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Salinity induced changes in esterase, peroxidase and alcohol dehydrogenase isozymes and leaf soluble proteins in salinity susceptible and salinity tolerant sugarcane genotypes

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ABSTRACT

The salinity susceptible CoC-671 and salinity tolerant sugarcane genotype CoM-265 were evaluated for Peroxidase (POX), Esterase (EST) and Alcohol Dehydrogenase (ADH) isozymes and soluble protein profiling by SDS and native-PAGE at salinity levels 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dSm^{-1}, and 8.01 dSm^{-1} maintained by NaCl solution. The plant height, number of leaves and seedling diameter got reduced in salinity susceptible sugarcane genotype CoC-671 as well as salinity tolerant sugarcane genotype CoM-265 with increase in salinity levels. However, reduction in plant height, number of leaves and seedling diameter was less in salinity tolerant sugarcane genotype CoM-265 as compared to salinity susceptible sugarcane genotype CoC-671. The POX isozyme profiling revealed that salinity susceptible CoC-671 and salinity tolerant sugarcane genotype CoM-265 had variation in soluble protein band intensity at different salinity levels with relative mobility (Rm) 0.137. The present study could be useful for genetic variability analysis in sugarcane genotypes differing in salinity stress tolerance capability.

1. Introduction

Sugarcane (*Saccharum officinarum* L.) plays an important role in world economy. Abiotic stresses such as drought, salinity, extreme temperatures (low and high), chemical toxicity and oxidative stress are serious threats to agriculture and major limiting factors for sugarcane productivity [1]. In the hot and dry regions, the soils are becoming saline with low agricultural potential with inadequate irrigation management practices that results salinization [2]. Soil salinity occurrence may be due to poor water management, high evaporation, heavy irrigation and pre-exposure to sea water at some extent [3]. Salinity stress alters various morphological, physiological and biochemical processes depending on severity and duration of the stress and ultimately adversaly impact sugarcane productivity [4,5]. The salinity tolerance limits of sugarcane ranges from 1.7 to 2.3 dSm^{-1} [6] . Sugarcane is considered highly sensitive to salinity and it has been reported earlier that EC of soil greater than 1.7 dS^{m-1} at critical growth stages significantly reduces cane length, girth, and ultimately yield [7]. Every unit increase beyond 1.7 dS m^{-1} limits 5.9 % yield in sugarcane [3]. Whereas EC > 8 dS m−1 caused significant reduction in growth and physiological traits and ultimately negatively impacted yield by 50 % [8–9]. In sugarcane, salinity at critical stages particularly at formative phase leads to physiological disorders due to increase of toxic salts in the root zone, reducing the osmotic potential of the soil and water uptake [10,11], which consequently hampers normal physiology and entire metabolic processes at cellular levels [12–13]. Dhansu et al. [4] evaluated plant growth and survival rate of sugarcance genotypes Co 0118, Co 05011, Co 12029, Co 15027 and Co 09022 under salinity stress conditions. He found that Co 0118 and Co 05011 showed >33.3 % survival, whereas Co 12029 and Co 15027 showed only 5 % survival and Co 09022 did not survive at 12 dSm^{-1} salinity level. The effects of salinity stress was characterized by stunted and slow growth, leaf area reduction and decrease in physiological traits particularly gas exchange attributes along with water status (RWC).

Preet et al. [5] determined salinity tolerance potential in sugarcane genotypes Co 13035, Co 0118, and Co 0238 based on

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morpho-physiological, biochemical, and yield traits. He found that plant height, leaf area, stem diameter, number of internodes, and internodal length were negatively impacted at 4, 8 and 10 dS/m salinity levels. Genotypes Co 13035 and Co 0118 maintained higher plant water status. Proline concentration increased in Co 0238 and Co 13035. The genotypes that were most resistant to salinity stress were Co 13035, Co 0238, and Co 0118, which had low Na+/K+ ratio [5] . Preet et al. [5] observed that genotype Co 13035 had highest survival rate, low Na+/K+, maintained higher water content and osmolyte accumulation, better chlorophyll content. Salinity also causes accumulation of Na^+ and Cl^- and inhibition of mineral nutrients uptake and ultimately causes ionic imbalance. Accumulation of soluble proteins in cultured plant cells occured when subjected to salinity stress [14–16]. The levels of protein differ in salinity tolerant and sensitive genotypes under salinity stress and salinity tolerant genotype revealed extra protein bands, while it was absent in salt sensitive genotype [17]. Protein-based method like SDS PAGE is cost effective, simple and extensively used biochemical technique to determine the protein levels [18]. Furthermore, SDS-PAGE is considered as a reliable technology that can be used to evaluate and characterize salinity affected and unaffected plants [19]. Salinity tolerant plants have lower rate of Na^+ and Cl^- transport to leaves as compared to salinity sensitive plants and these ions are compartmentalized into vacuoles to prevent salt toxicity [20].

Isozymes and soluble protein banding pattern and their intensity are considered critical for biochemical molecular markers [21]. Electrophoretic protein analysis technique has potential in characterizing sugarcane genotypes for genetic variability [22]. Biochemical markers such as isozymes have been used to distinguish homozygous and heterozygous lines and to estimate the level of genetic variability in plant population in several crops [14–16]. The isozyme analysis has been used in evolutionary systematic studies and allows the genomic study in certain species [23]. In the present study, we comprehensively evaluated sugarcane genotypes CoM-265 (salt tolerant), and CoC-671 (salt sensitive) for their salinity tolerance ability using peroxidase (POX), esterase (EST) and alcohol dehydrogenase (ADH) isozymes and soluble protein profiling in leaves at different salinity levels, i.e., 0.41, 2.31, 4.21 and 8.01 dSm^{-1}. Present study revealed that the isozyme profiling patterns of POX, EST and ADH got altered in sugarcane leaf under salinity conditions. The differential levels of isozymes was correalated with salt tolerance or salt susceptibility in sugarcane genotypes. Some isoforms of isozymes were increased significantly as salinity levels increased. This increase was prominent in salt-tolerant as compared to salt-sensitive sugarcane genotype. Reclamation of saline soils is tedious due to continuous expansion of saline area and also due to scarcity of good quality water. Considering these facts, identification of salinity tolerant sugarcane varieties would greatly help to reduce the loss in cane productivity under saline soils. Present study would provide the genetic basis to characterize and identify the sugarcane genotypes suitable for growing in saline soil.

2. Materials and methods

2.1. Planting materials

The salinity susceptible CoC-671 and salinity tolerant sugarcane genotype CoM-265 were used for isozyme and protein profiling in order to evaluate salinity tolerance ability of sugarcane genotypes at varying salinity levels. Sugarcane genotypes used in the present study are depicted in Table 1.

Table 1
Sugarcane genotypes used in the present study.

Sr. No	Parameters	Sugarcane genotypes	
		CoM- 265 (salinity tolerant genotype)	CoC- 671 (Salinity susceptible genotype)
1.	Maturity group	Mid-late	Early
2.	Pedigree	Co- 87044 GC	Q 63 x Co- 775
4.	Cane Yield (t/ha)	199.80	135.00
5.	CCS (t/ha)	26.68	17.50
6.	Sucrose (%)	19.33	19.50
7.	Sugar recovery (%)	13.41	14.20
8.	Special characters	Matures in 12–16 months, tolerant to drought and salinity, sugar recovery not affected even after late harvesting	Matures in 9–10 months, high sugar content, gives higher ratoon yield (265 ton/ha).

Plant salt tolerance can be analyzed based on the relative plant growth rate and the plant survival rate after exposure to a range of salt concentrations or after a treatment with a defined salt concentration. In the present investigation, four salinity levels, i.e., 0.41, 2.31, 4.21 and 8.01 dSm^{-1} attained by NaCl solution, were used. Fifty gram of soil was measured and 100 mL distilled water was added into it and stirred at regular interval for 1 h. The content was allowed to settle for 30 min and electrical conductivity was measured by dipping electrode into the supernatant and the procedure repeated twice to confirm the EC. The standard NaCl stock solution of 32 dSm^{-1} and 62 dSm^{-1} was used to optimize desired EC. Two eye bud sets of salinity susceptible CoC-671 and salinity tolerant genotype CoM-265 were planted and grown in pots (15 × 15 cm) containing 5 kg of soil and salinity treatment was imposed 60 days after planting. Third leaf samples of salinity susceptible CoC-671 as well as salinity tolerant sugarcane genotype CoM-265 were collected under no salinity stress as well as at different salinity levels, i.e., 0.41, 2.31, 4.21 and 8.01 dSm^{-1} after 75 days of planting.

2.2. Levels of salinity in soil, growing condition and morphological analysis of sugarcane plants under different salinity levels

Soil was maintained at salinity levels of 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dS m^{-1}, and 8.01 dS m^{-1} with NaCl solution. The two eye bud sets of sugarcane genotype CoC-671 (salinity susceptible) and CoM-265 (salinity tolerant) were planted in pots containing 5 kg soil (15 × 15 cm) and allowed to grow upto 60 days. After 60 days of planting salinity treatment was imposed and maintained for next 15 days. The plant height, number of leaves per plant and seedling diameter was measured 75 days after planting.

2.3. Leaf soluble proteins profiling by SDS PAGE and native PAGE

Extraction of protein from leaves was carried out by using 0.5 M Tris-HCl buffer. Soluble proteins in the leaves were determined by the colorimetric method using bovine serum albumin as a standard protein by Lowry et al. [24]. The soluble proteins were resolved by SDS and native PAGE. The band intensity was assessed as faint or intense bands and as absence or presence of specific bands. The relative mobility (Rm) of resolved protein bands were determined using the following formula:

$$Rm = \frac{\text{Distance migrated by protein band (cm)}}{\text{Distance migrated by tracking dye from the top of the separation gel (cm)}}$$

2.4. Peroxidase, esterase and alcohol dehydrogenase isozyme profiling

The peroxidase (POX), esterase (EST) and alcohol dehydrogenase (ADH) isozymes were extracted from the third leaf of salinity susceptible and tolerant sugarcane genotypes and estimated by Lowry et al. [24]. The peroxidase (POX), esterase (EST) and alcohol dehydrogenase (ADH) isozymes were resolved using polyacrylamide gel electrophoresis.

After electrophoresis, the peroxidase isozyme PAGE gel was immediately transferred into staining solution for a period of 60 min. Staining solution for peroxidase isozyme PAGE gel was prepared by dissolving 20 mg 3-amino-9-ethyl carbazole, 2.5 mL N, N-diethyl-formamide, 5 mL acetate buffer (1 M, pH 4.65), 1 mL of 0.1 M $CaCl_2$ solution in 50 mL distilled water. Just before use, 1 mL 0.7 % H_2O_2 solution was added and incubated for 60 min as described by Glaszmann et al. [25]. When the bands were stained sufficiently, the reaction was arrested by immersing the gel into 7 % acetic acid solution for 10 min and the bands in gel were visualized and photographed immediately using gel documentation system.

After electrophoresis, the esterase isozyme PAGE gel was immediately pre-soaked in 0.2 M Tris-HCl buffer, pH 7.1 for 10–15 min and then transferred into staining solution for a period of 30 min until the clear bands appeared. Staining solution for esterase isozyme PAGE gel was prepared by dissolving 2.8 g sodium dihydrogen phosphate, 1.1 g disodium hydrogen phosphate, 0.2 g fast blue RR salt and 0.03 g alpha-naphthyl acetate in distilled water and final volume was adjusted to 200 ml. The PAGE gels were fixed with 7 % acetic acid solution for 30 min and then gel was washed with distilled water and photographed immediately using gel documentation system.

After electrophoresis, the PAGE gel of alcohol dehydrogenase isozyme was immediately transferred into staining solution for a period of 30 min. The staining solution for ADH isozyme was prepared by mixing 0.25 mL ethanol, 5 mL 0.5 M Tris-HCl buffer, pH 8.5, 1 mL β-nicotinamide adenine dinucleotide (10 mg/mL) and the volume was adjusted to 50 mL. Just before use add nitroblue tetrazolium salt (10 mg/mL) and 1 mL phenazine methosulfate (1 mg/mL) was added and care was taken to avoid light exposure and incubated for 30 min at 40°C. The stained PAGE gel of ADH were visualized and photographed using gel documentation system.

2.5. Statistical analysis

The experiment was conducted three times. CD values were used to ascertain the significance among treatments. The plants were allowed to grow until they reached the vegetative stage, 60 days after germination and were then exposed to NaCl salinity levels of 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dSm^{-1}, and 8.01 dSm^{-1} for another 15 days (75 days after emergence of seedlings).

3. Results

3.1. Induction of different salinity levels and effect of induced salinity on plant height, number of leaves and seedling diameter

Two sugarcane genotypes *viz.*, salinity susceptible CoC-671 and salinity tolerant CoM-265 were exposed to salinity levels of 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dSm^{-1}, and 8.01 dSm^{-1} in order to study the morphology in terms of plant height, number of leaves and seedling diameter, sixty days after planting and salinity was maintained for next 15 days. The effect of induced salinity on plant height, number of leaves and seedling diameter were determined 75 days after planting (Fig. 1, Tables 2–4). Under no salinity stress, the vegetative growth pattern was almost similar in salinity tolerant sugarcane genotype CoM-265 and salinity susceptible sugarcane genotype CoC-671 (Fig. 1).

Table 2
Effect of different salinity levels on plant height in salinity susceptible genotype CoC-671 and salinity tolerant genotype CoM-265.

Sr. No.	Salinity levels (dSm^{-1})	Plant Height (cm)	
		CoC-671 (Salinity susceptible)	CoM-265 (Salinity tolerant)
1	0.41	107.67 ± 0.9	113.33 ± 1.3
2	2.31	103.00 ± 0.8 (4.4 %)	110.67 ± 1.1 (2.3 %)
3	4.21	94.00 ± 0.5 (12.7 %)	106.00 ± 0.8 (6.5 %)
4	8.01	88.33 ± 1.2 (18.1 %)	102.00 ± 0.9 (10 %)
LSD < 0.05		**1.5**	**2.1**

Mean ± SE. Experiments were repeated three times. n= 3. The LSD values at $P<$ 0.05 were applied to compare the significant differences among the mean values for plant height. Values in parenthesis indicate percent decrease over control.

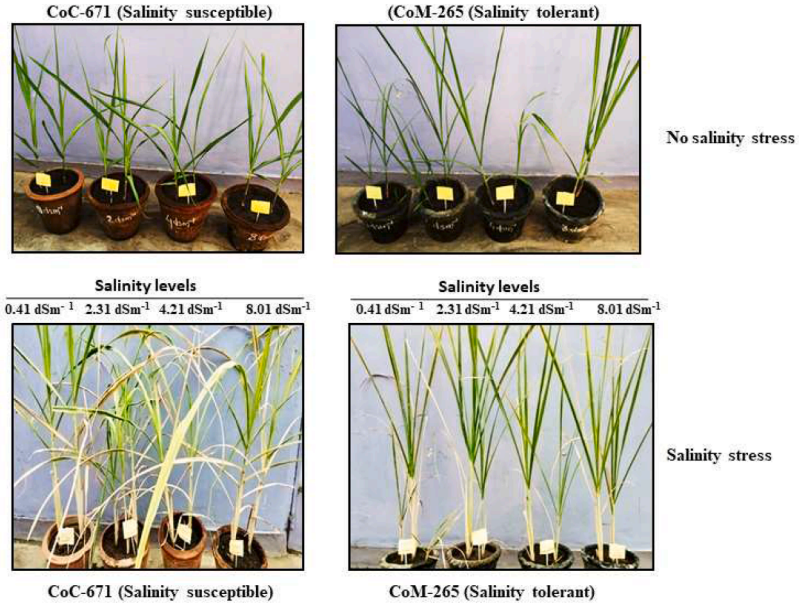

Fig. 1. Effect of salinity on salinity susceptible CoC-671 and salinity tolerant CoM-265 sugarcane genotypes.

Table 3
Effect of different salinity levels on number of Leaves per plant in salinity susceptible genotype CoC-671 and salinity tolerant genotype CoM-265.

Sr. No	Salt Stress (dSm^{-1})	Number of leaves per plant	
		CoC-671 (Salinity susceptible)	CoM-265 (Salinity tolerant)
1	0.41	7 ± 0.6	8 ± 0.7
2	2.31	7 ± 0.4	8 ± 0.5
3	4.21	5 ± 0.5	7 ± 0.6
4	8.01	5 ± 0.6	6 ± 0.4
LSD < 0.05		**1.4**	**1.5**

Mean ± SE. Experiments were repeated three times. n= 3. The LSD values at $P<$ 0.05 were applied to compare the significant differences among the mean values for number of leaves per plant.

Table 4
Effect of different salinity levels on seedling diameter in salinity susceptible genotype CoC-671 and salinity tolerant genotype CoM-265.

Sr. No	Salt Stress (dSm^{-1})	Seedling diameter (cm)	
		CoC-671 (Salinity susceptible)	CoM-265 (Salinity tolerant)
1	0.41	1.23 ± 0.10	1.37 ± 0.20
2	2.31	1.07 ± 0.09 (13.1 %)	1.30 ± 0.15 (5.1 %)
3	4.21	0.93 ± 0.08 (24.4 %)	1.07 ± 0.11 (11.9 %)
4	8.01	0.67 ± 0.07 (55.5 %)	0.73 ± 0.09 (46.7 %)
LSD < 0.05		**0.15**	**0.20**

Mean ± SE. Experiments were repeated three times. n= 3. The LSD values at $P<$ 0.05 were applied to compare the significant differences among the mean values for seedling diameter.

3.2. Effect of salinity on plant height

The plant height of CoM-265 salinity tolerant sugarcane genotype and susceptible sugarcane genotype CoC-671 at all salinity levels, i.e., 0.41. 2.31, 4.21 and 8.01 dSm^{-1} have been mentioned in Table 2. In salinity tolerant genotype CoM-265 at 0.41 dSm^{-1} salinity level, the plant height was 113.33 cm, while at 2.31 dSm^{-1}, 4.21 $dSm^{-1}$and 8.01 dSm^{-1}salinity levels, the plant heights were 110.67 cm, 106 cm and 102 cm, respectively. However, in salinity susceptible genotype CoC-671 at 0.41 dSm^{-1}, 2.31 dSm^{-1} at 4.21 $dSm^{-1}$and at 8.01 dSm^{-}1, the plant heights were 107.67 cm, 103 cm, 94 cm and 88.33 cm, respectively. In the present study, reduction in plant height was observed with increasing salinity levels, while more decline in plant height was observed at 8.01 dSm^{-}1 salinity level.

3.3. Effect of induced salinity on number of leaves

The data mentioned in Table 3 revealed effect of different salinity levels on number of leaves in two sugarcane varieties *viz.*, salinity susceptible CoC-671 and salinity tolerant CoM-265. The maximum number of leaf (8 leaves per plant) was recorded in CoM-265 salinity tolerant sugarcane genotypes as compared to CoC-671 salinity susceptible genotype (7 leaves per plant) at 0.41 and 2.31 dSm^{-1} salinity level. At salinity levels 4.21 and 8.01 dSm^{-1}, 5 leaves per plant observed in salinity susceptible genotype (Co-C671) as compared to salinity tolerant genotype CoM-265, 7 and 6 leaves per plant at 4.21 and 8.01 dSm^{-1} salinity levels, respectively (Table 3). From current study, it was observed that number of leaves got reduced as salinity levels increased, while more decline in leaves per plant was observed at 8.01 dSm^{-1} salinity levels.

3.4. Effect of induced salinity stress on seedling diameter

The data given in Table 4 showed the effect of salinity on seedling diameter in two sugarcane genotypes, *viz.*, salinity susceptible CoC-671 and salinity tolerant CoM-265. Slightly higher seedling diameter was recorded in salinity tolerant sugarcane genotype CoM-265 as compared to salinity susceptible CoC-671 at all salt stress levels, i.e., 0.41. 2.31, 4.21 and 8.01 dSm^{-1} (Table 4). In CoM-265 salinity tolerant genotype, the seedling diameter were 1.37, 1.30, 1.07 and 0.73 cm at salinity levels 0.41, 2.31, 4.21 and 8.01 dSm^{-1}, respectively. On the other hand, in salinity susceptible sugarcane genotype CoC-671, the seedling diameter were 1.23, 1.07, 0.93 and 0.67 cm, respectively. In the present study, it was observed that seedling diameter was reduced with increasing salinity levels, and more declines in seedling diameter was observed at 8.01 dSm^{-1} salinity level in both salinity susceptible and tolerant sugarcane genotypes. After exposure of plant under salinity condition, morphological changes were observed like poor growth and chlrosis in leaves. The intensity of the chlrosis of leaves increased with increase in salinity levels. While, under control condition, proper growth was observed and plants were found to be healthy and green. Between the two genotypes CoC-671 (salinity susceptible) was found to have more chlrosis in leaves than CoM-265 (salinity tolerant) sugarcane genotype under salinity conditions. Salt susceptible genotypes showed effects like chlorosis of leaves at 4.21 and 8.01 dSm^{-1} whereas, salt tolerant genotypes showed only at 8.01 dSm^{-1} salinity levels. The susceptible genotypes showed chlorosis earlier than salinity tolerant genotype. As the salinity levels increase, the height of plant decreases, while at 8.01 dSm^{-1} the height, number of leaves and stem diameter of plants was drastically reduced in CoC-671 (salinity susceptible)

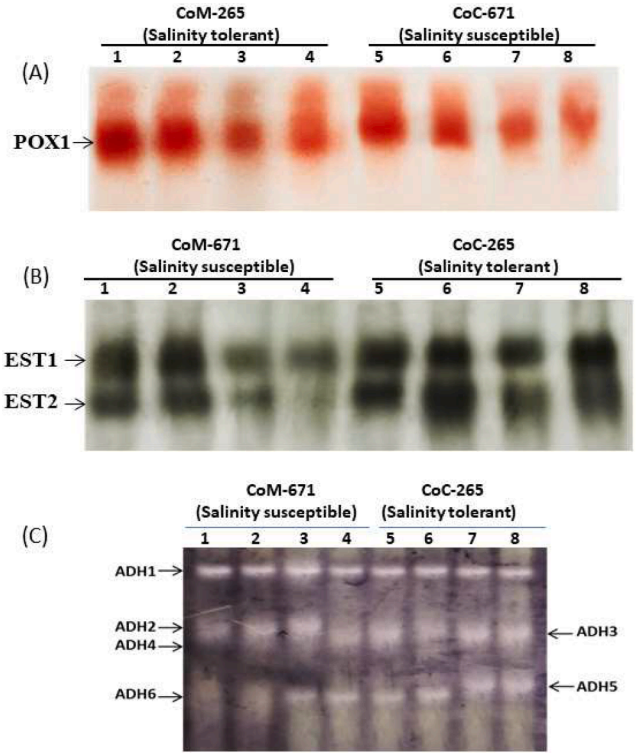

Fig. 2. Zymogram of isozymes. **A.** Zymogram of peroxidase isozyme (POX) extracted from leaves of sugarcane genotypes CoC-671 (salinity susceptible) and CoM-265 (salinity tolerant) grown under different salinity levels. Lane 1-4 and 5-8 represent 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dSm^{-1}, and 8.01 dSm^{-1} salinity levels, respectively. **B.** Zymogram of Esterase isozyme (EST) extracted from leaves of sugarcane genotypes CoC-671 (salinity susceptible) and CoM-265 (Salinity tolerant) grown under different salinity levels. Lane 1-4 and 5-8 represent 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dSm^{-1}, and 8.01 dSm^{-1} salinity levels, respectively. **C.** Zymogram of Alcohol dehydrogenase isozyme (ADH) extracted from leaves of sugarcane genotypes CoC-671 (salinity susceptible) and CoM-265 (salinity tolerant) grown under different salinity levels. Lane 1-4 and 5-8 represent 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dSm^{-1}, and 8.01 dSm^{-1} salinity levels, respectively.

Table 5
Peroxidase isozyme profile of sugarcane leaves in response to imposed salinity stress condition.

Band No.	Genotypes								Rm value
	CoC-671 (Salinity tolerant)				CoM-265 (Salinity susceptible)				
	Salinity levels (dSm^{-1})				Salinity levels (dSm^{-1})				
	0.41	2.31	4.21	8.01	0.41	2.31	4.21	8.01	
POX 1	+++	+++	++	++	+++	++	+	+	0.137
Total band	1	1	1	1	1	1	1	1	

(+++ Intense, ++ Moderately intense, + Faint).

sugarcane genotype as compared to salinity tolerant genotype CoM-265.

3.5. Effect of different NaCl salinity levels on peroxidase isozyme profile

The peroxidase isozyme (POX) was extracted from the leaves of CoM-265 (salinity tolerant) and CoC-671 (salinity tolerant) sugarcane genotypes exposed at 0.41, 2.31, 4.21 and 8.01 dSm^{-1} salinity levels. The salinity condition caused quantitative changes in POX isozyme profile (Fig. 2A, Table 5,). Band expression obtained from SDS-PAGE revealed that CoM-265 and CoC-671 showed the same number of isoforms, but the band intensity was different. Peroxidase enzyme band was more intense in salinity tolerant CoM-265 genotype as compared to salinity susceptible CoC-671 genotype and it was observed that band intensity decreased with increasing salinity levels. However, decrease is POX isozyme level was less in salinity tolerant CoM-265 with increase in salinity levels as compared to salinity susceptible CoC-671 geneotype. The POX isozyme pattern was observed in both the genotypes, salt susceptible (CoC- 671) and salt tolerant (CoM-265) at different salinity level with relative mobility (Rm) value 0.137. Less intense POX band was observed in salinity susceptible CoC-671 genotype at 4.21 and 8.01 dSm^{1} salinity level, while moderately intense POX band was observed at 0.41 and 2.31 dSm^{1} saliniy levels. Intense POX band was observed in salinity tolerant CoM-265 genotype under different salinity level, except 8.01 dSm^{-1} in which POX band was appeared moderately intense band.

3.6. Effect of different NaCl salinity levels on esterase isozyme profile

The esterase isozyme was extracted from the leaves of CoC-671 and CoM-265 sugarcane genotypes exposed at different salinity levels, i.e., 0.41, 2.31, 4.21 and 8.01 dSm^{-1}. The salinity condition caused the quantitative changes in esterase isozyme profile (Fig. 2B, Table 6). Electrophoretic profile of esterase (E) isozyme showed two types of bands with Rm value 0.152 (EST1) and 0.261 (EST2). More intense EST1 and EST2 bands were detected in salinity tolerant CoM-265 genotype as compared to CoC-671 a salt susceptible genotype (Fig 2B, Table 6).

3.7. Effect of different NaCl salinity levels on alcohol dehydrogenase isozyme profile

The alcohol dehydrogenase was extracted from the leaves of CoM-265 (salinity tolerant) and salinity susceptible CoC-671 sugarcane genotypes exposed at 0.41, 2.31, 4.21 and 8.01 dSm^{-1} salinity levels. The effect of salinity on alcohol dehydrogenase isozyme (Fig. 2C, Table 7). Alcohol dehydrogenase (ADH) isozyme system showed six isozymes patterns *viz*., ADH1, ADH2, ADH3, ADH4, ADH5 and ADH 6 with different bands intensity and Rm value. A high intense band ADH1 was observed in salt susceptible (CoC-671) and salt tolerant (CoM-265) genotypes of sugarcane at different salinity levels with relative mobility (Rm) value 0.189. The faint band of ADH 2 with relative mobility 0.377 was observed in salt susceptible CoC-671 genotype at 0.41, 2.31 and 4.21 dSm^{-1} salinity level except at 8.01 dSm^{-1}. However, ADH 2 remained absent in the salt tolerant CoM-265 genotype. ADH 3 as a faint band was present in salt tolerant CoM-265 genotype at 0.41, 2.31, 4.21 and 8.01 dSm^{-1} salinity level with a relative mobility 0.382, however remained absent in CoC-671 salinity susceptible genotype at all salinity levels. ADH 4 as faint and fast migrating band was observed at 8.01 dSm^{-1} salinity level in salinity susceptible CoC-671 genotype and not appeared in other stress levels in CoC-671 a susceptible genotype and all salinity levels in CoM-265 salt tolerant variety. ADH5 as a faint band was observed in salinity tolerant CoM-265 variety at 4.21 and 8.01 dSm^{-1}salinity level with migration distance 0.452. It was not observed in CoC-671 a salt sensitive genotype at all the salinity levels and in salt tolerant CoM-265 genotype at 0.41 and 2.31 dSm^{-1} salinity level. ADH6 a fast migrating band having relative mobility 0.49 was detected at all salinity levels in salinity susceptible CoC-671 genotype as a faint band and in salinity tolerant CoM-265 genotype at 0.41 and 2.31 dSm^{-1} salinity levels. These results suggested a positive correlation between ADH activity and genetic variation in salt tolerance in sugarcane genotypes CoM-265 and CoC-671.

3.8. Effect of different NaCl salinity levels on soluble protein content

Results obtained for soluble protein content is depicted in Table 8. The total soluble protein content ranged from 30 to 36.37 mg g^{-1}FW in salinity tolerant genotypes and 24 to 34 mg g^{-1}FW in salinity susceptible genotype. In susceptible sugarcane genotype CoC-671 less soluble protein content was found as compared to CoM-265 at 0.41, 2.31, 4.21 and 8.01 dSm^{-1} NaCl salinity levels. In salinity tolerant sugarcane genotypes CoM-265, the soluble protein content was 36.67, 35, 32.67 and 30 mg g^{-1}FW at 0.41, 2.31, 4.21 and 8.01 dSm^{-1} salinity levels, respectively. In salinity susceptible sugarcane genotype CoC-671, soluble protein content was 34, 31.67, 28, 24 mg g^{-1}FW, respectively at 0.41, 2.31, 4.21 and 8.01 dSm^{-1} salinity levels (Table 8). It was observed that soluble protein content got reduced with increasing salinity levels in salinity susceptible

Table 6
Esterase isozyme profile of sugarcane leaves in response to imposed salinity stress condition.

Band No.	Genotypes								Rm value
	CoC-671 (Salinity Susceptible)				CoM-265 (Salinity Tolerant)				
	Salinity levels (dSm^{-1})				Salinity levels (dSm^{-1})				
	0.41	2.31	4.21	8.01	0.41	2.31	4.21	8.01	
EST 1	++	++	+	+	+++	+++	++	+++	0.152
EST 2	++	++	+	–	++	+++	+	++	0.261
Total bands	2	2	2	1	2	2	2	2	

(+++ Intense, ++ Moderately intense, + Faint, - Absent).

Table 7
Alcohol dehydrogenase isozyme profile of sugarcane leaves in response to imposed salinity stress condition.

Band No.	Genotypes								Rm value
	CoC-671 (Salinity Susceptible)				CoM-265 (Salinity Tolerant)				
	Salinity levels (dSm^{-1})				Salinity levels (dSm^{-1})				
	0.41	2.31	4.21	8.01	0.41	2.31	4.21	8.01	
ADH 1	+++	+++	+++	+++	+++	+++	+++	+++	0.189
ADH 2	+	+	++	–	–	–	–	–	0.377
ADH 3	–	–	–	–	+	+	++	+	0.382
ADH 4	–	–	–	+	–	–	–	–	0.389
ADH 5	–	–	–	–	–	–	+	+	0.452
ADH 6	+	+	++	++	++	++	–	–	0.490
Total bands	3	3	3	3	3	3	3	3	

(+++ Intense, ++ Moderately intense, + Faint, - Absent).

Table 8
Soluble proteins in sugarcane leaves under different salinity levels in salinity susceptible genotype CoC-671 and salinity tolerant genotype CoM-265.

Sr. No.	Salinity levels (dSm^{-1})	Soluble protein (mg g^{-1} FW)	
		CoC-671 (Salinity susceptible)	CoM-265 (Salinity tolerant)
1	0.41	34.00 ± 1.77	36.67 ± 1.91
2	2.31	31.67 ± 1.64 (8.83 %)	35.00 ± 1.82 (4.55 %)
3	4.21	28.00 ± 1.55 (17.65 %)	32.67 ± 1.75 (12.2 %)
4	8.01	24.00 ± 1.65 (29.42 %)	30.00 ± 1.2 (18.19 %)
LSD < 0.05		1.2	1.5

Mean ± SE. Experiments were repeated three times. n= 3. The LSD values at $P<$ 0.05 were applied to compare the significant differences among the mean values for soluble protein levels.

CoC-671 as well as salinity tolerant sugarcane genotype CoM-265. However, decrease in solouble protein was less in salinity tolerant genotype CoM-265 as compared to salinity susceptible sugarcane genotype CoC-671.

3.9. Effect of different NaCl salinity levels on SDS-PAGE protein profile

The soluble proteins extracted from the leaves of CoM-265 (salinity tolerant) and CoC-671 (salinity susceptible) sugarcane genotypes exposed to different salinity levels were resolved on 12.5 % SDS-PAGE. It was observed 98.65 and 91.95 kD bands were detected only in salinity tolerant genotype CoM-265 at salinity level 2.31 dSm^{-1} and interestingly these bands were absent in salinity susceiptible genotype CoC-671 at salinity levels 0.41, 2.31, 4.21 and 8.01 dSm^{-1} (Fig. 3A). A 85.75 kD protein band was detected in salinity tolerant genotype CoM-265 at 0.41 salinity level, but not present in salinity susceptible genotype CoC-671 (Fig. 3A).

3.10. Effect of different NaCl salinity levels on Native-PAGE protein profiling

The soluble proteins from the leaves of CoM-265 (salinity tolerant)

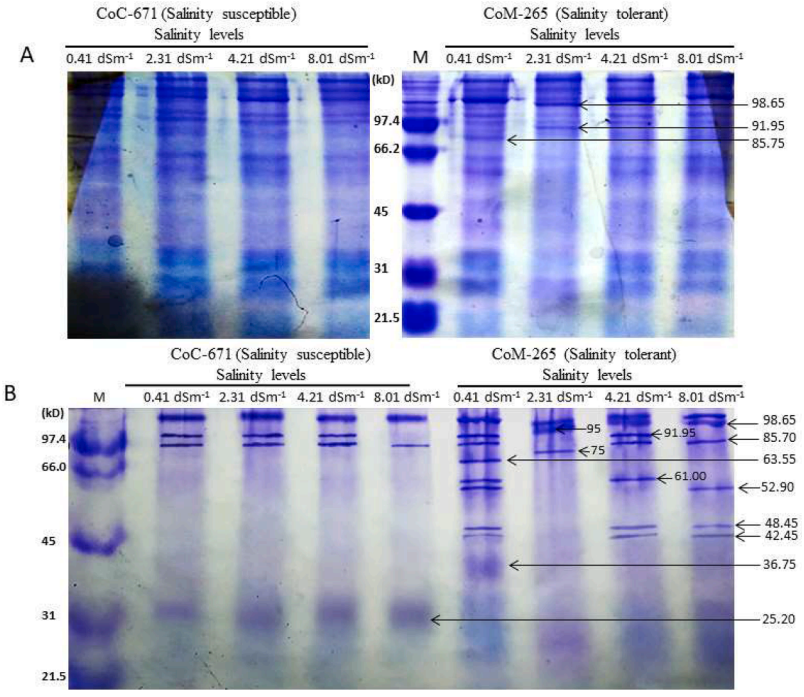

Fig. 3. Soluble protein profiling. **A.** SDS-PAGE protein profiling of sugarcane varieties CoC-671(salinity susceptible) and CoM-265 (salinity tolerant). Left panel and right panel represent profiling of soluble protein extracted from leaves of salinity susceptible sugarcane genotype CoC-671 and salinity tolerant sugarcane genotype CoM-265, respectively grown at salinity levels at 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dSm^{-1}, and 8.01 dSm^{-1}. Low range protein molecular weight marker of 14–97 kD was used to detect size of protein bands **B.** Native-PAGE protein profiling of sugarcane genotypes CoC-671 (salinity susceptible) and CoM-265 (salinity tolerant) grown at salinity levels 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dSm^{-1}, and 8.01 dSm^{-1}. Low range protein molecular weight marker of 14–97 kD was used to detect size of protein bands.

and CoC-671 (salinity susceptible) sugarcane genotypes under varying salinity levels and non-saline conditions were resolved on 7.5 % native-PAGE. It was observed that 98.65 kD band was present in salinity tolerant genotype CoM-265 at salinity levels 0.41, 2.31, 4.21 and 8.01 dSm^{-1}, however, this band was not detected in salinity susceptible CoC-671 genotype. A 95 kD band was present in salinity tolerant genotype CoM-265 at 2.31 dSm^{-1} salinity level, but not detected in salinity susceptible CoC-671 genotype. 91.95 and 61.00 kD bands were detected at salinity level 4.21 dSm^{-1} in salinity tolerant genotype CoM-265, but not present in salinity susceptible genotype CoC-671 (Fig. 3B). A 75 kD band was present at salinity level 2.31 dSm-1 in salinity tolerant genotype CoM-265, but not detected in salinity susceptible genotype CoC-671. 63.55 and 36.75 kD bands were present at salinity level 0.41 dSm^{-1} in salinity tolerant genotype CoM-265, but not detected in salinity susceptible genotype CoC-671 (Fig. 3B). 52.90 kD band was present at salinity levels 0.41 and 8.01 dSm-1 in salinity tolerant genotype CoM-265, but not detected in salinity susceptible genotype CoC-671. 48.48 and 42.45 kD bands were detected at salinity levels 0.41, 4.21 and 8.01 dSm^{-1} in salinity tolerant genotype CoM-265, but not present in salinity susceptible genotype CoC-671.

Interstingly, a 25.20 kD faint band was present in salinity susceptible genotype CoC-671 at salinity levels 0.41, 2.31, 4.21 and 8.01 dSm^{-1} salinity levels, but not present in salinity tolerant genotype CoM-265 (Fig. 3B).

4. Discussion

Sugarcane genotypes CoC-671 (salinity susceptible) and CoM-265 (salinity tolerant) were evaluated for plant height, number of leaves and seedling diameter at salinity levels 0.41 dSm^{-1}, 2.31 dSm^{-1}, 4.21 dS m^{-1}, and 8.01 dS m^{-1} 75 days after planting. There was significant reduction in plant height [88.33 cm $\pm$ 1.2 (18.1 %)], number of leaves per plant (5 $\pm$ 0.6) at 8.01 dSm^{-1} salinity level in salinity susceptible sugarcane genotype CoC-671. However, salinity tolerant genotype CoM-265 showed less reduction in plant height, number of leaves and seedling diameter. Plaut et al. [8] studied the effect of salinity on leaf growth, initiation and senescence and transpiration rate in two sugarcane cultivars H69-8235 and H65-7052 differing in salinity sensitivity. He found that leaf dry weight and area decreased with increasing salinity, however, the decrease was less in salinity tolerant cultivar H69-8235 as compared to salinity sensitive cultivar H65-7052. The average area per leaf was less impacted in salinity tolerant cultivar, H69-8235 under salinity, while in salinity sensitive cultivar H65-7052, the leaf area and initiation of new leaves were sharply reduced by salinity [8]. Khaled and Teixeira da Silva [21] reported decrease in the plant height, number of leaves/plant, and stem diameter when salinity was imposed. Rashed et al. [26] reported decrease in plant height with increase in salinity levels in maize and sorghum, respectively. Salinity resulted decrease in cell numbers in the meristem and a growth inhibition which negatively impacted the leaf development and ultimately reduction in plant height [27]. In the present investigation, the plant height of salinity tolerant sugarcane genotype CoM-265 and susceptible sugarcane genotype CoC-671 was studied at all salinity levels, i.e., 0.41. 2.31, 4.21 and 8.01 dSm-1 and it was found that salinity caused reduction in plant height as mentioned in Table 2. The observation made in the present investigations are in line with observation made by Mohamed [28] who reported altered morphology in plants exposed to 150 mM NaCl. In addition to poor growth of shoots, the leaves became yellow, while under control conditions, no growth retardation was observed. Salt induced reduction in the shoot length caused reduction in growth [29–30]. Santana *et al.* [9] reported that salinity negatively affects crop growth inversely proportional to the salt concentration. Earlier, sugarcane varieties have been evaluated for physio-biochemical parameters along with protein profile under normal and saline conditions [31]. Kasirajan et al. [32] assessed seven *Saccharum spontateum* clones for relative water content.

The POX isozyme pattern was determined in both the salt susceptible (CoC- 671) and salt tolerant (CoM-265) genotypes at different salinity level. The CoM-265 (salinity tolerant) and CoC-671 (salinity susceiptible) genotypes showed the same number of POX isoform at different salinity levels, but the band intensity was different. Low intense band was detected in salinity susceptible sugarcane variety CoC-671 at 4.21 dSm^{-1} and 8.01 dSm^{-1} salinity stress level, while moderately intense band was observed at 0.41 dSm^{-1} and 2.31 dSm^{-1} salinity levels. Intense bands were observed in salinity tolerant CoM-265 genotype at all salinity levels except 8.01 dSm^{-1}, where a moderately intense band was seen. Peroxidase is widely distributed in higher plants and plays crucial role in lignification, auxin metabolism, salt tolerance and heavy metal stress tolerance. Therefore, peroxidase has often served as a parameter of metabolism activity during growth alteration and environmental stress conditions [33]. The expression pattern of these isozymes varies in different tissues of healthy plants and is developmentally regulated or influenced by environmental factors [34]. Mohamed [28] studied peroxidase isozyme pattern in maize in response to induced NaCl stress condition and showed four activity zones among all samples. However, in the present study, only one POX isozyme pattern was observed. In wheat cultivar, three isoforms of POX was detected under salinity stress condition. However, there were no specific increasing or decreasing trend in the level of different isoforms of POX and also the interaction of salinity and wheat genotype for all POX isozymes was not significant [35]. In the present investigation, in salt tolerant CoM-265 genotype of sugarcane under salt stress condition, intense and moderately intense bands were observed indicating that increased in POX isozyme sustained stressed condition. High peroxidase isozymic activity in the tissue of salt stress reflects the changed mechanical properties of the cell wall which, in turn could be related to salt adoption process [28]. Increase in total peroxidase activity in salinity tolerant cultivar indicated involvement of peroxidases in cell membrane integrity [28]. The extent of damage of cells are controlled by the antioxidative systems like peroxidase which is an important defence system of plants against oxygen free radicals [28]. The peroxidase enzyme which exists in both cytosol and chloroplast, can effectively scavenge H_2O_2 which is produced under oxidative stresses [35]. In the present investigation, the intense and moderately intense bands observed in salt tolerant CoM-265 genotype under salinity stress level indicating increase in the activity of this enzyme under stress is probably a promising indicator of accumulation of H_2O_2 under salt stress. Jain et al. [36] reported that micropropagated sugarcane plantlets showed identical number and position of the bands of peroxidases as their donor plants. The similar results were observed in the present investigation in respect of band colour intensity that salinity tolerant CoM-265 variety had intense and moderately intense band under salinity stress levels. Gao *et al.* [33] reported that in *Jatropha curcas* seedlings, seven POX isoenzymes bands in cotyledons, and six POX isozymes bands in the hypocotyls and radicals were observed. These isozymes showed different intensities. In the present study, the POX band from SDS-PAGE revealed single isoform both in salinity tolerant genotype CoM-265 and salinity susceptible genotype CoC-671, but the band intensity was different. Interestingly, peroxidase enzyme band was more intense in salinity tolerant genotype CoM-265 as compared to salinity susceptible genotype CoC-671 genotype and band intensity decreased with increasing salinity levels. The decrease in POX isozyme level was less in salinity tolerant CoM-265 with increase in salinity levels as compared to salinity susceptible CoC-671 genotype. The POX band was less intense in salinity susceptible CoC-671 genotype at 4.21 and 8.01 dSm1 salinity level, while moderately intense POX band was seen at 0.41 and 2.31 dSm1 salinity levels. Abd El-baky et al. [37] showed that one POX band was manifested higher densities and intensities in the salt treated cultivars of onion than the cultivars grown under control condition. Sreenivasulu *et al.* [38] detected high POX isozyme activity in salt tolerant cultivar compared to salt susceptible cultivar of foxtail millet which was related to salt adoption process. Zeeshan et al. [39] compared wheat (salt-tolerant cv. Suntop and -sensitive Sunmate) and

barley (salt-tolerant cv. CM72) cultivars and concluded that higher activities of antioxidants (SOD, peroxidase; POD, APX, GR, and CAT) are strongly correlated with the higher salt tolerance depicting a clear role of antioxidant activities in mitigation of salt-induced oxidative stress. Peroxidase plays diverse function in the plant life cycles such as plant growth and development including cell wall metabolism, lignification, suberization, reactive oxygen species (ROS) metabolism, auxin metabolism and also in defense against pathogens etc. [40]. Cell wall peroxidase also contributes to ROS generation particularly H_2O_2, where H_2O_2 modulates NO, Ca^{2+} and MAPK pathways, which control plant growth and development, as well as other cellular and physiological responses under diverse abiotic stresses [41–44].

Electrophoretic profile of esterase (EST) isozyme showed two types of bands with Rm value 0.152 (EST1) and 0.261 (EST2). Intensity of EST1 and EST2 bands were higher in salinity tolerant CoM-265 genotype as compared to CoC-671 a salt susceptible genotype. In the present study, differences in band intensity were also observed between salt tolerant CoM-265 and salt susceptible CoC-671 sugarcane genotypes. Hassanein [45] observed that salinity increasde EST isozyme, the highest numbers of esterases isozymes were detected under high NaCl concentration. Mohamed [28] noticed the esterase isozymes differences in density and number of bands among control and salt treated samples. The results obtained in the present study are concomitant with these earlier reports. The intense band appeared at 8.01 dSm^{-1} in salt tolerant CoM-265 sugarcane variety and remained absent in salt sensitive CoC-671 genotype may have adoptive mechanism against salt stress.

The alcohol dehydrogenase from the leaves of CoM-265 and CoC-671 sugarcane genotypes of the stressed and unstressed plants were detected on 7.5 % native-PAGE. There was genetic variability in salinity susceptible CoC-671 and salinity tolerant CoM-265 with regard to presene or absence of ADH bands and their intensity. Alcohol dehydrogenase isozymes are widely distributed across all organism types [46–49]. These enzymes catalyze the interconversion between alcohols and aldehydes [50–51]. Salinity stress induces accumulation of ADH mRNA in soybeans, grass peas and *Arabiodopsis* [52–55]. Langston et al. [56] observed three alcohol dehydrogenase isozymes in the embryo of germinating *T. turgidum.* However, in the present investigation six bands were observed. Shi et al. [57] found that ADH1 overexpressing Arabidopsis plants conferred enhanced resistance to salt, drought, cold and pathogen infection. Overexpression of AtADH1 expression increased the transcript levels of multiple stress-related genes like dehydration-responsive element binding protein 2a (DREB2A), heat shock protein 17.6 (HSP17.6), responsive to desiccation 29 (RD29B) at 300 mM NaCl level, accumulation of soluble sugars and callose depositions. An Alcohol Dehydrogenase gene from Synechocystis sp. confered salt tolerance in transgenic Tobacco [52]. Yi et al. [58] analyzed wild-type (WT) and transgenic tobacco plants to investigate whether the constitutive expression of an Alcohol Dehydrogenase gene from Synechocystis sp. confreres salt tolerance. Yi et al. [58] also assessed plant growth in response to salinity in tobacco plants and he found that WT plants exhibited chlorosis and growth retardation, whereas alcohol dehydrogenase from Synechocystis sp. (sysr1-OX) expressing tobacco plants grew relatively well and demonstrated enhanced salinity tolerance. Yi et al. [58] also studied the effects of salinity stress on chlorophyll content using a floating leaf disk assay amd when leaf disks were floated on a 300 mM NaCl solution for 5 days, the disks of WT plants were bleached more intensely than those of sysr1-OX expressing tobacco plants. Decreases in leaf disk chlorophyll levels were greater in WT plants than in sysr1-OX tobacco plants [58]. In present investigation ADH 3 (Rm value 0.382) and ADH 5 (Rm value 0.452) present in CoM-265, a tolerant sugarcane variety under various salinity stress condition may have role insalt stress tolerance, since these two bands remained absent in CoC-671 susceptible genotype.

Salinity susceptible sugarcane genotype CoC-671 had less soluble protein content as compared to CoM-265 at 0.41, 2.31, 4.21 and 8.01 dSm^{-1} salinity levels. There was reduction in soluble protein content with increasing salinity levels in salinity susceptible CoC-671 as well as salinity tolerant sugarcane genotype CoM-265. However, decrease in solouble protein content was less in salinity tolerant genotype CoM-265 as compared to salinity susceptible sugarcane genotype CoC-671. In sugarcane, increase in a NaCl salinity level resulted decrease in soluble protein content [59]. The decrease in protein content with increase in salt stress may be due to the increase in proteolysis and increase in the level of amino acids particularly proline. The disruption in protein synthesis appears to be an important cause of damage by Na^+ [60]. One characteristics of saline stress is the removal of potassium ions by plant roots, which comes a physiological imbalance because potassium is necessary for protein synthesis. Thus, in present study decrease in soluble protein in sugarcane varieties may be due impairment of Na^+/K^{+-} channel in the plants. The decrease in soluble protein in response to salinity was also reported in *Oryzasativa* [61], *Vicia faba* [62], *Amaranthus tricolor* [63] and *Brugniera parviflora* [64]. A higher content of soluble protein has been observed in salt tolerant cultivars of barley, sunflower, finger millet and rice [65]. Murad and Muneer [66] examined the effects of different salinity levels on proteome level and antioxidant capability and salt responsive gene expression profiling also carried out profiling of isozymes of peroxidase enzyme by SDS and native PAGE profiling. Reduction in content of soluble protein was observed when plants were exposed to salinity stress. Passamani et al. [67] reported salinity stress induced changes in the proteomic profile of micropropagated sugarcane shoots.

A 98.65 and 91.95 kD bands were present only in salinity tolerant genotype CoM-265 at salinity levels 2.31 dSm^{-1} and were absent in salinity susceiptible genotype CoC-671 at salinity levels 0.41, 2.31, 4.21 and 8.01 dSm^{-1}. A 85.75 kD protein band was detected in salinity tolerant genotype CoM-265 at 0.41 salinity level, but not present in salinity susceptible genotype CoC-671. These band could be considered as a positive molecular marker for salt tolerance. This result is in line with Khaled and Teixeira da Silva [21] and they reported that SDS page electrophoretic pattern of water soluble protein fraction in the leaves at 72 days after planting for sugarcane varieties exhibited a maximum number of 15 bands, which were not present in all sample.

In the present study a 98.65 kD band was present in only in salinity tolerant genotype CoM-265 at salinity levels 0.41, 2.31, 4.21 and 8.01 dSm^{-1}. A 95 kD band was detected at in salinity tolerant genotype CoM-265 at 2.31 dSm-1 salinity level, but not found in salinity susceptible CoC-671 genotype. 91.95 and 61.01 kD bands were also detected at salinity level 4.21 dSm^{-1} in salinity tolerant genotype CoM-265, but not found in salinity susceptible genotype CoC-671. A 75 kD band was detected at salinity level 2.31 dSm-1 in salinity tolerant genotype CoM-265, but not present in salinity susceptible genotype CoC-671. 63.55 and 36.75 kD bands were present at salinity level 0.41 dSm^{-1} in salinity tolerant genotype CoM-265, but not detected in salinity susceptible genotype CoC-671. A 52.90 kD band was detected at salinity levels 0.41 and 8.01 dSm-1 in salinity tolerant genotype CoM-265, but not found in salinity susceptible genotype CoC-671. 48.48 and 42.45 kD bands were present at salinity levels 0.41, 4.21 and 8.01 dSm^{-1} in salinity tolerant genotype CoM-265, but not present in salinity susceptible genotype CoC-671. Presence of these bands could be considered as a positive molecular marker for salt tolerance. Interstingly, a 25.20 kD faint band was present in salinity susceptible genotype CoC-671 at salinity levels 0.41, 2.31, 4.21 and 8.01 dSm^{-1} salinity levels, but not present in salinity tolerant genotype CoM-265. Absence of this band could be correlated with salinity tolerance in sugarcane genotypes. Hurkman and Tanaka [68] studied change of protein banding pattern in barley roots under salinity and observed no specific polypeptide bands under salinity stress. Specific protein bands linked with salinity stress tolerance were detected in maize [29] and sorghum [69–70]. Sobhanian et al. [54] also reported that density of the polypeptide band of 54 kD decreased at high salinity concentration in all genotypes; however a band with 56 kD size decreased in all cultivars except at 210 mM salinity. One of the possible explanations for complete disappearance of some proteins under salt

stress is that the gene(s) responsible for certain proteins had been completely suppressed as a result of stress. It is also possible that the gene(s) had not been completely suppressed, but inhibited as the result of stress, and complete recovery of the inhibition was not achieved [28]. Talei et al. [71] reported that several proteins were differentially expressed in seedling exposed to high salinity. In the present investigation, it was observed that total number of expressed proteins in the salt treated leaves was decreased, which might reflect the adverse effects of salinity on growth and development of the plant. Many cellular and metabolic processes of plants are known to be affected by salinity, including the reductions in stromal value of chloroplast, generation of reactive oxygen species (ROS), photosynthesis, respiration, biosynthesis of proteins, nucleic acid, lipids and pigments [72]. Alamgir and Ali [61] reported decrease in number of proteins in response to salinity in some plant species such as *Oryzasativa* and *Bruguieraparviflora*.

5. Conclusion

The study of protein changes by electrophoretic analysis under salinity treatment may be useful for understanding the salinity tolerance in sugarcane. In esterase isozyme system, the intense band appeared at 8.01 dSm^{-1}in salt tolerant CoM-265 sugarcane genotype and was not detected in salt sensitive CoC-671 gentype. In the present investigation, salt tolerant genotype of sugarcane CoM-265 under salt stress condition, exhibited intense and moderately intense bands indicating that increased in POX isozyme activities might be involved to sustain stressed condition. The intense and moderately intense bands observed in salt tolerant CoM-265 variety under salinity level indicating increase in the activity of this enzyme under stress is probably a promising indicator of salinity stress tolerance in sugarcane. The presence of specific ADH 3 (Rm value 0.382) and ADH 5 (Rm value 0.452) isoforms in CoM-265, a tolerant sugarcane variety under various salinity stress condition may have involvement to overcome the salt stress, since these two banding patterns were absent in CoC-671 susceptible genotype.

CRediT authorship contribution statement

Manisha Rameshrao Patil: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **A.A. Kale:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Ajay Kumar Singh:** Writing – review & editing, Writing – original draft, Formal analysis. **Priyanka Rameshrao Patil:** Writing – original draft, Methodology. **Shaheen Badshah Inamdar:** Resources, Formal analysis. **R.D. Satbhai:** Methodology, Investigation, Conceptualization.

Declaration of competing interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data availability

Data will be made available on request.

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