

Effects of Salinity on Seedling Biomass Production and Relative Water Content of Twenty Sorghum (*Sorghum bicolor* L. Moench) Accessions

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Abstract: Salinity is a continuing problem in the arid and semi-arid tracts of the world. It could be alleviated using irrigation management and/or crop management. However, the former approach is outdated and very expensive. Nevertheless, the latter is economical as well as efficient and it enables to produce salt tolerant crop lines. But prior to that there is a need to confirm the presence of genetically based variation for salt tolerance among different species or varieties of a particular crop at different growth stages. Thus twenty lowland sorghum (*Sorghum bicolor* L. Moench) accessions were tested during early seedling biomass production at 2, 4, 8 and 16 dS/m salinity levels. Distilled water (0 dS/m) was used as a control. Data analysis was carried out using jmp5 statistical software (version 5.0). Seedling Shoot Fresh Weight (SFW), seedling Shoot Dry Weight (SDW), seedling Root Fresh Weight (RFW) and seedling Root Dry Weight (RDW) were measured. The two ways ANOVA for accessions found statistically insignificant with respect to most parameters recorded ($p > 0.05$) but it was significant with respect to RFW ($p < 0.001$). On the other hand, the two ways ANOVA for treatments displayed statistical significance for all parameters at $p < 0.0001$ except at $p < 0.01$ for RFW. However, it was insignificant for accession* treatment interaction ($p > 0.05$). Accessions such as 235461, 69239, 223550, 69029 and 23403 were found to be salt tolerant during seedling biomass production and in Relative Water Content (RWC). On the other hand, accessions 22885, 233247, 237264, 237265 and 237267 were found to be salt sensitive during seedling biomass production and in RWC. The rest sorghum accessions were intermediate in their salt tolerance. The study affirmed the presence of broad intraspecific genetic variation in sorghum accessions for salt stress with respect to their early biomass production and Relative Water Content (RWC).

Key words: Accessions, NaCl, relative water content, salinity, seedling biomass, sorghum

INTRODUCTION

Salt-affected soils are distributed through out the world and no continent is free from the problem (Brady and Weil, 2002). Salinization of soil is one of the major factors limiting crop production particularly in arid and semi-arid regions of the world (Ahmed, 2009). Globally, a total land area of 831 million hectares is salt affected. African countries like Kenya (8.2 Mha), Nigeria (5.6 Mha), Sudan (4.8 Mha), Tunisia (1.8 Mha), Tanzania (1.7 Mha) and Ghana (0.79 Mha) are salt affected to various degrees (FAO, 2000). Salt stress is known to perturb a multitude of physiological processes (Noreen and Ashraf, 2008). It exerts its undesirable effects through osmotic inhibition and ionic toxicity (Munns *et al.*, 2006). Increased salinity caused a significant reduction in germination percentage, germination rate, and root and shoots length and fresh root and shoots weights (Jamil *et al.*, 2006).

In Ethiopia salt-affected soils are prevalent in the Rift Valley and the lowlands. The Awash Valley in general and the lower plains in particular are dominated by salt-

affected soils (Gebreselassie, 1993). A significant abandonment of banana plantation and a dramatic spread to the adjacent cotton plantation of Melka Sadi Farm was reported (Abegaz, 1995). Moreover, of the 4000ha irrigated land of the above farm 57% has been salt-affected (Taddese and Bekele, 1996). Similarly, the occurrence of salinity problem in Melka Werer Research Farm was reported (Haider *et al.*, 1988). Another study also depicted that of the entire Abaya State Farm, 30% has already been salt-affected (Tsige *et al.*, 2000).

This problem is expected to be severe in years to come. Because under the prevailing situation of the country; there is a tendency to introduce and implement large-scale irrigation agriculture so as to increase productivity (Mamo *et al.*, 1996). In the absence of efficient ways of irrigation water management, salt-build up is an inevitable problem. To alleviate the problem, we need to look for a solution (Gebre and Georgis, 1988). It can be done either using physical or biological practice (Gupta and Mihas, 1993; Marler and Mickelbart, 1993). Since environmental management (physical approach) is not economically feasible (El-Khashab *et al.*, 1997) there

is a need to concentrate on the biological approach or crop management (Ashraf and McNeilly, 1988, Ashraf *et al.*, 2008; Ashraf, 2009). Nevertheless, to proceed with this approach, affirming the presence of genetically based variation for salt-tolerance in a particular crop is a prerequisite (Verma and Yadava, 1986; Marler and Mickelbart, 1993; Mahmood *et al.*, 2009). This could be done at different crop growth stages such as crop establishment (germination, emergence and seedling growth). There are reasonably adequate information on the effects of salinity on crops germination and emergence (Maas, 1986). However, there is only a little information on the impacts of salinity on crops biomass production during seedling growth (Katerji *et al.*, 1994).

Therefore, this research attempted to investigate the response of twenty sorghum (*Sorghum bicolor*, L. Moench) accessions for salt stress with respect to seedling biomass production and Relative Water Content (RWC). The reason for selecting sorghum for the research are: being a dual crop grown for both grain and forage, native to tropical regions (Azhar and McNeilly, 1987), and its resistance to environmental fluctuations and drought (Marambe and Ando, 1995). Moreover, previous reports on salt tolerance of sorghum are relatively few.

MATERIALS AND METHODS

Germination experiment: This study was carried out in Ethiopia from November 2006 to December 2007 at Mekelle University in the Biology Department Laboratory. Seeds of twenty lowland growing sorghum (*Sorghum bicolor* L. Moench) accessions were obtained from the Institute of Biodiversity Conservation (IBC), Ethiopia. The specific sorghum genotypes used in the research were accessions 69029, 69086, 69096, 69117, 69239, 69309, 223247, 223550, 228851, 231190, 234071, 234093, 235461, 237264, 237265, 237266, 237267, 237311, 239211 and 239237. They are adapted to altitudes ranging between 1400-1550 m above sea level. The NaCl concentrations used were 2, 4, 8 and 16 dS/m. These salinity levels were obtained by dissolving 1.12, 2.10, 4.95 and 9.9 g NaCl in one liter distilled water respectively. Distilled water (0 dS/m) was used as a control.

Germination experiment was conducted in a laboratory at room temperature following the procedures used by Mamo *et al.* (1996). Petri dishes with a diameter of 10 cm lined with Whatman No.3 filter paper were supplied with 10ml of each treatment solution and the control. Following this, twelve uniform seeds of each sorghum accession were placed on each petri dish and the petri dishes were arranged in a Randomized Complete Block Design (RCBD) with four replications. Eventually,

the petri dishes were covered with a 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 14th day. The seed was considered to have germinated when both the plumule and radicle had emerged >0.5 cm. After the 14 days, seedling shoot and root fresh weights were recorded and finally oven dried at 70°C for 48 h and the seedling dry weight was measured using sensitive balance.

Relative seedling water content was calculated (Shalaby *et al.*, 1993):

$$\frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Fresh weight}} \times 100$$

Statistical analysis: Data analysis was carried out by JMP5 (version 5.0) statistical software where two ways analysis of variance (ANOVA) and correlation analysis were done. Since all accessions were quite salt-sensitive at 16 dS/m with regard to SFW, RFW, SDW and RDW, the incomplete information obtained from this salinity level has not been included in data analysis.

RESULTS

Seedling Shoot Fresh Weight (SFW): The two ways ANOVA for accessions and treatment*accession interaction was insignificant ($p>0.05$). However, it was statistically significant for treatments ($p<0.0001$). Seedling Shoot Fresh Weight (SFW) was facilitated at 2dS/m in accessions 228851, 237266, 223247, 234093, 235461 and 69239 as compared to the control. Accessions 239211, 237266, 223247, 234071, 231190, 237264, 69117 and 69096 were more salt- affected but accessions 237311, 239237, 228851, 223550, 234093, 235461, 69239, 69086 and 69029 were least salt-affected at both 4 and 8 dS/m salt concentrations compared to the control (Fig. 1).

Seedling Root Fresh Weight (RFW): The two ways ANOVA for accession*treatment interaction was insignificant ($p>0.05$); however, it was significant for accessions ($p<0.001$) and also for treatments ($p<0.01$). Seedling Root Fresh Weight (RFW) was enhanced at 2 dS/m in accessions 223247 and 69309 as compared to the control. Nevertheless, this parameter decreased at 4 and 8 dS/m salinity levels in all accessions but the effect was pronounced at 8 dS/m. Accessions 237311, 237267, 237265, 228851, 234093 and 69309 were most salt-affected but accessions 239211, 239237, 237266, 223550, 234093, 235461, 237264, 69117, 69239, 69096 and 69029 were found to be least salt-affected at 8 dS/m in comparison with the control.

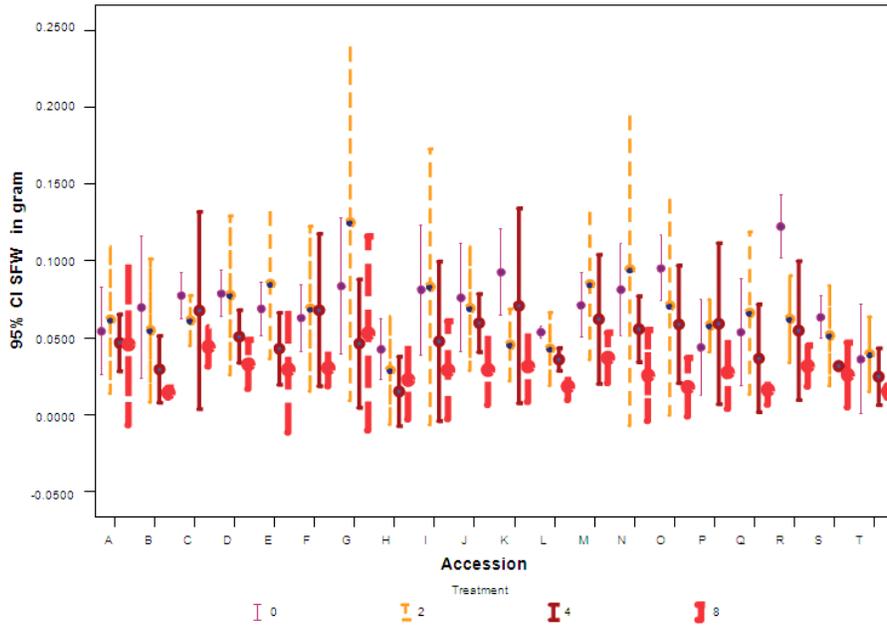


Fig. 1: Effects of different salinity levels (0, 2, 4d and 8 dS/m) on seedling shoot fresh weight (SFW) of *Sorghum bicolor* L. Moench accessions in gram. (Key to accessions: A = 237311, B = 239211, C = 239237, D = 237267, E = 237266, F = 237265, G = 228851, H = 223550, I = 223247, J = 234071, K = 231190, L = 234093, M = 235461, N = 237264, O = 69117, P = 69239, Q = 69309, R = 69096, S = 69086 and T = 69029)

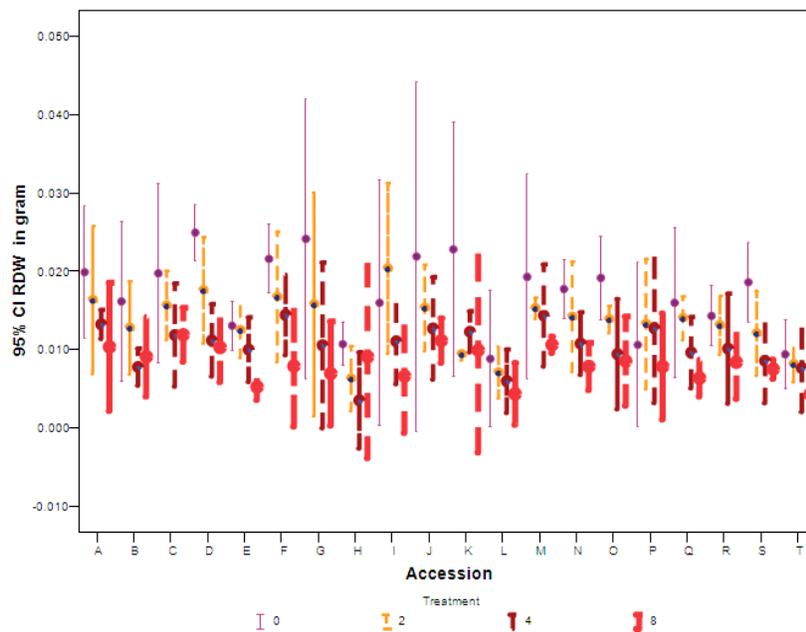


Fig. 2: Effects of different salinity levels (0, 2, 4 and 8 dS/m) on seedling root dry weight (RDW) of *Sorghum bicolor* L. Moench accessions in gram. (Key to accessions: A = 237311, B = 239211, C = 239237, D = 237267, E = 237266, F = 237265, G = 228851, H = 223550, I = 223247, J = 234071, K = 231190, L = 234093, M = 235461, N = 237264, O = 69117, P = 69239, Q = 69309, R = 69096, S = 69086 and T = 69029)

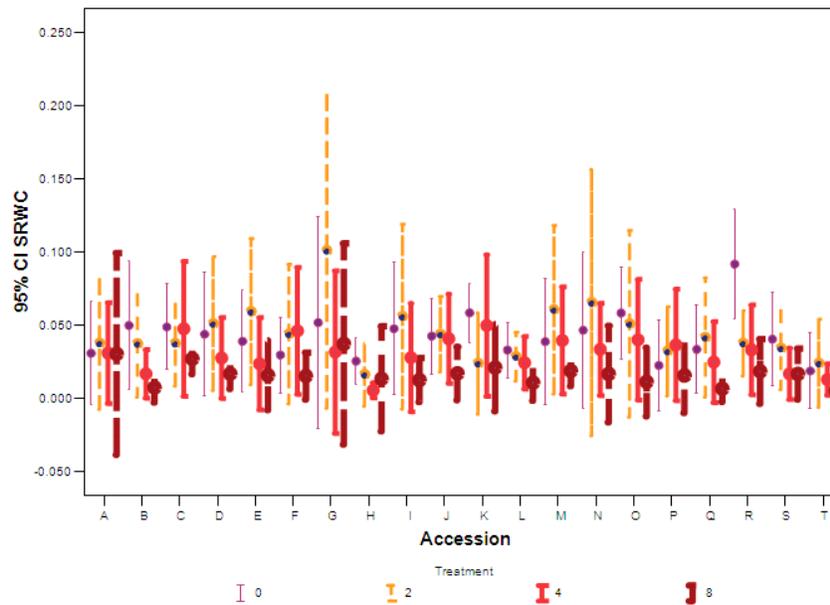


Fig. 3: Effects of different salinity levels (0, 2, 4 and 8 dS/m) on the seedling shoot relative water content (SRWC) of *Sorghum bicolor* L. Moench accessions. (Key to accessions: A = 237311, B = 239211, C = 239237, D = 237267, E = 237266, F = 237265, G = 228851, H = 223550, I = 223247, J = 234071, K = 231190, L = 234093, M = 235461, N = 237264, O = 69117, P = 69239, Q = 69309, R = 69096, S = 69086 and T = 69029)

Seedling Shoot Dry Weight (SDW): The two ways ANOVA showed statistical significance for treatment ($p < 0.0001$) but was insignificant for both accession and treatment*accession interaction ($p > 0.05$). Seedling Shoot Dry Weight (SDW) was enhanced in accessions 237311, 223550, 234093, 235461, 69239, 69309 and 69029 at 2 dS/m as compared to the control. Nevertheless, except these accessions, every salinity level decreased SDW but it was more profound at 8 dS/m. Accessions 239211, 237267, 239237, 237265, 228851, 223247, 231190 and 237264 were most salt-affected; however, accessions 237311, 223550, 234093, 235461 and 69029 were least salt-affected at 8 dS/m salinity level.

Seedling Root Dry Weight (RDW): The two ways ANOVA for accessions and treatment*accession interaction was found statistically insignificant ($p > 0.05$). However, it was significant ($p < 0.05$) for treatments. As compared to the control, seedling Root Dry Weight (RDW) was enhanced at 2 dS/m in accessions 223247 and 69239. It was reduced in all accessions at 4 and 8 dS/m but more profoundly at the latter salt concentration. At 8 dS/m, accessions 237267, 237265, 228851, 223247, 237311 and 69309 were more salt-affected; however, accessions 239211, 239237, 237266, 223550, 234093, 235461, 69239 and 69029 were least salt-affected in comparison with the control (Fig. 2).

Seedling Shoots Relative Water Content (SRWC): The two ways ANOVA showed statistical significance for treatment ($p < 0.001$) and accessions ($p < 0.01$) with respect to seedling Shoot Relative Water Content (SRWC). However, it was significant for accession*treatment interaction ($p > 0.05$). Seedling Shoot Relative Water Content (SRWC) was facilitated in accessions 237311, 237267, 237266, 228851, 223247, 235461, 237264 and 69309 at 2 dS/m as compared to the control. Moreover, it was facilitated both at 2 and 4 dS/m in accessions 237265 and 69239. Accessions 239211, 69096, 223247, 69117, 237264 and 237267 had the lowest SRWC and but accessions 237311, 237265, 228851, 223550, 69309 and 69029 possessed the highest Shoot Relative Water Content (SRWC) compared to other accessions (Fig. 3).

Seedling Roots Relative Water Content (RRWC): The two ways ANOVA for treatments was statistically significant ($p < 0.05$) but insignificant for accessions and treatment*accession interaction ($p > 0.05$) with respect to seedling Root Relative Water Content (RRWC). There was no significant variation among accessions in their RRWC except in accessions 237311, 237267, 237265, 234071, 231190 and 234093 its value being a bit higher than the rest accessions (Fig. 4).

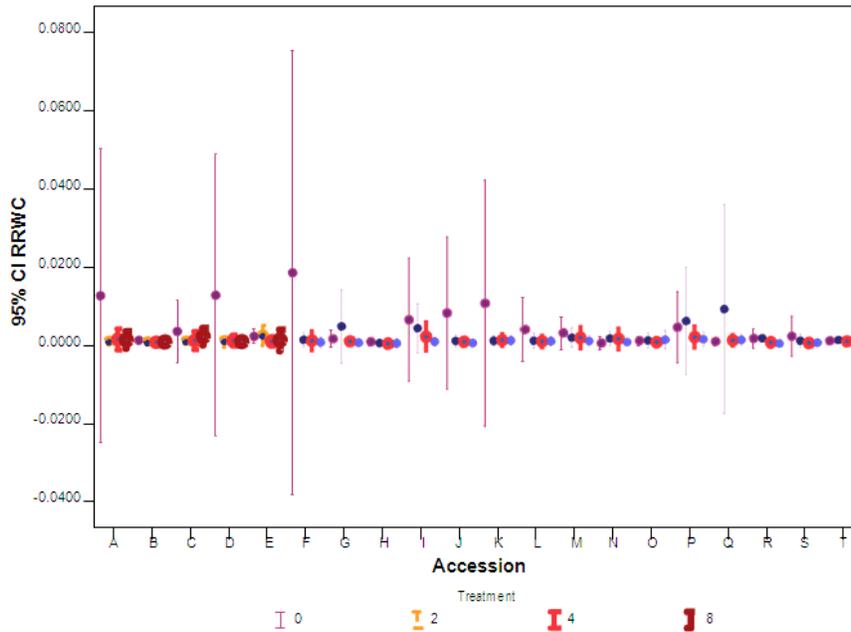


Fig. 4: Effects of different salinity levels (0, 2, 4 and 8 dS/m) on the seedling root relative water content (RRWC) of *Sorghum bicolor* L. Moench accessions. (Key to accessions: A = 237311, B = 239211, C = 239237, D = 237267, E = 237266, F = 237265, G = 228851, H = 223550, I = 223247, J = 234071, K = 231190, L = 234093, M = 235461, N = 237264, O = 69117, P = 69239, Q = 69309, R = 69096, S = 69086 and T = 69029)

DISCUSSION

In accessions 239211, 237266, 223247, 234071, 231190, 237264, 69117 and 69096 Shoot Fresh Weight (SFW) decreased as the salinity level increased. This does agree with previous studies conducted in durum wheat and tef (Mamo *et al.*, 1996) and phaseolus species (Bayuelo-Jimenez *et al.*, 2002). Increased salinity level caused simultaneous reduction of seedling shoots and roots fresh weights in accessions 237267 and 223247. This is in line with previous reports in wheat (Afzal *et al.*, 2005), and sugar beat, cabbage, amaranth and pak-choi (Jamil *et al.*, 2006). In general, Seedling Shoot Fresh Weight (SFW) was more salt affected than seedling Root Fresh Weight (RFW). This is in accord with previous research reports in wheat and triticale genotypes (Shalaby *et al.*, 1993), phaseolus species (Bayuelo-Jimenez *et al.*, 2002) and sugar beat, cabbage, amaranth and pak-choi (Jamil *et al.*, 2006).

Increased salinity level caused simultaneous reduction in seedling root and shoot dry matter production (SDW and RDW) in accessions 237267, 237265, 228851 and 223247. Similar research results were reported in sorghum (Boursier and lauchli, 1992), wheat and triticale genotypes

(Shalaby *et al.*, 1993), barely (Cho and Kim, 1998) and *prosopis alba* (Meloni *et al.*, 2004). On the other hand, both seedling fresh and dry biomass production (SFW, RFW, SDW and RDW) were concurrently reduced as a result of increased salinity level in accessions 237267, 237265, 228851 and 223247. This does agree with previous research findings in rice (Shannon *et al.*, 1998), phaseolus species (Bayuelo-Jimenez *et al.*, 2002), wheat (Afzal *et al.*, 2005) and *catharanthus roseus* (Jaleel *et al.*, 2008).

Seedling Relative Water Content (RWC) was drastically decreased at higher salinity level, 8dS/m. However, the impact was remarkable on Shoot Relative Water Content (SRWC) compared to the Root Relative Water Content (RRWC). This is in agreement with previous reports in lentil (Ashraf and Waheed, 1993), Maize (Çarkırlar and Çiçek, 2002), *Prosopis alba* (Meloni *et al.*, 2004). Salinity stress had no significant effect on seedling shoot and root Relative Water Content (RWC) in accessions 237311, 239237, 228851, 223550, 69239, 69029, 237266 and 235461. Similar result was reported in lentil (Ashraf and Waheed, 1993).

The two ways ANOVA for treatments was significant with regard to all parameters considered. On the one hand, the ANOVA for accessions was insignificant with respect

to SFW, SDW, RDW, RRWC and SRWC. This implies that there were no significant varietal differences among sorghum accessions in relation to the parameters considered. However, it was significant with respect to RFW. This reveals the presence of considerable varietal differences among sorghum accessions in respect of RFW. Accession* treatment interaction was insignificant for seedling Shoot Fresh Weight (SFW), seedling Root Fresh Weight (RFW), seedling Shoot Dry Weight (SDW) and seedling Root Dry Weight (RDW); reflecting that all accessions responded similarly to salt stress with respect to these parameters. In general, salt stress at 2 dS/m has enhanced growth with respect to SFW, RFW, SDW, RDW, RRWC and SRWC in some accessions. The impact of 2 and 4 dS/m salinity levels was not profound with respect to all parameters considered. Nevertheless, all accessions were quite salt-sensitive at 16 dS/m with regard to SFW, RFW, SDW and RDW.

Crop cultivar may germinate effectively under salt stress; nevertheless, its seedling growth may be salt-affected (Azhar and McNeilly, 1987). In line with this, accessions 237311 and 234071 were less salt-affected during germination (Geressu and Gezahagne, 2008) than subsequent growth (had inadequate seedling biomass production and relative water content). This implies that these accessions are salt tolerant during germination than subsequent growth like seedling biomass production and relative water content. On the other hand, crop genotype may be salt sensitive during both germination and seedling growth. This has already been reported in rice (Shannon *et al.*, 1998) and cowpea (Murillo-Amador and Troyo-Die'guez, 2000). Similarly, in this research, accession 223247 found salt sensitive at higher salinity levels during germination and seedling biomass production, and through its relative water content. Thus this sorghum accession could not be directly cultivated even on slightly saline soils.

On the other hand, accessions 235461 and 69239 were found salt tolerant with respect to seedling biomass production and Relative Water Content (RWC). These accessions also had the lowest germination rate (rapid emergence), the highest Final Germination Percentage (FGP) and facilitated seedling growth in another study (Geressu and Gezahagne, 2008). Their salt tolerance capability might emanate from their faster germination which allowed the emerging seedlings to accumulate more biomass relative to the control (Bayuelo-Jimeenez *et al.*, 2002). Thus due to the facilitated seedling root and shoot characteristics, these genotypes were enabled to possess the highest seedling Relative Water Content (RWC) which in turn enabled them to tolerate the toxic effects of salts through dilution (Lee and Senadhira, 1998). Since these two sorghum accessions had rapid

emergence, highest Final Germination Percentage (FGP), facilitated seedling growth, highest seedling biomass production and Relative Water Content (RWC); they could effectively establish themselves on moderately saline soils.

CONCLUSION

The study revealed the presence of broad intraspecific variation among sorghum (*Sorghum bicolor* L. Moench) accessions in response to salt stress during seedling biomass production and Relative Water Content (RWC). Specifically, the study proved that accessions 235461 and 69239 were the most salt tolerant of all sorghum accessions studied. Thus these two accessions could be cultivated on slightly saline soils directly. Because owing to their rapid emergence, highest final germination percentage, facilitated growth, highest biomass production and relative water content they could establish themselves effectively on such soils. However, this could not be a guarantee for them to be salt tolerant in later growth stages. Thus to recommend the cultivation of these two sorghum accessions in the field, there must be further profound greenhouse and field investigation at the whole plant growth level.

ACKNOWLEDGMENT

I would like to express my heartfelt thanks to NORAD II Research Award, for its financial assistance. Moreover, I am greatly indebted to Mekelle University (MU), Department of Biology, for providing the laboratory and also the Institute of Biodiversity Conservation (IBC) for supplying all the twenty sorghum (*Sorghum bicolor* Moench. L.) accessions. Finally, my great gratitude and thanks goes to Mr. Hayal Lemma and Mr. Getachew Gidey for their genuine technical support during the experiment. I also acknowledge the Ecole Normale Supérieure de Cachan (ENS cachan) and Professor Isabelle LEDOUX-RAK for funding the printing/handling fee of this manuscript

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