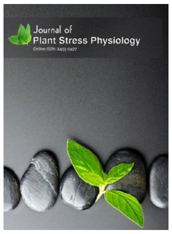
Journal of Plant Stress Physiology 2019, 5: 28-33
doi: 10.25081/jpsp.2019.v5.5643
http://updatepublishing.com/journal/index.php/jpsp

Regular Article



ISSN: 2455-0477

The response of sugarcane genotypes subjected to salinity stress at different growth phases

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Received: April 11, 2019
Accepted: June 19, 2019
Published: June 26, 2019

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ABSTRACT

A few commercial sugarcane genotypes were subjected to salinity stress at various growth phases of sugarcane to ascertain the critical growth stage for salinity stress and to assess the response of the genotypes. All the data were recorded and analysed during maturity phase. The salt treatments drastically reduced SPAD chlorophyll, chlorophyll fluorescence, RWC, stalk height, weight and other yield parameters in a few genotypes during T2 (salt treatment given during formative phase) & T5 (salt treatment given throughout crop cycle) but a few genotypes which are tolerant towards salt stress gave better results comparing other genotypes. The ions like sodium, potassium and chloride were analysed in the juice which showed higher elevation in the genotype Co 97010. Among the genotypes, Co 85019 and Co 99004 recorded significantly prime compared to rest of the genotypes. Treatment throughout the growth phases (T_5) followed by stress at formative phase (T2) were found to be critical for growth, physiological and yield responses in all the genotypes.

KEYWORDS: Salinity stress, sugarcane, physiological parameters, yield parameters

INTRODUCTION

In India nearly 9 million hectares area is occupied by salt-affected soils out of which 5.5 million hectares are saline soils. Salinity adversely affects crop production all over the world [1]. Saline soil is an inevitable component of arid and semi-arid region. Sugarcane is a typical glycophyte, which exhibit stunted growth or zero growth under salinity. About 40% of yield loss was reported in salinity affected areas. Sugarcane genotypes greatly differ in their ability to grow in saline condition. A report show a steep decline in growth once the soil EC rises above 3 dS m^{-1} [2]. There was also a report indicating 0% decrease in yield at an EC of 1.7 dS m^{-1}, 10% at 3.3, 25% at 6, 50% at 10.4 and 100% at an EC 18.6 dS m^{-1} respectively [3]. The EC value of 5.0 dS m^{-1} leads to overall reduction in germination (30.28%), cane weight (25.72%) cane yield (28.05%) and sugar yield (33.25%) [4]. Salinity levels (100 and 200mM NaCl) were shown to have detrimental effect on chlorophyll fluorescence [5].

The leaf growth parameters were reduced with increasing salinity and the concentration of Cl^- ions in the third visible dewlap leaf was 10 times higher than that of Na^+ ions [6]. A report has shown that the sucrose concentration was reduced by increasing KCl levels but not by K_2SO_4 levels [7]. Accumulation of high concentration ions in sink cells might have affected the expression of invertase gene [8].

Sugarcane grown in saline soil experiences the adverse growth and development behaviour throughout the crop cycle, as soil salinity is long lasting unless, soil reclamation has to be done to remove the salts. However, during monsoon rainfall periods, a temporary relief is experienced, as the soluble salts would be removed from root zone through run off effect. The present study focuses on identifying critical phenophase of the crop, once identified can be matched with monsoon periods to mitigate stress effect. The varietal response to salt stress at different growth phases was also a component of the study is to determine the tolerance level of commercial cultivars.

MATERIALS AND METHODS

Experimental Design and Imposition of Treatments

A pot experiment was conducted with eight commercial genotypes namely Co 8021, Co 85019, Co 2001-13, Co 97010, Co 99004, Co 94012 Co 95007 and Co 97009 with three replicates for each variety. Salt treatment was given with combination of three salts (sodium chloride, calcium chloride and sodium sulphate) in the ratio of 2:2:1 to raise soil EC up to 8 dS m^{-1} at different growth stages namely T_2 (formative phase 60 to 150 days), T_3 (grand growth phase 150 to 240 days), T_4 (maturity phase 2 40 to 360 days), T_5 (throughout the crop cycle) and a control with normal irrigation as T_1.

Physiological Traits

During the growth phases, physiological traits namely chlorophyll fluorescence, chlorophyll SPAD index and RWC (relative water content) were estimated. Chlorophyll Fluorescence (fv/fm ratio) was measured using Chlorophyll Fluorometer (model OS-30p) during 9:00am to 11:00am. Chlorophyll SPAD index was recorded with SPAD-502 (Konica Minolata Sensing,Inc.) on clear light between 9:00am to 11:00am.

RWC was estimated using the standard formula [9].

Data Harvest, Juice Quality Analysis

Plant characters namely plant height, number of stalk per pot were recorded. Cane yield, single cane weight was recorded at the completion of experiment. Sugarcane juice was extracted in crusher (65% capacity) and juice volume was measured in litres and juice was analyzed for brix (%), pol (%) and purity (%) as per standard methods [10]. Nutrients viz., potassium, sodium and chloride in the juice were estimated using flame photometer [11].

Statistical Analysis

The data on chlorophyll fluorescence, SPAD chlorophyll index, relative water content, plant height, single cane weight, number of stalks, cane yield, brix, sucrose, CCS and purity percent, nutrients such as sodium, potassium and chloride in juice were analyzed based on three replications and the results were expressed as mean values. Analysis of variance was performed and significance of each group was verified with two-way analysis of variance. The statistical analysis was performed using the software JMP 9.0.

RESULTS AND DISCUSSION

Physiological Traits

Chlorophyll fluorescence

Chlorophyll fluorescence varied from 0.659 (Co 2001-13) to 0.684 (Co 85019) among the genotypes in control (Table 1). In T_2 the highest was recorded in Co 85019 (0.647) and the least was found in Co 8021 (0.575), the same trend was also seen in the salt treatment (T_5) where the salt treatment was given throughout the growth stages. In T_5 Co 85019 it was 0.633 and in Co 8021 it was 0.542. So, T_2 (salt treatment given during formative phase) was found to be the critical stage. T_3 and T_4 have no ill effect when compared to T_2 and T_5. In T_3 it varied from 0.594 (C0 8021) to 0.662 (Co 85019). Therefore it was perceived that, the salt treatments at formative phase and throughout the crop cycle has similar declining trend with respect to chlorophyll fluorescence. Chlorophyll fluorescence is a very sensitive tool in the study of stress-induced damage to PSII [12]. There was also a review about the decrease in Fv/Fm ratio in salinity [13].

SPAD Chlorophyll Index

SPAD measurements are widely used to assess the absolute chlorophyll content per leaf area. SPAD index varied from 37.27 (Co 8021) to 40.18 (Co 97009) in T_1 (Table 1). It was decreased in T_2, the highest was observed in the genotype Co 85019 (37.7) and the least was recorded in the genotype Co 97010 (34.57). In treatments T3 and T4 reduction was marginal but in T5 there was a notable drop in SPAD index, where the least value of 29.09 was observed in the genotype Co 97010. The treatments T_2 and T_5 had similar trend for chlorophyll SPAD value again suggesting formative phase to be sensitive to salinity stress. It confirms formative phase as critical stage of crop growth under salinity [14].

Relative Water Content (%)

Relative water content varied from 69% to 80% among the genotypes in T_1 (control), while in T_2 it ranged from 48 to 70% with Co 97010 recording least turgidity; T_2 is in line with T_5 indicating stress severity on leaf moisture content during formative phase is effective (Table 1). Specific salt treatments during grand growth phase (T_3) and maturity phase (T_4) did not affect the relative water content as severely as in T_2 and T_5. Earlier report also had a similar finding [15].

Chlorophyll fluorescence an indication of active photochemical activity decreased in all the growth phases due to salinity and

Table 1. Effect of salt stress on physiological parameters

Treatments/ varieties	Chlorophyll Fluorescence						SPAD Chlorophyll						Relative water content (%)					
	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean
Co 8021	0.662	0.575	0.594	0.611	0.542	0.597	37.27	36.01	36.98	38.18	31.33	35.95	72.73	56.23	70.1	72.25	48.44	73.51
Co 85019	0.684	0.647	0.662	0.674	0.633	0.660	40.11	37.7	38.61	39.29	35.5	38.24	81.34	68.37	75.48	77	65.35	56.31
Co 2001-13	0.659	0.609	0.642	0.642	0.576	0.626	39.18	37.41	38.11	40.14	33.09	37.59	70.47	53.18	61.04	72.48	46.48	69.48
Co 97010	0.664	0.608	0.608	0.629	0.516	0.605	39.15	34.57	35.12	35.63	29.09	34.71	69.73	48.28	55.66	67.41	40.45	72.51
Co 99004	0.680	0.644	0.658	0.664	0.615	0.652	39.61	37.48	39.56	40.66	33.73	38.21	79.62	63.57	68.21	79.55	56.44	59.17
Co 94012	0.667	0.632	0.653	0.652	0.597	0.640	38.72	37.17	38.9	39.56	35.85	38.04	80.32	70.27	75.27	76.51	60.18	57.82
Co 95007	0.662	0.606	0.614	0.607	0.557	0.609	38.99	36.15	37.81	35.04	32.44	36.09	70.45	50.33	56.3	73.62	45.17	73.51
Co 97009	0.668	0.644	0.653	0.654	0.600	0.644	40.18	37.67	39.52	38.6	33.95	37.98	70.51	51.5	56.48	68.38	42.21	56.31
Mean	0.668	0.621	0.636	0.642	0.580		39.15	36.77	38.08	38.39	33.12		75.33	58.72	64.57	73.75	51.63	
		SEd			CD			SEd			CD			SEd			CD	
T		0.243			0.483			0.355			0.706			0.0021			0.0041	
V		0.192			0.382			0.281			0.559			0.0016			0.0031	
T x V		0.544			1.082			0.794			1.580			0.00478			0.0095	

genotype x treatment interaction was significant (Table 3). SPAD chlorophyll index and relative water content also showed similar trend and significant interactions suggest the physiological mechanisms adversely affected by salinity. Nevertheless, genotypes Co 85019, Co 94012 and Co99004 recorded higher fluorescence, SPAD index and RWC suggesting tolerance mechanism operating. Reduction in chlorophyll fluorescence and SPAD index are indications of impaired physiological function resulting in reduced physiological efficiency.

Harvest Traits

Plant height

There was a marked reduction in stalk length in all salt treatments (T_2 to T_5) when compared to control (Table 2). In control (T_1) it varied from 197cms (Co 8021) to 238cms (Co 85019). In treatments T_2 and T_5 the reduction in shoot length was elevated but in T_3 and T_4 the reduction was comparatively less. In T_2 it ranged from 124.59 (Co 97010) to 190.20 (Co 85019) and in T_5 higher reduction was noticed. In T_5 lowest value measure was found in the genotype Co 97010 (96.18 cm) and the highest length was recorded in the genotype Co 85019 (229.13cm). Shoot growth rate reduced even under mild salinity (EC of 2dSm-1) in sugarcane cultivars [16].

Number of Stalks

Number of stalks has decreased in all treatments (T_2 to T_5) when compared to control (Table 2). In control (T_1) it varied from 4.33 in Co 97010 to 7.67 in Co 85019. In T_2 it ranged from 2.67 in genotype Co 97010 to 6.33 in Co 85019. T_2 and T_5 severely affected the stalk number. In T_5, Co 85019 recorded the highest number (6.33) when compared to all other genotypes. The results pertaining to number of stalks and cane yield showed significant variation among the tested sugarcane varieties in saline condition [17].

Single Cane Weight

Single cane weight reduced due to salt treatment at all growth stages studied (Table 2). In T_5 ranged from 428.3g (Co 95007) to 1177.4g (Co 85019) in T_4. In control (T_1) the range was between 896.7g (Co 97010) to 1,343g (Co 85019). There was very sharp decline in single cane weight in both T_2 and T_5. In T_2 it ranged from 526.1g in Co 97010 to 777.9g in Co 99004 and in T_5 it was from 428.3g in Co 97009 to 937.1g in Co 85019. Treatments, T_3 and T_4 did not affect the single cane weight in with comparison with T_5. The results suggested that the effect of salt treatments during T_2 and T_5 were severe effect and also found to be critical. Reduction in single cane weight due to salt stress and considerable variation for salt tolerance in the sugarcane germplasm has been reported [18].

Cane Yield

The yield data (Table 2) indicated a remarkable variation among the genotypes. In control (T1) Co 85019 has recorded 10.31kg

Table 2. Effect of salt stress on cane yield and its related traits of sugarcane genotypes

Treatments/ Varieties	Cane height (cm)						Number of stalks						Single cane weight (g)						Cane yield (kg)					
	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean	T1	T2	T3	T4	T5	Mean
Co 8021	197	127.6	149.6	178.3	101.2	150.74	5.67	3.33	3.67	3.67	3	3.87	950	544.4	753.8	839.4	478.2	197	5.38	1.83	2.76	3.05	1.38	2.88
Co 85019	267.71	190.25	215.17	246.07	229.13	229.67	7.67	6.33	7	7.67	6.33	7.00	1343.3	774.8	1014.5	1177.4	937.1	1049.42	10.31	4.88	7.14	9.00	5.9	7.45
Co 2001-13	238.28	157.15	184.42	216.61	128.44	184.98	6	3.33	4.33	4	3.33	4.20	1123.3	677.4	846.3	996.1	518	832.22	6.71	2.24	3.69	3.98	1.75	3.67
Co 97010	197.38	124.59	146.51	175.39	96.18	148.01	4.33	2.67	2.67	3.33	2.67	3.13	896.7	526.1	668.8	765.1	463.5	664.04	3.88	1.40	1.80	2.51	1.25	2.17
Co 99004	245.22	172.06	193.99	225.3	149.61	197.24	6.33	4.67	5.33	6	4	5.27	1317.7	777.9	968.6	1151.5	735.6	990.26	8.30	3.59	5.19	6.97	2.98	5.41
Co 94012	229.99	153.35	182.29	209.66	129.08	180.87	6.33	4.33	4.67	5	4.33	4.93	1146.7	690	864.2	982.5	639.6	864.6	7.25	3.02	4.04	4.94	2.80	4.41
Co 95007	200.21	124.58	146.45	178.48	97.9	149.52	5	3.33	3.67	4.33	3.67	4.00	916.7	524.7	664.7	778	428.3	662.48	4.63	1.72	2.41	3.34	1.57	2.73
Co 97009	225.37	147.48	176.26	204.34	120.78	174.85	4.67	4.33	4.67	4.67	3.67	4.40	1133.3	646.8	851.3	973.5	591.3	839.24	5.31	2.78	3.93	4.51	2.15	3.74
Mean	225.15	149.63	174.34	204.27	131.54		5.75	4.04	4.50	4.83	3.88		1103.46	645.26	829.03	957.94	598.95		6.47	2.68	3.87	4.79	2.47	
		SEd			CD			SEd			CD			SEd			CD			SEd			CD	
T		0.242			0.481			30.11			60			272.74			543			0.112			0.222	
V		0.191			0.380			23.80			47.36			215.62			428			0.089			0.177	
T x V		0.542			1.078			67.33			133.98			609.86			1214			0.251			0.499	

which was the highest cane yield/pot among all the genotype and lowest was found to be 3.88kg in Co 97010. The treatments T_2 and T_5 has similar trend, the highest reduction was found in the genotype Co 97010 (1.40 kg in T_2 and 1.25kg in T_5), similarly the genotypes Co 8021 and Co 95007 recorded low weight below 2kg. In the treatments T_3 and T_4 the genotypes were not affected as compared to T_2 and T_5. From this result, it is inferred that the formative phase is critical apart from stress imposed at all other growth stages. Earlier finding also showed reduction in cane yield in sugarcane genotypes due to salinity [19].

At harvest the number of stalks/pot showed significant variation among genotypes as well treatments. Similarly, yield contributing traits viz, single cane weight and cane height also showed significant reduction due to salinity treatment imposed at different growth stages (Table 2). Highest reduction was recorded in treatment T_5 followed by T_2. The genotype x treatment interaction was significant (Table 3).

Juice Quality

Brix%, purity%, sucrose% and CCS%

Salt treatment had negative impact on brix% percentage, except for a few varieties which showed improvement in brix value. In T_5 very low birx was observed in genotype Co 8021 (17.03%) followed by Co 95007 (17.17%). In T_2 (salt treatment given during formative phase) it varied from 17.73% in Co 8021 to 24.03% in Co 94012. In T_3 highest brix% was recorded in Co 99004 (22.4%) and Co 2001-13 (21.03) and the lowest was observed in Co 8021(18.47%). In T_4 brix% ranges from 19% in Co 8021 to 22.7% in Co 99004. In T_5, Co 99004 has highest brix of 21.03% and Co 8021 has the lowest brix value of 17.17%. The same trend was also observed with juice purity, sucrose and CCS%. Effects of salinity, on brix, pol and conductivity were very similar, indicating that the response of juice quality to salinity is predictable [20,21]

Sodium, Potassium and Chloride

The amount of sodium, potassium and chloride in juice rose in salt treatment. The genotype Co 97010 showed highest salt content among the genotypes studied while, the genotype Co 99004 had least salt content. The genotype Co 85019 also has shown elevated levels of salts, which indicates storage of salts in juice.

Brix%, purity%, sucrose% and CCS% reduced in all the treatments as compared to control in all the genotypes (fig1a-d). The treatment x genotype interaction was significant (Table 4).

In juice, Na content increased in all the treatments and in all varieties indicating the higher uptake of Na. Na content in the juice was highest in T_5 and in genotypes Co 85019, Co 8021, Co 97010 and Co 95007. Sodium is a non-essential element which affects the quality of the produce in agricultural crops. Potassium and chloride also increased and the trend was similar to Na (fig 2a, b, and c). The results are in line with earlier reports on sugarcane [21]. The treatment x genotypes interactions were significant (Table 4). Plant tolerance to salinity was reported to be closely related to the Na^+: K^+ ratio in the cell than the absolute Na^+ concentration [22,23].

Figure 1: Effect of salinity on (a) Brix, (b) Purity, (c) CCS%, (d) Sucrose%. Error bars are indicated on the graph. Standard errors ($n = 3$)

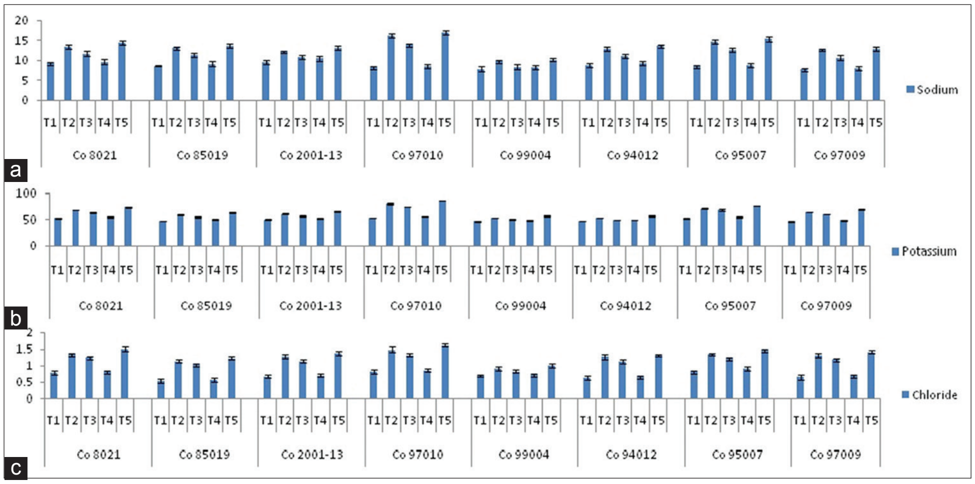

Figure 2: Effect of salinity on (a) Sodium, (b) Potassium & (c) Chloride .Error bars are indicated on the graph. Standard errors (*n* = 3)

Table 3: Two way ANOVA on NMC, SCW, Weight, Plant Height, Chlorophyll fluorescence, SPAD Chlorophyll & RWC

Factor	df	F Ratio probability NMC	Single cane weight	Weight/pot	Plant Height	Chlorophyll Fluorescence	SPAD Chlorophyll	RWC
Variety	7	46.40***	45.46***	78.58***	12128.47***	241.05***	28.25***	1560.49***
Salinity	4	30.21***	157.58***	115.77***	36072.20***	744.05***	143.70***	5616***
Var X Sal	28	1.620***	0.814***	1.674***	330.71***	17.09***	3.5***	64.06***
Error	80	35.33***	544064***	44632220***	78.29***	0.0027***	75.8***	36***
Total	119	244.80***	7150552***	636067442***	233447.30***	0.179***	900.61***	15683.18***

Level of significance: *** indicate significant difference at $P \leq 0.001$

Table 4: Two way ANOVA on Brix, Purity, CCS%, Sucrose%, Sodium, Potassium & Chloride

Factor	df	F Ratio probability Brix	Purity	CCS	Sucrose	Sodium	Potassium	Chloride
Variety	7	343.77***	1569.38***	279.51***	1224.28***	82.65***	1365***	85.15***
Salinity	4	261.47***	11570***	2695***	10015.43***	548.15***	3221***	754.15***
Var X Sal	28	17.27***	106.17***	42.30***	98.87***	14.6***	94***	6.19***
Error	80	7.62***	9.06***	1.54***	0.558***	19.06***	38***	0.239***
Total	119	382.52***	6832***	270.54***	359.71***	773***	11977***	11.551***

Level of significance: *** indicate significant difference at $P \leq 0.001$

CONCLUSION

In this study, a few sugarcane genotypes response towards phasic salt treatment was observed. From the treatments it was found that T2 (salt treatment during formative phase) and T5 (salt treatment throughout all the growth phases) were the critical phases. The genotypes Co 85019 followed by Co 99004 were found to be the best surviving when comparing all the other genotypes. These genotypes could be used in saline lands for occurring good yield.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, ICAR-Sugarcane Breeding Institute, Coimbatore for providing necessary facility and support to carry out the research facility and support to carry out the research work successfully.

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