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Effects of sodium chloride on seed germination and seedling establishment of sorghum genotypes

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Salinity and climate change are major threats that affect crop productivity in arid and semi-arid fields globally. Sorghum is a climate smart crop but wide range of sorghum genotypes grown is sensitive to salt. Sorghum was screened for salt tolerance using sodium chloride (NaCl) at different concentrations. There were 250 evaluated sorghum genotypes using factorial arrangement in a Completely Randomized Design (CRD) with 4 levels of NaCl concentrations (0, 3, 5 and 7) dSm^{-1} and three replications. Germinated seeds were determined on the 4th day after planting and transferred to nutrient saturated plastic cups in a greenhouse. Data on root hair numbers, root and shoot length were taken on the 6th day after transplanting. Data were subjected to R4.2.0 for ANOVA and SPSS V. 20.0 for cluster analysis. There were significant differences in root hair numbers, root length and shoot length among the genotypes and salt levels (P ≤0.001). Pearson correlation coefficient showed a high positive correlation (P ≤ 0.001) between root length and root hair numbers. The results revealed presence of tolerance among local sorghum genotypes with promise for use in crop improvement.

Key words: Climate change, salinity, tolerance, root length, root hairs.

INTRODUCTION

Sustainable crop production remains a concern as the world's population and food demand increase (Hunter et al., 2017, Griffiths and York, 2020). Strategies to improve food production in response to increasing demand are threatened by climate change occurrences. This calls for a deliberate effort to identify and promote climate-smart crops, among them being sorghum (*Sorghum bicolor* L. Moench). Sorghum is ranked 4th among major cereal crops globally and is grown on approximately 44 million ha with a production of about 58 million tonnes (FAOSTAT, 2021). It produces adequately well under conditions of relatively low soil moisture. However, salinity which is also referred to as a '*quiet crisis*' (Haw et al., 2000) limits the promotion and utilization of sorghum. Salinity is a major abiotic challenge to crop productivity with a total affected area approximated to be 800 M ha globally (Munns and Tester, 2008; Zheng et al., 2020; Xu et al., 2021). The increasing drought and irrational irrigation are expected to rapidly expand the saline land since most of the water being used contains about 3% sodium chloride (NaCl) salts (Robin et al., 2016).

Kenya's arid and semi-arid lands (ASALs) covers about 80% of the total area (Maina et al., 2019) with 50% of this predominated by saline soils (Mugai, 2004). The

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condition has been increasing over the years due to increased population and adverse encroachment to virgin lands for food and livelihood leading to the current accentuated climate change effects and reduction in food and feed crops production. The effects of climate change are compounded by salinity in the ASALs thus limiting the promotion of sorghum and other climate-smart crops as adoption mechanism (GoK, 2009; Robin et al., 2016). The government has widely promoted sorghum production among smallholder farmers owing to its resilience to soil water deficit unlike other cereals. Its promotion is a strategy to meet the 2030 sustainable development goals (SDGs) of improved food security, nutrition and increased rural income (GoK, 2009; Ochieng, et al., 2011) in the marginal lands. However, there are no known varieties which have been recommended for saline conditions in Kenya.

The main aim of this study was to identify sorghum genotypes that are tolerant to salt for the development of suitable and adaptable varieties to saline environments. It was hypothesized that NaCl has no effect on seed germination and seedling establishment of sorghum. The results were expected to generate useful information for sorghum improvement and selection for the saline environments.

MATERIALS AND METHODS

Evaluated sorghum genotypes

A total of 250 diverse sorghum genotypes were evaluated for salt sensitivity. The genotypes were sourced from the Kenya Gene Bank, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Farmers' collection and a few from Eastern Africa Region.

Experimental procedures

The 250 genotypes were subjected to 4 levels of NaCl concentration: 0, 3, 5, and 7 deciSiemens per metre (dSm^{-1}) with zero (0) being control. The experiment was laid in a Completely Randomized Design (CRD) arrangement and replicated 3 times. Different levels of NaCl were prepared by dissolving solid NaCl with distilled water until the desired treatment level was attained with the help of an Electrical Conductivity (EC) meter. Ten seeds per genotype were placed on Whatman's filter papers which was placed on Petri dishes and soaked with different NaCl treatments. The seeded petri dishes were placed in a multi-layered incubator chamber at a temperature maintained at 23ºC for four days. The number of seeds germinated was counted daily for four days after placement.

After four days, three seedlings of each of the germinated genotypes were transplanted into plastic cups (diameter 20 cm × 15 cm height) enriched with nutrients but containing NaCl treatments levels as their petri dishes. The nutrient used was Hydro A and Hydro B hydroponic nutrients.

Nutrient preparation and application

Nutrient solution was prepared by taking and dissolving 2 g each of Hydro A and Hydro B nutrients in 1L of distilled water. A known amount of NaCl was added to the prepared nutrient solution while checking the EC to ensure attainment of the desired level of concentration. The procedure was repeated for each of the salinity levels except for the control which was saturated with nutrients only. Enough quantity of the solution was added to each of the plastic cup to cover the roots of germinated seedlings and topping-up was done daily when necessary. The transplanted plants in the plastic cups were transferred to a greenhouse whose average day and night temperatures were 30ºC and 25ºC respectively. The plants were allowed to grow for six days after which, they were analyzed for root and shoot growth. The seedlings were gently washed with water over a 0.2 mm sieve to avoid loss of roots and shoots.

Data collection

Germination measurement

Final seed germination percentage was calculated from the final germination count on the fourth day using the following formula:

$$FGP\ (\%) = \frac{S_G}{S_T} \times 100\% \quad (1)$$

Where, *FGP (%)* = Final germination percentage, S_G = Total number of seeds germinated, and S_T= Total number of seeds taken for germination.

Root analyses

Root length was determined by measuring the root from its base to the tip. Root hairs were counted with the help of magnifying hand lenses.

Shoot measurement

Shoot length was determined by measuring the shoot length from crown to the topmost visible leaf color.

Statistical analysis

Data were analyzed for Analysis of Variance (ANOVA) using R Version 4.2.0. The effects of NaCl concentration was determined for seed germination and seedling establishment.

Salt tolerance indices were analyzed by cluster using SPSS V. 20.0 (SPSS Inc. 2007 Chicago, IL, USA) statistical software. Cluster analysis identifies variables which were further clustered into groups using Ward's Method (Kumar et al., 2014; Singh et al., 2015) to identify genotypes with certain levels of salt tolerance.

RESULTS

Influence of NaCl concentration on germination of sorghum genotypes

Germination of sorghum genotypes was negatively affected by salt concentration. Germination of sorghum progressively declined with increasing salt concentration (Figure 1). Despite the general decline in germination with increase in salt concentration, there was no significant difference between the control and low NaCl concentrations (3 and 5 dSm^{-1}).

Some sorghum genotypes sustained relatively high germination at 7 dsm^{-1} (high concentration) though their significance was negligible. Others showed a rapid decline in germination even under mild salt concentration indicating that they are susceptible to salt. Lesser decrement in germination percent (0%) from control to high salt concentration was evident in Seredo, BM 29, and BM 17, and high decrement of up to 25% was expressed in IESV 94076 DL, GBK 000382, IS 21158, and LARSVYT 58 85. The number of seeds germinated ranged from 8-10 in control and 4-10 in 7 dSm^{-1}.

Effects of NaCl on shoot growth

Shoot length for all the genotypes was negatively affected by salt concentration at P< 0.001 (Table 1). High shoot performance was evident in the control and progressively reduced with increasing NaCl concentration (Figure 2). The most susceptible genotypes showed a rapid reduction with some not showing the possibility of shoot emergence while others had a lesser reduction. Higher shoot length reduction was evident in BUSIA 28-1, IS 21158, IESV92022/1SH, EST 20 and SHAMBUKO (PP 290) all of which showed 100% reduction and lesser in AINAMOI (0%), IESH 22002 (0%), GBK 000098 (2%), IESV 23006 DL (5%), GBK 000070 (5%) and GBK 000111(10%). The rest of the genotypes had a medium reduction percentage.

Effect of NaCl on root hairs and root length

Root length and number of root hairs were significantly affected by NaCl concentration (Figure 2). The number of root hairs and root length reduced with increasing salt concentration at P<0.001 (Table 1). The reduction was genotype-specific where some showed drastic reduction compared to gradual response by others.

The reduction in root length as per cent of the control from 0-7 dSm^{-1} was noticeably small in GBK 000366 (0%), LABA (MW 5003) (5%), GBK 000027 (9%), EST 26 (10%) and ICSR93034 (12%). A high reduction in root length of up to 100% under the same treatments was observed in SIAYA 42, IS 21158, SIAYA 27 3, EST 20, and ICSR 24008. The rest exhibited medium change. Root length ranged from 0.2-18.1 cm in the control and 0-14.5 in the highest salt concentration treatment.

The number of root hairs for different genotypes varied dependent on root length at different salt concentrations. Using the number of root hairs as an indicator, EST 26(0%), MAKUENI LOCAL (1%), PP 290 (10%), BM 27 (13%), and IKINYARUKA (15%) were some of the genotypes which had high numbers with small percent reduction when compared to their control. IESV23008DL, GBK 000387, IS 2558, LARSVYT 58 85 and IS 25557 had 100 % root hair reduction relative to the control. The number of root hairs ranged from 1-90 in control and 0-55 in high concentration (7 dSm^{-1}). Parameters performance was high in the control and reduced as salt level concentrations increased.

Correlation of root hair numbers, root length, shoot length and final germination percentage

The Pearson correlation analysis using respective means from ANOVA showed a positive relationship between the variables at P<0.001 (Table 2). A high positive correlation was observed between root length and number of root hairs. The high correlation between root length and shoot length was expected given that increase in length is indicative of growth which means cell differentiation was taking place and hence the production of root hairs.

Cluster analysis for salt tolerance

Mean Membership Function value (MFV) was used to aggregate the genotypes into salt-sensitive and salt-tolerance groupings. The methods have been used widely in this regard (Ding et al., 2018; Wu et al., 2019). Data for root length, shoot length, and the number of root hairs was analyzed by cluster for salt tolerance indices. The mean MFV for each of the physiological parameters taken was calculated. The results were used to classify genotypes in a hierarchy for salt tolerance and sensitivity at different salt levels. The genotypes were divided into five clusters: highly salt tolerant (HST), salt tolerant (ST), moderately salt tolerant (MST), salt sensitive (SS) and highly salt sensitive (HSS) (Figure 3).

Hierarchical analysis for the 250 genotypes used showed that BM 17 was the highly salt tolerant genotype at all levels of salt concentration used followed by GBK 000049, GBK 000038, and BM 29, while the highly salt sensitive genotypes were EST 41, IESV 23007 DL, and GBK 000073, respectively.

The results showed that using individual parameters and cluster analysis to classify genotypes for salt tolerance has different outcomes which may or may not tally. However, using one parameter to classify crops for salt tolerance may not give enough information because the mechanisms and parameters for tolerance could be diverse and interrelated. These parameters therefore, need to be pooled and screened together to give a clear direction. After cluster analysis, a comparison analysis on the trend of root hair growth for the salt tolerant and salt sensitive genotypes at different NaCl concentrations was drawn (Figure 4).

It is clear that, salt have a significant effect on root hairs. The root hair numbers dropped rapidly from an

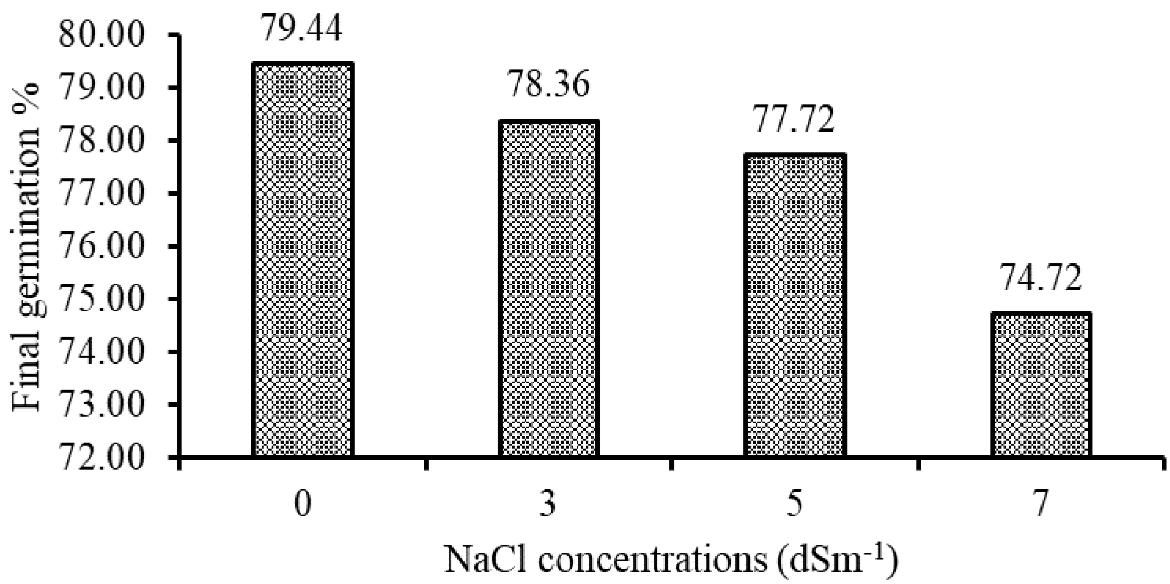

Figure 1. Effects of NaCl on germination of 250 sorghum genotypes.
Source: Authors' Results

Table 1. Mean squares from analysis of variance of 250 sorghum genotypes and four levels of salinity for number of root hairs, root length and shoot length.

Source of variation	df	Number of root hairs	Root length	Shoot length
Genotype	249	682.95***	59.84***	6.32***
Salinity level	3	45484.22***	4565.55***	99.96***
Genotype*Salinity level	747	352.31***	26.18***	1.79***
Error	1802	137.940	9.480	0.800
R^2		0.700	0.7	0.690
CV		11.75	3.10	8.90

***, Significant at ($P \leqslant 0.001$); CV= Coefficient of variation; d.f= degree of freedom.
Source: Authors' Results

average of 28 per root in control to zero at 7 dSm^{-1} of NaCl indicating 100% reduction for the salt sensitive genotypes.

The salt tolerant genotypes had a slow reduction from an average of 21 in control to 12 in 7 dSm^{-1} of NaCl indicating a 50% reduction, though there was a slight decline in root hair numbers at 3 dSm^{-1} of NaCl for the tolerant and rapid decline for the sensitive genotypes, respectively.

This may imply that salt influences some physiological process responsible for root formation and elongation leading to the observed trend (Figure 4).

DISCUSSION

The hierarchical clustering of 250 sorghum genotypes emphasized the genetic variability of sorghum to salt tolerance (Sagar et al., 2019) with a unique response being expressed by the highly tolerant genotypes. The ability of sorghum to adjust to different NaCl concentrations as shown in Figure 4 seems to point to a unique trait associated with salt tolerance in sorghum. However, a salt tolerance mechanism is yet to be fully understood. In this study, we found that salt tolerance in sorghum genotypes cannot be evaluated using a single parameter as done on *Brassica napus* (Wu et al., 2019). Based on the outcome of this study, root hairs appear to be a reliable screening trait for salt tolerance in sorghum.

Germination and seedling growth of sorghum had varied responses to NaCl concentrations. Results show germination of sorghum as being largely unaffected by salt concentrations. These results disagree with other studies that salinity limits seeds germination by limiting

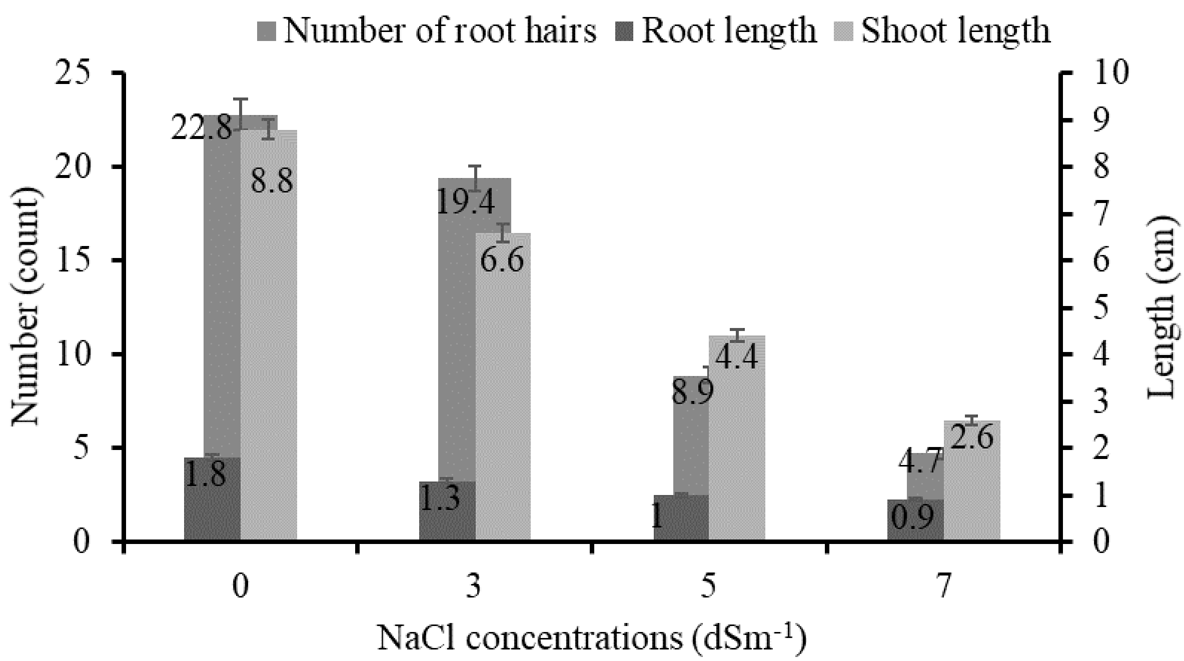

Figure 2. Effects of NaCl concentrations (dSm^{-1}) on number of root hairs, root length and shoot length for 250 sorghum genotypes.
Source: Authors' Results

Table 2. Pearson correlation matrix of number of root hairs, root length, shoots length and final germination percentage.

Variables	Number of root hairs	Root length	Shoot length	Final germination percentage
Number of root hairs	-	-	-	
Root length	0.73***	-	-	
Shoot length	0.27***	0.37***	-	
Germination	0.23***	0.25***	0.13***	

***, Significant at P<0.001.
Source: Authors' Results

water imbibition by the seed leading to increased absorption of sodium (Na^+) and chloride (Cl^-). Increased uptake of Na^+ and Cl^- creates ionic stress and toxicity (Mwando et al., 2020; Tobe et al., 2020) causing disruption of biochemical processes of germination. It was only at a higher NaCl concentration (7 dSm^{-1}) that a decline in germination was observed. In this regard, and due to the limitation of salt on growth, the seeds in the farmers' field may germinate but fail to emerge under saline soil. The adverse effect of NaCl was more visible at seedling stage and therefore, data on germination was not used in clustering genotypes for salt tolerance since it showed no significant contribution.

Though both shoot and root growth was limited by salt at the seedling stage, there was noticeable reduction in the roots. Root growth progressively reduced with increase in salt concentrations. Most of the sorghum genotypes tolerated salt concentration below 5 dSm^{-1} but a few could survive in NaCl concentration above 5 dSm^{-1}. Past reports point to a salt threshold of 1.9 to 4.5 dSm^{-1} for most crops that can allow about 70% yield output and a significant reduction with progressive salt increase above the range (Maas and Hoffman, 1977; Grieve et al., 2012). Sorghum genotypes that survived at 7 dSm^{-1} were considered tolerant to salt. Roots are primary organs exposed to salt in the growth media. The observed effect of salt on roots is no surprise since increased concentrations of salt retard root growth even in halophytes (Li et al., 2010). A more salt-sensitive root component in this study was the root hairs. A rapid decline in root hairs on exposure to NaCl could point to a process in root hair initiation in the trichoblast. Root exposure to NaCl leads to increased Na^+ in cytosol of root hairs (Halperin and Lynch, 2003). Root uptake of Na^+

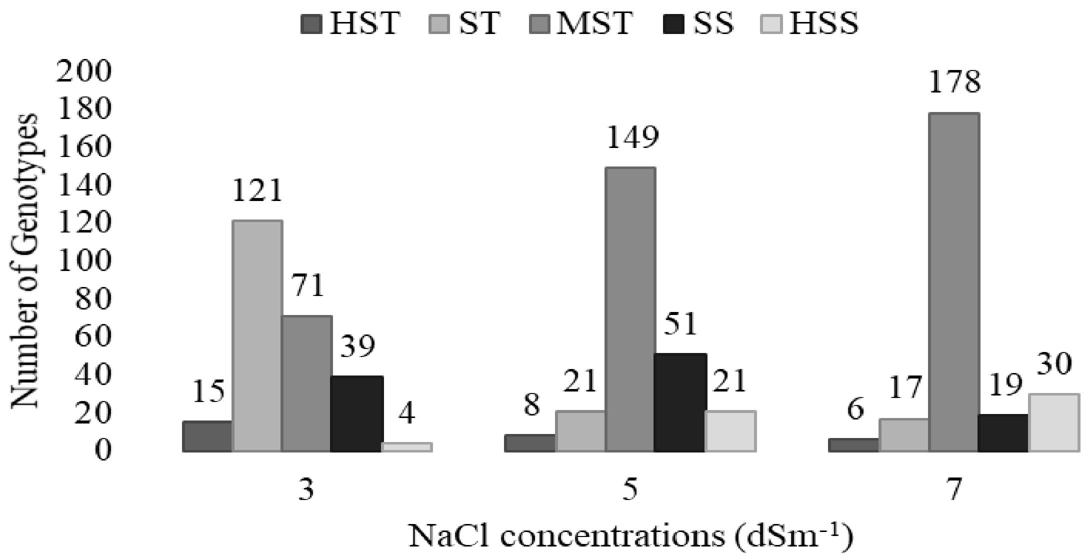

Figure 3. Hierarchical clusters of 250 sorghum genotypes evaluated based on mean MFVat different NaCl concentrations.
Source: Authors' Results

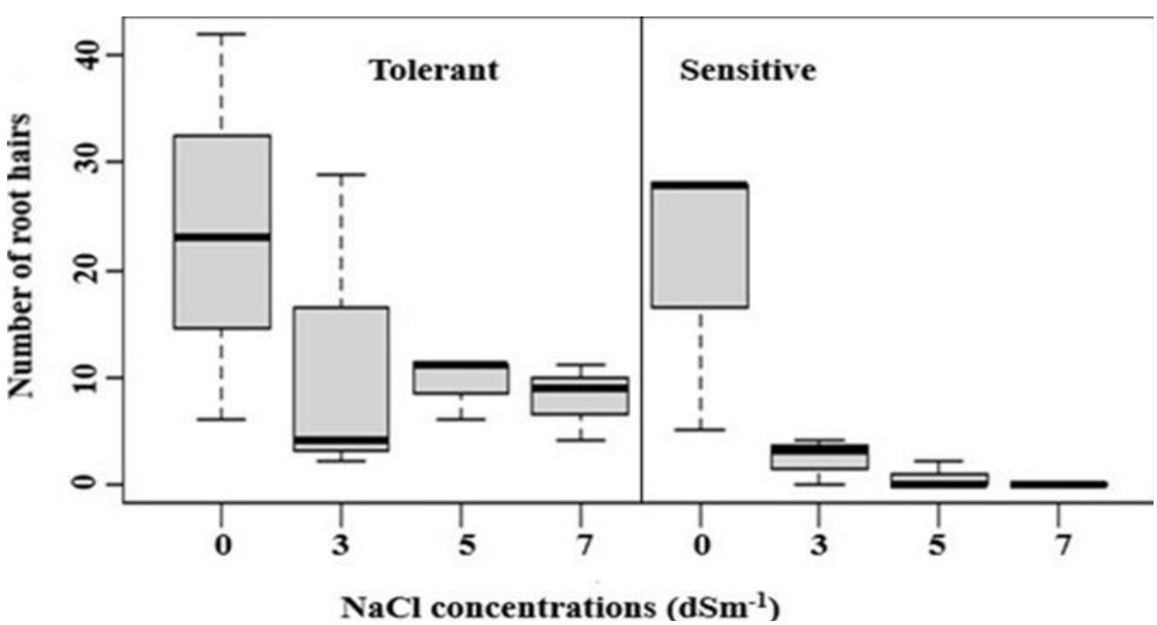

Figure 4. Box plots showing comparison of the effects of NaCl on number of root hairs of the salt tolerant and salt sensitive sorghum genotypes at 0, 3, 5 and 7 dSm^{-1}, respectively. The bars show the maximum and minimum range.
Source: Authors' Results

in *Arabidopsis* has been shown to reduce potassium (K^+) in root cells with a greater reduction in a salt sensitive cultivar (Guan et al., 2013). It has also been shown that high uptake of Na^+ in barley reduces the concentration of calcium (Ca^{2+}) in the cytosol (Shabala et al., 2003). Both K^+ and Ca^{2+} influence cell division and growth. It has been demonstrated that K^+ play a significant role in cell division and cell-cycle progression in plants among other functions (Sano et al., 2007; Xu et al., 2020) while Ca^{2+} is involved in cell division and elongation (Shabala et al., 2003). The reduction in Ca^{2+} and K^+ as occasioned by root exposure to NaCl could partly explain the reduced root hair numbers with increased salt concentrations.

Besides the possible limitation of growth due to reduced Ca^{2+} and K^+ in root cells, direct toxicity of emerging root hairs by NaCl salt was likely (Nxele et al., 2017). However, specific information on the ratios of these ions and root hair growth and/or inhibition as a mechanism of salt tolerance or sensitivity is scanty.

Reduction in shoot growth with increased salt concentration can be associated with shoot dependence on roots for nourishment. The transpiration stream causes increased concentration of Na^+ in the shoot (Tobe et al., 2020). High concentration of Na^+ is known to limit the transport of other ions such as K and nitrogen (N) which are essential for growth (Farooq et al., 2015; Sagar 2017; Sagar et al., 2019). However, shoot ion component and translocation in relation to root uptake as a mechanism of salt tolerance in sorghum is yet to be determined.

Conclusion

The present study demonstrates the existence of large genetic variation for salt tolerance among sorghum genotypes that can be used for the development of varieties suitable for the marginal areas. The variability can be explored during early seedling developmental stage. The root hairs appear to be a reliable parameter for screening for salt tolerance in sorghum. This may present a significant contribution to breeding for salt tolerance in sorghum.

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CONFLICT OF INTERESTS

The authors have not declared conflict of interests.

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