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Effects of salt stress on germination, seedling growth, and ion content of sweet sorghum

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ABSTRACT

Salinity is one of the most common abiotic stresses in the world. It negatively affects the growth and development of sweet sorghum (*Sorghum bicolor* L. Moench). It significantly reduces germination and seedling growth parameters. The present study was carried out to evaluate the impact of four salinity levels (0, 100, 200, and 300 mM) on the germination and seedling growth parameters of four sweet sorghum genotypes (Erdurmus, Uzun, Srg 156, and BSS 424) and on their ion content (Na, K, Ca, and Mg). The results indicate that under nonsaline conditions, the germination percentage (GP) of all genotypes was 100%, and Erdurmus was identified as the earliest germinating genotype. The BSS 424 genotype showed a significant reduction in germination index (GI), ranging from 8.33% at 100 mM to 0.89% at 300 mM, while Erdurmus and Srg 156 showed the lowest decreases, with mean values of 15.801 and 13.901, respectively. The highest root fresh weight (RFW) value was observed in the control for all the genotypes, while Erdurmus showed the lowest decrease. Moreover, the highest decrease in Mg (0.24%) and Ca (0.17%) content was observed in Uzun, and the lowest K content was identified in BSS 424 (0.5%), whereas the highest Na content was also determined in Uzun (3.12%). Considering all the results, salt stress above 200 mM significantly affected the germination and seedling growth parameters. Therefore, lower concentrations should be taken into consideration for sustainable sorghum production.

1. Introduction

Global warming and climate change, considered triggers for factors such as soil salinity, are two of the most important problems in the world (Özyazıcı and Açıkbaş 2021). The salinity of soil affects areas of agricultural production, particularly in arid, semi-arid, and coastal regions (Pankova and Konyushkova 2013; Corwin 2021). It is responsible for losing over 7% of arable land and 33% of irrigated land worldwide (Chele et al. 2021). It also contributes to the loss of 27.3 billion US dollars per year (Wichelns and Qadir 2015; Kumar and Sharma 2020). Salinity negatively affects plant growth and development (Rajabi Dehnavi et al. 2020; Sabagh et al. 2021), which is associated with some morphological, physiological, and biochemical aspects, particularly osmotic stress in young leaves and ion toxicity in older leaves (Munns and Tester 2008). It significantly reduces the length of the shoot and root, as well as the dry weight of the roots and shoots (Hamada and Al-Hakimi 2001; Ashrafuzzaman et al. 2002). In addition, salinity causes slower and dysfunctional germination (Ekmekçi et al. 2005) and reduces the absorption of mineral components by plants (Xu et al. 2016).

Sorghum (*Sorghum bicolor* L. Moench) is one of the most economically important cereals in the world (Tigabu et al. 2012). It ranks fifth in cereal production after wheat, rice, maize, and barley (Bakari et al. 2022). More than 100 countries (Hao et al. 2021) cultivate it on around 40.7 million hectares (FAOSTAT 2024), with an annual production of 57.58 million tons in 2022 (FAOSTAT 2024). Sorghum is an important crop cultivated for its seeds, fodder, and bioenergy production (Steduto et al. 1997; Bakari et al. 2022). Sweet sorghum is a type of sorghum characterized by high sugar concentrations in the stalks (Atokple et al. 2014). Salinity stress limits sorghum productivity (Wang et al. 2003). It represents a serious problem and a major obstacle for global agriculture (Flowers 2004). This reduces the germination ability and affects seedling growth factors, such as the length of the root and shoot and the weight of the fresh shoot (Norlyn and Epstein 1984). Germination and seedling characteristics remain the most commonly used factors in plant salt tolerance selection because of their importance to the crop (Tigabu et al. 2012).

Germination parameters, such as the germination index and germination percentage, are crucial and vulnerable stages in plant development (Hakim et al. 2010). Moreover, seedling growth parameters, such as dry and fresh shoot weight, dry and fresh root weight, and length of root and shoot are the factors commonly used in genotype selection, which is essential for the management of saline conditions (Bybordi and Tabatabaei 2009). Although sweet sorghum tolerates salt stress better than other cereals (e.g., wheat and rice) (Ratanavathi et al. 2004), its germination can be affected by severe salt stress, which reduces its productivity (Zhu et al. 2019). Many studies have shown significant differences in sorghum genotypes in response to various salinity concentrations. For example, Özyazıcı and Açıkbaş (2021) demonstrated that different varieties were more sensitive after a 100-mM salt dose. In contrast, Netondo et al. (2004a) and Gökkaya and Arslan (2023) reported that sorghum plants were sensitive to NaCl above 150 mM.

Selecting salt-tolerant sorghum genotypes by assessing different morphological, biochemical, and physiological plant characteristics contributes to the improvement of crop yields and the sustainability of agriculture (Sagar et al. 2023). In saline conditions, sorghum has the capacity to exclude certain minerals, such as Na (Yang et al. 2018), and reduce its transport from the roots to the leaves by discharging it from the root xylem (Shakeri and Emam 2017; Yan et al. 2015). Specific translocation and absorption of K and Ca compared to Na are determined as another mechanism of salt tolerance in the plant (Shakeri et al. 2020). High concentrations of sodium cause a toxic accumulation in sorghum leaves, affecting the absorption and translocation of K, Ca, and Mg (Netondo et al. 2004a; Bavei et al. 2011), which influences the development and photosynthetic activity of the plant (Netondo et al. 2004b; Joardar et al. 2018).

Generally, increasing salinity significantly reduces germination and seedling growth parameters (Jamil et al. 2006; Okumuş et al. 2023; Okumuş and Şekerci 2024). Identifying the genotype that exhibits resistance to various levels of salinity stress at the early seedling stage is crucial for the advancement of salt-tolerant plants and the achievement of optimal agricultural yields in saline environments (Hakim et al. 2010). Thus, the objective of our study is to investigate the response of four sweet sorghum genotypes to different levels of salinity for (i) germination and seedling growth parameters and (ii) to determine the ion contents (Na, K, Ca, and Mg) in the shoots of these genotypes.

2. Materials and Methods

2.1. Plant materials

In this study, four different sweet sorghum genotypes were used as a genetic material; Uzun, Erdurmus, BSS 424 and Srg156. Erdurmus and Uzun are sweet sorghum cultivars that are registered by the Western Mediterranean Agricultural Research Institute of Türkiye. The BSS 424 is a sorghum elite line originating from the United States (ID number IS 20697). Srg156 is an elite sweet sorghum line obtained by crossing sweet sorghum (Erdurmus) and grain sorghum (Ogretmenoglu) cultivars, reaching the F8 generation.

2.2. Germination experiment

A Petri dish experiment was conducted using a complete randomized design. The experiment involved a factorial design, with four salt treatments (0, 100, 200, and 300 mM NaCl) and four sweet sorghum genotypes. The experiment was performed in three replicates in a growth chamber at Akdeniz University, Faculty of Agriculture, in the Field Crops Department Laboratories. The growth chamber maintained a temperature of 25°C and a 16-hour light period. 48 Petri dishes were prepared with Whatman No. 2 filter paper and filled with varying NaCl solutions. Each dish contained ten sorghum seeds. Observations were performed in Petri dishes for ten days until germination was completed.

A modified version of seed sterilization process Özyazıcı and Açıkbaş (2021) was adapted. Firstly, the seeds were surface sterilized in a solution of 70% C_2H_5OH (ethanol) for 5 minutes, and the seeds were washed with ultra-distilled water. Then the seeds were kept in a solution of 10% NaClO (sodium hypochlorite) for 10 minutes. Afterwards, sorghum seeds were washed with ultra-distilled water. We added 10 ml of saline (NaCl) solution to each Petri dish, except for the control group.

2.3. Germination and seedling growth assessments

Germination number, root, and shoot length of the seeds were observed, and results were recorded daily. At the end of the study, fresh weights of roots and shoots were measured for each sample. Afterwards, the samples were dried at 65 ℃ for two days and their dry weight was recorded.

We evaluated the following variables:

Germination Percentage (GP): GP= (NGS/TS) x100, NGS: Number of normal germinated seeds, TS: Total number of utilized seeds (Scott et al. 1984).

Germination Index (GI): GI= $\sum$ (Gi/Tt), Gi: Germination percentage at the i^{th} day, Tt: Days of germination test duration (Wang et al. 2004).

Total Sodium, Magnesium, Calcium and Potassium Content**:** 0.5 g of the dried shoot samples were taken and 3 ml of HCl (37%), 9 ml of HNO3 (65%) were added. Wet combustion was performed in a digiblock (Labtech ED 36S) combustion unit (U.S. EPA 2007) The total concentration of Na, Mg, Ca and K was determined by ICP-OES (PEOptima 7000 DV).

2.4. Data analysis

The analysis of variance (ANOVA, PROC GLM) was performed with SAS version 9.2.

3. Results

All measured parameters showed statistically significant main effects of salinity and genotypes, as well as the combined effects of their interactions. However, there were no significant differences in genotypes for RDW, SDW and SL, and in the combined effects of genotypes and salinity interactions for RDW, SFW, GI and SL (Table 1).

3.1. Germination parameters

There were significant variations among sorghum genotypes in terms of germination parameters, GI and GP in saline conditions.

Statistical analysis showed that in non-saline conditions, there were no significant differences between genotypes in terms of GP; however, there were significant variations in GI (Table 1). The results indicated that in non-saline conditions, the GP of all genotypes achieved 100% (Table 2). In non-saline condition, Erdurmus was identified as the earliest germinating genotype, while BSS 424 was determined to be the latest genotype (Figure 1). With salinity increasing to 100 mM NaCl, all genotypes showed a decrease in GP between 3.4% and 43.4%. GP decreased significantly in all sweet sorghum genotypes that were exposed to NaCl concentrations of 200 mM and 300 mM. Under 300 mM NaCl, the highest decrease was observed in BSS 424, while the lowest was identified in Erdurmus.

Salinity also significantly decreased GI. Under saline conditions, the maximum reduction was identified in genotype BSS 424, ranging from 8.33% under 100 mM to 0.89% at 300 mM. Erdurmus had the lowest decreases, with a mean of 15.800, followed by Srg 156 with a mean of 13.900 (Table 2).

Table 1. Analysis of variance (*F* value) of four sweet sorghum genotypes in four salinity levels

Traits		NaCl	Genotypes	NaCl X Genotypes
Germination Parameters	**GI**	51.04**	38.04**	0.79ns
	GP	81.80**	39.07**	5.59**
Seedling Growth Parameters	**RFW**	153.89**	13.35**	3.50*
	RDW	13.39**	0.10ns	1.00ns
	SFW	136.34**	8.96*	1.00ns
	SDW	77.60**	5.08ns	5.54**
	RL	131.17**	5.22*	3.25*
	SL	122.41**	2.36ns	1.80ns
Ion Assimilation Parameters	**Mg**	1980.05**	302.18**	68.32**
	Ca	1044.57**	30.07**	27.17**
	Na	975057**	13272.30**	11135.80**
	K	2219.02**	5269.60**	226.55**

Root Fresh Weight; RFW, Root Dry Weight; RDW, Shoot Fresh Weight; SFW, Shoot Dry Weight; SDW, Germination Index; GI, Germination Percentage; GP, RL; RootLength, SL; Shoot Length *; P<0.05, **; P<0.001, ns; non-significant.

3.2. Seedling growth parameters

The salinity levels had a significant impact on the seedling growth parameters of the sweet sorghum genotypes (Table 1). All seedling growth parameters decreased significantly as salinity levels increased. No observations were obtained at the 300 mM level for RFW, RDW, and SDW, as well as at the 200 mM level for RDW (Table 2).

The highest value for RFW was observed in the control condition for all genotypes. BSS 424 had the highest decrease relative to the control in each level, followed by Srg 156, while the genotype Erdurmus showed the lowest decline. The lowest RDW was recorded with a 300 mM NaCl level, while the highest was recorded with 0 mM NaCl for all genotypes; however, there is no significant difference among genotypes.

Other measured seedling growth parameters, SFW and SDW, showed similar trends: there was a significant reduction in SFW and SDW with increasing levels of NaCl concentration (Table 2). However, there was no significant variation observed among the different genotypes for SDW. In the control concentration, the data on SFW showed that the cultivar Srg 156 achieved the maximum value, followed by Erdurmus and BSS 424, while the lowest value was obtained in Uzun.

In the control condition, RL among sorghum genotypes ranged between 4.02 and 6.26 cm, and Srg 156 was identified as having the highest value. (Table 2) The root length exceeded the shoot in Erdurmus, while in the BSS 424, the shoot length exceeded the root length in the control condition (Figure 2). Moreover, a drastic decrease in RL was identified at 200 and 300 mM NaCl for all genotypes. BSS 424 had the highest decrease relative to the control in each level, while the genotype Erdurmus showed the lowest decline. The SL also declined with the higher salinity effect; however, there is no significant difference among genotypes (Table 1).

3.3. Ion content

We identified Na, K, Ca, and Mg concentrations in shoot samples at two NaCl levels: 0 and 100 mM NaCl, because samples at other doses were insufficient. All Na, K, Ca, and Mg parameters were influenced by the salinity level (Table 3). The highest decrease in Mg (0.24%) and Ca (0.17%) content was observed in Uzun, and the maximum decrease in K content was identified in BSS 424 (0.5%), while the highest increase in Na content was also determined in Uzun (3.12%). Compared to other genotypes, Erdurmus and Srg 156 showed a relatively lower loss in ion content despite their high sodium accumulation.

4. Discussion

Seed germination is one of the most important indicators for the successful development of resistant cultivars under salinity stress conditions. In general, when the salt concentration increases, the water potential around the seeds drops. This leads to a smaller difference in water potential between the inside and outside of the seed, which restricts the absorption of water by the seeds (Munns 2002). The impact of salinity on seed germination and plant growth can differ based on the plant species and the various genotypes within a species (Rajabi Dehnavi et al. 2020). Therefore, it is crucial to assess various genotypes in order to understand the mechanism of salt tolerance under different salt concentrations (Ranjbar et al. 2008). For this reason, in this study, we evaluated four sweet sorghum genotypes to understand the response to various salinity concentrations. Our results indicated that the sweet sorghum genotypes exhibited different responses to salinity in germination and seedling growth parameters. The findings of our study indicate that as the level of salt stress increased, there was a noticeable decline in all the seedling growth parameters of the sweet sorghum genotypes. The study found that as the salt concentration increased, the sorghum genotypes experienced a decrease in SL and RL. In saline conditions, it is common for plants to experience a decrease in the lengths of their seedling shoots and roots. This is due to the fact that roots are the first organs to come into contact with salinity, as they are in direct contact with the soil (Asaadi 2009). This finding aligns with previous studies conducted by Bashir et al. (2011), Nimir et al. (2014), Rajabi Dehnavi et al. (2020) and Özyazıcı and Açıkbaş (2021). Furthermore, we discovered that the impact of salinity on root parameters was more severe than its influence on shoot parameters (Figure 2). For example, after the 6th day, the shoot continued to grow while the root length remained almost unchanged for Erdurmus. This difference might be attributed to the greater inhibitory impact of NaCl on root development compared to shoot growth (Rahman et al. 2001). Furthermore, the decrease in RFW, RDW, SFW, and SDW might perhaps be attributed to the toxic impact of Na+ on the rate of photosynthesis, particularly at elevated concentrations (Kawasaki et al. 1983). The results confirmed the observation of Rajabi Dehnavi et al. (2020) that salinity negatively affects plant growth parameters.

Table 2. Germination and seedling growth parameters of four sweet sorghum genotypes in four salinity levels

Genotypes	Salinity Concentrations (mM NaCl)				
	0	100	200	300	Mean
			GP (%)		
Erdurmus	100.00	96.66	86.66	63.33	86.66
Uzun	100.00	76.66	53.33	46.66	69.16
Srg 156	100.00	96.66	80.00	53.33	82.49
BSS 424	100.00	56.66	33.33	10.00	49.99
Mean	100.00	81.66	63.33	43.33	72.08
			GI		
Erdurmus	22.380	18.900	13.700	8.210	15.800
Uzun	13.960	12.740	6.060	5.470	9.550
Srg 156	19.520	17.980	11.570	6.520	13.900
BSS 424	10.380	8.330	3.640	0.890	5.810
Mean	16.563	14.492	8.747	5.277	11.265
			RFW (g)		
Erdurmus	0.110	0.080	0.026	0.000	0.054
Uzun	0.096	0.073	0.010	0.000	0.045
Srg 156	0.116	0.056	0.006	0.000	0.045
BSS 424	0.056	0.036	0.000	0.000	0.023
Mean	0.095	0.061	0.010	0.000	0.041
			RDW (g)		
Erdurmus	0.011	0.007	0.000	0.000	0.0045
Uzun	0.008	0.010	0.000	0.000	0.0045
Srg 156	0.012	0.007	0.000	0.000	0.00475
BSS 424	0.013	0.004	0.000	0.000	0.00425
Mean	0.011	0.007	0.000	0.000	0.0045
			SFW (g)		
Erdurmus	0.360	0.276	0.123	0.046	0.201
Uzun	0.320	0.190	0.026	0.003	0.135
Srg 156	0.406	0.236	0.113	0.016	0.193
BSS 424	0.340	0.153	0.006	0.000	0.125
Mean	0.356	0.214	0.067	0.016	0.164
			SDW (g)		
Erdurmus	0.030	0.027	0.012	0.000	0.0173
Uzun	0.025	0.021	0.001	0.000	0.0118
Srg 156	0.041	0.026	0.011	0.000	0.0195
BSS 424	0.030	0.017	0.003	0.000	0.0125
Mean	0.031	0.022	0.006	0.000	0.0153
			RL (cm)		
Erdurmus	5.620	2.830	1.060	0.590	2.520
Uzun	4.770	3.560	0.730	0.240	2.330
Srg 156	6.260	2.570	0.810	0.340	2.740
BSS 424	4.020	1.990	0.450	0.230	1.670
Mean	5.167	2.74	0.760	3.350	2.320
			SL (cm)		
Erdurmus	5.260	4.490	1.930	1.070	3.190
Uzun	5.340	4.060	0.780	0.250	2.600
Srg 156	6.490	3.640	2.120	1.320	3.390
BSS 424	6.860	4.020	1.080	0.400	3.090
Mean	5.980	4.050	1.480	0.760	3.070

GP: Germination percentage; GI: Germination Index; RFW: Root Fresh Weight; RDW: Root DryWeight; SFW: Shoot Fresh Weight; SDW: Shoot Dry Weight; RL: RootLength; SL: Shoot Length.

Seedling growth measures, such as GP and GI, exhibited a similar pattern to that reported for germination parameters. Salinity is well recognized to have a negative impact on GP and GI (Rehman et al. 2000). The effect of salinity varies depending on the degree of salt content. Low levels of NaCl induce seed dormancy, whereas high levels of NaCl impede seed germination due to the impact of increased osmotic potential and the toxicity of particular ions (Khan et al. 2008). In this study, we did not identify a salinity effect on GP at a relatively low salinity level of 100 mM NaCl in two genotypes, Sr 156 and Erdurmus.

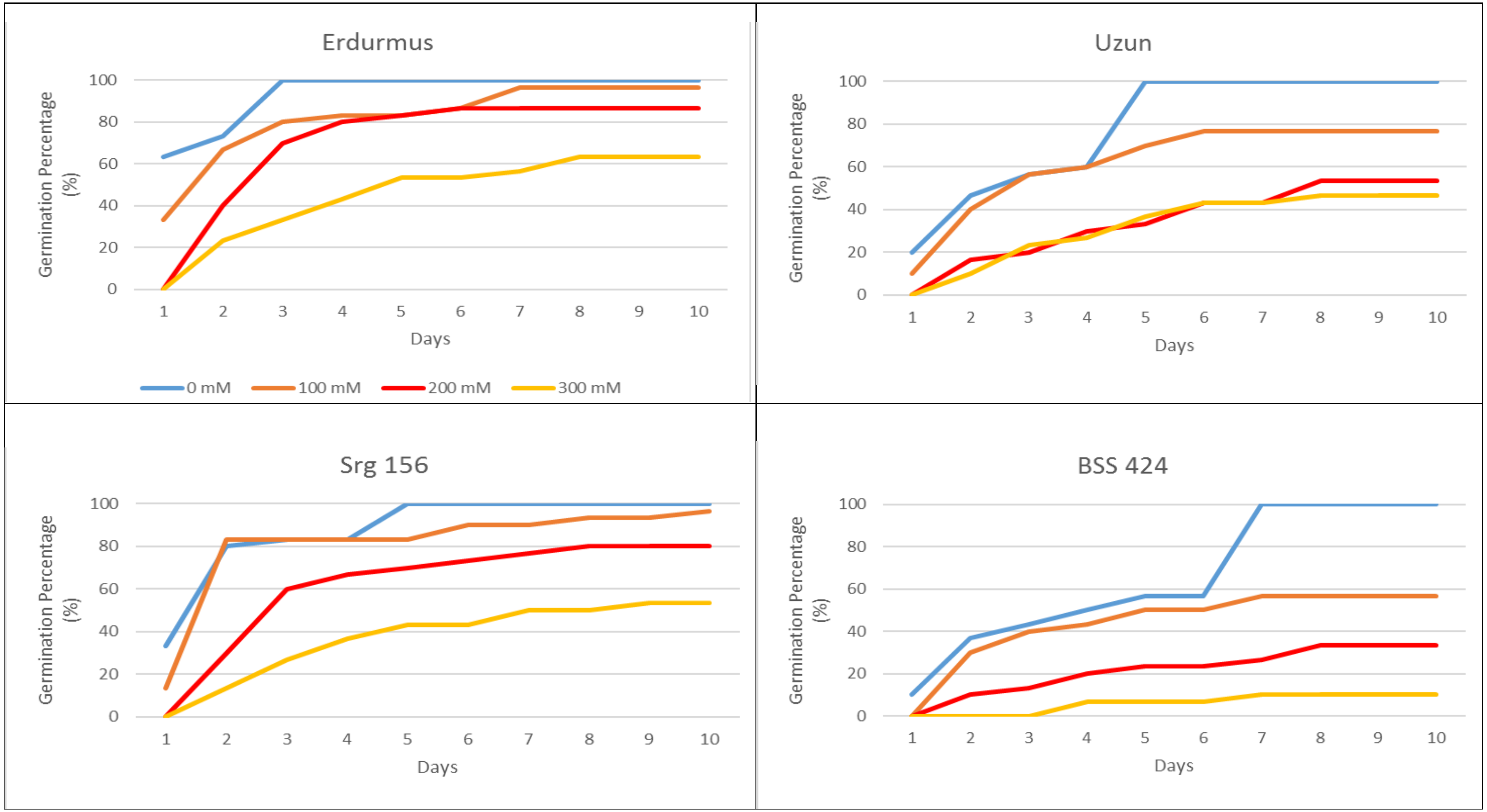

Figure 1. Effect of different levels of salinity stress on germination percentage.

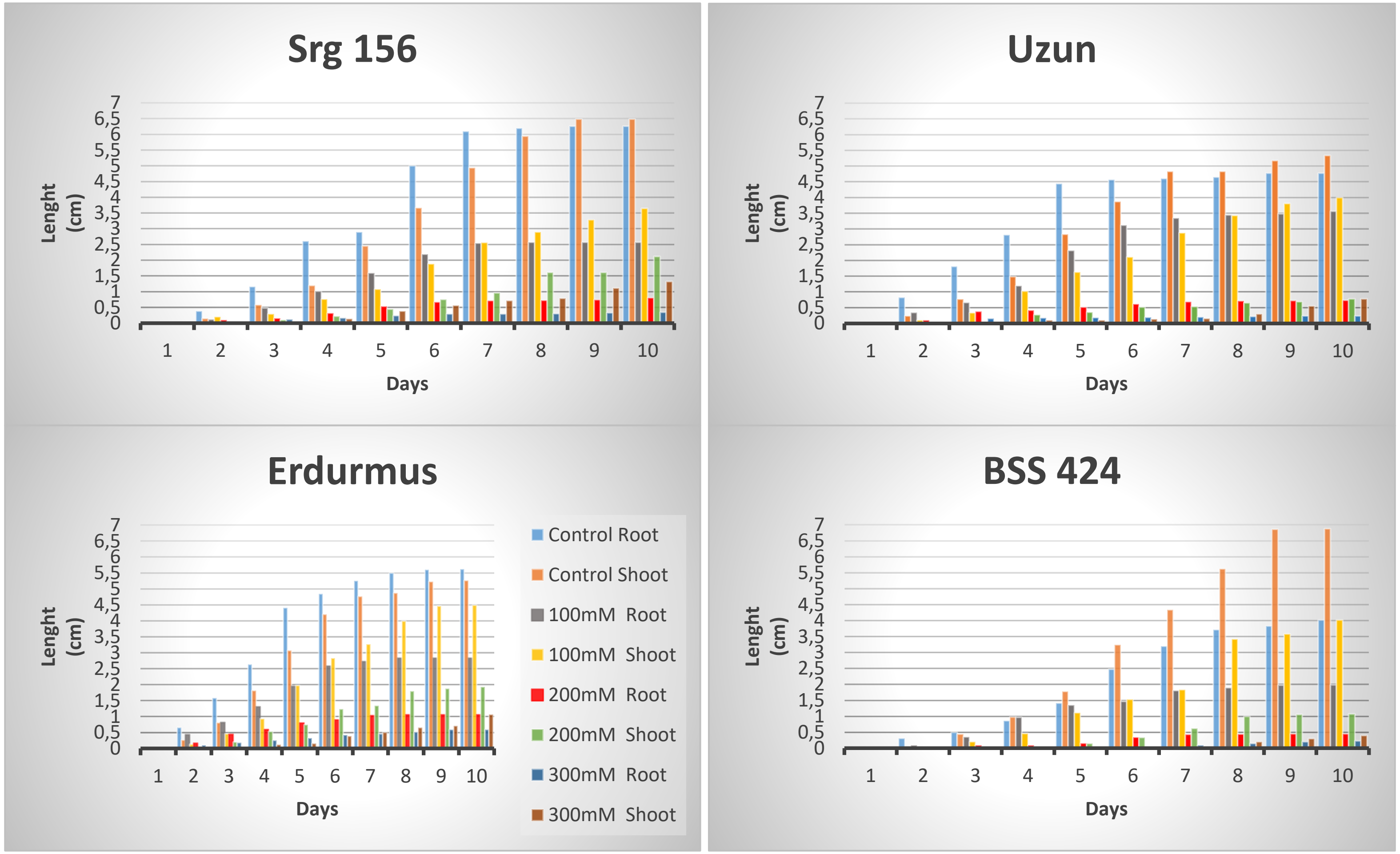

Figure 2: Effects of salt stress on root and shoot lengths of different genotypes of sweet sorghum.

Table 3. Mean Mg, Ca, Na, and K concentrations in shoots at two NaCl levels (%) for four sweet sorghum genotypes

Genotypes	Mg (%)		Ca (%)		Na (%)		K (%)	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Erdurmus	0.376	0.190	0.226	0.066	0.136	3.250	1.083	0.916
Uzun	0.420	0.180	0.220	0.050	0.160	3.280	1.016	0.893
Srg 156	0.260	0.120	0.156	0.066	0.090	2.890	1.146	0.883
BSS 424	0.220	0.123	0.146	0.050	0.090	1.973	2.050	1.550

However, salinity significantly influenced the germination parameter GI (Table 1). Moreover, among all genotypes examined in the present study, Erdurmus, Uzun, and Sgr 156 exhibited the lowest reductions in GP and GI, whereas genotype BSS 424 had the most reductions, in comparison to the control group. Several investigations have shown that genotypes that exhibit better germination rates in salty conditions are considered salt resistant and tend to have more biomass and yield (Ashraf et al. 2006; Shakeri and Emam 2017; Rajabi Dehnavi et al. 2020). Therefore, based on germination parameters, the genotypes Erdurmus, Uzun, and Srg 156 may be considered salt tolerant, whereas the genotype BSS 424 can be considered salt sensitive. The variations in germination parameters across sorghum genotypes seem to be attributed to genetic factors and variations in inheritance (Kausar et al. 2012).

Excessive levels of Na can result in an accumulation of toxic compounds in sorghum leaves (Netondo et al. 2004a) which can negatively impact the absorption and movement of essential nutrients such as K, Ca, and Mg (Bavei et al. 2011; Joardar et al. 2018). Uzun exhibited the highest salinity accumulation, while also demonstrating the lowest ion (except for K) uptake in comparison to the control conditions and other genotypes. This result aligns with the research conducted by Calone et al. (2020), which stated that higher Na accumulation was associated with lower Ca and Mg accumulation (Table 3). Moreover, Uzun was determined to be one of the genotypes showing low FSW in response to increasing salinity stress. Erdurmus and Srg 156 exhibited a comparatively lower decline in ion content despite their elevated sodium accumulation, and both had higher FSW as compared to other genotypes (Table 2, 3). These findings confirm the conclusion that plants exposed to salt stress have a lower biomass because of increased respiration (Ashraf 2004). Increased ethylene release during periods of stress may have hindered the development of both roots and shoots, resulting in a decrease in their growth rates (Sagar 2017).

5. Conclusion

The results of this study showed that as salinity concentration increased, germination and seedling growth parameters, as well as ion uptake, decreased. The genotypes Erdurmus and Srg 156 have shown better performances, particularly in terms of germination seedling growth parameters. These genotypes should be further assessed in field conditions to determine their agro-morphological characteristics.

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