

Article

Effect of Salinity on Seed Germination and Seedling Development of Sorghum (*Sorghum bicolor* (L.) Moench) Genotypes

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Abstract: Salinity is one of the most important abiotic stresses that negatively affects plant growth and development around the world. It has been reported that approximately 19.5% of all irrigated land and 2.1% of dry land is affected by salt stress, and these percentages continue to increase. Sorghum, a C4 plant, is the fifth most important cereal in the world. Numerous studies reported that there are high genetic variations in sorghum. These genetic variations can be monitored to search for the most salt-tolerant genotypes. Therefore, the aim of our study was to investigate the responses of ten sorghum genotypes to different levels of salinity. We focused on germination and seedling growth as the most critical stages of plant development. In our research we included germination percentage, germination index, mean germination time, seedling vigor index, seedlings' shoot and root lengths, fresh and dry seedling weight, and salinity tolerance indices. For data assessment we applied two-way ANOVA, non-metric multidimensional scaling, and hierarchical agglomerative classification. Our results demonstrate that salinity was responsible for 98% of the variation in assessed parameters, whereas genotype effect accounted for only 2% of the documented variation. It can be concluded that seedling traits can be used as a valid criterion for the selection of genotypes with a better tolerance to salinity stress.

Keywords: sorghum; salinity; crop plant; stress; cluster analysis; NMDS

1. Introduction

Salinity is one of the most important abiotic stresses that negatively affect plant growth and development around the world [1,2]. It has been reported that approximately 19.5% of all irrigated land and 2.1% of dry land is affected by salt stress [3]. Saline areas continue to increase in size because of mishandled irrigation [4]. In addition, in arid and semi-arid regions the salinization process occurs because of high evaporation and inadequate amounts of precipitation for considerable leaching [5]. Salinity inhibits crop growth and development, through complex traits that include osmotic stress, ion toxicity, mineral deficits, and physiological and biochemical defects [5–9]. In addition, under saline conditions, osmotic and ionic stress leads to the production of reactive oxygen species (ROS) in chloroplasts, mitochondria, and the apoplastic space [10,11]. This oxidative stress causes membrane peroxidation; ion leakage; and damage to nucleic acids, cell membranes, and cellular structure [12]; and ultimately, reduces the quality and total yield of the affected crop [13]. Additionally, it can adversely affect water quality and soil structure. To overcome salt stress, agriculture needs to look

for suitable methods that are economically feasible and cost-effective. One of the most important methods is monitoring genetic diversity within the germplasm of plant species for the trait of tolerance to salinity, which is used to identify genotypes that have more sustainability and better performance than other genotypes [14]. Numerous studies reported that high genotypic variation exists among genotypes for salt tolerance in different plants, such as wheat [15], alfalfa [16], oat [17], barley [18], and sorghum [19–23].

Sorghum (*Sorghum bicolor* (L.) Moench), a C4 plant, is the fifth most important cereal in the world [24]. This plant is well adapted to semi-arid and arid regions because of its tolerance to abiotic stresses such as drought and salinity [25]. Numerous studies reported that there are high genetic variations in sorghum genotypes in response to salinity [20,26]. These genetic variations can be monitored to search for the most salt tolerant genotypes. This monitoring analysis should be done at the most critical and sensitive stage of plant growth [27]. It has been reported that germination and emergence stages in sorghum development are the most informative stages of the plant's lifecycle to evaluate the effect of salinity [20]. For example, Nimir et al. [28,29], and Ali et al. [30] reported reduction in seedling emergence by increasing salinity levels in sorghum, but the responses varied depending on the genotype. In addition, Mbinda and Kimtai [31] reported that salinity substantially affects all traits associated with germination and early seedling growth, with the effect of salinity being dependent on the variety used and level of salinity stress applied. Seed germination can be described as an important and susceptible stage of plant growth, because the duration of this phase determines seedling establishment and future plant growth [32,33]. Tolerance to salt stress at the germination stage and at seedling emergence determines better plant establishment in saline soils [34,35]. Generally, increasing salinity significantly reduces germination percentage and rate, root and shoot length, and fresh and dry weights of the exposed plants [36]. Thus, study of the reaction of plants to salinity in the germination stage and seedling growth seems to be necessary for the development of salt tolerant plants and the production of appropriate crop yields in saline conditions. Therefore, the purpose of our research is to investigate the response of ten sorghum genotypes to different salinity levels during germination and seedling stages to determine their potential for salt tolerance. We assumed that: (1) genotypes differ in tolerance level to salinity; (2) different levels of salinity affect germination and seedling growth in different ways.

2. Materials and Methods

2.1. Germination Experiment

The ten sorghum genotypes included PEGAH, GS4, JUMBO, KGS23, SPEED FEED, MGS5, KIMIA, KGS29, SEPIDEH, and PAYAM. Some of the ten assessed genotypes are commercially cultivated by sorghum growers in Iran and were obtained from Seed and Plant Improvement Institute (SPII) Karaj, Alborz Province, Iran. We performed a Petri dish experiment as a complete randomized design in 4*10 factorial (four salt treatments: 0, 100, 150, and 200 mM NaCl) and ten sorghum genotypes with 3 replications in a growth chamber at 25 °C and with a 16-h light period. Fifty sorghum seeds were placed in each of the 120 Petri dishes, which were lined with Whatman No. 2 filter paper and watered with different NaCl solutions and monitored for 9 days to the end of germination.

2.2. Germination Assessment

We assessed the following parameters:

Germination percentage (GP):

$$GP = (\text{number of normally germinated seeds} \div \text{total number of seeds sown}) \times 100 \quad (1)$$

Germination index (GI):

$$GI = \Sigma(Gt/Tt) \quad (2)$$

where G_t is the number of seeds germinated on day t , and T_t is the number of days [33,34].

Mean germination time (MGT):

$$MGT = \Sigma(T_i \times N_i) / \Sigma N_i, \quad (3)$$

where N_i is the number of newly germinated seeds at time T_i [37,38].

Seedling vigor index (SVI) [39].

$$SVI = \text{mean germination percentage} \times \text{mean seedling length} \quad (4)$$

2.3. Seedling Growth Assessment

To assess seedling growth, ten seedlings were randomly selected from each petri dish at the end of the germination period. After selection, shoot (SL) and root length (RL) were measured in cm. We assessed fresh and dry biomass as seedling fresh weight (FW) and weight after drying the samples in an oven for 72 h at 80 °C to obtain seedling dry weight (DW).

2.4. Salinity Tolerance Indices

To assess the tolerance to salinity of each genotype, we adopted the stress susceptibility index (SSI) and stress tolerance index (STI) using the following equations [40,41]:

$$SSI = (1 - (Y_s/Y_p)) / (1 - (\bar{Y}_s/\bar{Y}_p)) \quad (5)$$

$$STI = (Y_p \times Y_s) / (\bar{Y}_p)^2 \quad (6)$$

In both the above equations, Y_p and Y_s are the average seedling dry weight of a given genotype under non-stress and NaCl-stress conditions, respectively. \bar{Y}_p and \bar{Y}_s are the average seedling dry weights of all genotypes under non-stress and stress conditions, respectively.

2.5. Statistical Analysis

Overall, effects were assessed by two-way ANOVA (salinity and genotype as factors) and non-metric multidimensional scaling (NMDS) with Bray–Curtis similarity as the similarity measure. To compare all treatments, we used analysis of variance (ANOVA), and for *post-hoc* comparisons, least significant difference (LSD, $p \leq 0.05$). To demonstrate similarity between genotypes, we applied hierarchical agglomerative cluster analysis with percent similarity as the similarity measure and the unweighted pair group method for classification tree construction. Statistical Analysis Software version 9.4 (SAS Institute Inc., Cary, North Carolina, USA), Multivariate Statistical Package 3.1 [42], and the Canoco 5.0 package [43] were used for calculations.

3. Results

3.1. Overall Effects of Genotypes and Salinity

The main effects of salinity and genotypes, and the interaction effects between them were significant in all measured parameters (Table 1). Salinity reduced germination percentage GP, germination index GI, seedling shoot length SL, seedling root length RL, seedling vigor index SVI, seedling fresh weight FW, and seedling dry weight DW, but increased mean germination time MGT in different sorghum genotypes (Figure 1). However, the response to salinity was dependent on genotype, which was demonstrated on the NMDS diagram (Figure 1), where the first ordination axis represents salinity gradient. Samples are distributed from the left (0 mM NaCl treatments) to the right side of the diagram (200 mM NaCl). This axis accounts for 98% of the variance in the measured parameters. The second NMDS axis represents differences in genotype responses and only accounts for 2% of the total variance. Genotypes are located from lower to upper positions.

Table 1. Analysis of variance (mean squares) for different parameters of ten sorghum genotypes in four salinity treatments.

Trait	Sources of Variations			
	S	G	S×G	Error
df	3	9	27	80
GP	5984 **	368 **	87.9 **	0.633
GI	3128 **	65.4 **	9.37 **	1.56
MGT	15.8 **	12.8 **	0.278 *	0.163
SL	63.7 **	8.00 **	0.053 **	0.012
RL	687 **	46.1 **	0.54 *	0.316
SVI	16,931,287 **	1,102,363 **	15,395 **	2805
FW	0.055 **	0.007 **	0.00004 *	0.00002
DW	0.002 **	0.0002 **	0.000001 **	0.0000003

GP = germination percentage; GI = germination index; MGT = mean germination time; SL = seedling shoot length; RL = seedling root length; SVI = seedling vigor index; FW = seedling fresh weight; DW = seedling dry weight; S = salinity; G = Sorghum genotypes; df = degrees of freedom; Error = within group variance; * = $p \leq 0.05$; ** = $p \leq 0.01$.

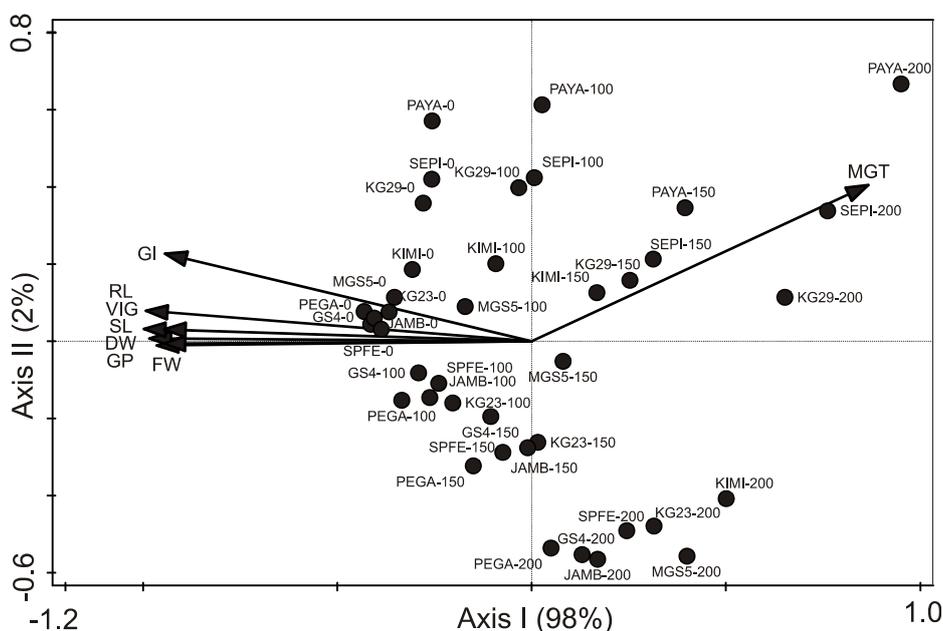


Figure 1. Non-metric multidimensional scaling ordination diagram (NMDS with Bray–Curtis similarity measure) of ten sorghum genotypes in four salinity treatments. Germination and growth parameters projected passively in ordination space. GP = germination percentage (%); GI = germination index; MGT = mean germination time (day); SL = seedling shoot length (cm); RL = seedling root length (cm); VIG= seedling vigor index; FW = seedling fresh weight (gr); DW = seedling dry weight (gr), PEGA-0–genotype abbreviation-salinity level (mM NaCl).

3.2. Germination Assessment

Sorghum genotypes differed significantly in terms of germination parameters, that is, mean GP, GI, MGT, and SVI, in both non-saline and saline conditions. Statistical analysis demonstrated that in none-saline condition, there were no significant differences between genotypes in terms of GP and GI. But differences were significant in terms of MGT and SVI, a finding that suggests significant initial differences between the genotypes in terms of seed germination rate. This can be due to genetic variation among sorghum genotypes or to the somatic quality of seeds. The results showed that in non-saline conditions, the GP of all genotypes was 100% (Table 2). With salinity increasing to 100 mM NaCl, GP in genotypes PEGAH, GS4, JUMBO, KGS23, and SPEED FEED did not change,

but in genotypes MGS5, KIMIA, KGS29 SEPIDEH, and PAYAM a decrease of between 5% and 9% was observed (Table 2). Under 150 and 200 mM NaCl, GP was significantly reduced in all sorghum genotypes. Compared to the non-saline condition, the highest decreases in GP were found in genotype PAYAM (25% and 57%, respectively), and the lowest decreases under 150 mM NaCl (9%) were found in genotypes PEGAH, GS4, and JUMBO, while under 200 mM NaCl the lowest (17%) was found in genotype PEGAH (Table 2).

Table 2. Germination parameters of ten sorghum genotypes in four salt treatments (means).

Trait	Genotypes	Salt Levels mM NaCl						
		0	100	% Change	150	% Change	200	% Change
GP	PEGAH	100 ^a	100 ^a	0	91 ^a	−9	83 ^a	−17
	GS4	100 ^a	100 ^a	0	91 ^a	−9	79 ^b	−21
	JAMBO	100 ^a	100 ^a	0	91 ^a	−9	79 ^b	−21
	KGS23	100 ^a	100 ^a	0	87 ^b	−13	75 ^c	−25
	SPEED FEED	100 ^a	100 ^a	0	87 ^b	−13	75 ^c	−25
	MGS5	100 ^a	95 ^b	−5	83 ^c	−17	71 ^d	−29
	KIMIA	100 ^a	95 ^b	−5	83 ^c	−17	67 ^e	−33
	KGS29	100 ^a	91 ^c	−9	79 ^d	−21	59 ^f	−41
	SEPIDEH	100 ^a	91 ^c	−9	79 ^d	−21	55 ^g	−45
	PAYAM	100 ^a	91 ^c	−9	75 ^e	−25	43 ^h	−57
GI	PEGAH	60.7 ^a	54.1 ^a	−10.9	47.6 ^a	−21.6	40.5 ^a	−33.3
	GS4	60.5 ^a	53.3 ^{ba}	−11.9	47.4 ^a	−21.6	39.7 ^{ab}	−34.4
	JAMBO	59.9 ^a	51.1 ^b	−14.7	44.0 ^b	−26.5	39.9 ^a	−33.4
	KGS23	60.0 ^a	49.9 ^{bc}	−16.8	42.3 ^{bc}	−29.5	37.6 ^{bc}	−37.3
	SPEED FEED	59.6 ^a	50.3 ^{bc}	−15.6	42.5 ^{bc}	−28.7	38.5 ^{abc}	−35.4
	MGS5	60.4 ^a	50.4 ^{bc}	−16.5	41.5 ^{cd}	−31.3	36.7 ^{cd}	−39.2
	KIMIA	59.8 ^a	48.6 ^{cd}	−18.7	39.9 ^{de}	−33.3	36.41 ^{cde}	−39.1
	KGS29	60.1 ^a	48.9 ^{cd}	−18.6	38.8 ^{ef}	−35.4	35.3 ^{de}	−41.3
	SEPIDEH	60.3 ^a	48.4 ^{cd}	−19.7	37.2 ^{fg}	−38.3	34.4 ^e	−42.9
	PAYAM	59.9 ^a	47.0 ^d	−21.5	35.3 ^g	−41.1	30.0 ^f	−49.9
MGT	PEGAH	3.09 ^c	3.28 ^e	+6.1	3.59 ^f	+16.2	4.03 ^f	+30.4
	GS4	3.12 ^c	3.47 ^{de}	+11.2	3.79 ^f	+21.5	4.14 ^f	+32.7
	JAMBO	3.11 ^c	3.52 ^{de}	+13.2	3.81 ^f	+22.5	4.28 ^f	+37.6
	KGS23	3.22 ^c	3.68 ^{de}	+14.3	4.01 ^{ef}	+24.5	4.67 ^{ef}	+45.0
	SPEED FEED	3.14 ^c	3.59 ^{de}	+14.3	3.96 ^{ef}	+26.1	4.46 ^{ef}	+42.0
	MGS5	3.47 ^c	4.11 ^{cd}	+18.4	4.57 ^{de}	+31.7	5.10 ^{de}	+46.9
	KIMIA	3.74 ^{bc}	4.53 ^c	+21.1	5.20 ^{cd}	+39.0	5.68 ^{cd}	+51.9
	KGS29	4.20 ^{ab}	5.22 ^b	+24.3	5.86 ^{bc}	+39.5	6.34 ^{bc}	+50.9
	SEPIDEH	4.42 ^{ab}	5.36 ^{fb}	+21.3	6.39 ^b	+44.6	6.80 ^b	+53.8
	PAYAM	4.82 ^a	6.13 ^a	+27.2	7.20 ^a	+49.4	7.71 ^a	+59.9
SVI	PEGAH	2740 ^a	2374 ^a	−13.3	17,191 ^a	−37.2	1219 ^a	−55.5
	GS4	2672 ^{ab}	2231 ^b	−16.5	1614 ^b	−39.6	1042 ^b	−61.0
	JAMBO	2619 ^{bc}	2144 ^{bc}	−18.1	1553 ^b	−40.7	968 ^c	−63.0
	KGS23	2484 ^d	1979 ^d	−20.3	1351 ^c	−45.6	776 ^e	−68.7
	SPEED FEED	2559 ^{cd}	2089 ^c	−18.4	1395 ^c	−45.5	857 ^d	−66.5
	MGS5	2476 ^d	1890 ^d	−23.7	1229 ^d	−50.4	662 ^f	−73.3
	KIMIA	2304 ^e	1662 ^e	−27.9	1085 ^e	−52.9	561 ^g	−75.6
	KGS29	2240 ^{ef}	1507 ^f	−32.7	936 ^f	−58.2	427 ^h	−80.9
	SEPIDEH	2155 ^f	1394 ^g	−35.3	855 ^f	−60.3	348 ⁱ	−83.8
	PAYAM	2197 ^f	1395 ^g	−36.5	754 ^g	−65.7	255 ^j	−88.4

GP = germination percentage (%); GI = germination index; MGT = mean germination time (day), VIG = seedling vigor index. Values within a group in a column denoted by different letters are significantly different at $p \leq 0.05$.

Salinity also significantly reduced GI (Table 2). Under saline conditions the maximum decrease was observed in genotype PAYAM (ranging from 21.5% under 100 mM to 49.9% at 200 mM) followed by SEPIDEH (from 19.7% to 42.9%, respectively) and the minimum decreases were obtained in genotype PEGAH (from 10.9% to 33%), GS4 (from 11.9% to 34.4%), and JUMBO (from 14.7% to 33.4%) (Table 2).

Salinity increased MGT of all sorghum genotypes (Table 2). At 100 mM NaCl, the highest difference compared to the control was observed in genotype PAYAM (27.2%) followed by KGS29 (24.3%), and the lowest increase was obtained in genotype PEGAH (6.1%) followed by GS4 (11.2%) (Table 2). At 150 and 200 mM NaCl, the highest increase was observed again in genotype PAYAM (49.4% and 59.9%, respectively) followed by SEPIDEH (44.6% and 53.8%), and the lowest increases were obtained in genotypes PEGAH (16.2% and 30.4%) and GS4 (21.5% and 32.7%) (Table 2).

Regarding vitality of seedlings assessed as the seedling vigor index, salinity significantly reduced SVI of all sorghum genotypes (Table 2). Under all treatments, the highest decrease was observed for genotype PAYAM followed by SEPIDEH, and the lowest decreases were obtained in genotype PEGAH followed by GS4.

3.3. Seedling Growth Assessment

Salinity significantly reduced SL of the investigated sorghum genotypes (Table 3). In each treatment the highest decreases compared to the control were observed in genotype PAYAM followed by SEPIDEH, and the lowest decrease was recorded in genotype PEGAH followed by GS4 (Table 3). The highest decreases were observed in 200 mM NaCl—in genotype PAYAM (70.4%) followed by SEPIDEH (67.3%)—whereas in genotype PEGAH the decrease reached 40.0%, and 44.2% was the decrease for GS4 (Table 3).

Table 3. Seedling growth parameters of ten sorghum genotypes in four salt treatments (means).

Trait	Genotypes	Salt Levels mM NaCl						
		0	100	% Change	150	% Change	200	% Change
SL	PEGAH	7.04 ^a	6.28 ^a	−10.8	5.03 ^a	−28.5	4.22 ^a	−40.0
	GS4	6.86 ^{ab}	5.85 ^b	−14.7	4.57 ^b	−33.4	3.83 ^b	−44.2
	JAMBO	6.81 ^b	5.68 ^{fb}	−16.6	4.40 ^b	−35.4	3.66 ^b	−46.2
	KGS23	6.08 ^d	4.83 ^d	−20.5	3.57 ^d	−41.3	2.62 ^d	−56.9
	SPEED FEED	6.43 ^c	5.23 ^c	−18.7	3.97 ^c	−38.2	3.15 ^c	−51.0
	MGS5	5.90 ^d	4.63 ^e	−21.5	3.35 ^e	−43.2	2.49 ^d	−57.8
	KIMIA	5.58 ^e	4.23 ^f	−24.2	3.01 ^f	−46.0	2.22 ^e	−60.2
	KGS29	5.44 ^{ef}	4.00 ^g	−26.5	2.83 ^{fg}	−47.9	1.93 ^f	−64.5
	SEPIDEH	5.39 ^f	3.86 ^g	−28.4	2.87 ^{fg}	−46.7	1.76 ^{fg}	−67.3
	PAYAM	5.41 ^{ef}	3.87 ^g	−28.5	2.72 ^g	−49.6	1.60 ^g	−70.4
RL	PEGAH	20.3 ^a	17.4 ^a	−14.3	13.8 ^a	−32.0	10.4 ^a	−48.8
	GS4	19.8 ^{ab}	16.4 ^b	−17.2	13.1 ^{ab}	−33.8	9.36 ^b	−52.7
	JAMBO	19.4 ^{abc}	15.7 ^{bc}	−19.1	12.6 ^{bc}	−35.0	8.59 ^{bc}	−55.6
	KGS23	18.7 ^c	14.9 ^c	−20.3	11.9 ^{cd}	−36.3	7.73 ^{cd}	−58.7
	SPEED FEED	19.1 ^{bc}	15.6 ^{bc}	−18.3	12.0 ^{cd}	−37.2	8.27 ^c	−56.7
	MGS5	18.8 ^c	15.2 ^c	−19.1	11.4 ^d	−39.4	6.83 ^{de}	−63.8
	KIMIA	17.4 ^d	13.2 ^d	−24.1	10.0 ^e	−42.5	6.16 ^{ef}	−64.6
	KGS29	16.9 ^{de}	12.5 ^d	−26.0	9.02 ^f	−46.6	5.30 ^{fg}	−68.6
	SEPIDEH	16.1 ^e	11.4 ^e	−29.2	7.95 ^g	−50.6	4.57 ^{gh}	−71.6
	PAYAM	16.5 ^{de}	11.4 ^e	−30.9	7.33 ^g	−55.5	4.34 ^h	−73.7

Table 3. Cont.

Trait	Genotypes	Salt Levels mM NaCl						
		0	100	% Change	150	% Change	200	% Change
FW	PEGAH	0.210 ^a	0.179 ^a	−14.7	0.143 ^a	−31.9	0.111 ^a	−47.1
	GS4	0.202 ^{ab}	0.169 ^b	−16.3	0.135 ^{ab}	−33.2	0.105 ^{ab}	−48.0
	JAMBO	0.197 ^b	0.161 ^{bc}	−18.4	0.126 ^{bc}	−36.0	0.099 ^{bc}	−49.7
	KGS23	0.186 ^{cd}	0.142 ^e	−23.6	0.112 ^{de}	−39.8	0.081 ^d	−56.4
	SPEED FEED	0.194 ^{bc}	0.153 ^{cd}	−21.1	0.120 ^{cd}	−38.1	0.094 ^c	−51.5
	MGS5	0.182 ^d	0.147 ^{de}	−19.2	0.104 ^e	−42.8	0.087 ^{de}	−52.3
	KIMIA	0.170 ^e	0.129 ^f	−24.1	0.090 ^f	−47.0	0.069 ^{ef}	−59.4
	KGS29	0.159 ^g	0.114 ^g	−28.3	0.087 ^f	−45.3	0.060 ^{fg}	−62.3
	SEPIDEH	0.147 ^g	0.106 ^{gh}	−27.9	0.073 ^g	−50.3	0.055 ^{gh}	−62.6
PAYAM	0.138 ^g	0.097 ^h	−29.7	0.060 ^h	−56.5	0.047 ^h	−65.9	
DW	PEGAH	0.038 ^a	0.031 ^a	−18.4	0.025 ^a	−34.2	0.018 ^a	−52.6
	GS4	0.037 ^b	0.030 ^b	−18.9	0.024 ^b	−35.1	0.018 ^a	−51.3
	JAMBO	0.036 ^c	0.029 ^c	−19.4	0.022 ^c	−38.9	0.016 ^b	−55.6
	KGS23	0.034 ^e	0.025 ^e	−26.5	0.020 ^e	−41.2	0.014 ^d	−58.8
	SPEED FEED	0.035 ^d	0.026 ^d	−25.7	0.021 ^d	−40.0	0.015 ^c	−57.1
	MGS5	0.033 ^f	0.025 ^e	−24.4	0.018 ^f	−45.4	0.013 ^e	−60.6
	KIMIA	0.031 ^g	0.022 ^f	−29.0	0.016 ^g	−48.4	0.011 ^f	−64.5
	KGS29	0.029 ^h	0.020 ^g	−31.0	0.015 ^h	−48.3	0.009 ^g	−68.9
	SEPIDEH	0.027 ⁱ	0.019 ^h	−29.6	0.012 ⁱ	−55.6	0.008 ^h	−70.4
PAYAM	0.025 ^j	0.016 ⁱ	−36.0	0.010 ^j	−60.0	0.007 ⁱ	−72.0	

SL = seedling shoot length (cm); RL = seedling root length (cm); VIG = seedling vigor index; FW = seedling fresh weight (g); DW = seedling dry weight (g). Values within a group in a row bearing different superscripts are significantly different at $p \leq 0.05$.

A similar pattern was observed in the other growth parameters assessed, including RL, FW, and DW (Table 3). The highest reduction in these parameters was determined for the genotype PAYAM at 200 mM NaCl: ca 74% for RL, ca 66% for FW, and ca 72%, for DW. At the same time, this reduction in the case of genotype PEGAH reached 14.3% for RL, 47% for FW, and 52.6% for DW.

3.4. Salinity Tolerance

Tolerance indices SSI and STI are shown in Table 4. The results demonstrate that under all salinity treatments, the highest values of the SSI index were observed in genotype PAYAM followed by SEPIDEH, and the lowest values of the SSI index were obtained in genotype PEGAH followed by GS4 (Table 4). Values of the STI index were the opposite—they were highest in genotype PAYAM followed by SEPIDEH, and lowest in genotype PEGAH followed by GS4 (Table 4).

The classification tree, which takes into account all germination and growth parameters, demonstrates that the most salt sensitive genotypes were PAYAM, SEPIDEH, KIMIA, and KG29 (Figure 2). These genotypes, when cultivated under control conditions, are clustered together with the remaining genotypes, increasing in their responses under the 100 mM NaCl stress treatment (group II). Samples grown under 100 mM have been clustered with other genotypes exposed to the 150 mM NaCl treatment, even in the case of PEGAH 200 mM NaCl (group III). PAYAM, SEPIDEH, and KG29 under 200 mM NaCl form a separate group (group V) and exhibited the lowest germination and growth parameters. The most salt tolerant genotypes after PEGAH were GS4, JAMBO, and SPEED FEED. These genotypes, when exposed to the 200 mM NaCl stress regime, were grouped in cluster IV (Figure 2) together with the sensitive genotypes under the 150 mM stress treatment.

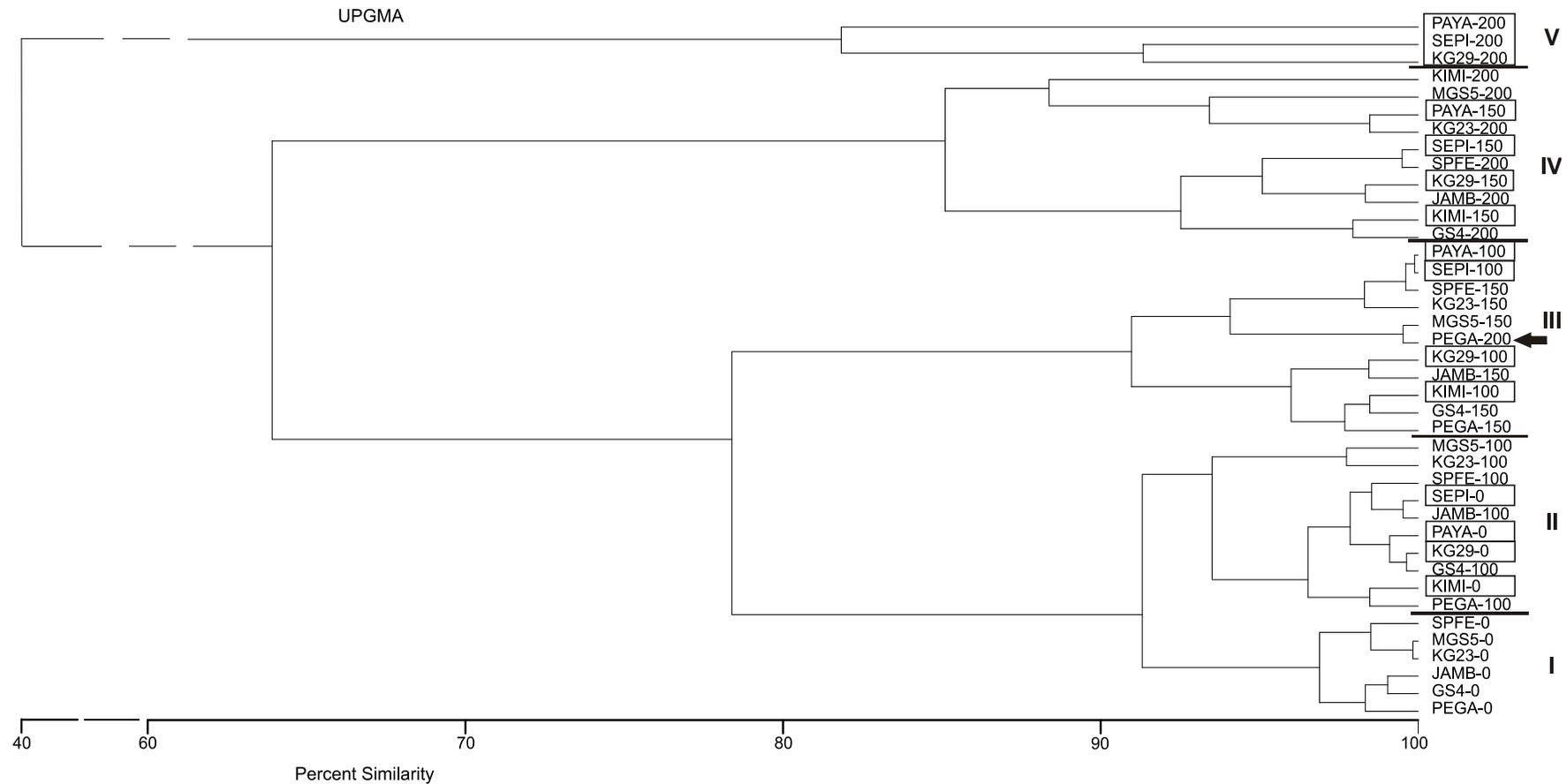


Figure 2. Results of hierarchical agglomerative cluster analysis based on all traits in all four salt treatments. Clusters I–V according to similarity in germination and growth parameters. The most salt sensitive samples in each cluster are in frame. PEGA-0 (genotype abbreviation)–salinity level (mM NaCl).

Table 4. Tolerance indices of ten sorghum genotypes in four salt levels.

Tolerance Indices	Genotypes	100 Mm NaCl	150 Mm NaCl	200 Mm NaCl
Stress susceptibility index (SSI)	PEGAH	0.736	1.36	2.10
	GS4	0.756	1.40	2.05
	JAMBO	0.777	1.55	2.22
	KGS23	1.05	1.65	2.35
	SPEED FEED	1.02	1.60	2.28
	MGS5	0.969	1.81	2.42
	KIMIA	1.16	1.93	2.58
	KGS29	1.24	1.93	2.75
	SEPIDEH	1.18	2.22	2.81
	PAYAM	1.44	2.40	2.88
Salt tolerance index (STI)	PEGAH	1.15	0.928	0.668
	GS4	1.08	0.867	0.650
	JAMBO	1.02	0.773	0.562
	KGS23	0.830	0.664	0.465
	SPEED FEED	0.888	0.718	0.512
	MGS5	0.805	0.580	0.419
	KIMIA	0.666	0.484	0.333
	KGS29	0.566	0.424	0.255
	SEPIDEH	0.501	0.316	0.210
	PAYAM	0.390	0.244	0.170

4. Discussion

The main aim of this study was to recognize salt tolerant and sensitive genotypes of sorghum during germination and seedling stages to determine their potential for salt tolerance. As was mentioned previously, the effects of salinity on plant growth can vary depending on the plant species and also on the different genotypes of a species. Thus, for improving salt tolerance it is important to monitor the genetic variability of plant species among genotypes [14,44] to study the mechanism of salt tolerance in different species [45]. We included early stages of plant development, because there is well documented that germination and seedling features are the most viable principles, and the final plant performance depends heavily on seedling specifications [20,46].

Based on our results, the sorghum genotypes varied in their response to salinity in germination and seedling characteristics. Our results demonstrate that with an increasing degree of salt stress, GP, GI, and VIG parameters of sorghum genotypes decreased, while MGT increased. However, these parameters varied depending on the assessed genotype, which is in line with our first assumption. These results are also in line with the results of Geressu and Gezahagn [47] obtained for sorghum. In addition, it has been reported previously that salt stress decreased the percentage and increased the duration of germination of sweet sorghum [48]. It is well known that salinity has a negative correlation with GP, GI, and VIG [49]. However, this negative correlation varies depending on the salt concentration, with low concentrations of NaCl inducing seed dormancy, and high concentrations of NaCl inhibiting seed germination due to the effects of high osmotic potential and specific ion toxicity (accumulation of Na⁺ and Cl⁻ ions) [50]. In this study we did not observe a salinity effect on GP at relatively low salinity (100 mM NaCl) in the case of only five of the ten investigated genotypes: PEGAH, GS4, JUMBO, KGS23, and SPEED FEED, but other germination parameters such as GI, VIG, and MTG were all significantly affected. In fact, salinity can influence the germination process by altering the imbibition of water by seeds due to the lower osmotic potential of germination media, which delays water absorption and thus reduces germination [36,50]. On the other hand, high accumulation of Na⁺ ions in medium causes osmotic and pseudo-drought stress, leading to a decrease in water absorption by plant tissues [51,52]. Moreover, salinity can cause changes in enzyme activity by the toxicity effect of ions [53]. This disruption of enzymatic activities causes major changes in plants during germination, such as altering the metabolism of nucleic acid and protein [53,54], disturbing the hormonal balance [55],

and reducing the use of seed reserves [56,57]. Additionally, it seems that by inducing disturbance of the metabolic process, salinity increases phenolic compounds which can reduce germination [58]. However, various internal factors of a seed, such as coat properties, age, polymorphism, dormancy, seedling vigor; and external factors, such as temperature, light, water, and gasses, can affect seed germination under saline conditions [59]. In the current study, of all genotypes, PEGAH and GS4 were determined to have the lowest reductions in GP, GI, and VIG, and genotypes PAYAM and SEPIDEH had the highest reductions, when compared to the non-stressed controls. Consequently, the lowest increase in MGT was observed in genotypes PEGAH and GS4 and the highest in genotypes PAYAM and SEPIDEH. Numerous studies reported that, under saline conditions, genotypes which maintain higher germination are salt tolerant and produce higher biomass and yield [14,20]. Therefore, based on germination parameters, the genotypes PEGAH and GS4 can putatively be said to be salt tolerant and PAYAM and SEPIDEH salt sensitive genotypes. It seems that the differences in germination parameters of sorghum genotypes may be due to the genetic factors and inheritance variation among them [60–62].

A similar pattern as with germination indices was observed for seedling growth parameters: SL, RL, FW, and DW all decreased in each assessed genotype with increasing salt concentration. However, the degree of reduction differed depending on the genotype. Under saline conditions the reduction of seedling shoot and root lengths is a common phenomenon in many plants, because roots are the first organs exposed to salinity and are in direct contact with the soil, absorbing water from the soil and supplying it to the shoot [63]. In addition, salinity can have a negative effect on the ultrastructures of cells, tissues, and organs [64,65]. Moreover, it is reported that salinity, by osmotic and specific ion toxic effects, inhibits the maintenance of necessary nutrient levels essential for plant growth, ultimately limiting root emergence and seedling growth [20,66,67]. The decrease in SL and RL of sorghum genotypes with increasing salt concentration observed in this study is in line with results reported by Bashir et al. [68], whereas the observed decreases in FW and DW are in agreement with previous studies conducted for sorghum by Asfaw [60], Netondo et al. [26,69], and Krishnamurthy et al. [20]. We observed that the effects of salinity on root length were more drastic than for shoot length. This difference might be due to the effect of NaCl being more inhibitory on root growth than shoot growth [70]. Moreover, the reduction in FW and DW may be due to the toxic effect of Na⁺ on photosynthesis rate at higher concentrations [71,72]. Specifically, it has been demonstrated previously that salinity reduces intercellular CO₂ concentration and then photosynthesis rate by stomatal closure [73]. In addition, under high salt levels, Na⁺ can cause lower transport rate of essential ions such as NO₃⁻ that reduce the N-containing compounds and ultimately inhibit plant growth and biomass accumulation [74,75].

Our results demonstrate that among all genotypes, the genotypes PEGAH and GS4 showed the lowest reductions in SL, RL, FW, and DW, and genotypes PAYAM and SEPIDEH had the highest. These findings confirm the results of germination parameters and the conclusion that genotypes PEGAH and GS4 can be assigned the status of salt tolerant, and PAYAM and SEPIDEH should be classified as salt sensitive. It has been reported for sorghum plants that Na⁺ accumulates more in the roots of these plants than in the shoots [76]. It can be argued that in roots of salt tolerant genotypes the mechanism of retention of Na⁺ ions occurred. Moreover, Ashraf et al. [77] reported that tolerant genotypes have lower uptake of Na⁺ than sensitive genotypes do.

For salt tolerance assessment, we also applied two tolerance indices (SSI and STI) according to quantitative criteria proposed for selection of genotypes based on their yield performances in stress and non-stress conditions. By using the SSI index, we can determine the stress tolerant genotypes based on the rate of yield changes compared to non-stress conditions, which means higher stability in yield [41]. STI reflects genotypes with high yield potential and stress tolerance [41]. In fact, higher STI indicates highest yield and highest tolerance to stress. Better performance and higher effectiveness for STI compared to SSI to distinguish higher yielding genotypes across different environments has been reported by others [41,42]. On the other hand, SSI allows for the identification sorghum genotypes with more stable yield under saline condition (lower changes). In fact, the lower values for SSI demonstrate

the lower difference in the yield between the stress and normal conditions, which means higher stability in yield. Thus, according to SSI, it can be concluded that genotypes PEGAH and GS4 have the highest stability under saline conditions. But this stability does not indicate better performance of these genotypes under saline conditions. However, according to STI genotypes PEGAH and GS4 have also the highest yield potential and salt tolerance. It should be emphasized here that we applied these indices taking into account the results of dry biomass of seedlings, not real yield. However, the results of this analysis reflect our findings based on germination and growth parameters: that PEGAH and GS4 can be considered as salt tolerant and genotypes PAYAM and SEPIDEH as salt sensitive.

Based on individual parameters, it is difficult to discuss the salinity tolerance of all ten investigated genotypes, because of problems interpreting the statistical significance of differences (Tables 2 and 3). Therefore, we applied cluster analysis to compare all parameters in all treatments simultaneously. Based on these results, we can define as salt sensitive not only the PAYAM and SEPIDEH genotypes, but also KIMIA and KG29. At the same time, we can claim JAMBO and SPEED FEED as salt resistant, together with PEGAH and GS4.

We observed differences between genotypes under non-saline conditions also, especially in MTG, VIG, and all growth parameters. It has been reported that these differences could be due to the genetic potential capability of each genotype [20,78,79].

5. Conclusions

In the present study, we proved significant differences between sorghum genotypes under saline conditions at germination and seedling stages. Identifying and selecting the most salt tolerant varieties of species is very important for agriculture. Sorghum has shown great potential for genetic variability under salt stress. These genetic differences are a good basis for providing information about sorghum genotypes that can be grown in areas affected by salt, and may be useful for better crop production and for determining the degree of salt tolerance in different genotypes for their further use in breeding programs. It can be concluded that seedling traits can be used as valid criteria for the selection of genotypes with a better tolerance to salinity stress.

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