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Evaluation of sugarcane (*Saccharum officinarum,* L.) genotypes for salt tolerance at germination and seedling establishment stages

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Abstract

Sugarcane is an important cash and industrial crop of Pakistan. Salinity limits the sugarcane crop growth and production, thereby, causing economic loss to the farmers. Through cultivation of salt tolerant sugarcane varieties saline lands can best be utilized for economic crop production. The pot experiment was carried out to explore the salt tolerance ability of sugarcane genotypes under salinity levels (0, 4, 6, 8 and 10 dS m^{-1}) at germination and seedling establishment phase. The experiment was undertaken at National Sugar and Tropical Horticulture Research Institute (NSTHRI), PARC, Thatta, Pakistan (24°45'32.27"N, 67°53'41.53E) during February 2021 and harvested in April 2021. The trial was laid out under completely randomized design having three replications. Ten sugarcane genotypes i.e. HoTh-127, HoTh-2109, HoTh-300, HoTh-311, HoTh-318, HoTh-326, HoTh-409, Th-910, YT-53 and YT-55 ("Ho" Houma, USA; "Th" Thatta, Pakistan and "YT" Yuetang, China) were selected for experimentation. All the salinity levels significantly affected germination and all other tested growth parameters of sugarcane genotypes. However, the decline in germination and growth traits was more prominent at higher salinity levels (6 to 10 dS m^{-1}). In case of genotypes performance, HoTh-326 was found better regarding germination and other growth parameters by showing maximum salt tolerance index values 87.76, 82.31, 71.60 and 61.38 as compared to HoTh-311 with minimum STI values 77.38, 64.72, 52.98 and 44.63 at EC level of 4, 6, 8 and 10 dS m^{-1}, respectively. It was concluded that the sugarcane genotype HoTh-326 was found tolerant and HoTh-311 salt sensitive to salinity at germination and seedling establishment phase.

Keywords: Early seedling stage, Genotypes, Germination, Salinity, Sugarcane

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Introduction

Salinity is prevalent environmental stress posing threat to crop productivity and damaging the world's 20 percent of cultivated land and 33 percent of the irrigated land (Zaman & Qureshi, 2018; Iqbal & Qureshi, 2021; Syed et al., 2021; Devkota et al., 2022). Saline soils are generally barren and getting enlarged with plodding pace in arid and semi-arid regions due to climate change effects. Untenable irrigation practices, poor drainage, and high evapotranspiration (Liu et al., 2020; Sanga et al., 2024). Because of being less productive, these soils are causing economic loss to the farmers in the shape of marginal returns from their produce (Kopittke et al., 2019). Salinity has been accepted to modulate the normal germination process and initial sprouting development (Lu et al., 2023) by restricting water accessibility to plants due to osmotic stress effect (Gonzáles et al., 2021). Moreover, reduced cell enlargement and division as well as distorted enzyme activity under saline stress leads to lesser seed reserve consumption (Bliss, et al., 2019). Under extreme salinity levels, greater accumulation of Na and Cl ions in plant tissues confer disorder in different physiological mechanisms ultimately grounds abridged growth and development (Hannachi et al., 2022). Shoot and root growth followed by their fresh and dry weight have been found to reduce due to salt stress effects (Gholizadeh et al., 2021; Irik & Bikmaz, 2024).

Sugarcane (*Saccharum officinarum* L.) is an important cash crop (Rasool & Arslan, 2019; Ali et al., 2021). The growth and productivity of sugarcane during the whole crop cycle are highly influenced by salinity (Brindha et al., 2019; Yunita et al., 2020). The salinity on sugarcane has been found to affect sprout emergence, nutritional balance, and growth, which lowers biomass production and sugar yield. The characteristics like cane height, leaf area, and biomass are primarily influenced. Early sugarcane growth stages, such as germination, tillering, and cane production, are more susceptible than later stages (Cham et al., 2024). According to Hussain et al. (2019), sugarcane exhibits contrasting behavior to damaging effects of salinity at germination and bud sprouting phase. Apon et al. (2023) observed that sugarcane seedlings subjected to salt stress levels of 4 and 8 dS m^{-1} during the early growth stage demonstrated a substantial reduction in plant height, total leaf area, shoot dry matter, and root dry matter accumulation. Yunita et al. (2020) found that salinity reduced root length, shoot, and fresh weight, and damage symptoms became more noticeable at salt concentrations higher than 100 mM. They further reported that in sugarcane germination and early growth periods are more prone to salt sensitivity than later stages of crop development.

Improved germination and continuance of growth, primarily during the initial growth period, has been recognized as a key marker for salinity tolerance (Hussain et al., 2018). In the crop life cycle, germination is an important aspect that promises the loss or thrives of crop establishment. Under salt stress, the subsistence of seedlings at the preliminary phase of development extends the possibility of plant population survival to move up to the maturity stage (Gholizadeh et al., 2021). Seedling establishment is a highly imperative segment in the plant life cycle that outlines the base for the later growth stages, Thus, well adopt genotypes both at germination and seedling growth stages is necessary for getting a good crop population stand on salt affected lands (Debez et al., 2019). Our hypothesis is that throughout the germination and early seedling growth phase sugarcane genotypes respond differently to salt stress. Finding possible sugarcane genotypes that can withstand salt stress during early stages of crop development is therefore essential to improving crop stands, which may contribute to better production on saline lands.

Therefore, the current study was carried out to screen the salt tolerance of sugarcane genotypes at germination and seedling establishment stage. The present investigation will make it possible for the growers to select salt-tolerant genotype for the attainment of improved crop productivity. Little literature has so far been documented regarding the selection of sugarcane genotypes as salinity tolerant at germination phase which is a novelty aspect of this study.

Materials and Methods

To evaluate the salt tolerance ability of sugarcane genotypes at the germination and early seedling growth stage, the study was carried out under pot culture at the research site of the National Sugar and Tropical Horticulture Research Institute (NSTHRI), PARC, Thatta, Pakistan (24°45'32.27"N, 67°53'41.53E) during February 2021.

Seed source

The seed cane of nine months age of different sugarcane (*Saccharum officinarum*, L.) genotypes i.e. HoTh-127, HoTh-2109, HoTh-300, HoTh-311, HoTh-318, HoTh-326, HoTh-409, Th-910, YT-53 and YT-55. ("Ho" Houma, USA; "Th" Thatta, Pakistan and "YT" Yuetang, China) were obtained from germplasm of NSTHRI, PARC, Thatta.

Soil collection and processing

A bulk sample of non-saline soil (plow layer) was collected from agricultural land in Thatta. The texture of the soil was sandy clay loam, with pH (7.4), EC (0.36 dS m^{-1}), OM (0.40%), total N (0.073%), K (29.0 mg kg^{-1}), P (6.57 mg kg^{-1}), Na (1.23 meq L^{-1}), Ca+Mg (2.61 meq L^{-1}), Cl (2.44 meq L^{-1}), HCO_3 (3.19 meq L^{-1}), SAR 1.53, and ESP 3.74.

Planting of material and growth conditions

Plastic tubs (13.97 cm height and 24.53 cm av. dia) were filled with 4.5 kg of soil. The required salinity (EC) levels i.e. 0, 4, 6, 8 and 10 dS m^{-1} in the tubs were developed using commercial salt of NaCl according to the following formula (US Salinity Laboratory Staff, 1954):

$$Amount\ of\ salt\ = TSS \times Saturation\% \times Mol.mass\ of\ NaCl \times Wt.of\ soil \div 100 \times 1000$$
$$Where\ TSS\ (Total\ Soluble\ Salts\ ) = (Required\ EC - Current\ EC) \times 10$$

Recommended fertilizer dose @ 230, 115, and 125 NPK kg ha^{-1} in the shape of urea, triple supper phosphate and sulfate of potash were given as follows; total P, K, and 1/3rd of N were well mixed with soil equally in all tubs. In control (no salt) and salt-treated tubs, five single bud sets of each sugarcane genotype were sown in an 'X' form. The sets' bud side was placed upwards and blanketed with a single layer of soil around one centimeter and left to germinate for sixty days. To keep the soil moist and facilitate better germination normal irrigation water was used as per crop water requirement through a hand sprinkler. A completely randomized design (CRD) was employed to arrange the tubs of each treatment in three replications and thus in each repeat 50 tubs were maintained.

Harvesting and data collection

The crop was harvested in April 2021, and the data of the following parameters were collected.

Germination percentage

After completion of germination (60 days after sowing) germinated buds of each sugarcane genotype in control and salt-treated tubs were counted to calculate germination percentage following the method given by Ruan et al. (2002):

$$Germination\ (\%) = \frac{Number\ of\ germinated\ buds}{Total\ number\ of\ buds} \times 100$$

Root and shoot height (cm)

After 60 days (final count day) normal seedlings were selected at random from all replications of each treatment. The seedlings after washing properly with distilled water and blotting with tissue paper were used for measuring the root and shoot length. The primary root's tip to the hypocotyl base was used to measure the root's length. From the base of the primary leaf to the base of the hypocotyls, the shoot length was measured, and the means were expressed in centimeters.

Seedling vigor index

The following formula as suggested by Abdul-Baki and Anderson, (1973) was used to calculate the seedling vigor index:

$$\textit{Seedling vigour index} = (\text{Root length} + \text{Shoot height} \times \text{Germination percentage})$$

Root and shoot fresh and dry weight (g plant^{-1})

Same seedlings were used for measuring root and shoot fresh weight. Their dry weight was measured after drying in the oven at 40°C for 72 hrs. The seedlings were cooled at ambient temperature followed by weighing on digital top loading balance.

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Relative water content (%)

For relative water content (RWC) assessment, fresh weight of leaf lumps was recorded; subsequently the same pieces were dropped in deionized water for five hours in order to gain complete turgidity and soon after the turgid weight were recorded. After that the bits were placed in hot air oven for drying to a constant temperature and dry weight was recorded. The RWC was determined using the formula of Schonfeld et al. (1988):

Salt tolerance traits index (STTI)

The formula outlined by Ali et al. (2007) was used to determine the salt tolerance index:

$$\textit{Relative Water Content }(\%) = \frac{\textit{Fresh weight} - \textit{Dry weight}}{\textit{Saturated weight} - \textit{Dry weight}} \times 100$$

$$\textit{Salt tolerance traits index (STTI)} = \frac{\textit{Value of trait under stress condition}}{\textit{Value of traits under control}} \times 100$$

Statistical analysis

Statistical analysis of the data was performed using Statistix version 8.1 software. One-way analysis of variance (ANOVA) was done for the characteristics under study. The treatment means were separated through Tukey's honestly significant difference (HSD) test at alpha 0.05 (Steel et al., 1997).

Results

Analysis of variance of different traits

The variance analysis data for germination, shoot height, root length, total fresh and dry weight demonstrated a highly significant impact of salinity and genotypes at $p<0.05$, except relative water content which had non-significant genotypes effect ($p<0.05$). The interactive effect of salinity × genotypes on all assessed traits persisted non-significant ($p<0.05$). In the case of relative water content, salinity had a highly significant effect, and genotypes had non-significant effect at a $p<0.05$ level of probability (Table 1).

Table 1 Error means square from analysis of variance of growth traits of sugarcane genotypes at germination stage under different salinity levels

SOV	df	Germination (%)	SH	RL	SVI	TFW	TDW	WC
Replication	2	0.41	0.1621	6.24	250	6.572	0.321	574.415
Genotype (G)	9	935.19 **	12.76 **	19.33 **	676786 **	56.914 **	1.122 **	99.136 (ns)
Salinity (S)	4	8703.33 **	42.61 **	75.81 **	3,537,840 **	174.464 **	2.857 **	424.276 **
G × S	36	32.96ns	0.1333ns	0.2705ns	16,275ns	0.546ns	0.020ns	1.769ns
Error	98	7.14	0.0686	0.5657	1,177	1.137	0.067	102.656

SOV = Sources of variation; df = Degree of freedom; SH = Shoot height (cm); RL = Root length (cm); SVI = Seedling vigor index; TFW = Total fresh weight (g); TDW = Total dry weight (g); WC = Water contents (%); ** = Significance levels (p<0.05); ns = Not significant

Germination percentage

The effect of salinity on germination percentage of sugarcane genotypes recorded 60 days after planting (DAP) is presented in Table 2. The germination percentage in all the ten sugarcane genotypes decreased significantly with increasing levels of salinity over control. Significantly highest mean germination was recorded in the control treatment, which tended to decrease progressively at salinity levels of 4, 6, 8 and 10 dS m^{-1}. Mean germination data under different salinity levels indicated that HoTh-326 exhibited significantly maximum mean germination followed by HoTh-2109, YT-55, and HoTh-300 with significantly differed values. In contrast, the genotypes HoTh-311 and HoTh-409 displayed the lowest performance and did not differ significantly.

Table 2 Effect of different salinity levels on germination (%) of sugarcane genotypes

Genotypes	Germination %					Mean
	Salinity levels (EC dS m^{-1})					
	Control	4	6	8	10	
HoTh-127	66.67	53.33	40.00	33.33	26.67	44.00 cd
HoTh-2109	80.00	60.67	53.33	46.67	33.33	56.00 ab
HoTh-300	73.33	60.00	53.33	40.00	33.33	52.00 bc
HoTh-311	66.67	46.67	33.33	26.67	20.00	38.33 d
HoTh-318	73.33	53.33	40.00	26.67	26.67	44.00 cd
HoTh-326	80.00	73.33	66.67	53.33	46.67	62.66 a
HoTh-409	66.67	46.67	33.33	26.67	20.00	38.80 d
Th-910	66.67	46.67	40.00	33.33	26.67	42.66 cd
YT-53	73.33	53.33	46.67	40.00	26.67	48.00 bcd
YT-55	80.00	60.00	53.33	46.67	33.33	54.66 ab
Mean	72.66 a	55.83 b	46.06 c	37.33 d	28.66 d	

Shoot height (cm)

The increasing salinity levels have an inhibitory effect on shoot height (Table 3). The overall mean of salt treatment showed maximum shoot height (SH) in control. While, in all of the other salt treatments (4, 6, 8, and 10 dS m^{-1}) a significant decrease in SH values was observed. In case of mean SH data of sugarcane genotypes under different salinity levels, the genotype HoTh-326 attained significantly highest mean SH followed by HoTh-2109, HoTh-300, and YT-55 which demonstrated statistically on par results. Contrary to this, the sugarcane genotypes HoTh-311 exhibited significantly lowest SH followed by HoTh-318, HoTh-127, HoTh-409, and YT-53 under different salinity levels.

Table 3 Effect of different salinity levels on shoot height (cm) of sugarcane genotypes

Genotypes	Shoot height (cm)					Mean
	Salinity levels (EC dS m^{-1})					
	Control	4	6	8	10	
HoTh-127	7.26	6.83	6.17	5.31	4.75	6.06 cd
HoTh-2109	9.54	9.12	8.70	7.46	6.29	8.22 ab
HoTh-300	9.61	9.05	8.45	7.60	6.38	8.21 ab
HoTh-311	7.10	6.22	5.82	3.92	3.44	5.30 d
HoTh-318	7.40	6.62	6.10	5.20	4.66	6.00 cd
HoTh-326	10.18	9.76	9.15	8.50	7.31	8.98 a
HoTh-409	7.65	6.91	6.23	5.40	4.62	6.16 cd
Th-910	8.48	7.70	7.11	6.33	5.50	7.02 bc
YT-53	7.81	7.14	6.45	5.78	5.13	6.46 cd
YT-55	9.32	8.80	8.19	7.25	6.50	8.01 ab
Mean	8.43 a	7.81 ab	7.23 b	6.27 c	5.46 d	

Values sharing the same letters in columns are not significantly different from each other at $P<0.05$.

Root length (cm)

The root length (RL) of all the ten sugarcane genotypes under salinity stress showed a similar reducing trend as in the case of shoot height (Table 4). On overall mean basis sugarcane genotypes exhibited a maximum mean value of RL at control treatment and the exposure to salt stress resulted in a significant reduction in RL in all salinity levels from 4 to 10 dS m^{-1}. The comparison of genotypes means in different salt treatments showed that the sugarcane genotypes differed considerably in this attribute. The genotype HoTh-326 had significantly higher means RL followed by HoTh-2109, HoTh-300, and YT-55 which displayed intermediate performance with statistically on par RL. Moreover, the sugarcane genotypes HoTh-311, HoTh-409, and HoTh-127 exhibited susceptibility to salt treatments and produced inhibitory results for this trait.

Table 4 Effect of different salinity levels on root length (cm) of sugarcane genotypes

Genotypes	Root length (cm)					Mean
	Salinity levels (EC dS m^{-1})					
	Control	4	6	8	10	
HoTh-127	5.80	4.35	3.64	3.17	2.28	3.84 de
HoTh-2109	8.39	6.46	5.72	4.69	3.33	5.71b
HoTh-300	8.04	5.61	5.87	4.52	3.26	5.46 b
HoTh-311	4.68	3.74	3.11	2.30	1.70	3.10 e
HoTh-318	6.32	4.85	4.35	3.12	2.45	4.21 cd
HoTh-326	9.45	7.55	7.22	5.64	4.71	6.91 a
HoTh-409	5.83	4.57	3.68	2.85	1.66	3.71 de
Th-910	7.60	5.38	4.65	3.71	3.11	4.09 bc
YT-53	6.28	5.19	4.52	3.35	2.62	4.39 cd
YT-55	7.72	6.30	5.44	4.29	3.41	5.43 b
Mean	7.01 a	5.40 b	4.82 c	3.76 d	2.05 e	

Values sharing the same letters in columns are not significantly different from each other at P<0.05.

Seedling vigor index

The data in Table 5 further revealed that all salinity levels in growth medium led to a significant reduction in seedling vigor index (SVI) as compared to control. In the case of different salt treatments, a prominent decrease in SVI was observed over control at salinity levels of 4, 6, 8, and 10 dS m^{-1}. Moreover, by increasing salinity, a significant decrease in SVI was also observed in all sugarcane genotypes. The lowest mean SVI was observed in the sugarcane genotype HoTh-311. However, compared to the other sugarcane genotypes, the HoTh-326, HoTh-2109, YT-55, and HoTh-300 genotypes progressed better because they maintained a high mean value of SVI at all salinity levels.

Table 5 Effect of different salinity levels on seedling vigor index of sugarcane genotypes

Genotype	Seedling vigor index					Mean
	Salinity levels (EC dS m^{-1})					
	Control	4	6	8	10	
HoTh-127	874.89	609.00	395.53	287.25	190.96	471.5 cde
HoTh-2109	1433.88	1032.95	769.50	561.73	312.43	822.8 b
HoTh-300	1290.85	888.56	768.45	490.35	325.08	752.7 b
HoTh-311	789.69	454.44	303.37	165.07	103.97	363.3 e
HoTh-318	1005.28	620.61	425.82	223.42	191.21	493.3 cd
HoTh-326	1567.85	1278.4	1093.12	755.20	486.75	1036.3 a
HoTh-409	905.09	529.09	347.82	218.79	128.04	425.7 de
Th-910	1076.85	604.88	471.95	335.94	229.36	543.8 d
YT-53	1022.67	646.00	521.57	382.91	200.70	554.8 c
YT-55	1362.85	898.86	732.30	540.73	313.40	769.6 b
Mean	1133.3 a	756.3 b	582.9 c	396.1 d	248.2 e	

Values sharing the same letters in columns are not significantly different from each other at P<0.05.

Total fresh weight (g plant^{-1})

A progressive reduction in mean total fresh weight (TFW) of sugarcane genotypes was observed with an increase in salt stress in the growth medium as compared to the control treatment (Table 6). The maximum and minimum mean reduction in TFW of genotypes was found in the case of 10 and 4 dS m^{-1} salt treatments, respectively. With regards to the comparison of sugarcane genotypes mean for this trait, considerable differences existed between the genotypes for tolerance to different levels of salinity. Among the sugarcane genotypes, HoTh-326, HoTh-2109, and HoTh-300 were found to be superior due to their highest mean total fresh weight under different saline conditions. However, sugarcane genotypes HoTh-311 and HoTh-409 were regarded inferior on account of their lowest performance for mean total fresh weight under different salinity levels.

Table 6 Effect of different salinity levels on total fresh weight (g plant^{-1}) of sugarcane genotypes

Genotype	Total fresh weight (g plant^{-1}) Salinity levels (EC dS m^{-1})					Mean
	Control	4	6	8	10	
HoTh-127	9.22	8.02	6.36	4.55	3.66	6.36 ef
HoTh-2109	13.54	10.85	9.37	8.19	6.56	9.70 b
HoTh-300	12.87	9.71	9.29	7.60	6.10	9.11 bc
HoTh-311	8.2	6.35	5.28	4.05	3.14	5.40 f
HoTh-318	8.74	7.75	6.06	4.95	3.89	6.27 ef
HoTh-326	15.43	12.53	11.47	10.03	8.57	11.60 a
HoTh-409	8.61	6.82	5.69	4.59	3.53	5.85 f
Th-910	12.25	9.65	7.95	6.15	5.07	8.21 cd
YT-53	10.71	8.75	7.18	5.48	4.61	7.34 de
YT-55	11.82	9.61	7.52	5.70	4.87	7.91 cd
Mean	11.13 a	9.00 b	7.61 c	6.12 d	5.00 e	

Values sharing the same letters in columns are not significantly different from each other at P<0.05.

Total dry weight (g plant^{-1})

The increase in salinity of the growth medium had imposed an adverse effect on the total dry weight (TDW) of sugarcane genotypes (Table 7). The sugarcane genotypes demonstrated highly contrasting behavior in different salt treatments for this trait. In the case of salt treatments mean, maximum TDW of sugarcane genotypes was recorded from the control treatment, which tended to decrease with an increase in salinity levels up to 10 dS m^{-1}. As regards the performance of sugarcane genotypes for TDW under different salinity levels, HoTh-326, HoTh-2109, and HoTh-300 emerged to be exceptional by surpassing the rest of the sugarcane genotypes with maximum mean TDW. However, the sugarcane genotypes HoTh-311, HoTh-409, and HoTh-318 showed the same trend of decreasing results for this parameter and appeared to be substandard.

Table 7 Effect of different salinity levels on total dry weight (g plant^{-1}) of sugarcane genotypes

Genotype	Total dry weight (g plant^{-1}) Salinity levels (EC dS m^{-1})					Mean
	Control	4	6	8	10	
HoTh-127	2.08	1.96	1.70	1.34	1.23	1.67 cd
HoTh-2109	2.49	2.17	2.07	1.96	1.75	2.08 b
HoTh-300	2.35	2.08	2.11	1.88	1.66	2.01 b
HoTh-311	1.97	1.67	1.51	1.24	1.11	1.50 d
HoTh-318	2.04	1.90	1.59	1.42	1.29	1.64 cd
HoTh-326	2.75	2.35	2.43	2.34	2.20	2.41 a
HoTh-409	2.05	1.79	1.55	1.37	1.18	1.59 cd
Th-910	2.27	2.07	1.90	1.67	1.45	1.88 bc
YT-53	2.23	2.02	1.78	1.53	1.40	1.80 bcd
YT-55	2.32	2.11	1.83	1.56	1.45	1.85 bc
Mean	2.25 a	2.01 b	1.84 b	1.64 c	1.47 c	

Values sharing the same letters in columns are not significantly different from each other at P<0.05.

Relative water content (%)

The effect of salinity on relative water content (RWC) of sugarcane genotypes in Table 8 indicated that all ten sugarcane genotypes witnessed a decrease in WC at salinity levels in the growth medium, however, the trajectory of the decline varied by genotype. Maximum RWC among sugarcane genotypes was recorded from control treatment, the drop in RWC across all examined entries was noticeable at increasing salt stress intensity levels and this characteristic in sugarcane genotypes was altered at salinity values of 4, 6 8, and 10 dS m^{-1}. Among the sugarcane genotypes under different salinity levels, HoTh-326 maintained maximum mean RWC followed by HoTh-2109 and HoTh-300. However, the sugarcane genotypes HoTh-311 uphold comparatively lowest RWC followed by HoTh-409, HoTh-127, and HoTh-318.

Table 8 Effect of different salinity levels on relative water content (%) of sugarcane genotypes

Genotype	Relative water content (%)					Mean
	Salinity levels (EC dS m^{-1})					
	Control	4	6	8	10	
HoTh-127	77.35	75.43	73.16	70.5	65.54	72.39
HoTh-2109	81.58	79.97	77.81	75.98	73.35	77.73
HoTh-300	81.73	78.45	77.26	74.96	72.74	77.02
HoTh-311	75.77	73.63	71.36	69.47	64.71	70.98
HoTh-318	76.53	75.41	73.75	71.25	67.18	72.82
HoTh-326	82.18	81.34	78.76	76.52	74.26	78.61
HoTh-409	76.13	73.73	72.69	70.61	66.25	71.88
Th-910	81.47	78.47	75.98	72.81	71.33	76.01
YT-53	79.14	76.59	75.05	72.26	69.90	74.58
YT-55	80.31	78.01	75.54	72.56	70.41	75.36
Mean	79.21 a	77.10 ab	75.13 ab	72.69 ab	69.56 b	

Values sharing the same letters in columns are not significantly different from each other at P<0.05.

Salt tolerance index

The salt-tolerance index (STI) was used to estimate the salt tolerance of each evaluated sugarcane genotype. According to the mean values of the recorded traits under EC 4 dS m^{-1} shown in Table 9, the sugarcane genotype HoTh-326, has emerged to be salt-tolerant on account of maximum salt-tolerance index followed by HoTh-127 and HoTh-2109, whereas the HoTh-311 was regarded salt-sensitive due to minimum STI values. Moreover, the rest of the genotypes were found intermediate. Under salinity level (EC 6 dS m^{-1}) the trend of higher STI values was observed from HoTh-326 genotype followed by HoTh-300, HoTh-2109, and YT-55 and minimum in HoTh-311 genotype (Table 10). The sugarcane genotype HoTh-326 surpassed the other genotypes by showing maximum STI value under EC 8 dS m^{-1} salinity level, followed by HoTh-2109, HoTh-300, and YT-55, while, HoTh-311exhibited same trend of lowest STI value (Table 11). The genotype HoTh-326 maintained its superiority over other genotypes for STI under EC 10 dS m^{-1} salinity levels, followed by HoTh-300, HoTh-2109, and YT-55, while HoTh-311 genotype came out to be salt-sensitive due to least STI value (Table 12).

Table 9 Salt tolerance traits indices (STTI) of five sugarcane traits studied under EC 4 (dS m^{-1}) salinity levels at germination and seedling establishment stage

Genotypes	Germination	SVI	TFW	TDW	RWC	STI	Rank
HoTh-127	79.99	69.60	86.98	94.23	97.51	85.66	2
HoTh-2109	83.33	72.03	80.13	87.14	98.02	84.13	3
HoTh-300	81.82	68.83	75.44	88.51	95.98	82.11	5
HoTh-311	70.00	57.54	77.43	84.77	97.17	77.38	10
HoTh-318	72.72	61.73	88.67	93.13	98.53	82.95	4
HoTh-326	91.66	81.53	81.20	85.45	98.97	87.76	1
HoTh-409	70.00	58.45	79.21	87.31	96.84	78.36	9
Th-910	70.00	56.17	78.77	91.18	96.31	78.48	8
YT-53	72.72	63.16	81.69	90.58	96.77	80.98	7
YT-55	75.00	65.95	81.30	90.94	97.13	82.06	6

SVI = Seedling vigor index; TFW = Total fresh weight (g); TDW = Total dry weight (g); RWC = Relative water contents (%); STI = Salt tolerance index

Table 10 Salt tolerance traits indices (STTI) of five sugarcane traits studied under EC 6 (dS m^{-1}) salinity levels at germination and seedling establishment stage

Genotypes	Germination	SVI	TFW	TDW	RWC	STI	Rank
HoTh-127	59.99	45.20	68.98	81.73	94.58	70.09	6
HoTh-2109	66.66	53.66	69.20	83.13	95.37	73.60	3
HoTh-300	72.72	59.53	72.18	89.78	94.53	77.74	2
HoTh-311	49.99	38.41	64.39	76.64	94.17	64.72	10
HoTh-318	54.54	42.35	69.33	77.94	96.36	68.10	8
HoTh-326	83.33	69.72	74.33	88.36	95.83	82.31	1
HoTh-409	49.99	38.42	66.08	75.60	95.48	65.11	9
Th-910	59.99	43.82	64.89	83.70	93.26	69.13	7
YT-53	63.64	51.00	67.04	79.82	94.83	71.26	5
YT-55	66.66	53.73	63.62	78.87	94.06	71.38	4

SVI = Seedling vigor index; TFW = Total fresh weight (g); TDW = Total dry weight (g); RWC = Relative water contents (%); STI = Salt tolerance index

Table 11 Salt tolerance traits indices (STTI) of five sugarcane traits studied under EC 8 (dS m^{-1}) salinity levels at germination and seedling establishment stage

Genotypes	Germination	SVI	TFW	TDW	RWC	STI	Rank
HoTh-127	49.99	32.83	49.34	64.42	91.14	57.54	7
HoTh-2109	58.33	39.17	60.48	78.71	93.13	65.96	2
HoTh-300	54.54	37.98	59.05	80.00	91.71	64.65	3
HoTh-311	40.00	20.9	49.39	62.94	91.68	52.98	10
HoTh-318	36.36	22.22	56.63	69.60	93.10	55.58	8
HoTh-326	66.66	48.16	65.00	85.09	93.11	71.60	1
HoTh-409	40.00	24.17	53.31	66.82	92.74	55.40	9
Th-910	49.99	31.19	50.20	73.56	89.37	58.86	6
YT-53	54.54	37.44	51.16	68.60	91.30	60.60	5
YT-55	58.33	39.67	48.22	67.24	90.34	60.76	4

SVI = Seedling vigor index; TFW = Total fresh weight (g); TDW = Total dry weight (g); RWC = Relative water contents (%); STI = Salt tolerance index

Table 12 Salt tolerance traits indices (STTI) of five sugarcane traits studied under EC 10 (dS m^{-1}) salinity levels at germination and seedling establishment stage

Genotypes	Germination	SVI	TFW	TDW	RWC	STI	Rank
HoTh-127	40.00	21.82	39.69	59.13	84.73	49.07	8
HoTh-2109	41.66	21.78	48.44	70.28	89.91	54.41	3
HoTh-300	45.45	25.18	47.39	70.63	89.00	55.53	2
HoTh-311	29.99	13.16	38.29	56.34	85.40	44.63	10
HoTh-318	36.36	19.02	44.50	63.23	87.78	50.17	6
HoTh-326	50.00	31.04	55.54	80.00	90.36	61.38	1
HoTh-409	29.99	14.14	40.99	57.56	87.02	45.94	9
Th-910	40.00	21.29	41.38	63.87	87.55	50.81	5
YT-53	36.36	19.62	43.04	62.78	88.32	50.02	7
YT-55	41.66	22.99	41.20	62.50	87.67	51.20	4

SVI = Seedling vigor index; TFW = Total fresh weight (g); TDW = Total dry weight (g); RWC = Relative water contents (%); STI = Salt tolerance index

Discussion

In the growth and development cycle of sugarcane, the germination period is thought to be extremely significant. Weak vegetative development and decreased seedling formation are the results of low germination, which eventually lowers the potential yield. There is a greater chance of total crop failure at this stage than at any other, making it one of the most vulnerable to environmental stressors of all kinds. Most of the crops exhibit greater salt tolerance in early growth stages than at later growth stages. For sugarcane, studies have made known about many genotypes which have displayed improved tolerance mechanisms. These genotypes have been selected based on their ability of tolerance to saline environments at the seedling stage.

Our present study aimed to identify salinity tolerance for sugarcane seedlings using traits such as germination percentage, shoot height, root length, seedling vigor index, shoot root fresh and dry weight, and relative water content. It was found that differences in salt stress tolerance existed amongst genotypes. In the case of germination percentage, our results of the experiment indicated variable reaction of sugarcane genotypes for bud germination and seedling vigor index. The sprouted seedlings were more in HoTh-326, HoTh-2109, HoTh-300, and YT-55 as compared to the rest of the sugarcane genotypes under salinity levels. It was observed that the germination and seedling vigor index in all ten sugarcane genotypes tended to decrease with an increase in salinity levels. The observed reduction in germination percentage of sugarcane genotypes was possibly owing to a specific ion effect under salt stress (Hussain et al., 2019; Yan et al., 2020) besides that the lowered osmotic potential of the germination medium, which made a reduced amount of water accessible for extraction by the sugarcane seed sets (El-Hendawy et al., 2019; Iqbal et al., 2020).

Salinity stress has been shown to affect various crops on seed germination and early seedling growth depending upon their extent of salinity (Bimurzayev et al., 2021). There is a significant reduction in germination percentage and germination rate in sugarcane genotypes with increasing levels of salinity (Kumari & Jha, 2018). The lower SVI value in sugarcane genotypes under salt stress is attributable to the reduced germination rate and initial growth of sugarcane seedlings caused by the reduced osmotic potential of the growth medium. Many workers have used the seedling vigor index as an important tolerance marker to determine the upshot of salinity on seedling growth and they found that salt-sensitive varieties exhibited significant decrement in SVI due to salt stress (Yohannes et al., 2020; Ergin et al., 2021). In our study, in contrast to control (0 dS m^{-1}), the shoot height and root length of sugarcane genotypes were decreased prominently when exposed to NaCl salt stress levels (4, 6, 8, and 10 dS m^{-1}). Significant genotype difference was observed as more shoot height and root length were recorded in sugarcane genotype HoTh-326, HoTh-2109, HoTh-300 and YT-55 as compared to HoTh-311, HoTh-127, HoTh-311, HoTh-409, YT-55 and Th-910. Variation in height among sugarcane genotypes under the same salt stress intensity could be attributed to the genetic makeup of the genotypes (Feven & Esayas, 2018). The osmotic stress and ion toxicity in consequence of salinity might have decreased the shoot and root growth (Alsahli et al., 2019). The reduction in root length and shoot length under salinity may be due to salinity induced reduction in turgor pressure of cells which results in slow cell division and production of small sized cells (Jameel et al., 2024).

Experimental results also showed that NaCl concentrations significantly affected the fresh and dry weight of sugarcane genotypes. The fresh weights of the shoots and roots of the sugarcane seedlings exposed to varying concentrations of salt were lower than those of the control. The highest measurement of the shoot and root fresh weight was obtained under the control treatment (0 dS m^{-1}), and this was significantly higher than all salt treatments (4, 6, 8, and 10 dS m^{-1}). As regards the genotype mean for these traits, the performance of HoTh-326, HoTh-2109, HoTh-300, and YT-55 was better as compared to the rest of the sugarcane genotypes. Significantly lowest shoot and root fresh weight was recorded in HoTh-311 in all salt treatments. This reducing trend was observed in the case of shoot and root dry weight among all tested sugarcane genotypes. The reduction in shoot and root dry weight was significantly greater at the higher salt stress level (10 dS m^{-1}) as compared to the control (0 dS m^{-1}).

Most of the plants at primary growth stages display sensitivity to salt stress thereby under medium and high salinity stress the fresh and dry weights of the shoot and root are reduced (Trotti et al., 2024). It has been reported that the higher salinity level has resulted in decreased dry weight in sugarcane plants because of higher sodium accumulation in the growth medium and its supply to shoots (Yunita et al., 2020). The trend of reduced DW of sugarcane as a result of salt stress has been evident by Zhao et al. (2020). The reduced seedling growth due to salt stress is further justified in other crops; Abdelrady et al. (2024) reported that salt stress level (12 dS m^{-1})) in growth medium resulted in restricted shoot length, fresh and dry leaf weight, fresh and dry stem weight, as well as total fresh and dry weight in barley. Ehtaiwesh et al. (2024) observed noticeable impairment in shoot height, fresh, and dry weights of the shoot of wheat plants as a result of salinity stress (120 mM NaCl). Farhangi-Abriz and Ghassemi-Golezani (2021) noticed reduced root length, root, and shoot dry weights in safflower consequence of raise in salt concentration under the growth medium. The reduction in growth parameters of plants under salinity stress, on the whole, is caused by the low osmotic potential that provokes ionic stress and results in nutritional imbalance in plants (Alharby et al., 2019).

In a previous research study, Abdallah et al. (2020) reported that salinity stress effects may confer disorder in plant cell functions, thereby enzymes and metabolism activities of plants are restricted which leads to inhibited growth in wheat cultivars. The formation of a strong root system under salt stress habitats ensures better growth establishment and higher biomass production (Iqbal et al., 2018; Zhao et al., 2021). The water content (WC) has been proposed as an easy agricultural attribute to select plants for their tolerance to salinity based on a high WC (Saeed et al., 2019). Our results showed that imposition of salinity non-significantly affected the water content of sugarcane genotypes. The genotypic difference was also non-significant for this trait as all the examined genotypes displayed the almost same behavior. However, the performance of HoTh-326, HoTh-300 and HoTh-2109 demonstrated better values for this measured trait as compared to other sugarcane genotypes under study. Kumar et al. (2023) reported that a decrease of WC in leaves of plants exposed to salt stress showed that salinity might have caused cellular water loss and the signs of dehydration were larger in plants under higher salt treatments. Sadiq et al., (2024) reported that excessive Na+ ion absorption in plants from salt-stressed environments can impair the plant's ability to absorb more water, leading to reduced RWC in plants. The WC is an important feature of salt tolerance in plants (Abrar et al., 2020).

Conclusion

According to the current findings, sugarcane genotypes exhibited decreased germination percentage, shoot height, root length, fresh weight, dry weight, relative water content, and vigor index as the concentration of NaCl increased. Based on comparisons across tested genotypes HoTh-326 appears to be more salt-tolerant and HoTh-311 salt sensitive at the germination and seedling establishment stage. The salt tolerance ability of sugarcane genotype HoTh-326 needed to be assessed at vegetative and reproductive stages to draw out its potential in whole crop cycle.

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