Screening Selected Genotypes of Cowpea [Vigna unguiculata (L.) Walp.] for Salt Tolerance During Seedling Growth Stage

A. Gogile, M. Andargie and M. Muthuswamy
Department of Biology, College of Natural and Computational Sciences, Haramaya University, P.O. Box: 138, Dire Dawa, Ethiopia

Abstract: The environmental stress such as, salinity (soil or water) are serious obstacles for field crops especially in the arid and semi-arid parts of the world. This study was conducted to assess the potential for salt tolerance of cowpea genotypes during the seedling stage. The experimental treatments were 9 cowpea genotypes and 4 NaCl concentrations (0, 50, 100 and 200 mM) and they were tested in greenhouse. The experimental design was completely randomized design in factorial combination with three replications. Data analysis was carried out using SAS (version 9.1) statistical software. Seedling shoots and root traits, seedling shoots and root weight, number of leaves and total biological yield were evaluated. The analyzed data revealed highly significant (p<0.001) variation among cowpea genotypes, treatments and their interactions. It is found that salt stress significantly decreased root length, shoot length, seedling shoot and root weight of cowpea genotypes. The extent of decrease varied with genotypes and salt concentrations. Most genotypes were highly susceptible to 200 mM NaCl concentration. The correlation analysis revealed positive and significant association among most of the parameters. Genotypes 210885, 211557 and Asebte were better salt tolerant. The study revealed the presence of broad intra specific genetic variation in cowpea varieties for salt stress with respect to their early biomass production.

Key words: Cowpea, salinity, seedling biomass, NaCl

INTRODUCTION

Cowpea (Vigna unguiculata L. Walp.), is one of the most important food grain legumes which is used as a food and animal feed. It is a crop which is grown in the semi-arid tropics covering Africa, Asia, Europe, United States and Central and South America. Cowpea production in Africa alone accounts for 64 percent of the world production (Timiko et al., 2007).

Cowpea is one of the most important food leguminous crop plant of great socio-economic, cultural, nutritional importance and a valuable component of the traditional cropping systems in the semi-arid tropics covering Asia, Africa and Central America. (Langyintuo et al., 2003). It’s high protein content, its adaptability to different types of soil and intercropping systems, its resistance to drought and its ability to improve soil fertility and prevent erosion makes it an important economic crop in many developing regions. The sale of the stems and leaves as animal feed during the dry season also provides a vital income for the resource poor farmers (Andargie et al., 2011).

Salinity is one of the most serious abiotic factors which impede crop production in many parts of the world and no climatic zone in the world is free from salinization (Hussain et al., 2009). Salinization of agricultural soils occurs primarily due to agricultural practices, including poor water management, high evaporation, heavy irrigation and previous exposure to sea water (Pitman and Lauchli, 2002). Salt-affected soils occur in more than 100 countries of the world with a variety of extents, nature and properties and around 930 million ha of the world’s land is salt affected (Munas, 2002; FAO, 2008). In addition, salinity affects 20% of the world’s irrigated land, which accounts for one-third of the world food production (Chinnusamy et al., 2005). According to (FAO, 2008) report, each minute salinity is affecting around 3 ha of additional arable land and if this continues it will bring a very serious global effect which could result in the loss of 30% of the arable land (Wang et al., 2003). This progressive loss of arable land has potentially serious consequences for the expanding global population, which is steadily increasing towards seven billion and set to increase by a further 50% by 2050 (FAO, 2009).

Ethiopia is reported to possess over 11 million hectares of unproductive naturally salt affected wastelands (Gebreselassie, 1993). The naturally salt
affected areas are normally found in the arid and semi-arid lowlands and in Rift valley and other areas that are characterized by higher evaportranspiration rates in relation to precipitation PGRC (1996). Melka Werer Research Farm, which is one of the largest state farms is facing a challenge due to salinity problem as it was reported by Geressu and Gezahagne, (2008). In another report by Tsige et al. (2000), 35% of the Abaya State Farm became out of use because of salinity. Under the prevailing situation of the country, the problem is likely to be severe in the years to come (Geressu and Gezahagne, 2008).

Germplasm screening for tolerance to salt under naturally occurring salt stress does not seem to be reliable because of spatial heterogeneity of soil, physicochemical properties and seasonal fluctuations in rainfall (Akhtar and Hussain, 2009). Therefore, selection must occur under controlled environments, where salt can be reliably induced to distinguish between tolerant and susceptible genotypes and can be compared to the variability of natural environment (Ashraf, 2010; Yamaguchi and Blumwald, 2005). Though there is no single definite morphological marker available for salt tolerance or sensitivity in any crop, but a combination of criteria like germination studies, seedling growth parameters, phenotypic expression and grain yield give a good indication toward the salt response of crop plants (Jamil et al., 2005; Pybordi and Tabatabaei, 2009). It is possible to see genetic differences to salt tolerance within certain species as well as among genera and species (Wimicov, 1993). Existence of genetic variability for salt tolerance within species is of paramount importance in crop improvement programme. Therefore, choice of germplasm for salt tolerance in breeding programmes is most crucial as the success lies on exploring the intra/inter-cultivar variation of a crop by screening its available germplasm. Though researchers have shown that among crop species, legumes are considered as the most salt sensitive (Mass and Hoffmann, 1977) but little information is available about the effect of salt on germination, seedling growth and yield and yield-related traits in leguminous plants in Ethiopia (EIAR, 2007).

Therefore, assessment of genetic variability among the crop genotypes under saline conditions is necessary for the attainment of good crop stand and hence for better yield on salt affected soils because of good seedling (Khan et al., 2007). Based on this, the research was intended to evaluate the salt tolerance of the selected cowpea genotypes during seedling growth stages under greenhouse conditions.

### MATERIALS AND METHODS

This research was conducted in Ethiopia between February 2012 to April 2012 in greenhouse at Haramaya University. The research station at Haramaya University was 1980 m above sea level and located at 9°26’N and 42°3’E. The site has a bimodal rainfall distribution and is representative of semi-arid tropical belt of eastern Ethiopia and was characterized by a sub-humid type of climate with an average annual rainfall of about 790 mm, annual mean temperature of 17°C with mean minimum and maximum temperature of 3.8 and 25°C, respectively (Tekalign and Hammes, 2005). The physical and chemical characteristic of the soil used for the pot experiment which is taken from a depth of 0-20 cm was analyzed at the soil science laboratory of the Haramaya University (Table 1).

<table>
<thead>
<tr>
<th>Content</th>
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<tbody>
<tr>
<td>Clay (%)</td>
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<tr>
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<tr>
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<td>Exchangeable Ca c mol kg⁻¹</td>
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<tr>
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<td>OM%</td>
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<tr>
<td>N (%)</td>
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</table>

For the experiment, seeds of nine cowpea genotypes (i.e., 222867, 216746, 221727, 211435, 210846, 211436, 211446, 211557 and Asebot) were collected from the Institute of Biodiversity and Conservation (IBC) and Melkasa Agricultural Research Center (MARC), Ethiopia. Sandy loam soil was collected from the research farm of Haramaya University, Dire Dawa and 3 kg of soil was filled in to 24×24 cm pots. NaCl solutions with different salinity levels of 50, 100 and 200 mM were prepared by dissolving 2.92, 5.84 and 11.68 g of NaCl in one liter tap water, respectively. The tap water was used as control and solvent. Then the seeds were first surface sterilized in 7% sodium hypochlorite solution for 20 min and washed twice with sterile distilled water. Following this ten pure seeds were sown in the pot in a uniform distance and depth. Then the pots were arranged in a Completely Randomized Design (CRD) in factorial combinations with three replications. Then the pots were irrigated with tap water, at a rate of 100 mL per pot for 15 days and the seedlings were thinned to six per pot to leave uniform plants. According to Taffouo et al. (2004) the pots were supplied with 80 mL of the 0, 50, 100 and 200 mM NaCl solutions for 45 days. Finally the plants were harvested during flowering period (at the age of 8 weeks) and number of leaves per plant (NL), Seedling Shoot Length (SSL), Seedling Root Length (SRL), Shoot to Root Ratio (SRR), shoot fresh weight (SWF), shoot dry weight (SDW), Root Fresh Weight (RFW), Root Dry Weight (RDW) and Total Biological Yield per plant (TBY) were collected.
**Data analysis**: The data was analyzed statistically for analysis of variance using the SAS (version 9.1) statistical software (SAS Institute Inc., USA). Whenever treatment differences were significant, means were separated using the Least Significant Difference (LSD) test at 5% level. Correlation analysis was also employed.

**RESULTS**

**Number of leaves (NL)**: The ANOVA result showed that both genotypes and treatments were highly significant (p<0.001) with respect to number of leaves. However, it was insignificant for treatment by genotype interaction (p>0.05). The mean number of leaves ranged from 5.00 to 6.56 at control, 4.07 to 5.56 at 50 mM, 2.26 to 4.04 at 100 mM and 0.33 to 2.28 at 200 mM NaCl concentrations. The result showed that increasing NaCl concentration led to reduction in leaf number in all the genotypes tested. However, there were differences in the extent of reduction of the number of leaves among the genotypes. Among the genotypes, 211557 followed by Asebot gave the highest number of leaves with 5.56 and 5.23 respectively at 50 mM salinity level while 211435 gave the lowest value with 4.07. Similarly, Asebot followed by 211557 gave the highest number of leaves at 100 and 200 mM NaCl level and were found to be salt tolerant whereas genotype 222867 gave the smallest value at the same concentrations and considered as salt sensitive. The remaining accessions had intermediate values and were found to be moderately salt tolerant (Fig. 1). These observed differences in number of leaves under salt stress conditions clearly indicated the presence of genotypic differences among cowpea germplasm.

**Seedling Shoot Length (SSL)**: The analysis of variance revealed significant (p<0.001) variation among accessions and treatments for SSL. Genotype by treatment interaction effect was also significant (p<0.05) reflecting all the genotypes responding differently to salt stress with respect to SSL. The result indicated the existence of substantial decrease in SSL among the cowpea genotypes in response to increased salinity level. Among the genotypes, the longest shoot length was observed in genotype 211557 followed by 210856 at all the treatments and were found to be salt tolerant while the shortest SSL was on genotype 222867 at all the treatments and considered as salt sensitive (Fig. 2).

**Seedling Root Length (SRL)**: The analysis of variance confirmed the presence of significant (p<0.001) variation in SRL among the genotypes, treatments and their interactions respectively. Among the genotypes, the longest SRL was observed in genotype 210856 at 50 mM, 100 and 200 mM NaCl concentration with 8.2, 5.9 and 4.97 cm while 222867 gave the shortest root length with 3.63, 3.43 and 2.60 cm at the same level of treatments, respectively. The remaining genotypes have intermediate SRL. Furthermore, the lower (50 mM) salt concentration stimulated the seedling root growth in genotype 210856 and 211557 (Fig. 3).
These results showed the presence of genetic variation among the cowpea genotypes in response to salinity stress.

**Seedling Shoot-to-root Ratio (SRR):** The analysis of variance for seedling shoot-to-root length ratio (SRR) showed significant ($p<0.001$) variations for genotypes, treatments and genotype x treatment interaction. This revealed the presence of considerable varietal difference among cowpea genotypes in seedling SRR in response to salt stress. Genotype 222867 had the highest SRR with 3.19 and 3.40 at 50 and 100 mM while the smallest SRR was recorded on genotypes 221727 and 211435 with 2.07 and 2.18 at 50 and 100 mM salinity level, respectively (Fig. 4).

**Root fresh weight (RFW):** Analysis of variance for seedling root fresh weight revealed highly significant ($p<0.001$) differences among NaCl treatments, genotypes and their interactions. The NaCl treatments caused a substantial reduction in seedling RFW. From all the genotypes considered, the smallest mean seedling RFW was recorded on genotype 222867 and the highest was on Asebot in all the treatments. Similarly, genotype 211557 gave significantly higher mean seedling RFW than the other genotypes while 211435 showed the lowest from the rest at all the salinity levels. At the lower (50 mM) salt concentration, genotypes 211436, 211557 and Asebot gave higher value of seedling RFW as compared to the control but it was insignificant. Nevertheless, higher salt concentrations resulted in a reduced seedling RFW in all genotypes (Fig. 5).

**Root dry weight (RDW):** The ANOVA for genotypes, treatments as well as genotype x treatment interactions appeared highly significant for root dry weight ($p<0.001$). As it can be seen in Fig. 6 below, RDW was diminished drastically with increasing salinity levels and the effect of salinity was varied with concentration and among the genotypes. At 50 mM NaCl, genotypes 211436, 210856, 211557 and Asebot showed slightly higher RDW as compared to the control but all the other genotypes had reduced RDW in varying degrees. Genotype Asebot followed by 211557 produced significantly highest RDW at all the treatments and were found to be salt tolerant whereas genotype 222867 produced the lowest RDW at all the treatments and considered as salt sensitive. Other genotypes had intermediate values of RDW and considered as moderately salt tolerant (Fig. 6).

**Shoot fresh weight (SFW):** The analysis of variance revealed highly significant variation ($p<0.001$) among genotypes and treatments for seedling SFW. Genotype by treatment interaction effect was also significant, reflecting all the genotypes responding differently to salt stress with respect to SFW. At lower (50 mM) NaCl level, genotypes 210856, 211436 and 211557 had showed 10.7, 4.3 and 4.5% of increment in SFW as compared to the control, respectively. All the remaining genotypes resulted in a decrease in seedling SFW as the salinity
Fig. 7: Effects of salinity on shoot fresh weight of cowpea (Vigna unguiculata L. Walp.) genotypes

Fig. 8: Effects of salinity on shoot dry weight of cowpea (Vigna unguiculata L. Walp.) genotypes

Fig. 9: Total biological yield (gm) of cowpea (Vigna unguiculata L. Walp.) genotypes under salt stress condition

**Total biological yield (TBY):** The genotypes showed significant differences (p<0.001) for TBY under salt stress conditions. The largest TBY was recorded on genotype 210856 with 12.01 g at the control whereas genotype 222867 attained the smallest TBY in all the treatments. Similarly, genotype Asebot attained the largest TBY with 10.52 and 8.76 g at 50 and 100 mM salinity level while 211557 attained the highest value at 200 mM. The result showed not only the significant reduction in total biological yield in stress compared to non-stress but also there was high genetic variation among the cowpea genotypes tested. From the result it is confirmed that genotype 222867 showed the smallest TBY in all the treatments and considered as the most salt sensitive whereas genotypes 210856, 211557 and Asebot produced the highest TBY relatively in all the treatments and found to be salt tolerant. The remaining genotypes produced intermediate values and considered as moderately salt tolerant (Fig. 9).

**Correlation analysis:** Pearson’s correlation coefficients (r) were computed for 9 parameters in the greenhouse experiment to assess the possible interrelationship between any two traits that have shown both positive and negative associations. The correlation coefficient among most of the quantitative characters was highly significant. From the 36 correlations, 31 (86.1%) correlation coefficients were statistically significant irrespective of directions of association. It showed a positive and significant correlation between seedling SRL with SRL, SRR, SFW, RFW, SDW, RDW and TBY (p<0.001). Similarly, SRL showed positive and significant correlation with SFW, RFW, SDW, RDW and TBY but it showed negative and significant correlation with SRR. SFW showed positive and significant correlation with RFW, SDW, RDW and TBY. Generally in this study the LN, SRL and RDW had significant correlation with most of the
Table 2: Pearson’s correlation coefficients for plant morphological and biomass parameters

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<th>LN</th>
<th>SSL</th>
<th>SRL</th>
<th>SRR</th>
<th>SFW</th>
<th>RFW</th>
<th>SDW</th>
<th>RDW</th>
<th>TBY</th>
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<td>0.6921**</td>
<td>-0.0776ns</td>
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** *, **: Significant at 0.001 and 0.05 probability levels, respectively. ns: Non-significant. LN: No. of leaves, TBY: Total biological yield SSL: Seedling shoot length, SRL: Seedling root length, SRR: Shoot/root ratio, SFW: Shoot fresh weight, RFW: Root fresh weight, SDW: Shoot dry weight, RDW: Root dry weight.

differently to salinity stress. Better SSL and SRL were observed at a lower salinity level as compared to higher salt treatments. This may be due to the presence of genetic variation among the cowpea genotypes. The SSL and SRL were diminished with increasing salinity levels in all of the genotypes, but statistically significant differences were found at the highest salinity level. Similar result was presented in different legumes by Taffouo et al. (2004, 2009) Stoeea and Kaymakanova (2008), Ndakidemi and Makoi (2009), Asfaw (2011) and Win et al. (2011).

The decrease in the SSL and SRL on plants that are grown in salinized environment was due to several reasons. One possibility is that salinity reduced photosynthesis, which in turn limited the supply of carbohydrate needed for growth. A second possibility is that salinity reduced root and shoots growth by reducing turgor in expanding tissues resulting from lowered water potential in root growth medium. A disturbance in mineral supply, either an excess or deficiency, induced by changes in concentrations of specific ions in the growth medium, might have directly affected growth (Murns, 2002; Alam et al., 2004; Gama et al., 2007). Concerning the SRR, it showed increment for some and decrement for other genotypes as the level of salinity increased. This was in line with the results of Abdelhamid et al. (2010) on faba bean and Gama et al. (2007) on common bean. The consequent increase in shoot to root ratio in some genotypes seems to be associated with increased salinity tolerance in these genotypes (Gama et al., 2007; Asfaw, 2011).

The outcome of the research showed that increasing NaCl concentration significantly reduced the root fresh and dry weights of cowpea genotypes. It also demonstrated the presence of clear genetic variation in root fresh and dry weights in response to salinity among cowpea genotypes. The mean value of RDW for entire genotypes of cowpea showed a strong reduction with the increasing salinity levels particularly at the highest (200 mM) salt concentration. This was in agreement with the results obtained by Bayuelo-Jiménez et al. (2002).
CONCLUSION

This study investigated the tolerance of nine Vigna species to four concentrations of NaCl (0, 50, 100 and 200 mM NaCl). On the basis of the growth parameters measured, the result demonstrated genetic variation in early seedling growth responses to salinity among and within the Vigna species. Among the nine Vigna genotypes studied, 210856, 211587 and Asebot showed better performance in all of the parameters considered and are recommended for general cultivation in salt affected areas due to their better tolerance to salt stress. The correlation result also showed that most of the parameters were significant and positively correlated among each other. The findings of the study must be regarded as preliminary and need of further confirmation on field conditions. For the future prospects, they can be utilized through appropriate selection and breeding for their improvement in salt tolerance.

ACKNOWLEDGMENTS

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REFERENCES


