Hydroponic Screening and Multi-Location Field Evaluation of Ethiopian Chickpea (*Cicer arietinum L.*) Genotypes for Tolerance to Soil Acidity

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INTRODUCTION

Soil acidity is one of the most important environmental factors which can influence plant growth, and seriously limit crop production (Beegle and Lingenfelter, 1995). Almost 50% of potentially arable land area of the world is known to be acidic in nature and this is commonly attributed to Al^{+3} toxicity worldwide (Kochian *et al*., 2004; Singh *et al.*, 2012). It reduces the average productivity of major crop plants by more than 50% (Reddy *et al*., 2012). In Ethiopia, vast areas of land in the western, southern and even the central highlands of the country which receive high rainfall are thought to be affected by soil acidity (Adane Buni, 2014). According to the Ethiopian Soil Information System (EthioSIS) (2014) report, about 40% of total land area or 28% of potentially agricultural land is estimated to be acidic. It causes a significant yield reduction in various crops and in more severe cases, complete loss of production have been reported in several parts of the country. It is also a major productivity problem that has not been addressed in depth. For both logistics as well as economic reasons, application of lime and/or mineral fertilizers is not a sustainable solution to mitigate the soil acidity problem. Besides, liming is only possible for the surface soil and does not remedy the sub-soil acidity (Tesfaye *et al*., 2001). Hence, development of genotypes tolerant to soil acidity and aluminum toxicity is a reliable and cost effective approach to enhance crop production on acidic soils.

Chickpea (*Cicer arietinum L.*) is the world's second most important pulse legume after dry beans, with particular importance in the semi-arid tropics of Sub-Saharan Africa. It is also one of the major annual pulse crops grown widely across the highlands and semi-arid regions of Ethiopia. Chickpea is one the grain legumes that are sensitive to aluminum toxicity (Choudhary and Singh, 2011). It reduces the productivity of chickpea by affecting the roots, root hairs and nodulation potential of chickpea. The information regarding identification and development of aluminum tolerant chickpea genotypes in Ethiopia is lacking. Hence, the overall objective of this research is to screen and identify acid tolerant chickpea (*Cicer arietinum L.*) genotypes for acidic soil regions of Ethiopia.
Furthermore, chickpea breeding programs in Ethiopia have been focused mainly on major biotic and abiotic stresses that adversely affects the yield of chickpea. However, genotype and environment interaction (G×E) hampers breeding by inducing variations in genotype performance and affecting selection (Zobel, 1990). Thus, stability and yield performance of advanced Desi type chickpea varieties and advanced lines were determined at multiple locations using GGE biplot analysis and AMMI model to identify stable high yielding cultivar(s) recommended for wider production in the test environments and similar agro-ecologies in Ethiopia.

MATERIALS & METHODS

Activity 1: Hydroponic Screening

Seed Preparation & Sterilization

29 chickpea genotypes: 24 nationally released improved varieties, four advanced lines and one local variety from Wollega zone were used for the hydroponic screening experiment. Seeds of improved varieties and advanced lines were obtained from Debrezeit Agricultural Research Center (DZARC). The experiment was conducted at Addis Ababa University, Institute of Biotechnology, Plant Genetics Research Laboratory. Seeds were disinfected with 1% sodium hypochlorite and germinated in filter paper for three days. Afterwards, seedlings with a uniform root and shoot length were transferred to a nutrient solution containing 500 µM KNO_3, 500 µM $CaCl_2$, 500 µM NH_4NO_3, 200 µM $MgSO_4.7H_2O$, 100 µM KH_2PO_4, 20 µM Fe:EDTA, 46µM H_3BO_3, 2µM $MnCl_2.4H_2O$, 1µM $ZnSO_4.7H_2O$, 0.3µM $CuSO_4.5H_2O$ and 0.5µM $NaMoO_4.2H_2O$ along with 0µM (control) and 120 µM (treatment) aluminum (Al) concentration. 120 µM Al concentration used in the present study was selected based on the optimization result done. The Al treatment was supplied as $Al_2(SO_4)_3.18H_2O$. The protocol used to prepare the nutrient solution was adopted from Simon *et al*. (1994) and Delhaize *et al*., (2004) with minor modification. For each genotypes, five seedlings were grown with two replication. The PH of both nutrient solutions was maintained at 4.5 using 1M HCl and 1M NaOH and was monitored daily. The nutrient solutions was renewed every three days to maintain the appropriate nutrient as well as Al concentration.

Phenotypic Data Collection & Statistical Analysis

After six days, phenotypic traits: tap root length, shoot length, root fresh weight and shoot fresh weight were collected. The phenotypic data was analyzed using Genestat statistical software package version 18 (Rayne *et al*., 2012) to evaluate the performances of the genotypes. The *Ryan–Einot–Gabriel–Welsch* (REGW) Multiple Range Test was used to estimate the threshold at which Al treatment induced a statistically significant response in the genotypes as well as to rank genotypes based Al tolerance level.

Activity 2: Genotype By Environment Interactions

Field experiment was conducted on sixteen desi type chickpea genotypes under field condition at five locations viz., Shambu, Hawa Galan, Mata, Alaku Belle and Badesso in western Ethiopia during the main cropping season of 2016/2017. The experiment was laid out in a randomized complete block design with three replication.

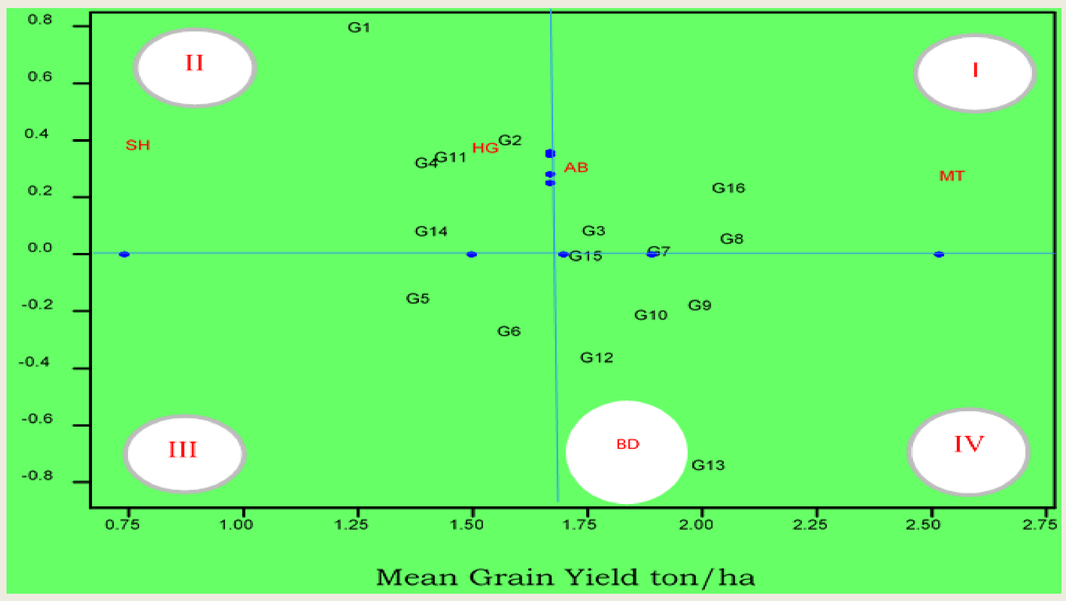
RESULTS & DISCUSSION

The hydroponic screening system was effective in differentiating the 29 chickpea genotypes at different aluminum tolerance levels. Analysis of variance (ANOVA) revealed a highly significant difference (*P<0.001*) among the twenty-nine chickpea genotypes evaluated for all phenotypic traits measured for both the control (0) and aluminum treatment (120µM Al^{3+}concentration) (Table 1). This indicates that the physiological response in each genotypes was different and the aluminum toxicity also induced difference in performance among chickpea genotypes. When we evaluate mean performance, all genotypes exhibited a decline in performance for all parameters taken in the Al^{3+} treated experiment compared to the control one. However, unlike to Tap Root Length, which was significantly reduced, there was only a slight reduction being observed for Fresh weight Root and Shoot in the Al^{3+} treated experiment as compared to the control (data not shown). This is because the root is the primary site of aluminum toxicity and our result is in line with Ryan *et al*. (1993), who reported that the primary symptom of aluminum toxicity in higher plants is inhibition of root growth. Among the 29 chickpea genotypes used, the advanced line DZ-2012-ck-20113-2-0042 was found to be the most tolerant genotype with respect to the trait tap root length followed by DZ-2012-ck-0233 while the released variety Yelebe was found to be the least tolerant (most susceptible) genotype followed by Akaki. Moreover, the advanced line DZ-2012-ck-20113-2-0042 was also found to be tolerant, high yielder as well as stable on field experiment conducted at acidic soil regions of western Ethiopia. In addition, in the previous optimization experiment, Local Variety and Akaki was selected as a reference tolerant and susceptible genotypes, respectively. Thus, all genotypes with a mean performance greater than that of Local Variety were selected as Al tolerant whereas, genotypes with a mean performance less that of Akaki were selected as the least tolerant genotype.

Table 1. Analysis of variance for four traits of twenty-nine chickpea genotypes tested at 120 µM Al^{3+} concentration.

Root length (RL)					
Source of variation	Df	Sum of Square	Mean square	F	P-value
Genotypes	28	24.5261	0.8759	5.61**	**P<0.001**
Residuals	29	4.5312	0.1562		
Total	57	29.0573			
Shoot length(SL)					
Source of variation	Df	Sum of Square	Mean square	F	P-value
Genotypes	28	186.6520	6.6661	8.11**	**P<0.001**
Residuals	29	23.8318	0.8218		
Total	57	210.4838			
Fresh weight for root (FWR)					
Source of variation	Df	Sum of Square	Mean square	F	P-value
Genotypes	28	0.1212140	0.0043291	7.96**	**P<0.001**
Residuals	29	0.0157785	0.0005441		
Total	57	0.1369924			
Fresh weight for shoot (FWS)					
Source of variation	Df	Sum of Square	Mean square	F	P-value
Genotypes	28	0.0934299	0.0033368	6.47**	**P<0.001**
Residuals	29	0.0149454	0.0005154		
Total	57	0.1083753			

Furthermore, AMMI biplot based on multi-location trial indicated Natoli (G8) and DZ-2012-CK-20113-2-0042 (G16) were higher yielder genotypes, and relatively stable. In the same manner, G16 (DZ-2012-CK-20113-2-0042) and Natoli (G8) variety were regarded as ideal varieties based on GGE biplot.



Key: - SH= Shambu, HG= Hawa Galan, MT= Mata, AB= Alaku Belle, BD= Badesso
Figure 2. Biplot of interaction principal component axis (IPCA-1) against mean yield of chickpea varieties evaluated across five environments.

CONCLUSION

In the present study, hydroponic screening system was effectively used to differentiate 29 chickpea genotypes based on their level of aluminum tolerance. The most tolerant and most susceptible genotypes found in the present study will be used for future field experiments and aluminum tolerance breeding of chickpea. DZ-2012-CK-20113-2-0042 (G16) and Natoli (G8) variety showed higher grain yield with better stability across environments and thus are recommended for wider production in test locations and similar agro-ecologies.

ONGOING ACTIVITIES

- Hydroponic screening of 500 chickpea germplasms obtained from EBI is an ongoing activity.

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