7 Sugarcane (*Saccharum* sp.) Salt Tolerance at Various Developmental Levels

Ch.B. Gandonou,[1,2]* and N. Skali-Senhaji[1]
[1]*Laboratoire de Biologie et Santé, Faculté des Sciences de Tétouan, Université Abdelmalek Essaâdi, Tétouan, Morocco;* [2]*Laboratoire de Physiologie végétale et d'Etude des Stress Environnementaux, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Cotonou, République du Bénin*

Abstract
Salt-stress affects plant growth and development at different stages. In this work, we evaluated the level of salinity tolerance of five sugarcane (*Saccharum* sp.) varieties: CP66-346, CP65-357, CP70-321, CP59-73 and NCo310 by using different NaCl concentrations (0, 17, 34, 68 and 102 mM). This evaluation was based on the *in vitro* bud emergency, young plants' survival and growth in hydroponic system, and finally on the aspect and the growth of calli issued from foliar explants. NaCl stress effects result in a reduction of the final bud emergency percentage. At bud emergence stage, varieties CP66-346 and CP59-73 appeared to be the most salt tolerant while NCo310 behaved as the most salt sensitive. Young plants' survival and growth are also reduced by salinity and at this stage variety CP66-346 seems to be the most tolerant while CP65-357 and CP70-321 are the most sensitive. Salinity causes calli necrosis and reduces their growth; varieties NCo310 and CP70-321 appeared to be the most salt tolerant while CP65-357 seems to be the most sensitive. These results indicate that the salt tolerance of a variety depends on the stage of development and the level considered. Consequently, salt tolerance of a given cultivar at whole plant level does not guarantee salt tolerance of tissue or cell cultures issued from this cultivar. Bud emergency stage seems to be the most tolerant stage. Variety CP66-346 appeared to be a salt-tolerant variety at both bud emergence and young plants stages.

7.1 Introduction

Salinity is a major abiotic stress increasingly affecting plant health and survival worldwide (Sakhanokho and Kelly, 2009). The cultivable areas affected by this stress were estimated to be 900 million ha (Flowers, 2004), half of which is irrigated (Zhu, 2001); this area increases continuously due to bad agricultural practices. Sugarcane culture which is generally carried out under strong irrigation is confronted with this problem. This plant is classified as a moderately salt-sensitive species with a threshold electrical conductivity of paste saturated extract of about 1.7 dS m^{-1} beyond which production decreases (Ayers and Westcott, 1989). This complex abiotic stress which presents osmotic and ionic components, poses a threat to agriculture (Munns *et al*., 2006). It causes many metabolic disturbances in higher plants without being always possible to distinguish effects due to the osmotic component from those related to specific ions toxicity. However, it is largely

*E-mail: ganchrist@hotmail.com

known that a substantial variation exists in salt sensitivity of various species and of various cultivars and ecotypes of the same species. In addition, several authors reported that NaCl affects seed germination (Cramer, 1994; Ghoulam and Farès, 2001; Debez *et al*., 2004), plant growth and survival (Lutts *et al*., 1995; Wang *et al*., 1997; Almansouri *et al*., 2001; Aghaei *et al*., 2008; Shafi *et al*., 2011) and cell growth and necrosis (Arzani and Mirodjagh, 1999; Basu *et al*., 2002; Alvarez *et al*., 2003; Htwe *et al*., 2011). It is generally admitted that the salt-tolerance of a given genotype depends on the developmental stage and the selected organization level (Lutts *et al*., 1995); therefore, a genotype tolerant at the germination stage can appear rather sensitive at the young plant stage and/or at cellular level. Salt effects on sugarcane plants are generally studied either at the stage of germination (i.e. bud emergence) (Kumar and Naidu, 1993; Chowdhury *et al*., 2001; Akhtar *et al*., 2003; Gandonou *et al*., 2008, 2011), whole plant level (Chowdhury *et al*., 2001; Wahid, 2004; Sebastian *et al*., 2009; Gandonou *et al*., 2012), or at cellular level (Gonzalez *et al*., 1995; Gandonou *et al*., 2005a; Errabii *et al*., 2006). Little work has dealt with the study of sugarcane NaCl tolerance combined with germination stage, whole plant level and cellular level. The aim of this study is to compare the average level of salt tolerance of five sugarcane cultivars at germination stage, young plant level and at cellular level in order to check if the average level of tolerance of a cultivar is the same at all three stages.

7.2 Material and Methods

7.2.1 Plant material

The experimental plant materials used are sugarcane cvs NCo310, CP70-321, CP65-357, CP59-73 and CP66-346 used by Gandonou *et al*. (2012).

7.2.2 Salt concentrations

NaCl was used as salt. For germination stage and *in vitro* callus culture stage studies, five NaCl concentrations were used: 0, 17, 34, 68 and 102 mM (0, 1, 2, 4 and 6 g l^{-1}, respectively) (Gandonou *et al*., 2005a, 2011) while for young plant-level study, only the first four NaCl concentrations were used because 102 mM NaCl appeared to be high enough to prevent young plant survival (Gandonou *et al*., 2012).

7.2.3 Germination stage study

The study was done *in vitro*. Young single bud setts (approximately 4 cm) were taken from the top of each plant and wiped with cotton saturated with ethanol 70%. Setts' disinfection conditions, medium composition and culture conditions are those described by Gandonou *et al.* (2008, 2011). Final germination percentage was determined for each variety and each NaCl concentration after 8 days.

7.2.4 Young plants-level study

The study was done in hydroponic medium. Stalk disinfection and germination, media composition, plant growth conditions, plant survival and growth determination methods are those described by Gandonou *et al*. (2012). Relative height growth of plants (RHG) was calculated as described by the former authors.

7.2.5 Cellular-level study

Calli were induced from young leaf cylinders. Medium composition, callus cultures and *in vitro* salt treatment conditions, calli necrosis and relative fresh weight growth (RFWG) determination methods are those described by Gandonou *et al*. (2005a, b).

7.2.6 Statistical analysis

All the experiments were repeated twice independently. The number of germinated buds, the number of dead plants and the number of necrotic calli were analysed as binomial distribution variates. For plants and calli growth, 1-way or 2-ways analysis of variance (ANOVA) were used to study the main effects of cultivars and/or stress intensity. All analyses were carried out using SAS program (SAS Institute, 1992).

7.3 Results

7.3.1 Effect of NaCl stress at germination stage

Practically no effect of salt stress was observed on final germination for the varieties CP59-73 and CP66-346 (Fig. 7.1). The final bud germination rate of variety CP66-346 was reduced for about 4% in presence of 17 and 68 mM of NaCl; a slight increase (no significant) was observed at 34 mM. For variety CP59-73, bud germination showed a slight reduction (3%, not significant) only at 34 mM of NaCl. The highest bud germination rate reduction was observed for cv. NCo310 with a reduction of 10% at 17, 34 and 68 mM NaCl. For cv. CP70-321, bud germination rate reduction under salt stress was about 6% at the three NaCl concentrations above while for cv. CP65-357, bud germination rate show a reduction of 3%, 9% and 6% at 17, 34 and 68 mM of NaCl, respectively. These reductions were not significant ($p < 0.05$).

Table 7.1 presents the percentages of final bud germination when means values were calculated from data collected for the three doses of NaCl (17, 34 and 68 mM) and expressed as percentage of that of the control. These data showed that salt stress reduced bud germination for about 10% compared to control for cv. NCo310 while this reduction was about 6% for cvvs CP70-321 and CP65-357; no reduction was observed for CP59-73 and CP66-346.

Thus, cvs CP59-73 and CP66-346 appeared to the most salt tolerant at germination stage while NCo310 behaved as the most salt-sensitive cultivar.

7.3.2 Effect of NaCl stress at whole plant level

Plant survival

The reduction of survival (plant death) due to the average effect of salt stress was lower for cv. CP66-346 (5%) and higher for cvs CP70-321 and CP65-357 (21.67% and 20%, respectively) (Table 7.2); this reduction was intermediary for cvs CP59-73 and NCo310 (13.33% and 16.67%, respectively). Thus, cv. CP66-346 presented the highest survival while CP70-321 and CP65-357 showed the lowest survival

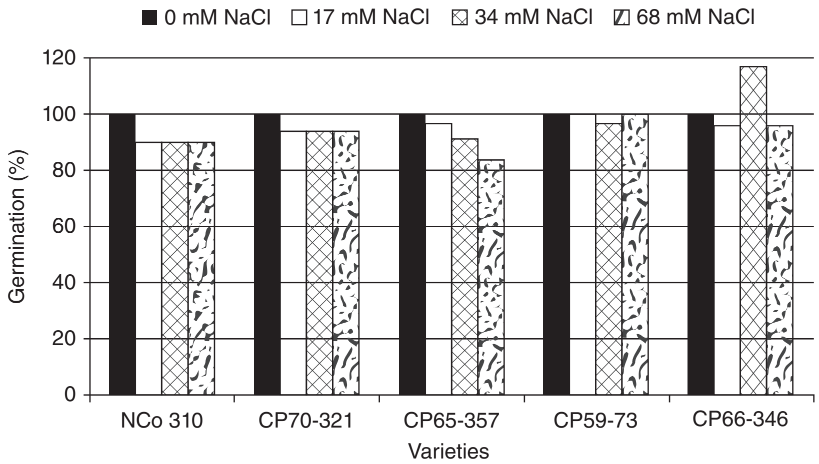

Fig. 7.1. Effect of NaCl salinity on five sugarcane variety buds *in vitro* germination percentage (cvs NCo310, CP70-321, CP65-357, CP59-73 and CP66-346) after 8 days of culture: germination percentages in presence of NaCl were expressed as percentage of that of the control (Gandonou *et al*., 2011).

Table 7.1. Mortality percentages of plants of five sugarcane cultivars as affected by different NaCl concentrations (Gandonou *et al*., 2012) (n = 20 or n = 30).[a]

NaCl concentration (mM)	Cultivars				
	NCo310	CP70-321	CP65-357	CP59-73	CP66-346
0	0 a	0 a	0 a	0 a	0 a
17	10 ab	10 a	10 ab	0 a	0 a
34	10 ab	10 ab	20 bc	10 ab	5 ab
68	30 bc	45 c	30 bc	30 bc	10 ab

[a]Values followed with same letter are not significantly different at $p < 0.05$

Table 7.2. Germination percentages, mortality percentages, relative height growth of plants, callus necrosis percentage and callus relative fresh weight growth of five sugarcane cultivars (CP65-357, NCo310, CP70-321, CP59-73 and CP66-346) as affected by NaCl stress: (Gandonou *et al*., 2005a, 2011, 2012).

		Cultivars				
		NCo310	CP70-321	CP65-357	CP59-73	CP66-346
Bud germination rate (%)	0 NaCl	100	100	100	100	100
	+ NaCl	90.78	94.12	94.29	98.96	103.08
Plant mortality (%)	0 NaCl	0 a	0 a	0 a	0 a	0 a
	+ NaCl	16.67 b	21.67 b	20 b	13.33 b	5 a
Plant growth (%)	0 NaCl	100 a	100 a	100 a	100 a	100 a
	+ NaCl	75.65 b	50.99 b	63.31 b	65.44 b	80.47 a
Callus necrosis (%)	0 NaCl	0 a	0 a	2.86 a	0 a	ND
	+ NaCl	8.33 ab	2.86 a	26.31 b	0 a	ND
Callus growth (%)	0 NaCl	100 a	100 a	100 a	100 a	ND
	+ NaCl	75.69 b	73.33 b	49.09 b	66.83 b	ND

0 NaCl, control; + NaCl, presence of NaCl: data in presence of NaCl were expressed as the average of the three (or four) values obtained in the presence of the three (or four) NaCl concentrations (17; 34, 68 and 102a mM) expressed in percentage of that of control.
[a]This concentration was used only for cellular level study.

in the presence of NaCl compared to NCo310 and CP59-73.

On the basis of plant survival criterion, cv. CP66-346 appeared to be the most salt-tolerant at the whole-plant level, while cvs CP70-321 and CP65-357 were the most salt-sensitive.

Plant growth

NaCl stress reduced significantly plant RHG for all cultivars (Fig. 7.2). For cv. NCo310, RHG reduction was significant ($p < 0.05$) for 17 mM NaCl (reduction was not significant at 34 mM NaCl) and 68 mM NaCl. These reductions correspond to 25%, 16% and 33% of the control, respectively. For cv. CP70-321, RHG reduction corresponds to 27%, 50% and 70% of control at 17, 34 and 68 mM NaCl, respectively. The reduction observed was significant ($p < 0.001$) at all NaCl concentrations used (Fig. 7.2). In cv. CP65-357, RHG reduction under salt stress was significant ($p < 0.01$) starting from 17 mM NaCl (Fig. 7.2) and corresponds to a reduction of growth of 29%, 40% and 41% compared to the control at 17, 34 and 68 mM NaCl, respectively. For CP66-346, RHG reduction was significant ($p < 0.001$) at 34 and 68 mM NaCl (Fig. 7.2) and corresponds to a growth reduction of 4%, 10% and 45% compared to the control at 17, 34 and 68 mM NaCl, respectively. For cv. CP59-73, plant RHG reduction was about 6%, 48% and 50% in the presence of 17, 34 and 68 mM of NaCl, respectively; this reduction was significant ($p < 0.001$) at 34 mM and 68 mM NaCl (Fig. 7.2). There is, thus, a significant difference in the behaviour of the studied cultivars. It is important to note that contrary to cvs NCo310 and CP65-357 for

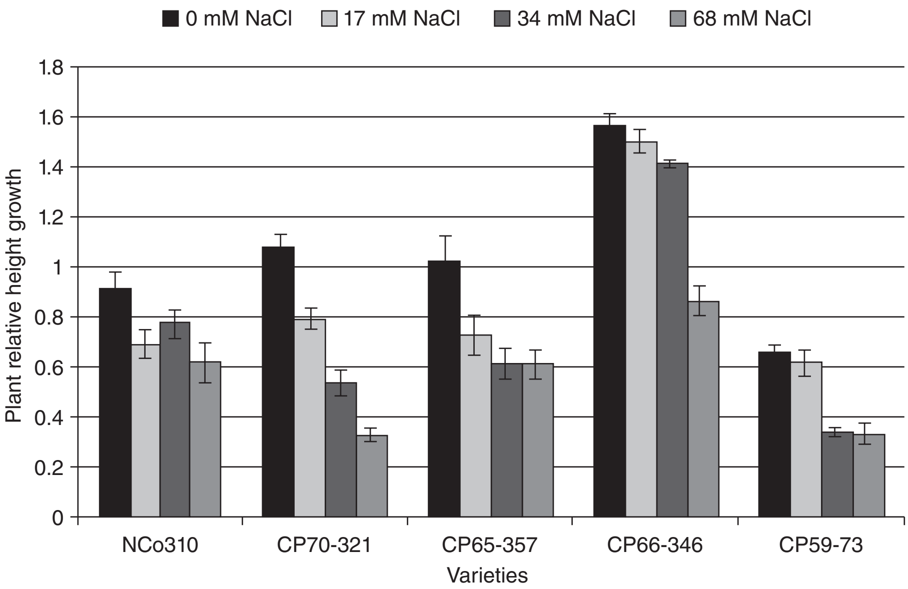

Fig. 7.2. Plant relative height growth of five sugarcane cultivars as affected by different NaCl concentrations (n = 4; vertical bars are standard errors): values within cultivar with the same letter are not significantly different at $p < 0.05$ (Gandonou *et al*., 2012).

which plant growth was significantly affected starting from 17 mM of NaCl (but not at 34 mM for NCo310), the growth of CP59-73 and CP66-346 was significantly affected only starting from 34 mM of NaCl.

The reduction of plant growth due to the average effect of salt stress was lower for cvs CP66-346 and NCo310 (19.53% and 24.35%, respectively) and higher for cv. CP70-321 (approximately 49%); this reduction was intermediary for cvs CP59-73 and CP65-357 (34.56% and 36.69%, respectively) (Table 7.2). Thus cvs CP66-346 and NCo310 presented a higher growth rate in the presence of NaCl compared to cvs CP59-73, CP65-357 and CP70-321.

On the basis of plant growth criterion, cv. CP66-346 appeared to be the most salt tolerant at the young plant stage (whole-plant level) while CP70-321 and CP65-357 behaved as the most salt-sensitive cultivars.

Effect of NaCl stress at cellular level

In the culture conditions used, cv. CP66-346 did not produce callus. Thus, the salt stress effect at cellular level was studied only for the four cultivars that produced calli in the culture conditions.

The addition of NaCl to culture medium caused an increase in calli necrosis for all cultivars (Table 7.3) and significant difference in calli necrosis was observed among genotypes. No callus of CP59-73 showed necrosis under NaCl concentrations used. For CP70-321, no callus showed necrosis below 68 mM NaCl, while for NCo310, the first callus necrosis was observed at 34 mM NaCl. For NCo310 calli, the effect of NaCl was significant ($p < 0.05$) only at the highest dose of NaCl used (102 mM) while this effect was significant ($p < 0.05$) at 68 mM for CP65-357 calli. These results revealed significant differences among cultivars for callus necrosis percentages.

The increase in callus necrosis due to the average effect of salt stress was lower for cvs CP59-73 and CP70-321 (0% and 2.86%, respectively) and higher for cv. CP65-357 (approximately 26%); this reduction was intermediary for cv. NCo310 (8.33%) (Table 7.2).

On the basis of callus necrosis criterion, cv. CP59-73 appeared to be the most salt-tolerant at cellular level followed by CP70-321; CP65-357 was the most salt-sensitive.

Callus RFWG decreased as the concentration of NaCl increased in the culture medium (Fig. 7.3). The highest reduction was observed in cv. CP65-357 with a reduction of callus growth about 27%, 45%, 59% and 63% at 17, 34, 68 and 102 mM NaCl, respectively. The reduction was significant ($p<0.001$) from 17 mM NaCl. Cultivars NCo310 and CP70-321 showed the lowest reduction with a reduction of 4%, 21%, 28% and 44%, and 10%, 21%, 35% and 41% respectively, at the same NaCl concentrations. The reduction observed was significant ($p<0.01$ in the case of NCo310, and $p<0.001$ in the case of CP70-321) only from 34 mM NaCl. Cultivar CP59-73 showed an intermediary behaviour with callus growth reduction about 12%, 28%, 36% and 56% in the presence of 17, 34, 68 and 102 mM NaCl, respectively. This reduction was significant ($p<0.001$) only from 34 mM NaCl.

The reduction of callus growth due to the average effect of salt stress was lower for cvs NCo310 and CP70-321 (24.31% and 26.67%, respectively) and higher for cv. CP65-357 (approximately 51%); this reduction was intermediary for cv. CP59-73 (33.17%) (Table 7.3). Thus, cvs NCo310 and CP70-321 presented the higher callus growth in the presence of NaCl while cv. CP65-357 presented the lowest growth; CP59-73 was intermediate.

Table 7.3. Necrosis percentages of callus obtained from four sugarcane varieties (CP65-357, NCo310, CP70-321 and CP59-73) as affected by different NaCl concentrations (partially from Gandonou *et al*., 2005a).[a]

NaCl concentrations (mM)	Cultivars			
	CP65-357	NCo310	CP70-321	CP59-73
0	2.86 ab	0 a	0 a	0 a
17	8.57 ab	0 a	0 a	0 a
34	13.33 bc	10.00 ab	0 a	0 a
68	40 cd	6.67 ab	2.86 ab	0 a
102	43.33 d	16.67 bc	8.57 ab	0 a

[a] Values within columns followed by the same letter do not differ significantly at $p=0.05$.

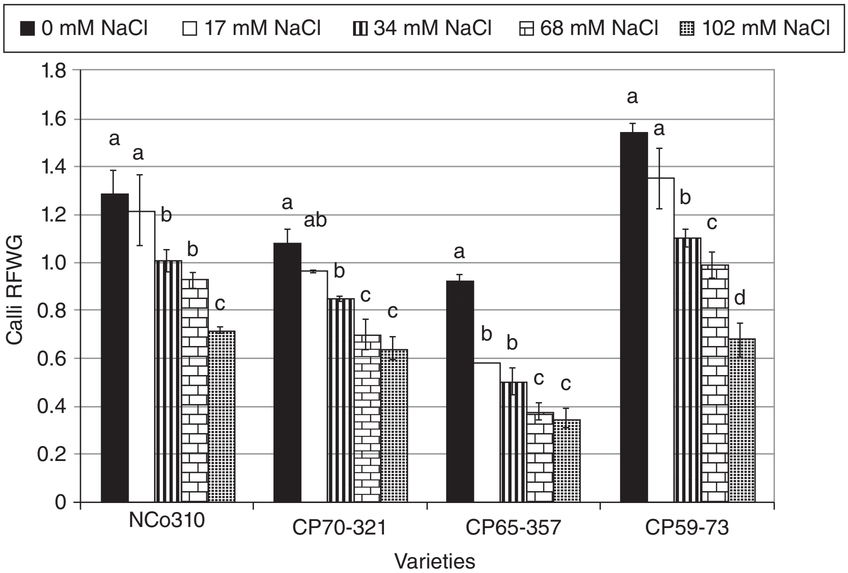

Fig. 7.3. Relative fresh weight growth (RFWG) of callus obtained from four sugarcane cultivars (CP65-357, NCo310, CP70-321 and CP59-73) as affected by different NaCl concentrations: vertical bars are standard errors ($n=4$) (partially from Gandonou *et al*., 2005a).

On the basis of the criterion of callus growth, cvs NCo310 and CP70-321 appeared to be the most salt-tolerant cultivars at cellular level while cv. CP65-357 appeared as the most salt-sensitive.

7.4 Discussion

NaCl stress reduced bud emergence rate, young plants survival and growth and callus growth. It also enhanced callus necrosis. Similar results were reported in several plants including rice (Lutts *et al*., 1995, 1996; Basu *et al*., 2002), durum wheat (Karadimova and Dyambova, 1993; Arzani and Mirodjagh, 1999), sunflower (Alvarez *et al*., 2003) and sugarcane (Kumar and Naidu, 1993; Gonzalez *et al*., 1995; Chowdhury et *al*., 2001; Akhtar et *al*., 2003; Hussain *et al*., 2004; Gandonou *et al*., 2005a, 2008). However, our results show a difference among cultivars' behaviour according to the stage or level of the study and the criterion used. For germination stage, cvs CP59-73 and CP66-346 appeared to be more salt tolerant whereas cv. NCo310 behaved as the most salt sensitive. Cultivars CP65-357 and CP70-321 were intermediaries or moderately sensitive. At young-plant stage, cv. CP66-346 was the most tolerant whereas cvs CP70-321 and CP65-357 behaved as the most sensitive. Cultivars CP59-73 and NCo310 appeared as moderately sensitive when based on plant survival rate criterion. Using plant growth criterion, cvs CP66-346 and NCo310 behaved as the most salt-tolerant whereas cv. CP70-321 was the most sensitive. Cultivars CP59-73 and CP65-357 were intermediaries. At cellular level, cvs CP59-73 and CP70-321 were the most tolerant whereas CP65-357 behaved as the most sensitive. Cultivar NCo310 appeared as moderately tolerant when based on callus necrosis criterion. Using callus growth criterion, cvs NCo310 and CP70-321 appeared as the most salt-tolerant whereas cv. CP65-357 was the most sensitive and cv. CP59-73 was intermediate.

Considering young plant stage, cv. CP65-357, which behaved as salt-sensitive based on plant survival criterion, appeared as moderately tolerant when plant growth was used as criterion. The same tendency was observed for cv. NCo310, which appeared as moderately sensitive when plant survival rate criterion was used, and as tolerant based on plant growth criterion. In the case of cellular level study, similar observations can be made for cvs CP59-73 and NCo310. These observations indicated that the relative salinity tolerance of the investigated sugarcane cultivars changed as a function of the criterion considered. These results are in agreement with those reported in rice by Lutts *et al*. (1995). For example, these authors have observed that at young seedling stage, cv. IR 31785 was the most sensitive variety according to root dry weight while it was viewed as rather resistant when root elongation was used as a criterion.

Overall, at the germination stage, among the investigated cultivars, cvs CP59-73 and CP66-346 were the most salt tolerant whereas cv. NCo310 appeared as the most salt sensitive while at young plant stage, cv. CP66-346 was the most salt tolerant whereas cv. CP70-321 was the most salt sensitive. At the cellular level, cvs CP70-321 and NCo310 were the most salt-tolerant whereas cv. CP65-357 was the most salt-sensitive. These data indicated variability in the relative order of tolerance of sugarcane cultivars according to the stage of development as reported previously (Heenan *et al*., 1988; Aslam *et al*., 1993; Lutts *et al*., 1995; Foolad, 2004) and that salt tolerance at a specific stage does not guarantee tolerance at another stage. Cultivar NCo310, for example, was sensitive during germination whereas it was rather tolerant at young plant stage and cellular level. A similar tendency was observed for cv. CP70-321, which was salt-sensitive at young plant stage but appeared as tolerant at cellular level.

Considering the four cultivars studied at both young plant stage and cellular level (i.e. cvs CP65-357, CP70-321, NCo310 and CP59-73), three tendencies were observed:

- Cultivar CP65-357 behaved as salt sensitive either at young plant stage or at cellular level;
- Cultivars CP59-57 and NCo310 behaved overall as moderately tolerant either at young plant stage or at cellular level; and
- Cultivar CP70-321, which was the most sensitive at young plant stage, appeared as the most tolerant at cellular level.

These findings revealed two types of correlation between salt tolerance of whole plant and salt tolerance of tissue or cell cultures derived from that plant. Positive correlation characterized by the same behaviour between whole plant and tissue or cell cultures as observed in this study for cvs CP65-357, CP59-73 and NCo310, and negative correlation characterized by a different behaviour between whole plant and tissue or cell cultures as observed in this study for cv. CP70-321. These findings corroborate data reported by Mills and Tal (2004) in tomato, which revealed these two types of correlation between whole-plant salt tolerance and salt tolerance of tissue or cell cultures issued from this plant. The fact that cvs NCo310 and CP59-73 are relatively tolerant, either at whole-plant stage or at cellular level, can be explained by the existence of a cellular basis, at least partial, of the tolerance of these cultivars.

Considering each specific stage, young-plant stage was more sensitive to salt than germination since the average effect of salt stress was significant and more accentuated at young-plant stage corroborating previous observations in rice (Akbar and Neue, 1987; Lutts *et al*., 1995). Although the high NaCl concentration (102 mM) was used only in the case of cellular study, the average effect of NaCl was similar to that obtained at young-plant stage. This observation indicates that cellular level was more salt tolerant than at the young-plant stage.

This study underlined the variability of relative salt-stress tolerance for some sugarcane cultivars during development and cellular level. It indicated that salinity tolerance at different development stages does not behave as an interdependent characteristic. Germination stage appeared as the most salt-tolerant stage in sugarcane. For the first time, we have demonstrated that, in sugarcane, salt tolerance of a given cultivar at whole-plant level does not guarantee salt tolerance of tissue or cell cultures issued from this cultivar.

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