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Character Association in Amaro Coffee (*Coffea arabica* L.) Landrace Using Morphological Traits at Awada, Southern Ethiopia

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Abstract

Fifty-eight Amaro Kele coffee (Coffea arabica L.) accessions and six standard check were evaluated for character association at Awada Agricultural Research Sub-Center, Southern Ethiopia using morphological traits. This work was done to determine the level of phenotypic and genotypic association of characters and their direct and indirect relation with coffee yield. The experiment was laid out in an 8x8 simple lattice design with two replications having eight coffee accessions per each incomplete block. Coffee yield has positive and significant genotypic association with number of primary branches(rg=0.704), number of bearing primary branch(rg=0.613), number of main stem node (rg=0.619), stem diameter (rg=0.335) and canopy diameter (rg=0.376), whereas average inter node length of main stem(1.083), number of main stem nodes (0.427), canopy diameter (0.414), height up to first primary branch (0.300) and number of bearing primary branch (0.294) had maximum direct effect on yield. Correlation analysis and path analysis confirmed presence of variation among tested accessions. However, additional traits of interest should be studied over year and locations including physiological, quality and biochemical analysis with the support of advanced molecular techniques.

Keywords: *Coffea arabica* L., Correlation, Path coefficient analysis

Introduction

Coffee belongs to the family *Rubiaceae* and to the genus *Coffea* (Berthaud and Charrier, 1988; Coste, 1992). *Rubiaceae* has over 6000 species and 500 genera (ITC, 2002). Ethiopia is the homeland and center of genetic diversity of Arabica coffee (Vavilov, 1951; Sylvain 1955). Ethiopia is the most important coffee producing and exporting country in Africa and the fifth most important coffee producer worldwide (ICO, 2019; USDA, 2019). But, if considering Arabica coffee alone, Ethiopia is the third largest producer (USDA, 2019). The Southern region is the second region next to Oromia regional state in coffee producing areas and production. Genetic relationships in crop species are an

important component of crop improvement programs, as it serves to provide information about genetic diversity, and is a platform for stratified sampling of breeding populations (Aleka, 2008).

Correlation coefficient measures mutual association without cause-and-effect relationship (Dewey and Lu, 1959). It is very useful in quantifying the size and direction of trait associations, can be ambiguous if the high correlation between two traits is a consequence of the indirect effect of other traits (Bizeti *et al*., 2004). A positive value of correlation shows that the changes of two variables are in the same direction, specifically high value of one variable is associated with high values of other and vice versa. When correlation is negative the movements are in opposite directions, that is, high values of one variable is associated with low values of the other (Yadav *et al*., 2011). Depending on the sign of genetic correlations between two traits can either facilitate or impede selection progress. Correlation value (r = 1) implies perfect (100%) correlation, where both traits vary hand in hand, (r = -1) means there is 100 % correlation between two characters, but they vary in opposite direction, and (r = 0) carries the implication that there is no correlation at all between the two characters (Falconer and Mackay, 1996).

Path coefficient analysis is a very important statistical tool that indicates which variables (causes) exert influence on other variables (responses), while recognizing the impacts of multi co linearity (Akanda and Mundt, 1996). Path coefficient analysis partitions the genetic correlation between yield and its component traits into direct and indirect effects and hence has effectively been used in identifying useful traits as selection criteria to improve yield (Akinwale *et al*., 2011; Sadeghi, 2011). Morphological characters related to yield potential have been studied to increase the indirect selection efficiency. For instance, Lemi *et al.*, (2017) reported that positive direct effect on coffee yield was exerted by plant height, canopy diameter and length of first primary branch so that they are effective for the improvement of coffee yield. Ermias (2005) also observed positive direct effect of plant height but negative direct effects of canopy diameter and length of primary branch on yield.

Moreover, Srinivasan (1980) reported that greater weight should be given for longer primaries and shorter internodes in selection for yield, as they had direct positive effects. On the other hand, internode length, number of primary branches, height up to first primary branch, main stem diameter and number of main stem nodes which had positive genotypic and phenotypic correlation coefficient with yield except height up to first primary branch exerted negative effect on yield (Lemi *et al.*, 2017).

In coffee, the outcome of yield depends on various growth characters,

and their combinations, such as stem girth, canopy width, number of primary branches and number of secondary branches (Dancer, 1964 and Srinivasan, 1982). In addition, a number of other agronomic characters; such as plant height, leaf area, number of nodes on primary branches, number of fruits, etc. can directly or indirectly influence yield (Mesfin,1982). So, it is crucial in the improvement of yield traits to have a clear understanding of the relationships between yield and other agronomic characters because it is influenced by all factors that determine productivity (Araus *et al.*, 2001). It is, therefore, valued to estimate the magnitudes of correlations among yield and yield component traits. Also, it is desirable to know the direct and indirect effect of yield related traits in coffee. These traits could be useful indicators in breeding programs to select coffee genotype for yield.

Since, the previous conventional approach largely focused on the development of widely adapted varieties, improved local varieties for each specific agro-ecology are lacking in most of the coffee growing areas. Therefore, collection was made in 2013 to address this locality through coffee genetic resources collection for further coffee improvement program that might help to develop coffee varieties that have paramount importance to promote and maintain the existing speciality coffee quality heritage of the area. Hence, fifty-eight coffee accessions were collected from Amaro Kele Woreda but not yet evaluated for phenotypic and genotypic association of character. Therefore, this work was initiated to determine the level of phenotypic and genotypic association of characters and their direct and indirect relation with coffee yield at Awada, Southern Ethiopia.

Materials and Methods

Description of experimental site

The experiment was carried out at Awada Agricultural Research Sub-Center that was established in 1997 on land area of 31 ha near Yirgalem town, 45 km south of Hawassa and 319 km from Addis Ababa. Awada is located at 06°44′57’’N latitude and 038°23’16’’E longitude and at an altitude of 1738 meters above sea level (m.a.s.l). The mean annual rainfall of the area is 1342 mm with an average maximum and minimum air temperatures of 28.4 ^{o}C and 11.^{0}C, respectively. The major soil types of the center are eutric-nitosol and chromotic-cambisols that are highly suitable for coffee production (Mesfin and Bayetta, 2008).

Planting materials

Fifty-eight coffee accessions were collected from ten representative peasant associations of Amaro woreda of Segen people zone. Hence, 64 coffee accessions including six pure line checks were used for the study. Experimental plants were established under uniform *Sesbania*

sesban temporary shade trees and the other management practices like: - pruning and slashing were also uniformly applied as per the coffee agronomic production practices.

Experimental design and field management

The experiment was laid out in an 8X8 simple lattice design with two replications and eight accessions per each incomplete block were planted. Each plot consists of six coffee trees. Spacing were 2mx2m for both between rows and plants in a single row.

Methods of data collection

Data from 19 different quantitative traits were collected using four sample trees of four-year aged tree per row on each plot as per the standard IPGRI (1996) coffee descriptor (Table 1).

Table 1 List of observed morphological traits and their descriptions

Traits	Abbreviation	Description of quantitative traits
Coffee bean yield (kg/ha)	YLD	Weight of fresh cherries in gram per plot were recorded, and converted in to red cherries of coffee in gram per tree (mean of six trees). Clean coffee bean (quantal/ha) = fresh cherries in gram per tree x 0.00417. Clean coffee bean (kg/ha) was calculated as (clean coffee bean (quantal/ha) x 100).
Plant height (cm)	PH	The length from the ground level to the tip of the tree per four trees was measured using tape meter.
Height up to first primary branch (cm)	HUFPB	Height from the ground up to first primary branch was measured, using tape meter.
Main stem diameter (mm)	SD	Measured as a diameter of the main stem at five cm above the ground using caliper.
Canopy diameter (cm)	CD	Was estimated as average length of tree canopy in east-west and north-south direction, using tape meter
Traits	**Abbreviation**	**Description of quantitative traits**
Average Inter-node length on orthotropic branch (cm)	AINL	computed per tree as (TH–HFPB)/TNN-1, where TH = total plant height, HFPB =height up to first primary branch, TNN = total number of main stem nodes
Number of bearing primary branches (no)	NBPB	Numbers of bearing primary branches were counted per trees
Number of primary branches (no)	NPB	Total numbers of primary branches were counted per trees.
Length of longest primary branch (cm)	LLPB	The lengths of longest selected first primary branches were measured using tape meter.
100 Bean weight (g)	HBW	Calculated as (bean weight at 0% moisture content x 100/ (bean No x 0.89)
Bean length (mm)	BL	Average of five normal beans was measured at the longest part.
Bean width (mm)	BW	Average of five normal beans were measured at the widest part
Bean thickness (mm)	BT	Average of five normal beans was measured at the thickest part.
Fruit length (mm)	FL	Average of five normal and mature green fruits were measured at the longest part, using digital caliper
Fruit width (mm)	FW	Average of five normal and mature green fruits were measured at the widest part using digital caliper.
Leaf width (cm)	LW	Average of five normal (node 3 from the terminal bud) leaves were measured at the widest part
Leaf area (cm^2)	LS	Calculated by multiplying leaf length and width by a constant 0.67

Coffee berry disease severity and Coffee leaf rust disease severity in percentage were also recorded through visual assessment on the four sample trees. Visual disease estimation per individual sample coffee tree was followed as described by Van der Graaff, (1981). In the visual assessments, four trees per accessions were randomly taken and diagnosed for presence and absence of the disease on each tree. There after, disease incidence was calculated using the following formula:

$$\text{Incidence of coffee berry per leaf disease (\%)} = \frac{\text{Total number of diseased trees}}{\text{Total number of observed trees}} * 100$$

Correlation analysis

The phenotypic correlation and genotypic correlation coefficients between two variables, including genotype were estimated, as described by Singh and Chaudhary (1985).

$\sigma g_{xy} = \frac{MSPg - MSPe}{r}$

Where: σg_{xy} = genotypic covariance between traits x and y,

MSPg= genotypic mean sum product of traits x and y,

MSPe= environmental mean sum product of traits x and y and *r*= number of replications.

$\sigma p_{xy} = \sigma g_{xy} + \sigma e_{xy}$

Where: σp_{xy}= phenotypic covariance between traits

x and y, σg_{xy}= genotypic covariance between traits x and y, σe_{xy}= environmental covariance between traits x and y. Correlation coefficients at genotypic level (rg_{xy}) were calculated as: $rg_{xy} = (\sigma g_{xy}) / \sqrt{\sigma 2\, \boldsymbol{g}x * \sigma 2 \boldsymbol{g}y}$

Where: rg_{xy}= genotypic correlation coefficient between traits x and y,

σg_{xy}= genotypic covariance between traits x and y,

$\sigma^2 \boldsymbol{g}_x$= genotypic variance of trait x, $\sigma^2 \boldsymbol{g}_y$=genotypic variance of trait y.

Correlation coefficients at phenotypic level (rp_{xy}) were calculated as;

$rpxy = \sigma pxy / \sqrt{\sigma 2px * \sigma 2py}$

Where: rp_{xy}= phenotypic correlation coefficient between traits x and y,

σp_{xy}= phenotypic covariance between traits x and y, $\sigma^2 \boldsymbol{p}_x$= phenotypic variance of trait x and $\sigma^2 \boldsymbol{p}_y$= phenotypic variance of trait y.

Path coefficient analysis

Path coefficient analysis was calculated as suggested by Dewey and Lu (1959) to determine direct and indirect effects of different variables on grain yield as: rij = Pij + ΣrikPkj

Where; rij is mutual association between the independent trait(i) and dependent trait (j) as measured by the correlation coefficient Pij is component of direct effects of the independent trait (i) on the dependent

variable(j); ΣrikPkj is summation of components of indirect effect of a given dependent trait via all other independent traits. The residual effect (U) was calculated using the formula: $\boldsymbol{U} = \sqrt{1 - R^2}$Where: - R^2=Σpijrijpij = component of direct effects ofthe independent character (i) on the dependent character (j) as measured by thepath coefficient.rij = mutual association between the independent character (i) and dependent character (j) as measured by the correlation coefficient. Path analysis correlation coefficients were computed using Microsoft Office Excel software.

Results and Discussion

Phenotypic and genotypic correlation of coffee yield with other traits

Genotypic (above diagonal) and Phenotypic (below diagonal) correlation coefficients of 19 quantitative traits were computed and presented (Table 2). The result showed that coefficients of phenotypic correlation were lower than the genotypic correlation coefficients for most of the traits (Table 1) which might be due to less influence of environments on the association of the characters, which might have not weaken the inherent genetic associations. The result agrees with the reports that phenotypic correlations were in most cases lower than the corresponding genotypic values (Abdi, 2009; Olika *et al.,* 2011; Getachew, 2012; and Lemi et *al.,* 2017).

Phenotypic correlation: The phenotypic correlation analysis exhibited that clean coffee bean yield in $kgha^{-1}$ was statistically significant and positive with stem diameter, canopy diameter, number of primary branches and number of bearing primary branches with correlation coefficient of (rp=0.30, 0.30, 0.50 and 0.44), respectively (Table 2). This is in agreement with the findings of average bean yield that exhibited significant and positive association with stem diameter, number of primary branches and canopy diameter (Ermias, 2005). However, in contrast to this Olika *et al*. (2011) reported nonsignificant phenotypic correlations of bean yield with all morphological characters.

Genotypic correlation: The genotypic correlation analysis result exhibited that clean coffee bean yield exhibited positive and significant correlation with number of primary branch, number of bearing primary branch, stem diameter and canopy diameter with correlation coefficient (rg=0.70, 0.61, 0.34 and 0.38) respectively (Table 2). These suggest that coffee yield would increase with the increase of these characters indicating the greater importance and reliability of these traits for the improvement of yield in coffee. The breeding implication is that selection of one of the characters might result in the improvement of other characters.

In studies of genetic divergence and the processes of evaluation and selection, it is important to maintain

traits that are correlated with the majority of traits (Ferrao *et al*., 2008). The close relationship between yield and yield attributing traits might be exploited in selection program, which might be helpful in developing high yielding genotypes. Thus, breeders might need to emphasize these characters, in selection and crop improvement program. This finding is in agreement with Yigzaw (2005); Olika *et al*. (2011) and Lemi *et al.*, (2017) who reported positive and significant correlation of most of the quantitative characters with yield. Srinivasan (1980) also reported high and positive correlation of stem diameter and length of primary branches with yield. Similarly, Walyaro and Van der Vossen (1979) also reported significant and positive genotypic correlations between yield and stem diameter at the base of the main stem. Walyaro, (1983) and Marandu *et al*. (2004) also reported that coffee yield is influenced by important characters, like number of primary branches and canopy diameter.

In general, genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic correlation coefficients for almost all of the characters indicating that there is a strong inherent association between the characters studied. Higher genotypic correlation might be due to the absence of masking or modifying effect of environment on the genetic association among traits (Johnson *et al.*, 1955). The clean coffee bean yield was significant and negatively correlated with coffee berry disease severity at genotypic level. This implied that through selection of tolerant or resistant coffee accessions, optimum yield of coffee accessions could be achieved in efforts of variety development in selection program. Therefore, it is suggested that independent selection may have to be carried out for improvement of each character.

Phenotypic and genotypic correlation among morphological traits

A positive and significant phenotypic and genotypic correlation among characters was obtained including 100 coffee bean weight, bean width, bean length, bean thickness, fruit length, and fruit width (Table 2). This study confirmed the existence of positive and significant phenotypic hundred coffee bean weight with fruit length and bean width (Atinafu and Mohammed, 2017). The other traits: plant height, length of longest primary branch, canopy diameter, number of primary branches, number of bearing primary branch and stem diameter had close correlation to each other. The result reveal that, the greater the number of primary branches, the larger will be the number of bearing primary branch, length of the longest primary branch, stem diameter and canopy diameter. This indicated that for those traits which were positively associated the improvement for one trait will simultaneously improve the other. Whereas, those traits, which were negatively correlated the improvement

for one trait antagonistically affects the other.

It was found that reaction to coffee berry disease were negative and significantly correlated with 100 bean weight, bean width and bean thickness with phenotypic correlation coefficient values of (rg=-0.257, -0.183 and -0.184), respectively. The number of primary branch (rg=-0.459) and number of bearing primary branch (rg=-0.37) were negatively and significantly correlated with coffee berry disease at genotypic level.

Therefore, selection for coffee berry disease could negatively affect the improvement of number of primary branch and number of bearing primary branch as these characters showed negative and significant correlation at genotypic correlation coefficients. This implied that, the selections for any one of these characters are not likely to result in improvement of the others. In such a situation, it is suggested that independent selection might be carried for improvement of each character. From this study, it was suggested that high yielding population in coffee may be selected by concentrating upon number of primary branches, number of bearing primary branch, length of the longest primary branch and canopy diameter. Since the four traits are correlated among themselves, selection in one of the traits can result in the improvement of the other traits.

Table 2 Genotypic (above diagonal) and phenotype (below diagonal) correlation coefficient among 19 significant characters of Amaro coffee accessions at Awada in 2017/18

Variable	YLD	HBW	BL	BW	BT	FL	FW	LW	LS	CBD	CLR	LLPB	PH	HUFPB	SD	NPB	NBPB	AINL	CD
YLD		0.23	0.11	0.20	0.18	0.22	0.04	-0.03	-0.05	-0.64**	-0.17	0.19	0.07	0.12	0.34**	0.70**	0.61**	0.03	0.38**
HBW	0.18		0.69**	0.59**	0.67**	0.58**	0.57**	-0.04	0.02	-0.24	0.06	-0.03	0.10	0.15	-0.29*	0.13	0.09	0.14	-0.23
BL	0.03	0.66**		0.42**	0.54**	0.67**	0.33**	0.17	0.24	-0.02	-0.13	-0.04	0.22	0.23	-0.12	0.04	0.10	0.287*	-0.12
BW	0.10	0.54**	0.42**		0.75**	0.32**	0.55**	0.21	0.17	-0.17	0.12	0.05	0.20	0.25	-0.18	0.06	0.14	0.23	-0.04
BT	0.07	0.58**	0.59**	0.71**		0.46**	0.57**	0.26*	0.28*	-0.13	0.23	0.17	0.18	0.20	-0.15	0.11	0.17	0.20	-0.03
FL	0.14	0.51**	0.56**	0.31**	0.39**		0.43**	0.16	0.19	-0.15	-0.05	0.13	0.20	0.14	0.08	0.16	0.21	0.21	0.10
FW	-0.04	0.51**	0.37**	0.49**	0.49**	0.46**		-0.03	-0.00	-0.15	0.30*	0.02	-0.00	0.13	-0.07	-0.10	-0.12	-0.02	-0.08
LW	-0.00	-0.04	0.15	0.16	0.24**	0.169	0.01		0.95**	-0.04	-0.21	0.16	0.16	0.03	0.08	-0.18	-0.13	0.33**	0.22
LS	-0.01	-0.04	0.15	0.12	0.23**	0.18*	0.00	0.93**		-0.07	-0.21	0.14	0.13	0.09	-0.01	-0.23	-0.14	0.31*	0.14
CBD	-0.53**	-0.30**	-0.11	-0.18**	-0.18*	-0.13	-0.15	-0.04	-0.05		0.39**	-0.14	-0.02	-0.06	-0.04	-0.46**	-0.31*	0.02	-0.15
CLR	-0.07	0.03	-0.18*	0.05	0.11	-0.05	0.19*	-0.08	-0.07	0.36**		0.06	-0.12	-0.128	-0.16	-0.04	0.06	-0.13	-0.15
LLPB	0.13	0.08	0.12	0.08	0.20*	0.19*	0.07	0.12	0.07	-0.07	-0.00		0.49**	0.09	0.46**	0.33**	0.36**	0.42**	0.65**
PH	-0.01	0.22*	0.31**	0.22*	0.23*	0.27**	0.19*	0.14	0.13	-0.10	-0.12	0.43**		0.33**	0.15	0.35**	0.33**	0.85**	0.32**
HUFPB	0.13	0.15	0.19*	0.21*	0.16	0.15	0.14	0.02	0.05	-0.07	-0.09	0.06	0.24**		0.100	0.09	0.09	0.11	0.18
SD	0.30**	-0.10	0.06	-0.09	-0.05	0.19*	0.06	0.07	-0.02	-0.03	-0.14	0.49**	0.22*	0.13		0.30*	0.28*	0.09	0.73**
NPB	0.50**	0.16	0.108	0.09	0.08	0.18*	-0.04	-0.17	-0.19*	-0.30**	-0.09	0.31**	0.36**	0.09	0.32**		0.83**	0.06	0.38**
NBPB	0.44**	0.18*	0.165	0.10	0.15	0.21*	-0.03	-0.12	-0.15	-0.19	0.01	0.42**	0.34**	0.05	0.36**	0.68**		0.09	0.34**
AINL	-0.09	0.21*	0.34**	0.26*	0.28*	0.24**	0.15	0.29**	0.28**	-0.07	-0.09	0.35**	0.84**	0.04	0.13	0.07	0.10		0.18
CD	0.30**	-0.03	0.06	-0.04	0.06	0.13	-0.03	0.19*	0.13	-0.13	-0.10	0.59**	0.36**	0.12	0.64**	0.34**	0.36**	0.21*	

*, ** = Significant at 5% and 1%, respectively.

Path coefficient analysis

As per path coefficient analysis, the highest direct positive effect was shown by average inter node length of main stem (1.08), followed by canopy diameter(0.41), height up to first primary branch (0.30), number of bearing primary branch(0.30), number of primary branch (0.18) and leaf width (0.17) (Table 3). Low magnitude and positive direct effects were recorded for fruit width (0.12), hundred bean weight (0.09), bean width (0.03), stem diameter (0.01) and bean length (0.004). The negative effect were recorded for plant height (-1.25), leaf size (-.0.26), coffee berry disease (-0.23), bean length (-0.06), coffee leaf rust (-0.05), length of the first longest primary branch (-0.19) and fruit length (-0.05). The positive direct effects of stem diameter and number of primary branches in this study were in agreement with findings of Johnson *et al.* (1955) and Atinafu and Mohammed (2017). Path coefficient analysis revealed that bean width (rg=0.20) and hundred bean weight (rg=0.23) had positive and direct effect on coffee bean yield, though exhibited positive and non-significant genotypic correlation with coffee yield. Average internodes length on main stem had the highest positive and direct effect on coffee yield, but exhibited negative phenotypic correlation (rp=-0.09) with coffee yield. The negative correlation it showed with coffee yield was mainly due to negative indirect effects via other traits: bean thickness, fruit length, fruit width, leaf size, CBD, length of longest primary branch and plant height. This indicated that restricted simultaneous selection has to be followed; as restrictions are to be imposed to nullify the undesirable indirect effects in order to make use of the direct effect of these traits.

The positive direct effect of stem diameter (0.09), bean width (0.03) and hundred bean weight (0.05) on coffee bean yield agreed with findings of Masreshaw(2018).Similarly, Masreshaw (2018) also reported the negative direct effect of yield contributing traits like: total plant height (-0.08), bean length (-0.31), coffee berry disease (-0.16)and coffee leaf rust (-0.06). In contrast, the findings of this study revealed positive and direct effect of number of primary branches, fruit width (0.07) and negative effect of canopy diameter (-0.06) on coffee bean yield(Table 3). The other scholar found that the positive highest direct effect on coffee yield was exerted by plant height (1.56) and canopy diameter (1.56**).**

Average internodes length (-1.86) and number of primary branches (-1.80) also exerted high negative effects on yield (Lemi *et al.*, 2017). Ermias (2005) reported positive direct effect of plant height but negative direct effects of canopy diameter on yield. Atinafu and Mohammed *(*2017) observed direct positive effect by plant height, hundred bean weights, coffee berry disease, stem diameter and average length of primary branches, number of primary branches, number of main stems nodes and bean yield.

On the contrary, plant height (-1.25), fruit length (-0.05), bean thickness (-0.05) and length of longest primary branch (-0.19) had negative direct effect and positive genotypic correlation coefficients of (rg=0.07, 0.22, 0.18and 0.19), respectively on coffee bean yield. Hence, the positive correlation coefficient was largely due to their respective positive indirect effects of other characters.

The main selection criterion in coffee is yield, quality and disease resistance. Other agronomic characters related to yield potential have been studied to increase the indirect selection efficiency. In this study, the positive direct effect on coffee yield was exerted by average inter node length of main stem, canopy diameter, height up to first primary branch, number of bearing primary branch, number of primary branches and leaf width, fruit width, hundred bean weight, bean width, stem diameter and bean length. This indicates that, with other characters kept constant, direct selection on the basis of average inter node length of main stem, number of main stem nodes, canopy diameter, height up to first primary branch, number of bearing primary branch, number of primary branch and leaf width, fruit width, hundred bean weight, bean width, stem diameter and bean length would be much effective for the improvement of coffee yield. This is usually happening and they are well known as the most important characters that influence the coffee yield directly. The residual effect permits precise explanation about the pattern of interaction of other possible components of yield. In other words, residual effect measures the role of other independent variables which were not included in the study on the dependent variable. In this study, the estimated residual effect was 0.35 indicating that about 65% of the variability in yield was contributed by the characters studied in path analysis. This residual effect towards yield in this study might be mainly due to the other characters which were not included in the investigation and environmental factor. Therefore, the aspects of intensive germplasm explorations in the Amaro Kele coffee considering additional characters were suggested in order to confirm the results. In general, the path analysis carried out in the present study revealed that the main components of bean yield which had positive direct effect of bean yield should be given high priority for making selection for high yielding accessions in Amaro coffee accessions.

Table 3 Direct and indirect effects of bean yield and 18 yield contributing characters of Amaro coffee accessions at Awada in 2017/18

Variable	HBW	BL	BW	BT	FL	FW	LW	LS	CBD	CLR	LLPB	PH	HUFPB	SD	NPB	NBPB	AINL	CD	rG
HBW	**0.085**	0.003	0.018	-0.037	-0.031	0.067	-0.007	-0.004	0.054	-0.003	0.006	-0.128	0.044	-0.002	0.023	0.027	0.152	-0.095	0.229
BL	0.059	**0.004**	0.013	-0.030	-0.036	0.039	0.029	-0.061	0.004	0.006	0.008	-0.280	0.070	-0.001	0.008	0.028	0.311	-0.051	0.109
BW	0.051	0.002	**0.030**	-0.042	-0.017	0.064	0.034	-0.044	0.038	-0.006	-0.009	-0.253	0.075	-0.002	0.010	0.040	0.248	-0.018	0.198
BT	0.057	0.002	0.022	**-0.056**	-0.025	0.067	0.044	-0.073	0.030	-0.011	-0.032	-0.225	0.060	-0.001	0.020	0.050	0.216	-0.011	0.179
FL	0.049	0.003	0.010	-0.026	**-0.054**	0.050	0.026	-0.049	0.035	0.002	-0.024	-0.246	0.041	0.001	0.028	0.060	0.226	0.040	0.216
FW	0.049	0.001	0.016	-0.032	-0.023	**0.117**	-0.005	0.001	0.033	-0.014	-0.004	0.004	0.038	-0.001	-0.017	-0.035	-0.020	-0.034	0.043
LW	-0.004	0.001	0.006	-0.015	-0.008	-0.004	**0.167**	-0.245	0.009	0.010	-0.031	-0.198	0.010	0.001	-0.031	-0.039	0.359	0.092	-0.030
LS	0.001	0.001	0.005	-0.016	-0.010	-0.001	0.159	**-0.257**	0.015	0.009	-0.027	-0.160	0.028	0.000	-0.040	-0.042	0.338	0.059	-0.045
CBD	-0.020	0.000	-0.005	0.008	0.008	-0.017	-0.007	0.017	**-0.227**	-0.018	0.027	0.024	-0.019	0.000	-0.081	-0.090	0.018	-0.062	-0.644
CLR	0.005	-0.001	0.004	-0.013	0.003	0.035	-0.035	0.053	-0.088	**-0.046**	-0.011	0.144	-0.038	-0.001	-0.007	0.017	-0.138	-0.064	-0.165
LLPB	-0.003	0.000	0.001	-0.010	-0.007	0.002	0.027	-0.036	0.032	-0.003	**-0.191**	-0.614	0.026	0.004	0.058	0.105	0.452	0.270	0.189
PH	0.009	0.001	0.006	-0.010	-0.011	0.000	0.026	-0.033	0.004	0.005	-0.093	**-1.254**	0.099	0.001	0.061	0.096	0.916	0.134	0.065
HUFPB	0.012	0.001	0.007	-0.011	-0.007	0.015	0.006	-0.024	0.014	0.006	-0.016	-0.416	**0.300**	0.001	0.015	0.025	0.115	0.075	0.117
SD	-0.025	-0.001	-0.005	0.008	-0.004	-0.008	0.013	0.003	0.008	0.007	-0.088	-0.185	0.030	**0.008**	0.052	0.083	0.092	0.303	0.335
NPB	0.011	0.000	0.002	-0.006	-0.008	-0.011	-0.030	0.059	0.104	0.002	-0.063	-0.434	0.026	0.002	**0.176**	0.243	0.068	0.158	0.704
NBPB	0.008	0.000	0.004	-0.010	-0.011	-0.014	-0.022	0.036	0.070	-0.003	-0.068	-0.409	0.025	0.002	0.146	**0.294**	0.096	0.139	0.613
AINL	0.012	0.001	0.007	-0.011	-0.011	-0.002	0.056	-0.080	-0.004	0.006	-0.080	-1.061	0.032	0.001	0.011	0.026	**1.083**	0.072	0.029
CD	-0.020	-0.001	-0.001	0.002	-0.005	-0.010	0.037	-0.037	0.034	0.007	-0.124	-0.405	0.054	0.006	0.067	0.099	0.189	**0.414**	0.376

Residual=0.35

Conclusion

The genotypic and phenotypic correlation analysis result exhibited that clean coffee bean yield has positive and significant correlation with number of primary branch, number of bearing primary branch, stem diameter and canopy diameter. These suggest that coffee yield would increase with the increase of these characters. In studies of genetic divergence and the processes of evaluation and selection, it is important to maintain these traits. The close relationship between yield and yield attributing traits might be exploited in selection program, which might be helpful in developing high yielding genotypes. Thus, breeders might need to emphasize on these characters, in selection and crop improvement program.

In general, genotypic correlation coefficients were higher in magnitude than the corresponding phenotypic correlation coefficients for almost all of the characters, indicating that there is a strong inherent association between the characters studied.

Higher genotypic correlation might be due to the absence of masking or modifying effect of environment on the genetic association among traits.

In path coefficient analysis, the highest direct positive effect was shown by average inter node length of main stem, followed by canopy diameter, height up to first primary branch, number of bearing primary branch, number of primary branch and leaf width. Hence, the path analysis carried out in the present study revealed that the main components of bean yield which had positive direct effect of bean yield should be given high priority for making selection for high yielding accessions in Amaro Kele coffee accessions.

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Detection of Quantitative Trait Loci for Seed Germination Traits in Tomato under Salt and High Temperature Conditions

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Abstract

The objective of the experiment was first to evaluate the Recombinant Inbred Lines (RILs) of tomato for seed germination under salt stress, high temperature and non-stress growth conditions and then to identify quantitative trait loci (QTLs) controlling different germination traits under these conditions. A total of 101 RILs along with their two parents were tested under three growths. Significant (P<0.05) differences were observed among the RILs for all germination traits under all growth conditions. Germination was more delayed under salt stress at -0.5 MPa of NaCl than two growth conditions. Out of all RILs, genotype 291 showed rapidly germinated seed under three growth conditions. The QTL analysis detected a total of 29 significant QTLs for five germination traits under the three different growth conditions. At least 1 to 4 significant QTLs per trait identified explaining variances ranging from 8.8 to 15.2%. Four significant co-located QTLs were found on chromosomes 9, two of each controlling for onset and rate of germination traits, each ranging from 9.9 % to 13.2 % per traits under salt and high temperature. Similarly, 12 significant QTLs clustered on chromosomes 1 and 11 for onset (t10), speed (t50) and the area under the germination curve *(AUC) traits under non-stress and high temperature conditions, ranging from 49.7 to 65.4% .This might be an indication that the common genes control different traits or a locus has pleiotropic effects that controls multiple traits. Further study is required on Co-located QTL through fine mapping that can identify the candidate genes tolerance to salt and high temperature conditions which is very useful to improve seed germination and the performance of seed production.*

Keywords: common QTLs, rate of germination, salt stress, high temperature

Introduction

Seed quality is composed of genetic, physical and physiological characteristics that enable germination and produce normal seedlings under wide environmental conditions. This means that seed quality and its traits are controlled by genomic and environment interactions (Koornneef *et al*., 2002). This interaction can also influence seed germination and subsequently post-germination characteristics; include germination rate, uniformity and percentage of normal seedling in the field.

Adverse environmental conditions such as salt stress and extreme temperature can affect tomato growth

and hence its yield. Seed germination and early seedling stages are more susceptible to salinity stress than other growth stage (Ashraf and Foolad, 2005). Salt stress delays the seed germination, decreases the rate and increases the dispersion of the germination process and afterwards could lead to poor performance of seedlings in the field resulted in low yield (Cuartero and Fernandez-Munoz, 1998; Ashraf and Foolad, 2005). Similarly, extreme temperature eventually inhibits seed germination and can decrease seedling emergence as well impact on the final yield (Hampson and Simpson, 1990). However, these environmental effects depend on the intensity, duration of stress and its interaction with the genetic background of the crop.

In the last decades, most studies tended to focus on the improvement of the economical outcome and nutritional quality of tomato by considering either the genetic or environmental factors rather than emphasising on the genetic and environmental interactions that governs seed and seedling quality traits. Thus, seed quality traits are complex, since they are governed by the genome of the crop and environmental interactions which primarily expressed germination traits. In such traits, several genes are involved and thus, are suitable for quantitative trait loci (QTL) analysis. In many crops including tomato studies were conducted to identify QTLs for seed germination, QTLs for germination rate and seedling vigor traits that have tolerance to abiotic stress and under optimum conditions (Foolad *et al*., 2007; Kazmi *et al*., 2012). For example, QTLs detected for seed germination traits under cold stress, salt stress and non-stress condition in tomato and rice (Foolad *et al*., 1996; Wang *et al*., 2011). These studies indicated that seed germination and vigor traits are controlled by many genes and are strongly affected by environmental conditions (Bettey *et al*., 2000; Koornneef *et al*., 2002; Finch-Savage *et al*., 2010).

Earlier reports revealed that most commercial tomato cultivars are sensitive to salt stress (Foolad and Lin, 1997) and limited genetic variation exists within cultivated tomato. But, genetic variation exists within tomato species including landraces, cultivated tomato and related wild species. Thus, this variability is a novel choice to study seed germination under adverse environment conditions. Subsequent study is required by crossing cultivated tomato with related wild species for QTL identification that regulate germination traits under fluctuation of environments. Therefore, the objective of this study was to assess the influence of salt stress and high temperature on seed germination of tomato and identify common QTLs that regulate germination traits under the aforementioned abiotic stress conditions.

Materials and Methods

Source of plant materials

The tomato Recombinant Inbred Lines (RILs) population derived from a cross between two parents: cv. Money maker (*Solanum lycopersicum)* and Pimp (*Solanum pimpinellifolium*) was used. In total 101 RILs along with two parents were used for seed germination experiments.

Experimental setup

The experiment was conducted at Wageningen University and Research Center in Seed Lab of the Plant Physiology laboratory during 2015 in the Netherlands.

Germination trays were arranged at random with two replications and a maximum of 35 trays were used for each repetition and one layer of white filter paper was laid on each tray. A total of 101 RILs along with two parents were used in a randomized complete design (RCD) with two replications for each environmental condition in separate experiments.

Three sets of experiments were independently conducted using non-stress (control), salt and high temperature experiments. Fifteen ml of water was applied on each tray for non-stress condition. For the salt stress condition, 15 ml of NaCl salt solution was added to each germination tray and for high temperature stress condition (35 ^{0}C) (Kazmi *et al*., 2012), 15 ml of water was added to each tray. Each tray (21 cm x 15 cm) contained three different sets of RILs and approximately 50 seeds of each line were sown under aseptic conditions for three conditions of treatment independently. Trays were piled up and each stack had one empty tray on the top and bottom, layers of white filter paper laid on each with 15 ml water applied to prevent unequal evaporation.

Growth conditions

The germination trays were mounted according to their order, tightly fitted with lids and the whole stack was wrapped in a closed transparent plastic bag and incubated at 4 ^{0}C for 3 days in the dark for stratification. Later, the plastic bags were put in incubator at 25 ^{0}C under continuous light for salt and non-stress conditions. For temperature stress, the bags were incubated at 35 ^{0}C (Kazmi *et al*., 2012)

Linkage analysis

We use the genetic linkage map by Kazmi *et al*. (2012), in which they used 5,529 SNPs to genotype the RILs tomato population. SNP markers with identical values were removed, leaving 2,150 polymorphic markers. Furthermore, co-segregating markers were also removed. The remaining 716 unique markers were used for generating the genetic linkage map, which contains 12 individual linkage groups corresponding to the 12 chromosomes of tomato and the average distance between markers was about 10 cM.

Data collection

Germination assessment

Seed germination was counted and scored when radical protrusion was observed. Germination was followed twice per day for non-stressed while daily for stressed conditions for 14 consecutive days during the germination. After 14 days, the remaining seeds were scored as non-germinated seeds.

Statistical analysis

Germination parameters

Statistical analysis was conducted by using the curve-fitting script of the Microsoft excel Germinator package. Cumulative germination data was analyzed by using this script to determine germination parameters (Joosen *et al*., 2010). Statistical output of germination curves were used to calculate five germination traits; t10 (the time required in hours to reach 10 % germinated seed), t50 (the time needed in hours to reach 50 % germination of seeds), Gmax (%) the maximum germination capacity of the seed batches, U8416 (%) the uniformity of germination, and AUC, the area under the germination curve:

QTL detection

The mean values per recombinant inbred lines of the germination traits were used for QTL detection. QTL analysis was performed by using mapping software; MapQTL®6.0 (Van Ooijen, 2009), to identify QTL positions on the genome of tomato for measured traits. Interval mapping is used for estimating the position of a QTL within two markers. A permutation test ($P<0.05$) per germination trait was performed to determine the significance of LOD threshold per chromosome. A LOD score of > 2.0 was used as a threshold level to declare the significance of QTLs. Additive effects of each detected QTL together with phenotypic variation explained by each QTL (explained variance, %) was determined.

Results

Mean of RILs population

The result showed significant difference among RILs for the different germination traits. It was not convenient to show all mean traits output on this paper but average and standard error values for germination rate under salt stress illustrated below (Figure 1).Tremendous genetic variation observed among for speed germination. For example: some of inbred lines were germinated between 50 and 100 hrs whereas other took more than 200 hours for germanium, indicated that a longer time for rate of germination.

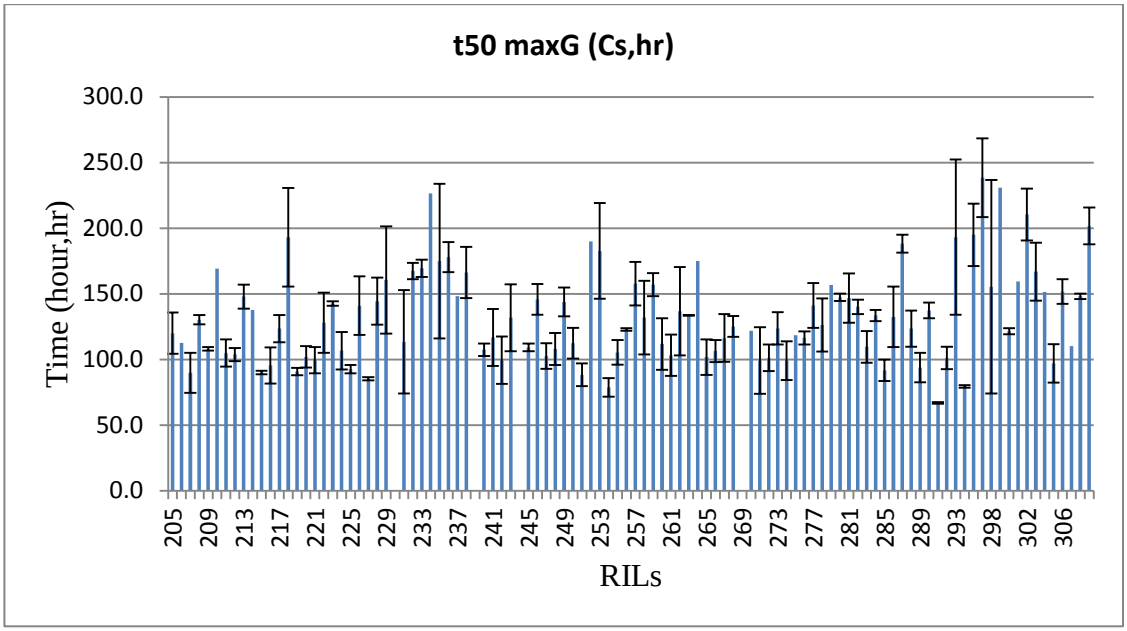

Figure 1. Average and standard error(± SE) of recombinant inbred lines and parents for t50 under salt stress condition

Comparing the two parents, seeds of Pimp germinated earlier (lower t10) and faster rate (lower t50) than seeds of money maker under salt stress, high temperature (35^0C) and non-stress conditions (figure 2 and 3). At -0.5 MPa of NaCl, salt stress delayed onset (t10), germination rate (t50) and other germination parameters compared to high temperature and non-stress conditions.

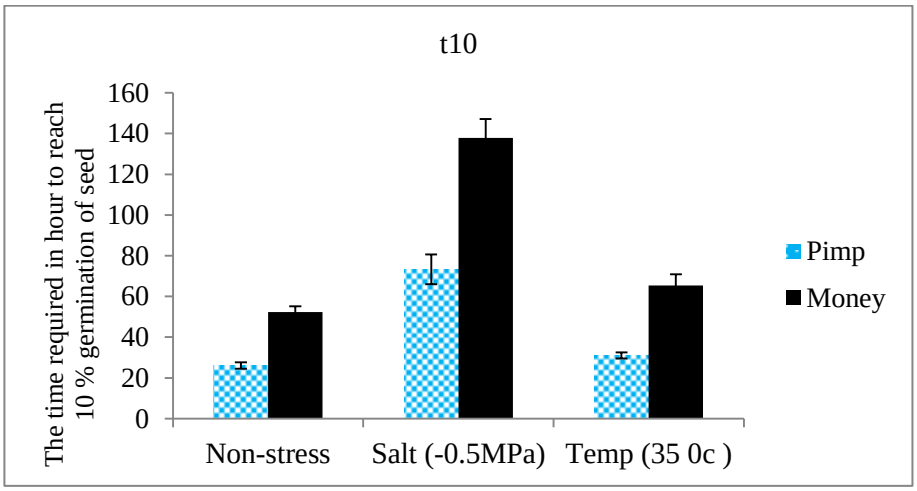

Figure 2. Average and standard error (± SE) of two parents, Pimp (*Solanum pimpinellifolium)* and Money maker (*Solanu mlycopersicum*) for t10 under control, salt (-0.5MPa) and high temperature (35 ^{0}c).

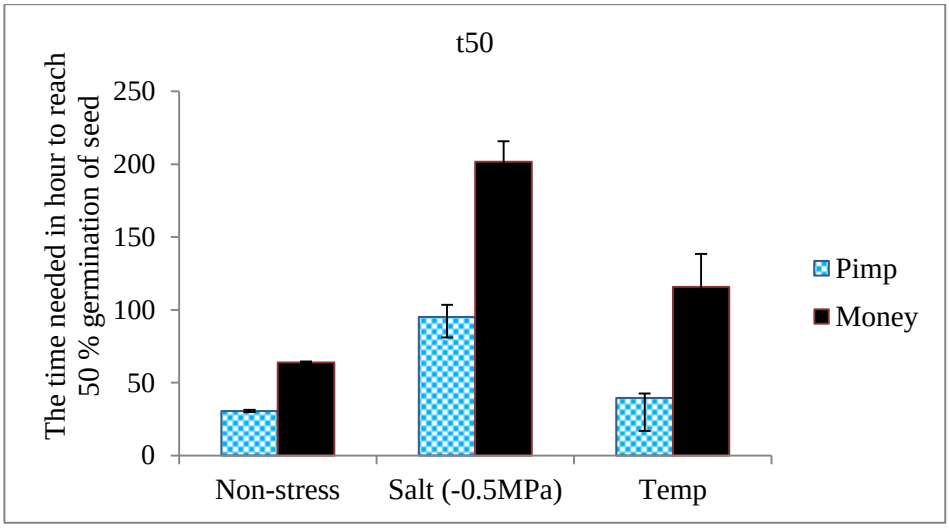

Figure 3. Average and standard error(± SE) of two parents, Pimp (Solanumpimpinellifolium) and Money maker (Solanumlycopersicum) for t50 under control, salt (-0.5MPa) and high temperature (35 0c)

Comparing the two parents, the seeds of pimp quickly achieved maximum germination percentage than money maker under non-stress conditions. For instance, the time to reach 98 % germination for pimp and money maker was 44 hrs and 127 hrs, respectively (Fig 4) under control. Also, pimp increasing sharply and tends to linear while money maker not (Fig 4).Similarly, money maker took longer time to achieve maximum germination percentage and had lower value than pimp under salt conditions (Fig 5). From germination curve, salt showed low germination of seeds compared to non-stress condition. This showed that the clear effect of salt stress on seed germination characteristics.

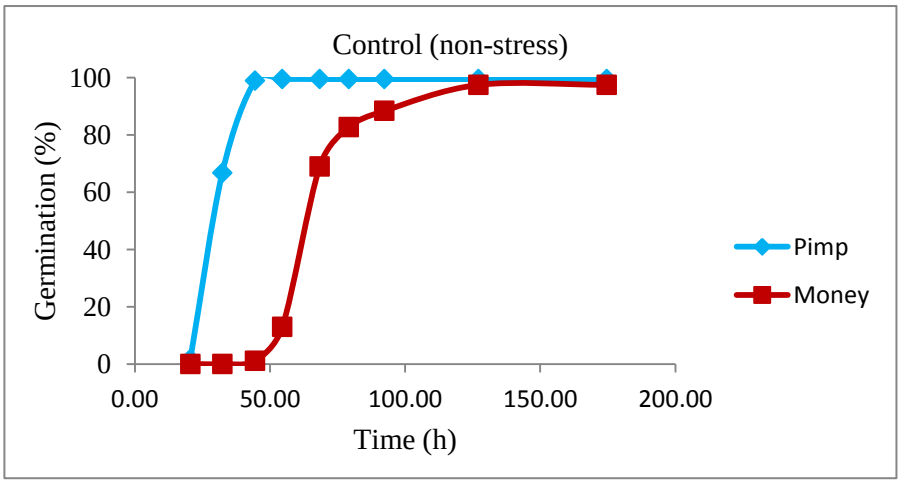

Figure 4.Germination curve of Pimp (Solanumpimpinellifolium) and money (Solanumlycopersicum) under control condition

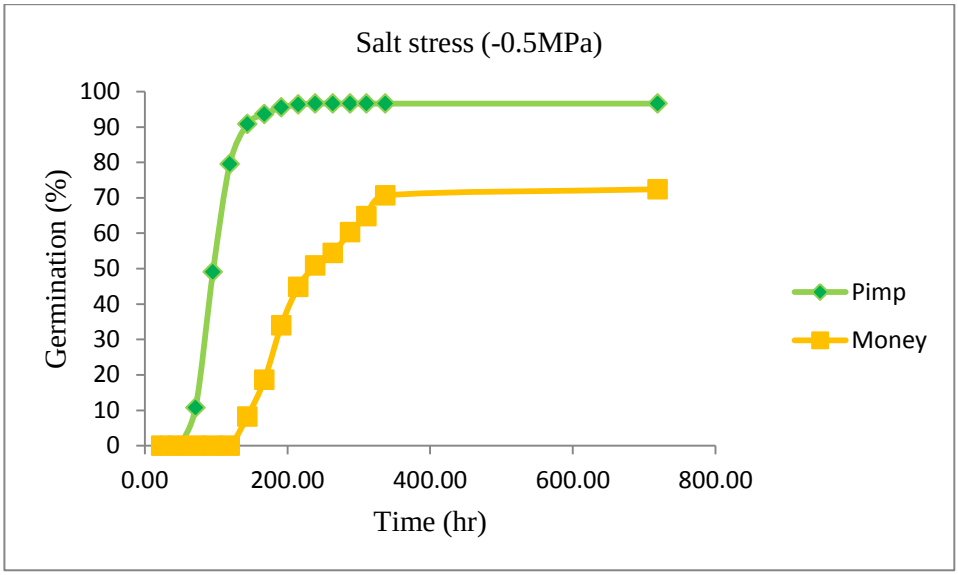

Figure 5. Germination curve of Pimp (Solanumpimpinellifolium) and money (Solanumlycopersicum) under salt stress

Detection of co-located QTLs for onset and germination rate under stress conditions

Several QTLs were found across common chromosomes for different traits under different growth conditions. For example, 4 significant co-located QTLs were found on chromosomes 9, two of each controlling for onset and rate of germination traits, each ranging from 9.9 % to 13.2 % per traits overlap under salt and high temperature (Figure 6). Also, 12 significant QTLs cluster on chromosomes 1 and 11 for t10, t50 and AUC traits under non-stress and high temperature conditions, ranging from 49.7 to 65.4% explaining variance (Figure 7). In addition, two significant co-located QTLs were found on chromosomes 2 for Gmax trait under salt and non-stress conditions, ranging from 9.8 to 10.1% overlap.

Comparison of QTLs affecting germination traits across environmental conditions

In total 29 significant QTLs were detected across three environmental conditions. Among these, 9 QTLs affecting onset of germination, 7 QTLs affecting germination rate, 10 QTLs; each five of QTLs affecting maximum germination and AUC, and one QTL contributes to uniformity of germination across three environmental conditions (data not shown). Therefore, the highest numbers of QTLs were detected for onset and germination rate across three environmental conditions. In comparison among environments, several QTLs (14 QTLs) were detected under high temperature, whereas, 3 QTLs were identified under salt stress conditions. This implies that QTLs might be sensitive to salt stress like at -0.5 MPa of NaCl.

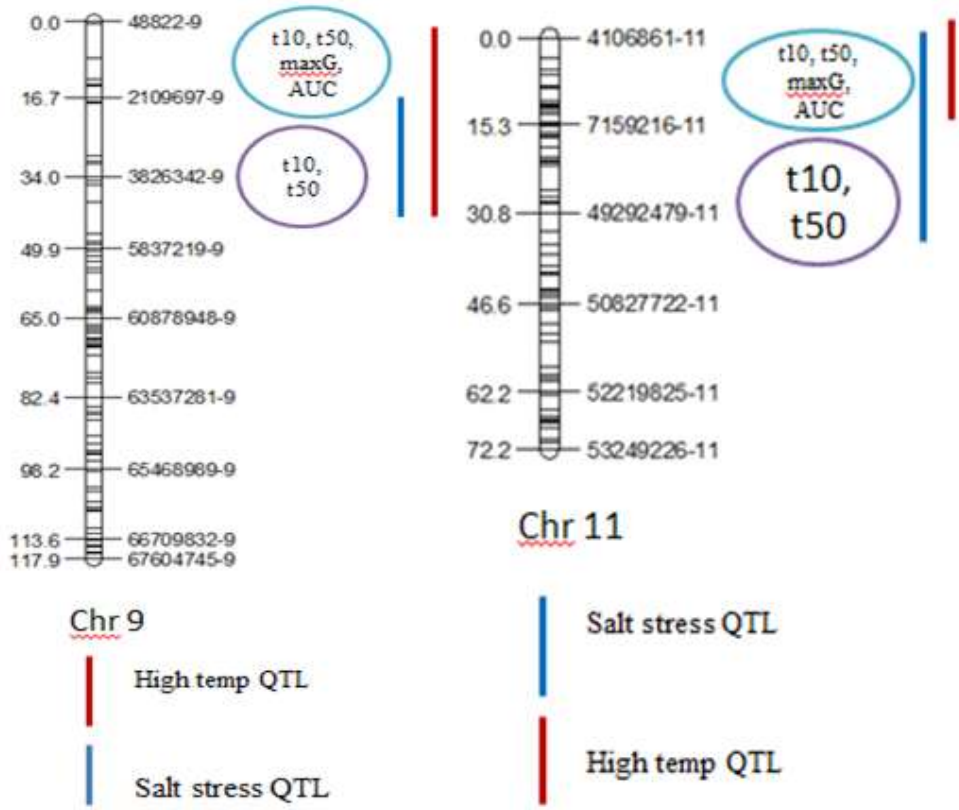

Figure 6. Co-located QTLs locations on chromosomes 9 and 11 for t10; onset and t50; rate of germination under salt and temperature growth conditions. The blue andred colours vertical lines at the right of the chromosomes indicate the approximate locations of QTL for germination traits under high temperature and salt conditions

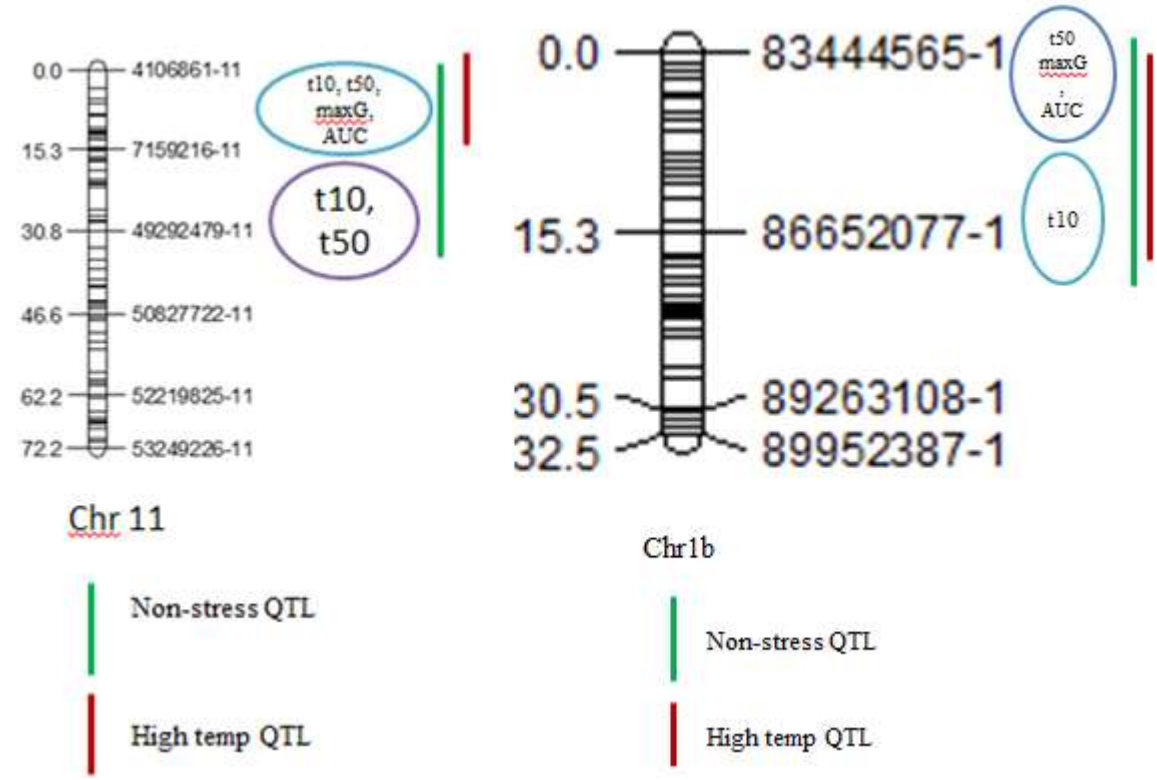

Figure 7. Co-located QTLs locations on chromosomes 11 and 1 for onset, germination rate, maxG and AUC traits under non-stress and high temperature growth conditions. The greenand redcolours vertical lines at the right of the chromosomes indicate the approximate locations of QTL for germination traits under non stress and high temperature growth conditions

Discussion

Effect of salt stress and high temperature on seed germination

Understanding the physiological mechanism how salinity and high temperature affect seed germination and seed quality is quite important. During seed germination, salinity may disorder physiological processes like decreasing the water uptake by seeds. There was evidence in this study under salt stress condition, some RILs population showed slower initial germination and longer time interval for germination traits. Foolad *et al*., (2007) observed that the delayed onset and slow rate of seed germination under salt conditions. Similarly, high temperature may disorder the germination process; like slow down and eventually inhibit seed germination (Hampson and Simpson 1990). This longer and or slower germination times affect seed quality in terms of uniformity of germination, rate of germination, seedling vigour and these attributes to impact on the whole value chain in tomato production.

This study was carried out to assess the effect of salt stress and high temperature on seed germination and identification of QTLs controlling seed germination traits under three growth conditions. Considerable genetic variation was observed for all physiological seed germination parameters (t10, t50, Gmax, U8416 and AUC) under three environmental conditions. Higher genetic variation was observed under stress conditions than in non-stress might be due to genotypes were exposed to stress environment,

The finding showed that salt stress at -0.5 MPa NaCl delayed all germination parameters such as onset germination, germination speed as well germination uniformity and decreased maximum germination as compared to high temperature and control. This statement agreed with Cuartero and Fernandez-Munoz (1998); Ashraf and Foolad (2005) who reported that salinity may delay the initiation of germination and elongate the time needed to complete germination. Thus salt stress directly influences the agronomic seed quality traits. But variation observed among RILs tolerance to salt stress. For example, genotype 291 showed rapidly germinated seed under salt and high temperature conditions. This genotype also efficiently performed for different germination traits under three growth conditions. Thus, this genotype might be the most economical useful to grow under saline soil and high temperature condition, i.e. This genotype is tolerance to salt stress and high temperature during seed germination, due to a mechanisms to regulate salt tolerance and revealing candidate genes or common QTLs that function in response to salt stress as well high temperature (Gong *et al*., 1999). On other hand, parent of Pimp had the lower t50 value indicates the faster in time to reach a 50 % germinating seeds. This could be due to the fact that the Pimp is a wild relative that might already be adapted to climatic change as well as buffering against changeable environmental conditions.

Identification of Co-located QTLs controlling seed germination traits

In this study, in total 29 significant QTLs were detected for different germination traits across three environmental conditions. Among these, 12 QTLs were detected under control, 14 QTLs detected under high temperature and the remaining 3 QTLs were found under salt stress.

The findings in the current study showed that several significant co-located QTLs were found for the different traits as well as under different growth conditions. For instance, for t10, t50 and AUC traits, six significant overlapped QTLs were found, two of each found on chromosomes 4 and 11 under control conditions. It is interesting that the common existence of genes also controlling different traits under control condition. Also, not all but most of the significant co-located QTLs were clustered for most

germination traits under high temperature condition. This implies that most of different germination traits were controlled by common generic QTLs under high temperature condition. This statement tends to agree with other studies (Clerkx *et al*., 2004; Khan *et al*., 2012) suggesting that due to common basis QTLs controlling such multiples traits. Similarly, some clustered QTLs were located on chromosomes 9 and 11 for onset and germination rate traits under salt and high temperature conditions. It is likely that the same QTLs were controlling the physiological characteristics of different germination traits across the two conditions. This is in line with Foolad*et al*., (2007) who suggested that the common QTLs control different physiological germination traits under different conditions. For possible explanation, common QTLs controlling for the onset and germination rate traits under salt stress might be contributed to early and speed germination traits under high temperature. It is likely that common genes for the tolerance to salt stress during onset and speed germination are also these genes encoding heat shock proteins associated with the tolerance to high temperature. Plants have different mechanism for salt tolerance. Zhang *et al*., (2012) reported that FAD2 genes regulate salt tolerance during seed germination and early seedling growth in some crops. For instance, Na^+ extrudes out from the cell and compartmentalizes it into vacuoles membrane using Na^+/H^+ anti-portal. However, we didn't detect such experiment in the present study.

High genetic variation was more observed under salt stress than two conditions. However, genotype by environment interaction was not expressed more number of significant QTLs under salt condition. This result does not support the hypotheses that the occurrence of more genetic variation would be expected to result in detection of more QTLs. No significant QTLs were detected for U8416 and AUC traits under salt conditions implying that some QTLs might highly sensitive to stress environmental conditions. Other study reported that salt stress might be damage the DNA activities and can induce the production of reactive oxygen species, ROS in plants (Zhu, 2002). Also, the ionic effect of salt can inhabit the enzyme activities during seed germination process (Ashraf *et al*., 2002). These might be the reason why seeds of a few RILs fail to germinate under salt stress in the present study. Furthermore, Many QTLs related to the salt tolerance have been reported in vegetable crops includes cucumber, watermelon and tomato (Li *et al*., 2011; Zhang *et al.*, 2003). However, we indentified very few significant QTLs detected under salt stress in RILs tomato.

Conclusion

Differences among RILs of tomato and genotype by environment interaction observed for all traits under

salt stress, high temperature and controlled conditions. at -0.5 MPa of NaCl, showed a major influence on seed germination than high temperature, 35 ^{0}c and control conditions. It was interesting that Line 291 showed rapidly germinated seed under salt and high temperature conditions as well efficiently performed for different germination traits under three growth conditions. In this study, significant co-located QTLs found on the same chromosome for the same traits and / or different traits under salt and high temperature stress. This might be an indication of the same genes that controlling different physiological processes which contribute to different germination traits expression or locus has pleiotropic effect that controls multiple traits. Further investigation is required on co-located QTLs through epistasis interaction, fine mapping and candidate genes detection in the QTL regions for target traits.

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Genetic Diversity of Yam (*Dioscorea* spp.) Landraces from Ethiopia Assessed by Morphological and Microsatellite Markers

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Abstract

Genetic diversity present within and between populations is crucial for breeding and conservation. The objective of this study was to assess the genetic diversity in yam landraces by using agro-morphological and microsatellite markers. Phenotypic diversity of 36 landraces collected from southwest Ethiopia was determined using diversity indices, principal component and cluster analyses. High phenotypic diversity indices were recorded, ranging from 0.53 to 1.50, with a mean of 0.985. Principal component analysis identified seven PCAs which contributed 88.4% of the total phenotypic variation among the landraces. The test primers amplified a total of 30 fragments, of which 80% was polymorphic. The number of alleles detected per locus ranged from 1 to 5, with a mean of 3. Number of effective alleles ranged from 1 to 3.57. Gene diversity ranged from 0.00 to 0.80 with a mean of 0.53. The mean polymorphic information content ranged from 0.00 to 0.72, with a mean of 0.30. The Simple Sequence Repeat markers and phenotypic traits showed similar clustering patters of landraces except some differences. The results obtained in this study are useful for future yam breeding and conservation program.

Keywords: Breeding, heterozygosity, microsatellite markers, phenotypic traits, yam

Introduction

Yam is a multi-species crop that belongs to the genus *Dioscorea* and family *Dioscoreaceae* (Tamiru et al., 2007). It is found in Africa, India, Southeast Asia, Australia and South America comprising of 600 species (Mignouna et al., 2002; Loko et al., 2015). All species are tropical origin and cultivated for their edible starchy tubers (enlarged, fleshy, usually underground storage stems) (FAO, 2010). Yam has great potential to combat food insecurity and for local and regional markets (Sesay et al., 2013). Globally, yam is the fourth most important tuber crop after potato (Solanum tuberosum L.), cassava (*Manihot esculenta Crantz*) and sweet

potato (*Ipomoea batatas* (L.) pori.) (Tamiru, 2006; Loko et al., 2013). West Africa is the predominant yam producing sub-region (FAOSTAT, 2006; FAO, 2010) contributing 95% of the world's yam production (Hamadina et al., 2009; Dansi et al., 2013).

In Ethiopia, yam is mainly used as a source of human food, medicine and cash income source through the sale of storage tubers (Hildebrand, 2003; Tamiru et al., 2007). Ethiopia is believed to be the centers of origin and diversity of yam (Rehim and Espig, 1991; Tamiru et al., 2011). Eleven species of yams have been described in Ethiopia (Miege and Demessew, 1997). Some of the species have both cultivated and wild forms in South, Southwestern and Western parts of the country. Edwards, (1991) reported that *Dioscorea* species are widely adapted in Ethiopia as cultivated and wild relatives. Further, Hildebrand, (2003) and Terauchi et al., (1992) reported that *D. abyssinica* is native to Ethiopia and grown over a wide range of agro-ecologies in Ethiopia.

Comprehensive phenotypic and genetic diversity analyses of the crop need to be undertaken to understand the population dynamics of yam landraces across the major growing regions in Ethiopia. Tamiru et al., (2011) sampled 84 yam accessions collected from Southern region of Ethiopia and reported the existence of a high level of phenotypic variation in accessions from the region of collections. Mulualem, (2016) also found morphological variation among yam landraces collected from Southwest region. Abebe, (2008), evaluated the genetic diversity of some 40 yam accessions collected from South and Southwest regions of Ethiopia.

Morphological characterization of germplasm is essential for crop improvement programs and conservation of existed genetic resources (Arnau et al., 2009; Alina et al., 2014). Germplasm characterization can be achieved using morphological traits and molecular markers (Paterne et al., 2019). Qualitative and quantitative traits are important agronomical traits that measured directly from the population (Mulualem et al., 2020). Nevertheless, this method is time consuming, requires phenotyping skills, multi-locations and multi-years experimentation, to account for environmental and genotype by environment effects (Spooner et al., 2005; Arnau et al., 2009). In yam, SSR (Simple Sequence Repeats) markers have been widely applied for the assessment of genetic diversity and characterization of germplasm and estimation of genetic distances between and within populations (Tamiru et al., 2011). SSR markers are currently the marker of choice for diversity analysis due to their ability to provide information on multi-allelic loci and greater genotypic differentiation (Abebe, 2008; Mulualem et al., 2018). The landraces used in this study were mainly collected from southwestern parts of

the country where the largest genetic diversity of yam is present (Hildebrand, 2003). In the region farmers developed and maintained large number of yam landraces for centuries based on their traditional knowledge (Demissew et al., 2003). Many authors indicated the presence of a wide morpho-agronomical variation in the Ethiopian yam landrace collections. However, most of the germplasm characterization in yam using phenotypic or limited molecular markers did not cover yam landraces from different growing areas of Ethiopia. The objective of this study was to assess genetic diversity present in yam landraces collections from southwest Ethiopia using morphological traits and SSR markers.

Materials and Methods

Study site, plant materials and experimental design

A total of 36 yam landraces collected across a wide altitudinal range (1171-1940 m.a.s.l from the southwestern parts of Ethiopia were used for this study (Table 1). The landraces were planted at Jimma Agricultural Research Center using a 6 x 6 simple lattice design with two replications with inter- and intra- row spacing of 1m and 1m, respectively. Tubers of the same size which started sprouting were used as planting material. All other agronomical practices were followed according to the recommendations and farmers practices of the areas. Each yam plant was tended using dried coffee sticks of 3.5-4.5 m long to provide support and induce good canopy and vine development. Five middle plants within a row were sampled and tagged for data collection and final harvest.

Five plants from each landrace were selected and tagged individually before sampling for DNA extraction. Genomic DNA samples were collected by using Whatman Flinders Technology Associates (FTA^{TM}) cards three weeks after planting. The FTA cards were labeled prior to sampling. Individual leaf was excised from the plant, wrapped round the FTA paper strip, and leaf sample extract were pressed onto the FTA paper until the FTA card was soaked with leaf sap. To prevent cross contamination in between samples, 70% of ethanol was used for cleaning materials.

Table 1. Names of the 36 yam landraces used for the study with collection zones, districts, geographical coordinates and altitude

Serial. No	Name of landraces	Zone	District	Latitude	Longitude	Altitude (m)
1	59/02	Jimma	Mana	$07^0$40'37N	$036^0$49'10E	1718
2	68/01	Jimma	Dedo	$07^0$30'63N	$036^0$53'45E	1784
3	6/02	Bench Maji	Sheko	$06^0$59'66N	$035^0$34'11E	1728
4	75/02	Jimma	Kersa	$07^0$40'43N	$036^0$48'76E	1734
5	3/87	Jimma	Manna	$07^0$40'58N	$036^0$48'75E	1731
6	56/76	Jimma	Manna	$07^0$41'89N	$036^0$48'06E	1837
7	54/02	Bench Maji	Sheko	$07^0$02'03N	$035^0$32'77E	1892
8	46/83	Jimma	Dedo	$07^0$31'28N	$036^0$53'59E	1771
9	08/02	Jimma	Kersa	$07^0$40'46N	$036^0$48'79E	1740
10	116	Jimma	Dedo	$07^0$31'28N	$036^0$53'63E	1683
11	01/75	Sheka	Yeki	$07^0$11'30N	$035^0$26'22E	1171
12	06/83	Jimma	Dedo	$07^0$31'32N	$036^0$53'64E	1692
13	17/02	Sheka	Yeki	$07^0$11'27N	$035^0$26'26E	1176
14	07/03	Jimma	Dedo	$07^0$31'50N	$036^0$53'60E	1733
15	45/03	Jimma	Mana	$07^0$41'86N	$036^0$48'08E	1810
16	27/02	Jimma	SekaChekorsa	$07^0$35'06N	$036^0$41'91E	1877
17	37/87	Jimma	Mana	$07^0$41'87N	$036^0$48'13E	1940
18	10/002	Bench Maji	Sheko	$07^0$02'91N	$035^0$29'76E	1668
19	76/02	Jimma	Kersa	$07^0$40'64N	$036^0$48'84E	1728
20	06/2000	Jimma	SekaChekorsa	$07^0$35'43N	$036^0$41'86E	1850
21	7/83	Jimma	Sekachekorsa	$07^0$35'06N	$036^0$41'91E	1898
22	58/02	Sheka	Yeki	$07^0$11'22N	$035^0$26'25E	1192
23	39/87	Jimma	SekaChekorsa	$07^0$35'42N	$036^0$42'94E	1885
24	32/83	Jimma	ShebeSombo	$07^0$26'74N	$036^0$24'01E	1372
25	24/02	Jimma	ShebeSombo	$07^0$26'75N	$036^0$24'07E	1379
26	2/87	Jimma	ShebeSombo	$07^0$26'76N	$036^0$24'12E	1365
27	60/87	Sheka	Yeki	$07^0$11'72N	$035^0$26'48E	1199
28	15/2000	Bench Maji	Sheko	$07^0$04'13N	$035^0$37'74E	1320
29	34/87	Jimma	Dedo	$07^0$31'37N	$036^0$53'44E	1911
30	21/02	Jimma	SekaChekorsa	$07^0$36'48N	$036^0$45'09E	1785
31	57/76	Bench Maji	Sheko	$07^0$02'88N	$035^0$29'74E	1654
32	0001/07	Jimma	ShebeSombo	$07^0$26'74N	$036^0$24'12E	1367
33	0004/07	Jimma	Kersa	$07^0$40'55N	$036^0$48'75E	1741
34	7/84	Bench Maji	Sheko	$07^0$02'88N	$035^0$29'74E	1661
35	7/85	Sheka	Yeki	$07^0$14'30N	$035^0$26'17E	1173
36	06/2001	Bench Maji	Sheko	$06^0$59'69N	$035^0$34'09E	1387

Phenotyping and data analysis

Thirteen phenotypic characters, the standard yam descriptor for characterization, were used for this study (IPGRI, 1999). Shannon-Weaver index (H') was computed for each phenotypic trait from frequency distributions observed in the different classes (Hennink and Zevan, 1991; Perry and McIntosh, 1991) as follows:

$$H' = -\sum_{i=1}^{n} pi\ loge\ pi$$

Where H' = Shannon diversity Index; pi= the proportion of landraces in the i th class of an n–class character; n = the

number of phenotypic classes of traits. Each diversity index value was divided by its maximum value ($\log_e n$) and normalized to keep the values between 0 and 1. The diversity index for each character was computed from the complete data set while the average diversity index was computed for each character.

In addition, the data was further subjected to principal component (PCA) and cluster analysis procedures using Genres (Genres, 2008) and SAS (SAS, 2000) statistical soft wares. Principal components (PC's) with eigen values > 1.0 were selected and morphological traits with load coefficient values > 0.5 were considered highly relevant to that PC (Morimoto et al., 2005).

Genotyping and data analysis

For molecular diversity assessment, a set of 10 microsatellite markers were used (Table 2). The markers used in this study were selected based on their polymorphic information content and diagnostic when used in yam (Tamiru et al., 2015). Genotyping was conducted at Incotec Biotechnology Laboratory, South Africa. All samples were used in bulk amplification, using DNA from five individual plants. A single punch of each card per submission was taken and homogenized in the Finnzymes dilution buffer. Two, micro-liters of each bulked sample were used in the polymerase chain reaction (PCR). Of the total of 36 yam landraces collected, three landraces namely, 59/02, 68/01 and 0001/07 were excluded from final analysis due to poor amplification. PCR amplification reaction contained 20 µl of PCR mix (1XPCR buffer, 3 mM MgCl, 1.25 U Taq polymerase, 0.2 mM dNTPs, 4pM each primer) and 2 FTA disc or 5 µl of CTAB extracted g DNA. A PCR profile of initial denaturation for 2 min at 94 ^{o}C, and 33 cycles of denaturation for 1 min at 94 ^{o}C, annealing temperature of 63 ^{o}C for 2 min, extension for 2 min at 72 ^{o}C was used. The PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3013 automatic sequencer (Applied Biosystems, Johannesburg, South Africa); analysis was performed using Gene Mapper 4.1. Product size was scored in base pairs based on the relative migration of the internal size standard. Information generated from the GeneMapper software was then used to determine the diversity parameters.

Table 2. Selected SSR primers for yam genetic diversity study

Locus	Repeat motif	Primers (5´to 3´) Forward	Primers (3´to 5´) Reverse	Tm ^{0}C		**GC (%)**		Product Size
				F	R	F	R	
YM02	$(AAG)_6$	TAGATTTCGCTTTTCCACTAGC	CCTAATCATCATCATCGTCATC	58	57	41	41	263
YM03	(GAT)6	TCACTCAAACAATGAGCGTAG	GATGGCTGCTGCATGACTG	60	60	58	58	202
YM05	(AAG)8	AGGATTATCACTGAAAGGGCT	CCTTCCAATTACTCTCCAAGA	57	56	43	43	140
YM09	(CTT)12	AGGAACATTCCCACTCAGTTA	ATTGGGCAAGTGTGGTGTG	59	59	43	53	193
YM12	AAC)8	TGAGCATTCTTGTTTTGCCG	CTTTCAGGGCGTGCATGG	58	60	45	61	215
YM13	(CTT)8	CCAATCACATCACGTCTAGTC	GACAATAGAAACTTCGAGACC	57	57	45	45	328
YM15	(CTT)7	CCATCTCCTCCCTTATCTACAC	GGGATTGAAGTTCCAGAGACT	57	57	50	45	485
YM17	(AC)8	TCCCTCAATTAAAGCATAGCC	AGCCACCAAACATCTTGCTC	59	60	43	50	181
YM18	(GT)19	GACATTGGGGATCTCTTATCA	TAGCAGCAGTAACGTTAAGGA	57	57	41	41	266
YM21	(GAT)5	AATGATGCATCTGAGGATAGT	GATGCTATTACGACAACCTTG	57	57	41	41	340

Genotypic data were subjected to analysis with various measure of genetic diversity within and among genotypes using GenAlEx software version 6.5 (Peakall and Smouse, 2012). Genetic diversity parameters such as total number of alleles per locus (N_a), observed fragment size (OFS) number of effective alleles per locus (N_e), observed heterozygosity (Ho), unbiased expected heterozygosity (gene diversity) (H_e) and polymorphic information content (PIC) were determined using the protocol of Nei and Li (1979). Further, phenotypic and genotypic relationships among yam landraces were determined by using neighbor-joining algorithm using the unweighted pair group method (UWPGM) in DARwin 5.0 software (Perrier and Jacquemoud Collet, 2006). A dendrogram was then generated on the dissimilarity matrix. Bootstrap analysis was performed for node construction using 10000 bootstrap values.

Results

Phenotypic diversity

From all traits considered, 20 (55.55%) and 28 (77.78%) of landraces exhibited medium and high leaf density, respectively (Table 3). Ten landraces (27.78%) produce spine on their tuber surfaces with variable shape; 22.22% had curved and 77.78% (28 landraces) had straight shape, and highly associated with the wildness of the landraces. In wild type landraces spines distributed all over the surface of vine and tuber in different amount with variable sizes. Tuber shape of the landraces varied from irregular (36.11%) to oval (8.33%). The following landraces have cylindrical tuber shape 68/01, 75/02, 3/87, 17/02, 07/03, 27/02, 10/002, 06/2000, 58/02, 24/02, 34/87, 21/02 and 7/85. The predominant tuber flesh colour was white with purple (25.0%) followed by purple (19.44%), purple with white (13.89%) and outer purple/inner white (11.11%), with dominant light and dark brown tuber skin colour. Most of the landraces (47.22%), considered in this study exhibited branched tuber with rough (77.78%) and smooth (22.22%) surfaces. The predominant tuber flesh colour at central transverse cross section was white (38.89%). Landraces such as 59/02, 75/02, 56/76, 46/83, 06/83, 27/02, 76/02, 06/2000, 58/02, 2/87, 34/87, 0001/07 and 7/85 recorded white fleshed colour. Other flesh colours observed include white with purple displayed by 19.44% and 13.89% of landraces producing similar colour, for example, light purple, purple and purple with white flesh colour.

All the phenotypic characters evaluated were highly polymorphic, with the maximum and minimum diversity index scores of 0.53 and 1.50 represented by leaf density, tuber surface texture and hairiness of tuber surface and flesh colour at central transverse, respectively (Table 3).

Table 3: Phenotypic variation and Shannon Weaver diversity indices of 36 yam landraces from Southwest Ethiopia

Landraces	Lsi	LD	Sp	SSh	CSpb	SVB	TS	TFC	TSC	TBr	TStex	HOTSu	F CC
59/02	Large	Medium	Absent	Straight	Present	None	Irregular	Purple with white	Light brown	Slightly branched	Rough	Small	White
68/01	Small	High	Absent	Curved	Absent	None	Cylindrical	White with purple	Dark brown	Branched	Smooth	Medium	Purple with white
6/02	Small	High	Absent	Straight	Present	Many	Irregular	White	Light brown	Highly branched	Rough	Medium	Purple with white
75/02	Small	High	Absent	Straight	Present	None	Cylindrical	White	Light brown	Highly branched	Rough	Medium	White
3/87	Medium	High	Present	Straight	Absent	Many	Cylindrical	Purple	Light brown	Highly branched	Rough	Small	Purple
56/76	Small	High	Absent	Straight	Present	Few	Oval	White	Dark brown	Highly branched	Rough	Medium	White
54/02	Small	High	Present	Curved	Absent	Many	Oval-oblong	Purple	Light brown	Branched	Smooth	Medium	Purple with white
46/83	Large	Medium	Absent	Straight	Present	None	Oval-oblong	Outer purple inner white	Light brown	None	Rough	Medium	White
08/02	Small	High	Absent	Straight	Present	None	Irregular	White	Dark brown	Branched	Rough	Small	White with purple
116	Small	High	Absent	Straight	Present	None	Flattened	Outer purple inner white	Dark brown	Branched	Rough	Small	Purple with white
01/75	Small	High	Absent	Straight	Present	None	Irregular	Purple	Dark brown	Branched	Smooth	Medium	Purple with white
06/83	Large	Medium	Present	Straight	Absent	Few	Oval-oblong	White	Light brown	Highly branched	Rough	Medium	White
17/02	Medium	High	Absent	Straight	Present	None	Cylindrical	Purple with white	Light brown	None	Rough	Small	White with purple
07/03	Medium	High	Absent	Straight	Present	Few	Cylindrical	White with purple	Light brown	Highly branched	Rough	Small	White with purple
45/03	Medium	High	Absent	Straight	Present	Few	Irregular	White with purple	Light brown	Branched	Rough	Medium	White with purple
27/02	Medium	High	Absent	Straight	Present	None	Cylindrical	White	Dark brown	Branched	Rough	Medium	White
37/87	Medium	Medium	Absent	Straight	Present	None	Irregular	Outer purple inner white	Dark brown	Highly branched	Rough	Medium	white with purple
10/002	Medium	High	Present	Curved	Absent	Few	Cylindrical	Purple with white	Light brown	Branched	Rough	Medium	purple with white
76/02	Medium	High	Absent	Straight	Present	None	Irregular	White	Light brown	Branched	Rough	Medium	White
06/2000	Medium	High	Absent	Straight	Present	None	Cylindrical	White with purple	Dark brown	Branched	Smooth	Medium	White
7/83	Medium	High	Absent	Straight	Present	None	Irregular	white	Light brown	None	Rough	Medium	Light purple
58/02	Medium	Medium	Absent	Straight	Present	None	cylindrical	White with purple	Dark brown	Branched	Rough	Medium	White
39/87	Large	High	Present	Curved	Absent	Few	Oval-oblong	White with purple	Dark brown	Branched	Rough	Medium	Purple
32/83	Medium	High	Present	Curved	Absent	Few	Irregular	Purple	Dark brown	Branched	Smooth	Medium	Purple
24/02	Large	Medium	Present	Straight	Absent	Few	Cylindrical	Purple	Dark brown	Highly branched	Rough	Small	White with purple
2/87	Medium	High	Absent	Straight	Present	None	Irregular	White	Light brown	Branched	Rough	Medium	White
60/87	Large	High	Present	Curved	Present	Few	Flattened	Purple	Dark brown	Highly branched	Rough	Medium	Light purple
15/2000	Medium	High	Present	Curved	Absent	Many	Oval	White with purple	Light brown	Branched	Rough	Medium	White with purple
34/87	Medium	High	Absent	Straight	Present	None	Cylindrical	White	Light brown	Branched	Rough	Medium	White
21/02	Small	High	Absent	Straight	Present	None	Cylindrical	Purple with white	Light brown	None	Rough	Medium	Light purple
57/76	Medium	High	Present	Curved	Absent	Many	Irregular	Purple	Light brown	Branched	Smooth	Medium	Purple
0001/07	Large	Medium	Absent	Straight	Absent	None	Oval	Outer purple inner white	Light brown	Highly branched	Rough	Small	White
0004/07	Medium	High	Absent	Straight	Present	None	Irregular	White with purple	Dark brown	Highly branched	Rough	Small	Light purple
7/84	Medium	High	Absent	Straight	Present	Many	Irregular	Purple	Dark brown	Slightly branched	Rough	Medium	Purple
7/85	Medium	Medium	Absent	Straight	Present	None	Cylindrical	White with purple	Dark brown	Highly branched	Smooth	Medium	White
06/2001	Medium	High	Absent	Straight	Present	None	Flattened	Purple with white	Dark brown	None	Rough	Medium	Light purple
H'	**0.99**	**0.53**	**0.61**	**0.79**	**0.61**	**0.96**	**1.39**	**1.59**	**0.69**	**1.26**	**0.53**	**0.53**	**1.50**

LSi=Leaf size, LD=Leaf density, Sp=, Spine on tuber surface, SSh= Spine shape, CSpb= colour at spine base, SVB=spine on vine base, TS=Tuber shape, TFC=Tuber flesh colour, TSC=Tuber skin colour, TBr= tuber branching, TStex= tuber surface texture, HOTs= hair on tuber surface, FCCS= flesh colour at central transverse. H'= Shannon-Weaver diversity index.

The mean phenotypic diversity index of genotypes was 0.985, showing high variability with respect to all phenotypic character classes.

The first seven PCAs, each with eigenvalues greater than one explained 88.4% of the total variation among the studied landraces for all morphological characters (Table 4). About 32.5% of the total variation was accounted for by PC1 which positively correlated to flesh colour at central transverse, spine on vine base and tuber flesh colour. Spines on vine base and tuber flesh colour had the highest loadings on PC2 and accounted 22.8% of the total variation, while PC3 associated with the spine on vine base and flesh colour at central transverse, explained 12.9% of the total variation. The remaining PCs accounted 20.2% of the total variation, which was mainly associated with spine on vine base, tuber shape, flesh colour at central transverse and tuber branching.

Table 4. Eigen values, proportion, cumulative variance and component scores of the first seven principal components for

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Leaf size	-0.003	-0.056	0.077	0.057	-0.091	0.128	-0.084
Leaf density	0.021	0.057	-0.034	-0.041	0.005	-0.023	-0.006
Spines on tuber surface	0.070	0.039	0.084	-0.008	0.081	0.108	-0.031
Spine shape	0.131	0.054	0.137	-0.016	0.124	0.164	-0.089
Colour at spine base	0.062	0.022	0.078	0.027	0.123	0.100	-0.024
Spine on vine base	**0.482**	**0.498**	**0.428**	**0.410**	-0.165	-0.144	0.076
Tuber shape	0.008	0.062	-0.255	-0.269	**-0.578**	**-0.458**	0.338
Tuber flesh colour	**0.461**	**-0.812**	0.258	0.094	-0.114	-0.103	0.083
Tuber skin colour	0.020	-0.078	-0.051	-0.090	0.238	-0.071	0.119
Tuber branching	0.010	0.067	0.052	0.159	0.207	0.087	**0.669**
Tuber surface texture	-0.036	0.008	-0.042	0.010	-0.024	-0.013	-0.051
Hair on tuber surface	0.026	0.080	0.091	-0.040	-0.031	-0.213	-0.075
Flesh colour at central transverse	**0.717**	0.155	**-0.496**	-0.317	0.125	0.157	-0.061
Eigen value	12.925	9.095	5.120	3.695	1.752	1.487	1.113
% total variance	32.50	22.80	12.90	9.30	4.40	3.70	2.80
% cumulative variance	32.50	55.30	68.20	77.50	81.90	85.60	88.40

13 qualitative traits in 36 yam collections.

SSR polymorphism

The 33 yam landraces evaluated in this study were differentiated uniquely, using 10 SSR markers (Table 5). A total of 30 putative alleles were detected from the population sampled. The observed fragment size (OFS) ranged from 155 to 495 nucleotides. The total number of polymorphic alleles per locus (N) varied from 1 (YM13 and YM18) to 5 (YM09) with a mean of 3.0. The number of effective alleles per locus (Ne) ranged from 1.00 to 3.57 and markers YM18 and YM09 had the lowest and highest numbers of effective alleles. This indicated the presence of genetic diversity among yam landraces from southwest Ethiopia.

Phenotypic and genetic relationships

Genetic relationships among the yam genotypes were studied using the Neighbour-joining dendogram constructed using unweighted pair group method of arithmetic means (UPGMA) algorithm based on morphological and SSR markers, which classified the landraces into four and three clusters, respectively (Figures 1 and Figure 2). Cluster analysis based on phenotypic traits grouped 24 (66.67%), 8(22.22%), 2(5.55%) and 2(5.55%) of landraces in Clusters I, II, III and IV, respectively (Figure 1). Cluster analysis based on SSR markers classified 14 (42.42%), 12 (36.36%) and 7 (21.21%) of the landraces into Clusters I, II and III, respectively (Figure 2). Phenotypic traits and SSR markers showed similar clustering patterns of yam landraces except some discrepancies.

Table 5. Genetic diversity within and among 33yam landraces based on 10 SSR markers

Locus	N_a	OFS (bp)	K	Ne	Ho	He	F_{IS}	PIC
YM02	3.0	237 - 242	0.471	2.22	0.71	0.47	-0.29	0.55
YM03	4.0	214 - 235	0.735	1.13	0.03	0.74	0.74	0.12
YM05	2.0	155 - 158	0.735	1.10	0.10	0.74	0.05	0.09
YM09	5.0	201 - 225	0.645	3.57	0.68	0.65	-0.06	0.72
YM12	4.0	221 - 232	0.657	2.34	0.70	0.66	-0.22	0.57
YM13	1.0	319	0.116	1.00	0.00	0.00	0.00	0.00
YM15	3.0	491 - 495	0.793	1.19	0.03	0.79	0.78	0.16
YM17	4.0	192 - 211	0.802	1.41	0.30	0.80	0.05	0.29
YM18	1.0	256	0.280	1.00	0.00	0.00	0.00	0.00
YM21	3.0	368 - 373	0.403	2.14	0.89	0.40	0.67	0.53
Mean	3.0	-	5.637	1.71	0.34	0.53	0.24	0.30
SE	0.42	3.47	0.567	0.27	0.11	0.10	0.12	0.08

Where, N_a= Total number of alleles per locus, OFS= Observed fragment size, K= Expected heterozygosity, Ne= Number of effective alleles per locus, Ho= Observed gene diversity within landraces, He= Average gene diversity within landraces, F_{IS}=Inbreeding coefficient, PIC= Polymorphic information content and SE= Standard error.

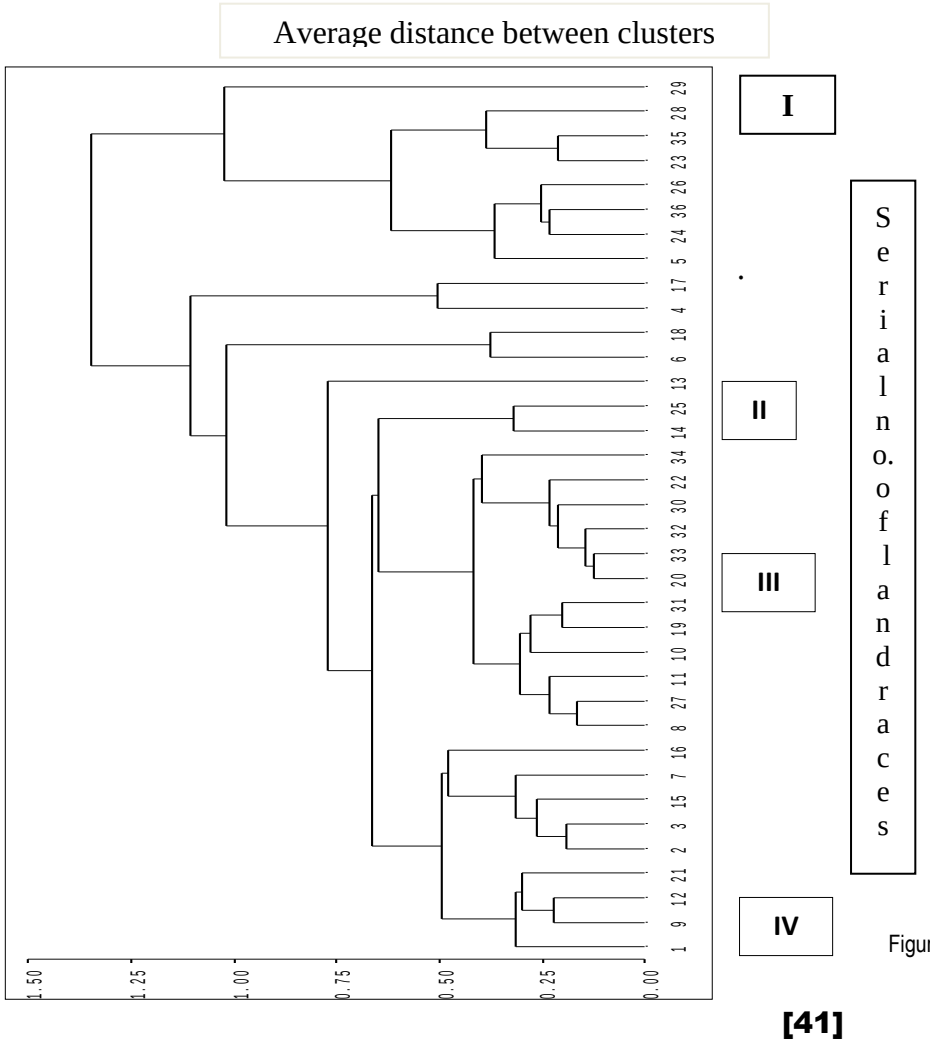

Figure 1. Neighbour-joining dendogram constructed using the unweighted pair group method of arithmetic means (UPGMA) algorithm depicting genetic relationship among 33 yam landraces based on phenotypic traits.

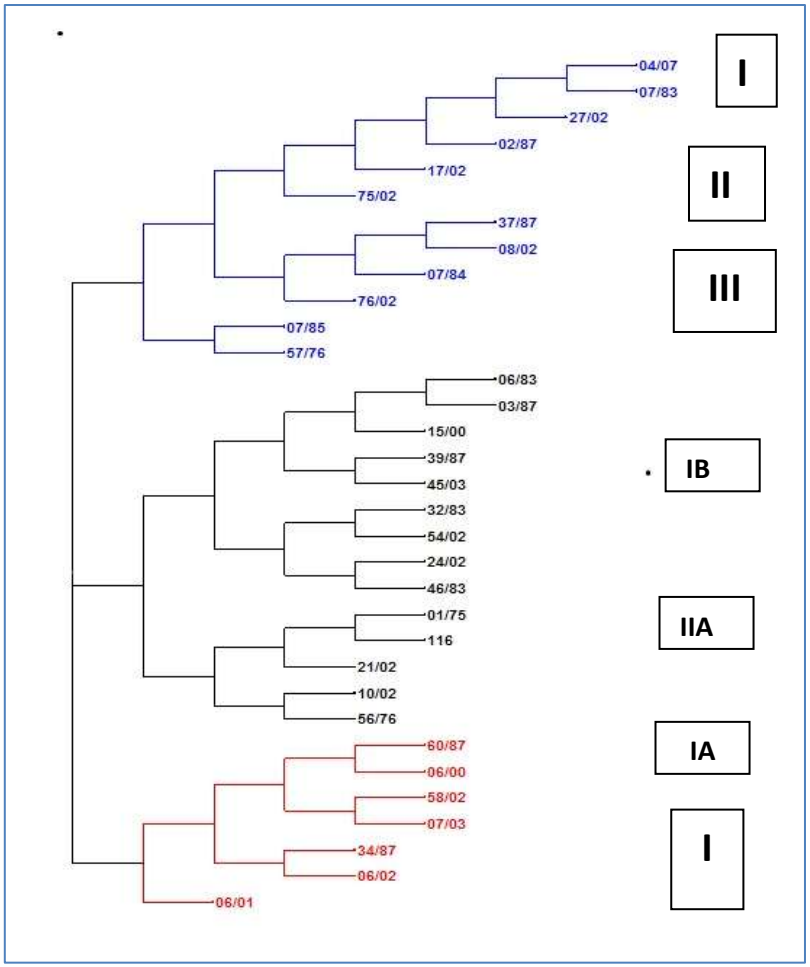

Figure 2. Neighbour-joining dendogram constructed using the unweighted pair group method of arithmetic means (UPGMA) algorithm depicting genetic relationship among 33 yam landraces based on SSR markers

Discussion

The present study analyzed genetic diversity of yam landraces collected from Southwest Ethiopia on the basis of phenotypic and SSR markers. Most phenotypic traits showed high genetic diversity on tuber characteristics such as: tuber colour, tuber flesh colour, tuber shape, tuber surface texture, tuber branching, tuber skin colour, hairiness of tuber surface and flesh colour at central transverse cross section. The present results are in agreement with the results of different reports who reported that yam exhibits significant variation on tuber characteristics (Obidiegwu et al., 2009; Dansi et al., 2013). The observed variation in tuber characteristics among the yam landraces could be partly due to the result of long-term selection by growers, the farming system, environmental effects and the mating system of the crop (Koffi et al., 2009; Mashilo et al., 2015). Probably farmers may have selected various unique tuber shapes for different uses (Loko et al., 2013). Yam is a dioecious plant and has high probability for cross-pollination, which result in considerable variation affecting the genetic identity of populations. Similar result was reported by Mulualem and Mohammed, (2013) on aerial yam (Dioscorea bulbifera).

The result of 'H' value for all observed phenotypic characters ranged from 0.53 for leaf density, tuber surface texture and hairiness of tuber surface to 1.50 for flesh colour at central transverse with the overall mean of 0.985. This result is in accordance with the report of Silvia and Gustavo, (2006) who found an average level of diversity in Colombian collections of water yam (Dioscorea alata) and Tamiru et al., (2011) in yams from South Ethiopia. High 'H' value indicates relatively high level of diversity and evenly distribution of landraces (Hennink and Zevan, 1991; Abebe, 2008). The low level diversity may also indicate the narrow genetic base of the plant and the lower probability of sexual reproduction in yam.

The number of alleles ranged from 1 to 5 with a mean of 3.0 per locus (Table 5). This result was lower than the mean alleles of 7.3 reported by Tostain et al., (2006) on yam, who used 17 polymorphic SSR loci. Zhigang et al., (2014) reported number of alleles of 6.09 per locus in 37 yam entries by using 7 polymorphic SSR loci, which is greater than the present findings. Further, Silva and Gustavo, (2006) reported the number of alleles per locus varying from 1.0 to 2.0 (mean 2.8), suggesting low allelic richness. In this study, the number of effective alleles per locus ranged from 1.0 to 3.57 with a mean of 1.71. Expected heterozygosity values in this study ranged from 0.116 to 0.80 with a mean of 0.567. The mean heterozygosity value observed in this study was quite smaller than the report of Obidiegwu et al., (2009) who

reported a mean value of 0.67 in Côte d'Ivoire using 13 SSR markers and comparing 89 water yam (Dioscorea alata L.) accessions from West African countries. The high level of heterozygosity observed among genotypes signified that landraces used in this study were collected from wide range of geographic areas with different levels of selection pressure and will enhance selection efficiency.

In the present study the PIC value ranged from 0.0 to 0.72 with a mean of 0.3. This value is similar with those reported by Emmanuel et al., (2015) who reported PIC values of 0.86 to 0.94; Marcos et al. (2011) with 0.39 to 0.78 (mean 0.4) and Obidiegwu et al., (2009) with 0.30 to 0.82. The PIC defines a relative measure of the in formativeness of a marker or discriminatory power of a polymorphic marker, which depends on the number of alleles and relative frequency of an allele in the population (Gaikward et al., 2008; Bekele, 2014). Four markers (YM02, YM09, YM12 and YM21) in this study had PIC values > 0.5, suggesting high discriminatory ability for classifying the landraces. The dendogram based on phenotypic traits and SSR markers classified the yam landraces into four and three main clusters, respectively. This indicated both SSR and phenotypic traits showed similar trend for clustering of studied landraces in southwest Ethiopia.

Conclusion

The phenotypic and SSR markers revealed high genetic diversity among yam landraces collections of south western Ethiopia. This variation would be attributed to the result of long-term selection and management of the yam by growers, the exchange of landraces between farmers and traders and environmental effects and the mating system of the crop. The variation obtained among the collection has good possibility to make selections for any of the traits in south west Ethiopia, assuming that a significant portion of the phenotypic variation is genetic. It was also found that the landraces showed a wide range of variation for tuber colour, tuber flesh colour, tuber shape, tuber surface texture, tuber branching, tuber skin colour, hairiness of tuber surface and flesh colour at central transverse cross section are useful morphological parameters for genetic analysis in yam. The detection of a significant number alleles that could be attributed to the high genetic diversity in the yam landrace. This conforms that Ethiopia was one of the primary centers for the domestication of yam. The diversity available in the studied landrace would allow for future breeding programs in the country.

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Evaluation of Drought Tolerance in Durum Wheat Genotypes Using Drought Tolerance Indices

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Abstract

Drought has become challenging globally, particularly under rain-fed crop production systems of Ethiopia. It is one of the major causes for wheat yield reduction. One hundred forty-four durum wheat genotypes were evaluated at Debre-Zeit experiment station in 2017 dry season, under drought stressed and non-stressed environments, to identify drought tolerant genotypes and suitable drought screening indices. The trial was conducted during the off-season. Based on grain yield under stressed and non-stressed conditions, various drought tolerant indices including Abiotic tolerance indices (ATI), Geometric mean productivity (GMP), Stress tolerance indices (STI), Stress stability indices (SSI), Tolerance indices (TOL), Yield index (YI) and Drought resistance indices (DRI) were computed. The combined analysis of variance showed that moisture, genotypes and their interaction had a significant ($p \leq 0.01$) effect on grain yield. Principal component and Correlation analysis indicated that DRI, YI, YSI and REI, as important indices highly correlated with grain yield under stress suggesting their suitability to identify drought tolerant genotypes. Cluster analysis also grouped the genotypes into five different clusters having various characteristics. Overall, evaluation of durum wheat genotypes for drought stress using different analysis identified G-16, G-763, G-31, G-119, G-63 and G-30 as drought tolerant genotypes. DRI, YI and YSI could be regarded as good indices for the identification of drought tolerant lines. MP, GMP, and REI were the best indices to identify suitable genotypes to both drought and non-drought stressed environment.

Keywords: Cluster analysis, Durum wheat, Drought tolerance indices, Drought stress, Principal component analysis

Introduction

Wheat is the fourth most important cereal crop after maize, tef and sorghum in terms of area coverage and production in Ethiopia. In Ethiopia, both bread and durum wheat is grown extensively, although separate area coverage and production are not known. Both are cultivated over an area of 1.69 million hectares with an annual production of about 2.46 million tones with average productivity of 2.74 tones ha^{-1} (CSA, 2018) which is below the world

average. There were several factors that can mention for low productivity of wheat in Ethiopia. Drought was one of the major causes of wheat yield reduction in the country. In Ethiopia, although durum wheat is traditionally grown in the highlands on vertisols with enough rainfall and extended growing season, the crop is usually grown late in the season due to the problem of water logging that exposed to terminal drought stress. Similarly, in lowland environments of Ethiopia, erratic rainfall with poor distribution as well as short growing seasons does not match with crop phenology resulting in terminal drought.

Several studies indicated the radical effect of drought stress on wheat. Exposure to terminal drought affects wheat grain by about 50% than drought at flowering **(**Chenu et. al., 2011), lower flag leaf and spike photosynthesis (Majid et.al. 2013), 1000 kernel weight and weight of kernels per spike by drought than heat (Plaut et. al. 2004) and crop phenology (McMaster, 1997**).** Thus, screening durum wheat for drought stress and identification of the right tolerant genotypes were among the important strategy to increase production and productivity. Breeding success and selection of genotypes under drought stress is often affected by low heritability of traits (Ceccarelli, 1994), high genotype by environment interaction (Hossain et. al., 1990) and lack of suitable screening techniques. The use of screening methods those are simple, cheap and repeatable are a pre-requisite and an alternative strategy to reduce factors that challenge the efforts of crop breeding for drought stress particularly when large numbers of genotypes are to be used for identifications of the right genotypes. Testing genotypes under stress and stress-free environmental conditions and the use of appropriate drought screening indices for associating the response of genotypes are suggested to be effective for identification of true field drought genotypes (Clark et.al. 1992; Trethowan et.al. 2002). The objective of this study was therefore to evaluate genotypes tested under drought stress and non- drought environments and identify tolerant ones using various drought tolerant and susceptible indices.

Materials and Methods

Description of experimental site

The field trial was conducted at Debre-Zeit Agricultural Research Center experimental station located in 8^0 41’36”latitude and 39^0 03’17” longitudes with an altitude of 1880 meter above sea level (masl). The station categorized as mid-highland that represents the major wheat growing areas of Ethiopia.

Experimental genotypes

One hundred forty four durum wheat genotypes were used in the study and the results for 90 of 144 genotypes were presented. Global wheat collections developed for dry

environment ,landraces collected from different parts of the country and obtained from Ethiopian biodiversity institute,, local breeding lines and improved cultivars were included (Table 1).

Table 1.Source, number of genotypes and names of genotypes tested under drought stress and non-drought stress environment at Debre-Zeit sandy clay soil during 2017 dry season.

Sources	No of genotypes	Names
Released cultivars	21	Quamy, Assasa,Ginchi,Ude,Werer,Mangudo,Mukiye,Gerardo, Utuba,Kilinto,Bichena, Yerer , Denbi , Tob-66, Ejersa, Toletu, Flakit, Arendato, Boohai, Hitosa and Cocorit
Global wheat collections	57	ICA -381, ICA-45, ICA -47, ICA- 55, ICA-33, ICA -32, ICA-360, ICA - 77, ICA -378, ICA - 54, ICA-46, ICA-61, ICA-383, ICA-359, ICA- 59, ICA- 353, ICA-26, ICA -50, ICA -60, ICA -23, ICA- 56, ICA -13, ICA - 382, ICA -39, ICA-357, ICA- 346, ICA-358, ICA- 48, ICA - 29, ICA -58, ICA-32, ICA-34, ICA-24, ICA-355, ICA -65, ICA-74, ICA- 51, ICA -44, ICA -53, ICA -73, ICA-57, ICA-20, ICA-64, ICA-41, ICA -354, ICA - 25, ICA- 49, ICA-384, ICA-38, ICA-356, ICA -28, ICA -43, ICA -30, ICA -62 and ICA-37
Breeding lines from DZARC nurseries	**27**	BI-1,BI-2,BI-3,BI-4,BI-5,BI-6,BI-7,BI-8,BI-9,BI-10,BI-11,BI-12,BI-13,BI-14,BI-15,BI-16,BI-17,BI-18,BI-19,BI-20,BI-21,BI-22,BI-23,BI-24,BI-25,BI-26,and BI-27
Landraces from EBI collections	**21**	EBI-1,EBI-2,EBI-3,EBI-4,EBI-5,EBI-6,EBI-7,EBI-8,EBI-9,EBI-10,EBI-11,EBI-12,EBI-13,EBI-14,EBI-15,EBI-16,EBI-17,EBI-18,EBI-19,EBI-20, and EBI21
Landraces from DZARC collections	**18**	GN-1,GN-2,GN-3,GN-4,GN-5,GN-6,GN-7,GN-8,GN-9,GN-10,GN-11,GN-12,GN-13,GN-14,GN-15,GN-16,GN-17 and GN-18
Total	**144**	

Experimental Design and Field Management

The plant materials were grown from January 14 to May 22, 2017 during the dry season when rainfall was not expected to affect the trial. The genotypes were arranged in 12 x12 simple lattice designs with two replications. Each genotype was grown in two rows of 2.0 m length and 0.20 meter width with a total plot area of 0.8m^2. Since drought stress is the only factor to be evaluated on genotypes expression, the cultural practices were the same in both conditions. The same amount of seeding rate and planting date were used for both experiments as per the recommendation. During planting, 50 kg/ha urea (46%N) as N sources and 100kg/ha DAP (46% %P_2O_5) as sources of phosphorus were applied. At the beginning of tillering, the remaining 50 Kg/ha urea (46%N) was applied by top dressing. To reduce the influence of biotic factors under both conditions, weeds were controlled manually as needed and tilt fungicides were sprayed twice to prevent the genotypes from stem and leaf rust infection.

In the drought stress treatment, genotypes were fully watered until 50 % of genotypes headed and then after irrigation stopped until time to maturity. The genotypes in non-drought conditions were irrigated

when soil moisture was depleted throughout the growing period.

Data collection

Based on grain yield under stressed and non-stressed conditions, various drought tolerant indices were computed (Table 2).

Table 2. Drought tolerance indices

Drought Tolerance induces	Formula	References
Yield stability index (YSI)	Ysi /Ypi	Bouslama and Schapaugh (1984)
Relative efficiency index (REI)	RDI= (Ys/Yp)/ (S Y / P Y	Raman *et.al*. (2012)
Abiotic tolerance indices (ATI)	ATI =[(Yp-Ys) / (P Y / S Y)]× (Yp × Ys)0.5	Moosavi*et.al*. (2008)
Drought resistance indices (DRI)	(DI) = Ys × (Ys/Yp)/ S Y	Lan (1998)
Stress tolerance index (STI)	(Ypi x Ysi)/Yp2	Hossain *et.al*. (1990)
Geometric mean productivity (GMP)	$\sqrt{(\mathrm{Ypi\ x\ Ysi})}$	Fernandez (1992)
Yield reduction percentages (YR)	$(\mathrm{GYNS} - \mathrm{GYMS})/GYNS$	(Fischer and Maurer 1978):
Tolerance index (TOL)	Ypi – Ysi	Rosielle and Hamblin (1981)
Mean productivity (MP)	(Ypi + Ysi)/2	Rosielle and Hamblin (1981)

Where, Ysi= Stress yield of a given genotype, Ypi=, none stress yield of a given genotype, PY=, Average of all genotypes under non-drought stress (Potential yield) SY=, Average yield of all genotypes under drought stress potential yield.

Data analyses

Analysis of variance was carried out using SAS version 9.0.Phenotypic and genotypic correlation coefficients were analyzed with Meta R statistical software. Principal component and cluster analysis were carried out using Minitab version16.

Results and Discussion

Analysis of variance

The combined analysis of variance for grain yield under stressed and non-stressed environments is given in Table 3. Moisture, genotypes and moisture levels by genotypes interaction were significant (P≤0.01). Moisture was the major source of variation on grain yield (56.7%) followed by genotypes (30.9%) and moisture by genotypes interactions (12.4%). This indicated that the intensity of drought stress was high enough to discriminate the genotypes and hence, the performance of genotypes on grain yield in each moisture environment varied in response to drought. This is in line with the finding of different authors with various durum wheat genotypes and environments (Habtamu et.al. 2016; Reza and Abdolvahab, 2017).

Table 3.Combined analysis on grain yield across 90 durum wheat genotypes tested under drought stress and non-drought stress environments.

Sources	DF	Mean squares	F-values
Moisture (M)	1	4184903.9	1771.3**
Genotypes (G)	89	22817.9	9.66**
Moisture x Genotype	89	10536.9	4.46**
Error	156	2362.7	

** Significant at 0.01

Reaction of durum wheat genotypes to drought

Drought stress reduced the yield of durum wheat genotypes and the genotypes respond differently to the effect of drought as revealed by drought indices (Appendix Table 1).According to TOL, genotypes 35, 29, 76, 77, 16, and 49 exhibited the most and 128, 8, 90, 74 and 50 were the least tolerance. For ATI the genotypes 35, 76, 77**,** 75 and 80, were the most and 58, 90, 128, 55, and 50 were the least tolerant genotypes. As per YSI, genotypes 76, 35, 77, 1 and 16were the most and 128,8,66,121 and 135 the least tolerant genotypes. Stress tolerance indices (STI) showed that genotypes 30, 55, 56, 58, 15 and 31 were the most, whereas genotypes 75, 64, 111, 22, and 35 the least tolerant genotypes. For Geometric mean productivity (GMP) genotypes 30, 55, 15, 58, 97, and 31 were the most and 75, 64, 111, 22, and 135 the least tolerant genotypes. Based on mean productivity (MP), genotypes 30, 15, 55, 58, 15, and 31 were found to be tolerant and the genotypes 75, 64,111, 22, and 35 were categorized as the least tolerant ones. Relative efficiency indices (REI) categorized the genotypes 75, 64,111, 22, and 135 as the most tolerant whereas genotypes 55,58,30,31 and 15 as the least tolerant ones. In Stress Susceptibility Index (SSI), the tolerant genotypes were genotypes 76, 35, 77, 1 and 16 whereas 128, 8, 66,121, and 35 were considered as the least tolerant genotypes.

Correlation analysis

Phenotypic and genotypic correlation coefficients of grain yield under drought stress, non-stress and drought tolerant indices are given in Table 4. The phenotypic and genotypic correlation coefficient of grain yield under drought stress in respective order were positive and strong with drought resistance indices (rp=0.92, rg= 0.94); relative efficiency indices (rp=0.90, rg=0.92); geometric mean productivity (rp=0.91, rg=0.94) and mean productivity (0.79, rg=0.85). The high correlation value of grain yield under drought with DRI, REI and GMP suggested that these indices could be used as drought tolerant selection traits and would able to discriminate drought tolerant genotypes. Hu et. al (2007) presented that high correlation of drought resistance indices with grain yield was an indication of drought tolerance. In contrast, grain yield under drought associated negatively with stress susceptibility indices (rp= -0.63, rg= -0.67) and tolerance (rp= -0.24, rg= -0.21). Similarly, yield under non drought stress was positive and strong with Abiotic tolerance indices (rp=0.94, rg=0.96); mean productivity (rp=0.91, rg=0.92); geometric mean productivity (rp=0.79, rg=0.81); and relative efficiency indices (rp=0.78, rg=0.82) but negatively correlated with yield stability indices (rp= -0.35, rg= -0.21). The existence of strong and positive association of grain yield under non-stress with abiotic tolerance

indicates that these indices would be more suitable to identify genotypes that can be utilized to drought free environments than the other indices. This result was supported by Farshadfar et.al. (2012) and Tauqeer et al (2013) that ATI had the highest positive phenotypic and genotypic correlation coefficients with grain yield under non-drought stress. It was also noted that MP, GMP and REI had positive and strong association with grain yield of both drought stress and non-stress conditions.

Positive and strong correlations were observed among each pair of Tolerance and SSI with phenotypic and genotypic correlation coefficients of (0.86 and 0.84); Geometric mean productivity had positive and strong phenotypic and genotypic correlations coefficient values of (rp=0.97, rg=0.98 and rp=0.99, rg=0.99) with Mean productivity and relative efficiency indices respectively. In contrast, Geometric mean productivity and Tolerance were correlated negatively with stress susceptibility indices (rp= -0.26, rg= -0.37) and Mean relative performance (rp= -0.86, rg= -0.84) respectively (Table 4).

Principal Component Analysis

Two principal components (PCs) with eigenvalues of 6.03 and 8.8 explained a cumulative of about 98.7% of the total phenotypic variability observed among the durum wheat genotypes (Table 5). PC1 alone explained 58 % of the total variation with high loading due to grain yield in the stress (Ys), drought resistance index (DRI) yield index (YI), yield stability index (YSI) stress. The genotypes which have a high value of first component (PC1) are expected to have a high yield under drought stress condition. Assefa et. al. (2019) presented YI and YSI as principal indices to identify superior genotypes under drought using 256 bread wheat genotypes. The correlation matrix of this study also revealed that these indices were highly correlated with grain yield under stressed condition. This result was supported by Guilherme et. al (2021) and Ayad et. al. (2021) that DRI significantly correlated with grain

Table 4. Phenotypic (Rp= above Diagonal) and genotypic (Rg= below Diagonal) correlation coefficients for grain yield under drought stress, non-drought stress and drought tolerant indices for 90 durum wheat genotypes tested at Debre-Zeit sandy clay soil in 2017 dry season.

Traits	GYNS	GYST	TOL	MP	SSI	GMP	YSI	REI	YI	DRI	ATI	SSPI
Grain yield non-stress		0.47	0.75	0.91	0.35	0.79	-0.35	0.78	0.47	0.12	0.94	0.75
Grain yield stress	0.56		-0.24	0.79	-0.63	0.91	0.63	0.90	1.00	0.92	0.21	-0.24
Tolerance	0.69	-0.21		0.40	0.86	0.18	-0.86	0.18	-0.24	-0.56	0.88	1.00
Mean Productivity	0.92	0.85	0.34		-0.06	0.97	0.06	0.96	0.79	0.51	0.75	0.40
Stress susceptibility indices	0.21	-0.67	0.84	-0.18		-0.26	-1.00	-0.25	-0.63	-0.86	0.55	0.86
Geometric mean	0.81	0.94	0.14	0.98	-0.37		0.26	0.99	0.91	0.67	0.59	0.18
Yield stability indices	-0.21	0.67	-0.84	0.18	-1.00	0.37		0.25	0.63	0.86	-0.55	-0.86
Relative Efficiency Indices	0.82	0.92	0.15	0.97	-0.35	0.99	0.35		0.90	0.67	0.60	0.18
Yield indices	0.56	1.00	-0.21	0.85	-0.67	0.94	0.67	0.92		0.92	0.21	-0.24
Drought Resistance Indices	0.28	0.94	-0.50	0.63	-0.88	0.77	0.88	0.75	0.94		-0.16	-0.56
Abiotic Tolerance Indices	0.96	0.34	0.84	0.78	0.44	0.64	-0.44	0.67	0.34	0.03		0.88
Stress susceptibility percentage index	0.69	-0.21	1.00	0.34	0.84	0.14	-0.84	0.15	-0.21	-0.50	0.84	

yield under drought stress**.** Similarly, the proportion of the total phenotypic variance of the genotypes accounted for by the second PC was 40.2 percent. The major trait included in the second PC was grain yield under non-drought, mean productivity, SSPI, STI, GMP andATI (Table5). Principal component analysis showed the relationship of different indices with grain yield under two moisture conditions. When all drought tolerant indices and grain yield under both conditions were considered as variables, three groups were formed (Figure 1). In the first group, grain yield under non-stress including all drought indices were categorized in the second PC except tolerance and SSPI. The second group comprised of SSI, drought intensity, tolerance and SSPI. In the third group traits included were similar to that of the first principal component. Hence, the use of these indices would enable to identify suitable genotypes for the respective moisture environments

Table 5. Eigenvectors and eigenvalues of the two principal components for 15 traits of 90 durum wheat genotypes.

Traits	PC1	PC2
Grain yield non-stress	0.020	-0.412
Grain yield stress	0.344	-0.172
Tolerance	-0.237	-0.319
Mean productivity	0.178	-0.365
Stress susceptibility index	-0.342	-0.166
Geometric mean productivity	0.246	-0.313
Yield stability index	0.342	0.166
Relative Efficiency Indices	0.242	-0.312
Yield Index	0.344	-0.172
Drought Resistance Index	0.374	-0.024
SSPI	-0.237	-0.319
Reduction percentage	-0.345	-0.160
Abiotic Tolerance Index	-0.081	-0.396
Eigenvalue	6.9596	5.8274
Proportion	0.535	0.448
Cumulative	0.535	0.984

Cluster Analysis

The cluster analysis resulted in the clustering of 92 durum wheat genotypes into five major groups' comprising 2-29 genotypes (Table 6 and Figure 2). The numbers of genotypes categorized were 26, 29, 28, 7 and 2 in cluster 1, cluster 2, cluster 3, cluster 4 and 5 respectively. Cluster five included only G-58 and G-90 which are characterized by the highest grain yield under non-drought stress and above-average grain yield under low drought stress conditions with moderate yield stability and considered as both drought tolerant with high yield potential.

The cluster analysis using mean values of 92 durum wheat genotypes generated five cluster groups (Table 6). Tauqeer et al (2013) evaluated 46 bread wheat genotypes for drought tolerance and found only two clusters. The relatively large number of cluster could be due to the variability of genotypes in response to drought

stress as well as different sources of origin while the genotypes found in the same group indicates the genetic affinity of the genotypes to respond to drought stress similarly than the genotypes in different group (Figure 2). The genotypes maintained under different groups had specific characters and it may give desirable genetic recombinants in developing drought tolerant varieties if they are used in hybridization.

The dendrogram presented in Figure .1 also showed similar result and trend with the correlation analysis and it classified the various indices in to three major categories such that YI, DRI and YSI were grouped with grain yield under stress. Similarly, grain yield under drought was also categorized with Abiotic tolerance indices indicating that the possibility of using alternative indices for identification of drought tolerant genotypes.

Table 6. Cluster numbers and lines grouped in each cluster for the 92 durum wheat genotypes tested at Debre-Zeit sandy clay soil in 2017 dry season.

Cluster no	No of genotypes	Genotypes	%
I	26	G-136, G-94,G-113,G-91,G-125,G-30,G-142,G-132, G-115, G-53,G-84,G-110,G-85,G-61,G-9,G-69,G-112,G-134,G-120,G-79,G-123,G-25,G-59,G-43,G-60 and G-37	28.3
II	29	G-121, G-26, G-127,G-63,G-137,G-97,G-89,G-104,G-98,G-135,G-133,G-103,G-105,G-18,G-39,G-31,G-66,G-33,G-144,G-32,G-102,G-17,G-140,G-13,G-3,G-36,G-4,G-138 and G-5	31.5
III	28	G-1,G-126,G-86,G-118,G-49,G-78,G-52,G-119,G-114,G-141,G-14,G-81,G-16,G-29,G-117,G-64,G-22,G-93,G-34,G-57,G-80,G-75,G-77, G-116,G-35,G-76,G-111, and G-71	30.43
IV	7	G-55,G-128,G-44,G-74,G-8,G-50 and G-15	7.6
V	2	G-58 and G-90	2.17
Total	92		

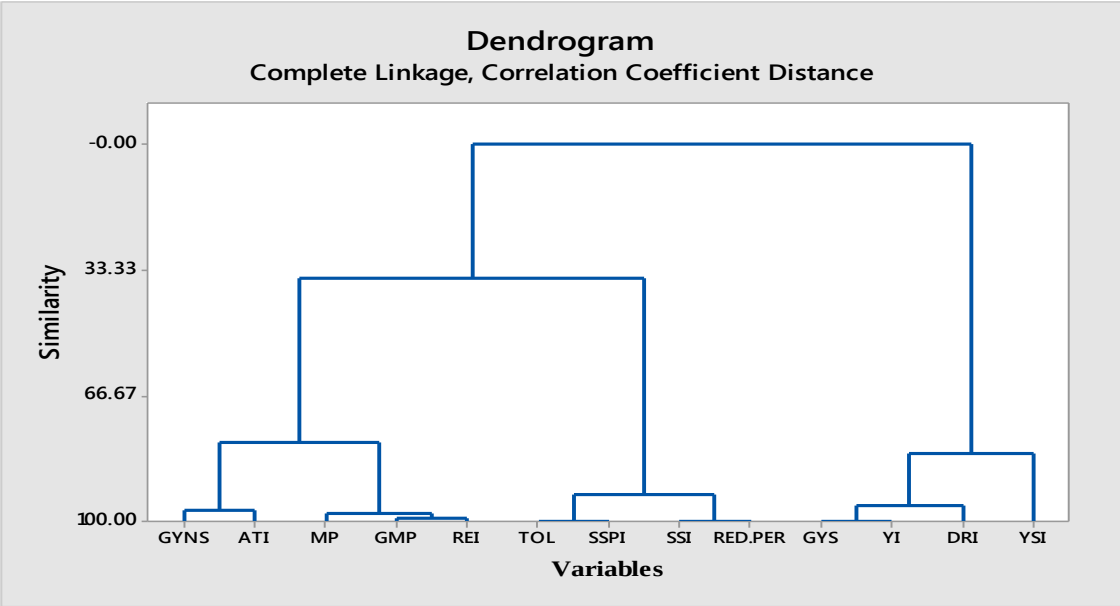

Figure . 1. Dendrogram of different drought screening indices on durum wheat

GYNS=Grain yield under no drought stress; GYS= Grain yield under low drought stress; ATI=Abiotic tolerance indices; MP=Mean productivity; GMP=Geometric mean productivity; REI=Relative efficiency index; TOL=Tolerance; SSPI=Stress susceptibility percentage index; SSI=Stress susceptibility index; RED.PER=Reduction percentage; YI=Yield index; DRI=Drought response index; YSI=Yield stability index

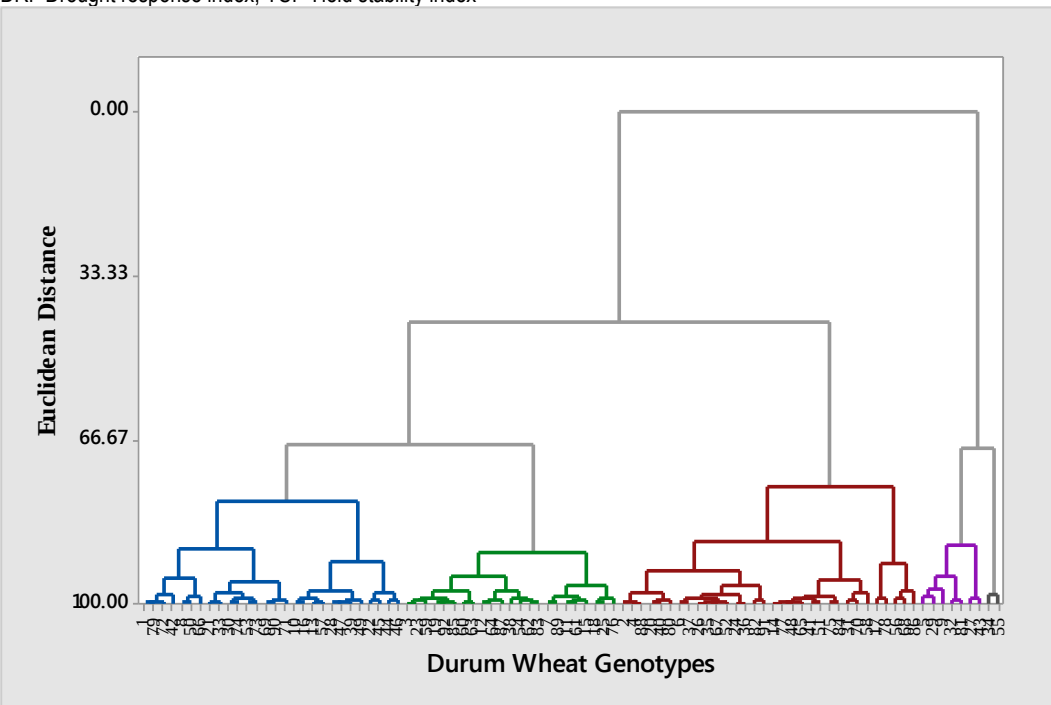

Figure . 2.Dendrogram categorizing durum wheat genotypes into five clusters

Conclusion

The results of the study based on the correlation matrices; cluster and principal analysis YI YSI and DRI were highly correlated to each other and to grain yield in stressed condition and could be used to identify drought tolerant genotypes. In contrast, ATI and MP had strong positive association with grain yield under non drought. MP, GMP, and REI associated with both grain yield under both drought and non-drought condition probably is the best indices for identification genotypes suitable for both environments. The durum breeding lines G-16, G-76, G-31, and G-119, G-63 and G-30 with better yield potential under drought stress with high drought resistant indices (DRI) and yield stability index could be used to identify drought tolerant genotypes.

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Appendix 1.Drought tolerance indices for durum wheat genotypes

GEN	YNS	YST	SSI	RDI	TOL	MP	STI	GMP	YI	YSI	ATI	DRI	SSPI	DIN
1	444.35	278.50	0.68	1.40	165.85	361.43	0.71	351.78	1.49	0.63	26117.19	0.93	19.82	0.37
2	493.20	226.00	0.98	1.02	267.20	359.60	0.64	333.86	1.21	0.46	39933.55	0.55	31.93	0.54
3	449.25	226.00	0.90	1.12	223.25	337.63	0.58	318.64	1.21	0.50	31843.84	0.61	26.68	0.50
4	369.05	135.60	1.15	0.82	233.45	252.33	0.29	223.70	0.72	0.37	23377.71	0.27	27.90	0.63
5	425.00	167.00	1.10	0.88	258.00	296.00	0.41	266.41	0.89	0.39	30768.63	0.35	30.83	0.61
6	252.00	168.00	0.60	1.49	84.00	210.00	0.24	205.76	0.90	0.67	7736.95	0.60	10.04	0.33
7	404.60	132.00	1.22	0.73	272.60	268.30	0.31	231.10	0.70	0.33	28200.80	0.23	32.58	0.67
8	653.40	139.55	1.42	0.48	513.85	396.48	0.52	301.96	0.75	0.21	69458.69	0.16	61.41	0.79
9	483.15	149.85	1.25	0.69	333.30	316.50	0.41	269.07	0.80	0.31	40145.83	0.25	39.83	0.69
10	423.95	234.00	0.81	1.23	189.95	328.98	0.57	314.97	1.25	0.55	26781.83	0.69	22.70	0.45
11	301.25	144.50	0.94	1.07	156.75	222.88	0.25	208.64	0.77	0.48	14639.99	0.37	18.73	0.52
13	451.45	260.85	0.76	1.29	190.60	356.15	0.67	343.16	1.39	0.58	29279.18	0.80	22.78	0.42
14	320.95	196.00	0.70	1.36	124.95	258.48	0.36	250.81	1.05	0.61	14028.74	0.64	14.93	0.39
15	671.05	256.00	1.12	0.85	415.05	463.53	0.98	414.47	1.37	0.38	77007.60	0.52	49.60	0.62
16	374.40	305.50	0.33	1.82	68.90	339.95	0.65	338.20	1.63	0.82	10431.06	1.33	8.23	0.18
17	425.35	186.00	1.02	0.98	239.35	305.68	0.45	281.27	0.99	0.44	30136.92	0.43	28.60	0.56
18	472.85	254.50	0.84	1.20	218.35	363.68	0.69	346.90	1.36	0.54	33907.40	0.73	26.09	0.46
19	157.50	68.20	1.03	0.97	89.30	112.85	0.06	103.64	0.36	0.43	4143.04	0.16	10.67	0.57
20	235.25	42.00	1.49	0.40	193.25	138.63	0.06	99.40	0.22	0.18	8598.94	0.04	23.09	0.82
21	389.55	240.00	0.70	1.38	149.55	314.78	0.53	305.76	1.28	0.62	20469.60	0.79	17.87	0.38
22	276.65	153.50	0.81	1.24	123.15	215.08	0.24	206.07	0.82	0.55	11360.30	0.45	14.72	0.45
23	415.40	94.00	1.40	0.51	321.40	254.70	0.22	197.60	0.50	0.23	28430.12	0.11	38.41	0.77
24	220.30	96.00	1.02	0.97	124.30	158.15	0.12	145.43	0.51	0.44	8091.89	0.22	14.85	0.56
25	667.00	209.00	1.24	0.70	458.00	438.00	0.80	373.37	1.12	0.31	76548.60	0.35	54.73	0.69
26	477.75	294.50	0.69	1.38	183.25	386.13	0.80	375.10	1.57	0.62	30769.66	0.97	21.90	0.38
27	486.00	298.00	0.70	1.37	188.00	392.00	0.83	380.56	1.59	0.61	32027.26	0.98	22.47	0.39
28	112.95	65.00	0.77	1.29	47.95	88.98	0.04	85.68	0.35	0.58	1839.18	0.20	5.73	0.42
29	374.35	303.00	0.35	1.81	71.35	338.68	0.65	336.79	1.62	0.81	10756.97	1.31	8.53	0.19
30	670.60	384.50	0.77	1.28	286.10	527.55	1.47	507.79	2.05	0.57	65032.96	1.18	34.19	0.43
31	563.65	334.50	0.74	1.33	229.15	449.08	1.08	434.21	1.79	0.59	44540.84	1.06	27.38	0.41
32	454.15	263.00	0.76	1.29	191.15	358.58	0.68	345.60	1.40	0.58	29572.47	0.81	22.84	0.42

33	483.10	279.60	0.76	1.29	203.50	381.35	0.77	367.53	1.49	0.58	33480.14	0.86	24.32	0.42
34	382.95	240.00	0.68	1.40	142.95	311.48	0.52	303.16	1.28	0.63	19399.77	0.80	17.08	0.37
35	237.85	174.00	0.49	1.63	63.85	205.93	0.24	203.44	0.93	0.73	5814.64	0.68	7.63	0.27
36	373.10	122.00	1.22	0.73	251.10	247.55	0.26	213.35	0.65	0.33	23981.42	0.21	30.01	0.67
37	505.00	198.50	1.10	0.88	306.50	351.75	0.57	316.61	1.06	0.39	43440.23	0.42	36.63	0.61
38	418.50	223.50	0.84	1.19	195.00	321.00	0.53	305.83	1.19	0.53	26696.65	0.64	23.30	0.47
39	469.05	212.50	0.99	1.01	256.55	340.78	0.57	315.71	1.13	0.45	36257.41	0.51	30.66	0.55
40	581.50	66.00	1.60	0.25	515.50	323.75	0.22	195.91	0.35	0.11	45207.58	0.04	61.60	0.89
41	219.45	156.00	0.52	1.59	63.45	187.73	0.20	185.02	0.83	0.71	5255.30	0.59	7.58	0.29
42	559.00	105.00	1.47	0.42	454.00	332.00	0.34	242.27	0.56	0.19	49237.06	0.11	54.25	0.81
43	463.60	147.00	1.24	0.71	316.60	305.30	0.39	261.05	0.78	0.32	36997.89	0.25	37.83	0.68
44	526.35	269.65	0.88	1.14	256.70	398.00	0.81	376.74	1.44	0.51	43291.15	0.74	30.68	0.49
45	306.80	65.00	1.43	0.47	241.80	185.90	0.11	141.22	0.35	0.21	15285.38	0.07	28.90	0.79
46	465.50	79.00	1.50	0.38	386.50	272.25	0.21	191.77	0.42	0.17	33178.64	0.07	46.19	0.83
47	409.10	89.50	1.41	0.49	319.60	249.30	0.21	191.35	0.48	0.22	27375.92	0.10	38.19	0.78
48	264.65	173.25	0.63	1.46	91.40	218.95	0.26	214.13	0.92	0.65	8761.02	0.61	10.92	0.35
49	340.70	246.50	0.50	1.62	94.20	293.60	0.48	289.80	1.32	0.72	12220.28	0.95	11.26	0.28
50	497.40	205.55	1.06	0.92	291.85	351.48	0.58	319.75	1.10	0.41	41774.10	0.45	34.88	0.59
51	491.75	138.50	1.30	0.63	353.25	315.13	0.39	260.97	0.74	0.28	41268.15	0.21	42.21	0.72
52	377.60	244.00	0.64	1.44	133.60	310.80	0.53	303.54	1.30	0.65	18153.20	0.84	15.97	0.35
53	480.85	206.00	1.03	0.96	274.85	343.43	0.57	314.73	1.10	0.43	38723.08	0.47	32.84	0.57
54	236.15	76.00	1.23	0.72	160.15	156.08	0.10	133.97	0.41	0.32	9604.25	0.13	19.14	0.68
55	639.30	366.00	0.77	1.28	273.30	502.65	1.34	483.72	1.95	0.57	59179.09	1.12	32.66	0.43
56	699.35	291.50	1.06	0.93	407.85	495.43	1.16	451.51	1.56	0.42	82433.31	0.65	48.74	0.58
57	389.15	200.50	0.88	1.15	188.65	294.83	0.45	279.33	1.07	0.52	23588.93	0.55	22.54	0.48
58	638.90	316.00	0.91	1.10	322.90	477.45	1.15	449.32	1.69	0.49	64947.70	0.83	38.59	0.51
59	464.95	195.50	1.05	0.94	269.45	330.23	0.52	301.49	1.04	0.42	36365.57	0.44	32.20	0.58
60	502.80	195.50	1.11	0.87	307.30	349.15	0.56	313.52	1.04	0.39	43128.99	0.41	36.72	0.61
61	509.10	222.50	1.02	0.98	286.60	365.80	0.65	336.56	1.19	0.44	43179.58	0.52	34.25	0.56
62	233.60	86.50	1.14	0.83	147.10	160.05	0.12	142.15	0.46	0.37	9360.37	0.17	17.58	0.63
63	329.00	354.50	-0,14	2.41	-25.50	341.75	0.67	341.51	1.89	1.08	-3898.36	2.04	-3.05	-0.08
64	253.50	141.00	0.80	1.24	112.50	197.25	0.20	189.06	0.75	0.56	9521.09	0.42	13.44	0.44

65	245.75	126.00	0.88	1.15	119.75	185.88	0.18	175.97	0.67	0.51	9432.86	0.34	14.31	0.49
66	447.90	106.00	1.38	0.53	341.90	276.95	0.27	217.89	0.57	0.24	33348.65	0.13	40.86	0.76
67	311.10	31.50	1.63	0.23	279.60	171.30	0.06	98.99	0.17	0.10	12390.20	0.02	33.41	0.90
68	410.85	194.00	0.96	1.05	216.85	302.43	0.46	282.32	1.04	0.47	27405.50	0.49	25.91	0.53
69	483.75	186.00	1.11	0.86	297.75	334.88	0.51	299.96	0.99	0.38	39981.07	0.38	35.58	0.62
70	270.25	164.00	0.71	1.36	106.25	217.13	0.25	210.53	0.88	0.61	10013.12	0.53	12.70	0.39
71	327.00	201.00	0.70	1.37	126.00	264.00	0.38	256.37	1.07	0.61	14460.33	0.66	15.06	0.39
72	269.65	184.30	0.57	1.53	85.35	226.98	0.28	222.93	0.98	0.68	8517.30	0.67	10.20	0.32
73	371.45	158.50	1.04	0.95	212.95	264.98	0.34	242.64	0.85	0.43	23130.14	0.36	25.45	0.57
74	604.50	200.50	1.21	0.74	404.00	402.50	0.69	348.14	1.07	0.33	62961.12	0.36	48.28	0.67
75	206.65	129.95	0.67	1.40	76.70	168.30	0.15	163.87	0.69	0.63	5626.48	0.44	9.17	0.37
76	313.90	281.00	0.19	2.00	32.90	297.45	0.50	296.99	1.50	0.90	4374.02	1.34	3.93	0.10
77	283.25	240.50	0.27	1.90	42.75	261.88	0.39	261.00	1.28	0.85	4994.76	1.09	5.11	0.15
78	385.50	238.05	0.69	1.38	147.45	311.78	0.52	302.93	1.27	0.62	19995.25	0.78	17.62	0.38
79	427.05	222.50	0.87	1.16	204.55	324.78	0.54	308.25	1.19	0.52	28225.37	0.62	24.44	0.48
80	295.15	203.00	0.57	1.54	92.15	249.08	0.34	244.78	1.08	0.69	10097.19	0.75	11.01	0.31
81	326.65	231.00	0.53	1.58	95.65	278.83	0.43	274.69	1.23	0.71	11761.64	0.87	11.43	0.29
82	297.05	131.00	1.01	0.99	166.05	214.03	0.22	197.27	0.70	0.44	14663.07	0.31	19.84	0.56
83	420.50	190.50	0.99	1.01	230.00	305.50	0.46	283.03	1.02	0.45	29140.30	0.46	27.49	0.55
84	514.20	203.33	1.09	0.88	310.87	358.77	0.60	323.35	1.09	0.40	44996.65	0.43	37.15	0.60
85	401.50	171.00	1.04	0.95	230.50	286.25	0.39	262.02	0.91	0.43	27036.31	0.39	27.54	0.57
86	346.60	196.55	0.78	1.27	150.05	271.58	0.39	261.01	1.05	0.57	17531.64	0.60	17.93	0.43
87	440.05	306.00	0.55	1.55	134.05	373.03	0.77	366.95	1.63	0.70	22019.85	1.14	16.02	0.30
88	490.80	114.00	1.39	0.52	376.80	302.40	0.32	236.54	0.61	0.23	39898.02	0.14	45.03	0.77
89	387.05	167.50	1.03	0.97	219.55	277.28	0.37	254.62	0.89	0.43	25024.19	0.39	26.24	0.57

90	502.80	248.50	0.92	1.10	254.30	375.65	0.71	353.48	1.33	0.49	40238.60	0.66	30.39	0.51
91	572.85	332.50	0.76	1.30	240.35	452.68	1.09	436.43	1.78	0.58	46956.54	1.03	28.72	0.42
92	196.05	138.15	0.53	1.57	57.90	167.10	0.15	164.57	0.74	0.70	4265.53	0.52	6.92	0.30
93	232.70	214.50	0.14	2.06	18.20	223.60	0.29	223.41	1.15	0.92	1820.20	1.06	2.17	0.08
94	475.85	185.00	1.11	0.87	290.85	330.43	0.50	296.70	0.99	0.39	38630.09	0.38	34.76	0.61
95	431.20	130.80	1.26	0.68	300.40	281.00	0.32	237.49	0.70	0.30	31935.88	0.21	35.90	0.70
96	685.10	145.50	1.43	0.47	539.60	415.30	0.57	315.72	0.78	0.21	76263.40	0.16	64.48	0.79
97	505.10	278.00	0.81	1.23	227.10	391.55	0.80	374.72	1.48	0.55	38094.65	0.82	27.14	0.45
98	371.80	187.50	0.90	1.13	184.30	279.65	0.40	264.03	1.00	0.50	21782.93	0.50	22.02	0.50
99	374.35	90.50	1.37	0.54	283.85	232.43	0.19	184.06	0.48	0.24	23387.72	0.12	33.92	0.76
100	208.00	132.00	0.66	1.42	76.00	170.00	0.16	165.70	0.70	0.63	5637.26	0.45	9.08	0.37
101	323.15	71.50	1.41	0.49	251.65	197.33	0.13	152.00	0.38	0.22	17123.31	0.08	30.07	0.78
102	407.00	155.20	1.12	0.85	251.80	281.10	0.36	251.33	0.83	0.38	28329.21	0.32	30.09	0.62
103	369.60	199.00	0.84	1.20	170.60	284.30	0.42	271.20	1.06	0.54	20711.30	0.57	20.39	0.46
104	365.50	143.00	1.10	0.87	222.50	254.25	0.30	228.62	0.76	0.39	22770.76	0.30	26.59	0.61
105	395.80	119.00	1.27	0.67	276.80	257.40	0.27	217.03	0.64	0.30	26891.39	0.19	33.08	0.70
106	425.60	39.00	1.64	0.20	386.60	232.30	0.09	128.83	0.21	0.09	22296.19	0.02	46.20	0.91
107	317.45	131.00	1.06	0.92	186.45	224.23	0.24	203.93	0.70	0.41	17020.47	0.29	22.28	0.59
108	359.85	91.00	1.35	0.56	268.85	225.43	0.19	180.96	0.49	0.25	21778.46	0.12	32.13	0.75
109	401.75	61.00	1.54	0.34	340.75	231.38	0.14	156.55	0.33	0.15	23878.91	0.05	40.72	0.85
110	534.80	303.50	0.78	1.27	231.30	419.15	0.93	402.88	1.62	0.57	41714.44	0.92	27.64	0.43
111	278.50	132.50	0.95	1.06	146.00	205.50	0.21	192.10	0.71	0.48	12554.78	0.34	17.45	0.52
112	510.75	312.50	0.70	1.37	198.25	411.63	0.91	399.51	1.67	0.61	35455.05	1.02	23.69	0.39
113	528.85	164.00	1.25	0.69	364.85	346.43	0.50	294.50	0.88	0.31	48099.24	0.27	43.60	0.69
114	408.15	181.50	1.01	0.99	226.65	294.83	0.42	272.17	0.97	0.44	27614.66	0.43	27.08	0.56

115	516.80	265.00	0.88	1.15	251.80	390.90	0.78	370.07	1.41	0.51	41713.40	0.73	30.09	0.49
116	395.05	166.25	1.05	0.94	228.80	280.65	0.38	256.28	0.89	0.42	26248.14	0.37	27.34	0.58
117	324.00	160.50	0.91	1.11	163.50	242.25	0.30	228.04	0.86	0.50	16690.28	0.42	19.54	0.50
118	391.75	208.00	0.85	1.19	183.75	299.88	0.47	285.45	1.11	0.53	23480.06	0.59	21.96	0.47
119	472.70	374.00	0.38	1.77	98.70	423.35	1.01	420.46	2.00	0.79	18577.25	1.58	11.79	0.21
120	521.30	224.50	1.03	0.96	296.80	372.90	0.67	342.10	1.20	0.43	45451.86	0.52	35.47	0.57
121	405.30	118.50	1.28	0.65	286.80	261.90	0.27	219.15	0.63	0.29	28136.00	0.18	34.27	0.71
122	385.15	244.50	0.66	1.42	140.65	314.83	0.54	306.87	1.31	0.63	19321.01	0.83	16.81	0.37
123	527.40	223.00	1.04	0.94	304.40	375.20	0.67	342.94	1.19	0.42	46730.76	0.50	36.38	0.58
124	801.20	230.50	1.29	0.64	570.70	515.85	1.05	429.74	1.23	0.29	109786.65	0.35	68.20	0.71
125	561.10	210.50	1.13	0.84	350.60	385.80	0.67	343.67	1.12	0.38	53937.87	0.42	41.90	0.62
126	394.00	205.00	0.87	1.16	189.00	299.50	0.46	284.20	1.09	0.52	24044.88	0.57	22.59	0.48
127	456.90	207.50	0.99	1.01	249.40	332.20	0.54	307.91	1.11	0.45	34375.72	0.50	29.80	0.55
128	593.00	117.50	1.45	0.44	475.50	355.25	0.40	263.96	0.63	0.20	56186.57	0.12	56.82	0.80
129	294.65	118.00	1.09	0.89	176.65	206.33	0.20	186.46	0.63	0.40	14744.96	0.25	21.11	0.60
130	533.60	243.00	0.99	1.02	290.60	388.30	0.74	360.09	1.30	0.46	46842.75	0.59	34.73	0.54
131	515.95	148.50	1.29	0.64	367.45	332.23	0.44	276.80	0.79	0.29	45530.35	0.23	43.91	0.71
132	487.15	280.50	0.77	1.29	206.65	383.83	0.78	369.66	1.50	0.58	34195.50	0.86	24.69	0.42
133	310.00	162.50	0.86	1.17	147.50	236.25	0.29	224.44	0.87	0.52	14819.56	0.45	17.63	0.48
134	495.95	173.00	1.18	0.78	322.95	334.48	0.49	292.92	0.92	0.35	42346.06	0.32	38.59	0.65
135	376.90	112.50	1.27	0.67	264.40	244.70	0.24	205.92	0.60	0.30	24371.74	0.18	31.60	0.70
136	392.20	201.00	0.88	1.14	191.20	296.60	0.45	280.77	1.07	0.51	24031.20	0.55	22.85	0.49
137	487.30	282.85	0.76	1.30	204.45	385.08	0.79	371.26	1.51	0.58	33978.10	0.88	24.43	0.42
138	443.10	175.50	1.09	0.88	267.60	309.30	0.44	278.86	0.94	0.40	33404.98	0.37	31.98	0.60

139	433.30	42.50	1.63	0.22	390.80	237.90	0.11	135.70	0.23	0.10	23739.91	0.02	46.70	0.90
140	418.60	160.50	1.12	0.86	258.10	289.55	0.38	259.20	0.86	0.38	29947.52	0.33	30.84	0.62
141	359.65	144.00	1.09	0.89	215.65	251.83	0.30	227.57	0.77	0.40	21968.81	0.31	25.77	0.60
142	471.15	197.00	1.05	0.93	274.15	334.08	0.53	304.66	1.05	0.42	37388.38	0.44	32.76	0.58
143	506.45	82.00	1.52	0.36	424.45	294.23	0.24	203.79	0.44	0.16	38720.19	0.07	50.72	0.84
144	434.60	149.50	1.19	0.77	285.10	292.05	0.37	254.90	0.80	0.34	32531.07	0.27	34.07	0.66

Biochemical and Mineral Composition of Anchote (*Coccinia abyssinica* (Lam.) Cogn.) Accessions from Ethiopia

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Abstract

Anchote (Coccinia abyssinica (Lam.) Cogn.) is among few indigenous crops in Ethiopia that has a great potential to be a food and nutritional security and socioeconomically important crop, so far given lower attention in research and development in Ethiopian national research system considering it as a minor crop . In order to fill the knowledge gaps about the nutritional potentials of this crop, the present study was conducted to assess the biochemical composition and anti nutritional profile of 100 anchote accessions collected from western and southwestern parts of Ethiopia. Flour from storage roots of 100 anchote accessions were collected and the samples run in three replications. Data on 20 biochemical, nutritional and root flesh color traits were generated in laboratory and subjected to analysis. Analysis of variance indicated significant variation ($p \leq 0.01$) among the accessions for the 20 traits and root flesh color. The calcium contents ranged from 1.45 to 6.79 mg/g with a mean of 4.16mg/g. The ranges and means of iron (0.041to 1.12and0.15), zinc(0.02to 0.47and 0.06), dry matter(%) range and mean (7.71 to 13.73 and 11.79), crude fiber(0.2 to 8.07% and 3.55%), protein(7.21 to 15.93% and 11.81%), carbohydrate (66.56 to 78.14% and 72.13%) and total energy(3.24to 3.54kcal/g and 3.34kcal/g dry matter). The cluster analysis of biochemical traits and root flesh color showed the existence of six divergent groups. According to PCA analysis all the accessions were grouped into seven components based on root flesh color, and biochemical traits of which flesh color, Ca, K, Mg, S, P and Na contents were significant (eigenvalue > 1) and explained 78 % of the total variability. The total genetic variability explained was 84.30% and some traits were negatively correlated; root flesh color, Ca, B, Mn, Zn, crude protein, and crude fiber and the remaining mineral and biochemical compositions correlated positively. The variation exhibited in this experiment could be attributed to environmental and genetic factors. Owing to the potential of the plant nutritionally, further identification of its active compounds is recommended.

Keywords: Anchote, accession, root crop, biochemical, Ethiopia

Introduction

In many developing countries, food insecure people and subsistence farmers depend highly on root and tuber crops as food source and possibly principal source of nutrition, and cash income (Scott *et al*., 2000). For millions of people in the tropical humid regions of Africa root and tuber crops occupy a position of prestige among the staple foods (Lebot, 2009). Root and tuber crops provide an estimated average of 20% of the daily per capita calorie intake for the 640 million inhabitants of sub-Saharan Africa, where with the growing population there is increasing demand for these crops both for food and feed (Kenyon *et al*., 2006).

In Ethiopia, root and tuber crops have a great contribution as part of the traditional food system and income generation specifically in Southern, South Western and Western part of Ethiopia (Andargachew *et al*., 2011; Fantaw *et al*., 2014). Root and tuber crops are mainly used as food security crops during food shortage since they are drought-tolerant and high yielding (Wheatly, Scoot, and Wiersema, 1995).

Several million people rely on enset (*Ensete ventricosum)* as a staple or co-staple food; as many as 7 to 10 million (Alemayehu *et al* 2011; Pijls *et al*., 1995) and other minor root and tuber crops such as Anchote (*Coccinia abyssinica*), Taro (*Colocasia esculenta*), Yam (*Dioscorea alata*), Cassava (*Manihot esculenta*) and 'Oromo Dinich' (*Plectranthus edulis*) are also important in some areas, mainly in South and South Western Ethiopia (Abdissa 2000; FAO 2007). Although root and tuber crops are drought resistant and food security crops in drought prone areas, their potential as a human food and medicine has not yet been fully recognized and utilized (Gebremedhin, *et al*., 2008).

Anchote (*Coccinia abyssinica* (Lam.) Cogn.) is one of the tuber crops belongs to the *cucurbitaceae* family and *conccinia* genus having over 30 species, about eight of which are believed to occur in Ethiopia (Desta *et al*., 2011; Bekele *et al.*, 2013; Yambo and Feyissa, 2013; Yassin *et al.*, 2013). Despite its food and nutritional security and other functional potentials, anchote has been given lower attention in research and development, and not well developed and popularized (Habtamu *et al*., 2013).

Anchote [*Coccinea abyssinica* (Lam.) Cogn.] is an endemic root and tuber crop of Ethiopia cultivated for human consumption (Abera, 1995; Beruk and Fikre, 2015; Habtamu, 2014;Tilahun *et al*., 2014). Anchote is a vine like cucurbit with a high yield and short crop cycle used as an important dietary and medicinal plant, grown widely in the Western and South-Western parts of the Ethiopia, encompassing about

115 genera and 960 species better known for its tuberous root and tender leaves for food (Schaefer *et al*., 2009; Girma and Hailu 2007; Yassin *et al*., 2013 and Abera, 1995).

Studies indicate that Anchote has relatively higher crude protein, utilizable carbohydrate, crude fiber, energy, and ash content as compared to sweet potato, potato and cassava (Habtamu *et al*., 2013). The calcium content of Anchote is also reportedly high (Desta, 2011; Habtamu *et al*., 2013). Traditionally, Anchote is consumed to heal broken or fractured bones which could be attributed to its high calcium content (Abera, 1995; Habtamu and Kelbessa, 1997).

Anchote has a significant contribution for the cultural and social values of the Oromo's (the largest tribe in Ethiopia) since long ago (Abera, 1995; Desta *et al*., 2011; Daba *et al*., 2012). The plant has been grown over a wide range of environments for a long time, and its cultivation and utilization have been passed from generation to generation through oral tradition, with very little recorded information mainly by women farmers (Abera, 1995; Girma and Hailu, 2007). The unique characteristics of the plant is the edibility of its different parts such as its tuber, leaf, and fruit which makes the plant ideal as potential food security crop (Amare 1973; Endashaw 2007;Desta *et al*., 2011). The root yield ranges of anchote varies as reported by different authors; 42 - 76 t/ha(Daba *et al*., 2012), 10-20t/ha (Weyesa *et al*., 2008), 22.71 - 130.83t/ha (Desta *et al*., 2011)

The high prevalence of malnutrition and persistent food insecurity in Ethiopia is due to the highly selective and restricted food consumption habit of the population as well as less exposure to the important wild and indigenous food plants (Getachew, 2001). According to Dandena (2010), malnutrition in Ethiopia still exists due to poor dietary habit regardless of the favorable highly diversified agro-ecological conditions of the country, which are suitable for the production of various types of fruit and vegetables.

There are a number of studies on genotypes (Abreham, Tileye, and Kassahun, 2014; Tilahun *et al*., 2014) and agronomic characteristics (Girma and Hailu 2007; Daba *et al*., 2012; Folla *et al*., 2013; Yambo and Feyissa 2013; Yassin *et al*., 2013) of Anchote but limited studies on its nutritional value. The few existing studies indicate its potential for human nutrition and medicine (Desta *et al*., 2011; Habtamu *et al*., 2013; Habtamu, 2014; Habtamu and Kelbessa, 1997). For instance, the protein content of Anchote tuber ranges from 4.6-16.4% (Desta *et al*., 2011) which is high and with wide range compared to other root and tuber crops commonly consumed in Ethiopia, with protein values ranging from 1-2% (Gebremedhin *et al*., 2008). Hence, understanding of the nutritional importance of root crops particularly anchote has potentially far-reaching

implication in solving the nutritional need of children and women and for further research and development. Therefore, the objective of this study was to characterize genetic diversity in terms of biochemical and nutritional composition of anchote accessions for further breeding, conservation and utilization.

Materials and Methods

Description of Sampling Site

A total of 100 anchote accessions collected from major anchote growing areas of west and south western parts of Ethiopia; East and West Wollega, Horro Guduru Wollega, Jimma and Buno Bedelle zones of Oromia region having altitudes ranging from 1427 to 3025 m.a.s.l (Table 1 and Figure 1) and were planted at Debre Zeit Agricultural Research Center experimental field , located at 08°44'N latitude and longitude of 38°58' E with 1860 m.a.s.l. elevation, mean annual rainfall and temperature of 851mm and 19 ^{o}C, respectively. The major soil type in the area is vertisols and alfisols/mollisols and the experiment was conducted on vertisols in 2017 and 2018 under irrigated system.

Sampling and sample preparation

Five anchote tuber samples per accession were randomly selected per replications, replicated three times, were prepared for laboratory analysis. All laboratory procedures; washing, peeling, slicing to small pieces and mixing thoroughly in order to prepare 400g of each samples and put them in a paper bag and dried to a constant weight in a hot air oven (DHG-9055A, Memment, Germany) at 105 ^{o}C for 24 hours. The oven dried anchote tuber chips, were milled using an electric grinder to obtain fine powder of anchote flour. The flour was sieved through **in** 1 mm sieve, measured and packed into air tight plastic bag and stored in refrigerator until it **was** used for analysis. Mineral and proximate analysis was conducted at Horticoop Ethiopia (Horticultural) PLC, Soil, Plant and Water Analysis Laboratory and the National Veterinary Institute (NVI), at Bishoftu.

Table 1. List of 100 anchote accessions with areas of collection

Accessions	Zone*	Woreda	Kebele	Longitude	Latitude	Altitude
002/09	E.W	Sibu Sire	Burka Talo	9°01'49.00"N	36°50'52.04"E	1791
003/09	E.W	Wayu Tuka	Warra Babbo Migna	9°03'52.98"N	36°52'05.77"E	1889
004/09	E.W	Wayu Tuka	Warra Babbo Migna	9°03'37.54"N	36°52'34.88"E	1840
005/09	E.W	Wayu Tuka	Warra Babbo Migna	9°03'47.65"N	36°52'56.35"E	1877
008/09	E.W	Wayu Tuka	Kura Migna	9°02'15.02"N	36°51'10.74"E	1830
009/09	E.W	Chingi	Maja Ale	9°02'43.31"N	36°43'10.25"E	1823
010/09	E.W	Wayu Tuka	Gute	9°02'04.84"N	36°40'22.64"E	1884
011/09	E.W	Chingi	Gobu	9°02'55.33"N	36°43'18.10"E	1843
014/09	E.W	Chingi	Gordommo	9°03'06.28"N	36°42'10.24"E	1935
016/09	E.W	Chingi	Jarte/Birbo Gibbi	9°01'55.46"N	36°40'40.97"E	1857
024/09	E.W	Leka Dullecha	Horda Qawusa	9°01'47.65"N	36°25'39.68"E	2110
029/09	E.W	Leka Dullecha	Horda Qawusa	9°01'46.27"N	36°25'06.82"E	2103
032/09	E.W	Leka Dullecha	Horda Qawusa	9°02'01.91"N	36°25'20.10"E	2056
040/09	E.W	Leka Dullecha	Horda Qawusa	8°59'55.44"N	36°30'31.60"E	2209
041/09	E.W	Leka Dullecha	Horda Qawusa	8°59'21.87"N	36°30'25.81"E	2227
043/09	W.W	Gimbi	Jogir	9°10'10.87"N	35°46'31.44"E	1727
049/09	W.W	Gimbi	Chuta Goch	9°12'06.03"N	35°44'03.15"E	1870
050/09	W.W	Gimbi	Inango Dambali	9°10'08.88"N	35°41'45.15"E	1888
051/09	E.W	Digga	Demeksa	9°02'26.20"N	36°27'17.84"E	2199
053/09	W.W	Gimbi	Aba Sena	9°02'00.08"N	35°58'42.00"E	1637
056/09	W.W	Gimbi	Choli	9°12'55.57"N	35°49'27.30"E	1864
058/09	W.W	Gimbi	Lalisa yesus	9°24'43.28"N	35°35'50.16"E	1892
062/09	W.W	lalo asabi	Harojji Harowwa	9°27'57.57"N	35°32'26.10"E	1798
065/09	W.W	Mana Sibu Mandi	Gombo Kiltu Jale	9°50'50.05"N	35°04'29.09"E	1753
066/09	W.W	Mana Sibu Mandi	Gombo Kiltu Jale	9°50'45.38"N	35°03'35.58"E	1651
067/09	W.W	Mana Sibu Mandi	Gombo Kiltu Jale	9°50'16.38"N	35°02'38.49"E	1616
075/09	E.W	Gobbu Sayyo	Tibbe Hara	9°05'11.09"N	36°21'50.67"E	1441
080/09	E.W	Leka Dullecha	Shakko	8°57'26.16"N	36°32'30.67"E	1986
081/09	E.W	Nunnu Kumba	Kumba	8°46'10.08"N	36°37'51.14"E	2313
087/09	E.W	Leka Dullecha	Bal'o	8°57'53.82"N	36°27'57.20"E	2248
089/09	E.W	Sibu Sire	Home Baro	9°02'12.41"N	36°53'05.23"E	1842
090/09	E.W	Sibu Sire	Burka Talo	9°01'52.57"N	36°51'01.05"E	1800
091/09	E.W	Bonaya Boshe	Ejersa Gute	8°57'33.64"N	36°39'52.77"E	1759
092/09	E.W	Gudeya Bila	Gonka Ija	9°14'53.50"N	36°57'56.26"E	1943

093/09	E.W	Gudeya Bila	Gonka Ija	9°14'55.42"N	36°57'40.82"E	1934
094/09	E.W	Gudeya Bila	Gonka Ija	9°14'48.15"N	36°57'27.68"E	1949
095/09	E.W	Gudeya Bila	Kalala	9°15'49.79"N	36°59'41.92"E	1989
096/09	E.W	Gudeya Bila	Gonka Ija	9°14'41.02"N	37°00'26.13"E	1910
097/09	E.W	Gudeya Bila	Gonka Ija	9°15'01.93"N	37°00'31.92"E	1943
112/09	W.W	Gimbi	Kombo Mikael	9°05'36.96"N	35°48'32.01"E	1938
113/09	W.W	Gimbi	Kombo Mikael	9°05'20.43"N	35°48'38.52"E	1911
126/09	W.W	Gimbi	Garjo Bikilal	9°14'02.64"N	35°55'19.48"E	1729
131/09	W.W	Gimbi	Garjo Bikilal	9°14'34.03"N	35°54'11.09"E	1704
132/09	W.W	Gimbi	Garjo Bikilal	9°14'57.66"N	35°54'30.61"E	1661
134/09	W.W	Gimbi	Garjo Bikilal	9°12'46.20"N	35°54'39.48"E	1842
140/09	W.W	Gimbi	Garjo Bikilal	9°16'40.84"N	35°51'39.96"E	2052
148/09	W.W	Gimbi	Lalo Choli	9°14'13.95"N	35°48'38.07"E	1793
151/09	W.W	Gimbi	Lalo Choli	9°13'41.94"N	35°48'02.11"E	1838
153/09	W.W	Gimbi	Lalo Choli	9°13'02.91"N	35°48'13.15"E	1827
155/09	W.W	Gimbi	Lalo Choli	9°13'02.03"N	35°47'55.28"E	1814
156/09	W.W	Gimbi	Lalo Choli	9°13'29.23"N	35°47'57.03"E	1845
157/09	W.W	Gimbi	Lalo Choli	9°13'16.14"N	35°48'16.95"E	1846
158/09	W.W	Gimbi	Lalo Choli	9°13'40.52"N	35°48'15.06"E	1826
161/09	W.W	Gimbi	Lalo Choli	9°12'59.74"N	35°47'27.49"E	1811
169/09	W.W	Gimbi	Didisa Bikilal	9°16'07.72"N	35°44'03.88"E	1839
183/09	W.W	Gimbi	Didisa Bikilal	9°17'48.43"N	35°44'29.76"E	1766
206/09	W.W	Mana Sibu Mandi	Wajitu Mandi	9°48'07.68"N	35°04'40.67"E	1610
209/09	W.W	Mana Sibu Mandi	Wajitu Mandi 01	9°48'22.76"N	35°04'32.51"E	1610
211/09	W.W	Mana Sibu Mandi	Wajitu Kiltu Lubo	9°45'36.34"N	35°05'34.92"E	1622
214/09	W.W	Mana Sibu Mandi	Wajitu Kiltu Lubo	9°45'56.74"N	35°00'29.85"E	1543
218/09	W.W	Boji Dirmaji	Gumbo Boji	9°23'14.75"N	35°36'03.09"E	2004
219/09	W.W	Boji Dirmaji	Lata Bobine	9°23'23.60"N	35°35'57.02"E	2000
222/09	H.G.W	Horro	Doyyo Bariso	9°36'09.82"N	37°11'33.91"E	2399
223/09	H.G.W	Horro	Doyyo Bariso	9°36'12.85"N	37°11'33.27"E	2392
227/09	W.W	Boji Dirmaji	Lata Bobine	9°23'47.77"N	35°35'42.86"E	1964
256/09	Jimma	Dedo	Dedo	7°30'11.60"N	36°52'12.49"E	2294
262/09	E.W	Digga Leqa	Digga	9°02'25.58"N	36°29'03.58"E	2214
271/09	E.W	Gudeya Bila	Hena Jawaja	9°14'09.72"N	37°01'31.09"E	1883
280/09	W.W	Kiltu Kara	Dandi Gudi	9°32'04.39"N	35°22'30.93"E	1817
281/09	W.W	Kiltu Kara	Dandi Gudi	9°32'09.40"N	35°22'09.30"E	1793

286/09	W.W	Boji Dirmaji	Lata Bobine	9°24'20.62"N	35°36'18.70"E	1918
288/09	W.W	Najo	Humna Wakayyo	9°30'32.40"N	35°31'10.33"E	1901
290/09	W.W	Boji Dirmaji	Lata Bobine	9°27'13.65"N	35°33'15.48"E	1853
291/09	W.W	Boji Dirmaji	Gumbo Boji	9°28'42.70"N	35°32'21.80"E	1805
292/09	W.W	Najo	Humna Wakayyo	9°31'20.80"N	35°31'32.35"E	1914
296/09	W.W	Najo	Humna Wakayyo	9°31'09.60"N	35°31'28.18"E	1913
297/09	W.W	Najo	Humna Wakayyo	9°31'36.07"N	35°31'31.17"E	1890
300/09	W.W	Mana Sibu Mandi	Guyo Sachi	9°50'29.80"N	35°03'50.10"E	1655
302/09	E.W	Limmu	Sakata Kiltu Babbo	9°51'04.95"N	36°29'27.52"E	2181
304/09	E.W	Limmu	Sapera	9°51'49.78"N	36°31'30.58"E	2192
305/09	E.W	Limmu	Degem Silassie	9°51'46.82"N	36°30'46.08"E	2133
308/09	E.W	Limmu	Sapera	9°51'34.09"N	36°29'36.91"E	2115
309/09	E.W	Limmu	Muka Arba Dima	9°51'29.62"N	36°29'03.18"E	2149
313/09	E.W	Gida Ayana	Gute Gudina	9°53'13.79"N	36°37'13.93"E	2060
316/09	E.W	Gida Ayana	Gaba Jimata	9°53'07.17"N	36°39'51.96"E	2098
328/09	E.W	Leka Dullecha	Badh'o	8°55'28.47"N	36°35'39.29"E	1855
376/09	B.B	Didessa	Sasso	8°37'45.79"N	36°22'04.73"E	1459
377/09	B.B	Didessa	Sasso	8°37'46.82"N	36°22'02.86"E	1463
381/09	B.B	Didessa	Yembero	8°37'51.56"N	36°21'28.53"E	1427
385/09	H.G.W	Jimma Geneti	Bikila Nagaro	9°25'53.98"N	37°04'20.75"E	2945
388/09	H.G.W	Jimma Geneti	Gidami Dabsho	9°24'25.81"N	37°04'13.78"E	2703
393/09	E.W	Gudeya Bila	Lanfaji	9°18'10.48"N	37°02'19.92"E	2063
394/09	E.W	Gudeya Bila	Lanfaji	9°18'02.31"N	37°02'51.32"E	2060
397/09	E.W	Gudeya Bila	Walane Lemu	9°20'53.47"N	37°02'16.31"E	2289
400/09	E.W	Gudeya Bila	Walane Lemu	9°20'14.80"N	37°02'55.30"E	2238
402/09	E.W	Gudeya Bila	Walane Lemu	9°20'10.43"N	37°02'11.83"E	2252
406/09	E.W	Gudeya Bila	Walane Lemu	9°19'55.93"N	37°02'15.88"E	2216
407/09	H.G.W	Jimma Geneti	Gamo Nagaro	9°22'45.81"N	37°04'18.96"E	2509
413/09	E.W	Gudeya Bila	Gute Chacho	9°19'11.72"N	37°03'24.73"E	2237
416/09	H.G.W	Horro	Burkitu Oborra	9°27'08.97"N	37°03'43.07"E	3025

**E.W- East Wollega;W.W-West Wollega; H.G.W-Horro Guduru Wollega ;B.B-Buno Bedelle Zones*

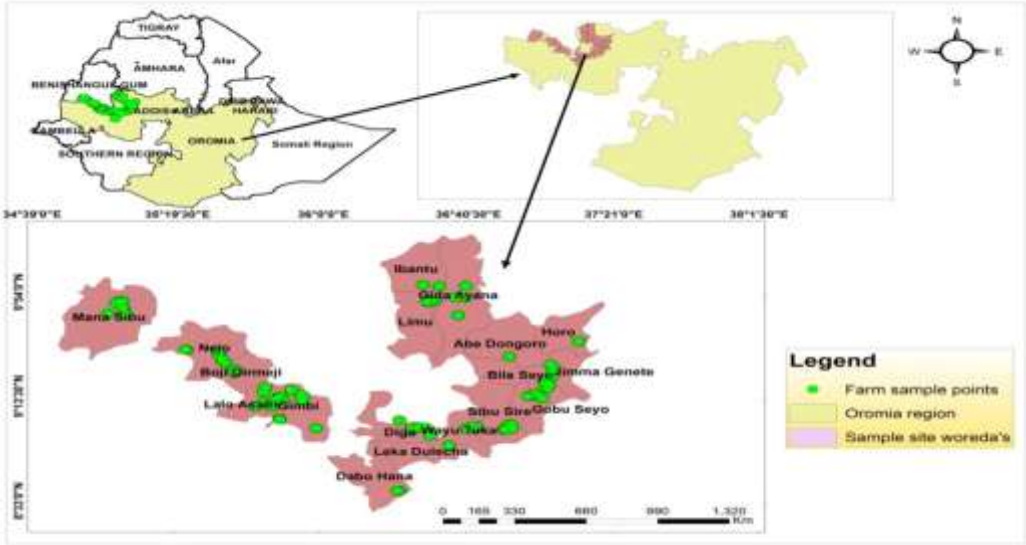

Figure 1. Anchote accessions collection areas used for biochemical and mineral analysis

Biochemical analysis

Analysis was carried out using the flour from the storage root of each 100 anchote accessions and the samples were run in three replications. The flour moisture, dry matter, organic matter, ash, crude fiber, crude fat, crude protein, carbohydrate and energy were determined accordingly with the standard methods of AOAC, 2000.

The flour moisture content was determined by the standard analytical method. Anchote flour samples (100 g) were weighed in aluminum dishes and oven dried at 65°C for 72 hours. The dried samples were cooled in a desiccator's room temperature and weighed. The flour moisture content was determined by loss of weight due to drying was converted to percent flour moisture content as follows: Moisture %=(weight of moisture evaporated/weight of flour sample) x 100. The dry matter content of tuber was calculated by taking a representative duplicate sample of about 100 g (W1) prepared by thoroughly mixing the sliced pieces of tuberous roots was oven dried at 65°C for 72 hours and weighed (W2) and the value was expressed in percentage(Cozzolino and Labandera, 2002; Egesi *et al*., 2003). The percentage dry matter content was calculated as: % Dry matter= (W2/W1) × 100 or % Dry matter=100 - % moisture content.

The ash content was determined following the instruction of AOAC(AOAC, 2000). Crucibles were rinsed and dried in hot air oven (SM 9053) maintained for 30 minutes at 105°C. These were cooled in desiccators and weighed. Five gram of

the sample was burnt on a heater inside a fume cupboard to get rid of the smoke. The samples were moved to preheated muffle furnace (SM 9080) maintained at 550°C until such a time when a light grey ash was noticed. The crucibles were cooled in desiccators and weighed. The ash content was calculated as:

$$\%\text{Ash} = \frac{(\textit{weight of crucible + Ash}) - \textit{weight of empty crucible}}{\textit{Ash weight of sample}} \times 100$$

The organic matter content was determined by subtract the percent ash from percent total dry matter and the value was expressed in percentage. The % organic matter content was calculated as:

Organic matter content (%) = % dry matter – % ash.

The amount of organic carbon is determined as;

$$\textbf{Organic carbon (g)} = \frac{\textbf{weight of ash}}{\textbf{Sample weight}}$$

Crude fiber of the sample was determined according to AOAC, 2000. Two gram of the sample was crushed with petroleum ether. The crushed sample was boiled in reflux for 30 minutes with 200 ml of a solution contain 1.25 g of H2SO4 per 100 ml of solution. The solution was then filtered through linen on a fluted funnel. Then the sample was washed with hot water, using a two-food muslin cloth to trap the particles, the washed sample was transferred quantitatively back to the flask and boiled again in 200 ml of 1.25 g of carbonate free NaOH per 100 ml for 30 minutes and washed before it transferred to a weighed Gooch crucible and dried in the oven at 105°C for three hours. After cooling in desiccators it was re-weighed. Then the percentage crude fiber was calculated as:

$$\%\ \text{CF} = \frac{(\textit{weight of sample + Crucible}) - (\textit{weight of crucible + Ash})}{\textit{weight of sample}} \times 100$$

The fat contents were determined by using fat extractor with automated control unit (FOSS Soxtec 2055) according to AOAC, 2000. The equipment has six extraction units with each unit accommodates the samples and aluminum cups for collection of the extracted fat. These units enable six samples to be analyzed at a time; within 75 minutes. Percentage of fat was considered as, the difference between weight of the pre-weighed cups and after extraction. One gram of the sample was weighed into the thimble and its mouth plugged with defatted cotton wool, after which it was inserted into the extraction unit. 18 ml of petroleum ether was dropped into each cup and maintained at 135°C aligning each cup with its corresponding thimble. The extraction and rinsing were done for 30 minutes each, after which the sample was aerated for 15 minutes and crude fat calculated as:

$$\%\ \text{Fat} = \frac{(W3-W2)}{W1} \times 100$$

Where: W1=weight of sample, W2= weight of empty cup and W3= weight of cup with the extracted oil.

The analysis of crude protein content was conducted with an aid of micro Kjedhal system in accordance with AOAC, 2000. A small quantity of the yam flour sample (1 g) was introduced into the digestion tube (Kjeltec 2200 FOSS) and, a catalyst (2 tablets of 5g K2SO4 and 5 mg of Se) and 12 ml of concentrated tetra oxo-sulphate VI acid (H_2SO_4) were added. The digestion was run for one hour at 42°C. Eighty (80 ml) and 40 ml of water and sodium hydroxide (NaOH) respectively were used in the distillation using 2200 FOSS distillation unit and the distillate was collected in 4% Boric acid. The percentage nitrogen (N) was calculated as:

$$\% N = \frac{(a-b)}{s} \times n \times 0.014 \times mcf$$

Where; a=ml of H2SO4 required for titration of sample, b=ml of H2SO4 required for titration of blank, S=air-dry sample weight in mg, n=normality of H2SO4 (0.1 N), 0.014= weight of nitrogen in g and mcf=moisture correction factor. Then the protein content of the sample was estimated by percent nitrogen multiplied protein coefficient (6.25). The protein content calculated as 'N' × 6.25 (Bressani, 1994 and Udosen, 1995).

Mineral composition analysis

The phosphorus content of each sample was determined by the dry ash extraction method following specific mineral element (AOAC, 1990). Five grams of the sample was burnt to ashes in a muffle furnace (SM9080) at 500°C. After complete ashing, the ash was diluted with 1% Hydrochloric (HCl) acid, then filtered into a 100 ml standard flask, and made up to the mark with deionized water. The solution was read with UV-visible spectrophotometer machine (model No: UV-1600, Shimadzu Corporation, Japan) for the determination of phosphorus in mg/100 g. The carbohydrate content of the sample was determined by estimation using arithmetic difference. The energy value was calculated by application of the thermal coefficients of Atwater and Rosa (Atwater and Rosa, 1899) with 4 calories for 1 g of carbohydrates; 4 calories for 1 g of protein and 9 calories for 1 g of crude fat. The available carbohydrate (CHO) and energy value were determined by using the formula as given below; CHO = [100 - (% moisture+% crude protein+% crude fat+crude fibre+% ash)].

Total energy (kcal)=[(% CHO × 4)+(% CP × 4)+(% CF × 9)]. Where; CHO, CP and CF are; carbohydrate, crude protein and crude fat, respectively (Hassan et al., 2008; Elinge et al., 2012; Tawheed and Monika, 2014).

Proximate Analysis

Tubers were peeled with a high-grade stainless steel handheld peeler, and were again washed with distilled water and dried using a paper towel before

they were sliced using a high-grade stainless steel knife. The peeled and washed roots were cut into pieces and oven dried at 105 °C for 24 hours. The dried samples were milled using a stainless steel mill and packed and stored at room temperature into sealed plastic bags for proximate and mineral analysis.

Proximate composition (in dry matter basis) of the oven-dried samples was conducted following the AOAC (2010) standard procedures at NVI and Horticoop. The micro-Kjeldahl procedure was used for the crude protein (N × 6.25) analysis following method 954.01, AOAC (2010). The moisture content (MC) was determined by the hot air oven method as described by 925.10, AOAC (2010). Fat content was determined using the Soxhlet extraction with hexane according to method number 2003.06, AOAC (2010). The method 923.03, AOAC (2010) was used to determine the total ash content. Method number 962.09, AOAC (2010), was used for crude fibre determination. Carbohydrate content was calculated by difference as shown: Total carbohydrate by difference = 100 – (water, protein, total lipid, ash in g/100 g). Standard energy conversion factors (protein 4 kcal/g, fat 9 kcal/g, and carbohydrate 4 kcal/g) were used to estimate energy (in kcal).

Mineral Analysis

Macro (Ca, Mg, P, K, Na, S) and micro (Cu, Co, Fe, Mn, Se, Zn) minerals analysis of anchote root were conducted at the Horticoop Ethiopia(Horticultural) PLC; Soil, Water and Plant Analysis Laboratory with the standard method, EPA (United States Environmental Protection Agency) 6010A of Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) (VARIAN VISTA RL CCD Simultaneous ICP-AES Spectrometer). Microwave digestion (100 g dry plant material using conc. HNO_3 and 30% H_2O_2) with wet ashing under pressure (for 75 min, 200 °C and 15 bar) was used to prepare samples prior to ICP-AES analysis. Mineral content was recorded in mg/kg of flour and later converted into mg/100 g of dry matter or fresh weight basis.

Data analysis

The data was subjected to analysis of variance (ANOVA), in three replications with Complete Random Design(CRD) using SAS statistical software package, SAS 9.3(32) (SAS Institute, 2000). The entire dataset was standardized by dividing each variable with its respective range, and was subjected to clustering based on Un-weighted Pair Group Method of Arithmetic mean (UPGMA) and cluster distance and principal component analysis (PCA) was analyzed to assess correlations between components and the parameters measured.

Results and Discussion

The result showed there were significant difference among the accessions on biochemical, mineral,

and root flesh color of Anchote in macro and micro minerals; calcium, potassium, magnesium, sulphur, potassium, sodium, boron, copper, iron, manganese, molbedinium, zinc, and other biochemical analysis; dry matter, moisture, ash, crude fiber, protein, fat, carbohydrate and total energy showed that the existence of sufficient genetic variability of these traits within anchote landraces from Ethiopia (Table 2). The variability among landraces also revealed wide chance of developing anchote varieties possessing desirable biochemical traits.

Crude protein: The range of protein content of anchote roots was 7.21 to 15.93% and a mean of 11.81 $\pm$ 2.19% which is higher than protein contents of other common edible roots such as sweet potato (1.4%), cassava (0.5%), yam (7.82%) (Mulualem *et al*., 2018) and taro (1.1%)(Bradbury and Holloway, 1988). According to Yenenesh *et al*., (2016), the protein content of the anchote tubers ranged from 5.82 to 13.72 % from 44 accessions collected from western parts of Ethiopia. On contrary, Habtamu *et al*., (1997), reported that peeled anchote root contains 3.9g of protein.

The crude protein content of anchote root in the present study was stuck between the range value (4.6- 16.4 %) reported by Desta et *al*., (2011), but higher than the values (3.00-3.90 %) reported by other scholars (EHNRI, 1997; Habtamu *et al*., 2013; Habtamu *et al*., 1997). The variations in genotype, geographical sources and agronomic practices could affect the protein content in root and tuber crops. The high crude protein content in anchote tubers are however comparable to those reported in yam (*Dioscorea alata*) (7.82 and 10.27%) (Mulualem *et al*., 2018; Ezeocha and Ojimelukwe, 2012); taro (*Colocasia esculenta*) (11.00%) (Temesgen and Negussie, 2015) but in contrary to Otegbayo *et al*., 2018 on yam collections from Nigeria (4.32%).

Crude fat: The crude fat content of anchote accessions was significantly influenced by the accessions and ranged from 0.01 to 2.25 % with the mean value of 0.59. Comparatively, this result was higher than the report of Habtamu *et al*., 1997 on anchote (0.12%) but comparable and in the range with fat contents of yam (0.32%), potato (0.09%), and cassava (0.03 to 0.05%),) (Mulualem *et al*., 2018; Montagnac *et al.*, 2009; Yirmaga, 2013).

Ash: The ash contents of anchote accessions were in the range of 3.82 to 6.98% with the mean value of 5.04%. This result is higher than ash contents of potato, guinea yam, and cocoyam (Montagnac *et al.*, 2009; Yirmaga, 2013; Ihediohanma *et al*., 2014; Kavitha and Parimalavalli, 2014). But it is comparable with enset (7.47 to 8.17%) (Mohammed *et al*., 2013). The ash content of the anchote accessions in this study (5.04%) is higher than yam (4.41 and 2.61%), Otegbayo *et al*., 2018 and Mulualem *et al*., 2018,

and 3.09% in cassava(Christopher *et al*., 2016).

The difference in ash contents among the accessions might be due to differences in the genotypes and collection areas' agro-ecology.

Carbohydrate: The carbohydrate contents of anchote accessions were in the range of 66.56 to 78.14% and with mean value of 72.13%. This result is comparable with carbohydrate contents of cocoyam (79.14-79.75%), but it is higher than that of sweet potato (25.74%), *enset* (32.75-35.53%), guinea yam (69.50%), yam (Discorrea *spp*.)(21.84%) and cassava (25.3-35.7%) (Montagnac *et al*., 2009; Yirmaga, 2013; Ihediohanma *et al*., 2014; Mulualem *et al*., 2018). In contrary, the mean carbohydrate content presented in this study is by far higher than the reported value (25.5%) by Habtamu *et al*., 2018 and lower than (80.52%) as reported by Yenenesh *et al*., 2016. This might be associated to higher moisture contents, which decrease the proportions of other proximate values, the genetic factor, maturity and management factors.

Energy: The gross energy of *anchote* accessions was in the range of 323.71 to 353.53 kcal /100g with mean value of 334.42 kcal. On contrary, the mean energy content presented in this study is by far higher than the reported value (117.50kcal) by Habtamu *et al*., 1997. On the other hand anchote's energy content is comparable with cereals such as corn (365kcal) and mung bean (342. kcal) (Montagnac *et al*., 2009; Blessing and Gregory, 2010).

Crude fiber: The crude fiber from this study was ranged from 0.20 to 8.07 % with the mean value of 3.55% which was higher than the reported value of 1.5% by Habtamu *et al*., 1997, 1.28% by Mulualem *et al*., 2018 and 0.68% in cassava(Christopher *et al*., 2016) but lower than 5.05% by Yenenesh *et al*., 2016. The crude fiber content of anchote accessions in this study is generally higher than yam(1.28%), potato (2.2%), carrot (2.8%), cassava(0.68%) and is comparable with other fibrous crops like kocho (*enset*) (3.37%), cassava (1.5%), and taro (3.9%) (Mulualem *et al*., 2018; Bradbury and Holloway, 1988; Yirmaga, 2013). The difference might be due to genotype differences and the maturity stage when they were harvested for analysis.

Moisture and Dry matter: The moisture and dry matter contents ranged from 7.71 to 13.73% and 13.73 to 7.70% with a mean of 11.52% and 11.79%, respectively. The mean dry matter content of 11.79% found in this study is the lowest with the value of 24.10% reported by Yenenesh *et al*.; (2016) might be due to the maturity stage of the roots and the genotype differences**.**

Mineral Elements Concentration in Anchote Tubers

The range and mean values of major mineral elements (Ca, P, K, Na and Mg) for 100 anchote root samples are

compiled in Table 2. The mean contents of Ca, P, K, Na and Mg in the roots were 416.15, 465.42, 1558.37, 71.1and 191.36mg/100g and the ranges were 144.62 - 679.89, 247.52-704.26, 999.83- 2732.02, 35.47-150.20, and 98.32-292.64 mg/100g on dry matter basis respectively. The mean value of trace elements (B, Fe, Zn, Co, Mo and Mn), were 1.72, 15.33, 5.74, 0.79, 0.72, and 0.95 mg/100g on dry basis. The macro mineral elements concentration in anchote roots in this study was by far better than Yenenesh *et al*., (2016) may due to the inclusion of limited number accessions in the study. On contrary, the mean Ca content presented in this study is comparable with the reported value, 327mg/100g by Habtamu *et al*., 1997 but the value of other mineral elements is by far higher may be due to genetic factors and the environmental conditions where the tubers were grown and the large number of accessions incorporated in this study. The calcium to phosphorous ratio is 0.89 to 1 and the potassium to sodium ratio is 22 to 1.

Correlation analysis was used to look into the patterns of variations of the traits associations and their relation patterns.

Table 2. Variance analysis of 20 mineral and biochemical traits of anchote

Qualitative character	Mean ± SE	Range	CV(%)	LSD(0.01)	P value	R^2
Calcium	416.15 ± 101.38	144.62- 679.89	5.65	498.18	<.0001	96.37
Potassium	1558.37 ± 292.85	999.83 - 2732.02	1.23	406.70	<.0001	99.72
Magnesium	191.36 ± 36.31	98.32 - 292.64	1.06	42.90	<.0001	99.79
Sulfur	119.79 ± 31.92	66.51 - 231.67	2.15	53.81	<.0001	99.23
Phosphorus	465.42 ± 75.11	247.52 -704.26	1.21	119.89	<.0001	99.59
Sodium	71.10 ± 20.46	35.47 - 150.20	0.87	13.21	<.0001	99.94
Boron	1.72 ± 0.71	0.43- 5.74	10.74	3.91	<.0001	95.46
Copper	0.79 ± 0.67	0.09 - 1.51	8.11	1.29	<.0001	95.89
Iron	15.33 ± 13.17	4.13 - 111.52	2.62	8.50	<.0001	99.94
Manganese	0.95 ± 0.33	0.28 - 1.99	8.52	1.71	<.0001	96.11
Molybdenum	0.72 ± 0.48	0.04 - 1.68	12.29	1.81	<.0001	96.50
Zinc	5.74 ± 0.52	1.57 - 46.73	3.1	3.76	<.0001	99.92
Dry matter	88.21 ± 4.60	86.27 - 92.30	0.14	0.25	<.0001	99.40
Moisture	11.52 ± 1.34	7.71 - 13.73	0.03	0.01	<.0001	99.99
Ash	5.04 ± 0.61	3.82 - 6.98	0.2	0.02	<.0001	99.98
Crude fiber	3.55 ± 1.58	0.20 - 8.07	0.03	0.002	<.0001	1.00
Protein	11.81 ± 2.19	7.21 - 15.93	0.06	0.02	<.0001	99.99
Fat	0.59 ± 4.24	0.01 - 2.25	0.29	0.002	<.0001	99.99
Carbohydrate	72.13 ± 15.50	66.56 - 78.14	0.08	0.12	<.0001	99.97
Energy	334.42 ± 17.32	323.71 - 353.53	0.05	0.38	<.0001	99.94

Cluster analysis

Grouping accessions based on their similarity is important for subsequent improvement and selection works. In this study 100 anchote accessions were clustered into six different groups based on 20 mineral and biochemical traits (**Figure 2** and Table 3). Each of the six clusters contributed differently with cluster number IV was the largest, containing 57 accessions and 57% of overall genetic similarity. The remaining clusters, I, II, III, V and VI totally contributed about 43% variation combined having 13, 18, 2, 9 and 1 accessions, respectively. The cluster mean for all the traits revealed that considerable differences were noticed between the cluster means (Table 4). Accessions from all clusters except for cluster II and IV have produced the highest Ca content than others. Accessions from cluster VI have produced the highest Fe content (0.3844 mg/g) and Zn was highest in clusters I and IV; 0.006 and 0.006mg/g, respectively. Generally, accessions from clusters I, III and VI produced the highest contents of most of the mineral elements than the remaining clusters. Accessions collected from west Wollega (Boji Dirmaji and Gimbi) and east Wollega (Gudeya Bila, Limmu and Wayu Tuka) exhibited wider distribution in four clusters than others (Table 5).

Table 3. Distribution of 100 anchote accessions in to six clusters based on mineral and biochemical traits

Clusters	No. of accessions in each cluster	Accession numbers	% of contribution
I	13	2, 153, 397, 29, 40, 308, 161, 291, 126, 313, 9, 222, 328	13
II	18	8, 406, 51, 11, 58, 183, 223, 131, 43, 75, 280, 416, 158, 16, 132, 93, 286, 155	18
III	2	3, 400	2
IV	57	5, 157, 209, 297, 10, 80, 262, 53, 81, 140, 227, 41, 151, 316, 56, 67, 148, 281, 62, 87, 305, 66, 91, 113, 96, 290, 393, 292, 95, 304, 402, 407, 97, 112, 24, 302, 377, 50, 32, 385, 388, 206, 256, 14, 134, 288, 94, 211, 394, 65, 300, 271, 92, 214, 376, 296, 169	57
V	9	4, 219, 49, 218, 90, 309, 89, 156, 413	9
VI	1	381	1

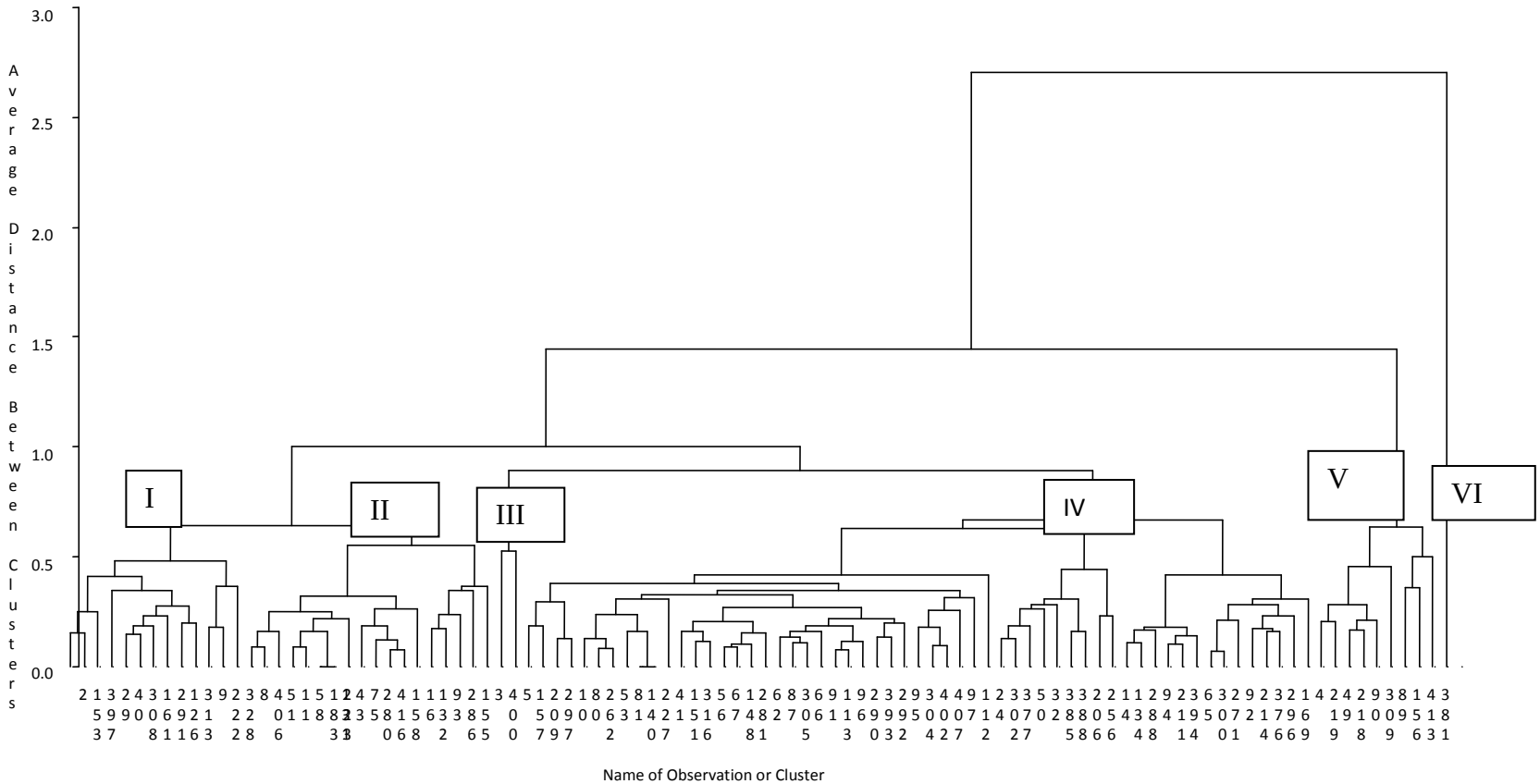

Figure 2. Dendrogram showing hierarchical clustering patterns of 100 anchote accessions (UPGMA) based on 20 qualitative characters

Table 4. Distribution of cluster means of mineral elements content of the 100 anchote accessions

Cluster	Ca	K	Mg	S	P	Na	B	Cu	Fe	Mn	Mo	Zn
I	552.90	1284.16	215.06	123.32	462.94	78.35	2.12	0.09	16.28	1.21	0.90	0.57
II	350.58	1214.62	172.68	105.38	402.03	56.24	1.50	0.09	11.87	0.76	0.52	0.51
III	598.18	1467.34	277.24	178.05	671.10	78.93	2.45	0.09	18.24	1.63	0.82	0.53
IV	397.99	1625.47	185.62	116.66	464.16	73.78	1.67	0.09	15.64	0.91	0.68	0.60
V	407.85	2070.16	207.58	129.74	530.53	75.00	1.48	0.09	15.13	0.95	0.77	0.55
VI	464.26	2707.08	204.62	136.18	700.75	60.26	2.88	0.09	38.44	1.10	1.08	0.53
MEAN	461.96	1728.14	210.47	131.55	538.59	70.43	2.02	0.09	19.27	1.10	0.79	0.55
S.deviation	87.81	518.76	33.08	22.95	110.93	8.87	0.52	0.00	8.78	0.28	0.17	0.03

Table 5. The distribution of anchote accessions in areas of collection based on cluster groups

Zone	District	Clusters						Total
		I	II	III	IV	V	VI	
West Wollega	Boji Dirmaji	1	1		2	2		6
	Gimbi	3	7		11	2		23
	Kiltu Kara		1		1			2
	Lalo Asabi				1			1
	Mana Sibu Mandi			1	7			8
	Nejo				4			4
East Wollega	Bonaya Boshe				1			1
	Digga	1						1
	Digga Leqa				1			1
	Gida Ayana	1			1			2
	Gobu Sayyo		1					1
	Gudeya Bila	1	2		10	1		14
	Leka Dulecha	3			5			8
	Limmu	1			3	1		5
	Nunu Kumba				1			1
	Sibu Sire	1				1		2
	Wayu Tuka	1		1	2	1		5
	Chingi	2	1		1			4
Jimma	Dedo				1			1
Buno Bedelle	Didesa				3		1	4
Horro Guduru Wollega	Horro	1	2					3
	Jimma Geneti				3			3
Total		16	15	2	58	8	1	100

Accessions grouped under clusters I, II and IV produced higher dry matter, clusters I and VI were superior in crude fiber, clusters II and VI in crude protein, clusters III and V better in carbohydrate and total energy(Table 6). Variability among clusters would be due to genetic factors than environmental influences as most of the clustered accessions exhibited variability in biochemical contents even though differ in collection areas except cluster VI which is accessed from Buno Bedelle, Didesa area. Accessions grouped under clusters III and V produced the lowest biochemical contents except for carbohydrate and total energy and cluster IV was better in dry matter but moderate in all other biochemical contents. Thus, utilization accessions grouped under clusters I for high dry matter and crude fiber; cluster II for dry matter and crude protein; cluster III and V for carbohydrate and total energy; cluster IV only for higher dry matter, and cluster VI for crude fiber and crude protein in the subsequent anchote improvement programs for better nutritional contents.

Table 6. Cluster means for seven biochemical traits of anchote accessions

Cluster	DM	Ash	CF	CP	Cfat	CHO	Energy
I	11.67	5.15	4.13	11.46	0.49	71.24	335.18
II	11.74	4.89	3.35	12.31	0.31	70.75	335.01
III	10.06	4.16	2.99	11.06	0.32	74.41	344.72
IV	11.66	5.08	3.42	11.86	0.33	71.09	334.71
V	10.83	4.93	3.35	11.29	0.28	72.68	338.36
VI	10.48	6.98	6.15	14.08	0.34	68.13	331.89
MEAN	11.07	5.20	3.90	12.01	0.35	71.38	336.64
S.Dev.	0.66	0.86	1.06	1.01	0.07	1.91	4.07

Principal component analysis (PCA)

The patterns of variation and the relative importance of each trait in explaining the observed variability assessed through principal component analysis. Principal component analysis was executed to characterize 100 anchote accessions based on their mineral and biochemical composition to explain the observed variability to be assessed. The accessions were grouped into seven principal components based on root flesh color, mineral elements and biochemical traits of which flesh color, Ca, K, Mg, S, P and Na contents were significant(eigenvalue > 1) and explained 78 % of the total variability(Table 7).

The first principal component (PC1) alone accounted for 23.79% of the total variation. Root flesh color, crude fat, crude protein and total energy had the highest loadings across all the seven components. The second principal component (PC 2), explaining 15.98% of the total variation and highly correlated with Ca, K, B, Fe, Zn, dry matter, crude fiber and carbohydrate while PC3 associated with root flesh color, Ca, K, Na, B, and crude protein explained 9.87% of the total variation. The remaining PCs (PC4-PC7) accounted 28.05% of the total variation, and mainly associated with all the variables except the crude fiber which was loaded lesser.

The variation exhibited in this experiment could be attributed to genetic and environmental factors.

Table 7. Variant component scores of root flesh color, minerals and biochemical traits of the first seven principal components for 100 anchote accessions

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Root flesh color	0.3006	0.2957	0.4375	0.3414	0.4642	0.3549	0.24
Ca	0.2754	0.3404	-0.4309	0.3346	0.3389	0.2903	0.2545
K	0.2409	-0.3793	0.5072	0.3946	0.3188	0.359	0.2525
Mg	0.3195	-0.2134	0.3269	0.5583	-0.4325	0.3539	-0.1506
S	0.3179	0.2323	-0.3782	0.2429	0.3166	-0.4064	0.4735
P	0.2697	0.4091	0.2465	0.178	-0.1586	-0.6478	-0.3115
Na	0.3005	0.2901	0.4123	-0.3081	-0.3282	0.3713	0.3773
B	0.3479	-0.3178	-0.4878	-0.5402	-0.2584	-0.2739	0.5447
Cu	0.2604	0.2899	-0.3221	-0.7028	0.2518	0.1665	0.1423
Fe	0.2959	-0.4118	0.1719	0.1482	-0.4895	0.1679	-0.5913
Mn	0.3607	-0.1789	-0.1902	0.1626	-0.8099	0.2542	0.1251
Mo	0.2794	0.1393	-0.1839	-0.1752	0.5848	0.2247	-0.6213
Zn	0.2493	-0.4429	-0.2003	0.2599	0.2177	0.4028	0.5304
DM	0.1583	-0.4578	0.1344	-0.2027	-0.1897	0.7794	0.1404
Moisture	-0.1576	0.4602	-0.1376	0.2031	0.1821	0.3428	0.7175
Ash	0.2345	-0.3678	0.2677	-0.5651	-0.2769	0.2607	0.2376
Crude fiber	0.6939	-0.5651	-0.2769	0.1484	0.1494	0.1565	0.1291
Crude protein	0.4899	-0.3221	-0.3564	-0.2852	0.1541	0.1584	0.5708
Crude fat	0.4216	0.2332	0.3305	0.2831	-0.3017	0.3166	-0.4946
Carbohydrate	-0.3394	-0.4151	0.2219	-0.1819	0.1923	0.6938	0.1477
Energy	-0.4971	0.1507	-0.1339	-0.1195	-0.4125	0.5692	-0.4052
Eigen value	4.9957	3.3556	2.0735	2.0174	1.5936	1.1978	1.0825
proportion	23.79	15.98	9.87	9.61	7.59	5.7	5.15
Cumulative	23.79	39.77	49.64	59.25	66.84	72.54	77.7

Conclusion and Recommendation

Anchote [*Coccinia abyssinica]* cultivation is quite common in western and southwestern parts of Ethiopia with its full utilization and production practices even though it is an underutilized root crop in other parts of Ethiopia and unknown in world's production and marketing. The result of analysis of variance indicated significant variations ($p \leq 0.01$) among the 100 anchote accessions for all the twenty biochemical and mineral traits considered in this study. There was a wide genetic variability among the anchote accessions tested in this study. According to the results of this study, anchote could be the best alternative source of protein (11.81%) and carbohydrate (72.13%) than any other root and tuber crops. The variations in biochemical and mineral contents among the tested 100 anchote accessions could be due to genotype, geographical sources and agronomic practices. Anchote's fiber content (3.55%) is the highest to other root and tuber crops even though *kocho* (enset) and taro are comparable. Among the most important contents of anchote, Ca (416.15), Fe (15.33), Zn (5.74) and Mn (0.95), in mg/100g, are the most dependable potentials which are found abundantly and could help in the efforts to solve nutrition insecurity of

Ethiopian population. The higher calcium content of anchote could be an alternative to milk allergic infants and adults and the higher Mn and Zn contents of anchote could be an alternative to control dehydration problems due to diarrhea.

The cluster analysis of biochemical and mineral traits revealed the existence of six divergent groups, 88% of the accessions represented by clusters I, II and IV, with the highest biochemical and mineral traits, regardless of the geographical locations where the accessions were collected, showing the reduced influence of environments on the genetic factor. All the variables were grouped in one principal component of which root flesh color, Ca, K, Mg, S,P and Na were significant(Eigen value> 1) explaining 78% of the total variability. All the traits except for fat, carbohydrate and total energy, contributed maximally to the principal component with calcium the most important contributor (20%). The variation is attributed to genetic and environmental factors. The results obtained from this study confirmed the existence of variability among the accessions which indicates huge potentials for selection and crossing of nutritionally superior anchote genotypes. Anchote could be used as a high potential crop to fight protein and calcium deficiency in countries such as Ethiopia. Accessions numbers 400,328,206,222 and 308 were superior in Ca content. Accession 304 was superior in crude protein and energy content. For dry matter content, accessions 62, 305,169,402, 58 and 262 were superior among the 100 accessions.

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Status and Relative Distribution of Maize Streak Virus in Western Ethiopia

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Abstract

Maize production in Ethiopia is constrained by both biotic and abiotic factors, and diseases of maize caused by viruses are serious threats to maize production. Maize streak (MS) is one of the most severe and widespread diseases that adversely reduces maize production. Hence, this study was conducted to assess the status and distribution of maize streak and to diagnose Maize streak Virus isolates. Surveys were conducted during the 2019 main cropping season in different agro ecological zones of Gambela, Benishangul-Gumez and Oromia regions. Maize and grasses in maize fields with suggestive MS-like symptoms were sampled for laboratory testing. Out of 127 maize fields visited, based on MS-like symptoms, the disease was prevalent in 100 (79%) fields assessed, from which 100 maize and 5 grass samples were collected for laboratory testing. High prevalence, incidence and severity of MS were recorded at low altitude areas; whereas low incidence and severity were recorded at high altitude maize growing areas. The mean incidences of 64%, 59% and 33%; and severities of 4.3, 4 and 3 (on 1-5 scoring scale) were, respectively, recorded in Gambela, Benshngul-gumeze and Oromia regions. Results of laboratory testing employing PCR showed that out of 100 symptomatic maize and 5 grass samples analyzed, MSV was recovered from 95 (90%) maize and none of the grass samples. Based on the results of the present study, MS is an economic disease of maize at low and mid altitude growing areas. Hence, disease management options involving development and deployment of resistant vanities along with cultural practices should be sought.

Keywords: Intensity, Maize, Maize Streak Virus, Mastervirus, Polymerase Chain Reaction

Introduction

Maize is one of the three leading global cereals that feed the world (Muiru *et al*., 2010; Shiferaw *et al*., 2011). Maize, together with rice and wheat, dominate human diets (Ignaciuk, 2014) and provide at least 30% of the food calories of more than 4.5 billion population. The crop provides high yields per unit of land, making it a key crop in ensuring food availability and security for the consumers (Mboya *et al*., 2011). Maize is a major staple food in Ethiopia and ranks first in production and second in area coverage next to tef (*Eragrostis tef*) (CSA, 2020). A total of 10.5 million tons have been produced across 2.5 million hectares

of land in the 2020 cropping season (CSA, 2020). Despite its importance, however, the national average yield (3.4 tons/ha) of maize in Ethiopia is below the world's average (8 tons/ha) (FAOSTAT, 2017).

Maize production in Ethiopia is constrained by both biotic and abiotic factors, and diseases of maize caused by viruses are serious threats to maize production. More than 50 virus species can infect maize (Redinbaugh and Zambrano, 2014) and among these, maize streak virus (MSV), genus Mastrevirus in the family *Geminiviridae* is one of the top ten economically important plant viruses (Rybicki, 2015). Virus species in the genus Mastrevirus have monopartite genomes of approximately 2700 nt encapsidated in geminate particles (King *et al*., 2011). Maize streak (MS) is one of the most severe and widespread diseases that adversely reduces maize production thereby posing a threat to food security (Dhau *et al*., 2018). The most extensively sampled and studied mastrevirus species is *maize streak virus*: the causal agent of maize streak disease (MSD), and the most significant viral disease of maize (Zea mays) in Africa (Oppong *et al*., 2015; Pande *et al*., 2017). Maize streak causes yield losses that range from trace to almost 100% if infection occurs in the first three weeks of planting maize (Alegbejo *et al*., 2002, Danson *et al*., 2006).

The disease is distributed throughout the African continent and surrounding islands (Monjane *et al*., 2011). Globally, MSD is regarded as the third most serious disease of maize after turcicum leaf blight (TLB) and gray leaf spot (GLS) (Pratt & Gordon, 2006). In Africa, however, MSD is a serious problem than both TLB and GLS (Martin & Shepherd, 2009). Epidemics resulting in economic losses of up to 100% have been reported in at least 20 African countries including Ethiopia, Nigeria, Ghana, Sudan, Cameroon, Zimbabwe, Tanzania, Togo, Benin, Burkina Faso, Sao Tome and Uganda (Lagat *et al*., 2008; Owor, 2008). MSD has been identified as a major constraint to maize production in Ethiopia (Alemu *et al*., 1997; Guadie *et al*., 2018).

Except for a few scattered information generated in localized areas (Alemu *et al*., 1997; Guadie *et al*., 2018). Major maize growing agro ecologies was not properly addressed and sufficient information generated regarding the distribution and status of MSD in Ethiopia. This paper therefore reports results of a comprehensive assessments made on the status and distribution of MSD in western regions, which represent the major maize growing areas in Ethiopia.

Materials and Methods

Assessment and Geographical Distribution of Maize Streak Disease

During the 2019 main cropping season, an intensive and extensive surveys for Maize streak (MS) were

carried out in major maize growing areas of Oromia (east Welega, west Welega, Jimma, Buno Bedele zones), Benishangule-Gumuz (Asosa zone) and Gambella (Agnuwak and Nuer zones) regions. In these regions maize, in most cases, is cultivated in monoculture as the major crop by small holder farmers (CSA 2020). The surveys were made from July to October 2019 main cropping season.

Most of the surveyed maize fields were at a vegetative growth stage ranging in size from knee height to early tasseling. The surveys were made along road-sides with approximately five to ten kilometer intervals wherever the crop was available. Sampling was done using simple random sampling technique, and assessment was made diagonally along field at five spots. At each spot representative symptomatic sample with characteristic MS symptoms were collected, bulked and desiccated in calcium chloride or silica gel for later laboratory testing. Suspected symptomatic alternative grass hosts of harboring MSV in each field were also collected and preserved as described above for laboratory analysis.

Data related to crop variables such as growth stage, variety, date of sowing, previous crops, crop performance, insect vectors and location, etc were recorded. Altitude and geographical position of survey locations were recorded using geographical positioning system (GPS) at each field. Information on agro-ecological zones was obtained from the Ministry of Agriculture (MOA, 2005).

Table 1. Major agro-ecological zones of surveyed areas for maize streak in Ethiopia

Agro-ecological zone	Altitude (m.a.s.l)	Regions*
Moist lowlands	<1000	Gambela
Moist lower &dry mid-altitudes	1000-1700	W. Oromia & BSG
Moist and semi-moist mid-altitudes	1701-2000	W. Oromia & BSG
Moist upper mid-altitudes	2001-2400	W. Oromia

*W, west; BSG, Benishangule-Gumuz
Adapted from (MOA, 2005)

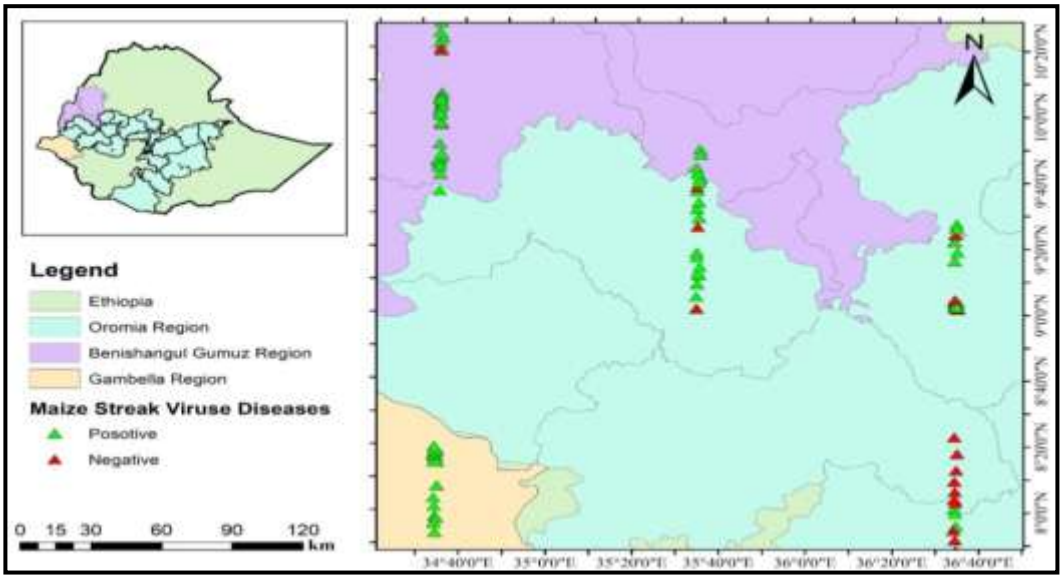

Figure 1.Spatial distribution of MSV in surveyed regions of Ethiopia, 2019 main cropping season

Assessment of MS Prevalence, Incidence and Severity in the Field

Disease prevalence was assessed by determining the number of fields where a particular disease was recorded in relation to the number of fields sampled from surveyed region.

The incidence of MS was determined by visually observing and recording the number of maize plants showing the disease symptoms and the percentage incidence was calculated as follows:

$$\text{Incidence}(\%) = \frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \text{x}100$$

Disease severity was assessed as the area of plant tissue affected by disease, expressed as a percentage of the total area at regular intervals using a 1–5 scale (Table 2) (Blankson *et al*., 2018).

Table 2. Visual rating scale for maize streak disease severity

Rating scale	Description	Expression in terms of Severity
1.	No symptoms	No infection
2.	Very few streaks on leaves, light streaking on old leaves gradually decreasing on young leaves	Mild infection
2.	Moderate streaking on old and young leaves Slight stunting	Moderate infection
4.	Severe streaking on about 60-75% of leaf area, Plants stunted	severe infection
5.	Severe streaking on more than 75% leaf area, severely stunted or dead	Very severe infection plants

Molecular Characterization of Maize Streak Virus

DNA extraction and Polymerase Chain Reaction

Young leaf samples collected from maize plants showing MS symptoms from different maize growing regions of Ethiopia were used for DNA extraction. Total DNA was extracted using modified CTAB extraction method as described by Doyle and Doyle (1991). The extracted genomic DNA was amplified by Polymerase Chain Reaction (PCR) using degenerate Primer pair of forward 5'-CCA AA (GT) (AGT) TC AGC TCC TCC G-3' and Primer reverse 5'-TTG G(CGA)C CG(AC) (ACG)GA TGT A(CG)AG-3'. The primers were expected to amplify a 1300 base pair (bp) fragment. PCR amplification of MSV was performed in 25 µl reaction volume containing 11µl Buffer, 10 µl SDH_2O, 1µl each primer, 1µl Taq polymerase and 1µl genomic DNA. PCR was run on standard thermal cycler (PCR sprit thermal cycler) at Ambo Agricultural Research Centre (AmARC), virology laboratory with reaction condition of 94 ℃ for 1min, followed by 30 cycles of 93 ℃ for 45sec, 54 ℃ for 30sec and 72 ℃ for 90sec, and a final extension step of 72℃ for 3 min.

Electrophoresis and UV visualization

PCR products were assessed by electrophoresis in 0.8% agarose gel in TAE (Tris-Acetate-EDETA) buffer, stained with ethidium bromide, and viewed under ultraviolet light trans-illuminator. For reference and comparison of PCR products, 100 bp DNA size markers were used along with the samples.

Data analysis

Descriptive statistics was performed for analysis of data related to prevalence, incidence and severity.

Results and Discussion

Prevalence, Incidence and Severity of MS across Locations and Varieties

A total of 127 maize fields were visited, and 105 maize and 5 grass samples with MS-like symptoms were collected. Most frequently encountered MS-like symptoms supposed to have

associated with MSV infection are: Broken to almost chlorotic strips centered on the tertiary leaf veins and uniformly distributed across the leaf surface, pale yellow streaks, mosaic and chlorosis on leaf lamellae, broken or longitudinal chlorotic streaks along leaf veins, wilting and drying of leaf margins, necrosis and mottling on the entire plant (Fig 2). The characteristic symptoms described in this study have similarly been reported in detail by various researchers (Shepherd *et al*., 2010; Mesfin *et al*., 1995). It is interesting to note that visual assessment of MS based on field symptoms, in most cases, corresponded with laboratory test result substantiating that characteristic symptoms are in most cases diagnostic and useful for field assessment.

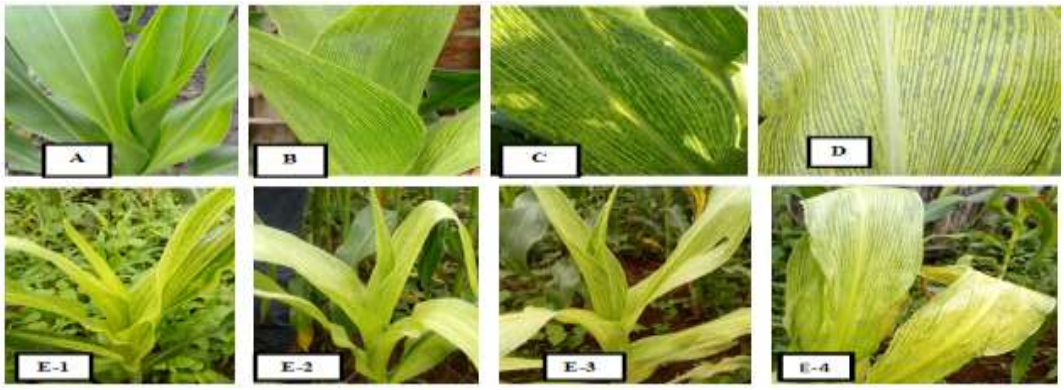

Figure 2. Shows different MS symptoms associated with plant growth stage at the time of infection: (A) no symptoms (no infection), (B) light streaking on old leaves (mild infection); (C) moderate streaking on old and young leaves, slight stunting (severe infection); (D) severe streaking on about 60-75% of leaf area, plants stunted (severe infection); (E1-E4) Severe streaking on more than 75% leaf area, plants severely stunted or dead (very severe infection).

Out of 127 maize fields visited, based on MS-like symptoms, the disease was prevalent in 100 (79%) fields assessed. Results of this investigation depicted that the disease prevalence, incidence and severity were highest in Gambella region followed by Benishangule-Gumuz and Oromiya regions, in this order (Table 3). This finding is in agreement with what reported by Mesfin *et al*. (1991) who indicated the exceptionally high MS outbreak in Gambella region during the 1986 crop season. In this work, other regions in the west other than Gambella are also succumbed to the disease. Mesfin *et al*. (1991) reported that MSV was prevalent at lower altitudes, this being attributed to higher population density of *Cicadulina spp* especially *C. mbila* found at lower altitude. This indicated that the altitude range was one of the deriving factors affecting MS distribution and prevalence by being favorable for the insect vectors build up and activity. Earlier, similar findings in Ethiopia reported the

distribution and importance of maize streak disease in some parts of the country (Guadie *et al*., 2018; Alemu *et al*., 1997).

The mean prevalence of MS was, respectively, 94 and 93% in Gambela and Benishangul Gumez regions, while the mean disease prevalence was 65% in Oromia region (Table 3). As indicated in Table 3, the mean prevalence was assessed in Agnuwak zone from three districts (Abobo, Abol and Gambela) and the disease had 100% prevalence in Abobo and Gambela while 75% in Abol distract and the average mean prevalence was 92% in Agnuwak zone. The prevalence of MS in Nuer zone was assessed from two districts (Itang and Lare) and had 100% prevalence in both districts and the average mean prevalence of MS in Nuer zone was 100%. Of four zones, namely Jimma, Buno Bedele, east and west Wolega Zones assessed in Oromiya, the mean prevalence of the MS was respectively 64, 0, 62 and 85%. Except Buno Bedele where the disease was not detected, MS was prevalent in three Oromia zones surveyed, the highest prevalence being recorded in west Wellega. The prevalence of MS was also assessed from one zone (Asossa) of Benishangul-Gumez region where the mean prevalence recorded was 93%.

Likewise, MS incidence was also higher in Gambella, followed by Benishangule-Gumuz and Oromia, where the mean incidences of up to 64% in Gambela, 59% in Benishangul Gumez and 33% in Oromia regions were recorded (Table 3). Of the surveyed areas in Gambella region, higher incidences of MS were recorded in Agnuwak zone, Gambela district and Itang and Lare districts of Nuer zone. In Oromiya, low incidence of MS was recorded in Buno Bedele and Jimma zones and relatively higher incidence recorded in east and west Wolega zones (Table 3). Recent report showed that MS incidence of up to 58% was also found in Ethiopian maize germplasm (Guadie *et al*., 2018).

The severity of MS was significantly higher in Gambela and Benishangul-Gumz compared to Oromia region. The severity score of MS was higher in Agnuwak, Nuer and Asosa zones and relatively low severity was recorded in Jimma, east and west Wolega zones where as no disease recorded in Buno Bedele zone. Mean severity of up to 4.3 (on 1-5 severity scoring scale) was recorded in all surveyed western regions of Ethiopia (Table 3) attributing to higher yield loss. Of the surveyed regions, the highest severities between 4 & 5 (on 1-5 scoring scale) were recorded in Gambella. This is followed by Benishangule-Gumuz and Oromia regions, in this order (Table 3).

The present study also disclosed the existence of variations in the level of disease prevalence and incidence in different altitude ranges assessed, where relatively higher prevalence and incidence were recorded at lower altitudes ranging from 400-1600 and

vice versa at higher altitudes ranging from 2000-2400 masl. For instance, the disease incidence was higher in Benishangul-Gumuz and Gambela regions at locations having 400 to 1500 masl (Table 4), which perhaps can be attributed to warmer climates favoring vector populations for virus transmission, which in turn contributing to more severe disease symptoms development (Redinbaugh and Zambrano 2014). The high disease incidence detected in the field is expected to cause a considerable reduction in yield and quality. In highly susceptible and late sown genotypes, MS reported to cause up to 100% yield losses in infected crops (Alegbejo *et al*., 2002; Kyetere *et al*., 1999; Lagat *et al*., 2008).

Table 3. The mean prevalence, incidence and severity of MS across district during the 2019 main cropping season

Region	Zone	District	Prevalence (%)	Incidence (%)	Severity (1-5)
Gambella	Agnuwak	Abobo	100	63	4
		Abol	75	45	4
		Gambella	100	85	5
	Mean		92	64	4
	Nuer	Itang	100	63	4
		Lare	100	65	5
	Mean		100	64	5
Grand Mean			94	64	4
Oromia	Jimma	Gumay	60	26	2
		Seka Chekorsa	67	33	3
		Shebe Sombo	75	42	3
		Kersa	80	29	3
		Mana	40	13	1
	Mean		64	28	2
	Buno Bedele	Didesa	0	0	0
		Gechi	0	0	0
	Mean		0	0	0
	East Welega	Guto Gida	78	44	3
		Diga	100	57	4
		Wayu Tuka	50	18	2
		Sibu Sire	20	14	2
	Mean		62	33	3
	West Welega	Gimbi	80	47	3
		Lalo Asabi	100	60	4
		Boji Dirmeji	67	35	3
		Nejo	100	40	4
		Leta Sibu	50	22	2
		Kiltu Kara	100	60	4
		Mene Sibu	100	58	4
	Mean		85	46	2
Grand mean			65	33	3
Benishangul-Gumuz	Asosa	Banbasi	100	67	5
		Homosha	80	51	3
		Asosa	100	58	4
Grand Mean			93	59	4

Table 4. Disease prevalence (%), Incidence (%) and severity (1-5scale) of Maize Streak across different Altitude ranges in Ethiopia, 2019 main season

S.N	Altitude Range masl	NFA	NFI	Prevalence (%)	Incidence (%)	Severity (1-5 scale)
1	<1000	23	22	96	63	4
2	1000-1700	23	22	96	63	4
3	1701-2000	52	36	69	33	3
4	2001-2400	8	1	13	5	1

NFA- Number of fields assessed
NFI- Number of fields infected

Table 5. Correlation of Altitude verses Maize Streak disease incidence and severity

	Altitude	Incidence	Severity
Altitude	1	-.510**	-.446**
Incidence	-.510**	1	.948**
Severity	-.446**	.948**	1

**. Correlation is significant at the 0.01 level (2-tailed).

From the present result, one can conclude that MS disease is distributed in almost all maize growing agro-ecologies with higher prevalence and severity recorded at low and mid altitudes. The high prevalence, incidence and severity of MS at lower and mid altitude maize growing agro-ecologies may partly attributed to the conducive environment for both the virus and vectors transmitting MSV. It is worthwhile to note that high level of correlations was observed between altitude ranges and MS disease distribution, incidence and severity. There were high percentages of disease incidence and severity at lower altitudes and vice versa at higher altitudes. The presence of several grasses in the family poaceae serving as alternate hosts to MSV along with high vector populations and activity at lower altitudes may also immensely contributed for high MS prevalence, incidence and severity. The present results support earlier findings that reported the virus was favored by warmer climate in sub-Saharan Africa (Monjane *et al*., 2011; Oluwafemi *et al*., 2014; Pande *et al*., 2017).

The exceptionally high MS incidence and severity recorded in the surveyed locations in Gambella region might have associated with high vector (s) population and activity. For example, earlier report by Mesfin *et al*. (1991) has shown that efficient MSV vector in the genus Cicadulina such as *C. mabila* is recorded at high intensity in surveyed areas in Gambella, whose activity and population has direct relation to MSV incidence and severity. Furthermore, Mesfin *et al*. (1991) has also reported cereal (Maize, Triticum sp, Avena sp. and sugar cane) and grass (Digitaria, Setaria, Hyparrhenia, Panicum, Pennisetum and Sorghum spp.) are hosts of

Cicadulina spp. vectoring MSV. MSV has also known to have wide host ranges. In addition to maize, cereals such as rice (Oryza sativa L.), wheat (Triticum aestivum L.), oats (Avena sativa L.), barley (Hordeum vulgare L.), rye (Secale cereale L.), finger millet (Eleusine coracana L.), pearl millet (Pennisetum typhoides L.), sorghum (Sorghum bicolor L.) and sugarcane (Saccharum officinarum L.) (Willment *et al*., 2001; Van Antwerpen *et al*., 2011) are infected by MSV.

A wide range of grasses in the genera Axonopus, Brachiaria, Coix, Eleusine, Paspalum, Imperata, Rottboellia, Eragrostis and Setaria (Willment *et al*., 2001; Lett *et al*., 2002; Fajemisin, 2003), most of which are naturally growing in valleys and irrigation schemes, are also attacked by MSV. All cereals and most grasses identified as hosts for MSV and its insect vectors are widely grown in Ethiopia (Bekele *et al*., 2017; Stroud and Parker, 1989). Furthermore, some of the grasses just identified as hosts of *cicadulina* spp. are also known as alternate hosts of MSV (Alemu *et al*., 1997). Hence, having common hosts for both the virus and its vectors simply imply the presence of suitable substrate/medium for their survival and multiplication serving as sources of inoculum and vectors from which the newly growing maize crop can get infected.

Similarly, prevalence, disease incidence and severity of Maize Streak were assessed across maize varieties evaluated. The study revealed that higher average MS incidence and severity were scored on varieties Gambela composite, local and BH540 than other varieties (Fig 3), implying that these varieties appear more susceptible to Maize Streak disease.

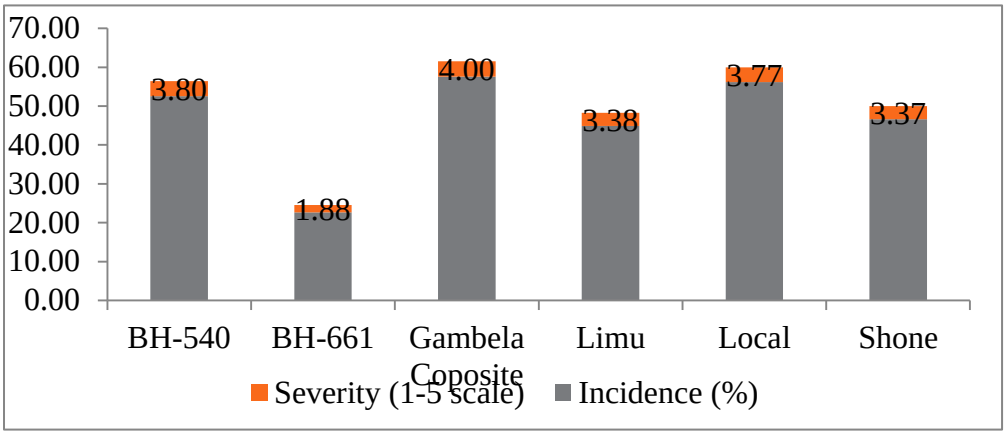

Figure 3. The mean incidence and severity of maize streak disease by varieties sampled across the surveyed locations during the 2019 main cropping season

Molecular Characterization of Maize Streak Virus

The PCR amplification using the degenerate primer pairs consistently yielded an approximately 1300-bp product for ninety five out of one hundred five samples tested (Figure 4). Absence of amplification for five symptomatic maize and five grass samples may be due to misdiagnosis in the field for other viruses which may develop similar or closely related symptoms with MSV such as Maize lethal necrosis (MLN). This primer was amplified nearly 95% of the samples at expected base products, which was comparative result with the reports of Willment, *et al*. (2001). Results of field assessments and molecular detection revealed that MS-causing viruses were distributed in major maize growing areas of Ethiopia surveyed during this study. Symptomatology and molecular diagnosis of MS showed that the disease was detected in nearly 95% of the fields surveyed, indicating that both diagnostic methods can be used interchangeably if attentively done. The bands of some isolates were weak, indicating low quantities of PCR products, which in turn attributed to low DNA extract.

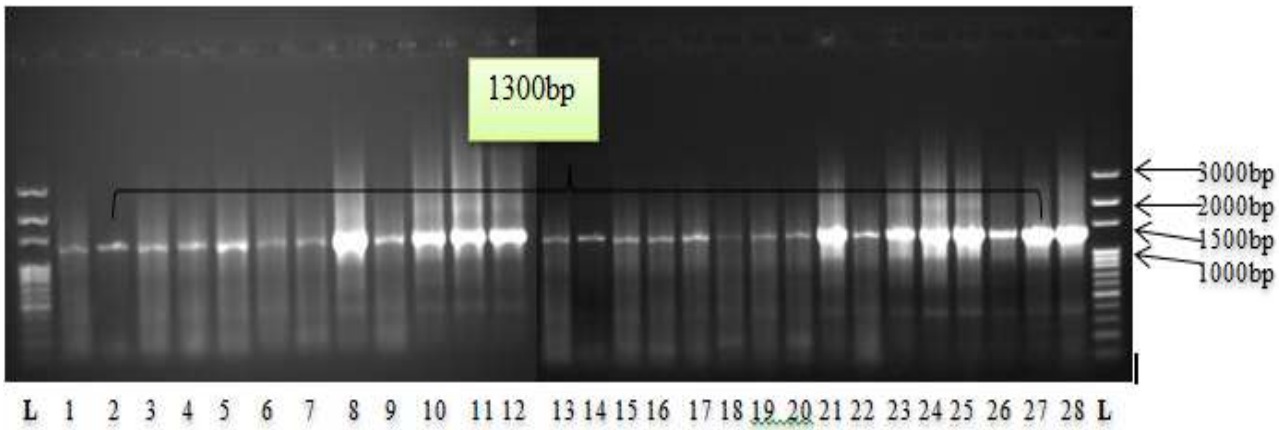

Figure 4. A 1.3-kilo base product was amplified using a MSV replicative form-specific degenerate primer pair, 215–234 and 1770–1792 (Willment et al., 2001). L=The 100 bp DNA Ladder ready-to-use molecular weight marker (Solis BioDyne, Estonia), Size range of 100 – 3,000 bp and 12 numbers of bands. Nos. 1-28 is representative samples collected from surveyed regions.

Conclusion

The study revealed that MS disease was distributed in almost all major maize growing agro-ecologies of western Ethiopia from low to high altitude areas assessed. The results of the present study provided broader information on the distribution, incidence, severity and molecular characterization of MSV in surveyed regions of Ethiopia.

High prevalence, incidence and severity of MS were recorded at low and mid-altitude areas surveyed where as low incidence and severity were recorded at high altitude maize growing areas. The disease recorded in all available local and commercial maize varieties.

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Effects of Maize and Mung Bean Intercropping on Performance of the Component Crops and System Productivity

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Abstract

Interaction effect of variety by cropping system is expected to be arising from morphological features of component crops thus Selection of compatible varieties of component crops can increase productivity of intercropping systems. Hence, a field experiment was carried out at two locations to identify the best compatible varieties of maize and mung bean that can increase the performance of the component crops and the productivity of the intercropping system. Factorial combinations of intercropping of three varieties of maize (Melkassa-1, Melkassa-2, Melkassa-6Q) and three varieties of mung bean (N-26/Rassa, NVL1, Shewa Robbit) with their respective sole crops were laid out as a randomized complete block design with three replications. The result showed that location and variety had a significant effect on growth parameters and yield components of maize and mung bean intercrops. The higher grain yield (4.1t ha^{-1}) was recorded from Melkassa Agricultural Research Center site. Regarding varieties, the highest maize grain yield (4.8t ha^{-1}) was obtained from variety Melkassa-2 intercropped with mung bean variety-26. The higher grain yield of intercropped mung bean was (530 kg ha^{-1}) recorded from Melkassa Agricultural Recearch Center. Among the different intercropping combinations, intercropping maize variety Melkassa-2 with mung bean variety N-26 had the highest Land Equivalent Ratio (1.57), maize equivalent yield (6390kg ha^{-1}) and monetary value (63900 ETBha^{-1}). Intercropping resulted in the highest Land Equivalent Ratio (LER) (1.30), maize equivalent yield (5270 kg ha^{-1}) and Gross Monetary Value (56300 ETB ha^{-1}). Thus, maize variety Melkassa-2 with mung bean variety N-26 can be used as compatible varieties of component crops to maximize the productivity of the intercropping system in drylands of central and eastern Ethiopia.

Keywords: Intercropping, economic yield, land equivalent ratio, maize, mungbean,

Introduction

Cereal-legume intercropping is the most commonly used practice by smallholder farmers in developing countries because of its environmental as well as economic advantages (Willey, 1979). For developing a feasible and economically viable intercropping system, planting pattern of the compatible crops are an important agronomic approach for enhancing system productivity. Intercropping cereals with legumes

provides an opportunity to harness the benefits of legumes sustaining the cereal based cropping system without adverse effect on yield. However, the advantage of intercropping is obtained when correspondent species have differences in crop architecture, maturity time, and nutrient use to optimize the use of natural resources and environmental factors when cropped together (Nurbakhsh *et al.*, 2013).

In Ethiopia, maize (*Zea mays* L.) ranks first among cereals in grain yield (4.2 t ha^{-1}) and in total grain production, while it is second to *teff* in total area coverage with 2.27 million ha in 2019/2020 cropping season (CSA, 2020). The major reasons for popularity of maize include: its highest yields per unit area, positive response to use of inputs, ease of production and food preparation, compatibility with many cropping systems, adaptability to major agro-ecologies. It is food security crop in the country where recurrent drought is a common phenomenon (Tesfa *et al.*, 2001).

Mung bean [*Vigna radiata* (L.) Wilczek] is an eco-friendly food grain leguminous crop of dry land areas with rich source of proteins, vitamins, and minerals (Keatinge *et al.*, 2011). It is of recent introduction to Ethiopian pulse crops production but its importance especially as an export crop is increasing. It is mostly grown by smallholder farmers under drier marginal environmental condition as food and cash crop due to its short growth duration and high market value. According to CSA (2020) report, the estimated area under mung bean in Ethiopia during the main cropping season of 2019/2020 cropping season was 49,123.52 ha with productivity of 1136 kg ha^{-1}. Mung bean is a quick crop, requiring 75–90 days to mature. It is a useful crop in drier areas and has a good potential for crop rotation, relay cropping and intercropping with cereals (Singh, 2014).

In maize based intercropping system, selection of an appropriate intercrop having desirable plant type and growth pattern assumes greater importance. Crops maturing well before the peak growth period of maize are ideal. Other studies on intercropping has indicated how niche difference in crop species can leading to increased biological efficiency and yield advantage (Mucheru-muna *et. a l.* 2010). Maize and mung bean intercropping can reduce the risk of crop failure that could result from terminal moisture deficit, as mung bean matures early relative to maize. However, the yield in intercropping system depends on selection of compatible genotypes with suitable characters for establishment of minimum competition and maximum complementarity (Mutungamiri *et al.*, 2001).

In Ethiopia, improved maize and different legume varieties have been recommended for sole cropping system and compatibility study of

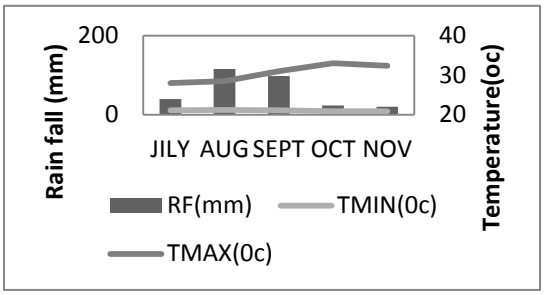
legume and maize varieties is not well-addressed (Tesfa *et al.*, 2012). Interaction effects of variety by cropping system are expected to be arising from morphological features such as leaf arrangement, canopy shape and growth habit. For sustainable intensification of maize and mung bean in maize-based cropping system of central and eastern Ethiopia, growth and morphological characters governing compatibility of varieties of the component crops under intercrop conditions need to be understood. Hence, this study was undertaken to identify the best compatible varieties of maize and mung bean that can increase the productivity of intercropping system.

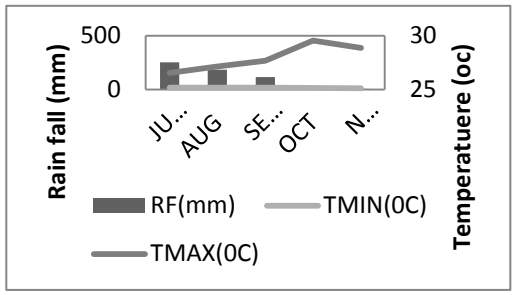
Materials and Methods

Description of the Experimental Site

The experiment was conducted at two locations, *i.e.* Melkassa in central Ethiopia and Fedis in Eastern Ethiopia in 2017 cropping season (July to November). Melkassa is located at $8^{o}24'$ N latitude, $39^{o}12'$ E longitude and an altitude of 1550 meters above sea level. The long-term mean annual rainfall is 791.69 mm with erratic distribution having peaks in July and August. The long-term (1977–2005) mean monthly maximum and minimum temperature was 28 and 14 ^{o}C, respectively (MARC, 2005). The soil of the experimental site is clayey loam. The total seasonal rainfall and mean temperature of growing season were 813.6 mm and 21.4 ^{o}C, respectively (Figure 1).

Fedis is located at of 9˚07'51.6"N latitude, 42˚04'24.3"E longitude, and an altitude of 1702 meters above sea level. The rainfall has a bimodal distribution pattern with small rains falling from March and May and the main rains falling from June to October, but with erratic distribution. The total seasonal rainfall and mean temperature of the growing season were 657.7 mm and 20.4 ^{o}C, respectively (Figure 1).





Melkassa Fedis

Figure 1. Monthly total rainfall (mm) and average maximum and minimum air temperatures (°C) of crop growing seasons during 2017 at Melkassa and Fedis

Soil sampling and analysis

Prior to planting, soil samples were collected from 0-30 cm depth from five spots across the experimental fields, in a *zig-zag* pattern, composited, and analyzed for selected soil physico-chemical properties. The composited soil sample was air-dried, ground and sieved to pass through a 2 mm sieve. Soil sample was analyzed at Melkassa Agricultural Research Center soil laboratory. Total nitrogen was determined following Kjeldahl procedure as described by Cottenie (1980); the soil pH was determined by using a digital pH meter (Page, 1982). Organic carbon was determined following wet digestion method as described by Walkley and Black, (1934); and the available phosphorous was measured using Olson II methods (Olsen *et al.*, 1954); cation exchange capacity (CEC) was determined by ammonium acetate method (Cottenie, 1980) and soil texture was determined by Bouyoucons hydrometer method (Bouyoucos,1962). The results of the soil analysis revealed that the soils of the experimental fields were clay loam in texture both at Melkassa and Fedis. The soils had moderate organic carbon (1.77%) and low total N (0.08%) at Melkassa and low organic carbon (1.4%) and low total N (0.11%) at Fedis. Available P was medium (6.84 ppm) at Melkassa and low (5.45 ppm) at Fedis (Table 1).

Table 1. Selected physicochemical properties of the experimental soils at MARC and Fedis (Boko site) in 2017 cropping season before planting

Soil parameter	MARC	Fedis
pH	7.73	8.05
Organic carbon (%)	1.77	1.4
Total N (%)	0.08	0.11
Available P (mg/kg)	6.84	5.45
Exchange K (c mol (+)/kg	2.85	0.92
CEC (cmol/kg)	36	35.2
Clay (%)	36	36
Silt (%)	32	35
Sand (%)	32	29
Soil texture	Clay-loam	Clay-loam

Description of the Experimental Materials

The maize varieties used for the experiment were obtained from Melkassa Agricultural Research Center (MARC). The varieties were selected due to their morphological differences (plant height, leaf area, number and size of leaves), maturity group, yielding potential and ecological preferences. Similarly, three varieties of mung bean were obtained from Melkassa Agricultural Research Center/MARC. Detailed description of the varieties used for the study is given on table 2.

Table 2. Maize and mung bean varieties used for the experiment

Maize Variety	Years of Release	Days to maturity	Plant ht (cm)	Yield(t/ha)		Altit (m)	Rf (mm)	Released institute
				On-station	On-farm			
Melkassa-I	2001	85	150-170	3.8-4.5	2.5-3.5	600-1700	450-570	MARC
Melkassa-2	2004	130	170-190	4.5-5.5	4-4.5	600-1700	-	MARC
Melkassa-6Q	2008	120	-	3-4	-	-	500-800	MARC

Source: MOAR 2008

Mung bean Variety	Years of Release	Days to maturity	Yield(t/ha)		Altitude (m)	Rainfall (mm)	Released institute
			On-station	On-farm			
(N-26)-Rasa	2011	65-80	0.8-1.5	0.5-1.0	900 – 1670	350-550	MARC
NVL-1	2014	60-70	0.75-1.5	-	450-1670	350-750	MARC
Shewa-robit	-	-	-		-	-	Local

Source: (MOARD, 2008; MOA, 2011)

Treatments and Expermental Design

Treatments consisted of factorial combinations of three varieties of maize (Melkassa-1, Melkassa-2 and Melkassa 6Q) and thee varieties of mung bean (N-26, NVL-1 and Shewa-Robit) and their respective sole crops. The treatments were laid out in a randomized complete block design with three replications. The gross plot size was 3.75 m x 3.5 m (13.125 m^2) accommodating five rows of maize. The space between plots and blocks were 0.5 m and 1m, respectively. The

net plot size was 1.5 m x 3.5 m (5.25 m^2) accommodating three rows of sole and intercropped maize. For the sole mung bean, the gross plot size was 3 m 1 m (3 m^2) accommodating ten rows and the net plot size was 2.1 m x 1.4 m (2.94 m^2) accommodating eight rows of mung bean. The total experimental area was 28*34 m (952 m^2).

Experimental procedure

The experimental field was ploughed and harrowed by a tractor to get a fine seedbed and leveled manually before the field layout was made. Both maize and mung bean varieties at two locations were planted simultaneously on July 9. Spacing for sole and intercropped maize was 75× 25 cm and for sole mung bean was 30×10 cm. For intercropped mung bean 50cm x 10cm, 25cm x 10cm and 105cm x 10cm for 1mz:1mn, 1mz:2mn and 2mz:1mn row arrangements, respectively. Two seeds per hill of both maize and mung bean were planted and thinned to one plant per hill one week after emergence. At planting, full dose of Di-ammonium phosphate (18% N, 46% P_2O_5) at the rate of 100 kg ha^{-1} was applied uniformly into all plots including sole maize. Half of nitrogen in the form of urea (46% N) at the rate of 50 kg ha^{-1} was applied into sole maize and maize/mung bean intercropped plots at the time of planting and the remaining half N was applied at knee height growth stage of maize. Nitrogen at the rate of 18kg/ha of Urea was applied to sole mung bean as starter since it cannot start N fixing immediately after planting. Hand hoeing and weeding were done as required. Both maize and mung bean were harvested from the net plot after they attained their normal harvest maturity.

Crop data collected

Phenological parameters

Maize component

Plant and ear heights were measured from the ground to the base of the tassel and from the ground to the base of the first cob, respectively. Leaf area was estimated by using the formula length x maximum width x 0.75 from leaves of five sample plants in each plot just after tasseling (Mokhtarpour *et al*, 2010). Then leaf area index was calculated as the ratio of leaf area to ground area occupied by the respective plants.

Mung bean component

Leaf area of five randomly selected plants from each plot was measured by using leaf area meter (model LI-3000A) at flowering for both locations. Leaf area index was calculated by dividing the leaf area to the ground area occupied by the plants. Plant height and number of primary branches per plant were recorded at physiological maturity from five randomly selected plants.

Plant growth parameters

Maize component: plant and ear heights were measured from randomly selected five plants. Measurement was

taken from ground to base of tassel and from ground to base of the first cob for plant and ear height, respectively. Leaf area was estimated by using the formula" length x width x 0.76" from leaves of five sample plants in each plot just after tasseling (Mokhtarpour *et al.* 2010). Leaf area index was calculated as the ratio of leaf area to the ground area occupied by the respective plants.

Mung bean component: leaf area of five randomly selected plants from each plot was measured by using leaf area meter (model LI-3000A) at flowering. The leaf area index was calculated by dividing the leaf area by the ground area occupied by the plants. Plant height and number of primary branches per plant were recorded during physiological maturity from five randomly selected plants.

Yield components and yield

Maize component

Cob diameter and number of kernels per cob were determined from five randomly selected cobs from each plot. Grain yield was taken from the three middle rows after shelling the sun-dried ears. Thousand kernels weight was measured from bulk of the threshed grains and the weight was adjusted to 12.5% moisture content. The grain was weighed using sensitive balance and the moisture content was determined by electronic moisture tester and the weight was adjusted to 12.5% moisture content. Aboveground biomass was also weighed after harvesting and sun drying to constant weight. Harvest index of maize was calculated as a ratio of the grain yield to the total biomass yield.

Mung bean component

Number of pods per plant was recorded from five randomly selected plants of each plot at harvest. Number of seeds per pod was recorded from 20 randomly selected pods of the five selected sampled plants. Grain yield and hundred grain weight were determined after grains were sun dried, weighed and adjusted to 10% moisture content. Aboveground biomass was also taken at physiological maturity stage from 10 plants randomly taken from each plot after sun drying to a constant weight. The dry biomass per plant was then multiplied by the total number of plants per net plot and was converted into kg ha^{-1}. This value was used to calculate the harvest index as well. Harvest index was calculated as a ratio of grain yield to the total biomass yield.

Evaluation of system productivity

Intercrop productivity was determined by land equivalent ratio (LER) (Willey, 1979), maize equivalent yield (MEY) (Anggarda *et al.*, 2015); and Gross monetary value (GMV). Land equivalent ratio is the amount of land required in sole cropping to obtain the same yield as in the intercrop. It was determined as:

$LER= (Y_{MB}/Y_M) + (YBM/Y_B)$

Where Y_{MB} is intercrop maize grain yield (kg ha^{-1}), Y_M is average sole crop grain yield of maize varieties (kg ha^{-1}), Y_{BM} is intercrop mung bean grain yield (kg ha^{-1}) and Y_B is average sole crop grain yield (kg ha^{-1}) of the mung bean varieties.

Maize equivalent yield is the sum of maize yield in the intercrop system and the converted mung bean yield and was compared with sole crop maize yield. Maize was the main crop; therefore, yield of the mung bean in the intercrop system was converted to maize yield by multiplying the mung bean yield with mung bean/maize price ratio. It was calculated using the following formula:

$$EY_M = Y_{MB} + (Y_{BM} \times P_B/P_M)$$

Where EY_M is maize equivalent yield, YML = intercrop maize grain yield (kg ha^{-1}); Y_{BM} = intercrop mung bean grain yield (kg ha^{-1}); P_M = price of maize grain kg $^{-1}$; P_B = price of mung bean grain kg^{-1}.

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Gross monetary value (GMV) is used to calculate the economic advantage of intercropping as compared to sol cropping. Gross monetary value was calculated as the product of yield of the component crops multiplied by their respective unit price. The total values obtained from the component crops were used to indicate the Gross Monetary Value. To estimate the GMV of component crops, maize grain yield was valued at an average open market price of 10.00 ETB kg^{-1}, and mung bean at 30.0 ETB kg^{-1} at the time of crop harvest.

Data analysis

The data were subjected to analysis of variance (ANOVA) using SAS version 9 software program (SAS, 2002). Homogeneity of variances was tested using the F-test as described by Gomez and Gomez (1984) and since the F-test showed homogeneity of the error variances of the parameters of the two locations, combined analysis of variance was used. Means were compared using Least Significant Difference (LSD) test at 5% level of significance where significant differences existed among the factors.

Results and Discussion

Maize Component

Phenology and growth parameters

The main effects of location and variety combinations did not show a significant effect on days to tasseling and silking however days to maturity highly significantly ($p<0.01$) affected by the main effects of location and varietal combination of component crops. The interaction of main effects showed non-significant effect on days to tasseling, silking and maturity of maize. Significantly longer days to maturity (115.7) was recorded from Melkassa (Table 4). The possible reasons for longer days to maturity at MARC might be related to extended vegetative growth as a result of

relatively adequate moisture availability.

Regarding varieties, the longest days to maturity (128) was recorded for maize variety Melkassa-2 intercropped with mung bean variety Shewa-Robit while the shortest days to maturity (84) was for maize variety Melkassa-2 intercropped with mung bean var. NVL-1. This might be due to the genetic difference of the crop on days to maturity (Table.1). Opposing this result, Wondimu *et al.*, (2016) stated that days to maturity of maize were not significantly influenced by intercropped soybean varieties.

On the other hand, cropping system had no significant effect on days to maturity of maize varieties which could be due to maize is more aggressive competent than mung bean for growth resources. In agreement with this result, Molla and Getachew (2018) did not found a significant effect of cropping system on maturity of maize when intercropped with fenugreek, field pea and haricot bean. Abraha (2013) also reported no significant effect of cropping system on days to maturity of maize in maize/cowpea intercropping

Table 3. Days to maturity (DM), plant height (PH), ear height (EH) and leaf area index (LAI) of maize intercropped with mung bean as influenced by the main effects of location, varieties and cropping systems.

Treatments	Days to Maturity(DM)	PH (cm)	EH (cm)	LAI
Location				
Melkassa	115.7^{a}	176.0^{a}	112.8^{a}	3.4^{a}
Fedis	104.9^{b}	150.9^{b}	93.3^{b}	2.9^{b}
Significance	**	**	**	**
LSD (0.05)	2.9	13.1	10.3	0.2
Intercrops				
Melkassa-1 x N-26	85^{c}	135.1^{b}	86.5^{c}	2.8^{bc}
Melkassa-1 x NVL-1	84^{c}	137.0^{b}	89.3b^{c}	2.7^{c}
Melkassa-1 x Shewa Robbit	85.2^{c}	135.0^{b}	86.5^{c}	2.7^{c}
Melkassa-2 x N-26	127.7^{a}	189.3^{a}	112.0^{a}	3.6^{a}
Melkassa-2 x NVL-1	127.6^{a}	182.6^{a}	106.4^{abc}	3.4^{a}
Melkassa-2 x Shewa Robbit	128^{a}	185.7^{a}	110.0^{ab}	3.5^{a}
Melkassa 6Q x N-26	118.7^{b}	164.9^{a}	110.5^{ab}	3.3^{a}
Melkassa 6Q x NVL-1	117.8^{b}	171.3^{a}	112.1^{a}	3.2^{ab}
Melkassa 6Q x Shewa Robbit	118.8^{b}	170.1^{a}	114.4^{a}	3.2^{abc}
Varieties				
Melkassa-1	85^{c}	124.8^{b}	86.6^{c}	2.9^{bc}
Melkassa-2	129.1^{a}	182.8^{a}	107.4^{abc}	3.6^{a}
Melkassa-Q	119.7^{b}	165.3^{a}	108.3^{ab}	3.3^{a}
Significance	**	**	*	**
LSD (0.05)	6.1	27.8	21.7	0.5
CV (%)		14.5	18	12.3
Cropping system				
Sole	115.2a	152.8	93.7	3.5^{a}
Intercropped	110.3	163.4	103.1	3.2^{b}
Significance	NS	NS	NS	*
LSD (0.05)	NS	NS	NS	0.2
CV (%)	18	17.9	17.9	13.9
Interaction (L x IC)	NS	NS	NS	NS

Means within the same column followed by the same letter or by no letters of each factor do not differ Ns, *, and ** = Non-significant, significant at 5% and significant at 1% probability

Combined analysis of data over locations revealed that main effects of location and varieties of intercropped mung bean had significant ($p<0.01$) effect on plant and ear height of maize varieties However, their interaction showed non-significant effect. The tallest plant (176 cm) and ear height (112.8 cm) were recorded at Melkassa than at Fedis (Table 3). Reasons for plant and ear height differences might be due to agro ecological variation

Among the varieties, the tallest plant and ear heights, 189.3 and 112 cm, respectively, were recorded for the maize variety Melkassa-2 intercropped with mung bean variety N-26, which were in statistical parity with same variety intercropped with the other mung bean varieties. However, 40.2% and 29.5% reductions in plant and ear height, respectively, were recorded for maize variety Melkassa-1 intercropped with mung bean variety Shewa Robbit (Table 3). The possible reason for reduction in plant and ear height in melkassa-1varieties might be due to genetic variation and less competitiveness of this variety than companion crop. Consistent with this result, Wubshet *et al*. (2020) reported significant effect varietal on plant height in sorghum when intercropped cowpea. In contrast to this result, Teshome *et al.* (2016), obtained that, plant height of intercropped maize was not significantly affected by the main effect of soybean varieties in maize/soybean intercropping due to compatibility of variety of companion crop to exploit common growth resources.

Moreover, cropping system had no significant effect on Plant and ear height of maize. In line with this result, Wolde (2015) also found a non-significant effect of cropping system on plant and ear height of maize in maize/haricot bean intercropping. In contrast to this result, Belsti *et al.* (2016) reported significantly taller height of maize from intercropped maize in maize/haricot bean intercropping.

Leaf area index (LAI) was significantly ($p<0.01$) affected by the main effects of location and varieties. However, their interaction showed no significant effect (Table 3) With this regard, 16.6% increments in LAI was recorded from MARC. The probable reasons might be related to favorable climatic and soil conditions. Among thee combinations of the varieties, the maximum LAI (3.62) was recorded for maize variety Melkassa-2 intercropped with mung bean variety N-26 and it was statistically at par with same variety and Melkassa 6Q intercropped with the three varieties of mung bean while the lowest LAI (2.73) was recorded for maize variety Melkassa-1 intercropped with mung bean varieties NVL-1 and Shewa Robbit. The possible reason for higher LAI for maize variety Melkassa-2 might be related to highest number of leaves produced per plant and extended maturity of this variety benefits to continue its vegetative growth after

companion crop harvested. In agreement with this result, Wubshet *et al.* (2020) found significant effect of varieties of intercropped cowpea on leaf area index of sorghum.

The analysis of variance showed that cropping system significantly ($p<0.05$) affected LAI of maize where significantly higher LAI (3.5) was recorded due to sole cropping. The higher LAI under sole cropping might be due to absence of inter-specific competition for growth resources. This result is in agreement with the finding of Alom *et al.* (2010) who stated that, LAI of maize decreased due to intercropping, but there was no remarkable difference between the sole and intercrop maize in maize/ground nut intercropping.

Yield components and yield

The main effects of location ($p<0.05$) and variety ($p<0.01$) showed significant effect on the number of ears per plant (Table 4). Number of ears per plant increased by 10.28% at MARC over Fedis due to favorable climatic condition resulted exceeded growth performances (Table 4). Among the varieties, the highest number of ears per plant (1.2) was recorded from maize variety Melkassa-2 intercropped with mung bean variety N-26 and it was statistically at par with same variety intercropped with the other mung bean varieties while the lowest number of ears per plant (1.0) was recorded from variety Melkassa-1 intercropped with mung bean variety NVL1. The reason for such difference in the number of ears per plant might be due genetic variability and all varieties have no equal competitiveness for growth resources with companion crop of which a variety with short maturity period (Melkassa-1) suffered the most with competition since no temporal complementary effect between companion crops. Supporting this result, Teshome *et al. (*2016) stated that effect of soybean varieties was significant on number of ears per plant. Jibril *et al.* (2015) also found significant effect of variety of intercropped common bean on cob number per plant of maize.

Table 4. Number of ears per plant, cob diameter and grains per cob of maize intercropped with mung bean as influenced by the main effects of location, varieties and cropping systems.

Treatments	No. of ears per plant	Cob diameter (cm)	Number of grains per cob
Location			
Melkassa	1.2[a]	4.3[a]	474.5[a]
Fedis	1.1[b]	3.8[b]	422.3[b]
Significance	*	**	**
LSD (0.05)	1.07[b]	3.8[b]	422.3[b]
Intercrops			
Melkassa-1 x N-26	1.07[cde]	3.5[b]	373.7[c]
Melkassa-1 x NVL-1	1.0[e]	3.5[b]	359.1[c]
Melkassa-1 x Shewa Robbit	1.0[de]	3.4[b]	369.9[c]
Melkassa-2 x N-26	1.2[a]	4.5[a]	516.0[a]
Melkassa-2x NVL-1	1.1[abc]	4.4[a]	502.4[ab]
Melkassa-2 x Shewa Robbit	1.2[ab]	4.4[a]	512.0[ab]
Melkassa-6Q x N-26	1.1[abcd]	4.3[a]	473.1[ab]
Melkassa-6Q x NVL-1	1.1[bcde]	4.1[a]	461.9[b]
Melkassa-6Q xNVL-1	1.1[cde]	4.2[a]	467.5[ab]
Varieties			
Melkassa-1	1.04[cd]	3.5[b]	378.5[ab]
Melkassa-2	1.2[a]	4.5[a]	5.5.6[a]
Melkassa-6Q	1.1[abc]	4.3[a]	469.4[ab]
Significance	**	**	**
LSD (0.05)	0.09	0.53	52.39
CV (%)	7.0	11.2	10.0
Cropping system			
Sole	1.14	4.37	472.7
Intercrop	1.09	4.04	448.4
LSD (0.05)	NS	NS	NS
CV (%)	1.09	4.04	4.48
Interaction (L x IC)	NS	NS	NS

Means within the same column followed by the same letter or by no letters of each factor do not differ Ns, *, and ** = Non-significant, significant at 5% and significant at 1% probability

There was no significant difference between sole and intercropped maize on number of ears per plant which might indicate that this parameter is more affected by varietal characteristics than management practices. In conformity with this result, Molla and Getachew (2018) did not find significant difference between sole and intercropped maize on cob number per plant in maize, fenugreek, field pea and haricot bean intercropping. Similarly, Demissie *et al.* (2018) also did not find significant difference on number of cob per plant between sole and intercropped maize in maize/common bean intercropping.

Cob diameter and number of grains per cob were significantly ($p<0.01$) affected by location and variety while the interaction of location and variety combination had no significant effect on cob diameter and grains per cob (Table 4). Fedis recorded 11.6 % and 11.0% reductions in cob diameter and grains per cob, respectively. This might be related to depressed growth performance mainly LAI, resulted lower rate of assimilate translocation to seed setting.

Among the varieties, 32.4% and 43.7% increments in cob diameter and grains per cob, respectively were recorded for maize variety Melkassa-2 intercropped with mung bean variety

N-26 and it was statistically at par with same variety intercropped with the other mung bean varieties while significantly lower cob diameter and number of grains per cob were recorded for maize variety Melkassa-1 intercropped with all the three mug bean varieties (Table 4). The reason for lower cob diameter and number of grains per cob for variety Melkassa-1 might be related to absence of temporal complementary which resulted in severe competition for growth resources. In agreement with this result, Teshome *et al.* (2016) reported that number of kernels per cob of the associated maize was highly significantly affected by the intercropped soybean bean varieties.

Cropping system had no significant effect on cob diameter and number of grains per cob (Table 4). In agreement with this result, Jibril *et al.* (2015) did not find significant effect of cropping system on number of kernels per cob. In contrast to this result, Tohura *et al.* (2014) obtained significantly higher number of grains per cob (376.70) from sole than intercropping (335.5) in maize/mung bean intercropping. Teshome *et al.* (2016) also found significantly higher number of ears per plant (2.33) due to sole cropping than intercropping (2.1) in maize/soybean intercropping.

Table 5. Thousand kernels weight, Grain yield, Biomass yield and Harvest index of maize intercropped with mung bean as influenced by the main effects of location, varieties and cropping systems.

Treatments	1000 kernels weight (g)	Grain yield (t ha^{-1})	Biomass yield (t ha^{-1})	Harvest index (%)
Location				
Melkassa	227.4^{a}	4.1^{a}	13.6^{a}	29.9
Fedis	211.8^{b}	3.5^{b}	11.3^{b}	30.6
Significance	*	**	**	NS
LSD (0.05)	12.87	0.22	0.63	NS
Intercropps				
Melkassa-1x N-26	214.6	3.0^{c}	9.6^{c}	31.6
Melkassa-1 x NVL	204	2.8^{c}	9.5^{c}	30
Melkassa-1 x Shewa Robbit	209.6	2.9^{c}	9.8^{c}	29.7
Melkassa-2 x N-26	228.4	4.8^{a}	15.2^{a}	31.4
Melkassa-2 x NVL1	221.5	4.6^{a}	15.0^{a}	31
Melkassa-2 x Shewa Robbit	226.6	4.7^{a}	15.2^{a}	31
Melkassa-6Q x N-26	226.6	3.8^{b}	13.9^{b}	28.9
Melkassa-6Q x NVL1	221	3.5^{b}	12.1^{b}	29.4
Melkassa-6Q x Shewa Robbit	223.9	3.9^{b}	12.7^{b}	29
Varieties				
Melkassa-1	214.2	3.0^{c}	10.2^{bc}	30.2
Melkassa-2	229.2	4.8^{a}	15.3^{a}	31.3
Melkassa-6Q	225.8	3.8^{b}	12.9^{b}	28.9
Significance	NS	**	**	NS
LSD (0.05)	NS	0.46	1.34	NS
CV (%)	10.6	10.5	9.2	8.6
Interaction (L x IC)	NS	NS	NS	NS
Cropping system				
Sole	233.5^{a}	4.2	13.8^{a}	29.9
Intercrop	220.9^{b}	3.8	12.5^{b}	30.2
Significance	*	NS	*	NS
LSD (0.05)	11.4	NS	1.32	NS
CV (%)	9.4	21.1	19	8.5

Means within the same column followed by the same letter or by no letters of each factor do not differ Ns, *, and ** = Non-significant, significant at 5% and significant at 1% probability

Thousand kernels weight was significantly ($p<0.05$) affected by one of main effects location, while neither varieties nor their interaction had significant effect (Table 5). Thousand kernels weight increased by 7.8% at MARC due to better growth performance mainly leaf area resulted maximum photosynthetic assimilate translocation to grain filling, resulted vigorous seed size. Similarly, Wondimu *et al.* (2016) stated that, soybean varieties non-significantly affected thousand kernel weights of maize in maize/soybean intercropping.

Cropping system had significant ($p<0.05$) effect on thousand kernels weight of maize (Table 5).
Where 5.4% reduction in thousands kernel weight recorded due to intercropping. Probable reasons for lighter kernels weight might be due to the fact, depressed growth performance mainly LAI, resulted low rate of assimilate translocation to grain

filling that leads to formation of shriveled and small sized kernels. In line with this result, Tohura *et al.* (2014) obtained the highest thousand kernels weight of maize (230.7 g) from sole over intercropping (225 g) in maize/mung bean intercropping. Similarly, Odigbo *et al.* (2013) obtained significantly highest thousand kernels weight of maize from sole (206.4g) than intercropped with cowpea (181.2g). Demissie *et al.* (2018) also obtained highest thousand grains weight (365 g) from sole cropped over intercropped maize (350g) in maize/common bean intercropping.

The grain yield and aboveground biomass yields of maize were significantly ($p<0.01$) affected by location and variety (Table 5). With this regard, grain and biomass yield was increased by 17.4% and 20.4%, respectively at MARC as result of exceeded growth and yield attributes. With respect to the effect of the varieties, 11.6% and 36.8% reduction in grain and biomass yield, respectively were recorded for maize variety Melkassa-1 intercropped with mung bean variety NVL and the values were in statistical parity with the values obtained with same maize variety intercropped with the other mung bean varieties. The possible reasons for reduction in grain and biomass yield might be the comulative effect of the least yield and yield related papameters mainly grians per cob, thousand kernels weight and cob diameter was recorded from this variety. In contrast, significantly lowest grain and biomass yields were recorded for the maize variety Melkassa-1 as result of poor competitive effect for common growth resources with companion crop. In line with this result, Teshome *et al*. (2016) obtained significant effect of varieties of intercropped soybean on aboveground biomass yield of maize. Similarly result that obtained by Wubshet *et. al*. (2020) stated that dry biomass of sorghum was highly significant among sorghum varieties in sorghum/cowpea intercropping. Opposing this, Zerihun (2011) reported no significant difference on dry biomass of the intercropped maize due to the associated soybean varieties.

Cropping system had also significant ($p<0.05$) effect on biomass yield of maize, but not on the grain yield (Table 5). With this regard, 9.4% reduction in biomass yield was recorded due to intercropping (Table 5). Probable reasons might be the cumulative effect of relatively depressed growth and yield attributes as result of sever inter and intra species competition between companion crops. In agreement with this result, Belsti *et al.* (2016) found 9.3% biomass yield reduction due to intercropping in maize/haricot bean intercropping. In contrast to this result, Tohura *et al.* (2014) stated that grain yield of maize reduced in intercropped situation (3966kg/ha) compared to sole maize(4488kg/ha) in maize/mung bean intercropping.

of variance showed that neither the main effect of location and variety nor their interaction had significant effect on harvest index of maize. Moreover, cropping system also had no significant effect on harvest index. This result was in agreement with the result of Tohura *et al.* (2014) who found non-significant effect of cropping system on harvest index of maize in maize/mung bean intercropping. Tamiru (2014); and Belsti *et al.* (2016) also found non-significant effect of cropping system on harvest index of maize under maize-haricot bean intercropping system.

Mung bean Component

Phenology and growth parameters

Days to maturity was significantly ($p<0.05$) affected by the main effects of location and variety while interaction had no significant effect (Table 7). Mung bean planted at Melkass took significantly extended maturity period (75.5 days) over Fedis (66.9 days). Probable reasons for extended days to maturity might be related to agroecological variation mainly soil and moisture.

Mung bean variety Shewa Robit intercropped with maize variety Melkassa-1 took extended maturity period (73.8) while the shortest days to maturity (69 days) was recorded for mung bean variety NVL-1 intercropped with maize variety Melkassa-2 (Table 7). Probable reasons might be relared to since Shewarobbit is local variety already adopted agroecology thus it became aggressive competator over other mung bean varieties. In line with this result, Tamiru (2013) found significant difference among the varieties of soybean on days to maturity in maize/soybean intercropping.

Cropping system showed significant ($p<0.01$) effect on days to maturity of mung bean. Sole cropped mung bean took extended days of maturity (75.2) was attributed to the absence of interspecific competition for water and nutrients thereby extending its vegetative growth leading to delayed maturity. In line with this result, Anisha *et al.*, (2015) reported significantly extended days to maturity of sole cropped cowpea in maize/cowpea intercropping.

Location had significant ($p<0.05$) effect on mean plant height, however, variety and interaction of location and variety were non-significant on mean plant height of mung bean. Significantly taller plants (48.0 cm) which 8.5% increase in plant was recorded at Melkassa than at Fedis (Table 6) which might be related to adequate rainfall during the growing season at the former site.

Cropping system had significant ($p<0.01$) effect on plant height of mung bean. Sole cropped mung bean increased by 11.7% over intercropping (Table 6) which could be due to sever shading effect and growth resources competition by the

associated maize in intercropping. In conformity with this result, Arshad *et al*. (2020) recorded the highest plant height of mung bean from sole cropping (56.7 cm) than in intercropping (45.2 cm) with maize. Onuh *et al*. (2011) also recorded the tallest mean plant height (47 cm) from sole cropped mung bean than in intercropping (41 cm) in maize/mung bean/melon intercropping.

Table 6. Days to maturity, Plant height, Leaf area index and primary branches per plant of mung bean as influenced by the main effects of location, varieties and cropping systems.

Treatments	Days to Maturity	Plant height (cm	Leaf area Index	Primary branches per plant
Location				
Melkassa	75.4[a]	48.0[a]	2.8	4.8[a]
Fedis	66.9[b]	44.2[b]	2.6	4.2[b]
Significance	**	*	NS	**
LSD (0.05)	2.2	4,1	NS	0.41
Intercrops				
N-26 x Melkassa-1	72.5[ab]	46.7	2.8	6.5[a]
N-26 x Melkassa-2	73.7[a]	49.1	2.6	5.6[ab]
N-26 x Melkassa-6Q	73.7[a]	45.2	2.6	5.3[bc]
NVL1 x Melkassa-1	70[ab]	45.1	2.7	4.1[de]
NVL1 x Melkassa-2	69[b]	42.7	2.5	3.7[def]
NVL1 x Melkassa-6Q	69.3[ab]	43.7	2.5	3.5[ef]
Shewa Robbit x Melkassa-1	69.7[ab]	47.6	2.8	4.5[cd]
Shewa Robbit x Melkassa-2	69.3[ab]	44.7	2.6	4.1[de]
Shewa Robbit x Melkassa-6Q	73.8[a]	50	2.6	3.8[def]
Varieties				
N-26/Rassa	74.4[a]	46.6	2.9	6.7[a]
NVL	70.3[a]	45.0	2.8	4.5cd
Shewarobbit	71.1[ab]	47.3	2.9	5.1[ab]
Significance	*	NS	NS	**
LSD (0.05)	3.6	NS	NS	0.87
CV (%)	5.5	15.5	12.8	16.6
Interaction (L x IC)	NS	NS	NS	NS
Cropping system				
Sole	75.2[a]	51.4[a]	3.6[a]	8.1[a]
Intercrop	70.9[b]	46.0[b]	2.7[b]	4.3[b]
Significance	**	**	**	**
LSD (0.05)	2.3	3.47	0.16	0.66
CV (5%)	5.9	13.5	10	23

Means within the same column followed by the same letter or by no letters of each factor do not differ Ns, *, and ** = Non-significant, significant at 5% and significant at 1% probability

The main and interaction effects of location and varieties showed non-significant effects on leaf area index (LAI) of mung bean. However, cropping system had significant effect ($p<0.01$) on LAI of mung bean. Leaf area index increased by 33.3% due to sole cropping (Table 6). The probable

reasons might be related to increased number and size of individual leaves production as result of adequate moisture and space. In line with this result, Belsti *et al.* (2016); and Alemayehu *et al.* (2018) obtained significantly higher LAI of sole haricot bean than intercropped haricot bean in maize/haricot bean intercropping.

The number of branches per plant was significantly ($p<0.01$) affected by the main effects of location and varieties. However, their interaction effect was not significant. Whereas 14.3% increase in branches per plant was recorded from MARC (Table 6) possibly due to relatively better soil moisture that resulted in better growth performance of the crop at Melkassa.

Among the mung bean varieties, the highest number of primary branches per plant (6.5) was recorded for variety N-26 intercropped with maize variety Melkassa-1 and it was statistically at par with same variety intercropped with maize variety Melkassa-2 while the lowest number of branches (3.5) was recorded for variety NVL-1 intercropped with maize variety Melkassa 6Q (Table 6). The probable reason for production of less number of branches per plant by the mung bean in association with maize variety Melkassa 6Q might be related to high competitive effect of intercropped maize variety Melkassa -6Q for common growth resources In line with this result, Masa *et al.* (2017) found significant differences between varieties of common bean on number of primary branches per plant in maize/common bean intercropping.

The number of primary branches per plant was significantly ($p<0.05$) affected by cropping system where 46.9% reduction in number of branches per plant was recorded due to intercropping (Table 6). The reason for reduction on number of branches under intercropping might be related to presence of adverse shading effect and sever inter and intra species competition for growth resources. In conformity with this result, Onuh *et al.* (2011 also obtained higher number of branches (7.5) in sole cropped mung bean than in intercropping (5.4) in maize/melon/mung bean inter-cropping.

Yield component and yield

The analysis of variance showed that number of pods per plant was significantly ($p<0.05$) affected by one of main effects variety. However, interaction of location and variety was non-significant (Table 7). The highest number of pods per plant (10.5) was recorded from variety N-26 intercropped with maize variety Melkassa-1 and it was statistically at par with mung bean variety Shewa Robbit intercropped with maize variety Melkassa-1 and Melkassa-2 while the lowest number of pods per plant (8.1) was obtained when mung bean variety NVL-1 was intercropped with maize variety Melkassa 6Q. The possible reason for few number of pods per plant on variety NVL might be relatively depressed growth performance when intercrop with all maize varieties as result of poor competitive effect with companion crop.

Similarly, Setegn *et al.* (2006) indicated that number of pods per plant was reduced by 65% and 55% when climbing bean was intercropped with maize varieties. Wondimu *et al.* (2016) also reported significant reduction in the number of pods per plant of the intercropped soybean varieties in maize/soybean intercropping.

Cropping system also significantly ($p<0.01$) affected number of pods per plant. With this regard intercropped mung bean recorded 38.9% reduction on number of pods per plant.(Table 7). The probable reasons for reduction in pod number might be related to adverse shading effect by taller plants and presence of inter and intra species competition for common growth resources. In agreement with this result, Azim *et al*. (2012) found significantly higher number of pods from sole mung bean (17.32) than intercropped mung bean (9.07) in maize/mung bean intercropping. Onuh *et al.* (2015); and Tuhura *et al.* (2014) also obtained significantly highest number of pods per plant of 6.5 and 15.67, respectively, in sole cropped mung bean than in maize/mung bean intercropping.

Table 7. Number of pod per plant, number of seed per pod and hundred seed weight of mung bean intercropped with maize as influenced by the main effects of location, varieties and cropping systems

Treatments	No. of pods per plant	No. of seeds per pod	100 grains weight (g)
Location			
Melkassa	9.4	7.8	6.1[a]
Fedis	8.8	7.2	5.8[b]
Significance	NS	NS	*
LSD (0.05)	NS	NS	0.35
Intercrops			
N-26 x Melkassa-1	10.5[a]	8.6[ab]	6.6[a]
N-26 x Melkassa-2	9.3[ab]	7.9[abc]	5.9[ab]
N-26 x Melkassa-6Q	9.0[ab]	7.7[bc]	5.8[b]
NVL1 x Melkassa-1	9.5[ab]	6.8[cd]	6.3[ab]
NVL1 x Melkassa-2	9.0[ab]	5.8[d]	5.8[b]
NVL1x Melkassa-6Q	8.1[b]	5.3[d]	5.6[b]
Shewa Robbit x Melkassa-1	9.7[ab]	8.4[ab]	6.1[ab]
Shewa Robbit x Melkassa-2	8.9[ab]	7.8[bc]	5.8[b]
Shewa Robbit x Melkassa-6Q	8.3[b]	9.4[a]	5.7[b]
Varieties			
N-26/Rassa	11.5[a]	9.0[a]	6.7[a]
NVL	9.8[ab]	6.9[cd]	6.4[ab]
Shewarobbit	10.4[a]	8.5[ab]	6.3[a]
Significance	*	**	*
LSD (0.05)	1.72	1.53	0.74
CV (5 %)	16.1	17.3	10.7
Cropping system			
Sole	14.9[a]	10.6[a]	8.0[a]
Intercrop	9.1[b]	7.5[b]	5.9[b]
Significance	**	**	**
LSD (0.05)	0.9	0.83	0.42
CV (5%)	15.7	18.5	11.9
Interaction (L x IC)	NS	NS	NS

Means within the same column followed by the same letter or by no letters of each factor do not differ Ns, *, and ** = Non-significant, significant at 5% and significant at 1% probability

Number of seeds per pod was significantly ((p<0.01) affected by one of main effects variety, however location and their interaction was non-significant (Table 7). The highest number of seeds per pod (9.4) was recorded for mung bean variety Shewa Robbit intercropped with maize variety Melkassa-6Q and it is statistically at par with variety N-26 intercropped with maize varieties while the lowest number of seeds per pod (5.3) was recorded for mung bean variety NVL-1 intercropped with maize variety Melkassa 6Q (Table 7). Probable reasons for reduction in seed per pod of variety NVL might be related to poor competitiveness over companion maize varieties. In line with this result Adigbo *et al.* (2013) stated that thousand seed weight of cowpea significantly affected by variety in maize/cowpea intercropping.

Number of seeds per pod was significantly ((p<0.01) affected by one of main effects variety, however location and their interaction was non-significant (Table 7). The highest number of seeds per pod (9.4) was recorded for mung bean variety Shewa Robbit intercropped with maize variety Melkassa-6Q and it is statistically at par with variety N-26 intercropped with maize varieties while the lowest number of seeds per pod (5.3) was recorded for mung bean variety NVL-1 intercropped with maize variety Melkassa 6Q (Table 7). Probable reasons for reduction in seed per pod of variety NVL might be related to poor competitiveness over companion maize varieties. In line with this result Adigbo *et al.* (2013) stated that thousand seed weight of cowpea significantly affected by variety in maize/cowpea intercropping.

Cropping system significantly (p<0.01) affected the number of seeds per pod where 29.3% reduction in seed per pod was recorded due to intercropping The reason for the reduction of seed per pod of mung bean might be attributed to shading of tall growing maize plants in which the receipt lower amount of incoming solar radiation adverse affected the rate of net photosynthesis and thereby poor translocation of photosynthates from source to sink leading to formation of few number of seeds per pod in intercropping. In agreement with this result, Tuhura *et al.* (2014) obtained higher number of seeds per pod (8.95) of mung bean due to sole cropping than intercropping (7.5) in maize/mung bean intercropping. Similarly, Azim *et al.* (2012) also found significantly higher number of seeds per pod of mung bean in sole (4.23) than in intercropping (3.51) in maize/mung bean intercropping.

Hundred grains weight of mung bean was significantly (p<0.05) affected by location and variety, but not by their interaction. With this regard, hundred grains weight was increased by 5.3% at MARC over Fedis (Table 7). Reasons for lighter hundred seed weight might be related to depressed growth performance mainly leaf area as result of moisture scarcity

throughout growth stages which resulted in low rate of photosynthetic assimilate translocation to grain filling and produced small seed size.

Among the varieties, mung bean variety N-6 intercropped with maize variety Melkassa-1 produced the heaviest hundred grains weight (6.6 g) while significantly lightest hundred seed weight (5.6 g) was for mung bean variety NVL-1 intercropped with maize variety Melkassa 6Q (Table 7). The heaviest hundred seed weight for variety N-6 might be because of inherent characteristics of the variety and due to mung bean variety N-26 benefited more from less competitiveness of maize variety Melkassa-1. Consistent with this result, Jibril *et al.* (2015) also reported a significant difference in hundred seed weight of common bean in maize-bean intercropping due to varietal difference. Likewise, Solomon (2015) also obtained significant differences in 100 seed weight among the soybean varieties in maize/soybean intercropping.

Cropping system also significantly ($p<0.01$) affected hundred grains weight of mung bean where 10.3% reduction in hundred grains weight was recorded due to intercropping (Table 7). This might be related to adverse shading effect and interspecies competition resulted depressed growth and low rate of assimilate translocation to grain filling and formation of shriveled and small sized seeds. In line with this result, Arshad *et al. (*2020), obtained the highest thousand grain weight of mung bean in sole cropping (43.5 g) than in intercropping with maize (36.8 g). Onuh *et al.* (2015); and Azim *et al.*, (2012) also obtained significantly higher thousand seed weight of 50 g and 39.3 g, respectively, of mung bean in sole cropping than in intercropping with maize.

The grain yield of mung bean was significantly ($p<0.05$) affected by main effects of location and varieties (Table 8). However, their interaction had no significant effect. Grain yield at MARC recorded 18.8% increments over Fedis due to favorable climatic condition (Table 8). With regard to variety, the highest grain yield (660.4 kg ha^{-1}) was obtained from mung bean variety Shewa Robbit intercropped with maize variety Melkassa-1 and it was statistically at par with mung bean variety N-26 intercropped with maize variety Melkassa-1 (638.3 kg ha^{-1}) while the lowest seed yield (360.4 kg ha^{-1}) was recorded for mung bean varieties NVL-1 and Shewa Robbit intercropped with maize variety Melkassa 6Q (Table 8). The possible reason for higher grain yield in mung bean varieties intercropped with maize variety Melkassa-1 might be related to less competitiveness of maize variety Melkassa-1 as evidenced from its low performance. In conformity with this result, Demissie *et al.* (2018) found significant differences among seed yields of common bean varieties in maize/common bean intercropping. Likewise, Wondimu *et al.* (2016) also

proved that grain yield per hectare of soybean was significantly affected by soybean varieties and nitrogen rates in maize/soybean intercropping.

With regard to variety, the highest grain yield (660.4 kg ha^{-1}) was obtained from mung bean variety Shewa Robit intercropped with maize variety Melkassa-1 while the lowest seed yield (360.4 kg ha^{-1}) was recorded for mung bean varieties NVL-1 and Shewa Robit intercropped with maize variety Melkassa 6Q (Table 10). This might be attributed to the inherent varietal characteristics in intercropping system and lifecycle of maize variety melkassa-1and companion crop mung bean was almost similar as result competition was sever throughout growing season with this regard maize variety melkassa-1 was less competitive than companion crop. This result was confirmed by Demissie *et al.*, (2018) who found significantly differences among seed yields of common bean varieties in maize/common bean intercropping. Wondimu *et al.*, (2016) also proved that grain yield per hectare of soybean was significantly affected by the main effect of soybean varieties and nitrogen rates in maize/soybean intercropping.

Cropping system had also significant ($p<0.01$) effect on grain yield of mung bean. Grain yield reduction by 60% was recorded due to intercropping. Probable reasons might be due to adverse shading effect by taller maize plants which depressed growth and yield attributes. In line with this result, Saleem *et al.* (2015) stated that, intercropping reduced the mung bean yield by 28% compared to sole cropping of mung bean in maize/mung bean intercropping. Similarly, Arshad *et al. (*2020); Onuh *et al.* (2015); and Tuhura *et al.* (2014) obtained significantly highest grain yield of sole mung bean in maize/mung bean intercropping.

Table 8. Grain yield, biomass yield and Harvest index of mung bean intercropped with maize as influenced by the main effects of location, varieties and cropping systems.

Treatments	Grain yield (kg ha^{-1})	Biomass yield (kg ha^{-1})	Harvest index (%)
Location			
Melkassa	530.9^{a}	1776.3^{a}	29.8
Fedis S	446.8^{b}	1525.1^{b}	29
Significance	**	**	NS
LSD (0.05)	45.6	144.2	NS
Intercrops			
N-26 x Melkassa-1	638.3^{a}	2141.8^{a}	29.9^{abc}
N-26 x Melkassa-2	534.4b	1793.3b	29.9^{abc}
N-26 x Melkassa-6Q	510.9b	1736.6b	29.3^{abc}
NVL1 x Melkassa-1	438.8^{bc}	1522.2^{bc}	28.5^{bc}
NVL1 x Melkassa-2	374.7c	1269.4c	29.3^{abc}
NVL1 x Melkassa-6Q	356.3c	1282.0c	27.8c
Shewa Robbit x Melkassa-1	660.4^{a}	2110.6^{a}	31.0^{a}
Shewa Robbit x Melkassa-2	530.9^{b}	1748.4^{b}	30.6^{ab}
Shewa Robbit x Melkassa-6Q	355.4^{c}	1251.9^{c}	28.4^{bc}
Varieties			
N-26/Rassa	748.4^{a}	1297.4^{a}	30.1^{ab}
NVL	578.5^{ab}	1953.4^{ab}	29.0^{abc}
Shewarobbit	738.4^{bc}	2350.0^{a}	31.2^{a}
Significance	**	**	*
LSD (0.05)	96.6	305.8	2.73
CV (%)	16.9	15.8	7.9
Cropping system			
Sole	1230^{a}	3900^{a}	31.5^{a}
Intercrop	490^{b}	1650^{b}	29.6^{b}
Significance	**	**	**
LSD (0.05)	0.74	2.11	1.19
CV (5%)	20.1	17.6	7.3
Interaction (L x IC)	NS	NS	NS

Means within the same column followed by the same letter or by no letters of each factor do not differ Ns, *, and ** = Non-significant, significant at 5% and significant at 1% probability

The aboveground dry biomass of mung bean component showed a highly significant ($p<0.01$) difference between the locations and among the varieties while the interaction effect was not significant. Significantly higher aboveground biomass (1776.3 kg ha^{-1}) was recorded at Melkassa (Table 8) than at Fedis. This might be due to the cumulative effect of higher growth and yield attributes recorded at Melkassa.

Among the varieties, the highest aboveground dry biomass (2141.9 kg ha^{-1}) was obtained for variety N-26 intercropped with maize variety Melkassa-1 while the lowest aboveground dry biomass (1251.8 kg ha^{-1}) was recorded for mung bean varieties Shewa Robbit intercropped with maize variety Melkassa 6Q (Table 8). The higher aboveground dry biomass of the mung bean varieties in association with maize variety Melkassa-1 could be attributed to less competitiveness from the associated

maize variety Melkassa-1 as observed from its poor growth and yield performance. In agreement with this result, Teshome *et al.* (2016) reported significant differences on aboveground biomass of soybean among the varieties in maize/soybean intercropping. Similarly, Zerihun (2011) reported variation on biological yield of soybean among varieties in maize/soybean intercropping.

Cropping system had highly significant ($p<0.05$) effect on the aboveground dry biomass of the mung bean. Significantly higher biomass yield (3900 kg ha^{-1}) was recorded from sole cropped mung bean than the intercropped mung bean (1650 kg ha^{-1}) (Table 8). In conformity with this result, Lyngdoh *et al.* (2020) stated a steep decline in biological yield of intercropped mung bean (0.6 t ha^{-1}) as compared to sole crop (1.5 t ha^{-1}) in maize/mung bean intercropping. Similarly, Azim *et al.* (2012) reported that maize-mung bean intercropping decreased mung bean biological yield by 21% as compared to mung bean mono cropping.

Harvest index of mung bean was significantly ($p<0.05$) affected by varieties while location and interaction of location by variety had no significant effect (Table 8). The highest harvest index (31.0%) was recorded for mung bean variety Shewa Robbit intercropped with maize variety Melkassa-1 while the lowest harvest index (27.8%) was recorded for mung bean variety NVL-1 intercropped with maize variety Melkassa 6Q (Table 8). The possible reason for highest harvest index for mung bean variety Shewa Robbit intercropped with maize variety Melkassa-1 might be related to less competitiveness of the maize variety which resulted in higher grain yield of mung bean. In line with this result, Teshome *et al.* (2015); and Zerihun (2011) reported highly significant difference among soybean varieties for harvest index in intercropping with maize.

Cropping system showed significant ($p<0.05$) difference on harvest index of mung bean. Higher harvest index (31.5%) was recorded for sole cropped mung bean than that of the intercropped mung bean (29.6%) (Table 8). The lower grain yield of mung bean in intercropping due to the shading of tall growing maize plants can be attributed to lower harvest index of the intercropped mung bean. In consistent with this result, Saleem *et al.* (2015) obtained higher harvest index (33.65%) for sole mung bean than the intercropped mung bean (30.52%) from maize-mung bean intercropping. Likewise, Berhane *et al.* (2015) reported higher harvest index (41.3%) for sole cow pea than the intercropped cow pea (28.4%) in sorghum/cow pea intercropping.

Productivity of the Intercropping System

Total land productivity is a basic consideration in evaluating intercropping system where land

holdings are very meager. For this purpose, Land equivalent ratio, maize equivalent yields and gross monetary return per hectare could be the better indicators of land productivity of inter cropping system.

Main effects of location and variety had significant effect on LER. Land productivity increased by 17.5 % at MARC due to relatively adequate moisture and soil fertility. With regarded to variety, the highest LER (1.57) was recorded from intercropping of maize variety Melkassa2 and mung bean variety N-26, and it was statistically at par with intercropping of maize variety Melkassa-2 and mung bean variety Shewarobbit, while the lowest LER (1.02) was from intercropping of maize variety Melkassa-1 and mung bean variety NVL-1 (Table 9). The probable reason for low land use efficiency might be related to highly competitive effect of mung bean variety NVL-1 over maize variety Melkassa-1 resulted in low grain yield of maize. In line with this result Yaa *et al.* (2017) ontained significant effect of sorghum varieties on LER in sorghum/common bean intercropping.

Table 9. Land equivalent ratio (LER), Maize Equivalent Yield (MEY) and Gross Monetary Return (GMR) as affected by location, varieties and cropping systems

Treatments	LER	MEY (kg ha^{-1})	GMR (ETB ha^{-1})
Location			
Melkassa	1.41^{a}	5690.0^{a}	56900^{a}
Fedis	1.2^{b}	4850^{b}	48500^{b}
Significance	**	**	**
LSD (0.05)	0.15	3687	5720
Varieties			
Melkassa-1xN-26	1.26^{c}	5020^{c}	50200^{c}
Melkassa-1xNVL	1.02^{e}	4120^{d}	41200^{d}
Melkassa-1xShewa Robbit	1.23^{cd}	4880^{c}	48800^{c}
Melkassa-2 xN-26	1.57^{a}	6390^{a}	63900^{a}
Melkassa-2 x NVL	1.4^{b}	5740^{b}	57400^{b}
Melkassa-2x Shewa Robbit	1.55^{a}	6290^{ab}	62900^{ab}
Melkassa-6Q x N-26	1.32^{bc}	5330^{bc}	53300^{bc}
Melkassa-6Q x NVL	1.13^{d}	4580^{cd}	45800^{cd}
Melkassa-6Q x Shewa Robbit	1.17^{cd}	4780^{c}	47800^{c}
Significance	**	**	**
LSD (0.05)	0.14	580	5880.5
CV (%)	9.4	11.5	10.2
Sole	1.0^{b}	4200^{b}	41600^{b}
Intercrop	1.3^{a}	5270^{a}	56300^{a}
Significance	*	*	*
LSD (0.05)	0.26	820.6	8200
CV (%)	7.9	12	11
Interaction (L x IC)	NS	NS	NS

Means within the same column followed by the same letter or by no letters of each factor do not differ Ns, *, and ** = Non-significant, significant at 5% and significant at 1% probability

Cropping system also significantly influenced LER, whereas higher LER (1.30) was recorded due to intercropping. Which means the intercrop system was 30% more productive compared to sole crop production. The possible reason for higher productivity of the intercropping system might be due to efficient utilization of growth resources by component crops and the intercropping advantage of nitrogen fixation and increased light use efficiency (Reddy, 2000). In line with this result, Tuhura *et al.* (2014) stated that intercropping maize with mung bean increased land use efficiency by 43%. Saleem *et al.* (2015) stated that, Maize/mung bean intercropping was more productive and efficient system in utilizing land compared to sole cropping.

ANOVA over locations indicated that there was significant effect of location and variety on maize equivalent yield. Fedis recorded 14.8% reduction in maize equivalent yield than MARC. The probable reasons might be related to sever resources competition for shortened growth resources. With regarded to variety, the highest maize equivalent yield (6390 kg ha^{-1}) was recorded from intercropping of maize variety Melkassa-2 and mung bean variety N-26 and it was statistically at par with intercropping of maize variety Melkassa-2 and mung bean variety Shewarobbit while the lowest maize equivalent yield (4120 kg ha^{-1}) was from intercropping of maize variety Melkassa-1 and mung bean variety NVL (Table 9). The probable reasons for reduction in maize equivalent yield might be related to aggressive competitive effect of mung bean variety NVL resulted lower yield of maize.

The intercrop system was significantly more productive than sole crop system with yield advantage of 20.3% when expressed as maize equivalent yield (Figure 2). The highest maize equivalent yield for the intercrop could be due to an additional yield of the mung bean and the relative increase in maize grain yield in the intercrop treatments compared to sole crop. Consistent with this result, Alemayehu *et al.* (2016) found higher maize equivalent yield (6.75 t ha^{-1}) of the intercropping system relative to sole-cropped maize in maize/common bean/lupin intercropping.

Main effects of location and variety had significant effect on gross monetary values. MARC recorded 17.3 % increment on gross income than Fedis. Significantly the highest gross monetary return (63900 ETB ha^{-1}) was recorded from intercropping of maize variety Melkassa-2 and mung bean variety N-26 and it was statistically at par with intercropping of maize variety Melkassa-2 with mung bean variety Shewarobbit, while the lowest gross monetary return (41500 ETB ha^{-1}) was from intercropping of maize variety Melkassa-1 and mung bean variety NVL. Moreover, significantly higher gross monetary return (56300 ETB ha-1) was recorded from intercropping due to due to additional benefits from mung bean without hampering the grain yield of maize. In line with this result, Tohura et al. (2014) obtained higher monetary returns from intercropping than sole maize, in

maize/mung bean intercropping. Kinde et al. (2015) also recorded higher monetary return from intercropping than sole cropping in sorghum/cowpea and sorghum/soybean intercropping.

Conclusion

Choosing of the right crop combination is very important in intercropping systems due to the fact that plant competition could be minimized not only by spatial arrangement, but also by combining those crops best able to exploit soil nutrients. The results of the study showed that maize variety Melkassa-2 in intercropping with mung bean produced significantly highest growth, yield components and yield. Similarly, mung bean variety N-26 in intercropping with maize produced significantly highest yield components and yield. Among the different intercropping combinations, intercropping maize variety Melkassa-2 with mung bean variety N-26 produced the highest Land Equivalent Ratio, maize equivalent yield and gross monetary return. Thus, maize variety Melkassa-2 with mung bean variety N-26 can be used for intercropping to maximize the productivity of the intercropping system in the study areas. Hence, maize and mung bean breeding programs need to consider selection criteria in developing varieties of maize and mung bean that can be used across range of cropping systems. Moreover, this experiment has to be conducted at least for one more season to account for seasonal variability.

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Soil Acidity Induced Land Use/Management Change, and its Impact on Soil Chemical and Biological Properties in the Central Highlands of Ethiopia

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Abstract

*Land use/management changes have become more common in the highlands of Ethiopia due to the prevalence of soil acidity, population growth and their demand for diverse products that might have positive or negative consequences on soil resources. In an attempt to explore the effect of soil acidity induced land use/management changes on soil properties, remote sensing and evaluation of selected soil chemical and biological properties in the different land uses was carried out. Analysis of remote sensing data categorized four major land use/cover classes: natural forest, Eucalyptus plantations, cropland/settlements and grasslands. In 1975, natural forest, Eucalyptus plantations, croplands/settlements and grasslands occupied 3.5, 7.5, 28.8 and 61.5 % of the total land area, respectively. Over a period of 39 years (1975 to 2014) of analysis, Eucalyptus plantations and cropland/settlements increased by 335 and 62.5 %, respectively. Soil chemical and biological properties under three land use (grassland, cropland, Eucalyptus) and two management systems (limed land and fallow land) all existing adjacent to each other were compared. Results of soil chemical and biological analysis showed considerable differences between the grassland and other land uses and management systems considered. A reduction of 53, 45, 46 and 47% in soil organic carbon (SOC) was observed under cropland, Eucalyptus, limed and fallow lands, respectively. However, cropland, Eucalyptus and limed fields showed similar values for most of the soil chemical properties studied. Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were also significantly higher under grassland, and lower under fallow land. Furthermore, r*esults clearly demonstrated that deterioration in soil chemical properties is more pronounced in 10-year-old *Eucalyptus* plantations than in 5-year-old *Eucalyptus*. *Soil organic carbon, the MBC to SOC ratio and MBN to total nitrogen could be considered as the three most important bio-chemical parameters to assess functional capacities of soils for soil acidity affected areas in the central highlands of Ethiopia.*

Keywords: *Low pH, land use change, MBC, MBN, Eucalyptus, central highlands*

Introduction

Soil acidification is a natural process which can be enhanced by human activity or can be controlled by appropriate soil management practices. Generally, acid soils have various constraints for crop production and are readily degraded when subjected to erosion, leaching, or contamination. The recently conducted soil fertility status maps produced by the Ethiopian Soil Information System (EthioSIS) have reported that 43 % of the agricultural land is affected by soil acidity, out of which 28 % of it being strongly acidic. The strongly acidic soils account about 3.7 million hectares of the currently cultivated land in the country. Several agricultural practices have been recommended to overcome the problem of tropical acid soil infertility worldwide. However, one of the immediate solutions to this chronic problem is amelioration through application of ground calcium and/or magnesium carbonates, hydroxides, and oxides aiming at raising the soil pH, modifying its physical, chemical and biological properties (Edmeades and Ridley, 2003; Temesgen *et al*., 2016). In the past, this practice had not been in use among Ethiopian smallholder farmers owing to technological, institutional and socio-economic related constraints. Instead, among few strategies that were in practice and still in use to mitigate the negative effects of soil acidity includes: fallowing croplands, conversion of grassland/cropland to short rotation *Eucalyptus* plantations and expansion of farming to communal grazing lands. The so called 'fallowing', the resting state of agricultural field (Szott *et al*., 1999) is common in some high-spot soil acidity areas, and areas with low soil fertility. This is intended to allow the soil to rest and regain some of its fertility by growing vegetation, usually consisting of naturally growing weeds and grasses.

Another strategy in response to the prevailing low soil pH and associated problems in the highlands of Ethiopia is conversions of natural vegetations and agricultural lands to *Eucalyptus.* This type of land use changes has become more common in the highlands of Ethiopia due to population growth and their demand for diverse products that led to the degradation of soil resources (Temesgen *et al.*, 2014). Particularly, the growing demand for construction and fuel wood and the wide adaptation of *Eucalyptus* to the different agro-ecological zones of the country are resulting in increased plantation of *Eucalyptus.* Consequently, *Eucalyptus* is voraciously integrated into farming systems in spite of a perception that this practice adversely affects soil quality and crop productivity. When soils come under increasing pressure to maintain a range of ecosystem services, there is interest in how soils change over time in response to factors such as change in land use (Tye *et al*.,

2013). Hence, timely detection of land use/management change and its potential effect on soil quality parameters is an essential prerequisite to take appropriate restorative measures, efficient land use planning and resource management. To date, however, there is insufficient information on the effect of soil acidity-induced land use change to eucalyptus on soil quality in the highland agro-ecosystems of Ethiopia. Hence, the objectives of this study were: 1) to show trends of LULC changes between 1974 -2014; 2) to assess and compare the changes in soil chemical and biological properties due to different land use changes; 3) appraise whether short-term fallowing would improve important soil properties such as SOC, MBC and MBN and; 4) investigate whether soil properties differ due to age of Eucalyptus and between soil depths.

Materials and Methods

Descriptions of the study area

The study was conducted in Wetabecha Minjaro peasant associations located in western central highlands of Oromia National Regional State, Ethiopia (Fig 1). It is situated at 9° 05′ 55″ N, 38° 36′ 21″ E, at an altitude of 2600 m.a.s.l. According to the local agro-climatic classifications, the study area belongs to moist highland agro-climatic zone with two rainy periods; the main rainy season occurs from June to mid-September, and the short rainy season extending from February to April. The area receives a total of about 1100 mm of rainfall annually. The mean maximum and mean minimum temperatures are 23.3°C and 8.7°C, respectively. The soils are classified as Nitisols with deep, red, well-drained tropical soils (IUSS, 2006). Most of the soils have a pH range of 4.5 to 5.5, contain low organic matter (<20 g kg^{-1}) and low nutrient availability. Agriculture is the main source of livelihood for the community, and subsistence type mixed barley-fallow-livestock best characterizes the farming system. Apart from croplands, the study landscape also comprises mosaic natural forests that belong to a tropical dry Afromontane Forest.

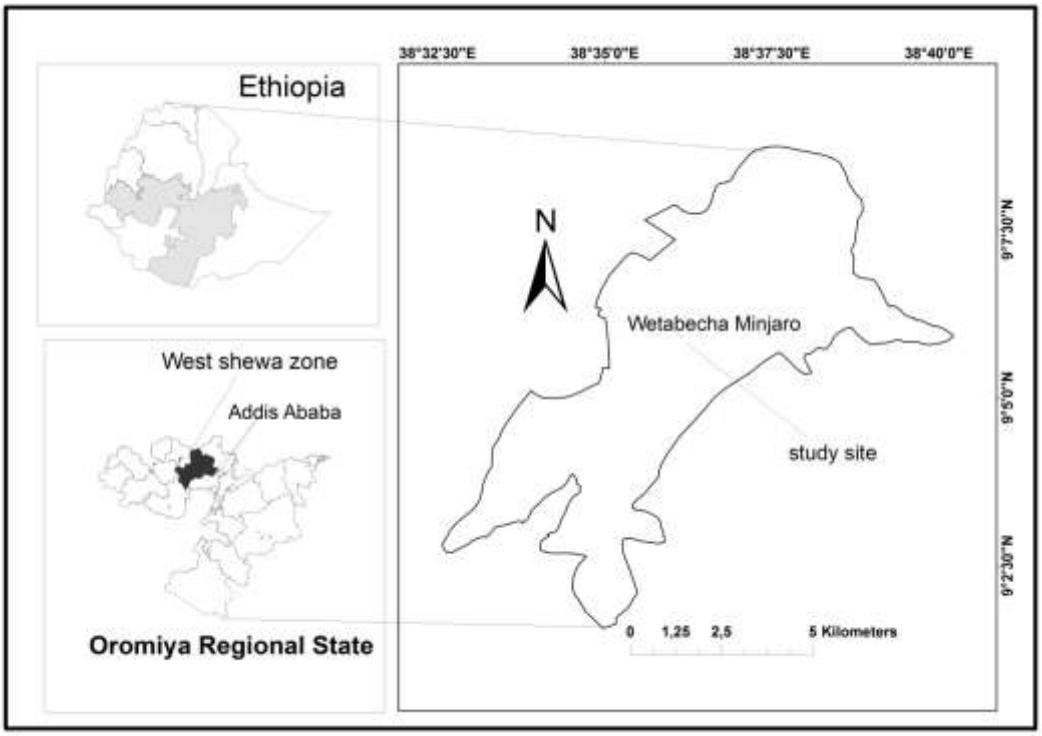

Fig.1. Location map of the study area

Land use/cover (LULC) change analysis

The main data source for LULC classification and change analysis were a series of Landsat imagery data. These include Landsat MSS, Landsat Thematic Mapper (TM), Enhanced Thematic Mapper (ETM+) and Landsat OLI scenes of the year 1975, 1986, 2000 and 2014, respectively. These datasets were obtained from the National Aeronautics and Space Administration (NASA) through their EOS Data Gateway Database. All the Landsat images were acquired in the same season (between October and January). All GIS data were projected to the Universal Transverse Mercator (UTM) projection system zone 37N and datum of World Geodetic System 84 (WGS84), ensuring consistency between datasets during analysis. Identifications and classifications of the land use/cover were supported by interpretation of aerial photographs and satellite images of the study area.

The satellite images were analyzed by utilizing data image processing techniques in ERDAS Imagine© 10.0 and ArcGIS© 10.0 software. A supervised signature extraction with a maximum likelihood was used in the analysis. Change analysis was conducted using post image comparison technique (Singh, 1989). Ground truthing was complemented with topographical maps of the study

area as well as several field visits, interviews with individuals and elderly people of the study site. As rural settlements are scattered and close to cultivated lands, croplands and settlements were classified together. The classified images were compared in three periods, i.e., 1975-1986, 1986-2000, and 2000- 2014. The identified land use/cover data were used for soil sample collection.

Soil sampling and analysis

A plot size of 10 m x 10 m (100 m^2) was demarcated in different land uses for soil sample collection. From demarcated area, composite soil samples were collected from eight randomly located sites where different land use and management systems (grassland, cropland, *Eucalyptus* plantation, limed land and fallow land) that exist adjacent to each other. Each of the soil samples consisted of ten sub-samples in a composite. Soil samples were collected from two depths; 0-10 cm and 10-20 cm for all land use types. A total of 80 soil samples (8 sites * 5 land use/management * 2 depths) were collected for laboratory analysis. Apart from these, soil samples were collected from 5- and 10-year-old *Eucalyptus* plantations and grassland that exist adjacent to each other. The collected samples were air dried and gently sieved through a 2 mm mesh to remove stones, roots, and were sealed in plastic bags before analysis. Soil chemical and biological parameters were analysed in duplicates. Soil biological analyses were done by wetting and keeping for one week of acclimatization period at room temperature. Soil organic carbon was determined by the Walkley-Black oxidation method (Walkley and Black, 1934). Total nitrogen (TN) was determined by Kjeldahl digestion method (Bremner and Mulvaney, 1982), and available phosphorous (AvP) by Olsen's extraction method (Olsen and Sommers, 1982). Exchangeable bases (Na^+, K^+, Ca^{2+}, and Mg^{2+}) were measured by atomic absorption spectrophotometer after extraction by ammonium acetate (Black *et al.*, 1965). The cation exchange capacity (CEC) was determined by extraction with ammonium acetate (Chapman, 1965). Exchangeable-titratable acidity was determined in 1 M KCl extracts titrated with 0.01 M NaOH. Microbial biomass carbon and microbial biomass nitrogen (MBN) were estimated by the classical chloroform fumigation extraction method (Brookes *et al.*, 1985; Vance *et al.*, 1987). Twenty five grams of dry weight-equivalent soil samples were fumigated with $CHCl_3$ for 24 hours in a dark in vacuum desiccators in two duplicates. After removal of chloroform by three repeated evacuations, the soil samples were extracted by 0.5 M K_2SO_4 (using a soil: extractant ratio of 1:4). Similarly, the unfumigated controls were also subjected to 0.5 M K_2SO_4 extraction. After shaking for 30 minutes in automatic shaker, the extracts were filtered through Whatman filter paper (N°.42). The filtrates were analysed for organic C

and total N by using SKALAR TOC/TN automatic analyzer. The difference in the C content of the extracts from fumigated and unfumigated samples was converted to biomass-C by dividing the value obtained by a factor (K_C) of 0.45 (Vance *et al*., 1987). The results were expressed as µg g^{-1} of oven-dried soil. The difference in the content of N of the extractants was also converted to biomass nitrogen by dividing the value obtained by a factor (K_N) of 0.54 (Brookes *et al*., 1985).

Statistical analysis

Analysis of variance (ANOVA) was performed to evaluate the main effects of land use/management, depth, and their interactions using SAS (version 9.1). A linear mixed model analysis with repeated measurements, considering two between-subjects factors (site with eight levels and land use/management with five levels) in a main effects design, and one within-subjects factor (depth with two levels). Tukey´s HSD procedure was used for multiple comparisons of mean chemical and biological properties of the soil under different land use systems.

Results and Discussions

Land use land/cover analysis

Analysis of remote sensing data categorized four major land use/cover classes: natural forest, Eucalyptus plantations, cropland/settlements and grasslands. Mapping of the land use and land cover changes of the study area has been published (Temesgen et al., 2014).In 1975, natural forest, *Eucalyptus* plantations, croplands/ settlements and grasslands occupied 3.5, 7.5, 28.8 and 61.5 % of the total land area, respectively. Over a period of 39 years (1975 to 2014) of analysis, *Eucalyptus* plantations and cropland/settlements increased by 335 and 62.5 %, respectively (Fig. 2). The increase in the area of *Eucalyptus* and croplands/settlements was at the expense of grasslands, where a corresponding 74% decrease in the area of grassland was observed in the same period. Between 1975 and 1986, the increases in *Eucalyptus* plantation and cropland/settlements were due to rapid population increases in the study area, and their demand for diverse products (food, feed, fuel and farm implements). This situation forced farmers to convert natural vegetations such as grassland to cropland/settlements and *Eucalyptus*. Numerous studies in Ethiopia and elsewhere (Dessie and Kleman, 2007; Kamusoko, 2007; Ningal *et al*., 2008; Paré *et al*., 2008; Zhao *et al*., 2006) also reported expansion of croplands and *Eucalyptus* plantations at the expense of grasslands. Similarly, Mengistie et al. (2013) also reported the highest conversion of grasslands into other land uses in sub-humid highlands of Ethiopia.

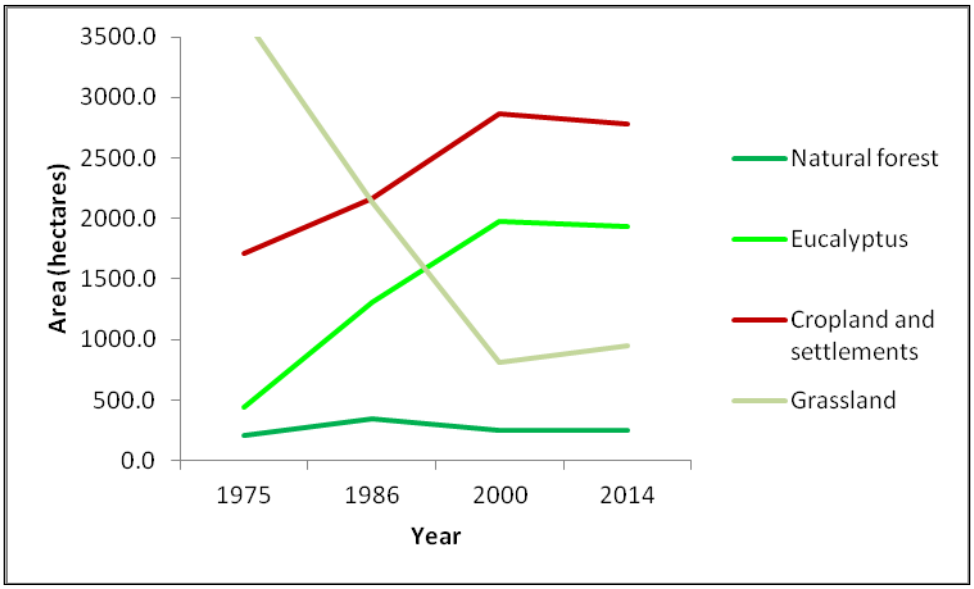

Fig.2. Summary of LULC changes between 1975 and 2014

Hierarchical evolution of Eucalyptus in the farming systems of the study area is presented in Fig. 3. In the past several decades, tropical meadow-type grassland and shrub lands were the dominant type of vegetation. Now, such types of vegetations are gradually replaced by croplands, settlements, and *Eucalyptus* plantations (Temesgen *et al.*, 2014). *Eucalyptus* plantations are widespread and 95% of the community grows *Eucalyptus* on private and communal land or around homesteads. As evidenced from a discussion with the community during soil sample collection, population growth and the growing demand for construction and fuel wood and depletion of soil fertility due to continuous cultivation without addition of external inputs are primarily resulted in increased plantation of *Eucalyptus* in the study area. As a result, the area of *Eucalyptus* is doubling every decade, and more and more smallholder farmers are growing it primarily on grassland or cropland. Even though the policy environment in Ethiopia discourages farmers from planting this exotic tree on productive land, many farm households still continued planting of *Eucalyptus*. Consequently, *Eucalyptus* is voraciously integrated into farming systems in spite of a perception that this practice adversely affects soil quality and crop productivity.

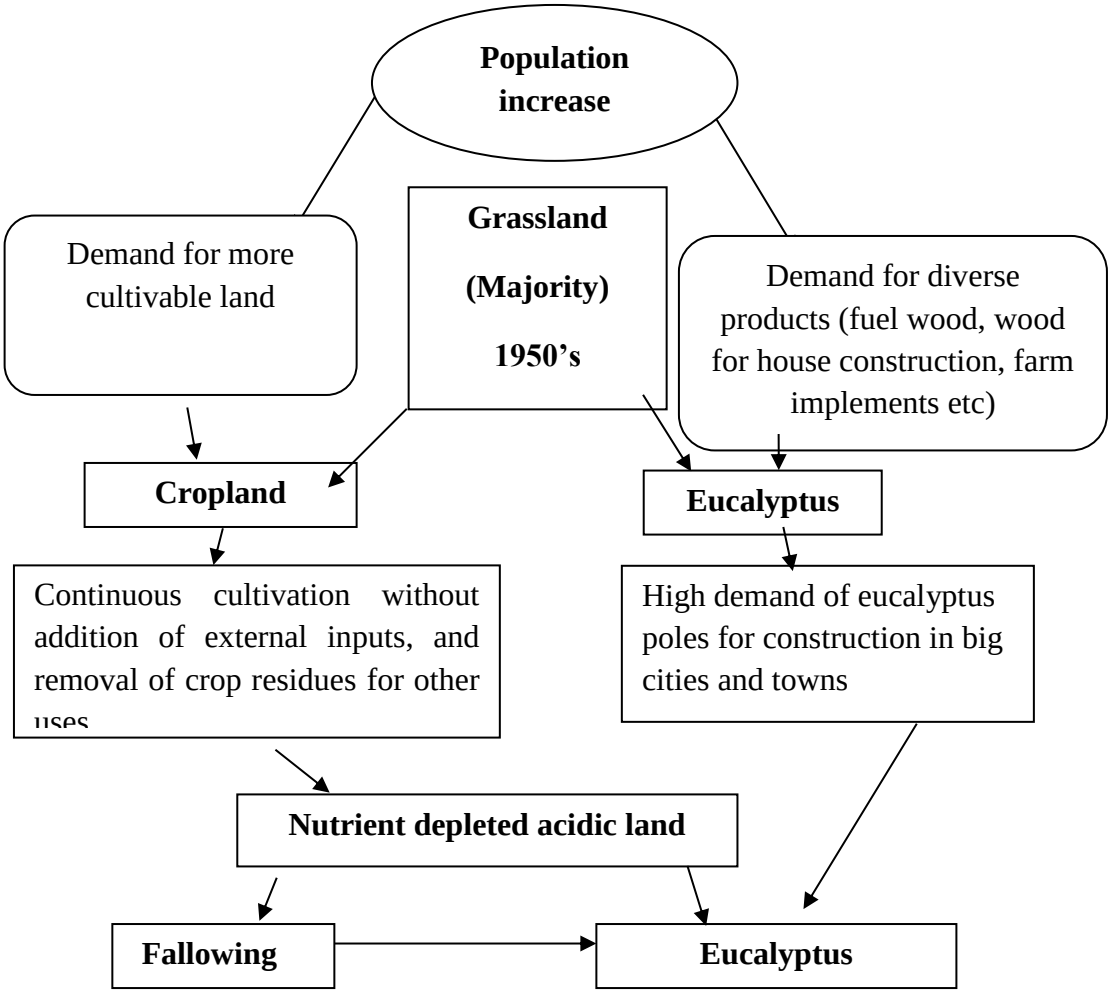

Fig.3. Hierarchical evolution of *Eucalyptus* in the farming systems of the study area (own analysis during soil sample collection)

Effect of land use/management change on soil chemical properties

Mean values of soil chemical properties as affected by different land use/management are presented in Table 1. The study clearly demonstrated that applications of lime significantly raised soil pH, and drastically reduced exchangeable acidity. When lime is added to acid soils that contain high aluminum and H^+ concentrations, it dissociates into Ca^{+2} and OH^- ions. The hydroxyl ions will react with hydrogen and aluminum ions forming water and aluminum hydroxide, respectively, and thereby increase soil pH in the soil solution. Numerous authors have reported decreases of Al in the soil solution as well as in the exchange complex upon liming (Álvarez and Fernández, 2009; Prado *et al*., 2007).

Higher amounts of SOC (4.3%) observed in grassland relative to the cropland, *Eucalyptus land*, limed land and fallow were contributed to the high amount of CEC in grassland. Grassland in our study area includes tropical type grasses and herbaceous vegetations which resulted in a higher litter input compared to the other land uses and management systems. Therefore, greater return of plant litter to soils and high root biomass of grasses could be the reason for the higher SOC in grassland. Tripathi and Singh (2009) also showed cultivation of soil previously supporting natural vegetation could lead to considerable losses of soil organic matter and microbial biomass. The lower values of SOC in other land use and management systems could be due to less physical protection, because tillage periodically breaks up macro-aggregates and exposes previously protected organic matter. The lowest mean values of soil chemical parameters, except for K^+, under fallow land might be associated with the current fallow management practices in the study area. Fallow fields are considered as grazing grounds for different species of livestock even though the primary purpose of fallowing by individual farmers is to restore soil fertility. Such practice leaves the land bare and exposes it to surface run-off during rainy season. In addition, crop residues are removed for domestic use, either as a source of fuel or animal feed. Many studies (e.g. Cai and Qin, 2006; Hati *et al*., 2007; Lemke *et al*., 2010) have shown that increases in SOC levels is directly related to the amount of organic residues added to soils. Therefore, the lowest soil fertility status (low pH, SOC, available P, Mg^{2+}, Ca^{2+}, MBC and MBN) in fallow lands was due to removal of crop residues and the washing away of nutrients by intense rainfall during fallow period. This clearly demonstrates that highly weathered acid soils in the study area would not recover rapidly after short-term fallow periods. Similar to this finding, short-term fallow (four years) in Senegal did not increase SOC or nutrient content (Masse *et al.*, 2004). On the other hand, even though, it is generally believed that plantations of *Eucalyptus* bring about a decrease in soil fertility, the absence of significant variation between *Eucalyptus* and cropland/limed lands in most of the soil chemical parameters is not clear, and needs further investigation. Danju *et al*. (2012) reported restoration of soil fertility following plantation of *Eucalyptus grandis* in south-western China. In their study, they found that soil organic matter content, SOC/ TN ratio, and MBC and MBN concentrations showed an initial phase of decline and then increased significantly over time in the upper soil layers of *Eucalyptus grandis* plantations aged from 1 to 4 or 5 years. Similarly, Tilashwork (2009) also reported that soils under cropland and *Eucalyptus* did not vary significantly in texture, bulk density, organic matter, pH, exchangeable K and available water content in the highland of north western Ethiopia.

Table 1. Mean values of soil chemical properties affected by different land uses and management systems (n=16) at two sampling depths (n=40).

		SOC	TN	AvP	K^+	Na^+	Ca^{2+}	Mg^{2+}	CEC	EXa
LUM†	pH (H_2O)	----------%---------		---- mg kg^{-1}----	----------(cmol (+) kg^{-1})----------					
Grassland	4.9a	4.4a	0.4a	5.1	0.8b	0.2	12.6a	2.6a	23.2	1.2b
Crop land	4.6b	2.3b	0.2b	5.6	0.9ab	0.2	8.9b	2.0b	17.5	1.2b
Eucalyptus	4.5b	2.5b	0.2b	6.0	0.8b	0.2	8.6b	2.3b	18.0	2.3a
Limed land	4.7ab	2.4b	0.2b	4.9	0.7b	0.3	9.6ab	1.9b	18.6	0.9b
Fallow land	4.3c	2.3b	0.2b	4.4	1.0a	0.1	0.18c	1.2c	18.9	1.5b
S.e	0.06	0.13	0.01	0.73	0.05	0.03	0.96	0.23	0.82	0.26
Depth(D)										
0 -10 cm	4.6 ± 0.02	2.8	0.26	5.6a ± 0.33	0.9	0.22	8.0	1.9b ± 0.11	19.1 ± 0.41	1.3b ± 0.11
10 -20 cm	4.6 ± 0.35	2.7	0.25	4.8b ± 0.35	0.8	0.19	7.9	2.1a ± 0.11	19.4 ± 0.42	1.5.a ± 0.13
ANOVA										
LUM	***	***	***	ns	**	ns	***	**	***	*
D	ns	ns	ns	***	ns	ns	ns	*	ns	*
LUM*D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

†LUM= land use/ management,, D= soil sampling depth, SOC= soil organic carbon, TN= total nitrogen, AvP= available phosphorus, CEC= cation exchange capacity, EXa= exchangeable acidity. Mean values with different letters within the same column indicate significant differences (Tukey's HSD procedure). The analysis of variances for the LUM, D and LUM*D are reported, $p < 0.001$***, $p < 0.01$** and $p < 0.05$, S.e= standard error, ns= not significant.

Effect of land use/management change on soil biological properties

Even though, various physical and chemical soil properties can be used to characterize soil quality, soil biological properties are more sensitive to changes than other indicators and could describe the soil quality in a broader picture (Bastida *et al*., 2008). The diversity of microorganisms and the related biochemical processes are also the most important components of soil quality, especially for the highly weathered acid soils, in which plant productivity is closely related to biological cycling (He *et al*., 2003). The effect of land use/management change on soil biological properties is presented in Table 2.

Table 2: Mean values of microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), MBC/MBN, MBC/SOC and MBN/TN as affected by different land uses and management systems (n=16) at two sampling depths (n=40).

LULC†	MBC	MBN	MBC/MBN	MBC/SOC	MBN/TN
	----------µg g^{-1} soil----------			*Computed as mean of the two depths (%)*	
Grassland	763.7a†	87.8a	9.0ab	2.56a	2.89a
Cropland	342.6b	71.28a	5.7c	1.03cd	2.30b
Eucalyptus	345.0b	37.9bc	10.2a	1.44bc	1.69c
Limed land	335.7b	47.2b	7.4b	1.54b	2.31b
Fallow land	190.8c	23.1c	8.8ab	0.80d	1.06d
S.e	35.25	4.16	0.40	0.14	0.16
Depth (D)					
0 -10 cm	485.4a ± 21.48	67.2a ± 2.99	8.00ns	1.62a±0.09	2.31a ±0.10
10 -20 cm	305.8b ± 15.45	39.7b ± 2.40	8.42ns	1.32b ±0.09	1.78b ±0.10
ANOVA	***	***	***	***	***
LULC					
D	***	***	ns	*	**
LULC*D	ns	ns	ns	ns	ns

†Means within a column with different letters between different land uses and management systems indicate significant differences ((Tukey's HSD procedure). The analysis of variance for each land use and management is reported, p <0.001***, S.e= standard error, ns= not significant

Results revealed that the highest values of MBC (763.7 µg g^{-1}) and MBN (87.8 µg g^{-1}) were recorded under grassland soils as compared to cropland, *Eucalyptus*, limed land and fallow land. Soils under fallows had the lowest MBC (190.8 µg g^{-1}) and MBN (23.1 µg g^{-1}). Values of MBC recorded under cropland, *Eucalyptus* plantations and limed land did not show significant differences. However, the MBN in grassland and cropland was comparable, and significantly superior to the other land

uses. The lowest values of MBN were observed in *Eucalyptus* and fallow land. Depth wise, MBC and MBN values were significantly higher in 0-10 cm relative to 10- 20 cm. In this study, changes in the ratios of MBC/SOC and MBN/TN due to land use changes were quite considerable. Accordingly, the values of MBC/SOC were in the following order: grassland >limed land >*Eucalyptus* > cropland >fallow land. Whereas, MBN/TN followed the order: grassland >limed land >cropland >*Eucalyptus* >fallow land. As expected, the mean values of MBC/SOC and MBN/ TN were significantly higher in 0-10 cm as compared to 10-20 cm soil profile. Higher mean values of both MBC and MBN in 0-10 cm depth as compared to 10-20 cm might be attributed to a decline in carbon availability and more occurrence of microbial biomass in the surface of 10 cm soil profile. The study demonstrated that more C mineralization happens in the first 0-10 cm than in 10-20 cm, indicating rapid activities of microbes that mineralize C within the surface of the soil profile. The decrease in the C mineralization in 10- 20 cm might be due to lower SOC content at 10-20 cm depth and relatively smaller number of microbes. These results are in line with the findings of Taylor (2002), who found reduced microbial and fungal activities in deeper *versus* surface soil layers.

The most important soil biological properties such as MBC/SOC and MBN/TN showed lower values in fallow land and croplands relative to grasslands or limed land. Similar to this result, cultivation of soils in the central highlands of Mexico with maize reduced MBC, and Reyes-Reyes *et al*. (2007) reported that converting soil under natural vegetation to arable soil was not only detrimental for soil quality, but also unsustainable when organic matter input is limited. A high MBC/SOC indicates an increased pool of available carbon in soil (Klose *et al*., 2004). Therefore, this work suggested that the MBC/SOC and MBN/TN could be useful tools to assess biological soil quality changes due to the conversion of grasslands to other land uses and management systems in highly weathered acidic soils of the central highlands of Ethiopia.

Effect of Eucalyptus age on soil chemical and biological properties

The effect of Eucalyptus age on soil chemical and biological properties is presented in Table 3 and 4, respectively. According to the results of a linear mixed model, the mean values of pH, SOC, and TN in adjacent grassland were significantly ($p<0.5$) higher than plantations of 5- and 10-year-old *Eucalyptus* (Tables 3). However, the two age groups of *Eucalyptus* did not differ from each other in the above parameters. Land use conversions from grassland to *Eucalyptus* resulted in higher soil acidity, and lower SOC and TN. The increased acidity under *Eucalyptus* plantations could be associated with

increased cation uptake by trees and consequent changes in the proportions of adsorbed cations to the soil exchange complex (Temesgen et al., 2016). Relative to SOC of grassland, the SOC of 5- and 10-years-old plantations decreased by 48% and 44% respectively. Similarly, TN was decreased by 50% and 42% in 5- and 10-years-old *Eucalyptus* plantations respectively. Lower SOC and TN in *Eucalyptus* plantations agree with reports from Balagopalan and Jose (1995), Animon et al. (1999) and Behera and Sahani (2003).

Table 3: Effect of Eucalyptus age on selected soil chemical properties

Depth	Land use type	pH (H_2O)	EXa (cmol(+)/kg	SOC (%)	TN (%)	AvP (mg/kg)
0 -10 cm	Grassland	4.9a†	0.56b	4.6a	0.41 a	5.8ns
	5-year-old *Eucalyptus*	4.3b	3.23a	2.4b	0.20b	3.9ns
	10-year- old *Eucalyptus*	4.4b	2.23a	2.3b	0.23b	6.0ns
10 -20 cm	Grassland	5.0a	1.0b	3.8a	0.36a	5.0ns
	5-year-old *Eucalyptus*	4.3b	3.6a	2.1b	0.18b	3.4ns
	10-year- old *Eucalyptus*	4.5b	2.2ab	2.4b	0.22b	5.0ns

†Mean values within a column with similar depth that share the same letters are not significantly different between land use type. EXa = exchangeable acidity; SOC=soil organic carbon; TN=total nitrogen; AvP=available phosphorus

Soil microbial biomass greatly depends on soil organic matter as substrate; a decrease in SOM causes a reduction in soil microbial biomass (Chen *et al*., 2005). Hence, the higher MBC and MBN in grassland is mainly attributed to greater availability of organic matter. The lower microbial biomass under *Eucalyptus* plantations could possibly be due to the toxic effects of harmful allelochemical compounds released from *Eucalyptus* leaf litter (Rice, 1984). Harmful effects of *Eucalyptus* leaf litter on microbes were also reported by Dellacassa *et al*. (1989) and Sankaran (1993). In this study, MBC/SOC and MBN/TN did not show remarkable differences among land uses. Generally, lower levels of MBN/TN in 10-year-old *Eucalyptus* indicate that the nitrogen supplying ability of soils is gradually decreasing as the age of the plantations progresses. Low ratios of microbial biomass and nitrogen in *Eucalyptus* plantations were also reported by Yu *et al*. (2008).

Table 4: Soil biological parameters as affected by age of Eucalyptus

Land use	MBC (μg g^{-1} soil)	MBN (μg g^{-1} soil)	MBC: SOC	MBN: TN
			Computed as mean of the two depths (%)	
Grassland	669.3a†	81.5a	1.7^{ns}	2.0^{ns}
5-year-old *Eucalyptus*	365.4b	30.5b	1.7^{ns}	1.6^{ns}
10-year-old *Eucalyptus*	280.2b	32.5b	1.2^{ns}	1.5^{ns}

†Means within a column that share the same letters are not significantly different within land use type. MBC=microbial biomass carbon; MBN=microbial biomass nitrogen; MBC: SOC= ratio of MBC to organic carbon; MBN: TN= ratio of microbial biomass nitrogen to total nitrogen; ns= non-significant

Conclusions

Remote Sensing and GIS data analysis clearly showed a dramatic decrease in the area of grassland from 1975 to 2014, accompanied by an increase in the area of croplands and settlements in the same period. Rapid population growth and their demand for diverse products and soil fertility deterioration over time are the major drivers to change part of a land to other forms of land use/management. Generally, conversions of grasslands to either cropland or *Eucalyptus* plantations or fallowing deteriorate the functional capacities of soils in the central highlands of Ethiopia. Even though, it is generally believed that plantations of *Eucalyptus* bring about a decrease in soil fertility, the absence of significant variation between *Eucalyptus* and cropland/limed lands in most of the soil chemical parameters is not clear, and needs further investigations. However, land use conversions from grassland to *Eucalyptus* resulted in higher soil acidity levels. The lowest soil fertility status (low pH, SOC, available P, Mg^{2+}, Ca^{2+}, MBC and MBN) in fallow lands clearly demonstrates that highly weathered acid soils in the study area would not recover rapidly after short-term fallow periods. Hence, improving soils conditions in the current land use system in sustainable way requires improving and maintaining soil productivity. This includes crop residues retention in crop fields after harvesting; avoiding bare fallows and judicious application of lime along other management practices would be sustainable options for the current soil acidity problem in the study area.

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Phosphorus and Sulfur Effects on Growth and Physiological Parameters of Snap Bean (*Phaseolus vulgaris* L.) Under Different Irrigation Regimes on Andosol in the Central Rift Valley of Ethiopia

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Abstract

Snap bean is a leading exportable vegetable crop and an important source of nutrition for consumers in many parts of Ethiopia including the Central Rift Valley. However, it's growth and physiology parameters are very stunted and poor due to inadequate moisture and poor soil fertility effect. Lack of phosphorus and sulfur fertilizers and irrigation regime are stunted and poor growth and physiology of snap bean. Therefore; field experiment was conducted at Melkassa Research Center off season in 2017 and 2018 to evaluate the effect phosphorus and sulfur fertilizers application under different irrigation regimes on growth and physiology of snap bean. The experiment was laid out in split-plot design with three replications. Four drip irrigation regimes (100, 85, 70 and 55% ETc) were assigned to the main-plots. Four fertilizer types (0 kg both P and S ha^{-1}, 21 kg P ha^{-1}, 30 kg S ha^{-1} and 21 kg P ha^{-1}+30 kg S ha^{-1}) were randomly assigned to the sub-plots. The analysis of variance revealed that the interaction effect of irrigation regimes and fertilizers application had a significant ($P<0.05$) effect on LAI and CGR. The highest LAI of snap bean was recorded from interaction of 100% ETc with control and S30 kg ha^{-1} with 3.32 and 3.18, respectively. Whereas, the lowest LAI was obtained from interaction of 55% ETc with S30 kg ha^{-1} and control with values of 1.44 and 1.46, respectively. The highest CGR of snap bean was recorded from interaction of 100% ETc with S30 kg ha^{-1} (17.45 g m^{-2} day^{-1}) followed by the control (15.66 g m^{-2} day^{-1}) and with P21 kg ha^{-1}+S30 kg ha^{-1} (14.46 g m^{-2} day^{-1}). The lowest CGR was obtained from interaction of 55% ETc with control (3.33 g m^{-2} day^{-1}). The main effect of various irrigation regimes had a significant ($P<0.05$) effect on NDVI, IPAR and stomata conductance parameters. On the other hand, application of different fertilizers types had no significant effect on all the parameters. The best growth and physiological parameters of snap bean was recorded from the interaction effect of 100 and 85% ETc with different fertilizers types. Therefore; 85% ETc irrigation regime without P and S fertilizers where scarcity of irrigation water is limiting and 100% ETc irrigation regime without P and S fertilizers where there are no water shortages, in the rift valley area with similar soil properties.

Keywords: fertilizers, irrigation, snap bean, growth

Introduction

Snap bean (*Phaseolus vulgaris* L.) comprises a group of common beans that has been selected for succulent pods with reduced fiber primarily grown for its young edible and fleshly pods (Kenneth, 2012). Snap bean is a major source of carbohydrates (39.7%), protein (28.9%), fibre (22%), fat (0.88%), calcium (1.8%), and phosphorus (0.13%) (Ghonimy *et al.*, 2009). It is most important for export with the highest share (94%) among all vegetables in Ethiopia (Lemma, 2011). Even though, snap bean is an important vegetable crop, its' phenology and growth parameters of snap bean is very stunted and poor due to lack of water and nutrient management practices and improved varieties in Ethiopia (Girma, 2009). Inadequate moisture and low soil fertility have been the major constraints to bean production in Ethiopia (Katungi *et al.*, 2010).

Water stress can reduce plant height, number of leaves plant^{-1}, number of branches plant^{-1}, and total fresh weight for snap bean when the supplied water was decreased from 80 to 60% of ETC (Saleh *et al.*, 2018). Under water stress conditions, snap bean vegetative growth is stunted and reduced at photosynthetic rates (Suzuki *et al.*, 1987). Efficient use of irrigation water is becoming increasingly important for enhancing profitability and resource conservation.

Poor soil fertility is a major problem that affects snap bean production in Ethiopia (Tesfaye, 2017). Among the essential nutrients; P and S are limiting nutrients (Jamal *et al*., 2010; Khan and Mazid, 2011). Phosphorus is one of the most sufficiently determinants of plant growth (Wang *et al.*, 1998). It is an important for intensify root growth, which improves the water and other nutrients absorption and translocation to the growing parts of the plants, resulting in an increased photosynthetic process and then better production. P deficiency reduces plant growth and crop productions especially in arid and semi-arid areas with calcareous soils (Bargaz *et al.*, 2016).

Sulfur is limiting nutrient nowadays due to nutrient mining under zero application of sulfur fertilizer, increases crop residue removal, and poor management practices (Alemu *et al*., 2016). Until recently, sulfur received a little attention due to conviction for many years, that fertilizers and atmospheric inputs supplied the soil with adequate amounts of sulfur (Jamal *et al*., 2010). However, nowadays arable soils in many areas are proved to be sulfur deficient (Goswam, 2014). Low soil S had effect on phenology, growth and yield and yield related parameters of snap bean. Similarly, various soil tests from sample collected in different farm fields and regions in Ethiopia showed that lack of sulfur nutrient (ATA, 2015). Thus, this experiment

was carried out with the following objectives:

- To evaluate the effect of phosphorus and sulfur fertilizers application on growth and physiology of snap bean.
- To determine the effect of irrigation regimes on growth and physiology of snap bean.

Materials and Methods

Description of the study area

The experiment was conducted at Melkassa Agricultural Research Center in 2017 and 2018 during off-season. The site is found at 117 km South East of Addis Ababa with geographic co-ordinate of $8^o24'$N latitude and $39^o12'$E longitude. It situated at an altitude of 1550 m.a.s.l. The area receives mean annual rainfall of 763 mm. The mean annual maximum and minimum temperature of the site is 28.6 and 13.8^0C, respectively. The average bulk density of the soil is 1.13 g cm^{-3}. The soil is slightly alkaline ranging from 7.4 to 7.8 pH.

Experimental design and treatments

The experiment was laid out in split-plot design with three replications. Four drip irrigation regimes (100, 85, 70, and 55% ETc) were assigned to main-plots. Four fertilizer types (0 kg both P and S ha^{-1}, 21 kg P ha^{-1}, 30 kg S ha^{-1} and 21 kg P ha^{-1}+30 kg S ha^{-1}) were to sub-plots. The area of each plot was 12 m^2. Three central rows were harvested, 3 rows were used for distractive samples, and the marginal rows were used as border rows. Spacing between plots and blocks were 1.5 m used.

Experimental procedures

Snap bean "Plati" variety was used for experiment at a spacing of 50 and 10 cm between rows and plants, respectively. P and S fertilizers were applied at sowing in the form of TSP (46% P_2O_5) and K_2SO_4 (51% K_2O and 18% S), respectively. KCl (60% K_2O and 48% Cl) was used to balance the supply of K applied through K_2SO_4. Low-head drip irrigation system was used for the experiment. Irrigation treatment was applied based on weather data obtained from meteorological station. Irrigation treatment was carried out when plants reached the first two true leaves stage.

Soil samples collection and analysis

Composite soil samples were collected from representative 12 spots using diagonal sampling method by using auger at a depth of 0-20, 20-40 and 40-60 cm for physico-chemical soil analysis. Soil texture (%) was determined by using hydrometer method (Bouyoucos, 1962). Bulk density (g cm^{-3}) was determined from undisturbed soil using core sampler. Electrical conductivity (dS m^{-1}) was analyzed on a 1:2.5 soil: H_2O suspension using a conductivity meter

(Sahelemedhin and Taye, 2000). Soil pH was determined on a 1:2.5 soil: H_2O solution suspension using a pH meter. Organic carbon (%) was determined using wet oxidation method (Walkley and Black, 1934). Organic matter (%) was calculated by multiplying the OC% by a factor of 1.724. Total N (%) was determined by using Micro-Kjeldahl digestion (Jackson, 1973). Available P (mg kg^{-1}) was determined by Olsen's method (Cottenie *et al.,* 1982). Exchangeable K was determined by using flame photometer (Hesse, 1971). Available S (mg kg^{-1}) was estimated by turbidity (Tandon, 1986). Soil samples were analyzed at MARC and DZARC soil laboratory.

Data collected

Growth anaysis parameters

Leaf area index (LAI) was calculated at 32, 46, and 60 DAS using the following equation (Thomas and Winner, 2000).

$$LAI = \frac{\text{Leaf area}}{\text{Ground area}}$$

(Eq. 1.1)

Crop growth rate (CGR) was estimated at 46 and 60 DAS using the following equation (Hunt *et al*., 2002).

$$CGR = \frac{W2 - W1}{T2 - T1} = g\ m^{-2}\ day^{-1}$$

(Eq. 1.2)

Where; W1, W2 = subscripts 1 and 2 indicate W (plant dry weight) on two occasions (g m-2), T1, T2 = subscripts 1 and 2 indicate consecutive samplings at time T2 and T1.

Physiological parameters

Normalized difference vegetation index (NDVI) was determined using Green Seeker 505 (Hand-held Manual NTech Industries INC., USA) portable model instrument. It measured green biomass from harvested rows at 40, 54 and 68 DAS and was taken at 11:00 am to 2:00 pm (Dobos *et al*., 2012). Stomata conductance (mmol m^{-2} s^{-1}) was measured using steady state porometer (Model Sc-1, Decagon Devices, INC.). It was measured from the mean of 3 plants $plot^{-1}$ at 40 and 54 DAS from fully expanded leaf at 3rd leaf from the top and was taken at 9:00 to 11:00 am. Intercepted photosynthetically active radiation (IPAR) (µmol m^{-2} s^{-1}) was measured using Ceptometer (Model LP-80, Decagon Devices, INC. WA, USA) on harvested rows at 44 DAS and was taken at 12:00 am to 1:00 pm.

Stastical data analysis

All data were subjected to analysis of variance using GLM procedure of the SAS software version 9.0 (SAS, 2004). The assumptions of ANOVA for normality of distribution and homogeneity of variance were checked, and statistical analyses where the F-ratios was found to be significant, mean separation was performed using Fisher's protected LSD at the 5% probability level.

Results and Discussion

Physico-chemical properties of the soil

Soil textural class and bulk density at all soil depths was loam and low, respectively in Table 1. Soil pH was 7.40 at all soil depths and is moderately alkaline (Motsara and Roy, 2008). Electrical conductivity of soil tested was free of salinity. Organic carbon at all soil depths, which could be rated as medium. Organic matter (OM) at a depth of 0-20 and 20-40 cm, which showed that high rating. However, OM at a depth of 40-60 cm, which could be rated as moderating (Hazelton and Murphy, 2007). Total N from all soil depths, which showed that low rating. Exchangeable K from a depth of 0-20, 20-40 and 40-60 cm (Table 2) was rated as very high. Available P at a depth of 0-20 cm was rated as medium, while at a depth of 20-40 and 40-60 cm, which was rated as low (Edossa *et al*., 2014). Available S at a soil depth of 0-20, 20-40 and 40-60 cm could be rated as medium (Goswam, 2014).

Table 1. Physical soil properties of experimental field before sowing

Soil depth (cm)	Bulk density (g cm^{-3})	Particles size distribution (%)			Textural classes
		Sand	Clay	Silt	
0-20	1.01	31.25	21.25	47.50	Loam
20-40	1.07	41.25	18.75	40.00	Loam
40-60	1.06	41.25	18.75	40.00	Loam

Table 2. Chemical soil properties of experimental field before sowing

Soil depth(cm)	pH	EC (dS m^{-1})	OC (%)	OM (%)	TN (%)	Ava. P (mg kg^{-1})	Exc. K (cmol (+) kg^{-1})	Ava. S (mg kg^{-1})
0-20	7.25	0.27	2.96	5.11	0.09	13.53	2.26	16.43
20-40	7.35	0.33	2.57	4.44	0.08	8.81	2.37	15.35
40-60	7.58	0.27	2.50	4.30	0.07	5.66	2.54	14.26

Growth analysis

Leaf area index

Use of various irrigation regimes, fertilizers and their interactions had significant *($P \leq 0.05$)* effect on leaf area index at 60 DAS. The highest leaf area index of snap bean was recorded from interaction of 100% ETc with control and S30 kg ha^{-1} with 3.32 and 3.18, respectively. Whereas the lowest leaf area index was obtained from interaction of 55% ETc with S30 kg ha^{-1} and control with values of 1.44 and 1.46, respectively (Table 3). Generally, as irrigation regimes decreased, leaf area index was decreased, which might be due to

reduction of leaf growth and accelerated leaf senescence (Table 3). Similar with El-Noemani *et al*. (2010) and Mohammad (2013) under low irrigation regimes, shrinking of leaves, acceleration in yellowing and aging of older leaves caused reduced leaf area index and also rate of light absorption was reduced.

Table 3. Interaction effect of various irrigation regimes and fertilizers application on leaf area index and crop growth rate of snap bean

Irrigation regimes (%)	Leaf area index				Crop growth rate (g m^{-2} day^{-1})			
	Fertilizers types (kg ha^{-1})							
	0	P21	S30	P21+S30	0	P21	S30	P21+S30
55 ETc	1.46^{f}	2.04^{b-f}	1.44^{f}	1.98^{b-f}	3.33^{h}	8.26^{efg}	7.94^{efg}	6.16^{fgh}
70 ETc	2.14^{b-f}	2.00^{b-f}	1.64^{ef}	2.20^{b-f}	11.43^{b-e}	9.66^{c-f}	8.42^{efg}	9.47^{c-f}
85 ETc	1.83^{def}	2.67^{ab}	2.34^{b-e}	1.84^{c-f}	8.50^{efg}	9.11^{d-g}	13.2^{a-d}	9.22^{c-g}
100 ETc	3.32^{a}	2.63^{abc}	3.18^{a}	2.46^{bcd}	15.66^{ab}	13.64^{abc}	17.45^{a}	14.46^{ab}
LSD (0.05)	0.71				4.47			
CV (%)	19.17				24.28			

Means within each column with different letters are significantly different using LSD at P ≤ 0.05

Crop growth rate

Use of various irrigation regimes and their interactions had brought significant *(P≤0.05)* effect on CGR at 60 DAS. However, the application of fertilizers had non-significant effect on CGR. The highest CGR of snap bean was recorded from interaction of 100% ETc with S30 kg ha^{-1} (17.45 g m^{-2} day^{-1}) followed by the control (15.66 g m^{-2} day^{-1}) and with P21 kg ha^{-1}+S30 kg ha^{-1} (14.46 g m^{-2} day^{-1}). The lowest CGR was obtained from interaction of 55% ETc with control (3.33 g m^{-2} day^{-1}) (Table 6). It indicated that as irrigation regimes increases, plants easily to take up minerals from the soil, then enhancing cell enlargement and elongation resulting in increases crop growth rate. Similar with El-Noemani *et al*. (2010); Ghassemi and Mardfar, 2008) low CGR was observed under low irrigation regime on common bean due to stomata closure, reduced minerals uptake by plants, and hence affected plant growth.

Physiological parameters

Normalized difference vegetation index

Irrigation regimes had brought highly significant (P≤0.01) effect on NDVI at 40, 54 and 68 DAS. However, application of fertilizers and their interactions had non-significant effect on NDVI at 40, 54 and 68 DAS. The highest NDVI was recorded from 100% ETc, while the lowest NDVI was obtained from 55% ETc at 40, 54 and 68 DAS (Figure 1). The highest irrigation regimes gave deep greener leaves, vigorous, and healthy plants with better biomass and pod yield. Similar with Nemeskeri *et al*. (2017)

NDVI was significantly lower under low irrigation regimes compared to those under optimal water supply condition due to retarded growth of snap bean.

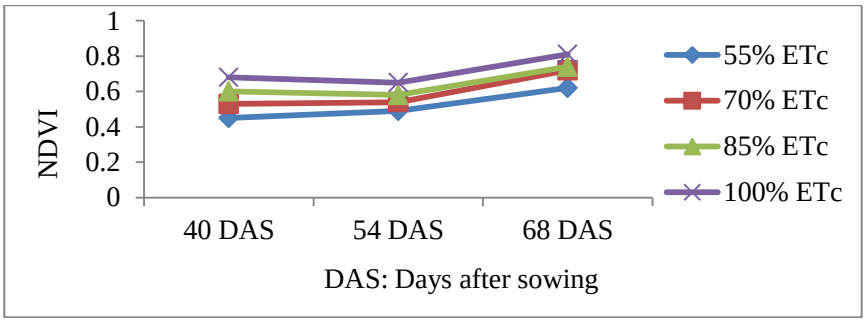

Figure 1. Normalized difference vegetation index of snap bean as affected by different irrigation regimes

Stomata conductance

Irrigation regimes had brought significant (P≤0.05) effect on stomata conductance at 40 and 54 DAS, but fertilizers and their interactions had non-significant effect on stomata conductance at 40 and 54 DAS. The highest stomata conductance was recorded from 100% ETc at both 40 and 54 DAS, while the lowest was obtained from 55% ETc in table 4. It indicated that at low irrigation regimes, stomata conductance was decreased. Similar with both Chaves *et al*. (2002) and Yokota *et al*. (2002) leaf stomata conductance decreases as soils get dry, either in response to drying of roots or leaves, or both and as result this decreased photosynthesis under mild to moderate water stress.

Table 4. Mean value of stomata conductance and IPAR parameters as influenced by different irrigation regimes and fertilizers applications on snap bean

Fertilizers types (kg ha^{-1})	Stomata conductance (mmol m^{-2} s^{-1})		IPAR (µmol m^{-2} s^{-1})
	40 DAS	54 DAS	44 DAS
0	386.32	192.02	950.25
P21	347.27	201.69	1141.58
S30	354.46	191.09	1057.83
P21+S30	337.09	222.05	1096.25
LSD (0.05)	NS	NS	NS
CV (%)	28.00	19.96	36.12
Irrigation Regimes (%)			
55 ETc	293.27^{b}	175.98^{b}	853.08^{b}
70 ETc	352.00^{ab}	187.18^{ab}	965.25^{b}
85 ETc	355.07^{ab}	205.17^{ab}	1015.50^{b}
100 ETc	424.88^{a}	239.33^{a}	1412.08^{a}
LSD (0.05)	83.34	40.21	210.48
CV (%)	23.42	20.04	19.52

Means within each column with different letters are significantly different using LSD at P≤0.05

Intercepted photosynthetically active radiation

Irrigation regimes had brought highly significant *(P≤0.01)* effect on IPAR at 44 DAS. However, application of fertilizers and their interactions didn't show significant effect on IPAR at 44 DAS. The result disagrees with Mario *et al.* (2006) reported that application of phosphorus gave higher NDVI values than zero phosphorus treatment. The highest IPAR of snap bean was recorded from 100% ETc with 1412.08 µmol m^{-2} s^{-1}, while statistically similar lower IPAR were obtained from 55% ETc, 70% ETc and 85% ETc with 853.03, 965.25 and 1015.50 µmol m^{-2} s^{-1}, respectively (Table 4). This indicated that as irrigation regime increased; larger leaves were produced leading to higher interception of photosynthetic active radiation. Low irrigation regimes, reduced IPAR owing to reduced light interception and decreasing photosynthetic rate. Similarly, Flexas *et al*. (2004) under low irrigation regime, stomata closure and reduction in leaf internal CO_2 concentration are the main causes for reduced leaf photosynthetic rates.

Conclusion and Recommendation

Snap bean is the most important exporting vegetable crop grown for young edible and fleshly pods. Its' production in Ethiopia has increased from year to year, but their growth and physiological parameters is very stunted and poor due to inadequate moisture and soil fertility problems. Lack of phosphorus and sulfur fertilizers and irrigation regime are stunted and poor growth and physiology of snap bean. Therefore; field experiment was conducted at Melkassa Research Center to evaluate the effect phosphorus and sulfur fertilizers application under different irrigation regimes on growth and physiology of snap bean. The analysis of variance revealed that the interaction effect of irrigation regimes and fertilizers application had a significant (P<0.05) effect on LAI and CGR. The highest LAI of snap bean was recorded from interaction of 100% ETc with control and S30 kg ha^{-1} with 3.32 and 3.18, respectively. Whereas, the lowest LAI was obtained from interaction of 55% ETc with S30 kg ha^{-1} and control with values of 1.44 and 1.46, respectively. The highest CGR of snap bean was recorded from interaction of 100% ETc with S30 kg ha^{-1} (17.45 g m^{-2} day^{-1}) followed by the control (15.66 g m^{-2} day^{-1}) and with P21 kg ha^{-1}+S30 kg ha^{-1} (14.46 g m^{-2} day^{-1}). The lowest CGR was obtained from interaction of 55% ETc with control (3.33 g m^{-2} day^{-1}). The main effect of various irrigation regimes had a significant (P<0.05) effect on NDVI, IPAR and stomata conductance parameters. On the other hand, P and S fertilizers application had no significant effect in all parameters. Lack of fertilizer types response could partially be attributed to the medium level of soil availability for nutrients,

P and S. Based on this current finding; 85 and 100% ETc without P and S fertilizers application, which gave better growth and physiology of snap bean. Therefore, 85% ETc without P and S fertilizers, where irrigation water is a limiting factor and 100% ETc without P and S fertilizer application, where there are no water shortages in the rift valley area with similar soil properties.

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Unraveling the Constraint of Lentil Productivity through Improved Agronomic Management for Sustainable Production in Ethiopia: A review

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Abstract

Lentil is the most vital grain legume crop in Ethiopia and ranking first in consumers' preference. It is primarily cultivated in the central highlands of Ethiopia during the main cropping system, with little care and management. There are significant limitations on the growth and production of lentil, which include lack of sufficient information, inherent lower output capacities of the local crops as well as unpredictable output due to various biotic and abiotic factors. As a result, researchers and farmers are facing a significant challenge in increasing lentil production to meet the national demand. Therefore, more focus needs to be paid to develop and encourage low-cost lentil production. Improved crop management practices, integrated soil fertility, and integrated pest, management, etc., that are not only increase productivity and profitability, but also ensure environmental and social sustainability besides nutritional security , are among the most potential technologies of lentil production. Thus, this review paper provides a critical assessment of the contribution of various improved agronomic practices to improve lentil productivity on one hand, while also highlighting future research priorities on the other, with the prime objective of ensuring lentil production in Ethiopia.

Keywords: agronomic practices, lentil, productivity, sustainability

Introduction

Agriculture is the backbone of Ethiopia's economy with diversification and intensification of farming systems because of the varied agro-ecologies that sustain its agricultural production and conserve the rich biodiversity of the region (World Bank, 2016). Agriculture contributes 42 percent of GDP and employs about 85 percent of the population who earn their living directly or indirectly from it (CSA, 2016), but how, when, and where to cultivate are the key issues that farmers and land managers face daily. On the other hand, Ethiopian farming is dominated by a rainfed scheme, which accounts for approximately 97 percent of the current cropland area and is widely regarded as a low-yield system (Korbu *et al*., 2020). The Ethiopian agriculture system generally subsistence primarily for the majority

of essential food crops, including lentils (Negussie, 2004). Thus, farmers grow a variety of legume crops for food and feed, as well as to earn money and, more importantly, to restore cropland fertility. Farmers' involvement in legume growing has risen almost twice over the last 20 years, increasing from 4.5 to 8.5 million farmers (Atnaf *et al*., 2015). Besides legumes have high protein content, they are economically and environmentally beneficial to use atmospheric nitrogen through biological nitrogen fixation (BNF) (Yirga *et al.*, 2016).

Lentil is among the principal grain legumes in the highland Vertisols of Ethiopia mainly involved in tef, wheat, and barley-based crop rotation systems. In this cropping system, the function of yield increment of the following cereal crop is realized as a result of the predecessor lentil that fixes nitrogen, as well as breaks the life cycle of essential diseases, insect pests, and weed populations (Geletu, 2006). Lentil is grown and harvested primarily in Ethiopia's northern, central, and eastern highlands mainly during the rainy season ('kiremt', June-December) and to some extent during the short rainy season (*'belg'*, February-May). In Ethiopia, lentil ranks 6th in terms of the number of growers; accounting for, 8.7% of total pulse growers, area, i.e., 0.79% of the total area under pulse crops and production for 0.45% of total pulse production (CSA, 2019). Over the last three decades, the production of lentils in Ethiopia showed an increasing trend regardless of the static nature of the area under cultivation (Figure. 1b). The annual growth rate, however, is lower than the other highland food legume crops in the country. Currently, the productivity of lentils has gone up to 1.4 t ha^{-1}, doubling since 1992 (Figure.1a). However, lentil production still faces numerous constraints due to pests, diseases and agroclimatic stresses, the lack of improved inputs, mechanization, and proper agronomic management (Frehiwot, 2009).

In Ethiopia, lentil research began on a small scale in the 1970s as a result of work by the National Committee for Crop Improvement, but efforts were intensified after the upgrading of the National Chickpea and Lentils Improvement Program in 1978 (Fikre & Bekele, 2020). Many research activities have been done so far to identify the agronomic requirement for the production of lentils and several site-specific studies have been carried out. However, much of the findings and information generated have been given less recognition by the users. Furthermore, the findings and research reports have not been adequately evaluated and not systematically reviewed in a usable manner. These papers, therefore, aims at reviewing and compiling the scattered findings of agronomic practices on lentil in the past decades and identifies the major yield gap and suggest future research direction.

Materials and Methods

This review applied reporting items for systematic literature review approach to search and select past and current publications relevant to the topic. The development of this review paper is based on the use of selected published articles in journals, proceedings, research centers annual reports, and technical reports available on the subject area that have been conducted by various researchers, institutions, and organizations from early 1970 until now.

Result and Discussion

Socio-economic significance of lentil

Ethiopian farmers in the agricultural system have been using grain legumes like chickpea, lentil, fababean common bean etc for many years to maintain soil fertility due to the capacity of the crops to fix atmospheric nitrogen in the soil. This reduces fertilizer costs in subsequent cereal crops and increases yields (Mashungwa *et al*., 2019). Lentil has major economic and ecological functions in Ethiopian low-input agriculture. The crop is mainly grown as a cash crop by smallholder farmers at the highest price in local markets in comparison to other food crops. Lentil is also one of the main pulse export crops that generate departmental income at the macro-level (Ferede *et al.,* 2014). Since 1997, Ethiopia has exported several lentil volumes to different areas of the world, with the lentil amount and value greatly increased until 2009. However, the trend in lentil amount and value has decreased since 2009 (Eyob & Baye, 2019).

Like many other food legumes, lentil is a valuable protein, and products contain no anti-nutrition or toxicity. It is the main pulse crop used as food in the country. whole seed or split seed is fried to produce a wot, which is consumed with injera. Lentil is a major component in making Sambusa' in Ethiopia. The dry seed consists of 25.0% protein, 1.0% fat, and 55.8% carbohydrate. From the fats, 82% are unsaturated fats, while 18% are saturated. From 100grams of lentil, we can get 353 kcal energy, 10.7 grams of dietary fiber, and though not complete, some amount of all the nine essential amino acids ranging from 0.22 to 2.05 grams. Also, 100-gram lentil meets the 76% Thiamine vitamin B1, 43% Pantothenic acid B5, 42% of vitamin B6, 100% of Folate vitamin B9, and 50% of our daily dietary requirement, assuming we have a daily need of 2000 calories (Edwards et al., 2019).

Due to its capacity to fix nitrogen, lentil enhances soil health, long-term soil fertility, and sustainable crop systems in cereals in the highlands of Ethiopia (Yirga *et al.,* 2016). Besides, lentils can be used to feed livestock, particularly poultry, although they are mostly human feeding. The walls of the stroke and pod have a high feed

weight, the waste from threshing. According to Mersha & Lemessa (2019), the nutritional values of lentil haulm including crude protein (6.9-8.1%), neutral detergent fiber (49.5-52.0%), metabolizable energy (8.0-8.2%), Ash (9.4-9.8 MJ kg-1), acid detergent fiber (36.8-38.0%) and in vitro organic matter digestibility (54.8-55.3%), which favors optimum feed intake and stimulating rumen function and cud-chewing for ruminant livestock.

Overview of lentil production constraints in Ethiopia

Due to increasing domestic demand, export potential, and high economic return, the area harvested for lentil increased from 39 thousand hectares in 1992 to 289 thousand hectares in 2019, increased by 86% (Fig.1b), it shows an increasing trend from 1992 to 2019 years. However, in the last five years (2014 to 2019) the area harvested for lentils has decreased by 30% and unfortunately, it was down by 47.5% in 2018 as it is compared to its previous year. The national productivity of lentils also shows an increasing trend starting from 1992 till 2019 as one can look in Figure 1a. However, Ethiopia's overall lentil production is lower than the average of the top five nations, owing to varieties of constraints (Fig.1a). The major constraints for the slow growth rate of lentil production and productivity are briefly discuses as follows:

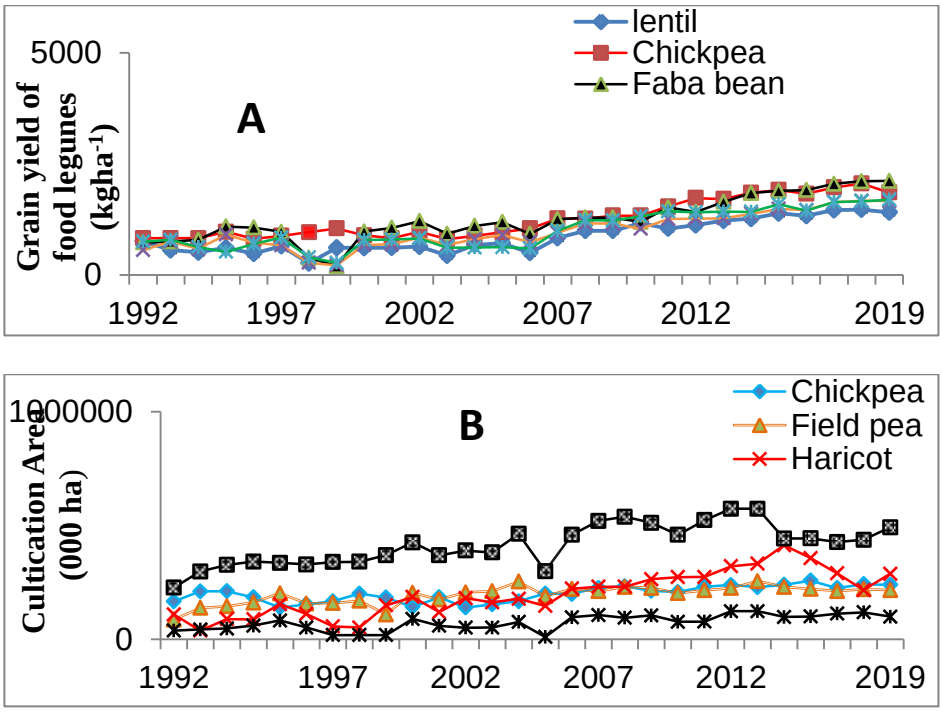


Fig 1 a & b. Ethiopian food legume production and productivity trends: (A) Region under cultivation of large food legumes grown in Ethiopia (Source: CSA (1992-2019)); (B) grain productivity (kg ha^{-1})

Agro-climate conditions and cropping systems in which lentil is cultivated differ across growing regions in Ethiopia. The productivity of this crop in this situation, therefore, depends on rainfall between mid-June and mid-October (Telaye *et al.*, 1994). Lentil is one of the main highland pulses and is suggested planting in mid to high-altitude areas of Ethiopia from late June to mid-July (Mebrate Tamrat & Admasu, 2018). Vertisols, on the other hand, are valuable agricultural soil in Ethiopia's highlands, where they are productive but difficult to maintain due to inadequate internal drainage and consequent waterlogging. As a result, the soil has inadequate aeration, lower soil microbial activity, depletion and unavailability of plant nutrients, and poor workability, all of which negatively impact crop growth, owing to reduced oxygen supply to the roots (Mekonen *et al*., 2013). Lentil is the most sensitive to waterlogging (Singh *et al.*, 2013). Waterlogging during germination can cause unsuccessful germination, late emergence, and suppression of root growth and at the vegetative stage can induce root system damage and lead to extensive leaf senescence and desiccation (Nessa *et al.*, 2013). Similarly, lentil is most susceptible to waterlogging at flowering and lead to flowers and pods abortion, retarded growth, and leaf senescence (turning from yellow to red). The crop will also face a rapid rise or lower in temperature and depleting soil moisture at grain filling stage, causing forced maturity. Dry and thermal seeds are normal in moisture stress areas during the seeding and blooming phases, intermediate drought during the crop season, and terminal drought and fire.

Lentil is mostly produced by smallholder farmers most of them follow conventional farming method based on indigenous knowledge. (Frehiwot, 2009). With this type of agriculture, the common agronomic practices of lentils mostly done by manual and seed handling and cleaning operations are not mechanized, even their produced grains/seeds are mostly consumed on-farm or sold to local markets. Farmers usually planted lentil in early July to mid-September and harvested without weeding at any stage of crop growth. Seeding rates are also extremely low: 30-35 kg^{-1}. However, research recommendations are a minimum of 50 kg ha^{-1} for small size lentils (Telaye *et al*., 1994). Moreover, farmers have never used fertilizer in food lentil production. Instead, this crop is used as the restorer of soil fertility for the following cereal crops. Threshing is done either through trampling by cattle or horses or in some sectors by groups of men or women who beat the dried plants with sticks. Winnowing is also done by throwing the chaff in the air on a windy day with a wooden fork or by hand. As a result, the returns from the above agricultural practices for lentil production are very low (Telaye *et al*., 1994).

Lentil is susceptible to a high prevalence of disease and insect pests .

The most common biotic factors causing the loss of lentil yield are the lentil wilt/root rot cluster, Ascochyta blight, and rust (Ahmed and Ayalew, 2006; Negussie *et al.,* 2006). Some insect-pests attack lentil during the various stages of development and cause damage by defoliation, tunneling of stems and pods, flowers, and floral parts. This may result in poor plant growth, losses in the quality and quantity of pods and seeds, and severe cases that result in plant mortality. Among these green pea aphids, and in some cases, trips and pod borer are the common important pests against lentil production (Regassa *et al.,* 2006). Weeds, both grass, and broadleaf, are a major impediment to increase lentil production and fast harvesting. From seedling establishment to early flowering stages, lentil is highly susceptible to weed competition. Weeds will drastically reduce lentil yield if left unchecked (Gerba, 2002). In general, lentil production in Ethiopia is influenced by farmers' land holding, agro-ecosystems, biotic and abiotic stresses, insufficient improved crop varieties, seed quality, and integrated nutrient management

Agronomic advancement of lentil in Ethiopia

To characterize agronomic technique, which affects crop production and performance, the terms "Best Management Practices," (Zavattaro *et al.,* 2015) and "Beneficial Management Practices (BMP)" (Baird *et al.,* 2016) have been used extensively. The first term shows how BMPs affect plant growth and maturity, crop production, quality, and efficiency of input, and the latter demonstrate how cropping practices are beneficial for non-cultivation issues. The lentil is one of Ethiopia's largest highlands, which is rotating on thick black soils (Vertisols) with tef, wheat, and barley (Geletu, 2006). In order to enhance and sustain lentil productivity at desired levels for better food and nutritional security, the development and refinement of low–cost lentil production technologies need greater emphasis so that these technologies are acceptable to resource-constrained Ethiopians farmers. Among the most potential technologies in lentil production include improved crop establishment and management practices, integrated soil fertility, and pest management practices, etc. which enhance not only productivity and profitability but also warrant environmental and social sustainability besides nutritional security. Agricultural activities should be economically feasible, ecologically sound, and socially acceptable together with food safety and quality dimensions. In this review, we use both BMP terms to describe key cultivation practices of lentils and highlight those that affect lentil production in Ethiopian.

Seedbed preparation

In the northeast and central Ethiopia, where lentil growing fields on Vertisols remain waterlogged during the main rainy season (June to

August), therefore, adequate provision of surface drainage is very important in land preparation. Preparation of land for lentil starts from May to July using traditional *Maresha* three or four times (Milliom, 1994). The bulk of lentil is mostly grown in Vertisols, where the main rainy season contains excess water. Since lentil is highly sensitive to waterlogging, farmers generally tend to cultivate lentil in on black soils having gentle slope and drain the excess water with ridge and furrow drainage systems (Geletu and Million, 1996). Studies conducted during the last several years have demonstrated the importance of improving drainage on lentil production. According to Tekalign (1992) report, pooled mean grain yield from the broad bed and furrow planting at two locations had an acceptable economic advantage over ridge and furrows planting, with a marginal rate of return of 1125%. Moreover, the broad bed and furrow method was more effective in draining excess water than the farmers' ridge and furrows method at Akaki, Dibandiba, and Keteba, which contributed 59, 102, and 99% for yield improvement, respectively (Figure,2). Likewise, Erkossa *et al*. (2006) reported that the lentil grain yield was significantly increased by 59% under large bed and furrow in comparison to control at the Chef Dona site (from 1029 to 1632 kg ha^{-1}).

More recently, Tolesa & Asrat (2019) observed that raised seedbed type sowing recorded significantly higher lentil yields and protect disease over other seedbed types at the Chefe Donsa site. Thus, drainage management is very important in the effort to boost the productivity of lentils per unit area of land. However, such practice requires strong effort to implement; as a result, farmers are becoming skeptical and reluctant to use these technologies for lentil production. Due to this productivity of lentils is highly hampered by the inherent properties of the soil. Thus, to increase lentil production to achieve food security in this region, more agricultural extension services, and future research should therefore concentrate in particular on the viability and drainage status of the Vertisols.

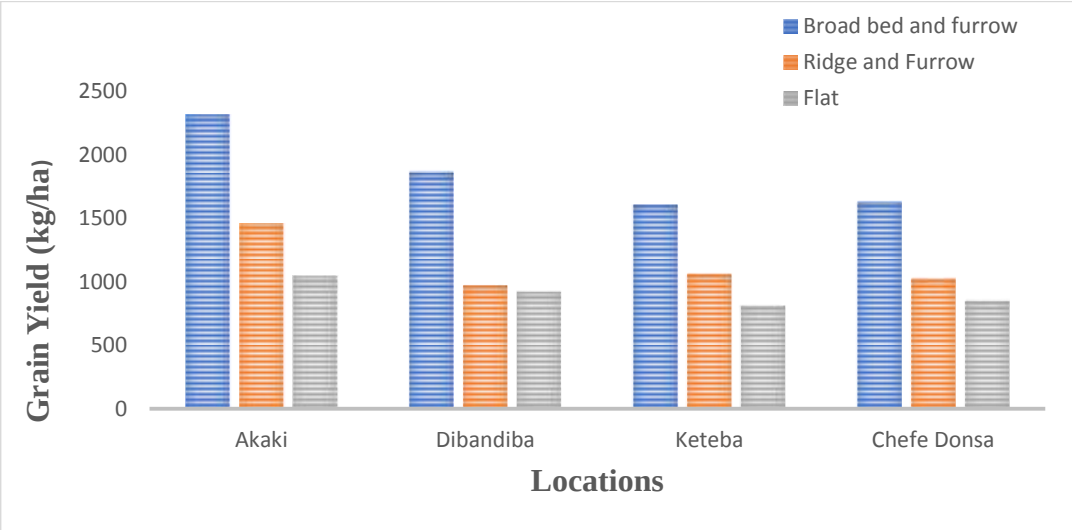


Fig 2. Seed yields of lentils as affected by drainage systems at four locations Source: DZARC, 1991 and Teklu *et al.*, 2006.

Planting method

The most widely used sowing method of lentil in Ethiopia is broadcasting. However, recently row planting by drilling method has been also started in some areas. The drilling method has an advantage over the broadcasting method, as it requires less seed, and facilitates mechanical weed control, rouging, and field inspection as well as giving better seed yield. The recommended lentil planting space using the drill method is 20 cm between rows and 2.5 to 5 cm between plants. Generally, the sowing method is an important factor that has a direct effect on seed requirement, plant establishment, and cultural operations, and efficiency of production inputs (Mitiku, 2016).

Sowing Window

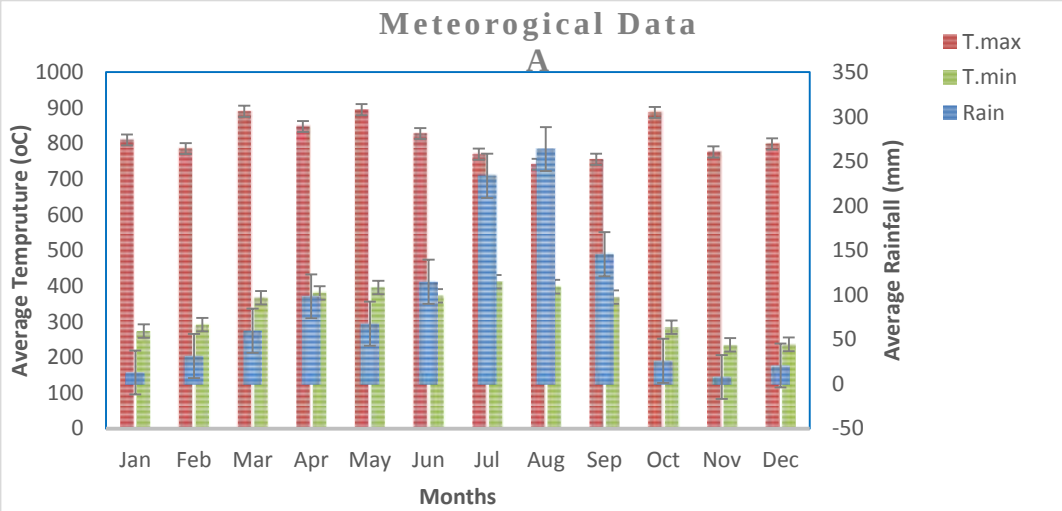
Seed development, phenological efficiency, insect-pest, weed dynamics, and crop productivity are all influenced by the time before planting. The environmental situations, topography, and elevation of the area, specific crop/lentil cultivar, soil types, etc. significantly changes with the time of sowing (Pooniya *et al.*, 2015). The choice of an optimal planting time can also compromise between the maximization of yield potential and minimization of the disease level. Early sowing of lentil may not result in higher yields, despite earlier flowering, due to the increased risk of disease and the impact of low temperatures on flowering but later sown can compensate through shortening their vegetative phase and flowering at temperatures more conducive to subsequent pod development (Ali *et al.*, 1998).

In Ethiopia, lentil planting varies across growing regions due to various agroclimatic conditions and cropping systems. Rains of the main growing

season always begin in June, and in July and August, the majority of the highlands are rained as low as possible. However, its amount and distribution significantly decline by a head of mid-August (Fig. 9a). Several studies have shown that planting time is among the most important factors in Ethiopia that affect lentil production in many lentil growing areas. It is more serious in low rainfall areas where it fluctuates from season to season... The first experiment was conducted in the late 1970s and early 1980s at several sites of DZARC and other center of the Institute of Agricultural Research. The study conducted at Welenkomi using six sowing dates and two lentil cultivars (small- and large-seeded) indicated that the second fortnight of August was found best for planting lentils (IAR, 1976). Later on, research at Kulumsa revealed that plantation in mid-July was the best time for rising Lentils (DZARC, 1977- 82). In comparison, planting at the DZARC station at the end of June to the beginning of July was higher than the late planting. Similarly, lentil planted at Akaki from late June to mid-July, Ejere from late June to early July, and Chefe Donsa for the second half of July were the best times for small and big crops (DZARC,1977-82). DZARC (1983) annual report showed that early July to mid-August planting was optimum for planting the local and exotic lentil at Tefki and Enwari. (Bejiga, 1991) also stated that the sowing date between the last week of June and the second week of July maximizes lentil yield. Million and Geletu (1998), on the other hand, found that the optimal date for sowing of the early maturing lentil varieties was until mid-July. In the same way, lentil sowing in July has been seen to have a profit over the conventional farmers planting time (late August to September sowing). The study conducted at Mehal-Meda showed that the lentil planted on BBF gave a 158 percent yield gain in early July compared to those on BBF in early August (ShARC, 2002).

Recently, Regassa *et al*. (2006) reported that lentil should be planted from July to mid-August on sloppy fields, but on flooded fields, better started in early September. In double cropping system at Dabat lentil planted in September after harvesting of barley, wheat and tef. It also proposed that in some areas of Bale and the East and West Hararghe areas a small portion of lentil has grown during the short rainy season (April-May). This result also suggested that the late maturing variety Alemaya had responded to a wider sowing date starting from July 19 until the end of August, but to escape the excess rainfall at early stages and the late coming frost on early planting (July 30 to August 09) found to be optimum. Figure 2bclearly stated that the first and the second weeks of August sowing dates were gave higher lentil yields than the third and fourth week of August sowing dates. This yield reduction in late-sown lentils was attributed to moisture stress at flowering and pod-filling stages due to

the declined amount of rainfall. However, early-sown in the first three consecutive weeks of July were not suitable for lentil grain yield. This might be early-sown lentils had luxurious vegetative growth accompanied by severe lodging and create favorable conditions for disease and pests due to the prolonged rainfall beyond the normal growing season. From the most obsolete studies above, it is clear that most authors were unable to justify the change in sowing date for the targeted area considering or incorporating physical-chemical characteristics of the soil and the trend rainfall.



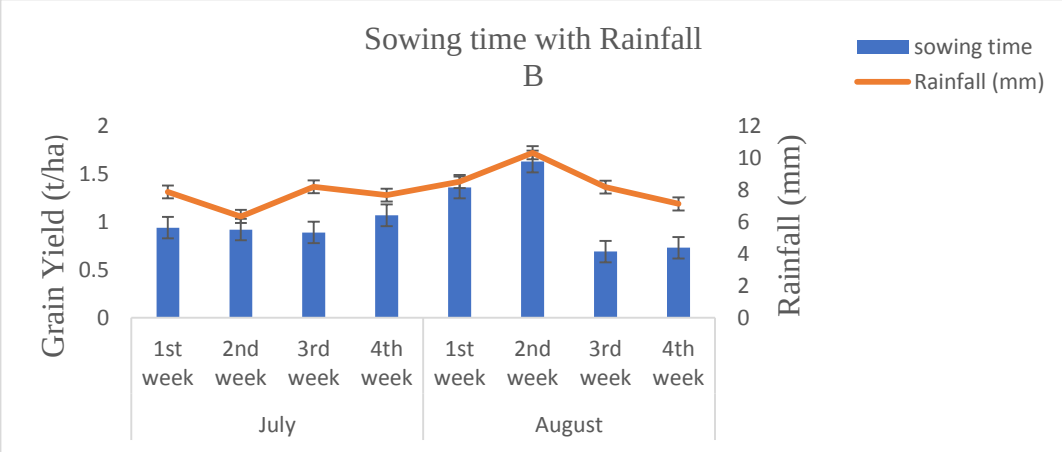


Fig. 9. A thirty-six (1984– 2019) average monthly rainfall pattern of the main lentil research station at Debre Zeit, Ethiopia, during lentil growing season. b Mean grain yield (t ha^{-1}) of lentil genotypes planted on different sowing dates (pooled into weeks) on experimental field conditions in Ethiopia. The data were extracted from results of different field trials conducted over the past several years (1977–2018) mainly on *Vertisols* testing sites (Debre Zeit, Akaki, Chefe Donsa, Inewari, Tefki, Ejeri, and Ginchi) representing the central highland Vertisols lentil growing areas.

Planting density

The number of seeds needed depends on the cropping system, the type of crop variety, the growing season, the test weight, and the seed material's germination percentage. The seed rate is also influenced by environmental conditions and the length of the crop's growth cycle. The seed/seedling rate is determined primarily by the target plant population per unit area (Prasad, 2012). A target plant stand of 130 plants is the suggested plant population for lentil per square meter. The seed rate is determined by the weight of 1000 kernels of each variety and can differ greatly based on the variety and the survival estimate (Fleury & Ag, 2016). In Ethiopia, lentil seed rates, such as those of sowing dates have been conducted at various DZARC and EIAR sites in the late 1970s and early and mid-1980s. The study conducted at Kulumsa showed that the maximum seed yield of lentil was obtained .from the middle two seeding rates (50-55 kg ha^{-1}) Similarly, at Debre Zeit and Chefe Donsa sites, the highest seed yields of lentil were obtained from 70 and 75 kg ha^{-1} seed rate , respectively (DZARC 1977- 82). In parallel tests, 70 kg seed $ha^{-1}$of lentil was beneficial at Akaki's compared to other seed rate. Based on these findings, a seed rate between 50 and 65 kg ha^{-1} was found to be ideal for large lentil cultivars for broadcasting lentil seeding and 65-75 kg ha^{-1} for small seed cultivars (Million and Benwal, 1988). Mitiku (2016) recommended a seed rates for the small lentil 50-65 kg ha^{-1}; for the medium 65-80 kg ha^{-1}, and the heavy or large seed lentil up to 120 kg ha^{-1}. However, the growing environmental and soil types may not be considered in these guidelines. Besides, the seed rate of lentils must also take into account the germination percentage of the seed, the growth habit of a particular cultivar, and, above all, the soil moisture balance and the disease load on the soil, to achieve the optimal plant density to optimize yield and ensure official exploitation of resources. In general, the model-based forecasts of interactions between genotype, climate, and cropping mechanism cannot be taken into account in all recommendations. Therefore, revisit of the seed rate of lentil is an important agronomic practice to rising of lentil productivity in the future.

Fertilizer management on lentil

The Ethiopian Vertisols areas are characterized by smallholder mixed grain farms with a distinct orientation towards livelihoods (Kebede, 2020). The region characterized as big crop production is carried out and high inputs such as DAP and Urea are used, in particular for cereals crop. Legumes are also considered as a secondary crop next to cereals and used in the following cereal crops to maintain soil fertility. According to Berhanu (1985), the pH of 61% of vertisols is 5.5-6.7, 70% of the available P is below 5 ppm and the overall total N content varies from 0.08 to 0.22 percent, which is low. Most growers, however, do not

use inorganic fertilizers, since they assume that legumes require no fertilizer and prioritize their application on other crops such as tef and wheat. However, the latest studies of national soil analysis showed that in most parts of Ethiopian highlands, the vast majority of agricultural soils showed low fertility (EthioSIS, 2014). Even if the above-mentioned facts are controversial, good crop production is a well-balanced supply of nutrients in appropriate quantities and forms. Fertilizer management ensures that an adequate fertilizer loss minimization approach allows for good nutrient use to improve crop production and sustain soil fertility at the right time (Dass *et al.*, 2014).

Response of lentil to N application

Like other legumes, lentil requires less nitrogen, since it can biologically fix atmosphere N through Rhizobial bacteria. Under good conditions for the symbiotic association, 70–90% of the N requirement for the lentil crop can be met through biological N fixation, with the remainder derived from soil N sources. Therefore, the application of inorganic N fertilizer would not necessarily react to a well-nodulated lentil culture inoculated with a proper lentil Rhizobium. On the other hand, if lentil is grown in low organic soils, dry environments, or seeded early into cool, wet soils may benefit from the application of low rates of N as a 'starter N' source. However, given that high inorganic N levels are known to have adverse effects on early nodulation, or that they prolong nodulation and lead to poor seedling development, the start N application rate should only be 10–25 kg ha^{-1}. Starter N rates higher than these rates can promote excessive vegetative growth and prolong maturity, both of which lead to poor seed set. In wet conditions, too much vegetative growth may create a microclimate suited for disease development. Research on the use of starter nitrogen has generally found advantages in greenhouse experiments but no or little advantage in the field (Togay *et al.*, 2008). Studies at Ginchi and Dembi in Ethiopia have shown that no apparent difference in lentil production was achieved in the application of N fertilizer in Dembi and the linear output was increased in Ginchi with increased N fertilization (Angaw and Desta, 1990). This may be because of high N soil availability. This research is therefore quite outdated and inadequate to widespread the effect of N applications on the production of lentil grain and in particular its nutrient requirements for vertisols. Most recent study at Northern Ethiopia also showed a non-significant effect of different level of N fertilizer on lentil yield. However, application of starter N at a rate of 20 kg/ha increased lentil yield 100% over nil application (Mesfin et al., 2020). In agreement to this finding a research conducted in USA under rain-fed revealed that application of an external N did not contribute to the yield enhancement of lentil (Huang et al., 2016). Therefore, by considering the

diverse agro-ecologies, current soil fertility status and climate variability, the recommendation on N reaction to lentils should be revised, to narrow the large yield gaps that exist between the real and achievable level for food security in the country.

Response of lentil to P application

Since lentils are effective N fixers under most conditions, P may be the most common nutrient limiting lentil growth and yield. To ensure optimum N fastening, optimum seed yield, and time for a harvest, adequate P-nutrition is necessary for lentil production. Phosphorus enhances root development and improves the ability of a lentil crop to tolerate stresses such as drought and frost. In Ethiopia, the first P fertilizer study lentil was conducted using three rates (45, 90, and 135 kg P_2O_5 $ha^{-1}$and two sources (TSP and DAP) at Debre Zeit station, the result indicated that neither the rates nor the sources showed marked differences for seed yield. Although neither supported nor confirmed by the research results, the use of 100 kg ha^{-1} of DAP has been recommended as optimum for lentils (DZARC 1977-82). Similarly, Angaw and Desta (1990) reported that lentils showed no response to P applications at Debre Zeit and Akaki. Angaw and Asnakew (1993) also found that there was no apparent lentil yield difference with P fertilizer application at Dembi and Ginchi sites. This might be the response of P fertilizer application are related to environmental factors, with the greatest responses typically occurring in cool-wet soils. While lentil has a relatively high demand for P, the response of seeds to the application of P fertilizer is inconsistent, even in soils of low soil P. Moreover, Ethiopian Vertisols have high P-fixing properties due to continuously cultivation as a result of a shortage of land (Hailu, 1991). This indicating that there is a high chance of getting a lack of crop response to P application. Thus, many investigators failed to find a positive response to P applied.

However, recent fertilizer studies indicated that lentil is significantly responsive to P application. A fertilizer study conducted at West Showa Vertisols sites indicated that the application of 20 kg P ha^{-1}, gave comparable grain yield and had a 64% yield advantage over unfertilized (Zike *et al*., 2017). Similarly, Gebrekidan *et al. (*2020) reported that combined application of 20/20 kg P/S ha-1 markedly improved the grain yield of lentil by 83% over the control at Debre Zeit on-farm field study. In comparison, Tesfahun (2007), the P rate of increase of up to 30 kg ha^{-1} in Haramay University Station, has found significant benefits in terms of seed return. Furthermore, lentil seed is extremely sensitive to the seed -row appliedto P fertilizer and the plant stand and seed yield can be substantially reduced at higher P levels. Small amounts of P 20 kg ha^{-1} based on a narrow seed row at 15–20 cm spacing) placed with the seed may

not be harmful and the adverse effect on plant stands may be more severe under drier soil conditions. Where P fertilizer application is necessary, banding the fertilizer to the side away from the seed is recommended. Overall, since the soil in the highlands of Ethiopia varies from farms to valleys and hillsides, crop output also varies and agronomic recommendations shift from appropriate to inappropriate. Therefore, P fertilizer application studies on lentils in Ethiopia have been further verified by considering soil type, soil depth, and the soil/crop/input histories of the farmers' fields, and the economic feasibility of fertilizer for small-scale farmers.

Response of lentil to S application

Unlike NPK fertilizer, sulfur is important for lentil production, but due to the long-term application of high input from DAP and Urea fertilizer, intensive cropping, high-yielding cultivars which extract higher S levels from soils have caused large nutrient deficits, including S (Kissi, 2014). Additionally, lentil being a leguminous crop, the requirement of N fulfill mainly from symbiotic N_2 fixation, which may be affected by S scarcity. Previously, there was no documented recommendation of S fertilizer for lentil production in Ethiopia. Recently, the use of 40 kg S ha^{-1}greatly increased the grain yield of lentil, but this was not statistically higher than 20 kg S ha^{-1} in the main rainfed conditions (Gebrekidan *et al.*, 2019). Gebrekidan *et al*. (2020) found that the combined application of S and P gave the highest grain yield, and hence, 20 kg ha^{-1}S and P were recommended for the study areas and other lentil production areas having similar agroecology. In general, the response of lentil to S application in most lentil growing areas is not well studied and its economic benefits are not yet determined. In the future, neglecting this fertilizer may be aggravated unless attention is given to reverse it. Besides, organic fertilizers such as farmyard manure (FYM) as the main source of soil nutrients by most Ethiopian smallholder farmers. However, the response of lentils to organic fertilizers and micronutrients application as well as the combined study of organic and inorganic fertilizer has not yet reported.

Rhizobium inoculation

In legumes, root nodules are highly specialized structures formed as a result of interactions between the host plant and the invading Rhizobium. The symbiosis between legume and rhizobia contributes to the BNF phase which can most much entirely satisfy the plant's N requirements (Santos *et al.*, 2019). Like other annual legumes, while inoculating seeds, lentils may provide a proportion of their N requirements through means of symbiotic N_2 fixation. FAOSTAT (2004) data showed that the annual nitrogen fixation by lentils was about 73 kg N $ha^{-1}yr^{-1}$ by the above-ground plant part or110 kg N $ha^{-1}yr^{-1}$ including the below-ground parts. The

average removal of nitrogen by lentil was approximately 65 kg N $ha^{-1}yr^{-1}$ in the harvested grain and lentil stored 8 kg N $ha^{-1}yr^{-1}$ in the soil for the crops (Rashid *et al.*, 2012).

In the past, only a few studies on rhizobiology have been done, with work on the rhizobiology of highland pulses such as Faba bean, chickpea, field pea, and lentil starting in 1982 at Nazret Research Center and moving to Holetta Research Center in 1986. (Tekalign and Asgelil, 1994). Little study has been conducted in Ethiopia about lentil growth and yield effects of Rhizobium inoculation. The first research on the need for Rhizobium inoculation in lentils was conducted at Ginchi and Dembi in 1989. . The result showed that there were no major variations in performance between the treatments (IAR, 1989). Similarly, the findings of a foreign lentil reaction test showed that no major variations were seen between treatments due to the soil fertility status of the areas (IAR, 1991).Recently, Tena *et al.* (2016) found that grain yields of lentil varieties were increased by 59%, 44%, and 40% over the control treatments when inoculated with Lt29, Lt5, and with N fertilizer, respectively under field condition. Likewise, inoculation with lt29, lt87, and lt5 improved grain yields in the pot experiment by 92%, 74%, and 67% over the control respectively. They also conclud that under field conditions, the amount of N fixed from lentil ranges from 16.9 to 31.2 kg ha^{-1}. A study made by Kassa (2018) reported that combined application of 150 kg NPSZnB ha^{-1} and rhizobium inoculation increased grain yield by 46% compared to the control. Gebrekidan *et al.* (2019) also confirmed S fertilization with the strain of Rhizobia inoculation enhances the grain yield of lentils by 8.5% over the control. Reports, therefore, indicated that the use of lentil inoculants favors yields, but BNF cannot fully substitute N-fertilizers, particularly in soils with very low N concentration. Thus, the use of 15 or 20 kg N $ha^{-1}$with sowing inoculation can boost grain output (Soares *et al.*, 2016), but increased sowing doses of N may cause reduced nodulation (Hungria *et al*., 2003).

Though the massive economic and environmental benefits of bio-fertilizers, the use in general has remained weak due to limited options of effective commercial BNF inoculants adaptable to diverse growing conditions and poor supply systems. Besides the infection process could fail due to several reasons including; no or few rhizobia in the soil, inability of the rhizobia to survive in the soil due to intrinsic limitations, inappropriate strains of rhizobia in the soil, efficient rhizobia being out-competed by less efficient/effective soil rhizobia, failure of the plant to invest in the symbiosis either through lack (e.g early in the seedling stage) of time or resources or through altered metabolism (e.g. towards nitrate reduction in soils high in available nitrogen). Many reports have highlighted that the potentials of the

native rhizobia population existing in the country is untapped. Therefore, extensive and systematic survey collection programs covering a wide range of lentil-growing agro-ecologies are highly needed to address the current research gaps.

Agronomic interventions for weed management

Weeds are a major constraint for legume production both in private mechanized and labor-intensive smallholder farming systems in Ethiopia. In general, grain legumes are more sensitive to biotic (weed) stresses than other crops and are often confronted with several major constraints simultaneously. Since lentil are sown at the beginning of the rainy season, weeds grow with them, and removing them is necessary. For this constraint, there is usually a research output available for its alleviation, but much of lentil research, and other pulses generally, involves studies on various levels of a single, or at best a few, factors keeping other possible yield-determining factors constant. This traditional, reductionist approach has increased our understanding of the major yield-limiting factors to lentil/pulse production. However, if farmers are to benefit from this component knowledge, it must be integrated into an overall crop management process, accounting for interactions between factors, which include integrated crop management (ICM), and integrated weed management (IWM) have been encouraged. Overall, there is a paucity of agronomic intervention research on lentil weed management in Ethiopia. Nevertheless, few studies shuch as Negussie *et al.* (1993) reported a higher grain yield of lentils with the combinations of conservation tillage, hand weeding, and herbicides application. The result showed that 70% yield increment could be realized through the integrated use of minimum tillage and twice weeding; or zero tillage, terbutryn and supplementary hand weeding. Similarly, the better yield of lentil and lower weed biomass were obtained from the application of herbicides coupled with hand-weeding, indicating better control of the weeds (DZARC, 1996, 1997).

Lentil cropping systems

A wide variety of cropping patterns is followed in different agro-ecological zones of Ethiopia depending on land characteristics and soil moisture regimes. Tef, wheat, maize, barley, and sorghum are the major cereal crops of Ethiopia and are grown extensively throughout the year. Hence, all the major cropping patterns are the above-mentioned crop-based. Lentil is grown as a summer crop largely under rainfed conditions in residual/ conserved moisture. However, in some parts of Ethiopia like Southweast Arsi, South Wollo, and North Shewa Zone, it is also cultivated in belg season.. Depending upon the annual precipitation pattern and demands of the local cropping cycle, lentil is grown to a large extent as a component of rotation with different cereals and to some extent as

intercropping with wheat. The inclusion of lentils in various cropping systems benefits the companion crop or succeeding crop by improving the physical and chemical properties of soil as a result of biological nitrogen fixation and other rotational effects. The major lentil-based rotation patterns are tef-lentil, wheat-lentil, and barley-lentil. Studies conducted so far indicated that nutrient status and the balance of nutrients were significantly influenced by the wheat- lentil intercropping system under supplementary irrigation in the semi-arid highland of Tigray. As a result, the levels of soil nitrogen, phosphorus, and intercropping with single wheat have been shown to marginally increase (Etany, 2016). Similarly, Annual research reports of DZARC (2012) proved that intercropping of wheat with various leguminous crops showed significant yield differences among them. Accordingly, grass pea, fenugreek, chickpea, lentil, and clover yielded in a declining amount. Almaz *et al*. (2021) reported that incorporation of lentil in wheat based cropping system at any combination was found to be more profitable and productive compared to sole wheat and lentil. Among crop combinations, 2:1 wheat-lentil intercropping combinations gave the highest LER, ATER, and MAI values compared to 1:1, 1:2 wheat lentil intercropping combinations. However, as compared to other legume, not much cropping system research work has been done on lentil in Ethiopia, therefore, research focus on lentil cropping system should be encourage inorder to assess the opportunities and challenges of lentil cropping system and identify best cropping syetm for an intensification of legume production in Ethiopia agriculture.

Yield improvement of lentil though agronomic intervention: where and how?

In the past, the discovery and implementation of new technology may have coincided with economic factors to create improvements in cropping practices that increased grain yield. While it is generally acknowledged that breeding and agronomic practices have contributed to increased yields, their relative contributions have varied depending on the crop species and the climate (Anderson *et al.,* 2005). In rainfed agriculture, there are two large areas for improving the yield of lentils.

Tactical management

Decisions concerning cultivar preference, sowing date, seed or plant population, fertilizers rates, and application techniques, weed, and pest control methods are considered important to the development of modern crops (Siddique *et al.,* 2012). Their contributions to improved yields have been modified and progressed through variation and crop technology (Ward & Siddique, 2015). Farmers' management decisions in Ethiopia are also influenced by seasonal variations in agronomic activities in rainfed regions, which are mainly related to rainfall. Seasonal variance nearly often

has a huge effect on how people respond to tactical management activities. Since lentil is grown mostly in the highland Vertisols of Ethiopia, the crop is usually affected by drainage problems. Thus, some tactical management practices used to reduce the effects of waterlogging in lentil involve sowing time, cultivar selection, seeding rate, and drainage types considering the biochemical and physiological responses for waterlogging tolerance. Future agronomic research can focus on enhancing farmers' ability to adapt tactical management using an agronomic model of cultivar-specific parameters to study and forecast genotype activity in a variety of ecosystems and cropping systems, as well as for a variety of objectives. Model-based predictions of the relationship between genotype, climate, and cropping mechanism are used in three ways:

1) Refining the definition of breeding targets, i.e., identifying the phenological, morphological, and physiological traits to breed for a given aim and environment/cropping system (ideo-typing).
2) Refining the analysis of multi-environment cultivar trials, by characterizing the environments to optimize networks, and understand the interactions between genotype, environment, and cropping systems that are observed in networks (agronomic diagnosis).
3) Helping in the use of new cultivars, by supporting the choice of the best cultivar to grow in a given cropping system, and/or a given environment (cultivar choice).

Strategic management

This mostly focuses on the improvement of the soil, even though decisions about the sequence of crops are also taken before the seed. Improved soil may include strategic activities such as acidity/salinity enhancement, soil compaction, water-logging, non-weathering, and poor SOC stocks, which are also linked to other soil physical deficiencies (Verhulst *et al.,* 2013). Latest remote sensing and global positioning system strategies including yield mapping, variable rate technology, auto-steering, and managed traffic (which can be a blend of tactical and strategic management) have shown promise in lowering manufacturing costs and increasing accuracy (Robertson *et al.*, 2012). On the other hand, components of the conservation agriculture system (zero or minimum tillage, residue retention), using alternative cropping system (crop rotation, intercropping and double cropping), bringing additional area through irrigated and beleg season might also be considered, wholly or partly, as strategic management. Even though the cultivation of lentils recorded a downward trend in most niche areas of lentils in the main season, few lowland areas of Ethiopia have emerged as potential areas of lentil cultivation and expansion. Therefore, searching for additional areas with full agronomic practices is one of the strategic managements to increase lentil production and productivity.

Conclusions and Future Outlooks

The improved agronomic practices and strategies about Ethiopia's lentil production would enhance the capacity of farmers to grow sustainable lentils in Ethiopia despite numerous production vulnerabilities, as discussed in this paper. As a result, an effort has been made in this study to address enhanced lentil production practices that can play a critical role in Ethiopia's long-term lentil production. Which is emphasized nearly all research and development efforts from their inception in the late 1970s to the present, with a focus on aspects of crop management practices that are a bottleneck for lentil productivity in Ethiopia. The paper also stressed that the majority of crop management recommendations for lentil cultivation are outdated and did not reflect the current agro-climatic setup or upcoming environmental changes. Similarly, many recent studies had significant shortcomings in portraying the diversity of emerging agro-ecologies and the dynamism of farming systems. Furthermore, developing a package of modern management practices and upgrading the existing packages would go a long way towards improving Ethiopia's lentil production potential. Thus, reseachers in Ethiopia have developed cutting-edge technology ranging from the varietal growth to crop and resource management practices to improve lentil production. As a result, future research should account for these constraints, and crop management studies should be conducted in a more systematic and organized manner. From this review, the following points reflect the main findings collected with some of the issues requiring immediate attention and others requiring further investigation:

1) In Ethiopia, not much lentil cropping system research have been done compared to other legumes. Therefore, lentil-based cropping systems should be developed for different regions together with information on genotypic compatibility of component crops, spatial arrangements, and fertilizer use.
2) More detailed investigations into the use of bio-fertilizers, inorganic fertilizer, and management practices are needed in lentil cropping systems. It is essential to identify the most successful rhizobia strains under various agro-ecological conditions. There is also further verified that crop-soil management aspects of the Vertisols, particularly agronomic requirements, are not yet adequately addressed, which may need research priority.
3) Improved lentil farm technology and agronomic practices, as well as the promotion and adoption of disease and insect pest resistant high yielding lentil cultivars, are all urgently required in the intensive lentil cropping systems. In addition to their other benefits, bed planting,

planting on ridges, and using resistant cultivars can help with waterlogging prevention.

4) Lentil production has risen dramatically over the last few decades in central parts of Ethiopia. However, maintaining this production trend is a major challenge for researchers, extension agencies, and farmers in these major production areas. Thus, lentil crop must be introduced to non-traditional areas and cereal fallow areas in various agro-ecological zones of the country.
5) Investigation of the physiological aspects of yield variation under different environmental conditions, which might be helpful for agronomic manipulations.

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On-Farm Performance and Farmer Participatory Evaluation of Newly Released Common Bean Varieties in Oromia Regional State, Ethiopia

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Abstract

Common bean is an increasingly important commodity in the cropping systems of smallholder farmers for food and income generation in drought-prone areas of Ethiopia. Despite its high yield potential on research stations, the actual farmer's yield is low. To minimize the yield gap, a range of high-yielding and disease-resistant common bean varieties were released. On-farm evaluation and demonstrations of newly released Awash-2 and SER-125 varieties with the recommended agronomic practices were conducted to select a best-fit variety for large-scale promotion of the technologies. The study was conducted in East Shewa and West Arsi zones of Oromia Regional state in 2017 and 2018. The field experiments were established on 54 farmers' fields. The new varieties were planted and evaluated alongside the checks (Awash-1 and Nasir). A mean yield of 2448 kg/ha and 2793 kg/ha were recorded from Awash-2 and SER-125 varieties, respectively. Given, similar investments per unit area to produce common beans, the new varieties have higher returns because of their yield advantages. The mean yield of SER-125 is significantly higher than that of the check–Nasir ($t_{(22)}$ =9. 236, p=.000). SER-125 gives a significant yield advantage of 363 kg per hectare. Similarly, Awash-2 gave a significantly higher yield than the standard check–Awash-1 ($t_{(30)}$ =8.049, p=.000) in which the mean yield difference of 464 kg per hectare. Sensitivity analysis shows that the financial profitability of common bean farming is more sensitive to reduction in yield than to increases in price. Farmers' common bean variety selection criteria were yield, drought tolerance, grain color, disease and insect resistance, food taste, and large seed size. Accordingly, Awash-2 and SER-125 were selected as the superior varieties as compared to Awash-1 and Nasir varieties respectively. Generally, common bean producers can earn higher returns if they produce the new varieties with the recommended agronomic practices. Therefore, we advise common bean farmers to pursue large-scale production of Awash-2 and SER-125 in the replacement of the old varieties in the moisture stress area such as West Arsi, East Shewa Zone and similar agro-ecologies.

Keywords: Common bean, Farmer's preference, On-farm performance, Yield gap, Participatory evaluation

Introduction

Common bean, *Phaseolus vulgaris L.*, is widely grown in Ethiopia and it is one of the important commodities in the cropping systems of smallholder farmers for food and income generation (Bedru and Nishikawa, 2012; Mulugeta et al., 2015). The crop is among the suitable grain legume crops for crop rotation and intercropping with maize/sorghum. Under rain-fed growing conditions, common beans can fit into various cropping systems: mono-cropping, sequential/relay-cropping, double-cropping, mixed-cropping, and inter-cropping. It can be grown twice a year in bimodal rainfall patterns. In the first season known as *Belg* (March to mid-May) bean is usually intercropped with maize and sorghum while in the main cropping season, *Meher* (end of June to September), is planted as the sole crop. Farmers prefer the crop because of its fast maturing that enables households to get additional cash income as a result of the possibility for double cropping (Berhanu et al., 2018).

Currently, it is produced by over 3 million smallholder farmers in Ethiopia. In the 2019/20 cropping season, the area planted to common beans was 281,083.49 hectares with 488,583.5 tons production. The major producing areas are concentrated in three regional states (Amhara, South Nations Nationalities and People (SNNP), and Oromia). The three regional states constitute more than 96.7% of the entire common bean area and make 96.8 % of the total common bean production (Figure 1). The produced common beans were utilized majorly for household consumption followed by sale, seed and feed, and in-kind payment for wages (Figure 2).

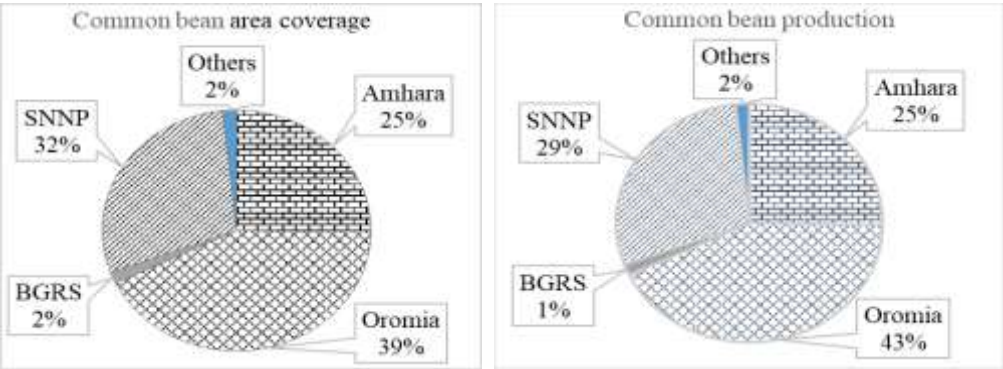

Figure 1. Total area planted and production of common bean across regional states, Ethiopia
Source: CSA, 2020

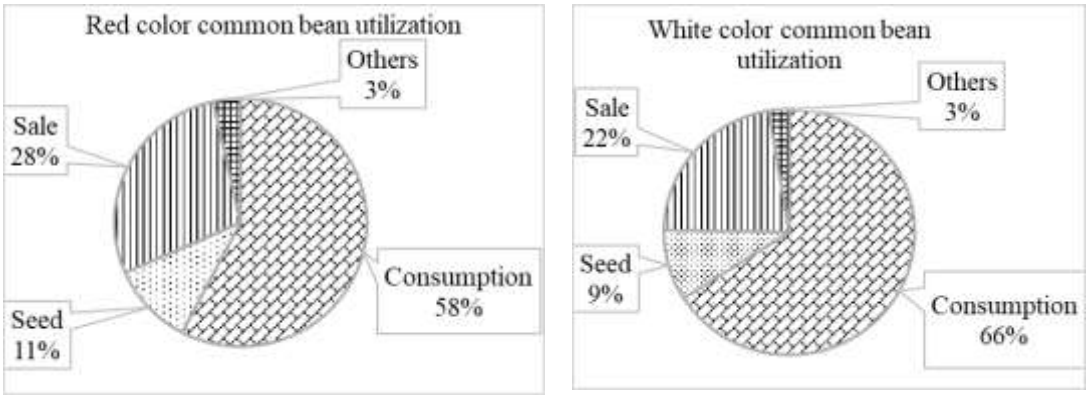

Figure 2. Common bean utilization in Ethiopia
Source: CSA, 2020

Despite the on-research center yield data of 3,500 kg/ha using improved varieties and improved management techniques while the national and East Shewa zone average productivity are 1,738 and 1,841 kg/ha which is significantly below the research result (MoANR, 2016; Berhanu et al., 2018; Endeshaw et al., 2018; CSA, 2020). According to Mulatwa et al. (2017), the low productivity can be attributed to environmental stresses such as drought, insect pests, and diseases. Limited access to the improved common bean seed is also one of the factors constraining productivity (Yitayal and Lema, 2019). The crop productivity can be increased substantially through investment in the dissemination and promotion of improved technologies (Enid et al., 2010). Aiming to enhance the productivity of common beans, the Ethiopian Institute of Agricultural Research (EIAR), has released a range of high-yielding and disease-resistant varieties. Common bean variety release in itself is not an end unless it is utilized as food or sold for cash. In turn, utilization needs the adoption of improved technologies. Hence, on farm improved common bean technology participatory demonstration and evaluation are vital to evaluate the performance of the new varieties to farmers and front-line extension agents to develop their confidence for enhanced adoption and increased yield.

Method and Approach

Description of the study area

The study was conducted in Adama and Adamitulu-Jidokombolcha (AJ) districts in East Shewa and Shalla district in West Arsi zones in Oromia National Regional State. The areas were selected based on their high production potential. The sites were selected in collaboration with the respective district agricultural and

natural resource offices. The description of the districts is presented as follows.

Adama district capital, Adama, is located 100 km to the southeast of Addis Ababa. Adama district is located between 08°33'35"– 08° 38'46"N latitude and 39° 10'57"– 39° 30'15"E longitude. The altitude ranges from 1450 to 2300 meters above sea level. The district receives an average annual rainfall of 600–1200 mm with an annual temperature between 15°C and 32°C. Agriculture is the mainstay of economic activities characterized by a mixed-farming of crop and animal production. Among the major crops, tef, maize and common bean are the major crops in area planted. Vegetables and fruits are grown mainly in areas where irrigation facilities are available (Habtamu et al., 2011)

AJ district capital, Batu, is located 168 km, South of Addis Ababa. The district is geographically located between 7° 35”–8° 05” N and 38° 20” –38° 55” E in the northern part of the Rift Valley. The area receives a mean annual rainfall of 690 mm, and it has an altitude between 1500 and 2300 meters above sea level. Its annual temperature varies between 14℃ and 27℃ respectively. The district is characterized by a bimodal pattern of rainfall; with a short rainy season running from February to April and a long rainy season from June to September. However, the pattern of rainfall is usually erratic with fluctuations in the start and end of the season, in addition to the total absence of rainfall at times (Tesfaye, 2008). Shalla district is one of the districts in the West Arsi zone of Oromia National Regional State. Its capital Aje is located 279 km south of Addis Ababa. The area receives annual rainfall ranging from 1000 to 1200 mm, and the main growing season is from June to September. The altitude of the district is between 1000 and 2300 meters above sea level. The mean annual temperature of the district is 22℃ and 25℃. Agriculture is the primary economic activity for 95% of the population. The major crops grown are maize, wheat, common bean, and tef (Mekonnen et al., 2015; Ahmed et al., 2018).

Farmer’s selection and consultation meeting

In consultation with the respective district agricultural experts and development agents, host farmers were selected based on land availability and willingness to host the experiment. Fifty-four farmers were selected (23 for the SER-125 and 31 for the Awash-2) to establish the demonstrations considering each farmer as a replication. In Adama and AJ districts, where farmers mainly cultivating the white-seeded common bean, Awash-2 were planted with the check (Awash-1). Whereas the red-seeded common bean, SER-125, was planted in AJ and Shalla districts with the check (Nasir). To develop an understanding of improved common bean production practices, a consultative meeting was organized

for farmers, agricultural development agents, and experts. On the occasion, 54 host farmers, 15 development agents, and 10 mid-level experts were participated.

Field layout and agronomic practices

Awash-2 and SER-125 were planted alongside the checks on 0.25 hectares of land, each 0.125 ha. The planting was done from June 25 to July 5. With a row spacing of 40 cm, 10 cm space between plants, 100 kg/ha of seed, 100 kg DAP/ha was applied during planting. Up to three times hand weeding was done depending on the prevalence of weed infestation. Researchers, extension agents, farmers visited the fields and evaluated during the emergence, flowering, and maturity stages of the crop, and evaluated based on farmers selection criteria. Finally, the yield of Awash-2 and SER-125 were weighed, recorded and compared with that of the checks-Awash-1 and Nasir respectively.

Varietal trait preference and ranking

Focus group discussions were held to identify farmers' common bean selection criteria for the preference of both white and red-seeded common bean types. The list of preference criteria for the common bean was ranked using pair-wise ranking to identify the priority traits of farmers. Based on the criteria set, the focus group discussants ranked the demonstrated varieties in each study area. For the focus group discussion, men and women farmers who have long experience in common bean production were selected in collaboration with respective district agricultural experts.

Method of yield gap analysis

The demonstration trials were conducted for two years at different districts/ locations and a mean yield value for the district yield was calculated. The demonstration data were averaged over the years to calculate the mean yield for the districts. The demonstration yields represented the achievable yields with improved management under farmers' conditions.

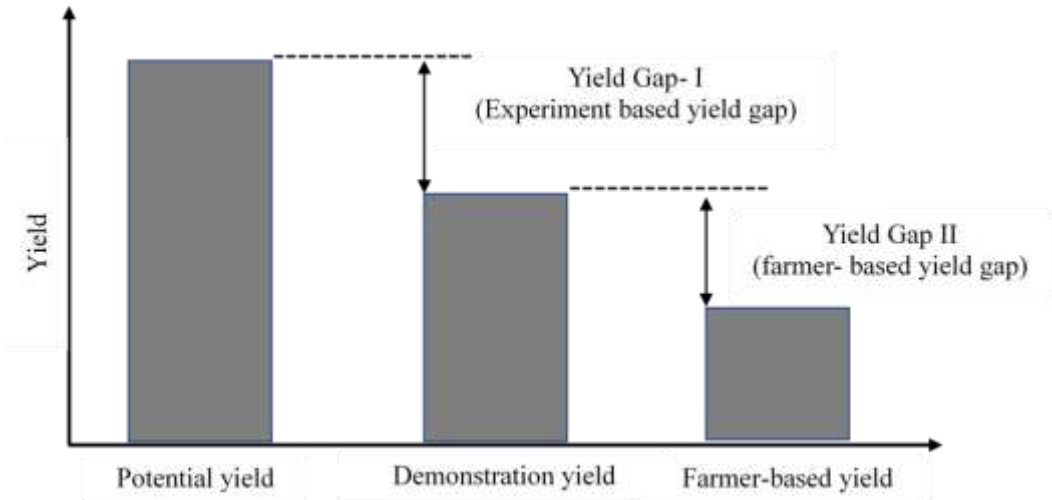

Figure 3. Yield gap as defined by Loblle et al., 2009

The farmer's yield represents yields under farmer management practices. The average production data of the crop in farmers' fields using farmer's practices were obtained directly from the participant farmers. Lobell et al. (2009) divided the yield gap into two types, the gap between research station (potential) and demonstration yield and the gap between demonstration yield and farmers yield (actual). The potential yield is the maximum possible rainfed yield. For the potential yield, the variety Registry Book was consulted for the demonstrated varieties. The crop yield data of the on-farm experiment were used for the demonstration yield.

Data collection and data analysis

Quantitative and qualitative data were collected. The yield was directly measured using a sensitive weight balance. Farmer preference on the variety and important traits were recorded using a data collection sheet. The data were analyzed using descriptive statistics, preference ranking, and yield gap. Finally, the benefit-to-cost ratio was calculated to assess the financial feasibility of improved common bean production.

Results and Discussion

Yield performance

The yield of Awash-2 and SER-125 were compared to the yield performance of their respective checks (Tables 1 and 2). A mean yield of 2,448 kg/ha and 1,984 kg/ha were harvested from Awash-2 and Awash-1 varieties respectively. From the study, we found that SER-125 gave a higher mean yield of 2,793 kg/ha compared with the check mean yield of 2430 kg/ha. This result clearly showed that new varieties have a higher yield. The result conforms with that of MoAL, 2016; Teame et al., 2017; Yitayal and Lema (2019).

Table 1. Summary of demonstration yield result of white-seeded common bean, 2017–2018 (N=31)

District	Variety	Productivity by year (kg/ha)						Combined Mean	SD
		2017 (n=15)			2018 (n=16)				
		Min.	Max.	Mean	Min.	Max	Mean		
Adama	Awash-2	2000	2800	2443	2000	2800	2425	2433	299.2
	Awash-1	1800	2000	1914	1700	2300	1963	1940	168.2
AJ	Awash-2	2100	3100	2513	2413	2900	2412	2463	350.0
	Awash-1	1800	2100	1925	2125	2300	2125	2025	161.2
Total	Awash-2	2000	3100	2480	2000	2900	2419	2448	321.3
	Awash-1	1800	2100	1920	1700	2300	2044	1984	167.5

Source: On-farm demonstration fields; SD=standard deviation

Table 2. Summary of demonstration yield result of red-seeded common bean, 2017–2018 (N=23)

District	Variety	Productivity by year (kg/ha)						Combined Mean	SD
		2017 (n=10)			2018 (n=13)				
		Min.	Max.	Mean	Min.	Max.	Mean		
AJ	SER-125	2600	2800	2748	2600	3333	2939	2852	220.8
	Nasir	2100	2800	2500	2100	2700	2400	2446	216.2
Shalla	SER-125	2560	3000	2816	2600	2833	2683	2739	140.8
	Nasir	2100	2500	2300	2400	2600	2500	2417	158.6
Total	SER-125	2560	3000	2782	2600	3333	2801	2793	188.2
	Nasir	2100	2800	2400	2100	2700	2454	2430	184.5

Source: On-farm demonstration fields; SD= standard deviation

Comparison of yield gaps

The yield gap was analyzed based on the potential yield, demonstration yield, and farmer-based yield. Table 3 presents the difference between the potential, demonstration, and actual farmers' yield. The experiment-based yield gap of red common beans was 707 kg/ha equivalent to a 25% mean yield gap. Under demonstration plots, the yield of common bean with improved variety (SER-125) was 2793 kg/ha, while the mean farmer-based yield of the check was 2430 kg/ha. Thus, the farmer-based yield gap was 363 kg/ha resulting in a 15% of yield advantage. Similarly, the yield gap was calculated for the white-seeded common beans in AJ and Adama districts. The mean value of the experiment-based gap was 652 kg/ha (27 %). The farmer-based mean yield gap was 464 kg/ha (19%) between the demonstration and actual farmers' yields. Due to differences in agronomic practices of smallholder farmers, biotic and abiotic factors, common bean yield differs from 1750 kg/ha, country average yield, (CSA, 2020) to 2793 kg/ha (on-farm demonstration yield). This indicates that there is option and high potential to increase common bean yield by 60% per hectare at the farmers' level by adopting improved varieties, improving the management practices and employing recommended agronomic practices. This is an indication that realized yields at the demonstration sites gave huge

potentials for improvement. If this gap is closed, the common bean production will be enhanced.

Table 3. Yield and yield gap of red seeded common beans (kg/ha)

Parameters	Location		Mean	Parameters	Location		Mean
	AJ	Shalla			Adama	AJ	
Potential yield (SER-125)	3500	3500	3500	Potential yield (Awash-2)	3100	3100	3100
Demo. Yield (SER-125)	2852	2739	2793	Demo. Yield (Awash-2)	2433	2463	2448
Nasir (check) yield	2446	2417	2430	Awash-1 (check) yield	1940	2025	1984
Farmer-based gap	406	322	363	Farmer-based gap	493	438	464
Experiment-based gap	648	761	707	Experiment-based gap	667	637	652

Source: On-farm demonstration fields and MoANR, (2016)

There is a significant mean yield difference among the varieties. The mean yield of SER-125 was significantly higher than that of the check–Nasir ($t_{(22)}$=9.236, p=.000). SER-125 gives a significant yield advantage of 363 kg per hectare. Similarly, Awash-2 gave a significantly higher yield than the standard check–Awash-1 ($t_{(30)}$=8.049, p=.000) in which the mean yield difference of 464 kg per hectare. This shows that the recently released improved common bean varieties have a statistically significant yield advantage over the checks.

Benefit-cost ratio (BCR) analysis

The total cost and benefits of common bean production using the new and old varieties are summarized in Table 4. BCR was calculated as the ratio of total revenue (TR) to total variable cost (TVC) which includes costs of inputs: fertilizer, seed, and operation costs like land preparation, planting, weeding, harvesting. The new varieties (SER-125 and Awash-2) have the highest benefit-cost ratio than the check (Nasir and Awash-1). The result showed that even if there is an equal cost of production within the same group of common beans, there is a positive difference in productivity which leads to higher income. For every one birr invested in the production of SER-125 and Awash-2, a farmer gets an additional 1.17 birr and 1.00 birr as a gross return respectively (Table 4). Thus, farmers will be at the high-profit level if they cultivate SER-125 and Awash-2 using associated production practices. In summary, common bean producer farmers can get a high-level benefit if they produce the relatively new common bean varieties in the study area and similar agro-ecologies.

Table 4. Financial returns of improved common bean production

Item	Awash-2	Awash-1	SER-125	Nasir
Overall mean yield in kg/ha	2448	1984	2793	2430
Price (Birr/kg)	16	16	13	13
Total Variable Cost	19,590	19,590	16,730	16,730

Total Return	39,168	31,744	36,309	31,590
Gross Margin	19,578	12,154	19,579	14,860
Benefit Cost Ratio (BCR)	2.00	1.62	2.17	1.89

Source: Demonstration host farmers

Sensitivity analysis

Agricultural is a risky business. Risk analysis consists of determining how sensitive the investment is to different economic assumptions. This is done by holding all other assumptions fixed and then applying the present value to each different economic assumption. It is a technique that highlights the consequences of changes in prices, volumes, rising costs, or additional investments on the value of projects. This helps us to identify which line of decision is most feasible or less risky. It gives the decision-maker more information to use in deciding whether or not to accept the advice of the original NPV analysis (Vernimmen et al., 2014; Lumby and Jones, 2003).

Table 5. Sensitivity analysis of gross margins in white bean for varying output price and yield

Parameters	Base	10% increase in price	10% decrease in price	10% increase in yield	10% decrease in yield
Yield (kg/ha)	2448	2448	2448	2692.8	2204.2
Price (birr/kg)	16	17.6	14.6	16	16
Total return	39168	43084.8	35740.8	43084.8	35267.2
Total variable cost	19590	19590	19590	19590	19590
Gross margin	19578	23495	16151	23495	15677
BCR	2.00	2.20	1.82	2.20	1.80
% change in gross margin		20.01	-17.51	20.01	-19.92

In the common bean cultivation, a 10% increase in price and yield resulted in a 20% and 19% increment in gross margin of the white and red beans respectively. While the decrease in price and yield by 10% resulted in an 18% and 20% decrease respectively in the gross margin of the white beans. Similarly, a 10% decrease in price and yield of red common beans brings a 20% and 39% reduction in the gross margin respectively (Tables 5 and 6). The implication is that common bean profitability is highly sensitive to yield decrease than price.

Table 6. Sensitivity analysis of gross margins in red bean for varying output price and yield

Parameters	Base	10% increase in price	10% decrease in price	10% increase in yield	10% decrease in yield
Yield (kg/ha)	2793	2793	2793	3072.3	2204.2
price (birr/kg)	13	14.3	11.6	13	13
Total return	36309	39939.9	32398.8	39939.9	28654.6
Total variable cost	16730	16730	16730	16730	16730
Gross margin	19579	23210	15669	23210	11925
BCR	2.17	2.39	1.94	2.39	1.71
% change in Gross margin		18.54	-19.97	18.54	-39.09

Trait preference and ranking

Based on the pair-wise ranking, farmers gave high priority for market preference, yield, resistance to disease, and drought tolerance for white-seeded common beans in the Adama district (Table 7). However, in Shalla district farmers gave priority to resistance to disease and insect, yield, and drought tolerance for the red-seeded common bean (Table 8). The result suggests that farmers give weight to qualitative traits on top of considering yield as an important parameter for varietal preference.

Table 7. Farmers' trait preference on the white-seeded common bean varieties in Adama district (n=10)

Selection criteria	A	B	C	D	E	F	G	H	Points	Rank
Resistant to disease (A)		A	C	D	A	F	A	A	4	3rd
Insect resistance (B)			C	D	E	B	B	B	3	4th
Market preference (C)				C	C	C	C	C	7	1st
High yield (D)					D	D	D	D	6	2nd
Drought tolerant (E)						E	E	E	4	3rd
Seed color (white) (F)							F	H	1	6th
Seed size (G)								H	0	7th
Good test (H)									2	5th

Source: Focus group discussion

Table 8. Farmer's trait preference on the red seeded common bean varieties in Shalla district (n=10)

Selection criteria	A	B	C	D	E	F	G	H	Points	Rank
Market preference (A)		B	C	D	E	F	A	A	2	5th
High yield (B)			C	C	B	B	G	B	4	3rd
Resistant to disease (C)				D	C	C	C	C	7	1st
Resistant insect (D)					D	D	D	D	6	2nd
Drought tolerant (E)						E	E	E	4	3rd
Good test (F)							F	F	2	4th
Seed color (red) (G)								G	2	4th
Seed size (H)									0	6th

Source: Focus group discussion

Awash-2 received the higher score weight at all locations (Table 10). The variety had an overall mean weighted evaluation score of 0.85 which is greater than the mean weighted evaluation score of Awash-1. This shows that Awash-2 is the preferred variety over the check. Similarly, the

SER-125 variety preferred more than the check by all traits set by the common bean farmers (Table 9).

Table 9. Preference ranking of the common bean varieties in AJ and Shalla districts (n=14)

Location	Variety	High Yield	Drought tolerant	Market	Grain color	Food tastes	Seed size	Total score	Rank
AJ	SER-125	3	3	2	2	2	2	14	1st
	Nasir	1	2	2	2	2	2	11	2nd
Shalla	SER-125	2	2	2	3	3	2	14	1st
	Nasir	1	2	2	2	3	2	12	2nd

Note: ranking scores out of 3 points with 1= low score, 2= Moderate score, and 3= high score.
Source: Focus group discussion

Table 10. Preference ranking for the varieties using different criteria (n=21)

District	Variety	Yield	Drought tolerant	Disease-insect resistance	Grain color	Food taste	Seed size	Total score	Weight	Rank
ATJK	Awash-1	1	2	2	2	2	3	12	0.67	2
	Awash-2	2	2	3	2	2	3	14	0.78	1
Dugda	Awash-1	1	2	2	1	1	2	9	0.50	2
	Awash-2	2	3	3	3	2	2	15	0.83	1
Adama	Awash-1	2	3	2	3	2	2	14	0.78	2
	Awash-2	3	2	3	3	3	3	17	0.94	1
Weighted mean score (Awash-1)		1.30	2.33	2	2	1.67	2.33	11.63	0.65	2
Weighted mean score (Awash-2)		2.33	2.33	3	2.67	2.33	2.67	15.33	0.85	1

Note: ranking scores out of 3 points with 1= low score; 2= Moderate score; and 3= high score.
Source: Authors' farmer feedback data

Conclusion and Recommendations

The new common bean varieties (Awash-2 and SER-125) found more productive than the older varieties on farmer's fields. Based on farmer on farm evaluation the new varieties SER-125 and Awash-2 were chosen over older ones—Nasir and Awash-1 because of their yield advantages and preferred traits. The traits that were preferred by the common bean farmers are market demand, high yield, resistance to disease and insects, and drought tolerance. Given, similar investments per unit area to produce common beans, the SER-125 and Awash-2 varieties are more profitable because of higher productivity. Hence, promotion and scaling of Awash-2 and SER-125 as the best replacements for Awash-1 and Nasir respectively is recommended in common bean producing areas in the moisture stress area such as West Arsi, East Shewa Zones and similar agro-ecologies.

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Traditional Production and Utilization of Cowpea in Ethiopia: A Showcase from Two Regional States

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Abstract

Ethiopia is believed to be the possible center of genetic diversity for cowpea but little is known about its production and utilization across diverse agro-ecologies and cultures. This paper is aimed at documenting cowpea production, utilization, variety preference criteria and production challenges. The data were collected using semi-structured questionnaire from 240 farm households in two major cowpea growing regions of Ethiopia, namely South Nations Nationalities and Peoples and Gambella regional states. The results showed that cowpea landraces are predominantly produced for food, market and feed. Preferences for cultivars grown were based on leaf texture, short cooking time, grain texture, early maturity, seed color, high yield and seed availability. Grain, leaves and green pods of cowpea are used as human food and the straw as animal feed. Despite multiple constraints faced by cowpea farmers, the level of technological intervention was minimal. Only 3% of the interviewed farmers responded to receive any information and training about improved cowpea production. Pests, drought, shortage of improved technologies, and limited extension services were among the major production bottlenecks that deserve research and development focus. Besides, to boost the production and productivity of cowpea for achieving household food and nutritional security, research and development endeavors need to capitalize on farmers' indigenous production and utilization experience.

Keyword: Cowpea, farmers' perception, constraints, selection

Introduction

Cowpea (*Vigna unguiculata (L.*) Walp.) is an economically important indigenous African legume crop contributing to food security of millions of small-scale farmers in Africa and maintenance of the environment (Tarawali *et al.*, 2002; Langyintuo *et al.*, 2003). It is a very versatile African crop. It is cultivated around the world primarily as a pulse but also as a vegetable (for its leaves as well as the green peas), as a cover crop and for fodder.

Cowpea is a highly nutritious grain legume well adapted to the stressful growing conditions of tropical Africa,

Asia, Latin America and Southern USA (Singh and Jackai, 1985; Matsui and Singh, 2003; Phillips *et al*., 2003; Singh *et al*., 2003; Trinidad *et al*., 2010). It plays a critical role in the lives of millions of people in Africa and other parts of the developing world where it is a major source of dietary protein that nutritionally complements staple low protein cereal and tuber crops (Langyintuo *et al*., 2003). It is not only rich in nutrients, but also nutraceuticals such as dietary fiber, antioxidants, and polyunsaturated fatty acids and polyphenols (Shetty *et al*., 2013). As a nitrogen-fixing legume cowpea improves soil fertility, and consequently helps to increase the yields of cereal crops when grown in rotation (Tarawali *et al.*, 2002). Cowpea grows in a wide range of soil pH and temperatures, tolerant to drought, shade, and low fertility and acid soils (Akyeampong, 1986; Badiane *et al*., 2012).

Cowpea grows in almost all administrative regions of Ethiopia; however, the Southern Nations, Nationalities and People and the Gambella Regional States are known for the utilization of the multipurpose nature of the crop (Bedru *et al*., 2019; Sisay *et al*., 2019). From the research system so far six varieties have been released for use in the country as grain (MoA, 2018) and all were introductions. So far, local collections (landraces) haven't been started for cowpea varietal development for grain or dual purpose legume. Despite its tremendous benefits, little attention has been given by research and development especially as a multipurpose legume. In order to ascertain the extent of these problems and propose a way forward for cowpea improvement, it was necessary to assess the production and utilization of the crop in the major cowpea production areas of Ethiopia. Information on cropping systems, cultural practices, production constraints, utilization, and benefits are pertinent to improve farmers' preferred varieties and production technology packages. Therefore, the objective of this study was to assess farmers' perceptions and experiences on production, utilization, preference criteria, and production challenges in intervention areas.

Material and Methods

Study area and data collection

The survey was conducted in two regional states of the Ethiopia (Figure 1). Since there was no sub-national or national data on cowpea production and area coverage, the study area was selected based on the discussion with the respective Ministry of Agriculture and Natural Resource (MANR) experts at national, regional, zone and wereda levels (Weredas are the third-level administrative divisions of Ethiopia, which are equivalent to district). Based on the discussions; 'Southern Nations, Nationalities and People Regional state' (SNNPRS) and Gambella Regional states were

selected based on production and consumption practice both as vegetable and grain crops. Accordingly, three zones from SNNPRS and two zones from Gambella were selected. Ten to twenty individuals that consisted of elders, women, and youth were interviewed from each *Kebele* (local lower level administrative structure in Ethiopia), making the total number of respondents 240 (Table 1).

Table 1. Number and distribution of respondents by regions

Regional state	Zone	District	Kebele	Number Respondents
Gambella	Anuak	Abobo	Kire	20
			Pinykew	20
		Itang	Dorong	20
			Bazyel	20
SNNPRS	Segen people	Dirashe	Agrobatena	19
		Konso	Jarso	20
			Nalya Segen	20
	South Omo	Bena Tsemay	Chali	20
			Goldia	20
		South Ari	Keyesa	21
	Wolaita	Humbo	Abela Forecho	20
			Ela Kebela	19
	Total			240

The survey was conducted using a semi-structured questionnaire completed as a personal interview with experienced and trained enumerators. A preliminary questionnaire was prepared, pretested with a group of farmers and modified before the actual data collection. The data were complemented with information obtained from focus group discussion, key informants comprised of cowpea growers from each Kebele, including local agricultural experts.

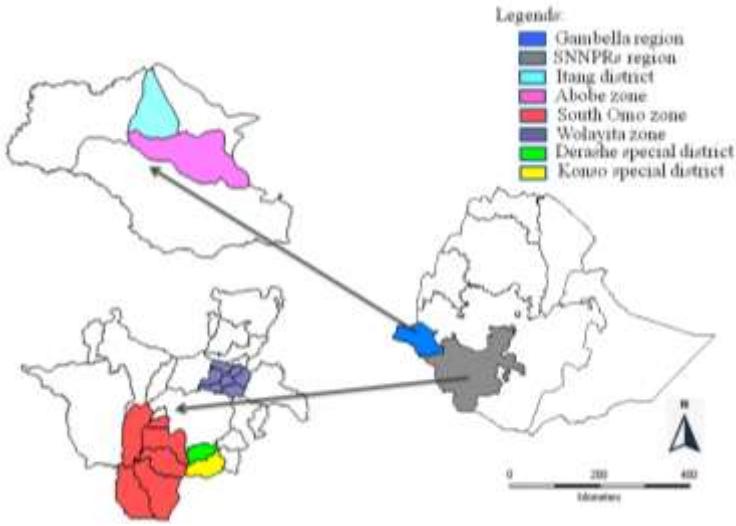


Figure 1. Map of cowpea survey areas in Ethiopia

The required socioeconomic information, production and consumption related information, cropping and seed systems, service delivery such as extension system and input usage as well as use of varieties, variety selection criteria for different traits and status of the crop in the society were collected using survey and focus group discussion. The interview was conducted in October 2017 by trained and experienced enumerators under the close supervision after pre-test of the questionnaire.

Data analysis

The collected data were coded, entered and analyzed using Statistical Package for Social Sciences (SPSS) software version 20. Data were cleaned before analysis. After data cleaning, 217 respondents were used for data analysis. Data analyses were carried out using descriptive statistics namely frequency, percentages and mean.

Result and Discussion

Socio-economic characteristics of sample households

Cowpea farmers were male-dominated but the participation of women were sufficiently high (38%) as compared to their participation in other field crops, for example, maize (14%) (Bedru and Nishikawa, 2017). The percentage of female-headed households' participation in cowpea production was high in Gambella (51%) than SNNPRS (29%) (Table 2). Cowpea here is mainly used as a vegetable mainly managed by women and used for home consumption and marketing in small amount.

Table 2. Socio-economic characteristics of the households

Characteristics	SNNPRS				Gambella Regional state			Total
	South Omo n=60	Wolaita n=29	Segen People n=48	Mean	Anuak n=40	Itang n=40	Mean	N=217
Sex (%)								
Female	32	21	35	29	58	45	52	38
Male	68	79	65	71	43	55	49	62
Year of cowpea growing experience (years)								
Less than 5 years	8.30	55.2	4.2	23	20.0	2.5	49.0	18.0
5-10	25.0	27.6	16.7	23	27.5	32.5	11.3	25.9
10-15	16.7	6.9	18.8	14	7.5	-	30.0	10
15-20	15.0	3.4	12.5	10	5.0	-	7.5	7.2
Above 20 years	35.0	6.9	47.9	30	40.0	65.0	5.0	39
χ^2=65***								
Landholding (ha)								
Total land size	1.38	1.76	0.66	1.27	1.35	1.30	1.33	1.29*
Over all cultivated land	1.25	1.68	0.64	1.19	1.27	1.21	1.24	1.21*
Own land cultivated	1.44	1.47	0.62	1.18	1.27	1.21	1.24	1.20
Rent in land	0.05	0.16	0.03	0.08	-	-		0.05*
Shared land	-	0.28	-	0.28	-	-		0.06**

*significant at p<0.05, **at p<0.01, ***at p<0.001

There was a significant ($p \leq 0.05$) difference in total land size and overall cultivated land size among zones (Table 2). The average landholding in Gambella regional was 1.16 ha (SD=0.27) whereas in SNNPR zones were 1.18 (SD=0.47). The average land holding of cowpea growing farmers in Wolaita zone in SNNPRS was higher than the other zones. In this zone, cowpea is produced by renting and sharing in land. The result of this study revealed that, cowpea growers have fairly enough land to cultivate different crops including cowpea, in both regions.

Of the total sample households, 35-65% has experience of cultivating cowpea for more than 20 years for all study area except Wolaita zone (Table 2). These shows a large number of respondents are well experienced showing the crop is one of the oldest crop in their farming system and the information obtained from the respondents would be reliable.

Farmland allocated to cowpea production

Overall farmland allocated to cowpea production was 0.10–0.27 ha or 20% of the total cultivated land. There was a difference in cowpea areas planted among the zones in 2017 growing year (Table 3). Relatively larger area was allocated to cowpea production in Gambella (0.20 ha) while relatively smaller area (0.18 ha) was allocated to the crop in SNNPRS (Table 3). This might be due to relatively large land holding and the importance of the crop in Gambella region.

There was also a difference among zones in the allocation of their land for cowpea production. Based on the perception of the respondents, they classified their land as fertile, medium fertile and poor or marginal land (Table 3). Accordingly, the result of the analysis of farm allocated revealed that, in Gambella regional state higher proportion (83%) of cowpea growers produce in fertile soil and the remaining 17% produce in medium fertile land. While in the SNNPRS only 25% of farmers allocated their fertile land for cowpea while 52% and 23% of the farmers produce in medium fertile and marginal land, respectively. The reason of allocating fertile soil in Gambella region could be due to availability land and/or the land is less explored. Additionally, Bena Tsemay, South Ari, and Dirashe districts from SNNP allocated fertile soil which may be attributed to intercropping or mixed cropping systems practiced in the area. While in the Humbo district, a high proportion of land for cowpea production was allocated from medium and marginal land. This might be due to high population pressure and degraded soil (Meseret and Dawit, 2019).

Table 3. Farmland allocation for cowpea

Characteristics	SNNPRS			Gambella Regional state		
	South Omo n=60	Wolaita n=29	Segen People n=48	Anuak n=40	Itang n=40	Total n=217
Cowpea planted in 2016/2017 (ha)						
2016 main season	0.19(0.22)	0.25(0.15)	0.19(0.18)	0.24(0.13)	0.22(0.15)	0.22(0.18)
2017 belg season	0.10(0.13)	0.12(0.18)	0.17(0.22)	0.22(0.18)	0.10(0.12)	0.14(0.17)
2017 main season	0.13(0.14	0.27(0.21	0.24(0.22)	0.23(0.13)	0.23(0.20)	0.22(0.19)
Perceived Soil fertility (%)						
Fertile	37.7	20.7	17.0	97.5	67.5	48.1
Medium	55.7	51.7	48.9	2.5	32.5	38.3
Marginal	6.6	27.6	34.0	0.0	0.0	13.6

Values in parentheses are standard deviation

Cropping system

Cowpea production was practiced under varying cropping systems. These include sole cropping, intercropping, mixed and relay cropping. The majority (60%) of cowpea growing households practices sole cropping while 15% of farm households practiced intercropping mainly with maize and sorghum (Table 4). Mixed cropping of cowpea was practiced to a lesser extent. There is variation on production system across the regional states. Cowpea was planted using sole cropping in Gambella regional state where both intercropping and sole cropping were used in SNNPRS. Intercropping of cowpea was usually done with cereals such as maize and sorghum though a few farmers in Gambella region reported intercropping cowpea with groundnut, linseed, tomato, and okra. In SNNPRS, the proportion of the number of farmers using mixed cropping was relatively small. A small proportion of farmers in South Omo (1.7%) and Segen people (8.3%) zone reported cowpea planted mixed with maize, sorghum, common bean, and mung bean. In both regions, small proportions of farmers practice relay cropping. Some farmers in Wolaita zone (10.3%) sow cowpea when the maize mature and in Itang (2.5%) when the sorghum mature. In both regions maize, common bean and sorghum are the major crops (CSA, 2018).

Table 4. Cowpea cropping system n SNNPRS and Gambella regions

	Administrative regions and zones (%)					Total (%)
	SNNPRS			Gambella regional state		
	South Omo N=60	Wolaita N=29	Segen People N=48	Anuak N=40	Itang N=40	
Cropping system (%)						
Solo cropping	46.7	58.6	45.8	82.5	75.0	59.9
Intercropping	18.3	24.1	31.3	0	0	15.3
Mixed	1.7	0	8.3	0	0	2.3
Rely cropping	0	10.4	0	0	2.5	1.8
Sole and Intercropping	33.3	6.9	14.6	17.5	22.5	20.7
Sum	100	100	100	100	100	100
Cowpea intercropping with cowpea (%)						
Maize	54.8	100.0	69.2	28.6	77.8	64.6
Sorghum	38.7	0	30.8	28.6	22.2	29.4
Tef	3.3	0	0	0	0	1.2
linseed	3.2	0	0	0	0	1.2
Groundnut	0	0	0	14.3	0	1.2
Tomato	0	0	0	14.3	0	1.2
Okra	0	0	0	14.3	0	1.2
Sum	100	100	100	100	100	100

Though sole cropping is the major cropping system in both regions, farmers also practiced intercropping manly with maize and sorghum. Thus, researchers should develop cowpea varieties that fit for both cropping systems. For intercropping the cowpea varieties should fit with the crop intercrop with such as with maturity time and growth habit.

Cowpea landraces and variety selection criteria

Almost all (99%) of cowpea grower farmers in the study area grow landraces. The name of the crop is different from area to area depending on the local languages, morphological characteristics, appearance, seed color and names of places where it is being produced. In South Omo zone they call it '*Zaka*,' '*Woqa'* and '*Aeqa'*, in Wolaita also call it '*Aeqa'*, in Segen People '*Ohoda'*, '*Alita'* and '*Elida'*, in Gambella regional state *Boho*, *Rapo* and '*Wenu*'. Often, farmers do not grow a single type of variety in a season. For example, they grow medium to long maturing varieties with good leaf and, grain yield mixed with the early maturing grain type varieties with market preferred seed color and texture (white, creamy and pink color and smooth textured). They indicated that in good season the late maturity type give better yield and if there is interruption of rain or in drought season the early maturing type escape the drought and gives better yield.

Varietal selection criteria of farmers were different from place to place mainly based on the use of cowpea part and marketability. Cowpea varieties are preferred on the bases of their leaf texture, short cooking time,

grain texture, early maturing, seed color, high yielding and accessibility of the seed (Table 5). These indicate the need for site-specific research and breeding approaches. Except for Wolaita zone, leaf quality (texture) was as important as grain yield in other locations. Thus, focus should be given to develop dual-purpose cowpea varieties.

Short cooking time was an important trait in all areas. Cooking time, an indication for cooking quality, is one of the most important factors responsible for consumers' choice for a particular food especially in grain legumes. Like other legumes, longer cooking time makes cowpea uneconomical and unacceptable to consumers (Demooy and Demooy, 1990; Bhokre and Joshi, 2015; Hamid *et al*., 2016). Often, women change their food consumption patterns (reducing food items that have long cooking times) when firewood supplies are limited. The changes can further compromise families' nutritional intakes. Short cooking time is one of the important criteria mentioned by the respondents for choosing a specific variety. Therefore, for maximum utilization of seed by food-insecure consumers, it is essential to address the long cooking times required to make seed palatable.

Ethiopia, as possible center of diversity for cowpea, understanding of their selection criteria is essential to understand the diverse need and challenges of farmers and, thus, to develop a sustainable cowpea breeding and management strategies.

Table 5. Farmers' variety selection criteria

	SNNPRS (percent)			Gambella Regional state (percent)			
Preference criteria	South Omo n=60	Wolaita n=29	Segen People n=48	Anuak n=40	Itang n=40	Total n=217	Over all Rank
Leaf texture	21.0	0	26.2	32.5	30.0	21.9	3
Seed texture	1.7	7.3	0	20.0	25.0	10.8	4
Seed color	6.7	10.3	2.1	5.0	7.0	6.2	5
Seed size	1.7	0	2.1	0	0	0.8	10
Short cooking time	21.7	30.4	29.3	22.5	15.5	23.9	2
Drought resistance	1.7	15.2	0	2.5	0	4.4	6
Early maturing	10.0	5.4	4.2	0	0	3.9	7
Disease resistance	3.3	0	0	5.0	5.0	2.7	8
High leaf yield	3.3	0	0	2.5	0	1.2	9
High grain yield	29.0	31.2	36.3	10.0	15.0	24.3	1
Sum	100	100	100	100	100	100	

Cowpea production constraints

Cowpea grower households in both regions face various production challenges. A household face one or multiple constraints in cowpea production. Insect, disease, weed, input availability (improved seed and pesticide), storage and moisture stress were the production constraints stated by the respondents (Figure 2). In SNNPRS, 77% of the respondents agreed as both field and storage insect were their number one constraint, while 45% of respondents in Gambella region indicated diseases as the number one production challenge. This might be due to the agro-ecological difference between the two regional states. Anuak and Itang zones in the Gambella regional state are characterized by a hot humid climate that favors the development of pathogens (Velásquez *et al*., 2019) whereas the selected zones in SNNPRS are hot dry area that creates conducive environment for insect pest multiplication (Jaworski and Hilszczański, 2013).

As perceived by the farmers, from the major constraints, damage by insect pests was moderate (52%), sever (31%) and mild (7%). The respondents mentioned different types of symptoms when the cowpea attacked by insect. The major symptoms were shrinking of leaf, holes in the pod and grains, reduced pod size and no grain filling, discoloration of grain, drying of leaf and pods, yellowing of leaves and shedding, deformation of leaf and pods, cutting of leaf, stem, and pod. To reduce the insect attack 84% of cowpea farmers use cultural practices such as mixed cropping, intercropping, crop rotation, and removal of infected leaves. Only 16% of farmers use insecticide and those farmers were from Segen people and Wolaita zone of SNNPRS. Several reports indicated that insect pest attack is a major constraint to cowpea production worldwide (Jackai and Daoust, 1986; Togola *et al*., 2017; Mishra *et al.*, 2018). Yield losses of up to 70%, from insect pests alone, have been reported (Livinus *et al*., 2012). Different reports have listed at least 20 major insect pest species in various cowpea producing regions of the world in which the number varies from region to region (Singh and Jackai, 1985; Jackai and Daoust, 1986). The most damaging of all the insect pests are the flowering and post-flowering insect pests. The major flowering and post-flowering insect pest of cowpea in tropical Africa are the flower bud thrips, (*Megalurothrips sjostedti* Tryb.), cowpea pod borer (*Maruca spp.*) and a complex of pod sucking bugs out of which *Clavigralla tomentosicollis* Stal is the dominant species (Jackai and Daoust, 1986). This result indicated the need to identify the major insect peats of cowpea in Ethiopia to devise control option for the growers.

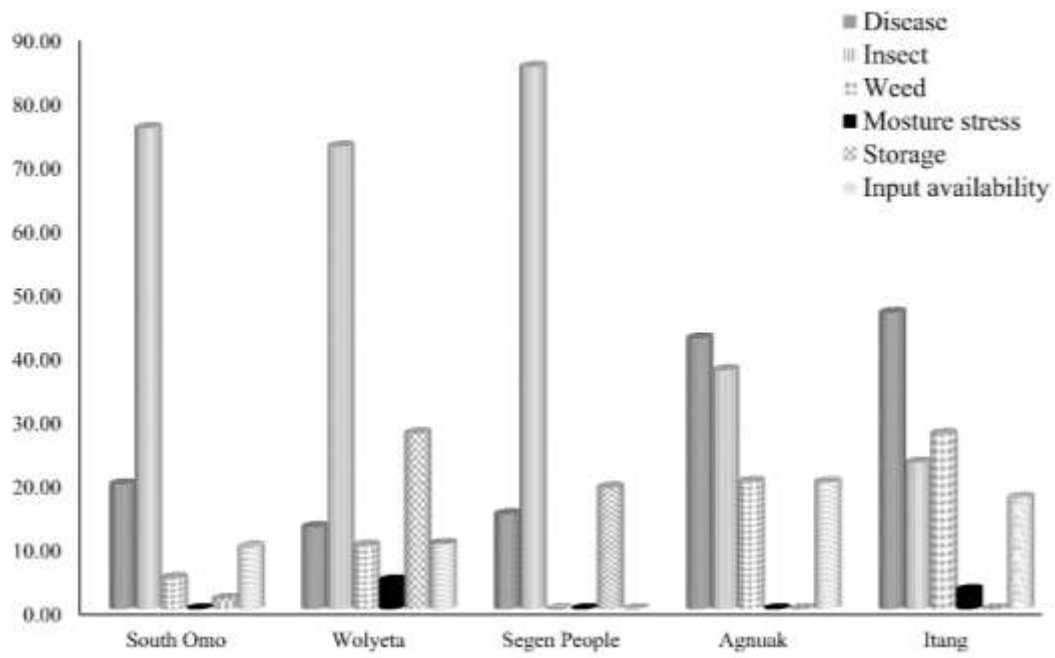


Figure 2. Constraints of cowpea production in Ethiopia by percentage of respondents

Farmers used different practices to control storage pests. Fifty percent of the farmers mix the grain with ash and 10% mix with small cereals (tef and/or millets), and 34% use chemicals (in Konso and Humbo district). The remaining 6% use different traditional practices namely hanging of dry pods around areas it can get smoke, keeping in cooler places, keeping in airtight containers such as plastic bottles, bag and sealed clay pots. Losses of up to 30-70% have been recorded on stored cowpea in the absence of insect pest control measures (Livinus *et al*., 2012). Cowpea bruchids (*Callosobruchus spp*.) are the most common and widespread insect pests in storage. Bruchid attack starts at the field and continued in storage. Bruchids damage might cause seed weight loss, reduce marketable seed quality and germination. At a small farming scale, grains are stored traditionally in different styles of containers, depending on the farmer's socio-economic status and environment. Thus, controlling this storage pest is indispensable.

The second challenge for cowpea production was a disease. Leaves and other parts of this crop disease are important production constraints, especially in the Gambella regional state. Cowpea is known to be affected by several fungal, viral and bacterial diseases. The diseases found on cowpea are mainly fungal diseases incudes anthracnose, ascochyta blight, black leaf spot, leaf smut, brown blotch, cercospora leaf spot, powdery

mildew, pythium stem rot, septoria leaf spot, spbaceloma Scab, and web blight are the common one (Emechebe and Florini, 1997; Nahunnaro and Ayuba, 2012). Thus, prioritizing the main diseases at cowpea growing areas is crucial to devise target control option for the growers.

The third challenge is both broad leaves and grass weeds. Weed is devastating in cowpea production since cowpea is a weak competitor of weed at the early stage and harbors insect pests which reduce the grain quality, and grain and fodder yield (Dugje *et al*., 2009).

In general, detail of insect pests, disease and weed species assessment including the level of damage, has to be done by competent experts since the oral interview hasn't given these detail information. This will help to devise integrated pest management options for the growers.

The fourth challenge is input unavailability (fertilizer and pesticide) shortage as part of cowpea production challenges. The fifth challenge was storage for keeping the seed of cowpea for a long time for keeping for next season planting and for long period of consumption. Moreover, some farmers reported as moisture stress is also one of production constraint. Although cowpea is considered a relatively drought-tolerant crop, previous reports also indicated its productivity is negatively affected by prolonged drought and high temperatures (Agbicodo *et al*., 2009; Hall, 2012).

Most of the farmers in the survey area use own saved seeds (Figure 4). Though seed unavailability and insufficiency were not reported as an issue in the study area, collecting the available landraces, evaluating based on their preference for different purpose and traits, and improving for different stresses and disseminating the improved variety is vital to improve productivity of the cowpea. In addition, despite its potential it hasn't been got research and development attention and it is still one of orphan crops in most African countries including Ethiopia. The Central Statistic Authority (CSA) of Ethiopia also does not generate data about the amount produced, area coverage, productivity, the number of household who produce cowpea and so on. Extension service of cowpea needs attention since the crop is nutritious and a climate smart. Moreover, extension service might need to pack holistic information (from production to consumption).

Cowpea management practice

To obtain high yield and quality product, effective crop management practices are important. Concerning tillage frequency in cowpea production, only 6.5% of cowpea grower households practice one-time tillage while about 87 percent of the farmers plow their cowpea plots two to three times. Only 7% of farmers plow more than three times (Table 6). Land preparation for cowpea production by

the majority of farmers falls within the research recommendation of 2–3 times tillage. Proper land plowing is important to break the soil to loosen it for easy penetration of roots, soil aeration, and water percolation and create a suitable medium for microbial activities within the soil. The result is in agreement with plowing frequency of farmers in Ethiopia, for other major crops (two to six times per planting depending on the crop) (Aune *et al.*, 2001). Cowpea yield increases with good land preparation *(Boukar et al.*, 2019). Among the major reasons for practicing intensive tillage are to prepare the seedbed, conserve soil moisture, reduce weed infestation and increase productivity (Temesgen *et al.*, 2008). Hence the frequency of land tillage is one of an indicator how cowpea is an important crop for the farm household in the study area. Thus, it is possible to say as cowpea is one of the main crops at the study areas.

Planting method is an important agronomic practice in food crop production. Half (51%) of the farm households primarily plant cowpea in broadcasting while the remaining half (47%) grow the crop in row planting (Figure 3). There was a difference among the administrative regional states in their adoption of row planting. The higher proportion (78%) of farm households in Gambella regional state grows cowpea using row planting. In SNNPRS, still the majority (58%) of the household plant in broadcasting. Farmers who planted cowpea in broadcasting reported that they do so because it is a long-held tradition (23%), or because they are not familiar with other planting methods (54%) while 23 percent reported that row planting is time-consuming, labor-intensive and land extensive. Some farmers from South Omo zone mentioned broadcasting distribute seeds better than broadcasting. In the group discussion in Wolaita zone, the participant mentioned that the reason for the use of row planting for cowpea in the area was due to the adoption of row planting of common bean through training and demonstration. Row planting of cowpea is recommended since it facilitates crop management practices during hoeing, weeding, fertilizer application, and harvest. In both regional states planting is mainly done in broadcasting which usually demand higher seed per unit areas.

Table 4. Cowpea management practices in the SNNPRS and Gambella regional state

	Administrative regions and zones (%)					Total (%)
	SNNPRS			Gambella regional state		
	South Omo n=60	Wolaita n=29	Segen People n=48	Anuak n=40	Itang n=40	
Tillage frequency (%)						
One time	6.7	0	14.6	7.5	0	6.5
Two times	23.3	20.7	79.2	62.5	82.5	53.5
Three times	65.0	51.7	6.3	22.5	15.0	33.2
More than three times	5.0	27.6	0	7.5	2.5	6.9
χ^2=102***						
Harvesting frequency for leaf yield (%)						
Less than 7 days	38.6	0	57.1	56.4	74.4	54.7
8 to 15 days	56.1	0	42.9	23.1	17.9	37.1
Throughout growing season	5.3	0	0	20.5	7.7	8.2
Crop stage to stop leaf harvest (%)						
Before flowering	29.8	-	5.7	84.6	56.4	43.5
At flowering	31.6	-	57.1	0	18.0	26.5
At mid pod filling stage	35.1	-	34.3	15.4	25.6	28.2
At maturity	3.5	-	2.9	0	0	1.8

The average seeding rate used for cowpea was 22 kg/ha with a range of 19 to 28 kg/ha. This is within the range of seeding rate of 12–25 kg/ha which is recommended by the International Institute of Tropical Agriculture–IITA (Dugje *et al*., 2009). The seed rate difference among zones was due to the difference in planting methods and cropping systems. Although row planting and intercropping needs relatively lower seed rate, in the study area for these cropping systems they use more amount of seed.

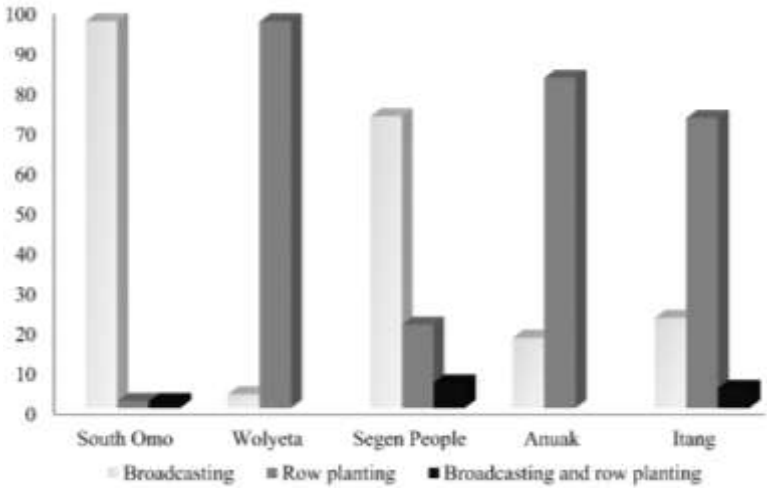


Figure 3. Planting methods of cowpea in different area of Ethiopia.

Cultivation and weeding are other management practices that affect the productivity of the crop. The survey result revealed that 92% of cowpea growers do hoeing and 97% do weed up their cowpea field. Twenty percent of the farmers weed once in the growing season, more than half of the grower weed twice and 25% of them said more than three times. These show how the farmers use gives attention for cowpea production.

The survey result revealed that only 11% of cowpea growers use fertilizer either organic or inorganic or both. Cowpea claimed to fix between 20 and 100 kg N ha^{-1} with an estimated N fertilizer replacement value ranging from 10 to 80 kg N ha^{-1} (Sanginga *et al*., 2000*;* Carsky *et al.,* 2002). This contributes to soil fertility improvement particularly in smallholder farming systems where little or no fertilizer is used. However, nitrogen fixation in legumes can be limited or enhanced by P availability and utilization (Vance *et al*., 2003; Waluyo *et al*., 2004). Because of its multiple effects on plant nutrition, P fertilizer is recommended to increase yields. The crop often responds favorably to phosphorus, because of its multiple effects on plant nutrition, a phosphorus fertilizer is recommended to increase yields. Hence, to improve the productivity of the crop, research should also give attention to select varieties with high phosphorus efficiency and determine optimum P and other important fertilizers for different soil types.

Leaf harvesting was done from the same plant that planted for grain. The majority of farmers stop leaf harvesting before flowering (43.5%), 26.5% and 28% of the farmers stop at flowering and at mid pod filing, respectively. Only 2% of farmers harvest up to maturity as long as there is young leaf in the plant. During group discussion farmers mentioned that leaf harvesting hasten maturity of the crop otherwise the plant stay long for maturity. This opinion needs further investigation.

Availability of inputs

Seed unavailability and insufficiency were not reported to be issues in the cowpea seed supply system in the study area. For instance, 55% of farmers use home-saved seed, 5% home saved and local market, 9% from neighbors or relatives and 25% get it from the local markets (Figure 2.4). Seventy-three percent of farmers get access within less than 30-minute walking distance and 15% farmer access seed from 30 to 60-minute walking distance (Table 7). Similarly, the majority (74.6%) reported that sufficient cowpea seed is obtained for their annual cowpea production. Seventy-three percent of respondents have access to their preferred (familiar) varieties of cowpea. Likewise, the majority of cowpea producers (78%) obtained cowpea seed fairly easily when the landraces are concerned. However, the farmers lack information about improved varieties of cowpea. This was a piece of clear evidence from farmers' heavy

reliance on the landraces for easy accessibility of their seeds.

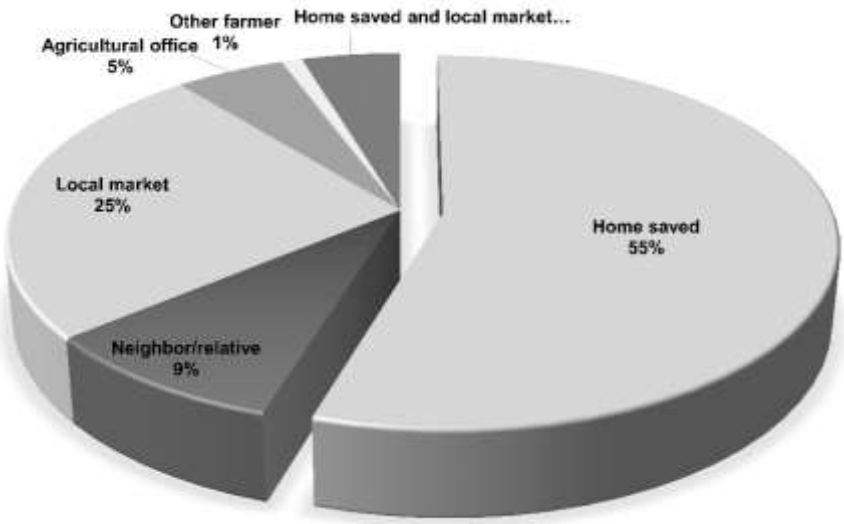


Figure 4. Cowpea seed source in selected zone of cowpea grower areas

Table 5. Distance to the major seed source and estimated price of cowpea in 2016 main season

	SNNPRS			Gambella regional state		Mean
	South Omo n=60	Wolaita n=29	Segen People n=48	Anuak n=40	Itang n=40	
Distance to the market (minute)						
Less than 30	55	72	79	85	73	73
30 to 60	34	24	13	8	0	15
more than 60	10	3	9	8	27	12
Seed price (Birr/kg)						
Seed price*	13.2	18.9	14.6	24.0	24.1	19.0
SD	4.3	6.7	4.2	5.5	4.4	5.0

*Estimated price of seed in 2016 main season, **SD=standard deviation

The price of seed in the local market is different from location to location. The overall mean price for 2016 was 19 birr (1birr=0.036 USD) (SD=5 birr) for one kilogram of cowpea seed. Seeds of cowpea in Gambella regional state was higher with the average price of one kilogram of seed with 24 birr (SD= 4.55 birr) whereas at SNNPRS the average price was 15.6 birr (SD=5.6 birr).

There was no difference in the use of fertilizer (organic or inorganic) to grow cowpea in both regional states. Only 10.6 percent of the respondent says they use fertilizer. Even though, pests are the major problem in cowpea production, in both regional states recommended pesticides were not available.

Purposes and consumption of cowpea

Ninety-two percent of cowpea grower households plant cowpea every year. Though they grow cowpea for a different purpose, the main purpose was food and income (Figure 5). To a lesser extent, they grow for animal feed and medicinal values. Grain and leaves were used significantly higher proportion in both regional states. From the selected zones, the grain harvest is the driving reason for growing cowpea in the Wolaita zone. Parts of cowpea and ways of consumption differ across regional states and across districts. Grain, leaves and green pods were used across all the study areas except Wolaita. Consumption of green pods was not important in itself as its use is related to growing cowpea for other purposes.

Growing cowpea as a feed was reported to be more significant in the Wolaita zone. As we understand from key informant in group discussion, in Wolaita zone of Humbo district cowpea was introduced to the area by Ministry of Agriculture for animal feed as forage crop. As reported by farmers, cowpea performed better than common bean in adaptation of poor soil and moisture stress condition; they started to grow cowpea using the package demonstrated for common beans.

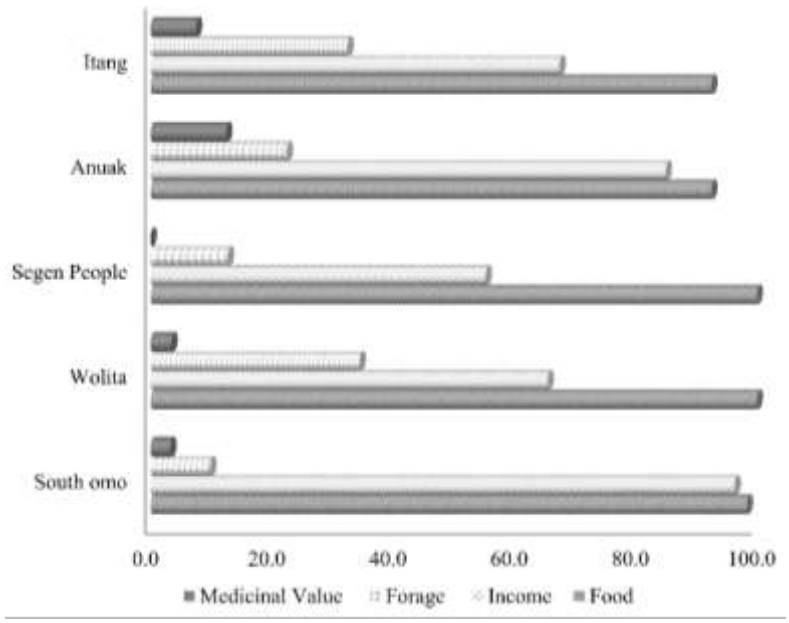


Figure 5. Major reasons for the production of cowpea (percent of the respondent)

Popular cowpea based recipes include stews, soups, and porridges consumed with other cereals like maize and sorghum. Others recipes are

'*fosese'* and '*kurkufa*; which is a cowpea-based product prepared by steaming. *Fosese* prepared by steaming maize or sorghum flour, mixed with cowpea grain and leaf whereas *kurkufa* only use the seed. '*Nefero'* is a popular nutritious food prepared from boiled whole grain cowpeas or mixed with maize grain. 'Kollo' is prepared from roasted cowpea fresh pea or dry grain while '*kita'* is prepared from cowpea flour like chapatti. The leaf is also cooked like any other leafy vegetable or mixed with meat and boiled seed. The roasted and milled cowpea grain flour or split-grain is also used to make stew ('*shero'* in Amharic). In other African countries, cereals are fortified with cowpea in weaning foods formulations (Elemo *et al*., 2011).

About 5% of farmers use cowpea for a medicinal purpose. Especially in the Gambella regional states, farmers used leaves and grain to control diarrhea, malaria, yellow fever and to enhance digestion. Sisay *et al*. (2019) also documented the medicinal value of cowpea ranked fifth in cowpea utilization whereas a quarter of the farmers were reported as they are using leaves and grains of cowpea for the treatment of gastric discomfort, malaria and liver diseases.

Table 6. Consumption of cowpea made food and preference among family members

	Percentage of users by Region					
	SNNPRS (%)			Gambella regional state (%)		
	South Omo N=60	Wolaita N=29	Segen People N=48	Anuak N=40	Itang N=40	Total (%)
Frequency of using cowpea food						
Daily	15	7	13	85	20	27
More than one per week	60	59	60	5	33	45
Once on two weeks	3	10	-	-	3	3
Once per week	17	21	25	10	35	21
Some times	5	3	2	-	10	4
χ^2=115***						
Family member that consume more cowpea (%)						
All family member	71.7	55.2	60.4	97.5	100.0	77.0
Children under 5	5.0	3.4	20.8	2.5	0.0	6.9
Breast feeding mothers	1.7	0.0	0.0	0.0	0.0	0.5
Sick family member	8.3	6.9	2.1	0.0	0.0	3.7
Children under 5,Pregenant mother and breast feeding mother	8.3	0.0	0.0	0.0	0.0	2.3
Children under 5 and pregnant mother	0.0	34.5	16.7	0.0	0.0	8.3
Children under 5 and breast feeding mother	5.0	0.0	0.0	0.0	0.0	1.4
χ^2=97***						

Cowpea was one of the most consumed crop in all study areas (Table 8). In Gambella regional state significant number (53%) of farm households consumes cowpea daily (Table 8) where as in SNNPRS region 60% of households consume more than once per week. There was also a significance difference in consumption of cowpea among family members but about 77% of the respondent said it is consumed by all members of the family (Table 8).

In SNNPRS, 38% of the respondents said there was a difference in consumption of cowpea based foods among the family members while in Gambella regional state 99% of the household said there was no difference. The reason indicated for the difference in consumption among family members was bloating of the stomach and taste preference. Reports elsewhere also indicated the major constraints associated with cowpea utilization are its long cooking time that increases opportunity cost for women's time and fuel wood consumption, flatulence factors and storage chemical residues (Phillips *et al*., 2003).

Cowpea leaves are among the top indigenous leafy vegetables that are marketed and consumed across the entire Africa continent (Barrett, 1990; Smith and Eyzaguirre, 2007). The production of cowpea as a leafy vegetable appears to have increased markedly in many areas in recent years as farmers shift to more drought-tolerant vegetable crops in light of repeated droughts facing many parts of Africa (Saidi *et al*., 2010). But consumption of cowpea as a leafy vegetable is not popular in Ethiopia as the other African countries (Saidi *et al*., 2010*;* Owade *et al.,* 2019). However, the study areas are known for the use of cowpea for both grain and leaf except Wolaita zone. Moreover cowpea ranks among the top 5 leafy vegetable next to Ethiopian kale (*Brassica carinata*) and moringa (*Moringa oleifera*) leaf except in the Anuak zone, where cowpea leafy vegetable is the leading vegetable (Table 9).

Sixty-three percent of farmers have more than ten years' experience of eating cowpea leaves as a vegetable (Table 9). Unlike leaves of other grain legumes, cowpea leaves may be more valuable than the seed because of its protein content and protein productivity exceed that of the seeds on a dry weight basis (Dube and Fanadzo, 2013). Protein concentrations of 29 to 43 % have been reported for cowpea leaves but only from 21 to 33 % in seed (Nielsen et al., 1997; Ahenkora et al., 1998).

The harvesting interval of cowpea leaves was different from farmers to farmers but almost half (49%) of farmers harvest the leaves within less than five days intervals. Harvesting leaves at a 7 days interval gives the highest leaf vegetable yields, but grain yields are depressed and harvesting leaves at a 14 days interval, on the other hand, minimizes grain yield reduction due to leaf harvesting (Saidi *et al*., 2010). Though farmers harvest within less than 5 days interval they might not be harvested from the same plant.

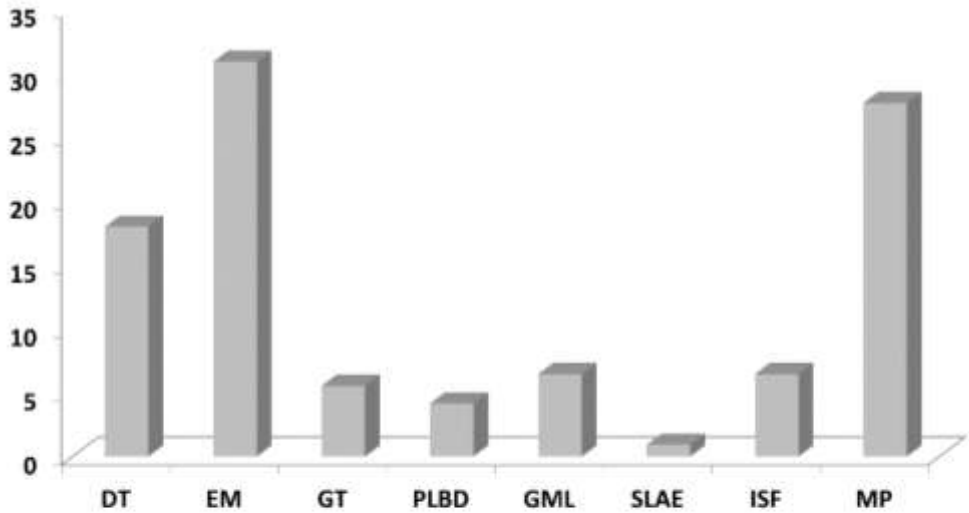
Table 7. Rank of cowpea among leafy vegetables and frequency of consumption of cowpea made food in selected areas of two regional states of Ethiopia

	Percentage of users by Regional states					Total (%)
	SNNPRS (%)			Gambella (%)		
	South Omo N=60	Wolaita N=29	Segen People N=48	Anuak N=40	Itang N=40	
Rank of cowpea leaf with other leafy vegetables (%)						
First	17	-	10	55	23	24
Top 3	77	-	90	33	73	70
Top 5	7	-	0	13	5	6
χ^2=191***						
Number of years consumed cowpea leaf						
1-5 years	17	-	21	23	20	20
6_10 years	15	-	10	25	23	18
11_15 years	22	-	13	8	8	13
16-20 years	17	-	13	5	20	14
Above 20years	30	-	44	40	30	36
χ^2=204***						
Harvesting frequency of leaf (%)						
Less than 5 days	29	-	51	54	69	49
5 to 10 days	61	-	37	46	31	46
11 to 15 days	11	-	11	0	0	6
Stage of cowpea to stop harvesting of leaf (%)						
Before flowering	30	-	6	85	56	44
At flowering	32	-	57	0	15	26
after flowering	0	-	0	0	3	1
At mid pod filling stage	35	-	34	15	26	28
At maturity	4	-	3	0	0	2

The majority of farmers stop leaf harvesting before flowering (44%) and 28% of farmers stop at mid pod filling stage. Another report also suggested that leaf harvesting is carried out before flowering (Dube and Fanadzo, 2013). Farmers in Zimbabwe start leaf harvesting at three weeks after the emergence and terminated harvesting once cowpea started podding, due to reduced leaf quality and not to affect seed yield (Matikiti *et al*., 2012).

To understand the position of cowpea as a dual-purpose crop, farmers were asked to mention the main reason why they are interested in this crop. The major reason mentioned was early maturity (31%), multipurpose nature of the crop (28%) and drought tolerance (18%) (Figure 6). The result support earlier research that described cowpea as one of the most important tropical dual-purpose legumes (Chweya and Eyzaguirre, 1999) and drought-tolerant crop (Agbicodo *et al*., 2009). Farmers also ascertained the high productivity of cowpea on marginal land and its advantage for soil fertility enhancement. Moreover, the farmers also stated as the leaf of cowpea can be preserved and used when there is shortage of food and the food made of cowpea stay long after eating. It shows the high potential of cowpea leaves for these purposes and, thus, more research, breeding, and promotion activities are necessary.

As we observed from interviewed farmers and in the focus group discussion, farmers were very much aware of health benefits in consuming cowpea grain and leaves, though they did not indicate as it is reach in vitamin, mineral, protein or fiber. They believed as cowpea helps for growth and maintenance for human body and also uses to treat gastric discomfort as well as serves as medicinal purpose. These results indicate an existing awareness about the value of cowpea by the farmers'. Nevertheless, further promotion activities are needed to enhance the knowledge of health benefits by consuming cowpea leaves.



DT= Give yield in drought season; EM= Early maturity; BT= Good taste; PLBD= Preserve the leaf by drying and use for long time; GML=Grow in marginal land; SLAE= Stay for long time after eating; ISF=Improve soil fertility; MP= It is a multipurpose crop

Figure 6. Advantage of cultivation of cowpea as perceived by farmers in selected areas of Ethiopia

Marketing

Marketing is a prime mover and stimulator of production. There was a difference in perception and practice among the two regional states concerning the cowpea parts to be marketed (Table 10). In Gambella regional state, 85% of farmers sell their cowpea in a different form while in SNNPRS grain is the only commodity for sale and only 36% of farmers sell the grain. In Wolaita zone cowpea leaves are not known as a vegetable, they produce for grain or forage purposes (Table 10). In South Omo and Segen people cowpea leave well known as leafy vegetables but it is not commercialized. Ninety-seven percent of cowpea growing farmers sell their cowpea products in local

markets. From these farmers who sold cowpea, 77% of them got market price information from their neighbors. Most of the farmers (61%) appreciated market prices (value) of cowpea and 35.5 % responded as cowpea has a modest market price value.

Table 8. Parts of cowpea marketed in the study area

Part marketed	Administrative Zones				
	South Omo N=60	Wolaita N=29	Segen People N=48	Anuak N=40	Itang N=40
Whole grain (%)	32	48	27	15	35
Fresh leaves (%)	—	—	—	52.5	30
Split grain (%)	—	—	—	2.5	2.5
Whole grain and fresh leaves (%)	—	—	—	20	10

Extension and training services

Only about 3 percent of cowpea growing households have participated in a cowpea demonstration or field days or received training relating to cowpea production. Extension service provides technical advice in crop production activities of the family farm and provides information to assist farmers in making sound production decisions. This can be information about improved varieties, agronomic practices, prices, and markets or about the availability of credit and inputs. At the study area however, the cowpea growers get information from experienced other fellow farmers. Practical extension works such as demonstration plot or field days are important to establish a farmer-to-farmer link and technology promotion. Although the farmer have enkowlage and experience on the traditional farming sytem, their skill and knowlage can be enhanced through extenstion servise and training.

Conclusions and Recommendation

Cowpea production in the selected regional states of Ethiopia is characterized by the widespread cultivation of landraces with differing preferred attributes among locations and farmers. Farmers' selection criteria for cowpea have implications for breeding activities in Ethiopia. Cowpea is used as multipurpose legume crop consumed as a vegetable or dry seed. It can also be prepared in different forms for consumption. Additionally, different parts of the crop are also use as animal feed. The present study provided insights on production constraints, preferred traits,

and utilization and farming systems of cowpea in the two regional states of Ethiopia. Breeding for high grain yields, resistance to field and storage pests and farmers-preferred cowpea varieties are the most important strategies to increase cowpea production and productivity by subsistence farmers in the regional states. Furthermore, the availability of improved seeds and production input need to be improved to boost cowpea productivity in the country. Hence, breeding efforts need to look at the area-specific needs of the user beyond focusing on seed yield. Breeding for high leaf and seed yield and farmers-preferred traits such as short cooking time is crucial to enhance the use of cowpea as dual purpose crop. Availability of seed and production input are issues that worth consideration to increase cowpea production, productivity and utilization by subsistence farmers in the selected regional states of Ethiopia.

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4. General outline for research articles (Full length paper) – Specific instructions

The format of the submitted manuscripts should conform to an acceptable standard editorial style that contains title, author(s) and institutions(s), abstract, key words, introduction, materials and methods, results and discussion, acknowledgement (if any), references, and tables and figures as major components.

4.1 Title Page

The title page must contain the following

1) ***Title:*** The title should be selected carefully. It should be concise, specific and descriptive enough to contain key words or phrases indicating the contents of the article.

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3) ***Keywords:*** Five to seven index words may be important to the journal editor and the audience for information storage, retrieval and to identify the article from among several other contributions. Important keywords may include phrases and nouns that characterize the study but the use of phrases longer than three words may not be recommended. Together with the title and abstract, keywords comprise most of the content of the article that will be available to people looking for it. Keywords must not repeat the title but they must amplify it.

4.3 Introduction: This part of the paper should state the justification or underlying hypothesis for doing the work, the major objectives of the research or investigation and provide a brief review of literature, which should be limited to information essential to orient the reader.

4.4 Materials and Methods: Under this heading a brief and concise description of the conditions under which the investigation was carried out and the procedures, techniques, and experimental designs used should be stated.

4.5 Results and Discussion: The major findings and assessment of the significance of the results of the investigation and the possible practical implications may be discussed with reference to the problems indicated in the introduction or stated as objective. Conclusions can also be presented as part of this section.

4.6 Acknowledgments: Acknowledgements, if any, may be given in a separate section immediately following the results and discussion.

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(a) Examples of Journal Articles

Collaku, A., Harrison, S.A., Finney, P.L. and D.A.van Sanford. 2002. Clustering of environments of southern soft red winter wheat region for milling and baking quality attributes. Crop Sci. 42: 58–63.

Gemechu Keneni, Belay Asmamaw and Mussa Jarso. 2001. Efficiency of drained selection environment for improving grain yield in faba bean under undrained target environments on Vertisol. Euphytica 122: 279–285.

(b) Example of a Book

Falconer, D.S. 1989. Introduction to quantitative genetics. 3rd ed. Longman, London, England.

(c) Paper in Proceedings

Edmeades, G.O., J. Bolanos and S.C. Chapman. 1997. Value of secondary traits in selecting for drought tolerance in tropical maize. pp. 222-234. *In*: Edmeades G.O., M. Banzinger, H.R. Mickelson, and C.B. Pena-Valdivia (eds.). Developing drought and low N-tolerant Maize. Proceedings of a symposium, March 25-29, 1996, CIMMYT, El Batan, Mexico. Mexico D.F.,

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processing software (such as WORD 2003, 2007 and above versions) for text manuscript processing for submission. Publications can be sent to:

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