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Full Length Research Paper

Effects of NaCl on Na^+, Cl^- and K^+ ions accumulation in two sugarcane (*Saccharum sp*.) cultivars differing in their salt tolerance

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The effects of salt on growth and ions accumulation were investigated in two sugarcane (*Saccharum sp*.) cultivars, CP66-346 (salt-tolerant) and CP65-357 (salt-sensitive). Young plants of these cultivars were exposed to four NaCl concentrations (0, 17, 34 and 68 mM). Na^+, Cl^- and K^+ ions concentrations were quantified after 2 weeks of stress. NaCl effect resulted in plant growth reduction in both cultivars but cv. CP66-346 plants were less affected compared to CP65-357 indicating that CP66-346 was more salt-tolerant than CP65-357. Na^+ and Cl^- concentrations increased significantly in leaves and roots under salinity while K^+ concentration significantly decreased in both cultivars. The highest accumulation of Na^+ and Cl^- occurred in young leaves of the salt tolerant cv. CP66-346 coupled with the lowest reduction in K^+ concentration. These results suggest that the salt tolerance of cv. CP66-346 is closely related to a high accumulation of Na^+ and Cl^-. K^+ ions also may play a key role in sugarcane salt tolerance.

Key words: Ions concentrations, sugarcane, *Saccharum* sp., salt-tolerance.

INTRODUCTION

Salinity is a major environmental factor limiting the crop productivity in the arid and semi-arid areas of the world (Dasgan et al., 2002). This complex abiotic stress, which affects osmotic and ionic component, induces a wide range of metabolic perturbations in higher plants but it is not always possible to distinguish those associated with the osmotic component to those due to ion toxicity. These metabolic perturbations result in growth reduction and alteration of nutritional balance. However, in order to survive in the presence of salt, plants have developed several adaptative mechanisms that are not yet well established (Lutts et al., 1996b) and there is a substantial variation in salt-tolerance among different species (Munns et al., 2002) and among cultivars of the same specie (Watanabe et al., 2000; Al-Karaki, 2000; Ghoulam et al., 2002). In General, the presence of NaCl in plant environment induces an increase in Na^+ and Cl^- and a decrease in K^+ concentrations in leaves, as well as in roots (Maggio et al., 2007). In many glycophyte species, such as rice (Lutts et al., 1996a; 1996b), wheat (Almansouri et al., 1999), cotton (Ashraf and Ahmad, 2000) and Lotus genotypes (Melchiorre et al., 2009), the most resistant genotypes accumulate the least toxic ions (Na^+ and or Cl^-) in growing tissues (leaves or calli). For other glycophytes, salt-tolerance is associated with inclusion of toxic ions; thus more tolerant types are those which accumulate more toxic ions (Na^+ and or Cl^-) in growing and photosynthetic tissues. In this category, we can quote *Vigna sp*. (Gulati and Jaiwal, 1993) and lens (Ashraf and Waheed, 1993); salt-tolerance of these types is due to a compartmentation of toxic ions in vacuoles to

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to keep only quantities compatible with enzymatic activities in the cytoplasm. In other plants, the response to salinity is expressed with a reduction in K^+ concentration. K^+ is the most abundant cation in higher plants (Mäser et al., 2002) and an important macro element for cellular metabolism. In General, tolerant genotypes maintain a high supply of K^+ in the presence of an excess of Na^+ (Piri et al., 1994; Lutts and Guerrier, 1995; Santa-Maria and Epstein, 2001; Ashraf et al., 2010). Contrary to other glycophytes such as rice, corn and wheat, little is known about the physiological mechanisms involved in sugarcane salt tolerance. Sugarcane plant is considered as moderately sensitive to salinity (Maas and Hoffmann, 1990); some studies analyzed salt effects on plant growth and metabolism (Meinzer et al., 1994; Rozeff, 1995; Lingle et al., 2000), without however highlighting the physiological mechanisms implied in salt tolerance. Recently, some reports concluded that in sugarcane, salt-resistant genotypes accumulate less toxic ions (Na^+ and or Cl^-) in leaves (Akhtar et al., 2003; Wahid, 2004) while Plaut et al. (2000) reported in other sugarcane cultivars that salt-resistant genotypes accumulate more Na^+ and or Cl^- in leaves than the salt-sensitive plants.

In the present study, we compared NaCl effects Na^+, Cl^- and K^+ accumulation in leaves and roots of plants of two sugarcane cultivars which differ in their response to salt stress. The study aimed to analyse the implication of ions accumulation in sugarcane plant salt tolerance.

MATERIALS AND METHODS

Plant material and culture conditions

Sugarcane (*Saccharum* sp.) cultivars were obtained from the "Centre Technique des Cultures Sucrières" (CTCS), Morocco. Cv. CP65-357 and CP66-346 are American cultivars (Canal Point) largely cultivated in Morocco. Cv. CP66-346 is more salt tolerant than CP65-357 at whole plant level (Gandonou et al., 2011). Stalk segments were cut at single bud sets (approximately 5 cm), surface desinfected with ethanol 70°, placed between humidified newspapers and transferred in drying oven at 30°C for germination. After 6 to 8 days, plants were transferred to pots containing tap water for 8 days in culture room characterized by a temperature of 28 ± 2°C, a photoperiod of 14 or 10 h, light intensity between 1100 and 1200 lux with artificial lamps, brand Philips, TL 40w/54-765 and a relative humidity of 50%. Then, tap water was replaced by modified Hoagland solution (Hoagland and Arnon, 1950) in which macro-elements concentrations have been reduced to half and added with Fe-EDTA of Murashige and Skoog (1962). Medium was prepared with distilled water and pH was adjusted to 6.5 with NaOH 4N. Plants were cultivated in this medium for 7 days.

Salt treatment

After 7 days in culture, stress was applied to the plants using four NaCl concentrations that are: 0 mM (0 mg/L); 17 mM (1000 mg/L); 34 mM (2000 mg/L) and 68 mM (4000 mg/L) corresponding, respectively to an electric conductivity of 0.983, 2.83, 4.26 and 6.63 mS/cm. Each pot contained 10 plants and 3 pots per NaCl concentration were used. Stress was maintained for 2 weeks.

Extraction and estimation of ion concentrations

For ions determination, leaves were rapidly rinsed with distilled water while roots were rinsed for 3 min. in distilled water to eliminate ions fixed on roots and those contained in the apoplasm (Bourgeais-Chaillou and Guerrier, 1992). The same number of leaves was collected from each plant. Then, the leaves were split in two groups; the young and the old leaves. If total number of leaves per plant is even, the inferior half was considered as old leaves while the superior half is considered as young. If the total number of leaves were odd, the repartition was the same except that the median leaf was ignored. Young leaves, old leaves and roots were oven-dried individually at 80°C for 72 h, ground in a poter in environmental conditions and the powder was dried for 24 h at 80°C. In order to evaluate the effect of NaCl stress on Na^+ and K^+; leaves and roots powders were digested in HNO_3 and analysis was conducted using a flame spectrophotometer (PHF 90 D). For Cl^- estimation, ions were extracted with hot distilled water (80°C for 2 h). Chloride was determined colorimetrically with ferric ammonium sulfate and mercuric thiocyanate as previously described (Guerrier and Patolia, 1989). Ions concentrations were evaluated based on dried matter and NaCl (Na^+ and Cl^-) and KCl (K^+) were used as standards.

Statistical analysis

All the experiments were repeated twice independently with similar results. Data are expressed as mean ± standard error with a reading of three samples per treatment. The analysis of the main effects of stress intensity and/or cultivars was based on a 1-way (K^+) or 2-ways (Na^+ and Cl^-) analysis of variance (ANOVA). All statistical analyses were performed by SAS 92 program (SAS Institute, 1992).

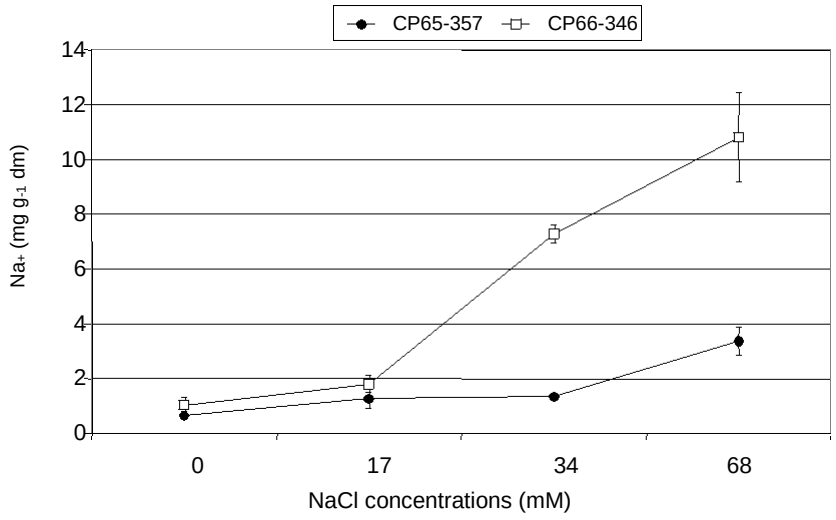
RESULTS

Na^+ concentration

A dose dependent increase in Na^+ concentration was recorded in young leaves (a), old leaves (b) and in roots (c) in the presence of NaCl (Figure 1). A two-way ANOVA showed a significant effect of NaCl ($p < 0.001$) on Na^+ concentration in both cultivars in old leaves, young leaves and in roots, and a significant difference ($p < 0.001$) between the two cultivars in leaves Na^+ content. Thus salt-tolerant cv CP66-346 accumulated more Na^+ in leaves (young and old) than salt-sensitive cv CP65-357 in particular at high NaCl concentrations, while salt-sensitive cultivar accumulated more Na^+ in roots than the tolerant cultivar in the presence of salt stress as revealed by the significant interaction ($p < 0.001$) between stress and cultivar (Table 1).

Cl^- concentration

In the present of NaCl, Cl^- concentration increased in young leaves (a), old leaves (b) and in roots (c) of both



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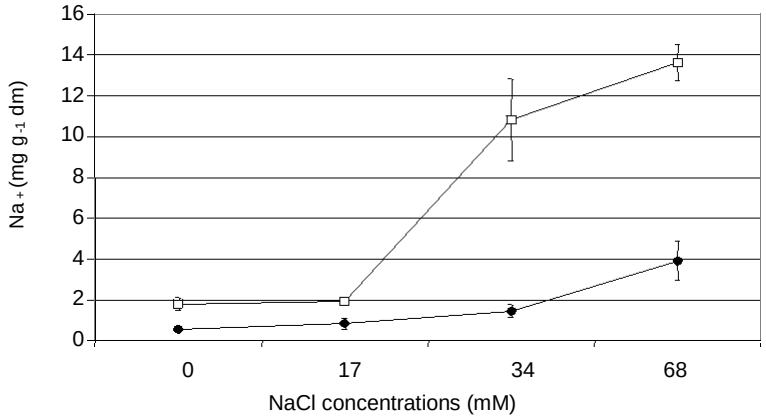

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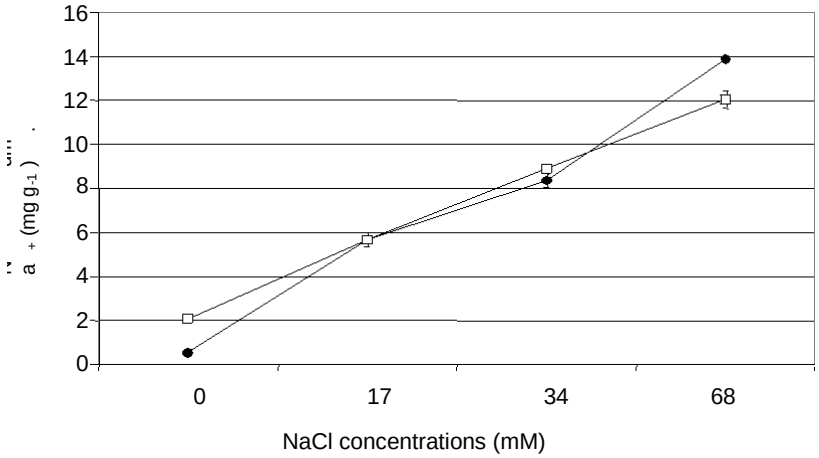

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Figure 1. Na^+ concentration [in mg g^{-1} (d. m.)] in young leaves (a), old leaves (b) and roots (c) of two sugarcane cultivars (CP65-357, salt-sensitive and CP66-346, salt-tolerant) as affected by different concentrations of NaCl after 2 weeks (n=3; vertical bars are S.E).

Table 1. Results of 2-ways variance analysis for Na^+ and Cl^- ions content of different parts of sugarcane plants (YL: Young leaves; OL: Old leaves; and roots); F- ratios are given for the main effects of the following levels of classification: stress intensity (i.e. NaCl concentration of stressing media) and cultivars and interaction between these levels of classification.

Parameters		Stress	Cultivar	Interaction (stress X cultivar)
Na^+	YL	38.77***	60.73***	15.82***
	OL	36.63***	75.56***	15.52***
	Roots	862.68***	0.12ns	17.84***
Cl^-	YL	1.34 ns	0.42 ns	0.53 ns
	OL	152.62***	1083.07***	137.35***
	Roots	93.92^^^	510.09^^^	68.04^^^

ns: not significant; *: significant at p=0.05; **: significant at p=0.01; ***: significant at p=0.001.

varieties as the NaCl concentration increase in the rooting medium (Figure 2). Statistical analysis revealed a significant effect of NaCl ($p < 0.001$) on Cl^- concentration in both cultivars in old leaves and in roots, and a significant difference ($p < 0.001$) between the two cultivars in old and roots Cl^- content (Table 1). So, salt- tolerant cv. CP66-346 accumulated more Cl^- in old leaves compared to salt-sensitive CP65-357 in particular at high NaCl concentrations as revealed by the significant interaction ($p < 0.001$) between stress and cultivar (Table 1).

K^+ concentrations

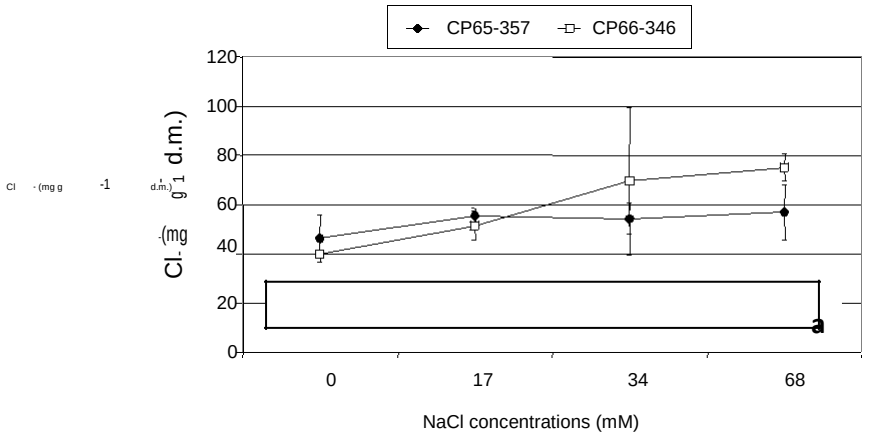
A dose dependent decrease was recorded for K^+ concentration in young leaves (a), old leaves (b) and in roots (c) in the presence of NaCl in the rooting medium in both varieties (Figure 3). A one-way ANOVA revealed that salt-effect in K^+ concentration was significant in the salt-sensitive cultivar CP65-357 in presence of 17 mM NaCl ($p < 0.001$), while in the tolerant CP66-346, the effect of salt on K^+ concentration was significant ($p < 0.01$) only in young leaves at 34 mM (Table 2). Thus, the reduction of K^+ content in leaves and roots in the presence of NaCl was more accentuated in the sensitive cultivar CP65-357 compared to the salt-tolerant cv. CP66-346.

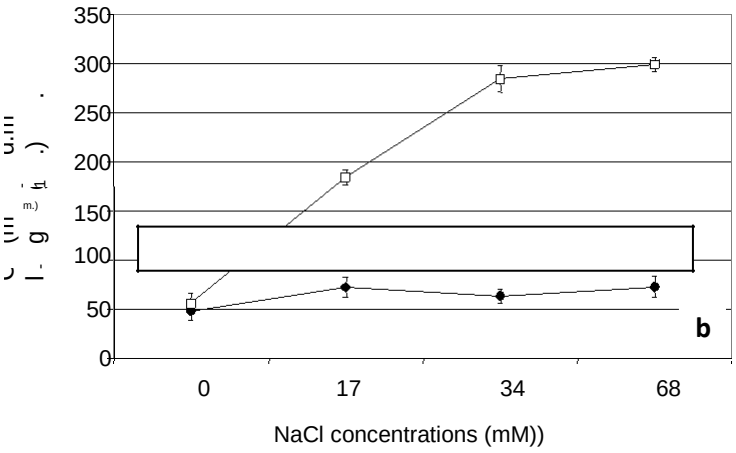
DISCUSSION

The effect of salt stress on plants can induce the following three responses: dehydration of the cells through the low water potential; nutritional imbalance caused by the interference of saline ions with essential nutrients in both uptake and translocation processes and toxicity due to the high accumulation of Na^+ and Cl^- in the cytoplasm.

Plants of the two cultivars accumulated high amount of Na^+ and Cl^- in old leaves and roots in comparison with young leaves. These results indicated that both cultivars are efficient in Na^+ and Cl^- exclusion from photo-synthetically active leaves as reported in other sugarcane varieties by Plaut et al. (2000) and in tomato varieties by Netondo et al. (2004).

Our results revealed that CP66-346 accumulated more Na^+ and Cl^- than cv CP65-357 both in young leaves (even if statistical analysis did not reveal a significant difference between cultivars for Cl^-) and in old leaves. In addition, salt-sensitive cv CP65-357 accumulated more Na^+ in roots compared to leaves. These ions are known to be toxic to cell metabolism. These results indicated that Na^+ and Cl^- ions toxicity should play a key role in NaCl adverse effects on sugarcane plants growth. Based on plants growth data, the detrimental effects of Na^+ and Cl^-on growth were more accentuated in aerial part of the sensitive CP65-357 compared to CP66-346 plants. These data suggest that the accumulation of these ions in young leaves apparently did not cause much injury to the aerial part in tolerant cultivar as reflected by the plant growth data. It is, therefore, logical to infer that much of the ions are sequestrated in the vacuoles (Jain and Selvaraj, 1997) causing very little or no interference with the cellular metabolism necessary for sustained growth. Our results are in agreement with those reported in other varieties of sugarcane (Plaut et al., 2000) and in varieties of other species (Ashraf and Waheed, 1993; Cramer et al., 1994; Chen and Zhao, 1996; Leidi and Saiz, 1997; Netondo et al., 2004). However, for most glycophytes (Lutts et al., 1996a; Almansouri et al., 1999) and other genotypes of sugarcane (Akhtar et al., 2003; Wahid, 2004; Ashraf et al., 2010), salt tolerant varieties accumu-lated less Na^+ and//or Cl^- in leaves in general and in young leaves in particular. It appears that in sugarcane two opposite tolerance mechanisms exist: some tolerant cultivars accumulated less Na^+ and//or Cl^- in leaves (Akhtar et al., 2003; Wahid, 2004; Ashraf et al., 2010) while some other tolerant cultivars accumulated more Na^+ and//or Cl^- in leaves as revealed by our results. It is well known that for a considered species, two different salt-tolerant genotypes could use opposite mechanisms as





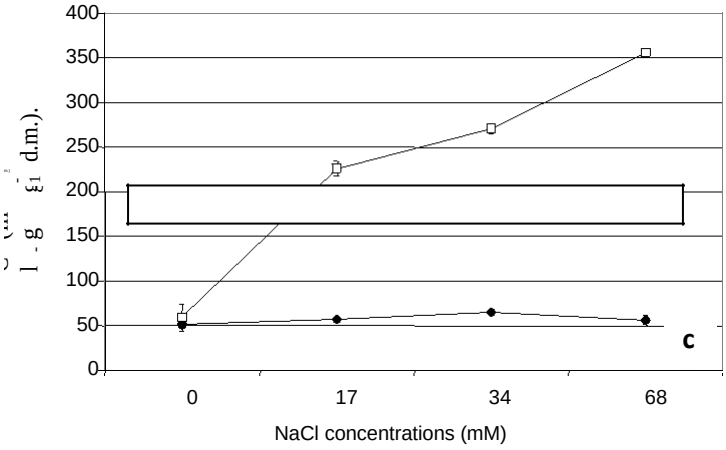
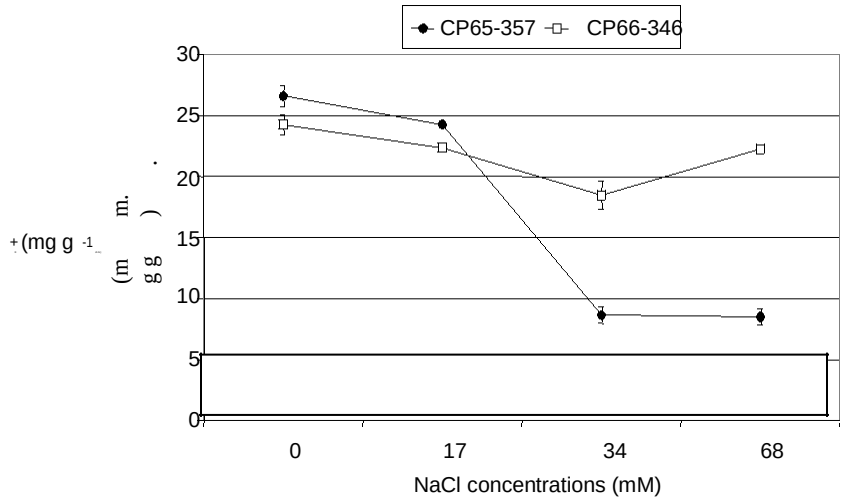


Figure 2. Cl^- concentration [in mg g^{-1} (d. m.)] in young leaves (a), old leaves (b) and roots (c) of two sugarcane cultivars (CP65-357, salt-sensitive and CP66-346, salt-tolerant) as affected by different concentrations of NaCl after 2 weeks (n=3; vertical bars are S.E).



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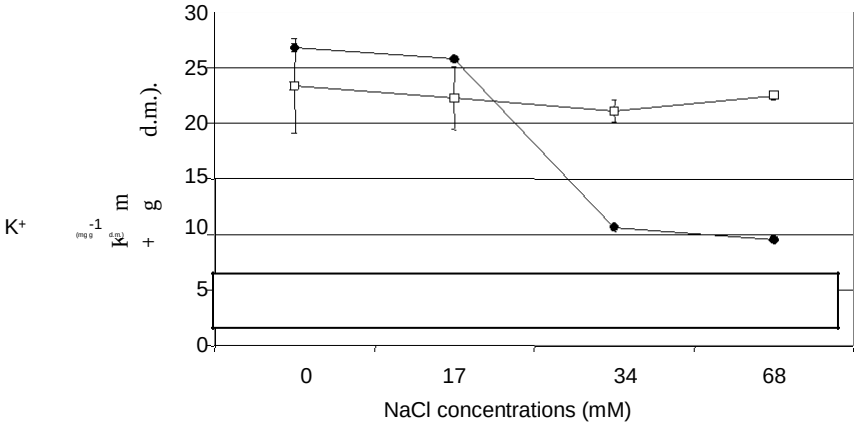

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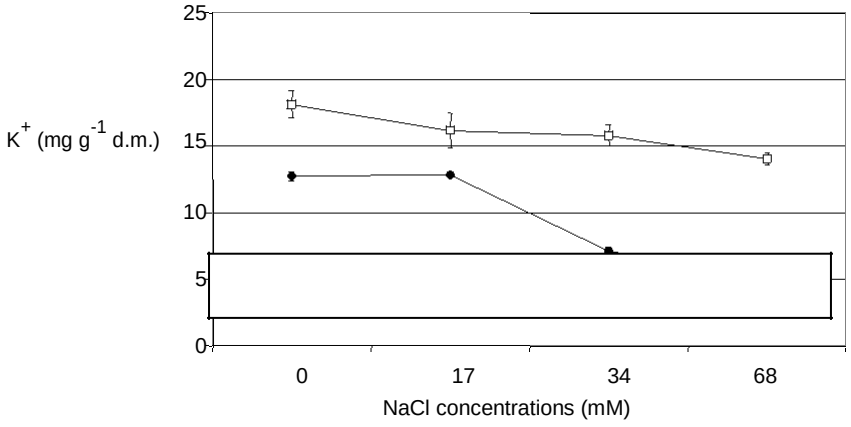

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Figure 3. K^+ concentration [in mg g^{-1} (d. m.)] in young leaves (a), old leaves (b) and roots (c) of two sugarcane cultivars (CP65-357, salt-sensitive and CP66-346, salt-tolerant) as affected by different concentrations of NaCl after 2 weeks (n=3; vertical bars are S.E).

Table 2. Results of 1-way variance analysis for K^+ content of different parts of plants (plants (YL: You ve and CP66-346, salt-tolerant); F- ratios are given for the main effect of stress intensity (i.e. NaCl ng leaves; OL: Old leaves; and roots) of two sugarcane varieties (CP65-357, salt-sensiti concentration of stressing media)

Parameter	CP65-357			CP66-346		
	YL	OL	Roots	YL	OL	Roots
Number of observations (n)	12	12	12	12	12	12
Degree of freedom (df)	3	3	3	3	3	3
F value	230.97***	1030.73***	283.82***	10.60**	0.13 ns	3.26 ns
Probability (p)	0.0001	0.0001	0.0001	0.0037	0.939	0.0804
Least significant difference (LSD)	2.0958	0.9496	0.7569	2.4285	8.4758	3.0332

ns: not-significant; **: significant at p = 0.01; ***: significant at p = 0.001.

reported in rice for example (Lutts et al., 1999). In cotton, Ahmad and Ashraf (2000) have reported that salt-resistant lines or cultivars accumulated less toxic ions (especially for Cl^-) in leaves than sensitive lines or cultivars, while Chen and Zhao (1996) and Leidi and Saiz (1997) have reported an opposite tendency (especially for Na^+). These observations were probably due to the genetically basis of salt-tolerance. Salt-tolerant cv CP66-346 appeared as a toxic ions includer since it accumulated more Na^+ and Cl^- in young leaves inpresence of NaCl than the salt sensitive cultivar.

Salt-stress decreased K^+ content in leaves and roots of both cultivars. They were significant differences between cultivars in K^+ concentration. The salt-tolerant cv CP66-346 maintained high amounts of K^+ in young leaves, old leaves and in roots in comparison with the sensitive cv CP65-357. The maintenance of high amount of K^+ was the main response of tolerant genotypes to salt stress in glycophytes plants and K^+ ions are known to be a major component of osmotic adjustment during stress (Wu et al., 1996). Thus, in rice, Lutts et al. (1996a) reported that salt tolerant variety maintained higher amounts of K^+ in leaves compared to the salt sensitive genotypes when both were confronted to salt stress. The same tendency was reported in durum wheat (Almansouri et al., 1999). Thus the salt tolerance of the cultivar CP66-346 was due, at least partially to the internal maintenance of high concentrations of K^+ in the presence of high amount of Na^+ in the medium.

The present study revealed that Na^+ and Cl^- toxicity is implied in salt detrimental effect in sugarcane plants. The results indicate that toxic ions accumulation combined to K^+ ion maintenance"are the main option to counteract the negative effects of salt stress in sugarcane plants. Complementary investigations are required to assess the physiological and biochemical basis of ion effects on stressed plants metabolism and the molecular basis of salt tolerance mechanisms.

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