on medium supplemented with 150 mM.

Conclusion

Growth in *C. schoenanthus* is affected by salinity, and the effect became more pronounced from 100 mM NaCl. The response of *C. schoenanthus* to saline stress helps us to conclude that this plant is an exclusive glycophyte moderately tolerant to salt. Besides, its tolerance is primarily related to its ability to transport Na⁺ to its shoots and use them for osmotic adjustment. Regarding the contents of proline and soluble sugars, it is necessary to highlight the significant accumulation of these compounds in the presence of salt stress. Finally, it is important to note that the osmotic adjustment implied an increase of proline and soluble sugar content essentially, thus reducing the shoots of plants subjected to treatment 150 mM NaCl.

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