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Short Communication

In vitro culture techniques as a tool of sugarcane bud germination study under salt stress

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Germination was the first stage confronted to soil salinity and it is important to determine salt effects on this stage. In this study, we reported an *in vitro* procedure for studying sugarcane bud germination and shoot growth under salt stress with different NaCl concentrations (0, 17, 34, 68 and 102 mM) using cultivar NCo310. Germination percentage of control was about 92% after 8 days. Germination of buds, plant fresh and dry mass decreased with increasing salinity. Data indicated that *in vitro* culture techniques could be used to evaluate salt stress effects in sugarcane at the germination stage.

Key words: *in vitro* culture, sugarcane, buds germination, salt stress, shoot growth.

INTRODUCTION

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. We proposed in this study an *in vitro* procedure which can limit this problem by maintaining constant NaCl concentration in the medium.

The experimental plant material used is cultivar NCo310 provided by Centre Technique des Cultures Sucrières (C.T.C.S., Gharb, Morocco). Young single bud setts (approximately 4 cm) were taken at the top of each plant (3 or 4 setts/plant) and wiped with cotton saturated with ethanol 70%. 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 i.e. 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 gl^{-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.

After 10 days, sprouts were cut and their lengths were determined. After that, they were weighed for fresh mass determination; they were then oven-dried at 80°C for 72 h and weighed again for dry mass determination. For each treatment, sprouts length, fresh and dry mass of four ran-

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Table 1. Effect of NaCl salinity on sugarcane (cv. NCo310) buds germination after 8 days of culture, and shoots length, shoot fresh mass and dry mass after 10 days of *in vitro* culture (means ± standard errors, n = 4).

Parameter	NaCl concentrations (mM)				
	0	17	34	68	102
Bud germination (%)	91.67	83.33	83.33	80.55	72.22
Shoot length (cm)	3.6±0.389^{a}	3.125±0.075^{ab}	3.025±0.350^{ab}	2.275±0.048^{c}	2.775±0.149^{bc}
Shoot fresh mass (g)	0.3741±0.0288^{a}	0.3742±0.0215^{a}	0.3252±0.0102^{ab}	0.2822±0.0196^{b}	0.3338±0.0052^{ab}
Shoot dry mass (mg)	0.0319±0.0018^{a}	0.0317±0.0014^{a}	0.0268±0.0006^{bc}	0.0296±0.0014^{ab}	0.0246±0.0008^{c}

Means in the same row followed by the same letters do not differ significantly ($p < 0.05$).

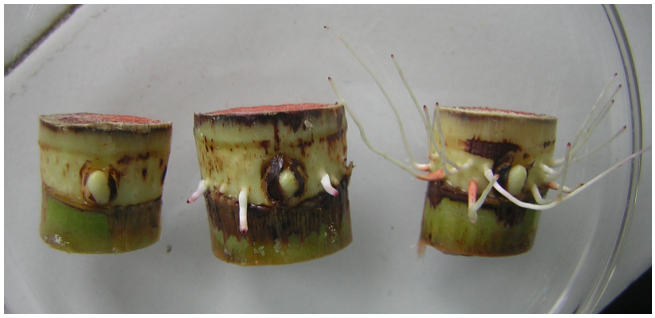

Figure 1. Different types of germinated buds of sugarcane in *in vitro* conditions.

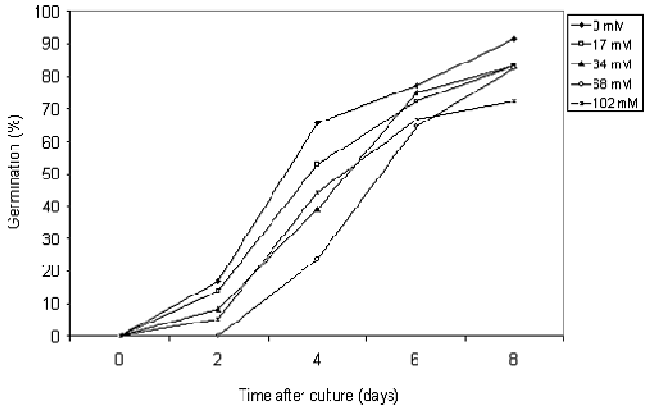

Figure 2. Rate of *in vitro* germination of sugarcane buds (cv.Co310) under saline conditions.

domly chosen plants from different jars were measured.

The analysis of the main effects of NaCl stress intensity was based on a one-way analysis of variance (ANOVA) and means were compared using LSD test. All statistical analyses were performed by SAS (1992) program.

Two types of germination were observed *in vitro*: bud germination without root and bud germination with roots (Figure 1). However, roots appeared generally after bud germination and the majority of sets showed germination without roots. In the absence of stress, the percentage of germination was 91.67% after 8 days (Figure 2). In other genotypes of sugarcane, Chowdhury et al. (2001) found a percentage of 82.0% in the control after 7 days while Kumar and Naidu (1993) showed that the percentage ranged from 44 to 95% according to the temperature. NaCl stress affects both the rate and the percentage of germination after 8 days. Figure 2 shows that after 2 days of culture, no bud in medium with 68 mM NaCl germinated while 18% of buds were germinated in the control. At this time, only 5.5% of buds germinated in the case of the medium with 102 mM NaCl. High salinity levels significantly decreased the rate of germination. In the control, about 65% of buds germinated after 4 days but at 34 mM and 68 mM NaCl, only 38.89 and 24% of buds, respectively, germinated (Figure 2). The percentage of germination after 8 days was 91.67, 83.33, 83.33, 80.55 and 72.22% at 0, 17, 34, 68 and 102 mM NaCl, respectively (Table 1). This result indicated that salt stress decreased the percentage of germination and that this decrease becomes more pronounced as NaCl concentration in the medium increases. The work of Chowdhury et al. (2001) showed that NaCl stress (25-200 mM) decreased the rate of germination and the average germination in sugarcane. Similar results were reported by Kumar and Naidu (1993) in this plant.

Shoot length, fresh and dry mass decreased with increasing salinity (Table 1). Shoot length was not significantly affected before 68 mM NaCl (LSD = 0.731); shoot length decrease becomes significant ($p < 0.05$) at 68 mM and above (Table 1). Shoot length was 3.6 cm for the control; this length decreased by 13, 16, 37 and 23% at 17, 34, 68 and 102 mM NaCl, respectively. Similarly, salt stress decreased shoot fresh mass significantly ($p < 0.05$) after 10 days. This reduction was observed at high NaCl concentrations (Table 1). Fresh mass decreased from 0.374 g in the control to 0.325 and 0.282 g, respectively, at 34 and 68 mM NaCl. The decrease at 102 mM NaCl is less than that at 68 mM NaCl. Dry mass also decreased significantly ($p < 0.01$) in the presence of NaCl salinity. No reduction was observed at 17 mM NaCl while application of 34 or 102 mM NaCl decreased shoot dry mass (LSD = 0.0039) (Table 1). This reduction was about 16% at 34 mM and 23% at 102 mM NaCl. Our results are in agreement with those reported by Akhtar et

al. (2003).

The results of this study show that NaCl salt stress delayed sugarcane bud germination, decreased germination percentage and reduced sprouts growth. This study leads us to suggest that *in vitro* techniques can be used to screen sugarcane genotypes for their response to salt stress at the germination stage. However, further studies in several genotypes are needed to confirm this proposition.

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