

## Research Article

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## Different Approaches for Developing of Salt Tolerant Sugarcane (*Saccharum officinarum*)

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### ABSTRACT

Sugarcane is a very important crop mostly cultivated in many regions of the world. Sugarcane is an important crop in tropical areas of the world, often being uncovered to environments with high salinity, but little is recognized of genetic variant in salt tolerance. The purpose of this work was once to examine the performance of two genetically various cultivars of sugarcane underneath specific concentrations of salinity (0, 40, eighty and a hundred and sixty mM NaCl) over a period of 30 days. SP 81–3250 was extra salt-tolerant and maintained its charge of biomass production, photosynthesis and leaf place up to one hundred sixty mM NaCl, whereas IAC 87–3396 was a sensitive to 80 mM NaCl. SP 81–3250 maintained very low concentrations of Na<sup>+</sup> in both leaves and roots with increasing time and salinity, whereas in IAC 87–3396 the Na<sup>+</sup> concentrations had been 2–5 instances higher. This suggests that the tolerance of SP 81–3250 to excessive salinity was due to its ability to leave out Na<sup>+</sup> whilst taking up water from the soil, and that measurements of Na<sup>+</sup> awareness in leaves should be used to pick out salt-tolerant genotypes for saline areas.

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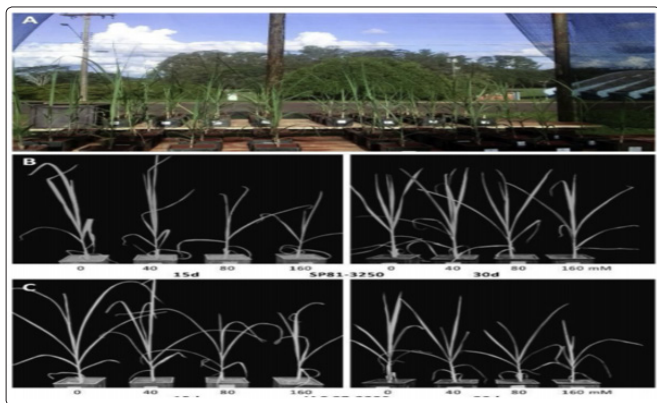
### Introduction

Reduction within the biomass had occur when plantation materialized in an exceedingly soil contain accumulation of salts. Sugarcane was a very important crop cultivated for the assembly of sugar. The residue was also use as a biofuel. Sugarcane was very sensitive about the chemical composition of soil. If the buildup of salts had in a very soil then the expansion of sugarcane was strongly prohibited. In sugarcane both osmotic and ionic affects occurred because of high soil salt concentration. It had decreased water uptake, increased salt uptake, and had increased cellular concentrations of Na<sup>+</sup> and Cl<sup>−</sup>. The proper amount of nutrients in soil was very necessary for the most effective adaptation of sugarcane. If any change was occur within the chemical composition of such soils then many complications passed off. Such complication caused harmful effects on sugarcane growth. Salinity of soil was also a facet that created many problems for them. Industrialization altogether over the globe had a source to expel out polluted water. When that water reached into the formation then created harmful effects. When sugarcane suffer from salt environments, the buildup of sodium and chloride ions had played negative role for the variation of sugarcane in saline soil. The quality of sugarcane was badly disturbed because of the salts transposition in sugarcane from roots. So as to extend food production and to produce the requirements of a growing population within the world, agriculture has expanded into soils that are salinized, either by their geological origin in arid and semi-arid regions or by management practices of land clearing

or irrigation [1-5]. Salinity was a significant abiotic factor that constrains agricultural productivity. Salt stress retarded sugarcane growth through many physiological, biochemical and metabolic processes. The reduced growth of the sugarcane under salinity was thanks to nutrient disturbances. This may well be attributed to the competition of Na<sup>+</sup> and Cl<sup>−</sup> with nutrients like K<sup>+</sup>, Ca<sup>2+</sup> and NO<sup>−3</sup>. Polluted water was additionally major source to reinforce the salts during a very bad way and is commonly occur in that area where salty water had employed as a chief source of irrigation due the shortage of fine water. Salt affected soils cover a district of nearly 13.8 Million ha in Rajasthan. In Pakistan & other countries, salinity of soil was increasing rapidly. Now causes the decline of soil productivity [6-8]. (Numerous environmental stresses like drought, flooding, salinity, heat, bloodless, and heavy metals had adversely affected the improvement and productiveness of crop vegetation.

Salinity had a significant abiotic element that constrains agricultural productivity. Salt stress retarded plant increase by means of affecting many physiological, biochemical and metabolic processes. The decreased boom of the flowers under salinity was due to nutrient disturbances, affecting the availability, shipping and partitioning of nutrients. This may be attributed to the opposition of Na<sup>+</sup> and Cl<sup>−</sup> with nutrients including. To embark upon that example, along with the traditional breeding and biotechnology of plant for salt tolerance, the use of plant increased selling rhizobacteria (PGPR) could also be disbursed as a vital method to boost cultivation in saline soils. Protein was similar in pleasant with soy protein, as pronounced by using the arena health corporation. The usage of oat grains as human

meals has multiplied swiftly because it consists of the soluble fibers having hypo cholesterolemic properties. Although, oat was thought to be moderately salt tolerant yet it had been far extra touchy to salinity in assessment to other cereals which include barley and wheat. PGPRs were regarded to push plant increase and improve vitamins either immediately or in a very route. The direct facilitation of plant growth via PGPR includes supplementation critical vitamins inclusive of nitrogen, phosphate, zinc, potassium and iron and manufacturing of phytohormones. The effect of PGPR inoculation in ground nut and wheat on various parts had increased; biochemical and physiological parameters beneath NaCl stress had been promoted.



An investigation showed that salinity had control by introducing genetic modification in sugarcane. Genetically modified sugarcane plants then sustain the ability to grow best in saline soil and acquire the characteristics to meet required nutrients through such soil [9]. In saline soil, sugarcane had experienced dehydration, nutrient deficiency, membrane dysfunction, and metabolic and photosynthetic activity reduction. To decrease the negative effects of salt stress on sugarcane growth and development, large efforts had required to developing salt tolerant sugarcane genotypes through conventional breeding or genetic engineering. Approximately 7% of the total land surface in effected by soil salinization in all over the world [10]. Brazil is the world's largest sugarcane producer and stands out in the commercialization and export of sugar and ethanol from this crop. Sugarcane was consider as moderately salinity sensitive, and put an estimates that one million hectares of sugarcane cultivation were salinized. Sugarcane needed soils with high water content and nutrients in order to reach maximum yield, so cultivation in salinized soil would result in a drastic reduction of growth, with losses of 50% or more in comparison to the productivity in unsalted soils. Most plants were susceptible to salt stress and unable to yield well in soil salt concentrations above 100mM of NaCl. Salt tolerance was a complex mechanism, involving tolerance to both the osmotic and ionic stress caused by high soil salinity, and sugarcane species had different role in biochemical and physiological responses.

Differences also occurred within species, especially those species with high genetic variability, as in sugarcane, and were not undergone persistent selection pressure for individual traits for salt tolerance. In case of sugarcane under salinity, both osmotic and ionic effects had occurred as the high soil salt concentration, decrease water uptake, increases salt uptake, and increases cellular concentrations of Na<sup>+</sup> and Cl<sup>-</sup>. Biotechnologists were taking interest to introduce the genes of interest in different species of sugarcane. Due to the working of biotechnologists to make sugarcane adapted in saline soil, two new varieties SP-81 and IAC 87-3396. SP 81-3250 had a greater accumulation of proline. These new varieties were playing their role to best adaptation of sugarcane in soil containing accumulation of salts. Genetically

modified species of sugarcane were producing high yield of sugar and other derived products. So, in IAC -81 and IAC 87-3396 were playing their role for the adaptation of sugarcane in such a saline soil. Accumulation of NaCl in roots and leaves had been decreased due to genetic modifications in sugarcane. Nearly 79% sugar was obtained from sugarcane while the remaining part was obtained from sugar beets.

Salt pressure surroundings had played a horrific role for the right variation of sugarcane. Because of the buildup of NaCl, proper characteristic of sugarcane roots was affected. Polluted water had also possessed Cd and different heavy metals. These heavy metals then reacted with other additives to make risky salts. Salts had reduced the right functioning of sugarcane in saline environment. Salt tolerance had a complicated mechanism, involving tolerance to both the osmotic and ionic pressure as a result of excessive soil salinity, and plant species. Different techniques had adopted to create biochemical and physiological responses. Variability had occurred within species, in particular those species with high genetic variability. As in sugarcane, an undergone persistent selection strain for individual tendencies for salt tolerance had reduced. Sugarcane changed into an crucial crop in tropical areas of the world, often being uncovered to environments with excessive salinity, but little become known of genetic variation in salt tolerance.

The aim of that paintings became to compare the overall performance of two genetically various cultivars of sugarcane beneath different concentrations of salinity (0, forty, eighty and 160mM NaCl) over a duration of 30 days. SP 81-3250 was more salt-tolerant and maintained its charge of biomass manufacturing, photosynthesis and leaf region up to 160mM NaCl, while IAC 87-3396 become sensitive to 80mM NaCl. SP eighty one-3250 maintained very low concentrations of Na<sup>+</sup> in both leaves and roots with growing time and salinity, whereas in IAC 87-3396 the Na<sup>+</sup> concentrations were 2-five times better. SP 81-3250 had a more accumulation of salts soil, and decrease lipid per oxidation, whereas glycine betaine and sucrose concentrations had been comparable in the cultivars. This suggests that the tolerance of SP eighty one-3250 to excessive salinity became because of its ability to exclude Na<sup>+</sup> even as taking over water from the soil.

Salinity had affected greater than 20% of global agricultural production and its miles expected to boom in its extent and severity within the coming a long time. Salinization takes place thru each natural and anthropogenic procedure. Predictions based totally on unprecedented versions in rainfall and temperatures suggest that many assets of freshwater for irrigation are at extreme risk for salinization. It has been envisioned that salinization renders three ha of arable land unproductive every minute. Salinity has major influences on meals production and constitutes a growing task for developing sustainable agricultural systems. High concentrations of salt had precipitated each ionic and osmotic stresses. Salinity induces osmotic strain by lowering the soil water ability, hence accelerated the energy required for uptake of water and nutrients.

## Materials and Methods

### Growth Conditions & Salt Concentration

The fundamental scan was once a definitely randomized 2×4 factorial scheme (2 cultivars and four treatments: 0, 40, 80 and one hundred sixty mM NaCl), with 4 replicates per treatment. The plants from mini-totes have been transplanted in two L pots filled with soil. Soil Dystrophic Red Latosol was once amassed at 0-20 cm soil depth, with chemical characterization of 5.7 pH; 10 mg dm<sup>-3</sup> of natural matter, 0.1 mg kg<sup>-1</sup> of Na<sup>+</sup>; 26 mg dm<sup>-3</sup> of P (resin); 1.6 mmol dm<sup>-3</sup> of K; 34 mmol dm<sup>-3</sup> of Ca;

21 mmol dm<sup>-3</sup> of Mg; 341 g kg<sup>-1</sup> of clay; 28 g kg<sup>-1</sup> of silt; 310 g kg<sup>-1</sup> of high-quality sand; 321 g kg<sup>-1</sup> of coarse sand; suggest textural density and density of 1.24 kg dm<sup>-3</sup>. Soil fertilization used to be carried out in three levels every 10 days, the usage of 0.3 g of NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and 0.35 g of KH<sub>2</sub>PO<sub>4</sub> per pot (incorporated to soil earlier than transplanting) and 0.45 g of urea and 0.35 g of K<sub>2</sub>SO<sub>4</sub> (second and 1/3 stage, overlay) and the irrigation used to be carried out daily, in order to restoration the water loss via evapotranspiration. At 30 d, the salt concentrations in the soil answer was once expanded to 40, eighty and a hundred and sixty mM NaCl and the opinions had been performed after 15 and 30 d of treatment. At both harvests, biometric measurements had been made on roots and shoots. Leaves and roots were analyzed for Na<sup>+</sup>, K<sup>+</sup>, proline, and glycine betaine. Leaves had been measured for water potential, malon di aldehyde (MDA) content and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), chlorophyll content and quantum yield of PSII.

### Biometric Measuring System

Height of the flora used to be measured from soil floor to the tip of the longest totally expanded leaf. Leaf location and root density have been measured after separation of the vegetative components the usage of the Delta-T Devices LTD image analysis system. The complete dry biomass of shoot and root used to be bought after drying in a compelled ventilation oven at temperature from 65 °C to regular weight. Biochemical and physiological opinions were carried out on +1, two and three leaves, active photosynthetically, according to Kuijper (1915). The +1 leaf is distinctive by means of first leaf from top to bottom of the stalk with truly visible dewlap.

### Ion Concentrations in Tissues

Na<sup>+</sup> and K<sup>+</sup> concentrations in leaves and roots of the two sugarcane cultivars were determined by means of flame photometry after digestion in HNO<sub>3</sub> in accordance to Bloise et al. (1976), the use of 3 leaves or the entire root system.

### Proline, Glycine Betaine and Sucrose Concentrations

Proline was decided on leaf (+2 leaves) and root tissues (200 mg) by means of the method described by using Bates et al. (1973). Leaf samples were homogenized in aqueous sulfosalicylic acid (3% w/v) and the filtered homogenate was once reacted with acid ninhydrin at a hundred °C for 1 h. The response combination was once extracted with toluene and the absorbance at 520 nm was recorded using toluene as a blank. Glycine betaine was once measured on leaf (+2 leaves) and root tissue (500 mg) by way of shaking with 20 ml of deionized water for forty eight h at 25 °C and extracting with bloodless potassium iodide-iodine reagent in

1N sulphuric acid in accordance to Grieve and Grattan (1983). The periodate crystals have been dissolved in 1, 2-dichloro ethane and, the absorbance used to be measured at 365 nm.



**Figure 1:** Sucrose was measured on 50 mg of lyophilized material (+2 leaves) powder extracted with MCW (methanol, chloroform and water in the share of 12:5:3 (v/v)) at 25 °C for 30 min, and centrifuged at 10,000 g for 10 min at 20 °C. The residue used to be re-extracted and the supernatants combined. Sucrose quantification used to be carried out at 620 nm through the anthrone approach of Van Handel (1968), using sucrose as standard.

### Statistical Analysis for Experiment

Statistical analysis used to be performed via the statistical software by Muhammad Ishfaq, Fatima tu Zahra, Furqan and Momina Batool. The information have been submitted to variance evaluation with the aid of the F check and the good sized differences between the redress were in contrast through the Tukey take a look at 5% probability.

### Result and Discussion

The preliminary test showed that boom of sugarcane plants for 30 days in saline soil ranging up to a hundred and sixty mM NaCl had little effect on shoot biomass of SP 81–3250, but the biomass of IAC 87–3396 at eighty mM and a hundred and sixty mM NaCl used to be notably reduced. Consistent with this, the photosynthetic price of SP 81–3250 confirmed no discount with growing salinity in distinction to IAC 87–3396 which used to be decreased at eighty and one hundred 60 mM NaCl. The sample of stomatal conductance was once similar to that for photo synthesis. This is constant with the absence of a statistically giant exchange in Ci (intracellular CO<sub>2</sub>) underneath salinity. Leaf Na<sup>+</sup> concentrations had been greater in IAC 87–3396 than SP 81–3250, especially at a hundred and sixty mM NaCl.

Total chlorophyll (SPAD)								
NaCl (mM)	15 d	40	80	160	30 d	40	80	160
SP 81-3250	42.9Aa	42.5 Aa	41.3 Aa	39.2 Aa	43.4 Aa	44.3 Aa	43.4 Aa	38.5 Aa
IAC 87-3396	40.1 Aa	39.0Aa	38.0Aa	35.0Aa	40.9Aa	39.0Aa	41.0Aa	35.0Aa
Fv/Fm								
NaCl (mM)	15d	40	80	160	30d	40	80	160
SP 81-3250	0	0.79Aa	0.78 Aa	0.77 Aa	0	0.77 Aa	0.78 Aa	0.77 Aa
IAC 87-3396	0	0.78 Aa	0.78 Aa	0.79Aa	0	0.79Aa	0.80Aa	0.79Aa
Water potential (-MPa)								
NaCl (mM)	15d	40	80	160	30d	40	80	160
SP 81-3250	0	0.17 Aa	0.26 Aa	0.31 Aa	0	0.16 Aa	0.23 Aa	0.26 Aa
IAC 87-3396	0	0.10Aa	0.21 Aa	0.25 Aa	0	0.09Aa	0.17 Aa	0.21 Aa

**Figure 2:** These observations had been followed up in the subsequent principal scan when plants have been harvested at both 15 and 30 days after salt treatment.

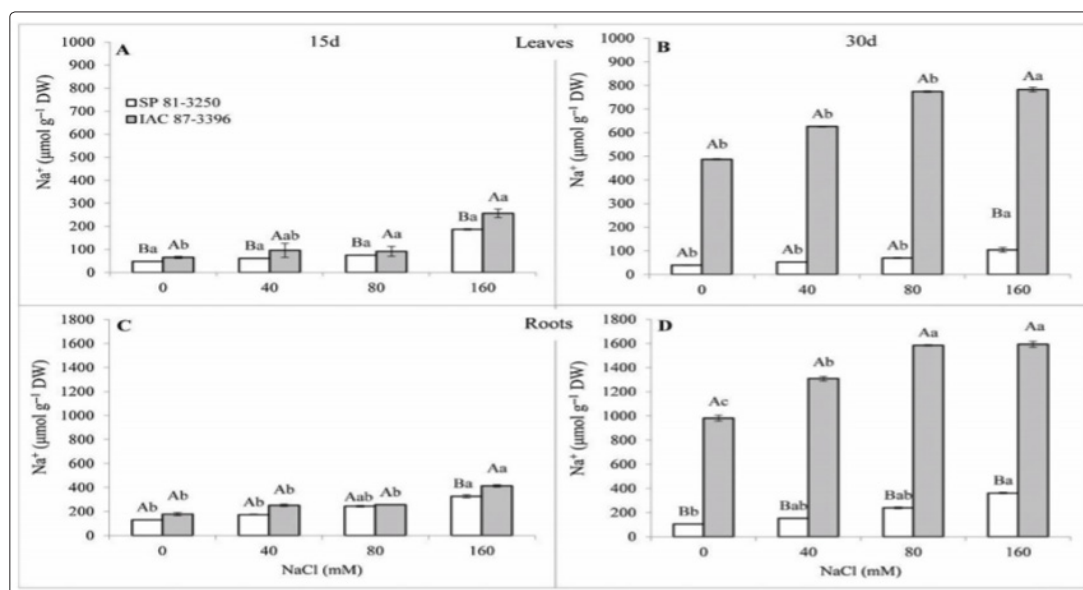


Differences between cultivars in response to salinity were visually obvious at 30 days however not at 15 days. Ion and metabolite concentrations were measured in roots and shoots, as properly as physiological parameters of leaves that would indicate ionic versus osmotic consequences on growth.

Photosynthetic response of plant life to salinity is complex, in view that it relies upon on the salt concentration in soil properly as the length of stress, so the symptoms and consequences will vary thus. The flora of cv. IAC 87–3396 confirmed a marked reduction in stomatal conductance, foliar vicinity and photosynthesis according to the severity of stress. Stomatal conductance reduction and the consequent lower CO<sub>2</sub> assimilation potential of photosynthetic apparatus is uncovered to excessive radiation, producing reactive oxygen species which may provide an explanation for the higher lipid per oxidation that happened in cv. IAC 87–3396. In low salinity (0 and forty mM NaCl) the photosynthetic price of cv. IAC 87–3396 used to be greater than SP 81–3250. However, in greater salinity concentrations (80 and a hundred and sixty mM NaCl), the plant life of this cultivar introduced a marked reduction of photosynthetic rate, in contrast to cv. SP 81–3250 which maintained similar prices in all treatments. Chlorophyll content material and quantum yield of photo system II (Fv/Fm) have been not notably altered via salinity in either cultivar, displaying that gasoline change was a extra sensitive indicator of the consequences of salinity on photosynthesis than was chlorophyll fluorescence. Salinity reduced the shoot dry mass, height, and leaf vicinity of cv. IAC 87–3396 with little or no effect on cv. SP 81–3250, indicating that the decrease productivity observed in cv. IAC 87–3396 is due to an aggregate of the lower photosynthetic vicinity as nicely as the lower charge of photosynthesis. The results of salinity are oftentimes evident in leaf place reduction, extensively impairing net assimilation and plant productiveness.

## Conclusion

The sugarcane cultivars showed excellent differences in boom response to salinity. It is in all likelihood that the greater Na<sup>+</sup> accumulation with the aid of cv. IAC 87–3396 triggered the greater minimize of productivity. Calculations showed that leaf Na<sup>+</sup> concentrations reached about 275 mM in plants grown at eighty and one hundred sixty mM in this cultivar whilst being solely 35 in cv. SP 81–3250.



**Figure 3:** Genetic variant in salt tolerance is essential for sustainable sugarcane manufacturing on saline soils as salt in the soil is difficult to remove, and the desire of salt-tolerant vegetation is the fine answer for each specific environment.

$C_i$ (intracellular CO <sub>2</sub> ) (μmol mol <sup>-1</sup> )				
NaCl (mM)	0	40	80	160
SP 81-3250	194 Aa	186 Aa	197 Aa	207 Aa
IAC 87-3396	186 Aa	177 Aa	186 Aa	198 Aa
$E$ (transpiration) (mmol m <sup>-2</sup> s <sup>-1</sup> )				
NaCl (mM)	0	40	80	160
SP 81-3250	1.65 Ba	1.66 Ba	1.97 Aa	1.49 Aa
IAC 87-3396	2.35 Aa	2.31 Aa	2.08 Aa	1.48 Aa

**Figure 4:** Our work printed biomarkers in younger sugarcane flowers for resolution for tolerance of saline conditions. These include the reduction of leaf location and gasoline change with consequent reduction in shoot dry count as vital factors to demonstrate relative sensitivity of cv. IAC 87–3396.



**Figure 5:** The lower Na<sup>+</sup> accumulation and higher Proline accumulation of cv. SP 81–3250 point out mechanisms allowing this cultivar to tolerate salt stress. Measurements of leaf Na<sup>+</sup> concentration would be a possible decision tool to identify salt-tolerant germplasm in breeding program.

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