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ORIGINAL ARTICLE

The Response of Some Cowpea (*Vigna unguiculata* (L.) Walp.) Genotypes for Salt Stress during Germination and Seedling Stage

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Salinity is one of the most significant abiotic factors affecting growth and yield of cowpea in arid and semi-arid areas of the world. Nineteen cowpea (*Vigna unguiculata*) genotypes were tested during germination and seedling stages of growth at 4 NaCl concentrations (0, 50, 100 and 200mM). The experimental design was completely randomized design (CRD) in factorial combination with three replications. Data analysis was carried out using SAS (version 9.1) statistical software. Germination percentage (GP), seedling shoot and root traits 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 and shoot length. The extent of decrease varied with accessions and salt concentrations. Most accessions were highly susceptible to 200 mM NaCl concentration. Genotypes 211557 and Asebot were better salt tolerant. The result showed the presence of broad intraspecific genetic variation in cowpea genotypes for salt tolerance.

Key words: cowpea, genotypes, NaCl, salinity, seedling growth

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Key words: cowpea, genotypes, NaCl, salinity, seedling growth

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of a multifunctional crop, providing food for man and livestock and serving as a valuable and dependable revenue-generating commodity for farmers and grain traders (Langyintuo *et al*. 2003, Muluemebt 2003). It has high value of protein content (23-29 %, with potential for perhaps 35%); high ability to fix atmospheric nitrogen, which allows it to grow in and improve poor soils and it is also more drought tolerant than other leguminous plants (Eaglesham *et al*. 1992). It belongs to class Dicotyledoneae, order Fabales, family Fabaceae alt leguminosae. All the cultivated cowpeas are grouped under the species *Vigna unguiculata,* which is subdivided into four cultivar groups: *unguiculata* (common cowpea), *biflora* (catjang), *sesquipedalis* (yard-long bean) and *textilis* (used for fibers) (Marechal *et al*. 1978; Ng and Marechal 1985).

Salinity is one of the most serious abiotic factors limiting crop production in the worldwide (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). It affects 7 % of the world's land area which is around 930 million ha (Munns 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). It has been estimated that salinity is affecting 3 hectares of additional arable land each minute worldwide (FAO 2008). This constant salinization of arable land is expected to have overwhelming global effects, resulting in 30% land loss within the next 25 years, and up to 50 % by the year 2050 (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 2009a).

Salt-affected soils are prevalent in the Rift Valley and the lowlands areas of Ethiopia. The Awash Valley in general and the lower plains in particular are dominated by salt affected soils (Tadelle 1993). Kinfemichael and Melkamu (2008) indicated that there had been report on the occurrence of salinity problem in Melka Werer Research Farm. According to Hailay *et al*. (2000), 30 % of the Abaya State Farm had already been salt affected. Under the prevailing situation of the country, the problem is likely to be severe in the years to come (Kinfemichael and Melkamu 2008).

Germplasm screening for tolerance to salt under naturally occurring salt stress does not seem to be reliable. The reason is due to spatial heterogeneity of soil, physico-chemical 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). Germination, seedling growth parameters and weight are the most commonly used criteria for cultivar selection for salinity tolerance (Jamil *et al*. 2005; Bybordi and Tabatabaei 2009). Differences in salt tolerance exist not only among different genera and species, but also within certain species (Winicov 1993). With this fact in mind, it is imperative to explore intra-specific (inter-cultivar) variation for salt tolerance of a crop by screening its available germplasm. Though researchers have shown that among crop species, legumes are considered as the most salt sensitive (Maas and Hoffman 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).

Generally, 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 study the genotypic variation of different cowpea genotypes in response to salt stress at germination and seedling growth stages.

MATERIALS AND METHODS

This study was conducted in Ethiopia between December 2011 to March 2012 at Haramaya University, in the Genetics laboratory of the Department of Biology. Seeds of nineteen genotypes of cowpea were collected from the Institute of Biodiversity and Conservation (IBC) and Melkasa Agricultural Research Center (MARC), Ethiopia. The specific cowpea genotypes used in the research were 211435, 211436, 215760, 211446, 211433, 222891, 222867, 216746, 216748, 211557, 211430, 211444, 210856, 223403, 211490, 211491, 211287, 221727 and Asebot. NaCl solutions with different salinity levels of 50, 100 and 200 mM were prepared by dissolving 2.92, 5.84 and 11.68 gm of NaCl in one liter of distilled water, respectively. Distilled water (0 mM) was used as control. In order to observe the response of the 19 cowpea genotypes to different concentration of NaCl, seeds were first surface sterilized in 7 % sodium hypochlorite solution for 20 min and washed twice with sterile distilled water. The seed germination experiment was conducted in a laboratory at room temperature followed by Taffouo *et al*. (2009b). Prior to experiment, Petri dishes were thoroughly washed and sterilized in hot air oven at 70 ^{0}C for 36 hours and Whatman filter papers at 70 ^{0}C for 24 hours. After sterilization, Petri dishes were lined with Whatman No.3 filter paper and treated with 10 ml of 0, 50, 100 and 200 mM concentrations of NaCl. Following this, eight uniform seeds of each cowpea genotypes were placed on each Petri dish in a uniform distance. The Petri dishes were arranged in a completely randomized design (CRD) in a factorial combination with three replications (Gomez and Gomez 1984). Each Petri dish was treated with 10 ml of the respective concentrations of NaCl in alternate days. The Petri dishes were put in a glass box to avoid the loss of moisture through evaporation. Germination started after three days of sowing and the germination count was continued until the 15th day. Germination was recorded daily and a seed was considered to have germinated when both the plumule and the radical had emerged greater than 0.5 cm. After the 15 days, the germination percentage (GP), overall shoot and the longest root length of three randomly selected seedlings from each replicate were measured using a draftsman ruler. Then their fresh weight was recorded and finally oven dried at 70^{0}C for 48 h and the seedling dry weight was measured using sensitive balance. The data was analyzed statistically for analysis of variance using the SAS (version 9.1) statistical software (SAS Institute Inc., USA). Means were separated using the least significant difference (LSD) test at 5 % level.

RESULTS

Germination percentage (GP): The two-way analysis of variance (ANOVA) exhibited highly significant ($p<0.001$) variation in seed germination percentage among genotypes, treatments and genotypes by treatment interaction. At 50 mM salinity level, genotypes 221727, 211436 and 211557 showed the highest percentage of germination whereas genotypes 215760 and 211430 showed the lowest percentage of germination. Moreover, 50 mM salt concentration stimulated seed germination in genotype 221727. At 100 mM salinity level, genotype 222867 followed by Asebot attained maximum percentage of germination. At the highest salt concentration (200 mM NaCl), genotypes 211435, 221727, 211436, 222867, 210856, 211557 and Asebot found to be salt tolerant whereas genotypes 211433, 211760, 211444, 211490, 211491, 222891 and 211430 were found to be salt sensitive at (Fig. 1). The remaining varieties were found to be intermediate in their response to salt stress with respect to GP.

Seedling Root Length (SRL): The analysis of variance (ANOVA) confirmed that there was a highly significant (p<0.001) variation in mean seedling root length among cowpea genotypes and treatments. The interaction between salt levels and genotypes were also found to be highly significant (p<0.001). The increase in NaCl level had significantly reduced the mean SRL of all the genotypes. However; the reduction in the SRL was not the same in all the genotypes at different concentration of NaCl. Genotypes 216746, 210856 and 211557 achieved more than 90 % SRL at 50 mM NaCl concentration as compared to the control. Moreover, this salt concentration stimulated SRL growth in genotypes 211436, 222867 and Asebot. At 100 mM salinity level, genotypes 222867, 210856 and Asebot attained more than 75 % SRL whereas the remaining accessions recorded between 13.2 and 64.6 % SRL as compared to the control. At 200 mM salinity level, genotypes 210856, 211557 and Asebot showed the least (45-53 %) reduction in SRL as compared to the control so these genotypes were salt tolerant at this salinity level where most of the genotypes were salt sensitive.(Fig. 2).

Seedling Shoot Length (SSL): The analysis of variance revealed significant (p<0.001) variation among genotypes and treatments for mean SSL. Genotype by treatment interaction effect was also significant (p<0.001) reflecting that all the genotypes responding differently to salt stress with respect to mean SSL. An increment in salinity level had significantly reduced the mean SSL in all of the genotypes. Seedling Shoot Length (SSL) was enhanced at 50mM in genotypes 221727, 211436, 211557 and Asebot as compared to the control.

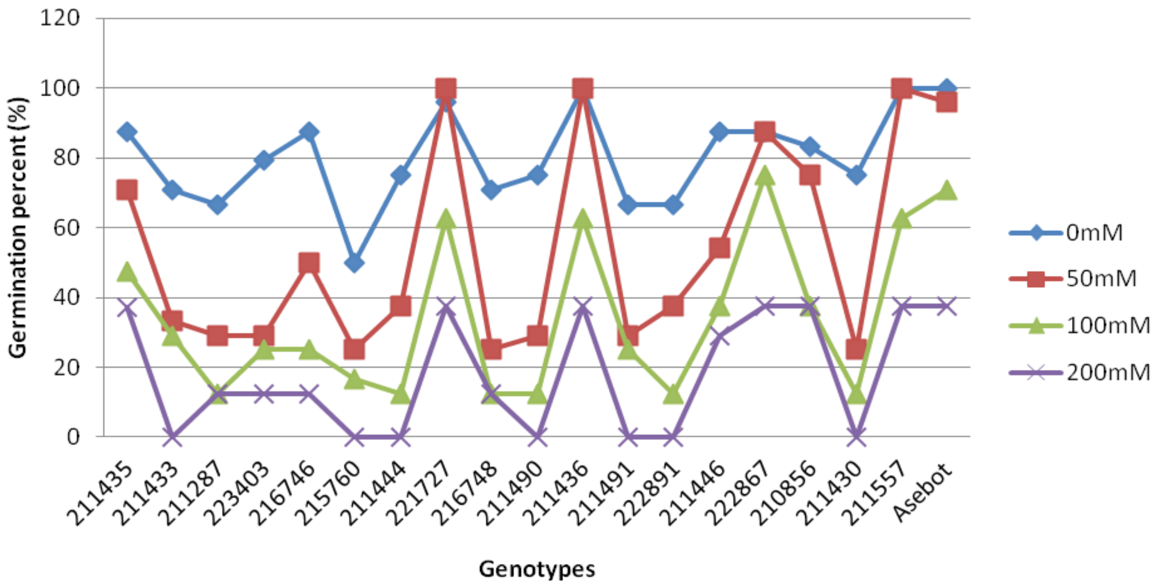

Figure 1. Effects of different salinity levels (0, 50, 100, and 200mM) on Germination Percentage (GP) of *Vigna unguiculata* genotypes

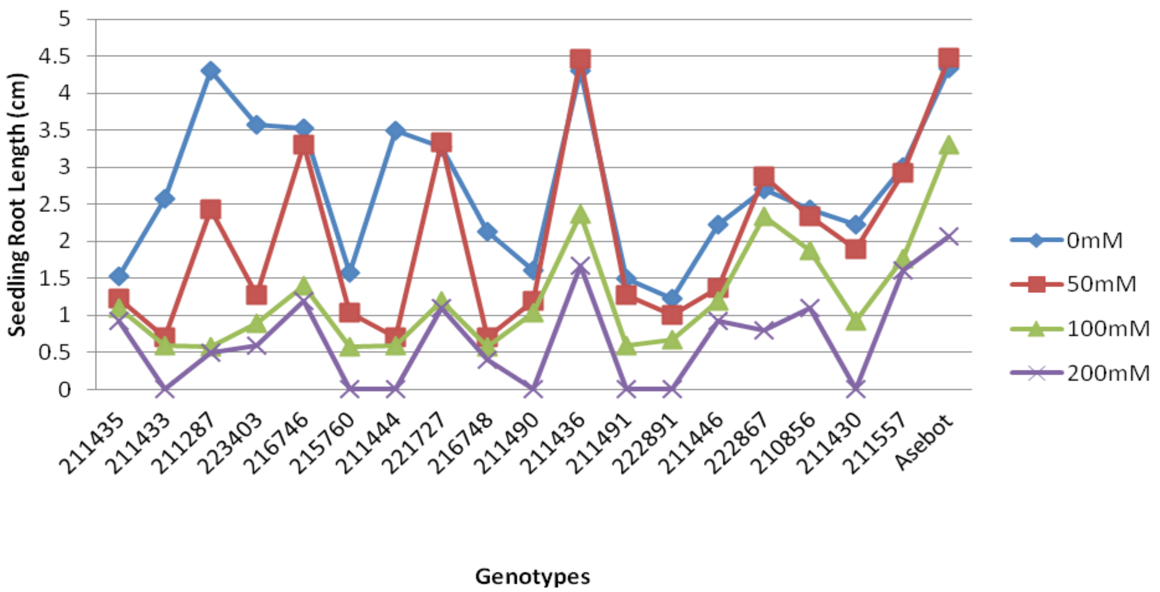

Figure 2. Effects of different salinity levels (0, 50, 100 and 200mM) on Seedling Root Length (SRL) of *Vigna unguiculata* genotypes (in centimeter).

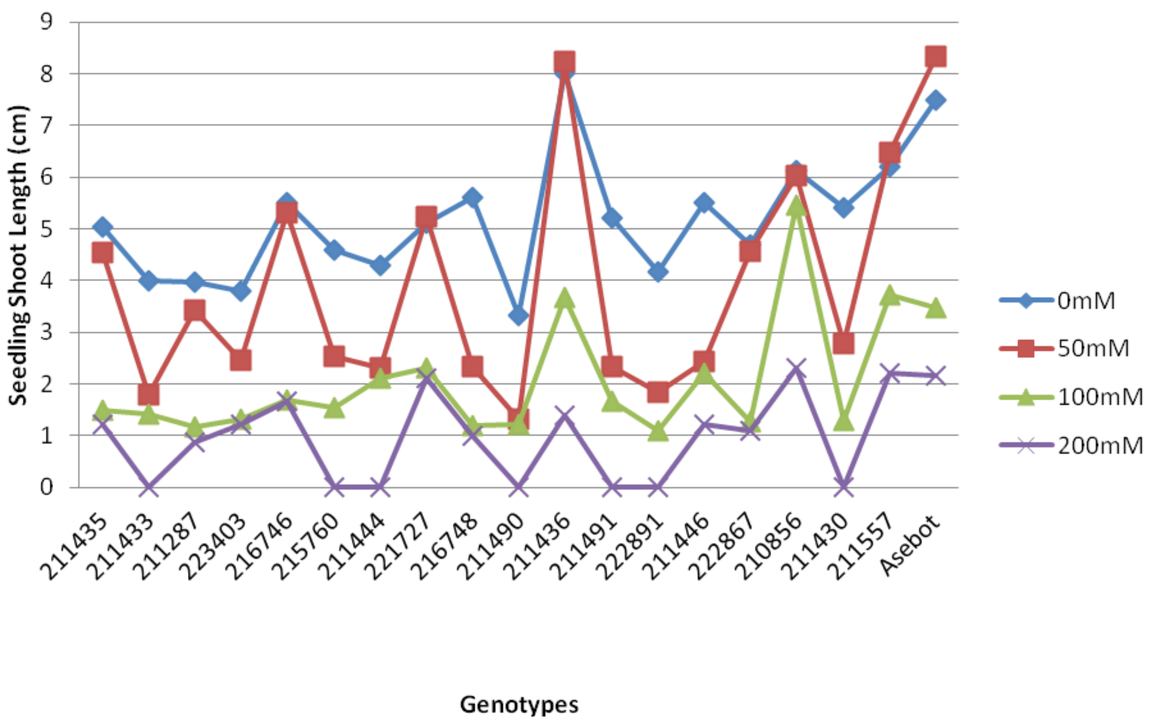

Figure 3. Effects of different salinity levels (0, 50, 100 and 200mM) on Seedling Shoot Length (SSL) of *Vigna unguiculata* genotypes (in centimeter).

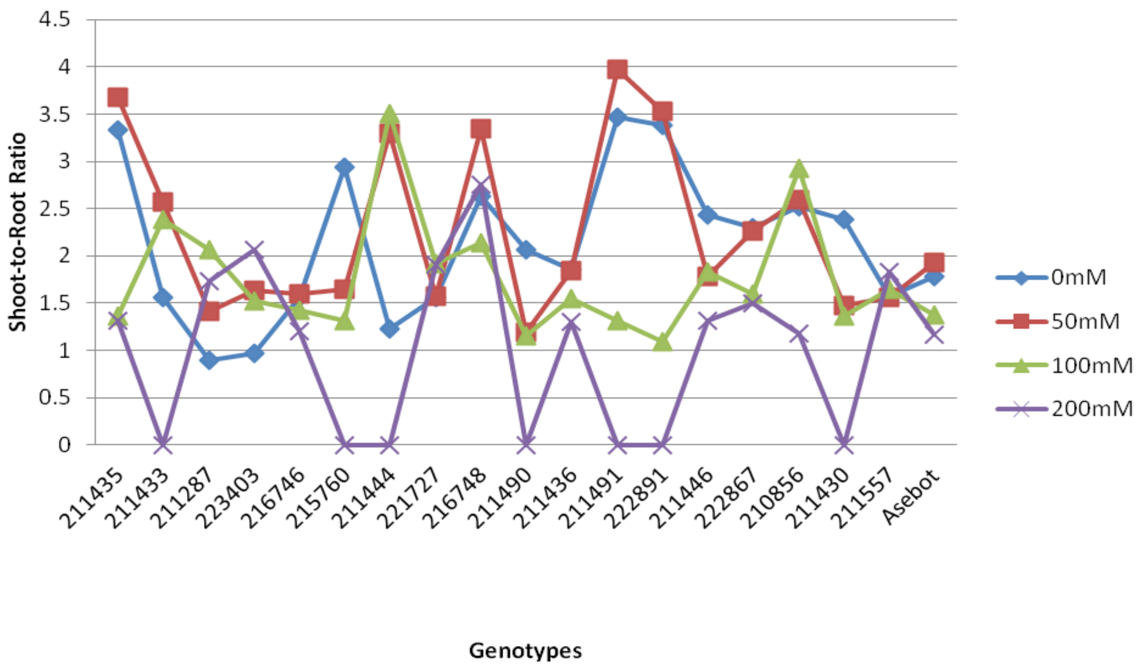

Figure 4. Effects of different salinity levels (0, 50, 100 and 200mM) on Shoot-to-Root Ratio (SRR) of *Vigna unguiculata* genotypes.

At 100 mM of NaCl concentration, genotypes 211557, 211436, 210856 and Asebot gave the highest mean SSL while the others showed a significant reduction in the mean SSL. The influence was much more pronounced at 200mM salinity level (Fig. 3). Genotypes 221727, 210856, 211557 and Asebot were found to be salt-resistant whereas 211433, 215760, 211444, 211490, 211491, 222891 and 211430 were found to be salt-sensitive at 200mM salinity level. However, the remaining genotypes remained intermediate in their salt tolerance with respect to seedling shoot length.

Shoot-to-Root Ratio (SRR): The two-way analysis of variance (ANOVA) for seedling shoot-to-root length ratio (SRR) showed significant ($p<0.001$) variations for genotypes and treatments. Genotype x treatment interaction effect was also depicted to be significant ($p<0.001$). This reflects that genotypes responded to salt stress differently with respect to seedling SRR. As the salinity level increased, SRR showed increment in some of the genotypes and decrement on the other genotypes as compared to the control. Genotypes 211287, 223403, 221727, 216748 and 211557 had a higher Shoot-to-Root Ratio at 200 mM NaCl concentration, whereas the remaining genotypes had lower Shoot-to-Root Ratio as compared to the control (Fig. 4).

DISCUSSION

Highly significant variation among cowpea genotypes, treatments and genotype by treatment interaction effect for Germination Percentage (GP), Seedling Shoot Length (SSL), Seedling Root Length (SRL) and Shoot-to-Root-Ratio (SRR) observed during germination and seedling growth reflects that the genotypes responded differently to salt stress with respect to the above mentioned parameters. In general, salt stress at 50 mM has enhanced growth with respect to GP, SSL, SRL and SRR in some genotypes. The impact of 50 mM salinity level was not fundamental with respect to all parameters considered. Nevertheless, most genotypes were quite salt-sensitive at 200 mM salinity level.

The result indicated that the seed germination percentage was generally decreased with increasing salt concentration and the degree of reduction was varied with the salinity levels and the cowpea genotypes. The mean value indicated that the highest germination percentage was observed on genotype Asebot followed by 211436 with 76.05% and 75.00% whereas the lowest was observed on genotype 215760 with 22.92 %. This result indicated that remarkable reduction in germination percentage was observed at higher levels of salt concentrations as compared to the control. Similar results were reported in cowpea (Murillo-Amador and Troyo-Die'Guez 2000), in chick-pea (Ashraf and Waheed 1992), *P. sativum* (Noreen *et al*. 2007) and red clover (Asci 2011). The reason is assumed that in addition to toxic effects of sodium ions, higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination (Neamatollahi and Bannayan 2009).

The SRL and SSL were generally decreased with increasing salinity level and degree of reduction varied with the salinity levels and among the genotypes. The outcome confirmed that better SRL and SSL was observed at lower concentrations (50 mM) and great reduction on seedling root length was observed at higher salt concentration (200 mM). Similar report was forwarded by Gama *et al*. (2007), Stoeva and kaymakanova (2008), Taffouo *et al*. (2009b) and Win *et al*. (2011) on different leguminous plants. The increment of SRR in response to increased salt concentration indicated that the mean seedling root length was more salt-affected than the mean seedling shoot length at higher salinity level. The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. It may also be due to the ability of the root system to control entry of ions to the shoot since it has a crucial importance to plant survival in the presence of NaCl (Maas and Poss 1989; Demir and Arif 2003). High salinity may also inhibit root and shoot elongation by slowing down the water uptake by the plant (Ghoulam *et al*. 2002; Zhu 2002).

Crop cultivar may germinate effectively under salt stress; nevertheless, its seedling growth may be affected (Azhar and McNeilly 1987; Francois *et al*. 1989; Miyamoto 1989). In line with this, genotypes 211436 and 222867 were less salt-affected during germination than later growth (had inadequate seedling growth). This implies that these varieties are salt tolerant during germination than subsequent growths like seedling growth. On the other hand, crop genotype may be salt-sensitive during germination and seedling growth. As it was previously reported by Murillo-Amador and Troyo-Die’guez (2000) similar finding was observed on this study. Genotypes 211433, 215760, 211444, 211490, 211491, 222891 and 211430 were found salt-sensitive at higher salinity levels during germination and seedling growth. Thus these cowpea genotypes can’t be cultivated even on slightly saline soils.

On the other hand, Asebot and 211557 were salt-tolerant during germination and seedling growth. Their extraordinary salt-tolerance ability might be achieved through reduced respiration (George and Williams 1964), dilution of toxic effects of salts (Lee and Senadhira 1998), and accumulation of compatible solutes (Kayani *et al*. 1990). It has already been reported that plant growth and development is dependent on crop stand establishment (Verma and Yadava 1986), in turn, the latter is a function of effective germination (Horst and Taylor 1983) and seedling growth (Ashraf and Waheed 1992). Crops with higher germination percentage can establish themselves effectively on moderately saline soils (Lee *et al*. 1998).

Generally, the detrimental effects of salts on plants were the consequence of both a water deficit that results from the relative high solute concentrations in the media as well as a stress specific to Cl^- and Na^+, resulting in a wide variety of physiological and biochemical changes that inhibit plant growth and development (Sairam *et al*. 2002). Thus even if, it is difficult to infer the research result obtained from controlled experiment directly to the field, relatively the above cowpea genotypes which had the highest Final Germination Percentage (FGP) could germinate and establish effectively on moderately saline soils.

CONCLUSIONS

In vitro screening method proves to be an ideal method to screen large set of germplasm accurately with minimum effort. An overall high level of dissimilarity was observed among the cowpea genotypes for most of the morphological traits analyzed. The result pointed out that all of the morpho-physiological traits considered were significantly decreased as the salinity levels increased. Seedling Root Length (SRL) was more salt affected than Seedling Shoot Length (SSL) in the nineteen cowpea genotypes studied. Lower salinity level promoted the germination and seedling growth in some genotypes. Genotypes 211433, 215760, 211444, 211490, 211491, 222891 and 211430 were the most salt sensitive. Thus these genotypes cannot be cultivated even on slightly saline soils. However, genotypes 211557 and Asebot were salt tolerant during germination and seedling growth. So it can be cultivated effectively on moderately saline soils.

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