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Phenotypic Diversity for Quantitative Characters in *Arabica* Coffee Landraces from Eastern Ethiopia

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ABSTRACT

A field-scale experiment established the agronomic performance of air-dried sewage sludge and composted sludge for winter wheat. The objective of the study was to initial measure the response of nutrient uptake once applying air-dried sewage sludge, composted sewage sludge and a commercial fertiliser to soil in grain and straw of winter wheat (Triticum turgidum L. cv. Vitron), to research the variations within the main soil characteristics in relevancy the initial soil once applying fertilizer treatments, and to determine the variations among treatments once harvest. Composted sewage sludge kept constant the organic matter level in soil after harvest and promoted higher Zn, Cu and Mn contents and yields than air-dried sewage sludge or the commercial fertiliser. No significant differences occurred among treatments for the studied parameters, except for nitrogen, organic matter and Zn in soil. Fe and Cu content in straw were higher when air-dried sewage sludge was used. No significant differences in grain nutrients content were observed.

Variations in soil composition were similar among treatments. Since no nutritional imbalances were observed in the grain or straw of durum wheat, sewage sludge from the Alcázar de San Juan WWTP (especially composted) is recommended as an alternative to standard commercial fertilisation.

Key words: *Sewage sludge, Agronomy*

Introduction

Coffee is a major cash crop in Ethiopia. In 2018, the country earned 839 million dollar from coffee export accounting for close to 30% income generated from all export commodities [1]. It is a means of livelihood for about 25 million people in the country. The country ranks 5th from world and 1st from Africa in coffee production [2]. Ethiopia is the center of origin and diversity for *Arabica* coffee [3]. Coffee research in Ethiopia was started in 1960s. But So far, only 40 (34 pure line and 6 hybrid) coffee varieties were released for production in different parts of the country. Efforts have been made to conserve the germplasm since 1967. Total of 12,654 *Arabica* coffee germplasm have been ex-situ conserved in field gene banks in the country [4]. Forty varieties (34 pure lines and 4 hybrids) were developed from collections and released for production in different parts of the country. In Hararge, Eastern Ethiopia, coffee is grown in diversified garden production systems. The crop is intercropped with maize, sorghum, beans, sweet potato and "chat" (Chata edulis). Hararge, eastern Ethiopia is known for production of best quality coffee known as Harar coffee [5]. Farmers grow coffee landraces having their own characteristic features. Number [6] recorded about 22 named coffee landraces in Hararge. In an effort to develop improved coffee varieties for the area, germplasm collection campaigns have been under taken since 1998. In 2004, 47 coffee accessions were collected from the area and maintained for evaluation at Mechara Agricultural Research Center. Genetic data are important for designing effective plant breeding programs.

Genetic diversity assessment methods could be categorized as morphological, biochemical and molecular markers. Of these, morphological characterization is considered as the first step in description and classification of crop germplasm [7]. It is also used for variety registration in Ethiopia. Accordingly, this study was conducted with objective to determine status of phenotypic diversity in coffee accessions from Eastern Ethiopia.

Materials and Methods

Description of Experimental Site: The study was conducted at the Mechara Agricultural Research Center in 2009. The Center is located at 8° 36'38.1'' North latitude, 40°019'29.8'' East longitude and at an altitude of about 1800 meter above sea level. It is situated at 434 km east of Addis Ababa, capital of Ethiopia and 110 km south of Chiro town, Zonal Town. The soil of the centre is deep, well-drained and slightly acidic nitosol, thus suitable for *arabica* coffee production. The area receives an annual rainfall of 1100 mm, of which about 85% is received from June to September, while the remaining amount is received from February to April. The annual average minimum and maximum temperature of Mechara are 14°C and 26°C, respectively.

Treatment and Experimental Design: A total of 49 *Arabica* coffee accessions consisting of 47 accessions collected in 2004, one accession collected in 1998 from West Harerge Zone district, Eastern Ethiopia and one improved variety from south-west part of Ethiopia. The 48 coffee accessions were collected from different coffee grower areas: Aba Aman (8), Bereka (3), Jeneti (6), Selama (4), Sham (18), Waltasis (8) and Oda Bultum district (1). The accessions were collected from altitude ranging from 1650 to 1916 m.a.s.l. All accessions were field planted in July, 2005 using simple lattice (7x7) design with two replications. The trees were planted with spacing of 2 m between plants and 3m between blocks. Field management practices were applied to all plots uniformly as per recommendation.

Data Collected: Total 15 quantitative morphological characters were recorded at second bearing stage (Table1) as per the standard coffee descriptor produced by International Plant Genetic Research Institute [8]. For most of data collection, five plants were selected at random from each accession and labeled. Then, data were recorded from each coffee tree and the average result was used for data analysis.

Table 1: Characters used for the study, their codes and descriptions.

	Characters	Code	Description
1	Plant height (cm)	PH	length from the ground level to the tip of the tree
2	Number stem nodes	NSN	Total number of main stem node counted per tree
3	Inter-node length of main stem (cm)	ILMS	Computed by dividing plant height to number of nodes of main stem, on five plants
4	Stem diameter (cm)	SD	Stem diameter of stem at five cm above ground measured using caliper
5	No. of primary branches	NPB	Number of primary branches counted per tree
6	Length of longest primary branches (cm)	LPB	Average of four primary branches at the middle of the stem, measured from point of attachment to main stem to apex of branch
7	No. of internodes of primary branch	NILPB	Average of four primary branches nodes counted
8	Inter-node length on primary branches (cm)	ILPB	Average of four primary branches at the middle of the stem per tree, calculated as length divided by the number of nodes
9	No. of secondary branches	NSB	Obtained by direct counting in four selected branches
10	Leaf length (cm)	LL	Average of five leaves, from petiole end to apex
11	Leaf width (cm)	LW	Average of five normal leaves measured
12	Leaf area (cm2)	LA	Average of five leaf area was calculated
13	Canopy diameter (cm)	CD	Average length of tree canopy measured twice, East- West and North- South; from broadest portion of tree
14	Hundred bean wt. (g)	HBW	Average weight of four samples of 100 beans
15	Yield per tree (g)	YLD	weight of green beans harvested per tree estimated

Data Analysis: Analysis of variance (ANOVA) was conducted using SAS [9]. Least significant difference (LSD) test was used for mean separation at 5% probability level. Clustering was done to separate the 49 coffee accessions in to different groups using SAS based on the generalized D2 distances by average linkage method of hierarchical clustering called Un-weighted pair group methods with arithmetic average (UPGMA). Appropriate number of clusters was determined from the values of pseudo F and pseudo T2 statistics calculated by SAS. Cluster analysis divides data in to meaningful groups based on the information found in the data that describes the genotype and their relationship [10]. Genetic distance between clusters was also calculated using the generalized Mahalanobis's D^2 statistics. The D^2 value obtained for pairs of clusters was considered as the calculated value of Chi-square (χ^2) and was tested for significance at the required level of probability against the tabulated values of χ^2 for p degrees of freedom, where p is the number of characters considered [11]. SAS software was employed for the analysis. The D2 is defined as: $D^2ij = (Xi\text{-}Xj)\ 1\ S^{-1}\ (Xi\text{-}Xj)$; Where, D^2ij = is the distance between two groups i and j; Xi and Xj are the two vector mean of the traits for ith and jth groups respectively, and S-1 is the inverse of the pooled covariance [12].

Result and Discussion

Analysis of Variance: Mean squares due to accessions were highly significant ($p< 0.01$) for all the quantitative characters studied except for number of internodes of main stem and average length of primary branch (Table 2), suggesting the presence of substantial variability among the 49 coffee accessions studied. Variation among accessions in some traits was also revealed by big difference between minimum and maximum values as well as high standard error (Table 3). For stem characters, plant height varied from 94.00 to 165.40 cm; number of internodes of main stem ranged from 17.90 to 24.80 cm; internode length of main stem varied from 5.30 to 8.25 cm; diameter of main stem from 3.40 to 5.35 cm. Genotype H26/04 was significantly taller (165.4 cm) followed by H-19/04 (163.1 cm), H-618/98 (162.2 cm) and H-11/04 (159.9 cm). Non-significant difference among accessions were observed for number of internodes on main stem (NIMS). However, among all accessions tested, H-41/04 had the greater number of internode (24.8), followed by H-25/04 (23.8), H-29/04 (23.6) and H-11/04 (23.5). Coffee genotype H-20/04 had significantly the longest internodes of main stem (8.25 cm) as compared to all other

Table 2: ANOVA table of 16 quantitative characters of coffee accessions at Mechara, 2009

SV	DF	PH	NIMS	ILMS	SD	NPB	LPB	NIPB	ILPB
Rep	1	973.48**	122.13**	5.27**	10.97**	687.68**	14.58^{NS}	50.07**	1.42**
Genotype	48	298.66**	4.24^{NS}	0.66**	0.43**	17.54**	201.92**	11.67**	0.53**
Error	48	115.39	2.76	0.11	0.1	7.9	49.1	2.34	0.12
CV (%)		7.37	7.97	4.66	6.75	7.77	7.97	7.25	8.15
R^2 (%)	-	73.43	71.07	87.6	86.91	80.13	80.47	84.44	82.66
Table2.ANOVA table of……..									
SV	**DF**	**NSB**	**LL**	**LW**	**LA**	**CD**	**HBW**	**YLD**	
Rep	1	5599.84**	3.46**	7.32**	77.08**	877.51*	0.69^{NS}	114.75*	
Genotype	48	749.32**	6.58**	2.21**	503.24**	564.90**	5.29**	2868.6**	
Error	48	269.34	0.01	0.01	0.01	179.02	0.22	168.28	
CV (%)		22.51	0.22	0.9	0.27	9.95	3.43	19.67	
R^2 (%)	-	76.28	99.99	99.87	99.99	76.51	96.1	94.46	
Abbreviation: PHT- plant height; NIMS- number of internodes of main stem; ILMS- internode length of main stem; SD- Stem diameter; NPB- number of primary branches; LPB- length of primary branch; NIPB- number of internodes of primary branch; ILPB- internode length of primary branch; NSB- number of secondary branches; LL- leaf length; LW= leaf width; LA- leaf area; CD- canopy diameter; HBW- hundred bean weight and YLD- yield per plant									
* indicates significant at 5%, ** indicates significant at 1% level of probability; NS- non-significant									

Table 3: Ranges and means values of 15 quantitative characters for 49 coffee accessions

SN	Traits	Min	Max	Range	Mean	CV (%)
1	Plant height (cm)	94	165.4	71.4	145.81	7.37
2	Number of internode of main stem	17.9	24.8	6.9	20.84	7.97
3	Internode length of main stem (cm)	5.3	8.25	2.95	7.06	4.6
4	Stem diameter (cm)	3.4	5.33	1.93	4.67	6.75
5	Number of primary branches	30.2	43.1	12.9	36.16	7.77
6	Length of primary branch (cm)	43.1	105.1	62	87.96	7.97
7	Number of internodes of primary branch	14	26.7	12.7	21.13	7.25
8	Internode length of primary branch (cm)	3.2	5.73	2.53	4.22	8.15
9	Number of secondary branches	41.1	133.2	92.1	72.91	22.51
10	Leaf length (cm)	9.2	18.43	9.23	13.4	0.22
11	Leaf width (cm)	3.49	8.77	5.28	6.24	0.9
12	Leaf area (cm^2)	24.63	105.45	80.82	57.96	0.27
13	Canopy diameter (cm)	76.7	164.6	87.9	134.46	9.95
14	Hundred bean weight (g)	11	16.5	5.5	13.53	3.43
15	Yield (g/plant)	10.36	176.39	166.03	65.93	19.67

accessions, followed by H-19/04 (7.91 cm), H-26/04 (7.86 cm) and H-48/04 (7.8 cm). Similarly, accessions were significantly different for their diameters on main stem. Of the tested accessions, H-19/04 had highly significant and the thickest diameter of main stem (5.35 cm), followed by H-08/04 (5.33 cm), H-15/04 (5.25 cm) and H-26/04 (5.2 cm).

For branch character, number of primary branches ranged from 30.20 to 43.10; length of primary branch from 43.10 to 105.10 cm; numbers of internodes on longest primary branch from 14 to 26.70; internode length of primary branch from 3.20 to 5.73 cm; numbers of secondary branch from 41.10 to 133.20. Differences between minimum and maximum values for other characters were also big. Accession H-41/04 had significantly more number of primary branches (43.1) per tree compared to all other accessions followed by H-25/04 (42.3), H-11/04 (42.1) and H-29/04 (40.9). Accessions were significantly different for length of primary branches. Among all accessions, H-16/04 had significantly the longest (105.1 cm) primary branch, followed by H-20/04 (99.4) and H-23/04 (99.2). Similarly, number of secondary branches of genotype the H-09/04 was significantly the highest (133.2) when compared to the other accessions, followed by H-17/04 (112.5), H-11/04 (111.5) and H-13/04 (109.1). Leaf characters also manifested significant differences among the accessions considered. Leaf length was from 9.20 cm to 18.43 cm and with mean of 13.40 cm. similarly, leaf width varied from 3.49 to 8.77 cm with mean vale of 6.24 cm. Average leaf area of all accessions was 57.96 cm2 with range from 24.63 cm2 to 105.45 cm2. Number [13-20] reported similar results. Regarding bean characters and yield, weight of hundred beans ranged from 11 g for five accessions (H-25/04, H-40/04, H-41/04 and H-618/ 98) to 16.5 for H03/04 with mean value of 13.53 g. There was no significant difference between accession (H-03/04) with the biggest bean weight and other 11 accessions (H-40/04, H-41/04, H618/98, H-17/04, H-24/04, H-31/04, H-33/04, H-37/04, H-39/04 and H-18/04). Similarly, accessions with smallest bean weight were H-03/04, H09/04, H-16/04, H-26/04 and H-38/04. On the other hand, accession H-11/04 gave significantly the highest average green bean yield (176.39 g/tree) as compared to all the other accessions followed by H-25/04 (146.22 g/tree), H-618/98 (140.21 g/tree), H-21/04 (131.85 g/tree) and H-18/04 (123.70 g/tree). The study further indicated that accession H=11/04, was the highest yielder and it can be advanced to the next breeding program though the other desirable traits remain for future studies. The coffee genotype 74110 (check) showed the least value for 13 of the 16 quantitative characters considered. The reasons for the low green bean yield in the present accessions could be explained by the age of the coffee accessions and the serious physiological disorder caused by moisture stress that occurred during the dry season.

Most of the findings of the present study are in agreement with reports of [21] and [22]. According to the [21], leaf length of *Coffea arabica* varied from 9.8 to 13.7cm; leaf width from 3.8 to 5.9 cm. Similarly, [22] reported values ranging between 10 to 15 cm for leaf length, 4 to 6 cm for leaf width, 0.15 to 0.20 g for bean weight among *arabica* coffee accessions. In addition, [23] who studied genetic diversity among 16 *arabica* genotype at Finoteselam, reported that average green bean yield per tree varied from144.6 to 566.7 g and 100 green bean weights ranged from 9.3 to 16.0 g. In his study, tree height also varied from 107.5 to 182.8 cm, canopy diameter from 137.1 to 246.5 cm, trunk diameter from 24.6 to 39.6 mm, number of primary branches per tree from 35.7 to 62.0 and number of secondary branches per tree from 21.3 to 117.7. Similarly, presence considerable to high phenotypic variation was observed in hybrid coffee from south western Ethiopia [13], in promising coffee cultivars from Wellega [14], on coffee landraces from Amaro at Awada center [16], in promising hybrid at highland Environments of Southwestern [22], on some specialty coffee cultivars [17], in coffee genotypes from Sidama [18], in coffee genotypes from Limmu area [19] and in coffee genotypes from Southwestern Saudi Arabia [12]. The slightest discrepancies in extreme values with the present study for some characters could be ascribed to differences in number and type of tested accessions, age of the tree and the location in which these studies were conducted. The presence of phenotypic variability among the accession reveals that there is a good chance of improving Hararge coffee accessions through selection and breeding. The prevalence of such variability in a predominantly self-pollinating species like *C. arabica* appears to be surprising. The variability may be attributed either to evolutionary tendencies, as the species is indigenous to Ethiopia, or to the natural mutations occurring to the population of the crop [24]. Cluster analysis. The results of cluster analysis confirmed the presence of substantial variation among accessions. The 49 coffee accessions were grouped into five clusters (Table 4). The size of cluster varies from one accession in cluster IV and V to 34 accessions in cluster II. Cluster II and Cluster I represented 69.39% and 20.41% of the accessions Cluster V with coffee accession H11/04 showed the highest results for the majority of the characters (Table 5). For example, it had highest plant height (159.90 cm), number of internodes of main stem (23.5), number of primary branches (42.1), length of longest primary branches (91.90 cm), number of internodes of longest primary. branches (23.2), average length of primary branches (84.1 cm), number of secondary branches (111.50), canopy diameter (139 cm) and average green bean yield per plant (176.39 g) [25-26]. The result is in agreement with previous work [13-20]. Based on Mahalanobis's D2 statistics, highly significant (p=0.01) inter-cluster distances were obtained except between cluster I and II (Table 6). Cluster III showed the maximum and significant genetic distance (181.80) from cluster V. Furthermore, the inter-cluster distances between clusters III and IV, I and III, II and V, II and III, I and IV, II and IV, IV and V in that order were found to be highly significant. Crossing of parental lines extracted from germplasm accessions belonging to different clusters of wide Mahalanobis distance (D2) could maximize opportunities for transgressive segregation as there is a higher probability that unrelated accessions would contribute unique desirable alleles at different loci. Therefore, the present

Table 4: Clustering patterns of 49 coffee accessions based on 16 qualitative characters

Cluster	No.	% of acc.	Name of Accessions	Cluster	No.	% of acc.
I	10	20.41	H-02/04	I	10	20.41
			H-18/04			
II	34	69.39	H-01/04	II	34	69.39
			H-03/04			
			H-04/04			
			H-05/04			
			H-06/04			
			H-07/04			
III	3	6.12	H-08/04	III	3	6.12
IV	1	2.04	H-09/04	IV	1	2.04
V	1	2.04	H-11/04	V	1	2.04
Cluster	**No.**	**% of acc.**	**Name of Accessions**	**Cluster**	**No.**	**% of acc.**
I	10	20.41	H-02/04	I	10	20.41
			H-18/04			
II	34	69.39	H-01/04	II	34	69.39

Table 5: Cluster means of the 49 coffee accessions studied for 15 quantitative traits

SN	Traits	I	II	III	IV	V
1	Plant height (cm)	150.02	146.80	114.40	150.50	159.90
2	Number of internode of main stem	21.34	20.70	19.73	21.30	23.50
3	Internode length of main stem (cm)	7.08	7.16	5.83	7.11	6.81
4	Stem diameter (cm)	4.66	4.68	4.63	4.65	4.78
5	Number of primary branches	37.06	35.93	33.17	38.10	42.10
6	Length of primary branch (cm)	89.97	89.65	62.93	81.30	91.90
7	Number of internodes of primary branch	22.55	21.03	16.83	21.10	23.20
8	Internode length of primary branch (cm)	4.06	4.32	3.73	3.89	3.97
9	Number of secondary branches	70.16	73.06	47.43	133.20	111.50
10	Leaf length (cm)	13.35	13.39	10.48	13.35	12.51
11	Leaf width (cm)	6.20	6.41	4.37	6.58	6.08
12	Leaf area (cm^2)	56.51	60.77	33.60	57.62	50.12
13	Canopy diameter (cm)	136.45	137.56	92.6	129.8	139.10
14	Hundred bean weight (g)	14.21	13.47	13.00	11.00	13.00
15	Yield (g/plant)	115.48	50.64	29.57	88.99	176.39

Table 6: Generalized squared distance among clusters for 49 coffee accessions at Mechara

Cluster	I	II	III	IV	V
I	-	13.68	88.42**	48.37**	37.69**
II			57.30**	43.58**	74.99**
III				117.52**	181.80**
IV					41.39**
V					

*= Significant at 0.05 probability level (X^2 15= 25.00) **= Significant at 0.01 probability level (X^2 15= 30.58)

study suggests that the germplasm accessions from cluster I and cluster V, I and IV and IV and V could offer relatively better potential parental lines that, when intercrossed, could produce hybrids with maximum heterotic value. Most of the high yielding accessions were grouped in cluster I. So, we can also cross accessions in this cluster with each other.

Conclusion and Recommendation

There is considerable phenotypic variation in coffee accessions grown in eastern Ethiopia. The presence of phenotypic variability among the accession reveals that there is a good chance of improving Hararge coffee accessions through selection and hybridization. The variability may be attributed either to evolutionary tendencies, as the species is indigenous to Ethiopia, or to the natural mutations occurring to the population of the crop. Evaluation for disease reaction, moisture stress tolerance, quality, biochemical composition and molecular marker is important.

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Declaration on conflict of interest

There is no conflict of interest.

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