

Is sweet sorghum (*Sorghum bicolor* L.) salt tolerant?

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Abstract: This study investigated how sodium chloride (NaCl) affected seed germination, plant growth, juice production, and ethanol yield in sweet sorghum (*Sorghum bicolor* L.). There were two sections for the experiment, the first is in a laboratory, five sorghum cultivars have been investigated for seed germination at 11 varied NaCl concentrations (0-1%), and second part is in greenhouse, four sweet sorghum genotypes were cultivated in a greenhouse at five different NaCl concentrations (0%, 0.2%, 0.4%, 0.6%, and 0.8%). The results revealed that increasing NaCl levels dramatically affected seed germination (first part), plant height, diameter, and stalk yield (second part). Germination was less than 80% at NaCl concentrations of 0.4% or higher, while stalk yield was reduced by 40% at 0.2% NaCl. At 0.8% NaCl, plants were unable to produce juice. As soil salinity increased, NaCl levels in the juice climbed, resulting in a considerable decrease in ethanol productivity. Ethanol production decreased by up to 14% at 0.6% NaCl, while plants at 0.8% NaCl produced no ethanol. Correlation research discovered that total soluble solids, plant height, and stalk yield were favorably associated with ethanol yield, however NaCl concentration in the juice had a negative effect on ethanol productivity. The findings suggest that sweet sorghum is extremely susceptible to salty soil conditions, making cultivation and ethanol generation in saline areas challenging. This work emphasizes the need for additional research on salt-tolerant sorghum varieties and yeast strains to boost bioethanol production in saline soils.

Keywords: Saline soil, Salt tolerance, Total soluble solids, Ethanol yield, Sodium chloride.

Abbreviation: Sodium chloride; NaCl, completely randomized design; CRD), Duncan's multiple range test; DMRT, Total soluble solids; TSS.

Introduction

Thailand's ethanol industry mainly uses agricultural crops such as cassava and sugarcane as its raw materials. Ethanol is produced from sugar cane molasses, which is not readily available in large enough amounts. The industry faces competition from other areas because to the increasing demand for cassava. In order to secure raw materials and prevent shortages, industry must therefore identify alternate raw material sources. When raw materials are also needed for human or animal feed, the ethanol industry in many nations also faces a lack of such materials. Since agriculture provides fuel and food for human habitation, competition for arable land is also inevitable.

Sweet sorghum (*Sorghum bicolor* L. Moench) refers specifically to genotypes that accumulate high levels of soluble sugar in the stalk (Yang et al., 2020) and it offers one of the best plant-based bioethanol productions from its sugary stalk (Disasa et al., 2018) and is considered a potential bioenergy crop throughout most of the tropical and temperate zones of the world, and it is also a leading contender for biofuel production in the southern United States (Viator et al., 2015), sweet sorghum has demonstrated a moderate degree of salt tolerance, making it a promising crop for cultivation in saline-affected soils (Yang et al., 2020). Salt-tolerant sorghum cultivars, notably

sweet sorghum, present a viable solution for agriculture in saline-affected areas. Their resilience, combined with economic and ecological advantages, positions them as strategic crops for enhancing productivity and sustainability in challenging environments (Xue et al., 2025). About 2.85 million hectares in the northeast of Thailand possess saline soils, of which 0.24 million hectares are extremely salty (Arunin and Pongwichian, 2015). The evidence from previous studies underscores the sensitivity of sorghum seed germination to high salinity levels, particularly at 300 mM NaCl (Hassanein and Tammam, 2019), while some cultivars demonstrate a degree of tolerance, many exhibits significant reductions in germination rates, with some failing to germinate entirely, these findings highlight the importance of selecting salt-tolerant cultivars for cultivation in saline-affected areas (Ali et al., 2014). The investigators also noted that salt stress lengthened the time required for germination to occur and that high salt concentrations reduced the weight of seedlings. Water stress (PEG) had a negative impact on shoot and root growth, but salt stress had less impact (Rao and Shahid, 2020; Patane et al., 2013). Asfaw and Dano (2021) reported that salinity up to 5 dS m⁻¹ has minimal effect on germination, while levels above 10 dS m⁻¹ lead to statistically significant reductions. A 30 dS m⁻¹ salt concentration lowered germination by 48.8%. For sorghum genotypes during the germination stage, the salt tolerance threshold value was 8.37 dS m⁻¹ (Fazelpoor and Syahbanu, 2021; Tabatabaei and Anaghali, 2012). To the best of the knowledge, the effects of salt on stalk production, juice quality, and ethanol content in sweet sorghum have not been investigated. However, Geng et al. (2020) found that both salt-tolerant and susceptible cultivars of sorghum accumulated NaCl ions in their stems, which may influence juice quality and ethanol yield.

Although sweet sorghum is a desirable crop for growth on salty soils, it may be premature to propose that farmers cultivate sweet sorghum. They will face additional risks if the technologies for growing sweet sorghum on saline soil are not sufficiently established. It is unclear how salinity affects seed germination, growth, juice quality, and ethanol yield in sweet sorghum plants. The research concerns whether sweet sorghum can survive in salty soil and how growth on saline soils impacts sweet sorghum's ethanol yield. The objectives of this study were to determine how sweet sorghum seed germination is affected by the degree of salinity in a laboratory study, to investigate the effects of salinity on growth, yield and juice quality of sweet sorghum grown in a greenhouse and to quantify the effect of salinity on ethanol yield. This information is important for future studies on the production of sweet sorghum in saline soils.

Results

Effects of salinity on seed germination of sorghum

Germination percentages of five sweet sorghum varieties at initial concentrations of NaCl (0%) ranged from 70 to 92% and were reduced with increasing concentrations of NaCl (Figure 1a). Urja and Sugar T did not germinate at a NaCl concentration of 1%, and there was a low percentage of germination for Suwan Sweet 5, KKKU 40 and KU 630 varieties. Although sorghum varieties were significantly different in their germination percentages at all concentrations of NaCl, there was high and significant interaction between sodium concentration and sweet sorghum variety (Data not reported). Therefore, identification of superior genotypes for high germination percentage was difficult. It seemed that KKKU 40 had the highest germination percentage at NaCl concentration of 1%.

Regression analysis indicated that the data explained 60% of expected values (Figure 1b). The b value of -54.58 also indicated a linear reduction in seed germination. It is expected that the germination percentage will become zero if the NaCl level is slightly higher than 1%.

Effects of salinity and sorghum variety on total soluble solids, plant diameter, plant height and stalk yield

These parameters were collected when sweet sorghum reached to harvesting stage (30 days after days to 50% flowering). Variances for all characteristics were homogeneous, and then the data of two greenhouses were combined and reported. Significant differences ($p \leq 0.01$) between seasons (greenhouses) were observed for TSS, plant diameter, plant height and stalk yield (Table 2). However, the effects of season were significant ($p \leq 0.01$) and low for TSS (0.4%), plant diameter (1.8%) and stalk yield (3.4%), but the effect was rather high for plant height (10.1%). Sweet sorghum variety contributed to significant ($p \leq 0.01$) and high variations in TSS (35.7%) and plant diameter (17.8%), but this source of variation had small effects on plant height (1.9%) and stalk yield (4.4%).

The contribution of NaCl to total variation was high for TSS (27.9%), plant diameter (58.2%), plant height (77.7%) and stalk yield (85.3%). There were high and significant interactions ($p \leq 0.01$) between sorghum variety and NaCl concentration, season and sorghum variety, season and NaCl level and NaCl level and season and sorghum variety for TSS, plant diameter, plant height and plant fresh weight.

NaCl had a minor effect on the TSS of sweet sorghum genotypes. A slight increase in TSS was observed at 0.2% NaCl; however, the levels declined as the NaCl concentration increased further (Figure 2a). Based on plant diameter, plant height, and stalk yield, it was concluded that these traits decreased with increasing NaCl concentration (Figure 2b, c, and d).

It is notable that the best genotypes for each characteristic were different between seasons because of high environment interactions with the genotypes for these traits. Therefore, identification of superior genotypes for each characteristic was difficult. However, KKKU 40 and Urja seemed to be better than other genotypes for TSS, stem diameter and stalk yield, whereas Suwan Sweet 5 was the best genotype for plant height followed by Urja (Table 3).

NaCl seemed to reduce TSS but the reduction was ambiguous, especially at a concentration of 0.8% which was 12.4 brix higher than 9.8 brix of the concentration at 0.6 (Table 3). However, clear reductions were observed in plant diameter, plant height and stalk yield, and the reductions were consistent across NaCl concentration levels from 0.0 to 0.8%.

Percent reductions (or increases) caused by NaCl levels were determined and are presented in Table 4. Percent reductions for TSS fluctuated especially for NaCl levels from 0.4 to 0.8%. However, percent reductions for plant diameter (-19.6 to -67.4%), plant height (-19.8 to -53.0%) and stalk yield (-40.3 to -83.8%) increased with higher concentrations of NaCl levels between 0.2 to 0.8% (Table 4). Increasing NaCl concentration had minimal effects on total sugar content but not for fructose and sucrose. Glucose and ethanol yield decreased notably even at 0.2% NaCl. TSS declined at 0.6% NaCl. Additionally, NaCl levels in both juice and soil increased starting from 0.2% NaCl (Table 5).

Effects of salinity and sorghum variety on juice quality and ethanol yield

Initially, Sugar T grown at a NaCl concentration of 0.8% was included in the experiment. However, Sugar T did not yield enough juice for analysis at the NaCl concentrations of 0.4, 0.6 and 0.8% and at a NaCl concentration 0.8%, only KKKU 40 yielded enough juice for analysis. Therefore, the data for the Sugar T variety grown at a NaCl concentration of 0.8% were considered extreme values and not used in regression analysis.

The mean sugar concentrations of the varieties were between 15.00-16.44 g/L for fructose, 14.83-24.18 g/L for glucose, 103.35-125.34 g/L for sucrose, and 143.97-158.07 g/L for total sugar. There was 6060.50-7149.75 ppm of NaCl in the juice and 0.61-0.75 ppm for NaCl

Table 1. Salinity of the soils in this study compared with the salinity levels				
NaCl (%)	pH	EC (dS/m)	EC (dS/m)	Na (mg/kg)
0	6.13	0.08	2	43.04
0.2	6.22	3.37	2-4	696.00
0.4	6.54	6.39	4-8	2,002.00
0.6	6.66	12.40	8-16	2,301.00
0.8	6.58	18.43	16	3,322.00

Table 2. Mean squares for total soluble solids, plant diameter, plant height and total fresh weight of four sweet sorghum varieties grown at five NaCl concentrations from 0 to 0.8% combined from two greenhouses in the early rainy season and late rainy season of 2011.

Sources of variation	df	Total soluble solids	Plant diameter	Plant height	Stalk yield
Season (S)	1	7.69**(0.4)	19.40**(1.8)	43776.70**(10.1)	56071**(3.4)
Rep within season	6	0.21(0.1)	0.09(0.0)	13.5(0.0)	9(0.0)
Variety (V)	3	221.57**(35.7)	64.03**(17.8)	2725.20**(1.9)	24545**(4.4)
NaCl (N)	4	129.95**(27.9)	157.42**(58.2)	84582.30**(77.7)	356064**(85.3)
S×V	3	38.38**(6.2)	12.67**(3.5)	3238.40**(2.2)	15466**(2.8)
S×N	4	34.90**(7.5)	9.51**(3.5)	1377.60**(1.3)	556**(0.1)
V×N	12	21.62**(13.9)	7.71**(8.5)	1520.90**(4.2)	2043**(1.5)
S×V×N	12	12.53**(8.1)	5.24**(5.8)	879.50**(2.4)	3374**(2.4)
Error	114	6.15(6.1)	8.76(10.5)	942.00(0.0)	1317(0.0)
C.V. (%) (a)		3.58	2.96	2.11	1.93
C.V. (%) (b)		1.84	2.82	1.65	2.19

** Significant at $\alpha=0.01$

Number in parenthesis is percentage of mean squares compared to total mean squares.

Table 3. Mean values of total soluble solids, plant diameter, plant height and stalk yield of four sweet sorghum varieties grown at five concentrations of NaCl in the early and late rainy season in a greenhouse.

Variety	Total soluble solid	Plant diameter (mm)	Plant height (cm)	Stalk yield (g/plant)
Urja	15.8 ^a	10.76 ^b	179.20 ^b	176.58 ^a
KKU 40	13.1 ^b	10.95 ^a	168.24 ^c	171.94 ^b
Suwan Sweet 5	11.1 ^c	8.26 ^d	183.55 ^a	122.44 ^d
Sugar T	10.6 ^d	9.38 ^c	166.68 ^d	149.66 ^c
NaCl (%)				
0	13.9 ^b	13.13 ^a	248.95 ^a	320.53 ^a
0.2	15.1 ^a	10.56 ^b	199.80 ^b	191.44 ^b
0.4	12.1 ^d	9.56 ^c	162.46 ^c	119.21 ^c
0.6	9.8 ^e	8.76 ^d	143.93 ^d	92.55 ^d
0.8	12.4 ^c	7.18 ^e	116.96 ^e	52.05 ^e

Means in the same column with the same letter(s) are not significantly different at $\alpha=0.05$ by DMRT.

Table 4. Changes in total soluble solids, plant diameter, plant height and stalk yield of four sweet sorghum varieties grown at five concentrations of NaCl in the early and late rainy season in a greenhouse (average).

NaCl (%)	Changes (%)			
	TTS	Stalk diameter	Plant height	Stalk yield
0 (control)	0.0	0.0	0.0	0.0
0.2	8.6	-19.6	-19.8	-40.3
0.4	-13.1	-29.5	-34.7	-62.8
0.6	-29.6	-36.3	-42.2	-71.1
0.8	-13.7	-67.4	-53.0	-83.8

Minus sign indicates reduction.

Table 5. Percent reduction (or increase) for fructose, glucose, sucrose, total sugar, NaCl (NaCl) concentrations in juice and ethanol concentration of three sweet sorghum varieties grown under different NaCl concentrations in a greenhouse in the early rainy season of 2011.

NaCl	Fructose	Glucose	Sucrose	Total sugar	NaCl in juice	NaCl in soil	Ethanol yield
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.2	9.0	-0.5	2.5	2.7	229.9	551.2	-1.2
0.4	13.4	-34.2	6.0	0.2	605.9	1095.9	-12.8
0.6	1.6	-54.3	8.7	-2.1	637.0	1318.4	-14.0
0.8 ^{1/}	NA	NA	NA	NA	NA	2157.4	NA

Minus sign indicates reduction. NA indicates not available data because the plants died or were not healthy. ^{1/} The data for NaCl level at 0.8% and NaCl in soil were not included in analysis of variance, but the data were shown in this table to indicate the patterns of reduction (or increase) in these parameters.

Table 6. Mean values for fructose, glucose, sucrose, total sugar NaCl (NaCl) in juice and ethanol yield of three sweet sorghum varieties grown at four concentrations of NaCl in the early rainy season of 2011 in a greenhouse.

Variety	Fructose (g/L) ^{1/}	Glucose(g/L) ^{1/}	Sucrose(g/L) ^{1/}	Total sugar (g/L) ^{1/}	NaCl in juice(ppm) ^{1/}	NaCl in soil (mg/kg) ^{1/1}	Ethanol yield (g/L)
Urja	15.00	17.73	125.34	158.07	7040.25	0.70	70.54 ^a
KKU 40	15.28	14.83	120.32	150.42	7149.75	0.61	67.51 ^b
Suwan Sweet 5	16.44	24.18	103.35	143.97	6060.50	0.75	68.29 ^b
NaCl							
0%	14.69	24.32	111.54	150.56	1441.67	0.081	73.95 ^a
0.2%	16.02	24.20	114.34	154.56	4756.33	0.530	73.03 ^b
0.4%	16.66	15.99	118.18	150.84	10177.33	0.973	64.51 ^c
0.6%	14.92	11.12	121.28	147.32	10625.33	1.154	63.62 ^d

Means in the same column with the same letter(s) are not significantly different $\alpha=0.05$ by DMRT.

^{1/}Single value for each treatment was averaged from at least three replications of chemical analysis, and analysis of variance was not performed.

Table 7. Correlation coefficients of total soluble solids, ethanol, fructose, glucose, sucrose, total sugar and NaCl (NaCl) concentrations in juice of three sweet sorghum varieties grown under six NaCl gradients in the early rainy season of 2011 in a greenhouse.

	Total soluble solids	Ethanol content	Fructose	Glucose	Sucrose	Total sugar
Ethanol content	0.505**					
Fructose	-0.166	-0.166				
Glucose	0.292*	0.737**	0.399**			
Sucrose	0.287*	0.364**	-0.403**	-0.696**		
Total sugar	0.671**	0.364**	0.039	0.125	0.614**	
NaCl in juice	-0.464**	-0.869**	0.136	-0.790**	0.447**	-0.156

*, ** indicate significance at $\alpha=0.05$ and 0.01, respectively.

Table 8. Correlation coefficients among total soluble solids, plant diameter, plant height and stalk yield of four sweet sorghum varieties grown under different NaCl concentrations in the early and late rainy season of 2011.

	Total soluble solids	Plant diameter	Plant height
Plant diameter	0.306**		
Plant height	0.310**	0.656**	
Stalk yield	0.386**	0.815**	0.891**

** indicates significance at 0.01 probability level.

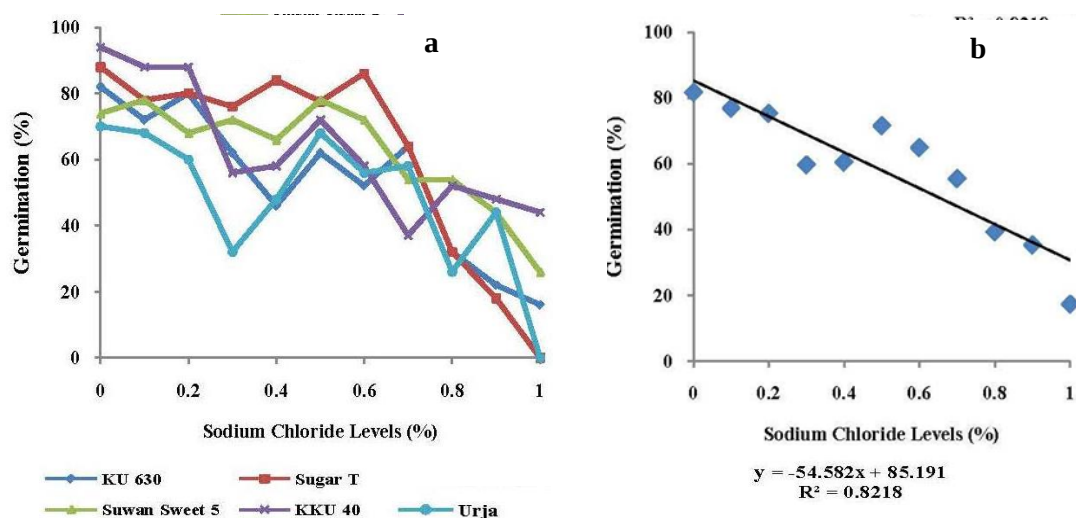


Figure 1 Mean (a) and regression (b) analysis for germination percentage of five sorghum varieties (one grain and four sweet sorghum) germinated under different salinity gradients in laboratory.

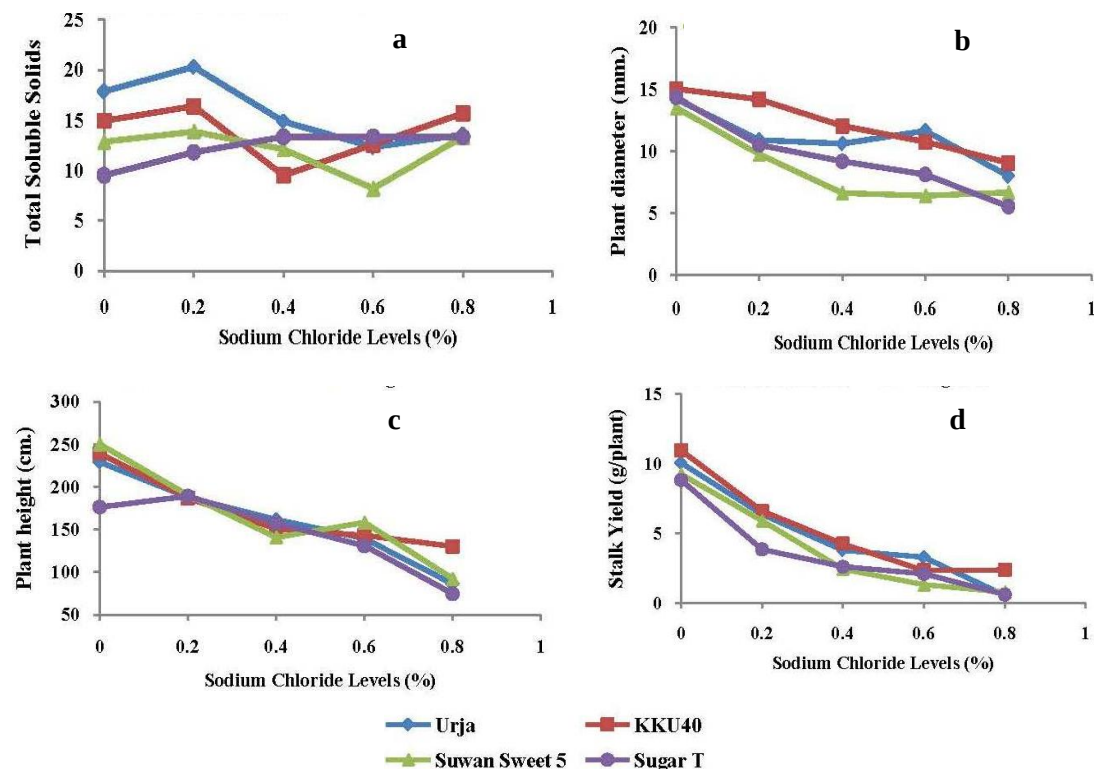


Figure 2. Mean for total soluble solids (a), plant diameter (b), plant height (c) and stalk yield (d) of four sweet sorghum cultivars grown under different NaCl concentrations for two seasons both early and late rainy season in 2011 under greenhouse condition.

in the soil (Table 6). The Urja variety yielded significantly higher ethanol (70.54 g/L) than the KKU 40 (67.51 g/L) and Suwan Sweet 5 varieties (68.29 g/L).

The means values among the various NaCl treatments ranged from 14.69 to 16.66 g/L for fructose, 11.12 to 24.32 g/L for glucose, 111.54 to 121.28 g/L for sucrose, 147.32 to 154.56 g/L for total sugars, 1441.67 to 10625.33 ppm for NaCl in the juice and 0.081 to 1.154 ppm for NaCl in soil. The mean values for ethanol yield ranged from 63.62 to 73.95 g/L with descending trend as sodium concentrations were increased from 0.0 to 0.6% (Table 6).

Correlation among traits

Correlation coefficients were determined among the values for TSS, ethanol content, fructose, glucose, sucrose and total sugar. It was clear that these traits were interrelated as the correlation coefficients were positive and significant ($p \leq 0.01$), ranging from $r = 0.287^*$ to 0.737^{**} ($p \leq 0.01$) (Table 7).

The correlation coefficients between TSS, ethanol yield, fructose, glucose, sucrose, total sugars and NaCl concentrations in juice were evaluated in the early part of the 2011 rainy season. TSS were closely associated with ethanol yield (0.505^{**} , $p \leq 0.01$), glucose (0.292^* , $p \leq 0.05$), sucrose (0.287^* , $p \leq 0.05$) and total sugar (0.671^{**} , $p \leq 0.01$), but it was negatively correlated with NaCl in juice (-0.464^{**} , $p \leq 0.01$) (Table 2). Ethanol yield was not correlated with fructose concentration (-0.166 , $p \geq 0.05$), but it was closely correlated with glucose (0.737^{**} , $p \leq 0.01$), sucrose (0.364^{**} , $p \leq 0.05$) and total sugar (0.364^{**} , $p \leq 0.05$) concentrations. However, ethanol yield was negatively associated with NaCl concentration in sweet sorghum juice (-0.869^{**} , $p \leq 0.01$). Fructose was positively correlated with glucose concentration (0.399^{**} , $p \leq 0.05$), but it is negatively correlated with sucrose concentration (-0.403^{**} , $p \leq 0.01$). Fructose was not correlated with total sugars (0.039 , $p \geq 0.05$) and NaCl in juice (0.136 ; $p \geq 0.05$). Glucose was negatively correlated with sucrose (-0.696^{**} , $p \leq 0.01$) and NaCl in the juice (-0.790^{**} , $p \leq 0.01$), but it was not correlated with total sugars (0.125 , $p \geq 0.05$). Sucrose was closely associated with total sugars (0.614^{**} , $p \leq 0.01$) and NaCl in the juice (0.447^{**} , $p \leq 0.01$), whereas NaCl in the juice was not associated with total sugars (-0.156 , $p \geq 0.05$) (Table 2).

Discussion

Sorghum is more tolerant of salinity than other grain crops such as maize, wheat and rice (Shrivastava and Kumar, 2015; Almodares and Hadi, 2009). It was expected that sweet sorghum could be successfully and profitably produced in saline soil for ethanol production. The results of previous investigation of salt tolerance of sweet sorghum were very encouraging. The current study experimentally tested the assumption that production of sweet sorghum in saline soil might be successful. As control of salinity levels under field conditions is difficult, this experiment was conducted in a greenhouse to quantify the effects of soil salinity on seed germination, growth, stalk yield and ethanol yield of sweet sorghum. The results of this study did not support previous claims that sorghum is a salt tolerant crop. Although sorghum is widely considered a salt-tolerant crop, the findings of this study contradict that generalization, showing that sweet sorghum genotypes experienced reductions in seed germination, growth, stalk yield, and ethanol yield under salinity stress. This raises the question: is the observed sensitivity due to the specific type of sorghum used i.e., sweet sorghum rather than sorghum as a species.

It was observed that sweet sorghum was not a salt tolerant species as it accumulated high concentration NaCl under saline conditions. In previous studies, salt tolerant sorghum varieties failed to exhibit an efficient ion exclusion mechanism of other salt tolerant species, but rather accumulate higher concentrations of Na⁺ and Cl⁻ ions over 72 h of salt stress (Jogeswar et al., 2008). The results in the current and some previous studies found that sweet sorghum is not salt tolerant.

A clear and severe reduction in seed germination was observed at 0.4% salinity. The results showed that sorghum can germinate successfully if sodium concentration was not higher than 0.4%. Concentrations of 0.4% NaCl or higher resulted in germination of less than 80% of the seeds. Salt stress also delayed germination time. Germination consistently declines as NaCl concentration increases, 200-300 mM NaCl typically results in severely reduced or zero germination for sensitive cultivars and cultivar-specific responses are well-documented, supporting the observed behavior in Soave, Rio, Sofra, and Keller (Kaur and Singh, 2023; Elhindi et al., 2022; Ahmed and Ghoneim; 2021). At salt concentrations of 0, 100, 200 and 300 mM NaCl, seed germination of four cultivars of sorghum (Soave, Rio, Sofra and Keller) decreased significantly as salt concentration was increased, and Sofra did not germinate at 200 and 300 mM NaCl (Almodares et al., 2007). Multiple recent studies confirm that salt stress delays the germination process in sweet sorghum, extending the time required for seeds to sprout, this is due to the osmotic effect of NaCl, which hampers water uptake, and ionic toxicity, which interferes with metabolic processes essential for germination (Kaur and Singh, 2023; Elhindi et al., 2022). Sorghum germination remained stable under salinity < 5 dS m⁻¹, and decreased sharply at >10 dS m⁻¹ and at 30 dS m⁻¹, germination dropped to around 48–52% depending on cultivar (Elhindi et al., 2022; Gorai, and Neffati, 2021).)

The results of the current study support theoretical expectations and previous findings that salinity reduces seed germination and delays germination times. The germination threshold in this study (0.4%) was also similar to previous findings. The EC values for salt concentration levels in this study were also similar to those reported previously (Rhoaded et al., 1999).

The effects of elevated salinity on the various sorghum varieties were significant. Therefore, identification of sorghum genotypes for better seed germination was not possible in this study. High interactions between sorghum genotypes and salinity levels were also observed for all characteristics under investigation. This study therefore was not focused on comparison of sorghum genotypes for salt resistance.

Salinity did not have significant effect on TSS as indicated by inconsistent effects of salinity across salinity levels. However, salinity did increase the NaCl concentration in the juice. The results implied that salinity reduced sugar content and at the same time increased NaCl concentration in juice. Since TSS includes both sugar and salt, salinity had a small effect on TSS.

In a previous study, differences among sorghum genotypes for salt tolerance were observed at germination and growth stages. Soave is a cultivar recommended for soils with high salinity (Almodares et al., 2007). However, a study on salt tolerance at the time of harvest is not available for sweet sorghum. To the best of our knowledge, the current study is the first report on the effect of salinity on TSS in sweet sorghum. The study revealed that sweet sorghum does not tolerate salinity as high accumulations of salt in its juice were observed, and there was no juice yield at very high salinity.

Most studies on salt tolerance in sweet sorghum have so far been conducted at germination and seedling stages less than 45 days after planting. Salt tolerance at germination and seedling stages might not be related to salt tolerance at harvest since this occurs about 120 days after sowing. This may have allowed more salt to accumulate in the stems rather than the plants. At harvest, most, if not all sorghum varieties are intolerant of salt stress.

Salinity significantly reduced plant diameter, plant height and stalk yield. These reductions were 19.6 to 67.4% for plant diameter, 19.8 to 53.0% for plant height and 40.3 to 83.8% for stalk yield. It is notable that stalk yield was very sensitive to salinity as it was reduced as much as 40.3% at concentration of 0.2% (3.37 dS m⁻¹).

Recent studies confirm that plant adaptation to salt stress involves enhanced capacity to regulate ion concentrations, particularly through selective uptake, compartmentalization, and exclusion of toxic ions like Na⁺ and Cl⁻ (Munns and Gilliam, 2015; Zhu, 2016; Roy et al., 2014). These recent studies confirm that KFS2 is among the most salt-tolerant forage sorghum genotypes at the germination and early seedling stages, as evidenced by higher shoot length and germination rates under NaCl or EC stress (Aref and Ghanbari, 2022; Mohammadi and Ghassemi-Golezani, 2021). Direct comparison of the results is not possible because there was no report evaluating sweet sorghum at harvest. Our results indicated that sweet sorghum did not adapt to salt stress as small concentrations of NaCl had great effects on sweet sorghum growth parameters and stalk yield.

Salinity significantly reduced glucose but it did not significantly affect sucrose, fructose and total sugar concentrations. The reason for the reduction in glucose content is not fully understood. Soil salinity increased NaCl in juice and NaCl in soil from 229.9 to 637.0% (at 0.6% of NaCl or 12.40 dS m⁻¹) and 551.2% to 2157.1% (at 0.8% of NaCl or 18.43 dS m⁻¹), respectively.

These results supported previous studies findings that sweet sorghum is not drought tolerant since it has no mechanism to eliminate NaCl. This was indicated by the very high NaCl concentrations in the juice at harvest. In the current study, sweet sorghum treated with salt concentration higher than 0.8%, had no juice in its stalks. Evaluation at harvest showed very high salt accumulation in sweet sorghum juice and it was intolerant of high salt concentrations.

The reduction and eventual loss of ethanol production in sweet sorghum under increasing NaCl concentrations is a multifaceted issue involving osmotic stress, ionic toxicity, metabolic inhibition, and sugar content reduction. These stressors impact both the production of fermentable sugars and their conversion to ethanol, necessitating the development of salt-tolerant genotypes. Salinity stress in sweet sorghum leads to increased Na⁺ and Cl⁻ ion concentrations, which are detrimental to plant growth and development. Salt-tolerant genotypes, such as SSV84, maintain higher K⁺/Na⁺ ratios, which is crucial for mitigating ionic toxicity and ensuring better growth under saline conditions (Almodares et al., 2014). Salinity affects the metabolic pathways in sorghum, leading to reduced growth and ethanol production. Tolerant genotypes exhibit specific metabolic changes, such as increased levels of spermidine and spermine, which are associated with better stress adaptation (Oliveira et al., 2020).

Salinity reduced ethanol yield from 1.2 to 14.0% (at 0.6% NaCl concentration). In a previous study, it was found that salts can be significant inhibitors of *S. cerevisiae*. The cell growth rate, glucose consumption rate, and ethanol production rate were all reduced by salt (Loannis et al., 2011). Different strains of *Saccharomyces* sp. showed variations in ethanol conversion efficiency from sweet sorghum juice (Bulawayo et al., 1996). The results of the current study indicated that sweet sorghum grown in saline soil is not suitable for ethanol production, but the use of salt tolerant *Saccharomyces* sp. may increase the efficiency of ethanol production.

The productivity of sweet sorghum is determined by stalk yield and sugar content. Under the salt stress in this study, TSS were well correlated with growth parameters such as plant height, stem diameter and stalk yield. In a previous study under normal conditions, plant height, TSS, fresh stalk yield manifested positive significant correlation with ethanol yield (Rani and Umakanth, 2012). The results indicated that the relationships between TSS and growth parameters were interrelated across cultivation conditions.

The relationship between TSS, sugar components, and ethanol yield in sugarcane is significantly influenced by salinity, particularly NaCl. Increased salinity negatively impacts sugarcane yield, extractable juice, and soluble solids, with these parameters showing a strong negative correlation with Na⁺ and Cl⁻ concentrations. Increased Na⁺ and Cl⁻ concentrations were associated with reduced biomass production and yield, as salinity disrupts nutrient uptake and osmotic balance (Morais et al., 2022). Increasing salinity significantly decreased juice recovery, brix, and pol in sugarcane, negatively correlating with Na⁺ and Cl⁻ concentrations. The results suggested that salinity also reduced sugar concentration in the juice of sweet sorghum (Kumari, 2017). Most sugar components were closely related to ethanol yield except for fructose, but it was negatively correlated with NaCl in juice.

Materials and Methods

Plant materials

Laboratory experiments: Effects of salinity on seed germination

Four sweet and one grain sorghum varieties were used in this study. Urja is a pure line sweet sorghum variety from Praj Co. Ltd., India. KKKU40 is a pure-line sweet sorghum variety, was developed by Khon Kaen University, Thailand. Suwan Sweet 5 is a pure-line sweet sorghum variety and was developed by the National Corn and Sorghum Research Center Kasetsart University, Thailand. Sugar T is a hybrid sweet sorghum variety, it was donated from Pacific Seeds Co., Ltd. Thailand. KU630 is a pure-line grain sorghum variety, was developed at the National Corn and Sorghum Research Center, Thailand and was used as a control variety.

Five sorghum varieties were set as factor A and 11 levels of NaCl (0% as a control, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0%) were assigned as factor B in a 5×11 factorial experiment. The 5×11 treatment combinations were arranged in a completely randomized design (CRD) with four replications. This research was done at the Seed Laboratory of the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, and there were 50 seeds per replicate. The seeds were germinated between two moist paper sheets and stored in a germination cabinet at 25°C. Germination percentage was observed at 7 days after treatment and at 10 days after treatment (ISTA, 1996). The data at 10 days after treatment were used for analysis.

Field experiment: Effects of salinity on growth and fresh stalk weight

Four sweet sorghum varieties (Urja, KKKU40, Suwan Sweet 5 and Sugar T) were used in this study. The details of these varieties are given in previous section. Four sweet sorghum varieties were assigned as factor A and five levels of NaCl (0% (control), 0.2, 0.4, 0.6 and 0.8%) (factor B). The experimental design is arranged in a 4×5 factorial in CRD with four replications. This research was studied in two seasons in the early rainy season during April to September 2011 and late rainy season during October 2011 to February 2012 in a greenhouse at the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University. The experimental unit consisted of 6 containers with one plant in each container for treatment in a replicate.

The soil is Yasothorn soil series (Oxic Paleustults), it was collected from the Field Crops Research Station, Khon Kaen University. The soil was air-dried in a shelter and sieved. Soil samples were analyzed for physical and chemical properties (Data not reported). The soil was then mixed with NaCl at concentrations of 0, 0.2, 0.4, 0.6 and 0.8% and filled into plastic bags containing 30 kg of soil for each bag. Soil properties after treatment are reported in Table 1. The bags were placed in containers that were 38 cm in diameter and 28 cm high. The soil surface was 10 cm below the rim of the containers.

Water was supplied to the containers at a field capacity level for 24 hours prior to sowing. N-P-K fertilizer with the formula 15-15-15 was applied to the containers at the rate of 1.2 g per container. Carbofuran 3%G was applied at the rate of 0.24 g per container to control sorghum shoot fly (*Atherigona soccata*). The seeds were over-planted at the rate of 4 seeds per container, and the seedlings were later thinned to obtain one seedling per container 7-10 days after sowing. N-P-K fertilizer formula 15-15-15 was applied at the rate of 1.2 g per container for the second split 30 days after sowing.

Water was carefully supplied to the crop daily to avoid water stress, and soil moisture was maintained at field capacity throughout the experiment. Pests and diseases were controlled when necessary.

Plant height, plant diameter, fresh and dry weight of the above ground part were recorded for randomly chosen two plants from each replicate at 60, 90 and 120 days after sowing. After 120 days, the data for TSS and NaCl concentration in juice were recorded.

Effects of salinity on juice quality and ethanol yield

After harvesting sweet sorghum and sweet sorghum stalks were then squeezing by sugarcane squeezer. The juice was taken to the laboratory for fermentation. The yeast *Saccharomyces cerevisiae* DBKKUY-53 was used in this study. One loop full of yeast in yeast extract malt extract (YM) agar was transferred into fresh YM broth. The culture medium was incubated on a shaker at 200 rpm for 18 hours. The broth culture was used as an inoculum into the juice of sweet sorghum at an initial sugar concentration of 12 °brix with yeast concentration of 1.0×10⁸ cells/ml. The sorghum yeast culture was incubated on a shaker at 200 rpm per minute for another 18 hours at 30 °C. The yeast culture growing on sweet sorghum juice was centrifuged (Sorvall RC 26 Plus) at 5,000 rpm at 20 °C. The supernatant was discarded, and the yeast cells were dissolved in an aqueous 0.85% NaCl (w/v) solution. The yeast suspension was used as an inoculum for ethanol production from sorghum juice.

Fermentation of the sweet sorghum juice of each sample to ethanol was carried out in a 250 ml flask with 150 ml of juice. The juice samples in their flasks were autoclaved at 110 °C for 28 minutes, and they were allowed to cool at room temperature. Then, yeast was inoculated into the juice to an initial concentration of 1×10⁸ cells/ml and incubated at 30 °C for 12, 24, 36 and 48 hours. Then the samples were centrifuged at 13,000 rpm for 15 minutes. The ethanol concentration in the supernatant was determined using gas chromatography (Shimadzu GC 14B, Japan) (Laopaiboon et al., 2009).

Total sugar, fructose, glucose, sucrose, and NaCl concentrations in the juice as well as ethanol yield were determined and reported as the average of four replicates. The experimental design for ethanol yield was a completely randomized design.

Statistical analysis

Data were analyzed statistically according to the experimental design for each experiment. Data for seed germination was analyzed according to a 5×11 factorial experiment in a completely randomized design. Data for plant growth, juice yield, NaCl concentration in juice and ethanol yield were analyzed for each season according to a 4×5 factorial experiment in a completely randomized design. Error variances were tested for homogeneity and a combined analysis of the data of the two seasons was performed. When the main effects were significant at a 95% (p≤0.05) probability level, Duncan's multiple range test (DMRT) was used to compare means.

The data for ethanol yield were analyzed in a completely randomized design, and DMRT was used to compare means.

Conclusions

This study demonstrated that increasing NaCl concentrations significantly reduced seed germination, growth traits, juice quality, and ethanol yield in sweet sorghum. Among the five varieties tested, KKK 40 exhibited the highest germination rate under mild salinity, while Urja and Sugar T were most sensitive, failing to germinate at 1% NaCl. Salinity had the most pronounced effects on stalk yield (85.3% variation) and plant height (77.7%), while genotypic differences contributed most to variations in TSS (35.7%) and stem diameter (17.8%). Strong genotype $\times$ environment interactions hindered consistent identification of superior varieties, although KKK 40 and Urja showed favorable performance across several traits.

Juice quality parameters (e.g., glucose, sucrose, total sugar) and ethanol yield declined with increasing salinity. Notably, Urja produced the highest ethanol yield (70.54 g/L), but ethanol production was negatively correlated with NaCl concentration in juice ($r = -0.869^{**}$, $p \leq 0.01$). Positive correlations between TSS and sugars suggest that salinity-induced ion accumulation directly impacts fermentable sugar availability and conversion efficiency. These findings emphasize the need for selecting salt-tolerant sweet sorghum genotypes with stable sugar profiles to ensure biomass productivity and ethanol yield under saline conditions.

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