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*Full Length Research Paper*

# Response of sorghum (*Sorghum bicolor* (L.) Moench) genotypes to NaCl levels at early growth stages

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**Salinity which affected approximately 7% of the world's total land area is one of the factors which reduce productivity of sorghum. Exploiting genetic variability to identify salt tolerant genotype is one of the strategies used to overcome salinity. Petri dish experiment was conducted to evaluate the response of eleven sorghum genotypes for NaCl salinity tolerance at germination and early seedling stages. The experimental treatments included five NaCl salinity levels (0, 2, 4, 8, and 16 dS $m^{-1}$) and eleven sorghum genotypes. The experimental design was completely randomized design with three replicates. Data was analyzed using statistical analysis system (SAS) (version 9.0) statistical software. Germination rate, final germination percentage, seedling shoot length and seedling root length were measured. The analysis of variance (ANOVA) for treatments, genotypes and their interaction was found to be highly significant ($p<0.001$) with regard to all parameters. Genotype ICSV-111 showed greater salt tolerance during germination stages while Teshale and 76T1#23 were better salt tolerant during seedling growth stages. However, genotypes ESH-2 and 97MW6130 were found to be salt sensitive based on all parameters. All parameters measured showed to have an inverse relationship with increase in NaCl salinity levels. The study affirmed presence of wide genotypic variation among the sorghum genotypes for NaCl salt tolerance.**

**Key words:** Germination, NaCl, salinity tolerance, seedling growth, sorghum genotypes.

## INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most economically important crop among cereals in the world. It is grown on approximately 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). From the total 9.23 million hectares of crop area under cereals, sorghum occupies 17.55% of the cultivated land. It is cultivated on 1.62 million hectares of land and about 29.71 million quintals are produced each year in Ethiopia (CSA, 2010). It is a staple food crop on which lives of millions of poor Ethiopians depend. It has tremendous uses for the Ethiopian farmer and no part of this plant is wasted (Asfaw, 2007b).

Despite its importance, sorghum productivity in Ethiopia 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 Africa, there are a reported 8.2, 5.6, 4.8, 1.8 and 1.7 Mha of salt affected land in Kenya, Nigeria, Sudan, Tunisia and Tanzania, respectively (FAO, 2000).

In Ethiopia, 44 million ha (36% of the country's total) land area is potentially susceptible to salinity problems.

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Out of the 44 million ha, 33 million ha have dominantly salinity problem (Hawando, 1995). Salt affected soils are prevalent in the Rift Valley and lowland areas of the country. The Awash Valley in general and the lower plains in particular are dominated by salt affected soils (Tadelle, 1993). Out of the 170,000 ha of land under irrigation by state farms in Awash Valley and in Central Rift-Valley lake area, 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; Wani et al., 2011; Wani and Gosal 2010), and millet (Yakubu et al., 2010).

Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in 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 criterias for genotype selection (Bybordi and Tabatabaei, 2009).

Therefore, our research attempted to investigate the response of sorghum (*S. bicolor*, L. Moench) genotypes which grow in the Central Rift Valley of Ethiopia for salt stress during germination and seedling stages. The reasons for selecting sorghum for the research are: being the third important cereal crop in Ethiopia (Asfaw, 2007a) and its tremendous use for Ethiopian farmers (Asfaw, 2007b). Moreover, previous reports on salt tolerance of sorghum are relatively few.

## MATERIALS AND METHODS

The study was conducted in the Botanical Sciences Laboratory of the Department of Biology of Haramaya University, Ethiopia during March to April, 2011. The geographical location of the study site is 9° 24' N and 42° 03' E. This experiment was carried out at room temperature following the procedures used by Geressu and Gezahegne (2008). Seeds of eleven sorghum [*S. bicolor* (L.) Moench] genotypes which grow in the Central Rift Valley of Ethiopia were obtained from Melkassa Agricultural Research Center (MARC), Ethiopia. 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.

Different salinity levels (2, 4, 8 and 16 dS $m^{-1}$) were used in this study. These salinity levels were obtained by dissolving 1.12, 2.10, experiment, Petri dishes were sterilized by autoclaving at 160°C for 2 h and Whatman No. 3 filter papers at 120°C for 1 h.

The sorghum seeds were surface-sterilized by soaking them into sodium hypochlorite (3%) solution for 5 min. The seeds were then rinsed with distilled water for 5 min and air-dried at room temperature in the laboratory. Following this, twelve uniform seeds of each sorghum genotypes were placed on each Petri dish and the Petri dishes were arranged in completely randomized design (CRD) with three replications. Eventually, the Petri dishes were covered with polyethylene sheet to avoid the loss of moisture through evaporation.

Treatment application continued every other day and germination count was started after 48 h of sowing and continued until the 14$^{th}$ day. The seeds were considered to have germinated when both the plumule and radicle had emerged $\geq$ 0.5 cm. Fourteen days after sowing, germination count was terminated and application of treatments continued until the 28$^{th}$ day. Germination rate (GR) which is the average number of days needed for plumule or radicle emergence was calculated using MAGUIRE's equation (Maguire, 1962):

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

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 28$^{th}$ day (14 days after the termination of germination count), the longest shoots and roots of six randomly selected seedlings from each Petri dish 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. Seedling shoot to root ratio (SSRR) was calculated as the ratio of seedling shoot length to seedling root length.

### Data 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

The ANOVA for genotypes, NaCl salinity levels and their interaction was found to be highly significant ($p<0.001$) with respect to germination rate. Data for mean germination rate revealed that germination rate was reduced by 1, 2.6, 15 and 38.2% due to 2, 4, 8 and 16 dS $m^{-1}$ salinity levels as compared to the control treatment, respectively. Genotype Meko followed by Teshale, Abshir and Gambella1107 gave significantly higher mean germination rate than the other genotypes in the control (Figure 1).

Meko, ICSV-111, Abshir and Teshale were the genotypes with significantly faster germination rate than the other genotypes in the 2 dS $m^{-1}$ treatment. In the 4 and 8 dS $m^{-1}$ treatments, Meko and Melkam showed significantly higher mean germination rate. ICSV-111 followed by Melkam showed significantly higher mean germination rate than the other genotypes tested in the 16 dS $m^{-1}$ treatment. Genotypes 97MW6130 and ESH-2 showed significantly slower mean germination rate than the rest of the genotypes at all salinity levels (Figure 1).

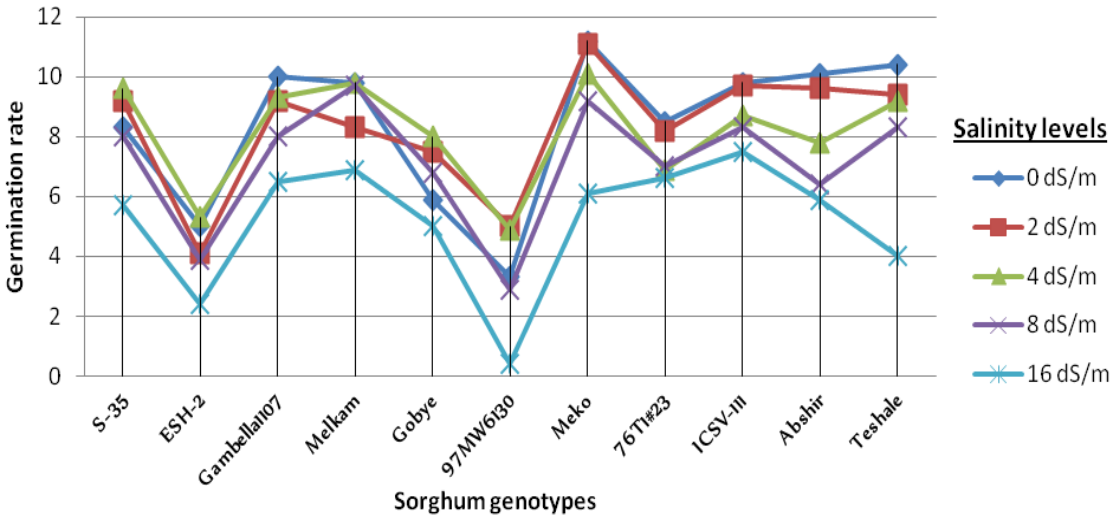


**Figure 1.** Effects of different salinity levels on germination rate (GR) of sorghum genotypes.

The remaining genotypes were intermediate in their response to NaCl salinity.

## Final germination percentage

The ANOVA for final germination percentage was found to be highly significant ($p<0.001$) with respect to genotypes and NaCl salinity levels. It was significant ($p<0.01$) for interaction effect. Compared to the control, relatively higher final germination percentage was observed at 2 and 4 dS $m^{-1}$ levels of salinity. However, final germination percentage was significantly reduced by 3.3 and 22.5% at 8 and 16 dS $m^{-1}$, respectively.

Genotypes Meko, Gambella1107 and Teshale showed significantly higher mean final germination percentage than the other genotypes in the control. Genotypes Meko, ICSV-111 and Abshir in the 2 dS $m^{-1}$ and Genotypes Meko, Teshale, Gambella1107, Abshir and S-35 in the 4 dS $m^{-1}$ treatment showed significantly faster mean final germination percentage.

In the 8 dS $m^{-1}$ treatment, significantly higher mean final germination percentage was recorded on genotypes Meko and ICSV-111. Genotype ICSV-111 showed significantly higher mean final germination percentage than the other genotypes in the 16 dS $m^{-1}$ treatment. However, genotypes ESH-2 and 97MW6130 showed significantly reduced final germination percentage at all salinity levels (Figure 2).

## Seedling shoot length

The ANOVA for seedling shoot length revealed highly significant ($p<0.001$) differences among NaCl treatments, genotypes and their interaction. Compared to the control, seedling shoot length was reduced by 2.3, 18.9, 81.2 and 100% at 2, 4, 8 and 16 dS $m^{-1}$, respectively. Genotypes Teshale and Meko produced significantly higher mean seedling shoot length than the other genotypes in the control while 97MW6130, ESH-2 and Gobye were found to have significantly shorter mean seedling shoot length (Figure 3).

Genotypes Teshale and Gambella1107 showed significantly taller mean seedling shoot lengths in the 2 and 4 dS $m^{-1}$ treatments whereas ESH-2, 97MW6130 and Gobye were found to be with significantly lower mean seedling shoot length. In the 8 dS $m^{-1}$ treatment, 76T1#23 followed by Teshale gave significantly higher mean seedling shoot length than the other genotypes tested. On the other hand, ESH-2 showed highly significant lower mean seedling shoot length. All the genotypes tested were unable to produce sufficient seedling shoot length at 16 dS $m^{-1}$ NaCl salinity level (Figure 3).

## Seedling root length

The ANOVA for seedling root length revealed highly significant ($p<0.001$) differences among NaCl salinity levels, genotypes and their interaction (Figure 4). Increasing the level of salinity by adding different amount of NaCl from 0 to 16 dS $m^{-1}$ significantly reduced mean seedling root length by 20, 37.5, 85.5 and 100% at 2, 4, 8 and 16 dS $m^{-1}$ compared to the control, respectively. Genotype ICSV-111 followed by Gambella1107 gave significantly higher mean seedling root length in the control while 97MW6130 followed by ESH-2 showed significantly reduced result than the other genotypes. Teshale and Gambella1107 were the genotypes with significantly higher seedling root length while ESH-2 is with significantly lower mean seedling root length in the 2 dS $m^{-1}$ treatment.

In the 4 dS $m^{-1}$ treatment, genotypes Teshale, Melkam

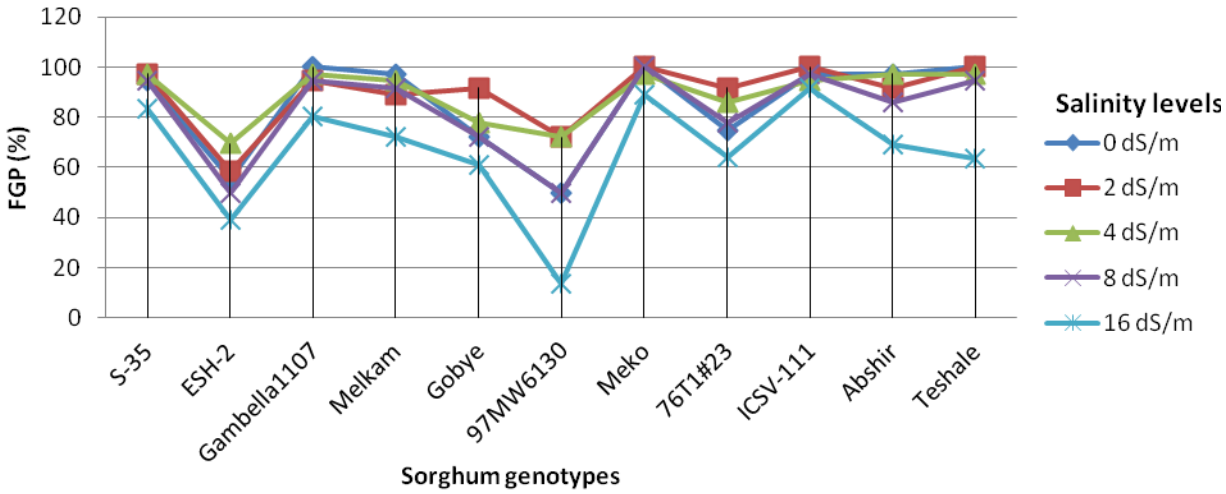


**Figure 2.** Effects of different salinity levels on final germination percentage (FGP) of sorghum genotypes.

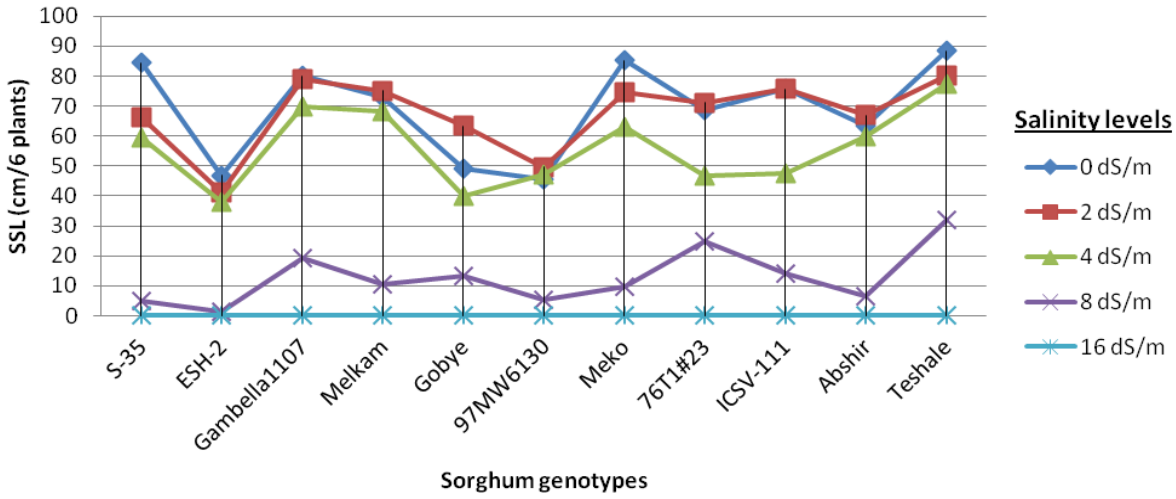


**Figure 3.** Effects of different salinity levels on seedling shoot length (SSL) of sorghum genotypes

and Gambella1107 produced significantly higher mean seedling root length than the other genotypes while ESH-2 showed the lowest value. In the 8 dS $m^{-1}$ treatment, 76T1#23 followed by Teshale showed significantly taller but ESH-2 had a lower mean seedling root length than the other genotypes. There was no sufficient seedling root length data recorded at 16 dS $m^{-1}$ treatment indicating salt sensitivity of genotypes at this level of salinity for seedling root length (Figure 4).

## Seedling shoots to root ratio

The ANOVA for seedling shoot to root ratio showed highly significant ($p<0.001$) differences among NaCl salinity levels, genotypes and their interaction. Genotype S-35 followed by Teshale and Meko gave significantly higher mean seedling shoot to root ratio in the control whereas ICSV-111 followed by Gobye showed the lowest result (Figure 5). Meko followed by S-35 was the genotype with significantly higher mean seedling shoot to root ratio in the 2 dS $m^{-1}$ treatment while genotype Gobye was found to be with significantly lower value than the other genotypes.

In the 4 dS $m^{-1}$ treatment, ICSV-111 followed by 76T1#23 showed significantly higher mean seedling shoot to root ratio while Gobye followed by Melkam showed significantly lower mean seedling shoot to root ratio. In the 8 dS $m^{-1}$ treatment, Teshale followed by Gambella1107 produced significantly higher mean seedling shoot to root ratio while ESH-2 showed the lowest value. There was no sufficient seedling shoot to

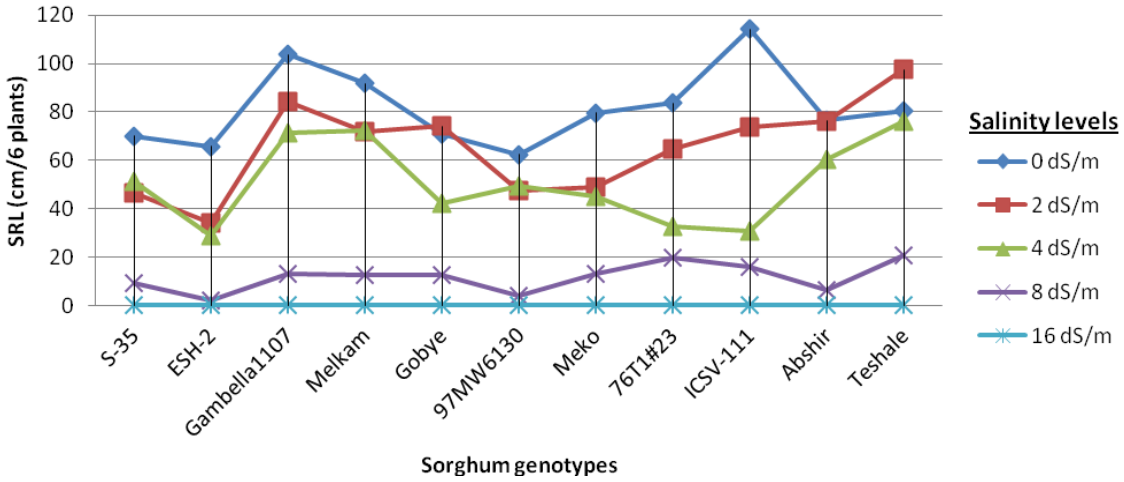


**Figure 4.** Effects of different salinity levels on seedling root length (SRL) of sorghum genotypes.

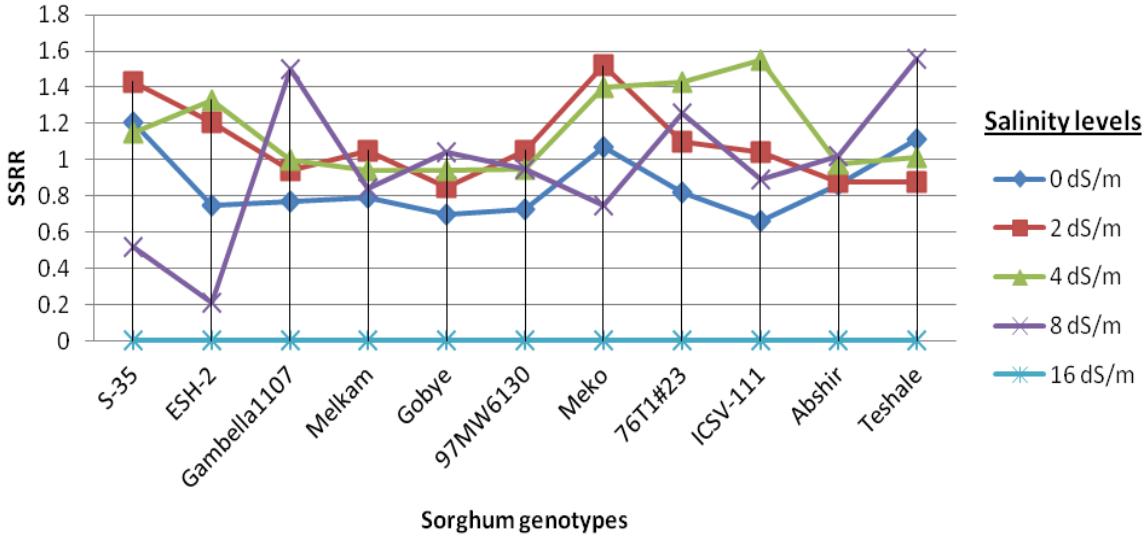


**Figure 5.** Effects of different salinity levels on seedling shoot to root ratio of sorghum genotypes.

root ratio data recorded at 16 dS $m^{-1}$ treatment (Figure 5).

## DISCUSSION

The ANOVA for genotypes, NaCl salinity levels and their interaction was found to be significant for all parameters; reflecting that all genotypes responded differently to salt stress with respect to all parameters.

Increase in NaCl salinity level caused significant reduction in germination rate of all sorghum genotypes. The reduction was sharp at 8 and 16 dS $m^{-1}$ NaCl salinity levels. This could be due to toxic effects of certain ions. In addition to this, higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination (Jameil et al., 2006). Similar report on sorghum genotypes has been reported by Geressu and Gezahagn (2008). This result is also in line with the findings of Abbad et al. (2004), who reported reduction in germination rate of plants by increasing salinity levels.

The ANOVA for germination rate revealed the presence of significant difference among sorghum genotypes for NaCl salinity tolerance. Genotypes ICSV-111, Melkam, 76T1#23 and Gambella1107 showed significantly higher GR than the other genotypes at the highest (16 dS $m^{-1}$) salinity level. However, genotypes 97MW6130 and ESH-2 were found to be with significantly slower GR. The other genotypes showed intermediate response to NaCl salinity levels. Generally, significant genotypic differences exist among sorghum genotypes in response to salinity stress (Netondo et al., 2004). The current result is in line

with Ashraf et al. (2006) who reported significant differences among grass accessions for germination rate.

Significant final germination percentage decrease was observed in most genotypes with increase in the application of NaCl salinity. This could be due to the fact that increasing salinity concentrations in germination media often causes osmotic and specific toxicity which may reduce or retard germination percentage (Abari et al., 2011). Similar results were reported in sorghum accessions (Gerressu and Gezaghegne, 2008) and vegetables (Jameil et al., 2006). The reports indicated that the mean time to germination percentage increased with the addition of NaCl and this increase was greater in higher NaCl salinity levels as compared to lower salinity levels.

The ANOVA for final germination percentage revealed that the tested genotypes showed significantly varied response to salinity which could be due to the presence of genetic variability amongst them. Genotypes ICSV-111, Meko, S-35 and Gambella1107 showed significantly higher final germination percentage in the highest (16 dS $m^{-1}$) NaCl salinity level while genotypes 97MW6130 and ESH-2 showed significantly reduced **f**inal germination percentage (FGP) than the other genotypes tested. Genetic variability within a species offers a valuable tool for studying mechanism of salt tolerance (Ates and Tekeli, 2007). Similar results to the current findings were reported by Jameil et al. (2005) who reported significant differences among *Brassica* species for germination percentage.

The results of the current experiment indicated that increased salinity levels caused significant reduction in mean seedling shoot length in most genotypes. This could be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. Another reason could be that high salinity may inhibit root and shoot elongation due to slowing down of water uptake by the plant (Werner and Finkelstein, 1995). Similar results had been reported by Hakim et al. (2010) and Bashir et al. (2011). They reported a general shoot length decline as NaCl salinity levels increased.

The ANOVA for genotypes with respect to seedling shoot length showed the presence of genotypic variation to NaCl salinity tolerance. Genotypes Teshale, 76T1#23 and Gambella1107 were the genotypes with significantly higher mean seedling shoot length in the 8 dS $m^{-1}$ NaCl salinity level whereas genotypes ESH-2, S-35 and 97MW6130 showed significantly reduced seedling shoot length than the rest genotypes in the same treatment. All the genotypes were salt sensitive that they could not produce sufficient SSL data at the 16 dS m-1 NaCl salinity level.

Generally, the results showed that different sorghum genotypes responded to NaCl salinity differently with regard to seedling shoot length. This could be due to the presence of genetic variation among genotypes. Reduction of seedling shoot length is a common phenomenon of many crop plants grown under saline conditions (Amin et al., 1996). The reason for reduced shoot development could be due the toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. Another reason may be that high salinity may inhibit root and shoot elongation by slowing down the water uptake by the plant (Werner and Finkelstein, 1995). The results of the current study agree with the findings of Geressu and Gezahagn (2008). They indicated as sorghum genotypes responded differently to salinity in terms of early seedling growth rate due to the existence of genetic variation under salt stress condition.

The seedling roots are the first organs exposed to salinity, and root growth is particularly sensitive to increased salt concentrations. As a result, root growth is prevented or rapidly reduced by salinity (Bilgili et al., 2011). The results showed that increasing the amount of salt caused significant reduction in seedling root length of most of the genotypes. Excess salinity with the plant root zone has a deleterious effect on plant growth (Datta et al., 2009). Salinity affects both water absorption and biochemical processes resulting in reduction of plant growth (Parida and Das, 2005). Neumann (1995) indicated that salinity can rapidly inhibit root growth and hence capacity of water uptake and essential mineral nutrition from soil. It can be concluded that to select cultivars for better salt stress tolerance at seedling stage, root elongation may be used as a breeding criterion (Moud and Maghsoudi, 2008).

This study showed better seedling root length at non-saline treatments and significant reduction at higher saline treatments. The same result was reported by Atak et al. (2006). They reported that the control had the longest root length, while the shortest value was at higher (13.2 dS $m^{-1}$) salinity level and generally, root length decreased as NaCl concentration increased.

The findings of this study showed that different sorghum genotypes responded to salinity differently. Genotypes Teshale, 76T1#23 and ICSV-111 produced significantly higher seedling root length than the other genotypes at the 8 dS $m^{-1}$ NaCl salinity level. Genotypes ESH-2 and 97MW6130 produced significantly reduced seedling root length in the same salinity level. There was no sufficient SRL data recorded at the 16 dS $m^{-1}$ NaCl salinity level indicating that the genotypes tested were sensitive to salinity at this level. Similar findings were reported by Geressu and Gezahagn (2008). They indicated the presence of considerable varietal difference among sorghum genotypes in seedling root length with regard to their response to salt stress. This can be due to the presence of genetic variation among the genotypes. Similar results were reported by Moud and Maghsoudi (2008). They indicated that wheat (*Triticum aestivum* L.) cultivars responded differently to salinity in terms of early seedling growth rate due to the existence of genetic variation under salt stress condition. Similar root length reduction due to increased salinity was reported by

Jameil et al. (2005).

The ANOVA for seedling shoot to root ratio showed significant differences among NaCl treatments, genotypes and their interaction; indicating that the sorghum genotypes tested responded to NaCl salinity levels differently. Genotypes Teshale, Gambella1107 and 76T1#23 showed significantly higher and genotypes ESH-2 and S-35 showed significantly lower mean seedling shoot to root ratio in the 8 dS $m^{-1}$ NaCl salinity level. There was no sufficient SSRR data obtained at the 16 dS $m^{-1}$ NaCl salinity level. The results in this study are in line with Abdelhamid et al. (2010) who reported significant differences due to salinity levels and genotypes among faba bean genotypes to seedling shoot to root ratio. According to their findings seedling shoot to root ratio was highly reduced at higher salinity levels and genotypes showed variation in their response to salinity. The results are also in agreement with the results of Geressu and Gezahagn (2008). They reported significant difference for salinity treatments and treatment interactions for seedling shoot to root ratio of sorghum genotypes.

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 (Bybordi and Tabatabaei, 2009). Based on these results, the sorgum genotypes tested in this experiment vary in their response to salinity. Hence, even if it is difficult to conclude from research results obtained from a single laboratory experiment, the previous sorghum genotypes which had significantly better results at both germination and seedling growth stages could germinate and establish themselves effectively on moderately saline soils. However, further field study should be carried out to test their response to salt stress during later growth.

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