

# Salinity tolerance of some sugarcane (*Saccharum* sp.) cultivars in hydroponic medium

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## abstract

Three-weeks-old sugarcane (*Saccharum* sp.) cultivars CP66-346, CP65-357, CP70-321, CP59-73 and NCo310 were exposed to NaCl concentrations of 0, 17, 34 or 68 mM for two weeks in a hydroponic system (Hoagland modified medium), corresponding to an electric conductivity of 1.00, 2.83, 4.26, and 6.63 mS/cm, respectively. Salinity stress variously impacted both plant growth and survival. Plant mortality increased with increasing salinity stress, with cultivar CP66-346 (5%) having the lowest mortality rate, cultivars CP70-321 (21.7%) and CP65-357 (20%) had the highest. In response to the salinity stress, reduction in shoot growth was lowest for cultivars CP66-346 (19.5%) and NCo310 (24.3%) and highest for cultivar CP70-321 (about 49%). Salinity impact on root elongation was variable depending not only on cultivar concerned but also ambient growing conditions. Considering the criteria based on plants survival and shoot growth, findings indicate that CP66-346 was the most salt tolerant, followed by NCo310 and CP59-73. Cultivars CP70-321 and CP65-357 were the most sensitive.

Keywords: cultivars, *Saccharum* sp, salinity, salt-tolerance, sugarcane, plant growth, plant survival

## Tolerancia a la salinidad de algunos cultivares de caña de azúcar (*Saccharum* sp.) en medio hidropónico

Se expusieron cultivares de caña de azúcar (*Saccharum* sp.) de tres semanas de edad, CP66-346, CP65-357, CP70-321, CP59-73 y NCo310, por dos semanas a concentraciones de NaCl de 0, 17, 34 o 68 mM, correspondiendo a conductividades eléctricas de 1.00, 2.83, 4.26, y 6.63 mS/cm respectivamente, en un sistema hidropónico (medio de Hoagland modificado). El stress salino impactó de diversas maneras al crecimiento y supervivencia de las plantas. La mortalidad de las plantas aumentó con el aumento del stress salino, presentando la menor tasa de mortalidad el cultivar CP66-346 (5%) y las más altas los cultivares CP70-321 (21.7%) y CP65-357 (20%). La reducción del crecimiento del tallo en respuesta al stress salino más baja fue para los cultivares CP66-346 (19.5%) y NCo310 (24.3%) y la más alta para el cultivar CP70-357 (cerca del 49%). El impacto de la salinidad en la elongación de la raíz varió en respuesta no solo al cultivar sino a las condiciones ambientales de cultivo. Considerando los criterios basados sobre la supervivencia de las plantas y el crecimiento del tallo se encontró que CP66-346 era el más tolerante a la sal, seguido por NCo310 y CP59-73. Los cultivares CP70-321 y CP65-357 fueron los más sensibles.

## Tolerância a salinidade de algumas canas (*Saccharum* sp.) no meio hydroponico

Cultivares de cana de três semanas (*Saccharum* sp.) CP66-346, CP65-357, CP70-321, CP59-73 e NCo310 foram expostos a concentrações de NaCl 0, 17, 34 ou 68 mM por duas semanas em um sistema hidropônico (meio modificado Hoagland), correspondente a uma condutividade elétrica de 1.00, 2.83, 4.26 e 6.63 mScm, respectivamente. Estresse de salinidade variadamente impactaram no crescimento e sobrevivência das plantas. A mortalidade das plantas aumentou com o aumento de estresse de salinidade, com cultivar CP66-346 (5%) tendo os cultivares de taxa de mortalidade mais baixas CP70-321 (21,7%) e CP65-357 (20%) mais altas. Em resposta ao estresse de salinidade, a redução no crescimento foi mais baixa para cultivares CP66-346 (19,5%) e NCo310 (24,3%) e mais alto para cultivar CP70-321 (cerca de 49%). Impacto de salinidade no alongamento de raiz foi variável dependendo não só do cultivar mas também condições do ambiente de crescimento. Tendo em conta os critérios com base no crescimento e sobrevivência de plantas, as conclusões indicam que CP66-346 era mais sal tolerante, seguido pelo NCo310 e CP59-73. Cultivares CP70-321 e CP65-357 foram mais sensíveis.

## Introduction

Salinity adversely impacts agricultural production (Wei *et al.*, 2003). Around 900 million hectares (Flowers, 2004) including half of irrigated areas (Zhu, 2001) are saline. There is a substantial variation in salt sensitivity among cultivars of the same species (Lutts *et al.*, 1995, Wei *et al.*, 2003). In sugarcane production, which by and large is irrigated, salinity stress is common.

However, data related to salt effects on sugarcane are scarce. Moreover these effects were generally studied in pots containing sand which is regularly irrigated with NaCl solution (Chowdhury *et al.*, 2001; Akhtar *et al.*, 2003; Wahid, 2004). With these methods, salt concentration in sand can not be easily controlled and could accumulate beyond desired treatment concentrations. Sometimes, studies are carried out in field (Sebastian *et al.*, 2009). These researchers conducted field experiments on sandy

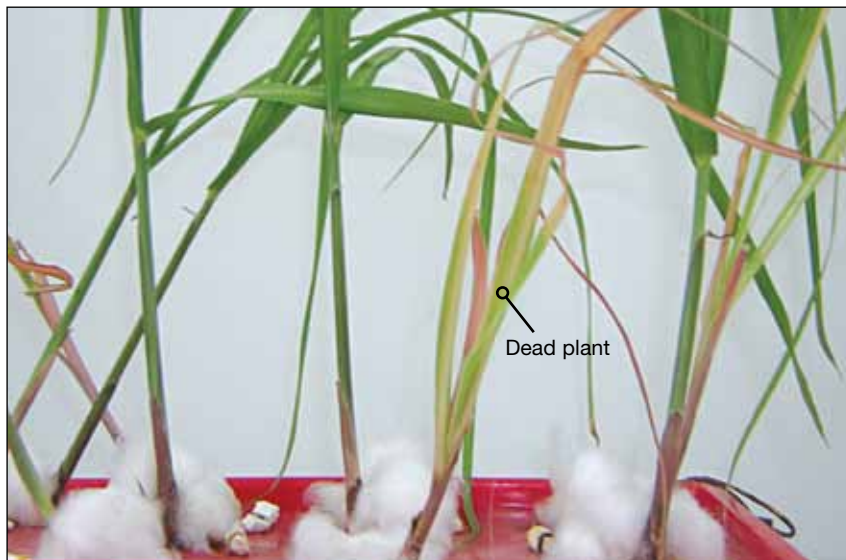
**Figure 1a.** NaCl symptoms in sugarcane plants after 2 weeks of stress: Marginal chlorosis



**Figure 1b.** NaCl symptoms in sugarcane plants after 2 weeks of stress: Advanced chlorosis accompanied with necrosis



**Figure 1c.** NaCl symptoms in sugarcane plants after 2 weeks of stress: Dead plant after all leaves' necrosis



clay loam soil with saline groundwater to screen four saline tolerant sugarcane cultivars [COC(SC)23, COG(SC)5, COSi(SC)6 and CO86032]. Their findings revealed that among these cultivars, COC(SC)23 had the highest sprouting percentage and produced more tillers under saline groundwater irrigation than the other cultivars.

Further, little work has focused on salt tolerance of sugarcane cultivars produced in Morocco. A hydroponic medium system could limit salt accumulation by maintaining its concentration constant in a culture medium. Indeed, the present study aims to evaluate NaCl stress effects on plants survival and growth of five sugarcane cultivars grown in Morocco, and to compare the mean level of salt tolerance of these cultivars at a young plant stage (corresponding to three weeks old plants).

### Materials and methods

Five sugarcane cultivars, grown in Morocco were used (CP65-357, CP70-321, CP59-73, CP66-346 and NCo310) including four American cultivars (CP65-357, CP70-321, CP59-73, CP66-346) and one Indian cultivar selected in South Africa (NCo310). Cultivars are obtained from "Centre Technique des Cultures Sucrières" (CTCS), Morocco. Only NCo310 is quoted in the literature as a salt tolerant cultivar (Heinz, 1987; Zérega *et al.*, 1991) and is not grown in Morocco. However it is preserved in a varietal collection in the technical center and was used as a reference cultivar. Stalk segments were cut in single bud setts (about 5 cm), surface disinfected with ethanol 70%, placed between humidified journal papers and transferred to a drying oven at 30°C for sprouting. After 6 to 8 days, plants were transferred to pots containing tap water for 8 days in a culture room characterized by a temperature of 28±2°C, a photoperiod of 14h/10h, light intensity between 1100 and 1200 lux with artificial lamps and a relative humidity of 50%.

After this period, the tap water was replaced by modified Hoagland solution (Hoagland & Arnon, 1950) in which macro-nutrients concentrations were reduced to half and added with Fe-EDTA of Murashige & Skoog (1962). Medium was prepared with distilled water and pH was adjusted to 6.5 with concentrated NaOH. Stress application was done after 7 days (plants were then three weeks old). Treatments consisted of 0, 17, 34, and 68 mM NaCl, corresponding respectively to an electric conductivity of 1.00, 2.83, 4.26, and 6.63 mS/cm. Electric conductivities were adjusted every two days with a conductimeter at the same time with the water volume lost due to evapotranspiration. Each pot contained 10 plants and 2 or 3 pots were used per NaCl concentration, equivalent to 20 or 30 plants for each treatment. Stress was maintained for 2 weeks.

Plant mortality was evaluated by counting the dead plants for each treatment. We considered a plant as dead when all leaves

showed a large necrosis. The results are expressed as mortality percentage. Plants height (aerial part) was measured before transferring to the media (H0); height was measured again after 2 weeks of treatment (H1). Relative height growth of plants (RHG) was calculated as (H1- H0)/ H0. Roots lengths were measured as for the aerial part and relative length growth (RLG) were calculated for roots as (L1 – L0)/L0.

All the experiments were performed twice independently. The number of dead plants were analysed as binomial-distribution variates. For growth parameters, each value was presented in the form of mean ± standard error with a reading of four independent samples per treatment. The analysis of the main effects of stress intensity was based on a 1-way analysis of variance (ANOVA). All statistical analyses were performed by SAS program (SAS Institute, 1992).

## Results

Salt effect resulted in a progressive leaf chlorosis and necrosis. Chlorosis can be marginal, (i.e. limited at the edge of the leaf) (Figure 1a) or located at the end of the leaf accompanied with necrosis (Figure 1b). Progressive leaf necrosis could lead to plant death when necrosis spreads in all leaves (Figure 1c).

In the absence of stress, all plants survived two weeks after the experiment. Exposure to NaCl increased plant mortality, but the responses amongst the five cultivars differed (Table 1). Cultivars CP70-321 and CP65-357 were most adversely affected by NaCl. Cv. CP70-321 exhibited a mortality percentage of 10, 10, and 45% in the presence of 17, 34, and 68 mM of NaCl. (increase significantly different from the control only at 68 mM NaCl,  $p < 0.05$ ), respectively. The mortality percentages were 10,

20, and 30% for CP65-357 (increase significantly different from the control at 34 and 68 mM NaCl). Cultivar CP66-346 was not significantly affected by salt concentrations. Cultivars NCo310 and CP59-73 showed an intermediate response with mortality percentages of 10, 10, and 30% for NCo310, and 0, 10, and 30% for CP59-73 in the presence of 17, 34, and 68 mM of NaCl (increase significantly different from the control 68 mM NaCl), respectively. For a better comparison of cultivars on the basis of their survival in the presence of NaCl, the average percentage of mortality in the presence of NaCl was calculated as the average of the three values obtained in the presence of the three NaCl concentrations (17, 34, and 68 mM) (Table 2). The reduction in survival (plant death) due to the average effect of salt stress was lower for cultivar CP66-346 (5%) and higher for cultivars CP70-321 (21.7%) and CP65-357 (20%). For cultivars CP59-73 (13.3%) and NCo310 (16.7%), the response was intermediate. The reduction was significant ( $p < 0.05$ ) for all cultivars except for CP66-346. Thus, cultivar CP66-346 had the highest survival rate, whereas CP70-321 and CP65-357 had the lowest survival in the presence of NaCl, compared with NCo310 and CP59-73.

The Figure 2 presents NaCl effect on plants relative height growth (RHG) after two weeks of stress. Salinity stress adversely impacted RHG in all cultivars. For cultivar NCo310, RHG reduction was significant ( $p < 0.05$ ) at 17 and 68 mM NaCl (reduction was not significant at 34 mM NaCl). These reductions corresponded to 25, 16 and 33% of the control, respectively. For cultivar CP70-321, RHG reduction corresponds to 27, 50, and 70% of control respectively at 17, 34, and 68 mM NaCl. The reduction observed was significant ( $p < 0.001$ ) at all NaCl concentrations. Considering the cultivar CP65-357, RHG reduction under salt stress was significant ( $p < 0.01$ ) – compared with the control, reduction of growth was by 29, 40, and 41% at 17, 34, and 68 mM NaCl, respectively. For the cultivar CP66-346, RHG reduction was significant ( $p < 0.001$ ) at 34 and 68 mM NaCl, resulting in growth reduction of 10, and 45%, respectively. For the cultivar CP59-73, plant RHG reduction was 6, 48, and 50% at 17, 34, and 68 mM NaCl, respectively. This reduction was significant ( $p < 0.001$ ) only at 34 mM and 68 mM NaCl.

It is apparent to note the varied response of the cultivars to the stress. It is worth noting that while cultivars NCo310 and CP65-357 were significantly and adversely affected at 17 mM of NaCl (but not at 34 mM for NCo310), the growth of CP59-73 and CP66-346 was adversely affected only with 34 mM of NaCl and higher. For better comparison of cultivars on the basis of their relative growth in the presence of NaCl, Table 2 notes the average of the three values obtained at the three NaCl concentrations. The reduction in plant growth was lowest for cultivars CP66-346

**Table 1.** Mortality percentages (%) of plants of five sugarcane cultivars after 2 weeks of culture in the presence of different NaCl concentrations

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

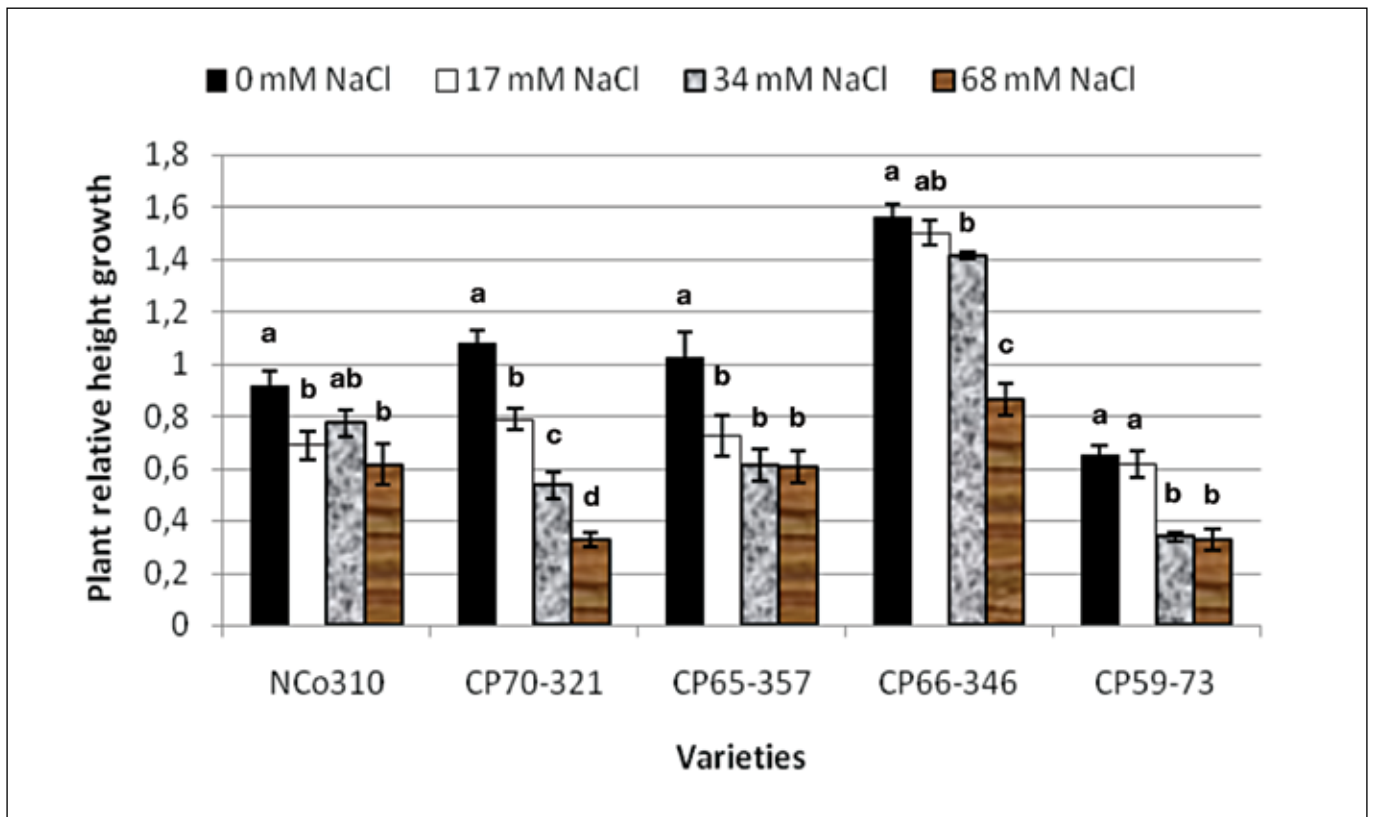
Values within columns followed with same letter are not significantly different at  $p < 0.05$

**Table 2.** Mortality percentages (%) and relative height growth (%) of plants of five sugarcane cultivars after 2 weeks of culture in the presence of different NaCl concentrations: 0 NaCl: control; + NaCl: presence of NaCl: Data in presence of NaCl were expressed as the average of the three values obtained in the presence of the three NaCl concentrations (17; 34 and 68 mM) expressed in percentage of that of control

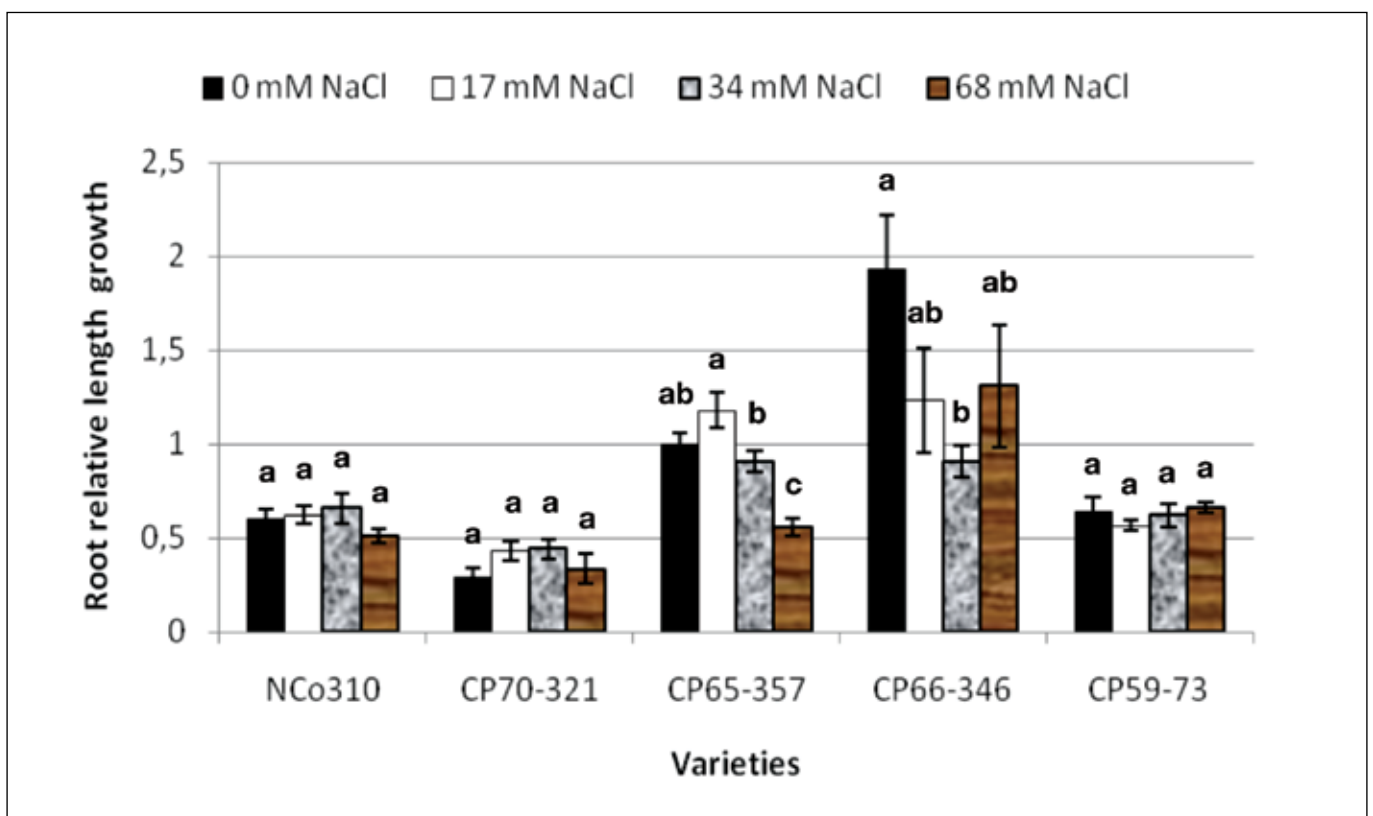
	NCo310	CP70-321	CP65-357	CP59-73	CP66-346
Plant mortality (%)	0 NaCl	0a	0a	0a	0a
	+ NaCl	16.7b	21.7b	20b	13.3b
Plant growth (%)	0 NaCl	100a	100a	100a	100a
	+ NaCl	75.6b	51b	63.3b	65.4b

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

**Figure 2.** Plant relative height growth of five sugarcane varieties after 2 weeks of culture in presence of different NaCl concentrations (n = 4; vertical bars are standard errors): Values within variety with same letter are not significantly different at p < 0.05



**Figure 3.** Root relative length growth of five sugarcane varieties after 2 weeks of culture in presence of different NaCl concentrations (n = 4; vertical bars are standard errors): Values within variety with same letter are not significantly different at p < 0.05



(19.5%) and NCo310 (24.3%) and highest for cultivar CP70-321 (about 49%). The reduction was significant ( $p < 0.05$ ) for all cultivars except CP66-346.

Salinity stress did not adversely impact root growth in NCo310, CP70-321, and CP59-73 (Figure 3). For CP65-357, a slight stimulation was observed at 17 mM of NaCl followed by a significant reduction ( $p < 0.001$ ) at 68 mM NaCl, whereas root growth of CP66-346 was significantly reduced ( $p < 0.05$ ) only at 34 mM NaCl.

## Discussion

Hydroponic culture system was used to evaluate NaCl effects on sugarcane young plants. This type of culture has already been used for salt stress on other sugarcane varieties (Huwyzeh *et al.*, 2008). In this study, salt stress caused progressive leaf chlorosis (yellowing), necrosis, and plant death. NaCl stress resulted in an increase in plant death rate. For the surviving plants, it caused plant aerial part growth reduction. In rice plants, Lutts *et al.* (1995) reported that salt stress increases plant mortality and inhibits growth of surviving plants. Our results are in agreement with those reported in sugarcane by Akhtar *et al.* (2003). These authors showed that NaCl stress reduced plants length in two sugarcane cultivars CPF-213 and L-116. Similar results were also reported in barley and corn by Cramer (2003) who found that salt stress reduced aerial part elongation. In other sugarcane cultivars, Hussain *et al.* (2004) showed that NaCl reduced stem length, stem diameter, internodes, and leaves length. Similar results were reported in triangle orache (*Atriplex prostrata*) (Wang *et al.*, 1997).

In this study, difference among cultivars in terms of salt tolerance was apparent. CP66-346 was the most NaCl tolerant cultivar, while CP70-321 and CP65-357 seemed to be the most sensitive, while cultivars CP59-73 and NCo310 showed an intermediate response.

While there was a distinct response to overall plant growth in response to salinity stress, it was apparent that root growth does not seem to be a good criterion for sugarcane salinity tolerance evaluation. Gerald (1978) reported that salt stress stimulated sugarcane roots elongation (cultivar NCo310), but the level of this stimulation depended on the season period when the experiment was conducted. Akhtar *et al.* (2003) reported that salinity inhibited root lengthening for cultivars CPF-213 and L-116 with a significant reduction for the sensitive cultivar L-116. It seems likely that the effect of salinity on root growth not only depends on cultivars, but also on culture conditions, particularly room temperature. At this young plant stage, salt stress effect caused a progressive leaf chlorosis and necrosis leading to a reduction of plant survival and growth, while the response at the root level was variable, depending on the cultivar. At a whole plant level, roots were less affected compared to the aerial parts. However, there was a differing responses amongst the five cultivars.

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