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ORIGINAL ARTICLE

Genotypic Variation for Salinity Tolerance in Sorghum (*Sorghum bicolor* (L.) Moench) Genotypes at Early Growth Stages

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Sorghum (*Sorghum bicolor* L. Moench) is the fifth most economically important crop among cereals in the world. Salinity is an abiotic factor which reduces productivity of sorghum. Exploiting genetic variability to identify salt tolerant genotype is one of the strategies used to overcome salinity. Pot experiment was carried out to evaluate the genetic variation of eleven sorghum genotypes for NaCl salinity response at germination and early seedling stages. The experimental treatments were five NaCl salinity levels (0, 2, 4, 8, and 16 dS m^{-1}) and eleven sorghum genotypes (Gambella1107, Melkam, S-35, ESH-2, Gobye, 97MW6130, Meko, 76T1#23, ICSV-111, Abshir and Teshale). The experimental design was completely randomized design with three replicates. Data was analyzed using SAS (version 9.0) statistical software and means were separated by LSD. Germination rate, final germination percentage, seedling shoot length and seedling root length were measured. The ANOVA for treatments, genotypes and their interaction was found to be highly significant ($p<0.001$) with regard to all parameters. Genotypes Meko, Gambella1107, ICSV-111 and Melkam were found salt tolerant during germination and seedling growth stages. However, genotypes ESH-2 and Gobye were salt sensitive during both stages. The rest sorghum genotypes were intermediate in their salt tolerance. The study affirmed the presence of wide genotypic variation among the sorghum genotypes for NaCl salt tolerance.

Key words: Germination, NaCl, salinity, seedling, sorghum

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Key words: Germination, NaCl, salinity, seedling, sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most economically important crop among cereals in the world. It is grown on about 44 million hectares of land (Prakash *et al*., 2010), in 99 countries (ICRISAT 2009) with an annual production of 60 million tons (Iqbal *et al.,* 2010).

In Ethiopia, sorghum ranks third among major cereal crops in terms of area and production next to tef (*Eragrostis tef*) and maize (*Zea mays*) (Asfaw 2007a). It is cultivated on 1.62 million hectares of land and about 2.97 million ton is produced each year in Ethiopia (CSA 2010). It is a staple food crop on which lives of millions of poor Ethiopians depend and has tremendous uses for the farmer and no part of this plant is wasted (Asfaw 2007b).

Despite its importance, sorghum productivity is far below the genetic potential of the crop (Kidane *et al*., 2001). Salinity is one of the major factors that reduce the productivity of sorghum (Wang *et al*., 2003). The amount of worldwide salt affected land is about 900 million ha with most of its water containing about 30 g of sodium chloride (NaCl) per liter (Flowers 2004). In African countries like Kenya, Nigeria, Sudan, Tunisia and Tanzania there is a reported 8.2, 5.6, 4.8, 1.8 and 1.7 Mha of salt affected land, respectively (FAO 2000).

Ethiopia has 44 million ha (36% of the country's total) of land area potentially susceptible to salinity problems. Out of the 44 million ha, 33 million ha have dominantly salinity problem (Hawando 1995). Out of the 170,000 ha of land under irrigation by state farms in Awash Valley and in Central Rift-Valley lake area of Ethiopia, almost 10% (11,000 ha) is feared to have been salinized and have already gone out of production (Hawando 1995).

One of the important strategies plant scientists adopted to overcome salinity is to exploit genetic variability of the available germplasm to identify tolerant genotype that may sustain a reasonable yield on salt affected soils (Ashraf *et al.,* 2006). This approach involves understanding the response of plants at different growth stages under saline conditions as reported in different crops such as maize (Khatoon *et al*., 2010), sorghum (Geressu and Gezahagn 2008), rice (Momayezi *et al*., 2009), and millet (Yakubu *et al*., 2010).

Germination and seedling characteristics are the most viable criteria used for selecting salt tolerant plants due to the fact that the final plant stand of a crop primarily depends on seedling characteristics. Germination percentage, germination rate and seedling growth are most commonly used criteria for genotype selection (Bybordi and Tabatabaei 2009, Endalew *et al*., 2012). Therefore, this research attempted to investigate the genetic variability of sorghum (*Sorghum bicolor,* L. Moench) genotypes which grow in the Central Rift Valley of Ethiopia for salt stress during germination and seedling growth stages.

MATERIALS AND METHODS

Plant material and growth conditions

The experiment was conducted in a greenhouse at Rare Research Station of Haramaya University, Ethiopia during March-April, 2011. The experiment was conducted following the modified procedures used by Ahmed (2009) using plastic pots with widths 15 cm at the base and 20 cm at the top and 18 cm height. The experiment consisted of 11 sorghum genotypes and 4 salinity levels in the form of NaCl solutions and one control. The specific sorghum genotypes used in the research were Gambella1107, Melkam, S-35, ESH-2, Gobye, 97MW6130, Meko, 76T1 #23, ICSV-111, Abshir and Teshale. The seeds of eleven sorghum genotypes which grow in the Central Rift Valley of Ethiopia were obtained from Melkassa Agricultural Research Center (MARC), Ethiopia. The four NaCl salinity levels used were 2, 4, 8 and 16 dS m^{-1}. This NaCl salinity levels were created by dissolving 1.12, 2.10, 4.95 and 9.9 g NaCl in one liter distilled water, respectively. Tap water was used as control. The

sorghum seeds were surface-sterilized by soaking them in sodium hypochlorite (3%) solution for 5 minutes and then rinsed with distilled water for 5 minutes. The rinsed seeds were then air-dried at room temperature. The experimental soil was taken from Haramaya University's Rare Research field and had (according to the soil analysis done at the Soil Sciences Laboratory of Haramaya University) 0.746% organic carbon, 1.3% organic matter, 6.18 pH, 0.064% total nitrogen, and 0.65 dS m^{-1} EC (in 1:2.5 water extract). Prior to the experiment, the collected soil was adjusted to 2, 4, 8 and 16 dS m^{-1} soil salinity by adding 1.08, 2.16, 4.32 and 8.64 g NaCl in 3.5 kg soil as described by Mamo *et al*., (1996). After the soil was adjusted, 3.5 kg soil was filled into 165 pots. There was no NaCl added to the soil of the control pot. Before adding soil into the pots, pots were lined with polyethylene bags to avoid salt leaching. Then twelve surface sterilized uniform seeds of each sorghum accessions were sown in the plastic pots at uniform depth. Pots were placed in a Completely Randomized Design (CRD) placement and replicated 3 times. Pots were irrigated with equal 100 ml of NaCl solutions of 2, 4, 8 and 16 dS m^{-1}, and tap water as control. The EC of the tap water was measured by EC meter and was 1.52 dS m^{-1} during the experiment period. Treatment application with the same amount of salt solution continued every other day and germination count started after 72 hours of sowing and continued until the 14th day. The seeds were considered to have germinated when both the plumule and radicle had emerged ≥ 0.5 cm. Fourteen days after sowing, germination count was terminated and application of treatments continued until the 28th day. Germination rate and final germination percentage were obtained from germination counted until the 14th day and seedling growth parameters were obtained after 28th day as described below.

Germination rate (GR) which is the average number of days needed for plumule or radicle emergence was calculated using MAGUIRE's equation (Maguire 1962) as:

$GR(M) = n_2/t_2 + n_4/t_4 + n_6/t_6 \ldots + n_{14}/t_{14}$;

where n_2, n_4, n_6 .., n_{14} represent the number of germinated seeds at times t_2, t_4, t_6 …, t_{14} (in days).

Final germination percentage (FGP) was calculated as a percent of the total number of seeds germinated during the 14 days over the total number of seeds planted. At the 28th day (14 days after the termination of germination count), the longest shoots and roots of six randomly selected seedlings from each pot were measured using a draftsman ruler to obtain seedling shoot length (SSL) and seedling root length (SRL), respectively. Before measurement, the seedlings were uprooted and washed carefully to remove soil and debris.

Statistical analysis

Data analysis was carried out using SAS (version 9.0) statistical software (SAS Institute Inc., USA) where two way analysis of variance (ANOVA) was done. Whenever treatment differences were significant, means were separated using the Least Significant Difference (LSD) test.

RESULTS

Germination rate

Analysis of variance exhibits significant differences among the sorghum genotypes, NaCl salinity levels and their interaction with respect to germination rate. Our results indicated that genotype ICSV-111 followed by S-35 and Meko gave significantly higher mean germination rate than the other genotypes in the control (Table 1). Genotypes Meko, ICSV-111, Teshale and Abshir showed

significantly faster germination rate than the other genotypes in the 2, 4 and 8 dS m^{-1} treatments. In the highest NaCl salinity level (16 dS m^{-1}), Meko, Gambella1107 and ICSV-111 showed significantly higher mean germination rate than the other genotypes tested. On the other hand, genotypes ESH-2 and Gobye showed significantly slower mean germination rate than the rest of the genotypes at all salinity levels (Table 1). The remaining genotypes were intermediate in their response to NaCl salinity.

Final Germination Percentage

Analysis of variance for final germination percentage was found to be highly significant with respect to genotypes, NaCl salinity levels and their interaction. Table 2 indicates that the percent germination generally decreased with increasing salt concentration and degree of reduction varied with the salinity levels and genotypes of sorghum. Remarkable reduction in germination percent was observed at higher levels from 8 to 16 dS/m of salt concentrations as compared to control. Tolerance of the sorghum genotypes against salinity stress showed marked differences.

Genotypes S-35, ICSV-111, Meko, Melkam and Teshale showed significantly higher mean final germination percentage than the other genotypes while ESH-2 and Gobye showed slower mean final germination percentage than the rest in the control, 2 and 4 dS m^{-1} treatments. The sorghum genotypes such as Gambella1107, Abshir and ICSV-111 showed tolerant behavior and rest of the genotypes seemed to be salt sensitive at 8 dS m^{-1} salt concentration. Sorghum genotypes such as Meko, Gambella1107, ICSV-111 and Melkam showed significantly higher mean final germination percentage than the other genotypes in the 16 dS m^{-1} salinity level. A large reduction is observed in ESH-2 and 97MW6130 as salt concentration increase from 8 to 16 dS m^{-1} (Table 2).

Effect of salinity on seedling growth

The experiment was conducted to observe the influence of salinity on the seedling growth of sorghum genotypes. The results obtained indicate that increasing salt concentration caused delayed emergence of plumule and redicle as compared to control. At the early seedling stage, reduction in root length and shoot length clearly demonstrated genetic variation in vegetative growth responses to salinity among sorghum genotypes. The average length of root and shoot for 11 genotypes of sorghum shows a strong inhibition with the increasing salinity levels particularly at high salt concentration (8 to 16 dS m^{-1}). Tables 3 and 4 indicate that the reduction of shoot and root growth in ESH-2, Gobye and 97MW6130 was highest in comparison with Abshir, Melkam and 76T1#23 at 2, 4 and 8 dS m^{-1} salinity levels. These results showed sign of great inhibition of shoot and root growth with salt treatments. Genotypes Meko, ICSV-111 and Gambella1107 had more length of shoot while genotypes Gambella1107, Meko and Melkam had more length of roots as compared to other genotypes and showed tolerant behavior up to 16 dS m^{-1}.

DISCUSSION

The main purpose of the study was to identify salt tolerant genotypes in sorghum germplasm at early vegetative growth stages under different levels of salinity. The analysis of variance for genotypes, NaCl salinity levels and their interaction was found to be significant for all parameters; reflecting that all the genotypes responded differently to salt stress with respect to all parameters.

Table 1 Mean germination rates of sorghum genotypes under different NaCl levels

	Salinity (NaCl) level (dS m^{-1})					
Genotype	0	2	4	8	16	Mean
S-35	7.95	7.49	8.19	7.15	1.31	6.42
ESH-2	2.03	1.81	2.26	1.69	0.03	1.56
Gamb*	6.55	5.23	6.28	7.00	2.07	5.43
Melkam	7.18	7.56	7.67	6.21	1.56	6.04
Gobye	2.68	3.65	2.55	2.55	0.39	2.36
97M*	4.59	6.07	5.08	4.18	0.43	4.07
Meko	7.78	9.07	8.68	6.57	2.31	6.88
76T1#23	4.55	6.40	5.96	5.15	1.54	4.72
ICSV-111	8.37	8.25	8.89	7.77	1.72	7.00
Abshir	7.49	8.07	7.45	8.21	0.71	6.39
Teshale	7.72	8.24	8.32	6.94	1.22	6.49
Mean	6.08	6.53	6.48	5.76	1.21	

LSD ($P \leq 0.05$)
S=0.23 G =0.35 S x G=1.07 CV(%)=9.15
S=salinity levels; G=genotypes; CV=coefficient of variation; 97M*=97MW6130; Gamb*= Gambella1107

Table 2 Mean final germination percentage (%) of sorghum genotypes under different NaCl levels

	Salinity (NaCl) level (dS m^{-1})					
Genotype	0	2	4	8	16	Mean
S-35	94.47	88.87	89.57	80.53	22.17	75.12
ESH-2	36.07	24.97	24.97	24.97	2.77	22.75
Gamb*	75.00	72.23	66.67	91.67	36.10	68.33
Melkam	80.43	88.90	86.13	75.00	27.73	71.64
Gobye	41.67	41.67	36.10	36.07	11.07	33.32
97M*	69.43	74.97	63.90	61.07	8.30	55.53
Meko	83.37	97.23	91.67	80.53	41.63	78.89
76T1#23	69.47	72.17	72.20	63.87	22.20	59.98
ICSV-111	91.67	88.90	97.23	83.30	27.73	77.77
Abshir	80.57	86.10	83.30	86.10	11.10	69.43
Teshale	83.33	86.10	94.47	77.73	19.27	72.18
Mean	73.22	74.74	73.29	69.17	20.91	

LSD ($P \leq 0.05$)
S=1.65 G =2.45 S x G=1.07 CV(%)=6.41
S=salinity levels; G=genotypes; CV=coefficient of variation 97M*=97MW6130; Gamb*= Gambella1107

Table 3 Mean seedling shoot length (cm 6 $plants^{-1}$) of sorghum genotypes under different NaCl levels

	Salinity (NaCl) level (dS m^{-1})					
Genotype	0	2	4	8	16	Mean
S-35	205.67	199.67	191.67	132.00	0.00	145.80
ESH-2	137.83	130.67	117.00	38.00	0.00	84.70
Gamb*	214.83	203.00	153.67	104.33	26.33	140.43
Melkam	206.67	211.67	204.67	155.00	23.00	160.20
Gobye	139.67	140.00	96.33	60.33	0.00	87.27
97M*	209.50	180.67	197.67	119.00	0.00	141.37
Meko	215.00	204.33	187.00	122.67	31.00	152.00
76T1#23	254.00	250.33	177.67	144.33	16.33	168.53
ICSV-111	200.33	222.17	163.67	130.67	28.67	149.10
Abshir	220.83	203.67	205.67	145.33	0.00	155.10
Teshale	192.67	192.00	185.00	130.67	11.33	142.33
Mean	199.73	194.38	170.91	116.57	12.42	

LSD ($P \leq 0.05$)
S=5.13 G=7.61 S x G=10.8 CV(%) =9.12
S=salinity levels; G=genotypes; CV=coefficient of variation; 97M*=97MW6130; Gamb*= Gambella1107

Table 4 Mean seedling root length (cm 6 plants^{-1}) of sorghum genotypes under different NaCl levels

	Salinity (NaCl) level (dS m^{-1})					
Genotype	0	2	4	8	16	Mean
S-35	144.33	133.67	124.33	84.67	0.00	97.40
ESH-2	93.67	54.33	57.00	10.67	0.00	43.13
Gamb*	166.67	134.50	87.67	46.33	10.00	89.03
Melkam	140.33	119.67	134.17	50.67	7.67	90.50
Gobye	65.83	75.00	50.00	35.17	0.00	45.20
97M*	132.67	78.67	103.67	39.33	0.00	70.87
Meko	161.33	142.50	104.67	59.00	9.67	95.43
76T1#23	146.67	144.00	82.00	34.67	0.00	81.47
ICSV-111	140.00	133.33	140.00	68.67	3.33	97.07
Abshir	123.83	102.17	102.00	35.00	0.00	72.60
Teshale	167.33	171.33	130.67	58.67	3.00	106.20
Mean	134.79	117.20	101.47	47.53	3.06	

LSD ($P \leq 0.05$)

S=2.95 G=4.37 S x G=29.16 CV(%)=7.96

S=salinity levels; G=genotypes; CV=coefficient of variation; 97M*=97MW6130; Gamb*= Gambella1107

Increment in NaCl salinity level caused significant reduction in germination rate (GR) of sorghum genotypes. However, the reduction was sharp at 8 and 16 dS m^{-1} NaCl salinity levels. On the basis of germination rate for the soils having 16 dS m^{-1}of salinity, the genotypes Meko, Gambella1107 and ICSV-111 can be recommended. It is a well documented fact that if a crop stand is good then the yield will be higher than that crop with poor stand. There are many reports which indicate that the genotypes which maintained higher germination under saline conditions produced higher biomass and yield (Ashraf *et al.,* 2006; Krishnamurthy *et al*., 2007). The reduction in germination rate could be due to toxic effects of certain ions and also higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination (Jamil *et al*., 2006). Results of Abari *et al*., (2011) also confirmed the present findings. Higher salinity level retard seed germination and root emergence due to osmotic effect which is deleterious and prevent the plants from maintaining their proper nutritional requirements necessary for their healthy growth (Krishnamurthy *et al*., 2007).

The data regarding final germination percentage indicated that the genotypes Meko, Gambella1107, ICSV-111 and Melkam showed significantly higher FGP in the highest (16 dS m^{-1}) NaCl salinity level while genotypes ESH-2 and 97MW6130 showed significantly reduced FGP than the other genotypes tested. These differences might be due to the genetic variation among genotypes (Krishnamurthy *et al*., 2007). Overall, final germination rate of all genotypes exhibited decline with all increasing level of salinity. Reduction in plant growth due to the adverse effect of salinity has been also reported in many other crops (Abari *et al.,* 2011; Hakim *et al*., 2010; Ates and Tekeli 2007).

In the present experiment, effect of salinity on seedling growth particularly that of the shoot length showed that Meko, ICSV-111 and Gambella1107 could be grown as salt tolerant varieties up to 16 dS m^{-1} NaCl salinity because they produced the maximum shoot length and it is well known fact that tolerant genotypes have a higher mean seedling shoot length under saline environment than sensitive genotypes (Bashir *et al*., 2011; Hakim *et al*., 2010). Reduction of seedling shoot length is a common phenomenon in many crop plants that are grown under saline conditions

(Amin *et al*., 1996). The reason for the reduced shoot development could be due to the toxic effects of the NaCl used as well as the unbalanced nutrient uptake by the seedlings. Another possible reason may be slowing down of the water uptake by the plant because of inhibition of root and shoot elongation by high salinity (Werner and Finkelstein 1995).

The results of the current study also showed increasing the amount of salt caused significant reduction in seedling root length of most of the genotypes. Genotypes Gambella1107, Meko and Melkam produced significantly higher seedling root length than the other genotypes in the 16 dS m^{-1} NaCl salinity level though most of the genotypes could not produce sufficient root growth. Neumann (1995) indicated that salinity can rapidly inhibit root growth and hence capacity of water uptake and essential mineral nutrition from soil. Plants vary in their tolerance to salinity that depends on their efficiency of root system with regards to nutrient absorption and K, Na uptake discrimination (Khan *et al*., 1995) or mainly the translocation of sodium and chloride ions. In sorghum sodium mainly remained in roots as compared to shoots (Khan & Ashraf 1990) that might be due to the mechanism of retention more of Na+ in roots (Khan *et al.,* 1995) as compared to other plant parts. Keeping in view the above results it is clear that salinity reduced all growth parameters at all levels. Reduction in growth may be due to lower transport rate of essential ions like NO_3^- that reduced the N compounds and increased Na+ in plant under high salinity (Hamid *et al*., 2008).

Generally, one of the important strategies plant scientists adopted to overcome salinity is to exploit genetic variability of the available germplasm to identify tolerant genotype that may sustain a reasonable yield on salt affected soils (Ashraf *et al.,* 2006). Germination and seedling characteristics are the most viable criteria used for selecting salt tolerant genotypes due to the fact that the final plant stand of a crop primarily depends on seedling characteristics (Bybordi and Tabatabaei 2009). Hence based on the parameters evaluated; the above sorghum genotypes which had significantly better results at both germination and seedling growth stages could germinate and establish themselves effectively on moderately saline soils. These genetic differences presents a good basis to provide information about genotypes of sorghum that could be grown in salt-affected areas to chance crop productivity, and also for determining degree of salt tolerance in different plant species to further utilize them in breeding programme.

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