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Bio-Molecular Analysis of the Effects of Salinity Stress on Sugarcane Genotypes at Elongation Stage

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Abstract
Bio-molecular analysis often Sugarcane Genotypes under salinity stress conditions at Elongation stage was studied. Three replications of ten sugarcane varieties vizCoSe 03234, CoS 03251, CoS 95255, CoSe 01424, CoSe 01434, CoS 03261, CoS 07250, CoSe 96436, CoS 97261 and UP 49 were exposed to normal (0.4 dsm^{-1}) and saline (8.0 dsm^{-1}) soil treatment. Different morphological and biochemical parameters were evaluated to understand the irregularities in plant growth at their elongation stage due to salinity of soil. Morphological characters, such as mother shoot height, leaf surface area and the number of green leaves, wereconjointlyfound reduced in saline soil condition plants as compared to normal ones. Among the evaluated biochemical parameters,protein, free amino acids and proline contents were found to be increased in saline soil condition plants as compared to normal. Further,CoSe 03234, CoS 95255and CoS 03251 sugarcane varieties emerged as early maturing varieties and CoSe 01424, CoSe 01434, CoS 03261, CoS07250, CoSe 96436, CoS 97261 as well as UP 49 were observed as mid-late maturing varieties based ontheir morphological and biochemical traits at the elongation phase under saline conditions.
Keywords: Sugarcane varieties, elongation stage, biochemical parameters, saline soil conditions, proline content, salt stress.

Introduction

In most parts of the world, salt-affected soil is one of the principle cause of soil degradation[1], consequently resulting in reduction of biomass production. Increasing soil salinity and sodicity are serious worldwide land degradation issues which may even increase more rapidly in the future[2,3]. It is estimated that 1.5 billion hectares of lands, all over the world, are salt-affected[4]. As per latest reports salt has affected 20% of agricultural land and 33% of the irrigated land in the world[5].In India20% cultivable lands are mainly affected by salinity including the forest area in Rajasthan, coastal Gujarat and Indo-Gangetic plains[6-7].Cultivable land could suffer a 30% loss in next 25 years which is expected to grow more by 50% by 2050[8].Further, about 25% of ground water resources are affected by salt water in India. Salt affected soil in India is about 13 million hectares. 6.0 million hectares of cultivated land area are saline and sodic in the country[9].

Salinity is an environmental stress factor affecting plant magnification and development. It is a destructive threat to ecumenical agricultural engenderment, which damages more than 400 million hectares of land over 6% of the world's total land area[10]. In India, the yield of wheat, rice, sugarcane and cotton crops on salt affected land in Gangetic Basin is 40%, 45%, 48%, and 63%, respectively[11]. Soil salinity is a type of abiotic factor that affects the productivity of crops all over the world[12].

Therefore, scientists are working continually to explore new salt tolerant varieties in different crops. Salinity of the soil does impart a negative effect on sugarcane crop like all other crops. The problem of increasing salinity in tropical and subtropical countries not only inhibits sugarcane growth but also indicates a decrease in its sucrose content and quality[13]. Salt stress exhibits a wide impact on both the quality and production of sugar by reducing the growth rate of sugarcane and affecting the quality of jaggery. All varieties of sugarcane vary in qualitative and quantitative losses under

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biological and abiotic conditions[14]. Due to this, farmers do not get sufficient returns from the yields of sugarcane grown[15,16,17]. For every 100 tons of sugarcane in the sugar mill, there is a loss of Rs 2000 per day.

Although the salt tolerance of many crops has been widely studied, little work has been done on the biochemical and molecular determination of salt tolerance in sugarcane crops[11]. Therefore, in the present work we are presenting investigations related to overall morphological changes as well as biochemical changes related to nitrogenous contents under salt stress in some selected varieties of sugarcane viz. CoSe01424, CoSe01434, CoSe 03234, CoS 03251, CoS 03261, CoS 07250, CoS 95255, CoSe 96436, CoS 97261 and UP 49.

Materials and methods

The required reagents and solvents were sourced from the commercial suppliers and purified/distilled/crystallized before use wherever was found necessary.UV-1800 spectrophotometers (Shimadzu, Japan) were used for all biochemical studies. Centrifuge: Genie company, SKU: MC - 4, (Rotor speed: 1000-14,000 rpm) with digital timer, Microcentrifuge: Genie company, SKU: MC - 3, (Rotor speed: fixed 10000 rpm) with digital timer, Microcentrifuge: Genie company, SKU: MC - 1, (Rotor speed: fixed 6000 rpm) with digital timer and Potter-alvehgenum Homogenizer were also used to carry out the research work.

Experiment design and treatment

An experiment was carried out in cement pots (80X80X60 cm) at the Sugarcane Research Institute farm of Shahjahanpur in the spring planting season. Shahjahanpur is located at 27.58 N latitude with 79.54 S longitude and at an altitude of 154.53 meters above sea level. Sixty cement pots were taken to perform the experiment. All pots were divided into two equal parts. Each pot was filled with 80 kg of soil. The soil in the first thirty pots was allowed to remain normal and in the second part 30 pots of soil were treated with saline. Ten cane varieties viz. CoSe 01424, CoSe 01434, CoS 03234, CoS 03251, CoS 03261, CoS 07250, CoS 95255, CoSe 96436, CoS 97261 and UP 49 were taken to experiment. Three replications of ten varieties were sown in normal and saline soil.

Setting up of physical properties of the experimental soil

The soil taken for the experiment was a sandy loam in normal condition with pH 7.7 and 0.4 dsm^{-1} electrical conductivity. The desired amount of sodium chloride, sodium sulphate and calcium chloride were added to the soil to provide saline treatment and saline level 8dsm^{-1} was artificially created. Five budded sets of each variety were placed in each pot. Thinning was removed after germination and only two clumps were left in each pot for further study. The EC level was checked in saline soils at an interval of every 20 days and was adjusted repeatedly as and when required. Irrigation operations as per the requirement were given in the normal and saline pots. Nitrogen was given as urea in all the pots as per 15 kg/ ha requirement. Nitrogen was given half at the time of planting and the remaining half was given in two equal divided doses before the onset of monsoon.

Phenotypingand collection of leaf samples

During the last phase of the elongation stage, we recorded the growth characters such as area of fresh open leaves, height of the mother's shoot and increase in the number of green leaves of the cane stalk. To analyze genomic nitrogenous contents viz. proteins, free amino acids and proline, leave samples were taken from the normal and saline pots of each variety.

Protein determination

The protein determination is one of the most important parameters for a better understanding of plant immunity[18-22]. Total protein concentrations were determined by Lowry's method (1951)[23]. In this method 500 mg of leaf sample was crushed with 05 ml Tris buffer and was centrifuged at 10,000 rpm for 10 minutes. The supernatant was separated and the volume was made 5 ml again by adding more Tris buffer. An equal amount of 0.5 ml supernatant and trichloro acetic acid was mixed and left overnight. Supernatant was discarded and to the obtained solidpallets was added 0.5 ml (0.1 N) NaOH. Then vertex was done. 200 µl. of this sample was added to 800 µl NaOH(0.1 N). It was the protein suspension. The absorption of the sample was recorded by UV spectrophotometer at 640

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nm and protein estimation was done.

Free amino acids determination

Determination Free amino acids concentration was determined by Yemm and Cocking's(1955)method[24-27]. Leucine was used as the standard. In this method, 500 mg fresh leaf sample from each pot was taken and homogenated with 10 ml of 50% ethanol. Then 100 gm of activated charcoal was added to each sample and they were centrifuged at 5000 rpm for 10 minutes. Later added 1% of 2.5 ml ninhydrin to each one of them and acetate buffer was also added to the sample tubes. The mixture was heated to boiling temperature on a water bath for 30 minutes and after cooling 5 ml aqueous iso-propanol (1:1) was added. The final supernatant was obtained and absorption was recorded at 850 nm.

Proline determination

Proline content was measured using Batesmethod (1973)[28]. Each 50 mg fresh weight leaves were crushed with 2.0 ml of 40% methanol. 01 ml of its extract was mixed with 01 ml of Glacial acetic acid and orthophosphoric acid (3:2, v/v). 25 ml ninhydrin solution was added to every sample after 20 minutes. Then their solutions were incubated for 1 hour at 100˚ C. After that, all tubes were cooled down and 5 ml toluene was added to each. Their absorption was recorded at 528 nm through spectrophotometer.

Results and Discussion

Different types of morphological and bio-molecular parameters were studied in ten sugarcane varieties (CoSe 03234, CoS 03251, CoS 95255, CoSe 01424, CoSe 01434, CoS 03261, CoS 07250, CoSe 96436, CoS 97261, UP 49)under normal and saline conditions. Morphological characters of all the sugarcane verities grown under normal and saline conditions are mentioned below (Table 1, Fig. 1).

Table 1.Morphological characters of different sugarcane varieties grown under normal and saline conditions

S. No	Varieties	Mother shoots height (cm)		No. of green leaves		(Leaf area cm^2)	
		Normal Soil	Saline Soil	Normal Soil	Saline Soil	Normal Soil	Saline Soil
1	CoSe01424	138.00	95.50	10.00	9.00	312.00	233.70
2	CoSe01434	153.00	134.00	11.00	10.00	262.80	253.90
3	CoSe 03234	93.00	80.00	8.00	7.00	419.10	406.80
4	CoS 03251	102.00	96.00	7.00	6.00	364.30	317.40
5	CoS 03261	112.00	94.00	8.00	7.00	345.00	212.50
6	CoS 07250	102.00	80.00	8.00	7.00	325.10	277.70
7	CoS95255	105.00	98.00	7.00	6.00	336.70	285.90
8	CoSe96436	105.00	100.00	9.00	8.00	288.10	160.50
9	CoS 97261	133.00	121.00	10.00	9.00	293.20	267.40
10	UP 49	105.00	95.00	8.00	6.00	289.80	246.30
	Mean	**114.80**	**99.35**	**8.60**	**7.50**	**323.60**	**266.21**
SE/CD Variety (V)		0.25/0.51		0.17/0.36		0.27/0.45	
SE/CD Soil (S)		0.11/0.22		0.08/0.16		0.05/0.11	
SE/CD (V) X (S)		0.35/0.72		0.25/0.51		0.26/0.53	

*SE- Standard Error, CD- Critical Difference

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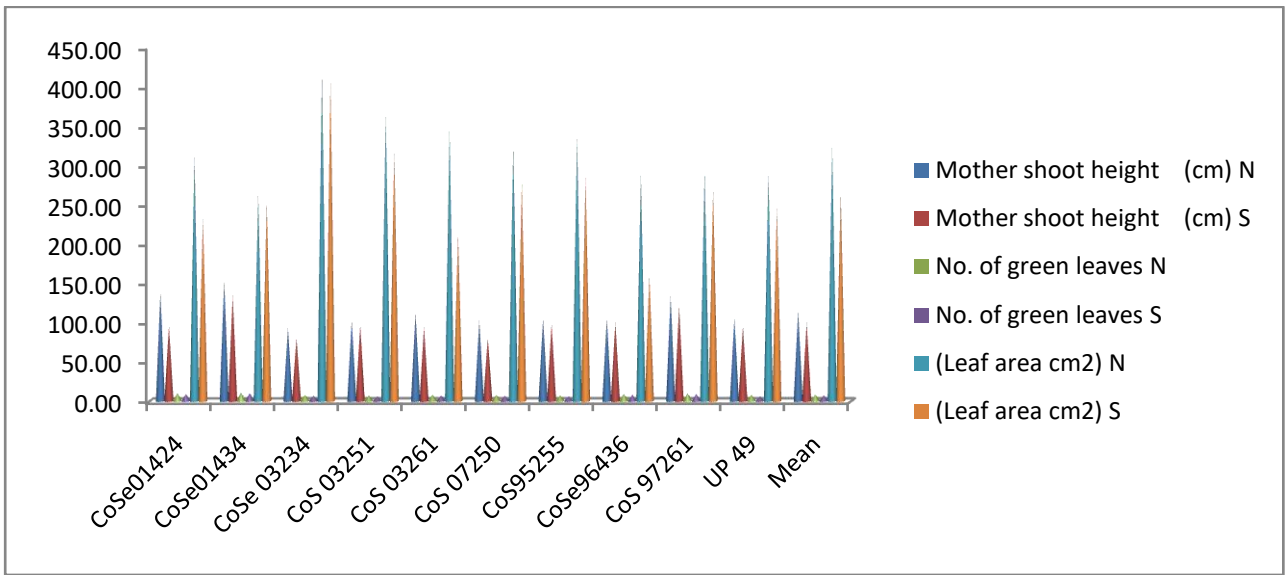

Fig 1. The graphical representation of the morphological data obtained under the normal and saline conditions.

It has been found that the growth rate was decreased in almost all the sugarcane varieties grown under saline conditions as compared to normal. Various morphological growth characters of all the sugarcane varieties such as mother shoot height, number of green leaves and first open leaf area as shown in Table 1 were found to be lower in the saline conditions as compared to normal. The graphical representation of the obtained data is given in Fig 1. The picture of the elongation stage of 10 sugarcane varieties under normal and saline soil conditions is shown in Fig 2.

Fig 2. The photograph of the elongation stage of 10 sugarcane varieties grown under normal and saline soil conditions

The average height of mother shoot was found to be 114.8 cm in all the varieties grown in normal soil. On the other hand, average height of mother shoot was found to be 99.35 cm in plants grown under saline conditions. It was observed that the growth rate of mother shoot height came down by 13.5% in the saline conditions as compared to the normal. The highest mother shoot height under normal conditions was found in variety CoSe 01434(153 cm) followed by CoSe01424(138 cm) and CoS 97261(133 cm). However, under saline conditions the highest mother shoot height was found in variety CoSe 01434(134 cm) followed by CoS 97261(121 cm) and CoSe96436 (100 cm).

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In almost all the varieties, the average number of green leaves in the normal condition sugarcanes was found to be 8.6 as compared to 7.5 in saline soil condition plants. The results showed a decrease of 12.8% in the number of green leaves under saline conditions as compared to normal. The maximum number of green leaves in normal condition was found in CoSe 01434 (11) variety followed by CoS 97261(10) and CoSe 01424(10). The maximum number of green leaves in saline condition was found in CoSe 01434 (10) variety followed by CoS 97261(9) and CoSe 01424(9). Further, it was seen that number of green leaves were found to be the same in varieties 97261 and CoSe 01424 under the normal and saline condition.

The average of the first open leaf area was found to be 323.6 cm^2 in normal condition plants and 266.21 cm^2 in saline condition plants in almost all the varieties. The maximum first open leaf area in normal condition plants was found in CoSe 03234 (419.1 cm^2) variety followed byCoS 03251(364.3 cm^2) and CoS 03261(345 cm^2). The maximum first open leaf area in saline conditioned plants was found in varietyCoSe 03234 (406.8 cm^2) followed by CoS 03251 (317.4 cm^2) and CoS95255 (285.9 cm^2).

Overall, it was estimated that that various growth characters of sugarcane varieties such as mother shoot height decreased by13.5 %, the number of green leaves decreased by 12.8 % and first open leaf area was found to be decreased by17.8 % in the sugarcane varieties grown under saline conditions as compared to normal.

Many other scientists also have evidenced through their own experiments that all morphological characters of the plants such as growth and development, biomass production, germination rate, seedling growth rate are found decreased under saline conditions[29]. Number of productive tillers, panicle length and number of primary branches per panicle of plants have also found reduced under saline soil conditions[30].

Further, results showing determination of biochemical parameters related to nitrogenous contents viz. proteins, free amino acids and proline are given below (Table 2):

Table 2: Bio-molecular characters of sugarcane varieties under normal and saline conditions

S. NO.	Varieties	Protein (µg / g)		Free amino acids (µg / g)		Proline (µg / g)	
		Normal Soil	Saline Soil	Normal Soil	Saline Soil	Normal Soil	Saline Soil
1	CoSe01424	131.70	133.90	0.072	0.283	0.247	0.483
2	CoSe01434	124.41	136.60	0.040	0.050	0.309	0.574
3	CoSe03234	172.60	278.40	0.054	0.566	0.234	0.481
4	CoS 03251	84.30	93.00	0.025	0.438	0.238	0.553
5	CoS 03261	120.30	137.20	0.057	0.498	0.259	0.583
6	CoS 07250	189.50	229.00	0.022	0.643	0.341	0.463
7	CoS 95255	68.90	88.90	0.072	0.339	0.209	0.287
8	CoSe96436	72.60	125.50	0.044	0.865	0.104	0.244
9	CoS 97261	66.80	193.00	0.037	0.759	0.249	0.290

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10	UP 49	62.70	66.30	0.026	0.559	0.340	0.363
	Mean	**109.38**	**148.18**	**0.044**	**0.501**	**0.253**	**0.432**
SE / CD Variety (V)		0.511/ 1.03		0.0019/ 0.0038		0.25/0.51	
SE / CD Soil (S)		0.228/ 0.463		0.0008/ 0.0017		0.11/0.22	
SE / CD (V) X (S)		0.72/ 1.48		0.0028/ 0.005		0.35/0.72	

*SE- Standard Error, CD- Critical Difference

Effect on protein content

Proteins are the macromolecules which are polymers of amino acids. Protein content was estimated in all the sugarcane varieties grown in normal and saline conditions by recording the absorbance at 640nm[23]. A comparative analysis of protein concentrations in different varieties revealed that the average of protein concentration in normal condition remained109.38 µg/g. However, it was found increased to 148.18 µg/ g in sugarcane varieties with saline affected soil. The protein concentration increased approximately 35% in sugarcane varieties with saline affected soil as compared to normal At varietal level, it was observed that the protein concentration in normal condition plants was found to be highest in varietyCoS 07250 (189.5 µg/g) followed by CoSe 03234 (172.6 µg/g) and CoSe 01434 (124.41µg/g) varieties. Protein concentration in saline conditions was found to be highest in CoSe 03234 (278.4 µg/g) followed by CoS07250 (229 µg/g) and CoS 97261 (193 µg/g). While in normal conditions the protein concentration was found to be lowest in varietyUP 49 (62.7µg/g) followed by CoS 97261 (66.8 µg/g) and 95255 (68.9 µg/g). In saline conditions it was found to be lowest in variety UP 49 (66.3 µg/g) followed by CoS 95255 (88.9 µg/g) and CoS 03251 (93 µg/g).Overall, it was estimated that protein concentration of approximately each variety of sugarcane increased under the saline conditions as compared to normal.

Effect on free amino acids content

Sugarcane derives amino acids from primary elements and absorb through its stomas. Amino acids have an important role in increasing the yield and overall quality of sugarcane crop. The estimation of free amino acids content was done at850 nmby an already reported method[27] and has been shown in table 2. It was found that the average of free amino acids concentration remained 0.044 µg/g and 0.501µg/g in normal and saline condition plants respectively. At varietal level, it was observed that the free amino acid concentration in normal condition was found to be highest in CoSe 01424 and CoS 95255 varieties (0.072µg/g) followed by varieties CoS 03261 (0.057µg/g) and CoSe 03234 (0.054µg/g)respectively. Free amino acids concentration in saline condition plants was found to be highest in variety CoSe 96436 (0.865µg/g) and followed by varietiesCoS 97261(0.759µg/g) and CoS 07250 (0.643µg/g) respectively. Lowest free amino acid concentration was found in sugarcane variety CoS 03251 (0.025µg/g) under normal soil conditions and in saline soil conditions lowest free amino acid concentration was found in variety CoSe 01434 (0.050µg/g). An overall increase was observed in the free amino acids content in all the plant varieties grown under saline conditions as compared to the normal.

Effect on proline content

The comparative analysis of proline concentration in both normal and saline conditions has been shown in table 2. The proline concentration has been found increased in sugarcane varieties grown in saline soil as compared to normal soil. It was found that the average proline concentration under normal and saline conditions came out to be0.253µg/g and 0.432 µg/g respectively. At varietal level, the proline concentration was found highest in variety CoS07250 (0.341 µg/g) followed by UP 49 (0.340 µg/g)under normal condition and under saline condition it was found to be highest in CoS 03261 (0.583 µg/g) variety followed by CoSe01434 (0.574 µg/g). Lowest proline concentration was found in sugarcane variety CoSe 96436 (0.104 µg/g)in normal soil conditions and in saline soil conditions lowest proline concentration was found in variety CoSe 96436 (0.244 µg/g.

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Proline is a proteinogenic amino acid in the biosynthesis of proteins. Proline protects sugarcane plants from saline stress and helps them recover quickly from any stress. Lots of work has been done to know the role of proline in helping plants to survive under salt stress[31]. It has been reported that proline is an osmolyte that acts as a metal chelator as well as an anti-oxidative defence molecule which enhances stress tolerance activity among plants. Salt stress conditions lead to disturbances in cell homeostasis. This leads to the biosynthesis of proline that acts like an osmolyte and fights against the stress. The exogenous application of proline is to improve the tolerance ability of crops that are under any adverse conditions, especially those which are saline affected crops[32]. Therefore, it can be said that higher contents of proline lead to increase in salt tolerance capacity of sugarcanes.
Standard errors and critical differences were also calculated for various studied parameters using suitable statistical tools and were found within the permissible limits. Further, the obtained results revealed that CoSe 03234, CoS 95255 and CoS 03251 sugarcane varieties have emerged as early maturing varieties. However, CoSe 01424, CoSe 01434, CoS 03261, CoS07250, CoSe 96436, CoS 97261 and UP 49 were observed as mid-late maturing varieties based on their morphological and biochemical traits at the elongation phase under saline conditions. A number of other groups have also reported that sugarcane crops respond to the adverse effects of salinity like other plants[33]Whenever, sugarcane plants face salt stress, their growth, metabolism and the overall crop yield gets negatively affected[34].

Conclusion

From the above results it is evident that the growth of sugarcane plants under saline soil conditions exhibits a decrease in the morphological growth parameters i.e., Reduction in the mother shoot height, leaf surface area and the number of green leaves on the stalk. Also, the nitrogenous contents viz. protein, free amino acids and proline concentrations were found increased in the plants grown under salt stressed soil as compared to the normal soil. Further, out of ten sugarcane varieties under investigation, CoSe 03234, CoS 95255 and CoS 03251 emerged as early maturing varieties. However, remaining sugarcane varieties i.e.,CoSe 01424, CoSe 01434, CoS 03261, CoS07250, CoSe 96436, CoS 97261 and UP 49 were observed as mid-late maturing based on their morphological and biochemical traits at the elongation phase under saline conditions.
The reason behind the irregularities in the morphological characters and nitrogenous biochemical parameters concentrations may be attributed to some kind of damages at nucleic acid levels in the sugarcane crops due to salinity stress which needs further probing into. Nevertheless, these findings may prove beneficial for developing the new and better salt tolerant sugarcane varieties.

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