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Full Length Research Paper

NaCl effect on *in vitro* sugarcane bud emergency

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It is important to determine precisely salt effects on bud germination. In this study, an *in vitro* procedure was used for studying sugarcane bud germination under NaCl salinity stress with different concentrations (0, 17, 34 and 68 mM) using five cultivars: NCo310, CP70-321, CP65-357, CP66-346 and CP59-73. Germination percentage of control ranged from 75% for variety CP66-346 to 100% for variety CP65-357 after 8 days of culture. Salt stress reduced the rate of germination in all varieties. At the end of the 8th day, salt stress decreased the percentage of final germination at all NaCl concentrations used and for all varieties except variety CP66-346 which showed a stimulation of germination at 34 mM NaCl. However, NaCl stress effects on bud germination of the five varieties used in this study were very low with an average reduction of 9.22; 5.88; 5.71 and 1.04 % in the presence of NaCl, respectively, for NCo310, CP70-321, CP65-357 and CP59-73; a little increase (3.08 %) was observed for CP66-346. Thus, NaCl stress delayed buds germination and globally reduced the percentage of final germination. Varieties CP59-73 and CP66-346 appeared to be more salt tolerant at this stage than the three other varieties.

Key words: *In vitro* culture, sugarcane, *Saccharum* sp., buds germination, salt stress.

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). High concentrations of salt in soils account for large decrease in the yield of a wide variety of crop all over the world (Tester and Davenport, 2003). Approximately 5% of the cultivated land is affected by salt (Munns et al., 1999). Sugarcane is a glycophyte confined to tropical and sub-tropical irrigated regions, where salinity is an ever-increasing problem (Wahid et al., 1997).

Salt-induced stress deters sugarcane and sugar productivity in many parts of the world (Shrivastava et al., 1993) and several studies have shown that salinity affects both germination and plant growth (Lutts et al., 1995; Chowdhury et al., 2001). Data related to salt effects on sugarcane germination are scarce; moreover these effects were generally studied in pot containing sand (Kumar and Naidu, 1993; Chowdhury et al., 2001) which is regularly irrigated with NaCl solution. With these methods, salt concentration in the sand could not be easily controlled and could reach higher amounts after its accumulation. In our previous paper (Gandonou et al., 2008), we have proposed an *in vitro* procedure which can limit the problem of salt accumulation in the soil by maintaining constant NaCl concentration in the medium. In the present study, we used this *in vitro* procedure to evaluate salt stress effects on bud germination for five

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sugarcane varieties.

MATERIALS AND METHODS

The experimental plant materials used are sugarcane cultivars NCo310, CP70-321, CP65-357, CP59-73 and CP66-346 provided by Centre Technique des Cultures Sucrières (C.T.C.S., Gharb, Morocco). Young single bud setts (approximately 4 cm) were taken in the top of each plant (3 or 4 setts/plant) and wiped with cotton saturated with ethanol 70%. Setts disinfection was done as described by Gandonou et al., (2008). Setts were then disinfected with 0.03% of chloride mercuric solution supplemented with tween 80 for 15 min, followed by rinsing with three changes of sterile distilled water (10 min each). Each disinfection and rinsing solution contains the same NaCl concentration of the medium in which setts will be transferred, that is, 0; 17; 34; 68 or 102 mM. Disinfection and rinsing were done under continuous agitation. After drying on sterile filter paper, setts were aseptically placed on the medium constituted by distilled water supplemented with different concentrations of NaCl. All media were solidified with 8 g l^{-1} agar before autoclaving for 20 min at 120°C. Four setts were cultivated per jar and cultures were kept in darkness at 25 ± 1°C. For each NaCl concentration, 32 or 36 setts were used and the germinated buds were recorded every 2 days for 8 days.

All the experiments were carried out twice independently. The number of germinated buds was analysed as binomial-distribution variates with 32 or 36 buds for each repetition. Analysis was performed using SAS program (SAS Institute, 1992).

RESULTS

Effect of NaCl on bud germination kinetics

Two types of bud germination were observed *in vitro*: bud germination with roots production and bud germination without roots production as reported by Gandonou et al. (2008). Figures 1, 2, 3, 4 and 5 present the effect of NaCl on bud germination rate after 2, 4, 6 and 8 days in the presence of NaCl at 0, 17, 34 and 68 mM, respectively for varieties NCo310, CP70-321, CP65-357, CP59-73 and CP66-346. In absence of stress, the behaviour of varieties are different: after 2 days of culture, approximately 18% of the buds of NCo310 stripped, whereas for varieties CP66-346 and CP70-321, respectively, 25 and 30% of the buds stripped for the same period; this rate is about 60% for CP59-73 and CP65-357. After 4 days, the percentages of bud germination were about 66, 69, 94, 53 and 97%, respectively, for NCo310, CP70-321, CP65-357, CP66-346 and CP59-73. At the end of the experiment (after 8 days of culture), the percentages of bud germination are about 91, 94, 97, 75 and 100%, respectively, for NCo310, CP70-321, CP65-357, CP66-346 and CP59-73. NaCl stress effect results in a reduction of bud germination speed, visible throughout all varieties. Indeed, a reduction of the percentages of bud was observed for all varieties at the various NaCl concentrations used as well after 2, 4, 6 as after 8 days (Figures 1 - 5). For variety NCo310, the percentage of bud germination after 2 days passes from 18% in absence of NaCl, to 14 and 0%, respectively at 17 and 68 mM of NaCl. Similar observations can be made concerning varieties CP70-321, CP65-357, CP66-346 and CP59-73 where buds germination percentage passes, respectively from 30, 60, 25 and 60% on the control to 3, 22, 11 and 44% at 68 mM of NaCl. Similar tendencies were observed for 4 and 6 days. These observations indicate that salt stress delays bud germination for all varieties.

Effect of NaCl stress on the percentage of final germination

Figure 6 presents the percentages of final bud germination at the end of 8 days of five sugarcane genotypes under various NaCl concentrations. In the absence of stress (controls), final germination percentages were 100, 97.22, 94.44, 91.44 and 75%, respectively for varieties CP59-73, CP65-357, CP70-321, NCo310 and CP66-346. The difference among these values was significant ($P < 0.05$). These results indicate that varieties CP65-357 and CP59-73 present a high capacity of bud germination, while variety CP66-346 presents a weaker capacity; varieties NCo310 and CP70-321 are intermediary. Salt stress reduces the percentage of final bud germination. This reduction is marked slightly for variety NCo310. From 91.44% for control, it passes to 83.33% at 17, 34 and 68 mM of NaCl. The reduction observed was not significant ($P < 0.05$). Practically, no effect of salt stress was observed on final germination for varieties CP59-73 and CP66-346. For variety CP66-346, the percentage of bud germination final passes from 75% for control to 72.22% in the presence of 17 and 68 mM of NaCl; a light increase (no significant) was observed at 34 mM, whereas for variety CP59-73, bud germination showed a light reduction (no significant) only at 34 mM of NaCl passing from 100 to 97%. For varieties CP70-321 and CP65-357, bud germination percentages show a little reduction from 94.44% for control to 88.89% at the various NaCl concentrations for variety CP70-321, and from 97.22% for control to 94.44%, 88.89 and 91.67%, respectively, at 17, 34 and 68 mM of NaCl for variety CP65-357. These reductions were not significant ($P < 0.05$).

To compare the five varieties on the basis of the rate of bud germination after 8 days, mean values were calculated from data collected for three doses of NaCl (17, 34 and 68 mM) and expressed as percentage of that of the control as shown by Table 1. These data showed that salt stress reduces bud germination for about 10% compared to control for variety NCo310 while this reduction was about 6% for varieties CP70-321 and CP65-357; practically, no reduction was observed for varieties CP59-73 and CP66-346.

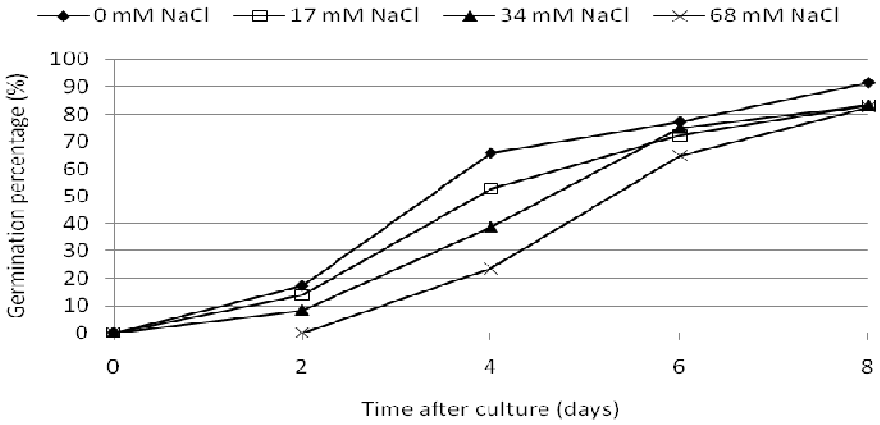

Figure 1. Rate of *in vitro* germination of sugarcane buds under saline conditions for cultivar NCo310.

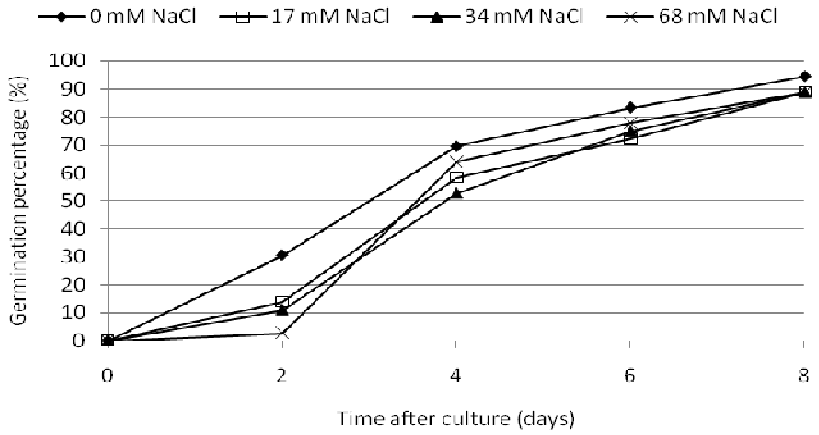

Figure 2. Rate of *in vitro* germination of sugarcane buds under saline conditions for cultivar CP70-321.

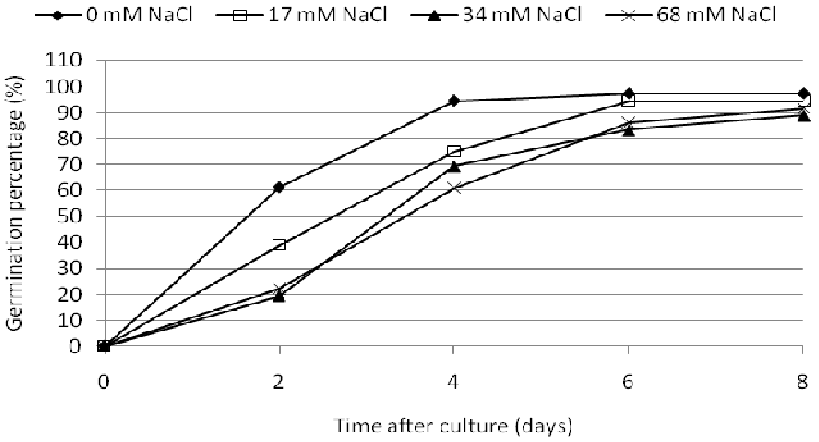

Figure 3. Rate of *in vitro* germination of sugarcane buds under saline conditions for cultivar CP65-357.

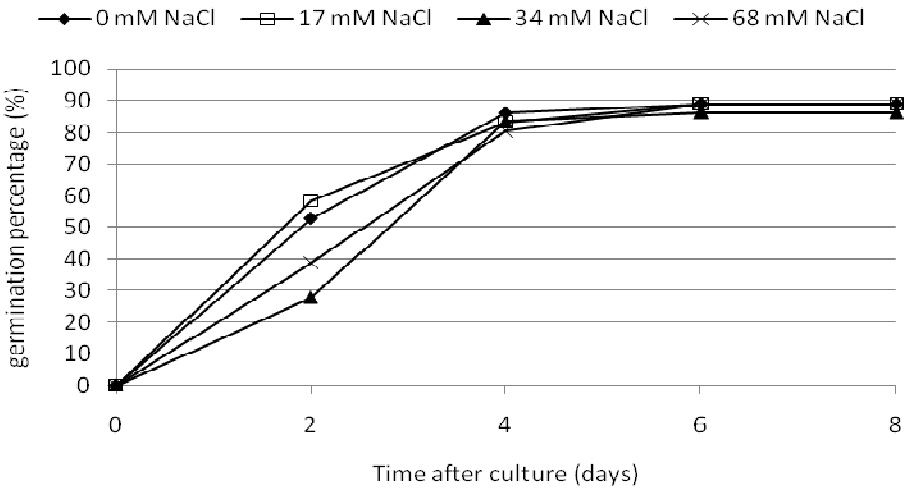

Figure 4. Rate of *in vitro* germination of sugarcane buds under saline conditions for cultivar CP59-73.

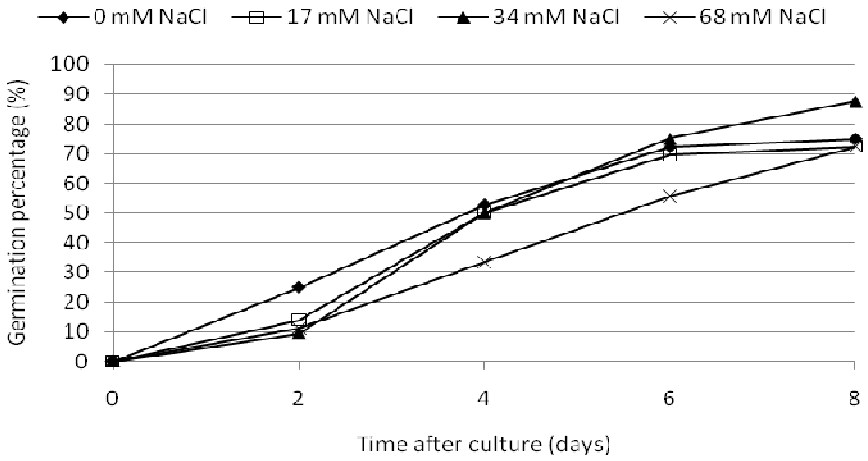

Figure 5. Rate of *in vitro* germination of sugarcane buds under saline conditions for cultivar CP66-346.

DISCUSSION

The varieties tested in this study present different capacities of bud germination in absence of salt stress. With 100 and 97% of buds stripped after 8 days, varieties CP59-73 and CP65-357 present the best capacities of bud germination, while CP66-346 shows the weakest capacity of bud germination with only 75% of stripped buds; variety CP70-321 and NCo310 present intermediary capacities of bud germination with, respectively 94 and 91% of stripped buds. Our results are in agreement with those of the Centre Technique de la Canne à Sucre of Morocco (CTCAS, current CTCS, Rapport 1986) which indicated that CP66-346 presents a low capacity of bud germination compared to CP65-357. At other genotypes of sugarcane, Chowdhury et al. (2001) found a percentage of bud germination of 82% in absence of stress after 7 days, while Kumar and Naidu (1993) found that the percentage of bud germination varied between 44 and 95% according to the ground temperature. Salinity induces a reduction in the rate of bud germination, in particular during the first 4 days of culture, and this for all varieties and at all NaCl concentrations used. However, the percentage of final germination is not very affected by NaCl concentrations used. It thus appears that the NaCl concentrations used in our study affect mainly the kinetics of bud germination; the the percentage of final germination can be significantly

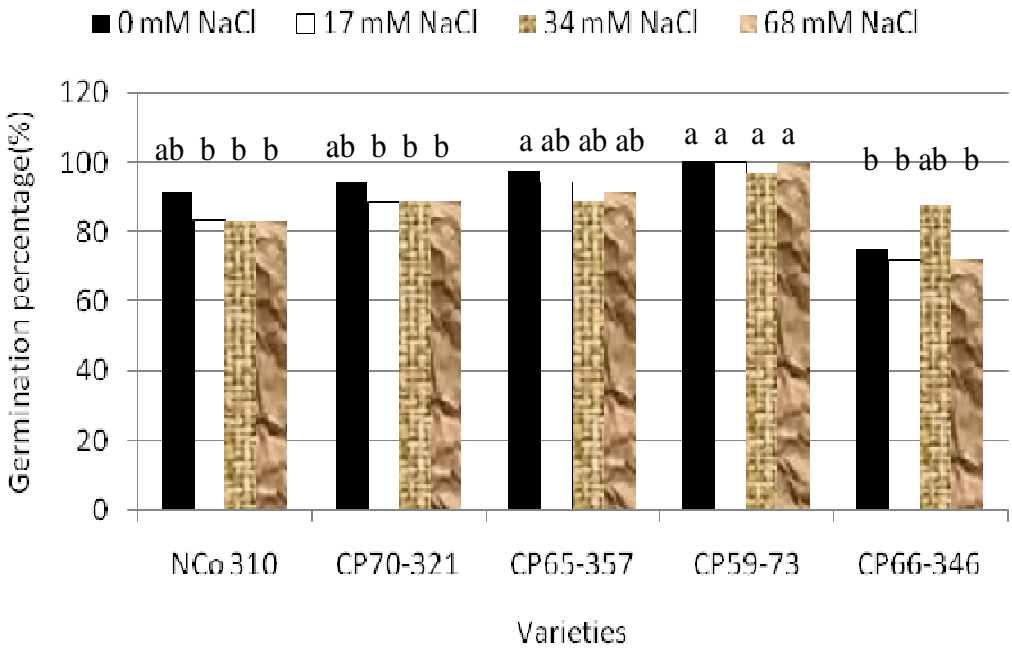

Figure 6. Effect of NaCl salinity on five sugarcane variety buds *in vitro* germination percentage (cultivars Nco310, CP70-321, CP65-357, CP59-73 and CP66-346) after 8 days of culture. Values with the same letters are not significantly different at P = 0.05.

Table 1. Bud germination percentage (in %) of five sugarcane varieties after 8 days of salt-stress exposure *in vitro*

Cultivar	0 NaCl	+ NaCl
NCo310	100	90.78
CP70-321	100	94.12
CP65-357	100	94.29
CP59-73	100	98.96
CP66-346	100	103.08

0 NaCl: controls; + NaCl: mean of the three values collected for the three doses of NaCl used (17, 34 and 68 mM).

affected with higher NaCl concentrations. These results agree with those reported by Cramer (1994). This author reported that for the majority of corn genotypes, the percentage of germination is reduced only beyond an electric conductivity of 12 dS/m, whereas the speed of germination is decreased with a conductivity as low as 1 dS/m. In addition, at *Cakile maritima*, Debez et al. (2004) found that the percentage of germination is significantly affected only with concentrations of NaCl higher or equal to 200 mM. The reduction of germination under salt stress was also reported in durum wheat (Almansouri et al., 2001), sugar beet (Ghoulam and Farès, 2001) and in barley (El-Dardiry, 2007). For sugarcane, several authors found that salt reduces bud germination. Thus, Kumar and Naidu (1993) reported that NaCl reduces the percentage of bud germination with buds cultivated in ground, and that this reduction is as more important as the NaCl concentration is high. Similar results were also reported by Chowdhury et al. (2001) for other sugarcane varieties. In addition, Akhtar et al. (2003) found that salt delays bud germination and decreases the percentage of bud germination at other sugarcane varieties. In other sugarcane varieties, Patade et al. (2009) have shown that NaCl (100 mM) improved both the percent and rate of germination of the sets of the tolerant (Co 62175) and moderately tolerant (CoM 265) varieties compared to sensitive (CoC 671) and test (Co 86032) varieties.

On the basis of our results, varieties CP59-73 and CP66-346 appeared to be more salt tolerant at germination stage than the three other varieties.

This study confirms our previous suggestion that *in vitro* techniques can be used to screen sugarcane genotypes for their response to salt stress at germination stage (Gandonou et al. 2008). NaCl salt stress delayed bud germination and reduced the percentage of final germination in sugarcane. In addition, considering the percentage of final germination, which is the parameter most easily quantifiable and most largely used in the literature to compare varieties at germination level, our results do not allow to clearly separate the varieties according to their level of salinity tolerance; however, varieties CP59-73 and CP66-346 appear more salt-tolerant at the stage of germination than the three other varieties used.

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