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In vitro response of promising sugarcane varieties for salinity tolerance through shoot tip culture

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Abstract
Sugarcane is one of the most important industrial crop in both tropical as well as subtropical regions of the world and a major export product of many developing countries. Reduction in sugarcane productivity and sugar recovery is mainly due to abiotic stresses. In India most of the sugarcane growing areas are under the influence of salinity. The present investigation was carried out on experimental shoot apex portion of two sugarcane varieties CoN-13073 and CoN-13072, were exposed to different NaCl levels to assess salinity tolerance under *in vitro* condition. Among the shoot apex portion, undergone to different NaCl concentrations maximum survival per cent was found in untreated shoot apex (37.20%) in genotype CoN-13073. Maximum regeneration per cent (78.40%) was observed without NaCl concentration in genotype CoN-13073. In case of number of days for shoot formation genotype CoN-13072 regenerated within 15.40 days without NaCl concentration. The highest number of multiple shoots were obtained (28.40) and (24.20) from the treatment where 0.0% NaCl application was done in CoN-13073 and CoN-13072 respectively.

Keywords: Promising sugarcane varieties, salinity tolerance, shoot tip culture

Introduction
Sugarcane is an important cash crop in all the countries of tropical Asia. Sugarcane belongs to the genus *Saccharum* spp. L. of the tribe andropogonae, family poaceae. It is a highly polyploidy species with complex genome, with variable chromosome number ranging from 40 to 128 and a genome size range from 3.36 to 12.64 GB. It is the main sugar producing crop that contributes more than 77 per cent to the total sugar pool at the global level. The world's largest producer of sugarcane is Brazil, followed by India and China. The importance of sugarcane has increased because it provides industrial raw material for sugar allied industries such as, paper, plywood, industrial enzymes, animal feed and as a source of renewable energy. Sugarcane crop generally prone to abiotic stresses like moisture stress, water logging, salinity, high or low temperature and micronutrients deficiencies. Salinity is one of the major abiotic stress which greatly affects the sugarcane productivity and recovery. The soils with electrical conductivity (EC) less than 4 dsm^{-1} are generally considered as salt-free, whereas soil with EC range between 4-8 dsm^{-1} are generally considered as salty soil. Salinity is a significant factor that affects crop production and agricultural sustainability worldwide, since about 10% of the land surface and 50% of all irrigated land in the world are prone to salinity (Flowers *et al*., 2010) [2]. Sugarcane is a typical glycophyte (salt sensitive plants) and hence exhibits stunted growth or no growth under salinity, with its yield falling to 50% or less than its true potential (Subbarao and Shaw, 1985) [9]. Salinity in the root zone of sugarcane decreases sucrose content, through its effect on both biomass and juice quality (Lingle and Wiegand, 1996) [4]. Salt tolerance is a complex trait both physiologically as well as genetically. The technique of plant tissue culture is used for producing large number of clonal plants by *in vitro* culture of explants. It has become now a viable and effective alternative to the conventional breeding and clonal propagation method. So, present investigation was carried out to screen two promising sugarcane genotypes CoN 13072 and CoN 13073 for salinity tolerance through shoot tip culture at sugarcane tissue culture laboratories, Main Sugarcane Research Station, Navsari Agricultural University, Navsari. Various physiological and morphological parameters was studied.

Materials and Methods
The commercial cultivars of sugarcane CoN 13072 and CoN 13073 grown in Gujarat were used as the source of explants in this experiment. The explants were obtained from Main

Sugarcane Research Station, Navsari Agricultural University, Navsari. The shoot apex of sugarcane were used as explants. Disease-free, genetically true-to-type and actively growing cane tops were selected from 5 to 7 months old sugarcane crop. Cane tops with the growing apices were cut approximately 10 cm long and washed thoroughly in running tap water for 30 minutes. Outer sheaths of cane tops were removed by wiping the sheath with rectified spirit. The shoots were then washed with soapy water (2 drops of Labonin into 250 ml of water) for about 5 to 6 minutes in a sterile 1-litre conical flask, followed by cleaning the materials with distilled water. The shoots were rinsed in 5 per cent sodium hypochlorite for 10 minutes. Then shoots were thoroughly rinsed in 70 per cent ethanol for 30 seconds followed by sterilizing double distilled water for 4-5 times till ethanol was completely washed out from the surface of the material. Surface sterilization was performed using 0.1 per cent mercuric chloride solution. Shoots were shaken vigorously for 5 minutes. Then the container was taken to the laminar clean air station. They were rinsed 3 to 4 times with sterile double distilled water to remove all traces of chemicals. The isolation of shoot apex of 1 to 1.5 cm was done by carefully removing the 2-3 outer whorls of the developing leaves with the help of a sterile sharp blade and inoculated on autoclaved MS medium.

Good quality plantlets generated from the explants was selected for further experimentation to impose different levels of NaCl concentration and to check the response to salinity tolerance on the basis of *in vitro* evaluation procedure in both the genotypes. In regeneration medium, NAA 2 mg/l + BAP 1mg/l kept constant in all the treatments. Transfer the regenerated shoots on rooting medium i.e., MS + NAA (2mg/l) + BAP (1mg/l) + different concentration of NaCl. Incubate the culture either in the incubator or growth room maintained at temperature 25+2 °C, with fluorescent light (3000-5000 Lux), 16 hrs light/ 8 hrs dark regimes and possessing good relative humidity (60-80%). After successful regeneration of multiple shoots at different levels of NaCl concentration in MS medium, the best and healthy plantlets were evaluated for salinity tolerance considering various parameters.

Observation recorded during experiment

Plantlets were raised from treated shoot apex on MS medium allowed to regenerate and the following observations were recorded. Survival per cent was calculated after 35 days of NaCl treatment on the basis of number of plantlets survived from the total number at each treatment combination. Regeneration per cent recorded on the basis of number of treated cultures undergone regeneration process for shoot formation. The number of days required for shoot formation of shoot apex on MS medium supplemented with different cytokinins and enzymes. The numbers of shoots were counted after 30 days of shoot apex inoculation on regeneration medium.

Statistical analysis

The data generated from the *in vitro* experiment were subjected to statistical analysis in Completely Randomized Design with factorial concept (FCRD) technique as suggested by Panse and Sukhatme (1985) [5].

Results and Discussion

Survival per cent

Genotype CoN-13073 registered maximum survival per cent (37.20%) in treatment (0.0% NaCl). On the other hand, shoot tips treated with (3.0% NaCl) registered minimum survival per cent (12.40%). Genotype CoN-13072 registered maximum survival per cent (32.20%) in treatment (0.0% NaCl). On the other hand shoot tips treated with (3.0% NaCl) registered minimum survival per cent (8.60%) (Table 1).

Overall, shoot tips without NaCl in the nutrient media registered maximum survival per cent (37.20%) in CoN-13073 and (32.20%) in CoN-13072 (Plate no. 1). In both the genotypes, higher concentrations of NaCl above 2.0% showed poor survival per cent. Similar findings was observed by Akhtar *et al.*, (2003) [1] and Shomeili *et al.* (2011) [8].

Regeneration percent

In genotype CoN-13073, maximum regeneration per cent (78.40%) was observed in treatment (0.0% NaCl). Whereas minimum regeneration per cent (38.6%) was noticed in treatment (3.0% NaCl). In genotype CoN-13072, highest regeneration per cent (72.60%) was observed in treatment (0.0% NaCl). Whereas minimum regeneration per cent (24.6%) was noticed in treatment(3.0% NaCl) (Table 1).

Overall, incorporation of NaCl solution to the nutrient media was more influential to shoot regeneration (Plate no. 2). Response to higher NaCl concentrations was varied in both the genotypes. Increase in NaCl concentration resulted in reduction of regeneration per cent in both the genotypes. These results are in agreement with Patade and Suprasanna (2009) [7]; Gandonou and Senhaji (2015) [3].

Number of days for shoot formation

In genotype CoN-13073, shoot apex incubated in MS medium with (0.0% NaCl) registered minimum number of days (18.40) for shoot formation. Shoot apex portion incubated in MS medium with (3.0% NaCl) registered maximum number of days (38.60) for shoot formation. In genotype CoN-13072, shoot apex portion incubated with (0.0% NaCl) registered minimum number of days (15.40) for shoot formation. Whereas shoot apex portion incubated with (3.0% NaCl) took miximum number of days (32.40) for shoot formation (Table 1).

Overall, untreated shoot apex portion took minimum number of days for shoot formation in both the genotypes. Application of higher concentrations of NaCl to the nutrient medium resulted into more days for shoot formation and poor shoot formation in both the genotypes. Inhibitory effect was observed due to higher NaCl concentrations. Similar results were reported by Patade and Suprasanna (2009) [7]; Shomeili *et al.* (2011) [8] and Parmar *et al.* (2017) [6].

Number of multiple shoots

In genotype CoN-13073, among the NaCl treated culture highest number of shoots (28.40) were observed in treatment (3.0% NaCl). Whereas minimum number of shoots (4.20) were observed in treatment (3.0% NaCl). In genotype CoN-13072, among NaCl treated culture highest number of shoots (24.20) were observed in treatment (0.0% NaCl) and minimum number of shoots (2.60) were observed in treatment (3.0% NaCl) (Table 1).

Overall comparision, at higher concentrations of NaCl in the nutrient media indicated that genotype CoN-13073 showed more number of multiple shoots than genotype CoN-13072.

Among the two genotypes, CoN-13073 found to be more tolerant to salinity for multiple shoot formation upto 2.0% NaCl concentration (Plate no. 3). These findings are in agreement with Akhtar *et al.* (2003) [1]; Patade and Suprasanna (2009) [7] and Shomeili *et al.* (2011) [8].

Table 1: Response of sugarcane varieties CoN 13073 and CoN 13072 to salt stress

T (Concentration of NaCl)	Survival per cent			Regeneration per cent			Number of days for shoot formation			Number of multiple shoots		
	G_1 (CoN-13073)	G_2 (CoN-13072)	Mean T	G_1 (CoN-13073)	G_2 (CoN-13072)	Mean T	G_1 (CoN-13073)	G_2 (CoN-13072)	Mean T	G_1 (CoN-13073)	G_2 (CoN-13072)	Mean T
T_1 (NaCl-0.0%)	37.20	32.20	34.70	78.40	72.60	75.50	18.40	15.40	16.90	28.40	24.20	26.30
T_2 (NaCl-1.0%)	28.60	24.60	26.60	69.20	61.80	65.50	20.40	18.60	19.50	21.40	18.20	19.80
T_3 (NaCl-1.5%)	20.40	20.80	20.60	64.60	56.20	60.40	28.60	20.20	24.40	20.60	14.10	17.35
T_4 (NaCl-2.0%)	18.80	14.20	16.50	58.40	50.40	54.40	32.80	24.40	28.60	16.80	12.20	14.50
T_5 (NaCl-2.5%)	16.80	10.80	13.80	44.60	32.80	38.70	36.40	26.80	31.60	08.60	06.80	07.70
T_6 (NaCl-3.0%)	12.40	08.60	10.50	38.60	24.60	31.60	38.60	32.40	35.50	04.20	02.60	03.40
Mean G	22.37	18.53	-	58.97	49.73	-	29.20	22.97	-	16.67	13.02	-
Effect	S.Em.±	C.D. @ 5%	CV %	S.Em.±	C.D. @ 5%	CV %	S.Em.±	C.D. @ 5%	CV %	S.Em.±	C.D. @ 5%	CV %
G	0.06	0.18	1.25	0.06	0.17	0.45	0.05	0.14	0.77	0.14	0.41	4.01
T	0.10	0.30		0.10	0.29		0.08	0.24		0.24	0.71	
G x T	0.15	0.43		0.14	0.41		0.12	0.34		0.34	1.00	

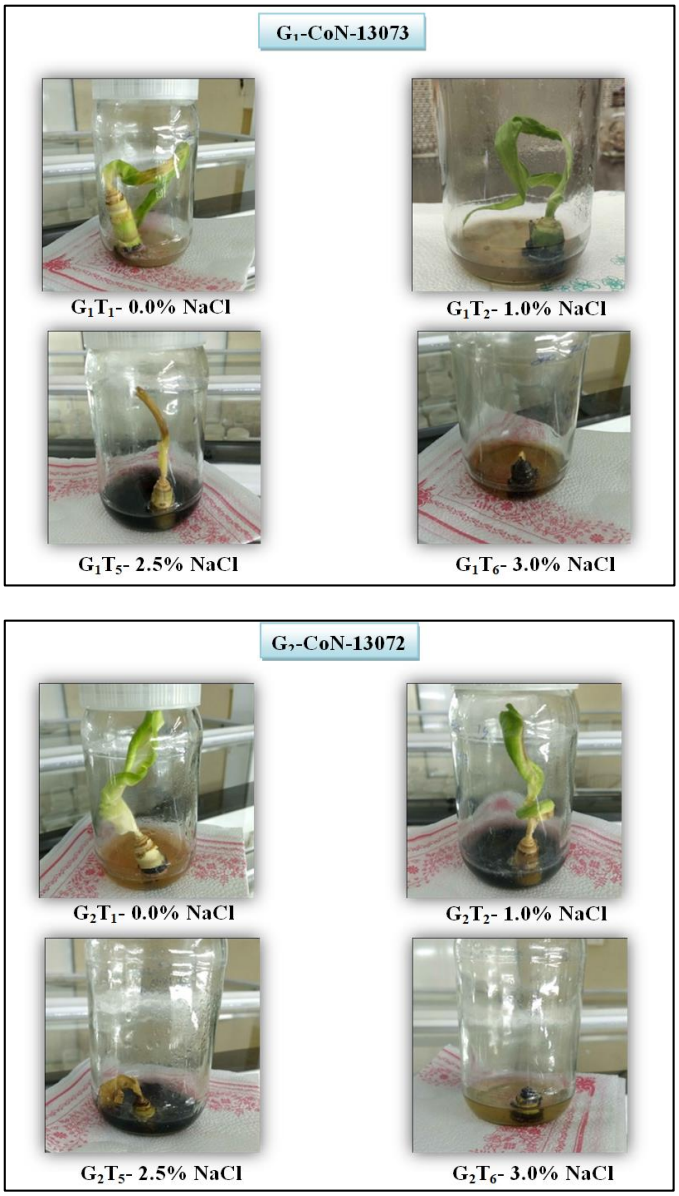


Plate 1: Effect of different NaCl concentrations on survival per cent of two sugarcane genotypes (after 35 days)

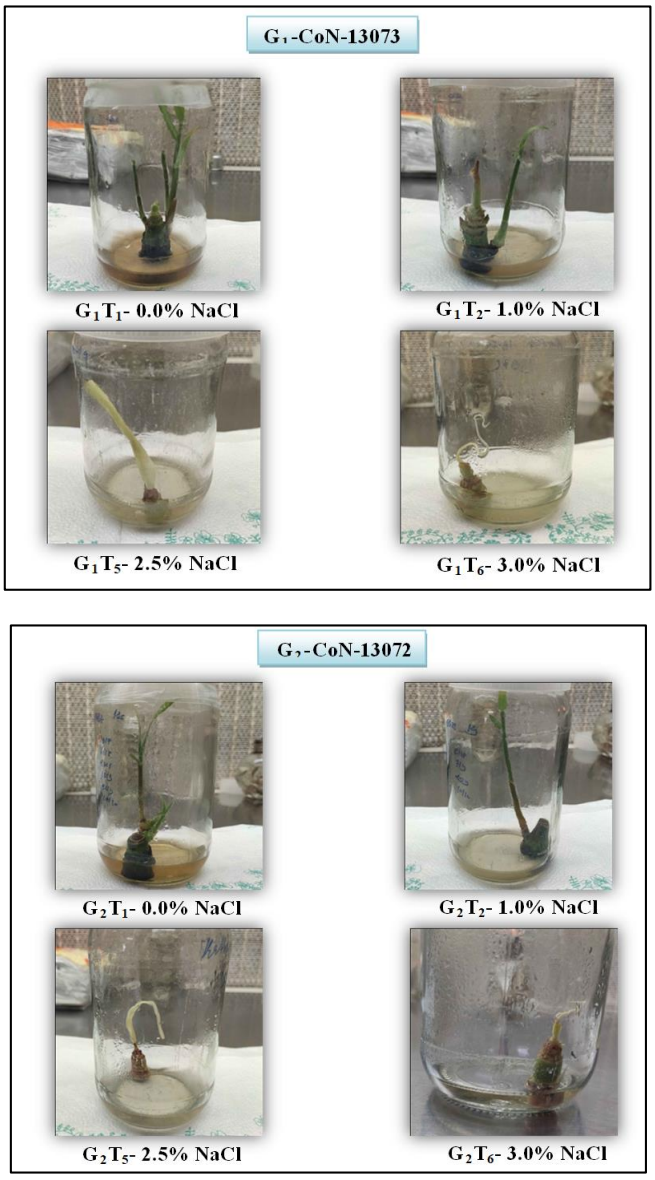
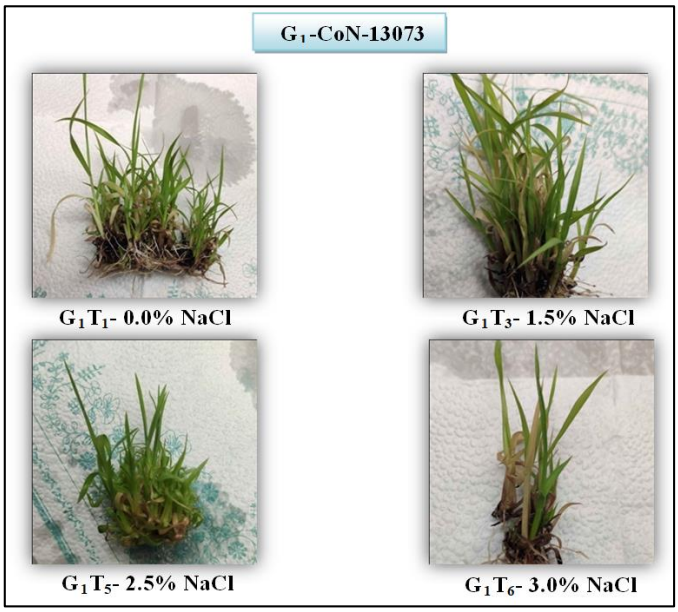


Plate 2: Effect of different NaCl concentrations on regeneration per cent of two sugarcane genotypes (after 35 days)



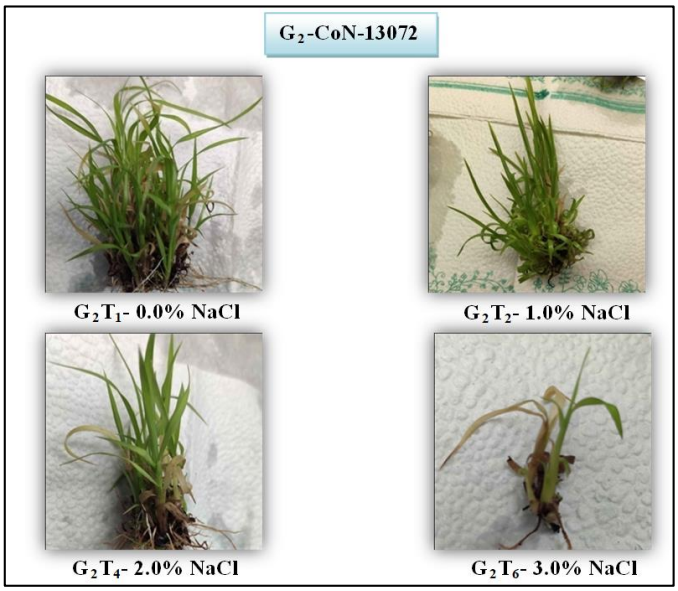


Plate 3: Effect of different NaCl concentrations on number of multiple shoots of two sugarcane genotypes

Conclusion

Form the study it is concluded that *in vitro* selection can be used to identify salt tolerance clones in sugarcane and also to study the various parameters. The depletion of an excess amount of soluble salt in cultivable land directly affects the crop yield. The uptake of high amount of salt inhibits diverse physiological and metabolic process of plants even impacting their survival. Clones derived from genotype CoN 13073 showed higher tolerance towards NaCl up to 2.0% than genotype CoN 13072. The study also suggests that *in vitro* cultured tissue or cell and plantlets can be useful as a system to screen for salinity stress in sugarcane. Overall, genotype CoN 13073 showed better performance in respect of all the characters in the study as compare to genotype CoN 13072.

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