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Full Length Research Paper

Phenotypic Diversity Studies of Coffee (*Coffea canephora* Pierre ex. Froehn) Landrace Accessions and an Accession from Germplasm Using Morphological Markers

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Six landrace accessions of robusta coffee and one accession from CRIN coffee germplasm were used for this study. Ate-kekere (ATK), Shekoni (SEK), Iyamuye (IY1), Kabba bunu (KB3), Omu-Alagbede (OMA), Oranre (ORA1) were farmer`s cultivars, while C105 is the accession from coffee germplasm. GPS was used to take coordinate of collection areas. Experiments were conducted at two locations for two years 2018 to 2019, in randomized complete block design, with three replicates. Data was collected on morphological traits: plant height, stem diameter, numbers of branches, numbers of leafs, inter node length, leaf length and leaf width. All observations were recorded and subjected to ANOVA using SAS package V. (9.1) and Minitab package V. (17). There were variations in all morphological characters, with ATK (67.25) being the tallest among all the accessions. OMA (48.22) was observed to be the shortest among other accessions. ATK had significant highest mean number of branches (15.83) and of leaves (38.67) when compared to other accessions. Locations tend to have significant effect on the performance of accessions. Plants at Ibadan show significant better performance in morphological traits than plants at Owena. Year 2019 revealed significant improvement in morphological traits as compared to 2018, but number of branches was not different significantly. Dendrogram hierarchically grouped the accessions based on geographical area of collection (North Central and Southwest), implies that morphological traits could be used to classify cultivars for characterization based on area of core collection and for establishment of germplasm bank.

Key words: landrace robusta coffee, accessions, morphological markers, diversity studies and locations.

INTRODUCTION

Coffea canephora is among the two commercially cultivated varieties of coffee in Nigeria (Adepoju *et al*., 2017), as it constitute about 95% of coffee production in Nigeria while the remaining 5% comes from *C. arabica*. (Anagbogu *et al*., 2019). *Coffea canephora* also known as Robusta coffee as it is being refer to (Tshilenge *et al*., 2009), was said to have originated from Central to West Africa (Nugussie and Dererse, 2007). According to Tornincasa *et al*., (2010) and Davis *et al*., (2011), earlier studies revealed that genus Coffea, has more than 100 species and among all the species, *C. arabia* and *C. canephora* are the two economic important varieties (Mishra and Slater, 2012 and Zamir 2014). Studies carried out by Eghe *et al*., (2008), indicated that cultivation of coffee started in Nigeria as far back as 1940`s to mid 1950`s. Findings by Lashermes *et al*., (2011),

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Mishra and Slater, (2012) shows that about 125 million people or more are in to cultivation of coffee worldwide which serve as a source of income to them. In Nigeria, cultivation of coffee is being done mainly by small scale farmers and most of the planting materials are landrace cultivars.

The geographical spread of robusta coffee and its cultivation by many Nigeria farmers was due to its better adaptability to diverse soil and climatic conditions (Maurin *et al*., 2007), which contribute to variation in its phenotypic traits (Tadesse Benti, 2017). In Nigeria, planting of landrace robusta coffee, is done through the use of seeds in spite of effort on vegetative and micro-vegetative propagation. Effort by plant breeders in developing high yielding varieties to meet the growing population demand do lead to genetic erosion of landraces varieties and wild species of crop plants which constitute the major gene pool of genetic diversity. Genetic diversity of natural coffee has been grouped into three namely: landrace, wild species and developed cultivars.

Morphological traits such as plant height, stem diameter, variation in charries sizes, shape, beans size, colour among others, provide information regarding to diversity studies in coffee (Gichimu and Omondi, 2010b) as well as other crops (Dah-Nouvlessounon *et al*., 2016). However, species discrimination would be more informative if molecular evaluation is incorporated (Duminil and Di Michele, 2009), since it is a complementary tool to phenotypic markers and being a genetics and genomics based. Evaluations and characterization of morphological traits are of important for documentation in coffee breeding programs, as plant breeders use this tool in selecting and discarding accessions that are of less interest (Teixeira *et al*., 2013). According to Gessese *et al*., (2015) use of morphological markers is useful in coffee characterization, for diversity, as it distinguish variation based on external observable differences and potential for genetic improvement (Paul *et al*., 2019). Many researchers have used morphological diversity to determine the existence of diversity among newly developed varieties with the existing commercial coffee genotypes (Gichimu and Omondi 2010b).

In spite of the economic important of coffee, the crop has numerous challenges, ranging from poor market outlet, (Sanusi *et al.,* 2004), yield instability, slow growth, abandoning of coffee farms for other crops (Agbongiarhuoyi *et al*., 2006), non improvement on the existing cultivars, lack of proper method of classification and genetic erosion and so forth (Hammer and Teklu, 2008). These challenges have led to low coffee production in Nigeria (Willians, 2008). In addition, according to Van de Wouw *et al*., (2010), challenges of landraces or locally adapted varieties are due to its lower yield, susceptibility to insect pest and disease attack, when compared to improve varieties. These has contributed to genetic erosion being faced over 100 years ago (Negri, 2003; Hammer and Teklu, 2008). Information on morphological characteristics of robusta coffee accessions at juvenile stage in Nigeria is very scarce. Farmers identify their coffee landraces using traditional methods such as: leaf colour, weight of beans, and shape of fruits among others. In view of these, characterization of cultivated landraces accessions of robusta coffee becomes necessary as well as their evaluation on the field. The study aimed to ascertain the importance of morphological trait for diversity studies and to provide basic information for exploring the potentials associated with accessions of landrace robusta coffee. This is to give an insight for conservation and crop improvement for the purpose of future classification and selection in Nigeria.

MATERIALS and METHODS

Landrace coffee accessions were collected from areas with history of coffee cultivation. Seven landrace robusta coffee accessions were collected from North central and Southwestern for this study, (Table 1). Out of seven accessions, three accessions were from North central part of Nigeria, while four accessions were from Southwestern part of Nigeria of which One of them was from coffee germplasm of Cocoa Research Institute of Nigeria, Ibadan. The codes for the population used in this study were related to their collection site. The field experiment ware carried out at the Cocoa Research Institute of Nigeria, Ibadan, Oyo state and Owena substation of Cocoa Research Institute of Nigeria, Ondo state. GPS was used to determine coordinate of the sample collection areas. Sampling methods was used for the collection to ensure representative of each collection within a population as well as geographical patterns of distribution. All the accessions collected were processed using wet processing method and letter pre-germinated using saw dust as a germination media. Healthy and well developed seedlings were transplanted into a polythen bag filled with moist top soil. The coffee seedlings were transplanted into well prepared field. The experiment was laid out in a Randomizes complete block design with three replicates and plants were spaced at 3M x 3M as suggested by Anim-Kwapong *et al*., (2010) and maintained uniformly under rain fed.

Data on morphological traits were collected and recorded from three randomly selected coffee plants from each replicate representing each population on the trial. The seven morphological traits evaluated were: plant height PH (cm), stem diameter SD (cm), numbers of branches NB (cm), number of leaves NL, internode length IL (cm), leaf length LL (cm) and leaf width LW (cm). Morphological traits collected from 2018 to 2019, were based on descriptors for coffee in accordance with IPGRI (International Plant Genetic Resources Institute,

Table 1. shows the accessions number, sources of collection, latitude, longitude and elevations.

Plants	Accessions	Source	State	Geographical zone	Latitude	longitude	Elevation
1	ATK	Ate-kekere	Ondo	South west	N6.923	E3.442	163.63
2	SEK	Shekoni	Ondo	South west	N7.489	E5.638	1076.68
3	IY1	Iyamuye	Kogi	North central	N7.793	E5.807	1595.50
4	KB3	Kabba bunu	Kogi	North central	N7.824	E6.079	1385.77
5	C105	germplasm	Ibadan	South west	N7.204	E 3.862	422.25
6	OMA	Omu-Alagbede	Ogun	South west	N6.924	E3.448	268.50
7	ORA1	Oranre	Kogi	North central	N7.864	E5.742	1746.89

1996). Data were subjected to analysis of variance (ANOVA) and cluster analysis, using SAS (Statistical Analysis System) package (Version: 9.1) and Minitab package (Version: 17).

RESULTS

Plant characteristics

Data on plants growth traits were shown in Table 2. Results indicated significant different ($p \leq 0.05$) in all the growth traits observed (Table 2). Plant height varies significantly among the landrace accessions. ATK was tallest having the highest mean of (67.25cm). SEK and IY1 were similar to C105 followed by KB3 and ORAI although both were equally statistically similar. OMA1 presented the lowest height (48.22). For stem diameter, highest mean were observed in IY1, ATK and SEK which were statistically similar, but they differ significantly from the other four accessions. ATK and IY1 were similar although both revealed highest number of branches comparison to the others landraces. They were immediately followed by SEK, KB3 and OMA that showed lowest mean number of branches than others. ATK recorded highest numbers of leaves (38.67) that was significantly different from other accessions. KB3 and OMA were statistically similar with lowest number of branches which were significantly different from other accessions. ATK and C105 recorded highest mean internode length that were similar to ORA1. OMA recorded the least mean internode length. IY1 revealed longest mean leaf length (22.59). It was followed by ATK and SEK which were statistically similar to ORA1. OMA recorded shortest leaf length (17.61) than other accessions. IY1 and ATK revealed highest mean leaf width which was significantly not different from SEK. OMA recorded narrowest leaf width (6.98) than other accessions.

Means with the same letters are significantly not different Where: Plant height (PH), Stem diameter (SD), Number of branches (NB), Numbers of leave (NL), Internodes length (IL), Leaf length (LL) and Leaf Width (LW)

The effect of locations and years is presented in Table 3. The result indicates two locations that have significant effects on morphological traits of robusta coffee. Ibadan shows significant better performance in all the traits studied as compared to Owen location. Ibadan recorded tallest mean plant height (66.16), wider mean stem diameter (1.13), number of branches (18.55), highest mean number of leaves (105.58) internode length (8.10), leaf length (21.20) and leaf width (9.09).

The result shows year 2019 had significant effects on plants performance, than 2018 (Table 3). Tallest plant height (84.35), wider stem diameter (1.44), highest number of branches (15.81), number of leaves (84.17), internode length (8.87), leaf length (23.40) and leaf width (9.85) were significantly higher in year 2019, than year 2018.

Where: Plant height (PH), Stem diameter (SD), Number of branches (NB), Number of leave (NL), Internode length (IL), Leaf length (LL) and Leaf Width (LW).

The mean of square values of the interactions, accessions × year, accessions × locations and year × location is shown in Table 4. They revealed significant variation for all the traits studied at ($p \leq 0.05$). Variability in traits is a proof of existence of different accession within the population and could use as a factor for clustering in population.

Table 4: The mean of square value for accession, accession × year, accession × location and year × location interactions component of variation for quantitative traits of robusta coffee accessions grown at two locations during 2017 and 2019 seasons.

CLUSTER ANALYSIS

Cluster analysis (Figure 1), shows the relationship among accessions of robusta coffee collected at different locations as shown in Table 1. The overall cluster analysis grouped accessions into two major clusters based on geographical locations. Cluster group 1 revealed KB3 and IY1 were closer than ORA1. Under second grouping, SEK and OMA were most closely related than ATK and C105. Cluster group 1 were

Table 2. Effects of plants growth characters on the accessions.

Characters	ATK	SEK	IY1	KB3	C105	OMA	ORA1
PH (cm)	67.25a	61.92ab	61.63ab	56.39bc	58.92ab	48.22c	54.42bc
SD (cm)	1.12a	1.12a	1.16a	0.77b	0.81b	0.72b	0.82b
NB	15.83a	14.42ab	15.50a	10.67c	12.75abc	10.42c	11.50bc
NL	38.67a	27.78abc	36.33ab	16.75c	26.67abc	16.25c	18.33bc
IL(cm)	7.42a	6.61ab	6.93ab	6.81ab	7.49a	5.37b	7.13a
LL(cm)	22.03ab	22.18ab	22.59a	17.96c	19.63bc	17.61c	20.86ab
LW(cm)	9.18a	8.90a	9.39a	7.48bc	8.42ab	6.98c	8.53ab

Table 3. Effects of locations and years on the performance of coffee plants.

Characters	Location		Year	
	Ibadan	Owena	2019	2018
PH (cm)	66.16a	50.62b	84.35a	32.44b
SD (cm)	1.13a	0.73b	1.44a	0.43b
NB	18.55a	10.79b	15.81a	13.52b
NL	105.58a	32.28b	84.17a	1.89b
IN (cm)	8.10a	5.54b	8.87a	4.77b
LL (cm)	21.20a	19.61b	23.40a	17.42b
LW (cm)	9.09a	7.73b	9.85a	6.97b

Means with the same letters are significantly not different.

Table 4. The mean of square value for accession, accession × year, accession × location and year × location interactions component of variation for quantitative traits of robusta coffee accessions grown at two locations during 2017 and 2019 seasons.

Trait	Accession	Accession × year	Accession × location	year × location
Plant height	450.02**	158.29**	250.54**	3537.31**
Stem girth	0.44**	0.18**	0.16**	2.48**
Number of branches	68.92**	31.63**	31.72**	697.19**
Number of leaves	6712.66**	1387.94**	4909.10**	20795.32**
Internode length	6.15**	3.60**	6.59**	53.60**
Leaf length	50.29**	13.40**	16.06**	16.65**
Leaf width	9.47**	4.66**	3.84**	2.50**

** donated significant at the P≤0.05 level.

accession collected from North central part of Nigeria, while cluster group 2 comprises of accession collected from Southwestern part of Nigeria. The distance among the seven population ranges from 0.00 to 1.00. Cluster 1 comprises of three accessions at a distance of 0.35 (KB3, IY1and ORA1). Cluster 2 comprises of four accessions at a distance of 0.30 (OMA, SEK, ATK and C105). In cluster analysis, minimum dissimilarities were observed between accessions ORA1 and KB3 they were closely followed by IYAI with moderate distance. Also OMA and SEK were less dissimilar when compared to ATK and C105 is the highest distance from OMA and SEK.

DISCUSSION

Morphological characters evaluation of cultivated landrace species of robusta coffee and one accession from germplasm exhibit differences in all evaluated traits. Accession ATK showed highest numbers of leaves, branches and inter node length which contributed to the tallness observed compared to other accessions. Accessions from OMA were observed to be the shortest among other accessions. This suggest that ATK, could posses the trait of tallness while OMA could be regarded as dwarf cultivar. IY1 was better than other accessions in

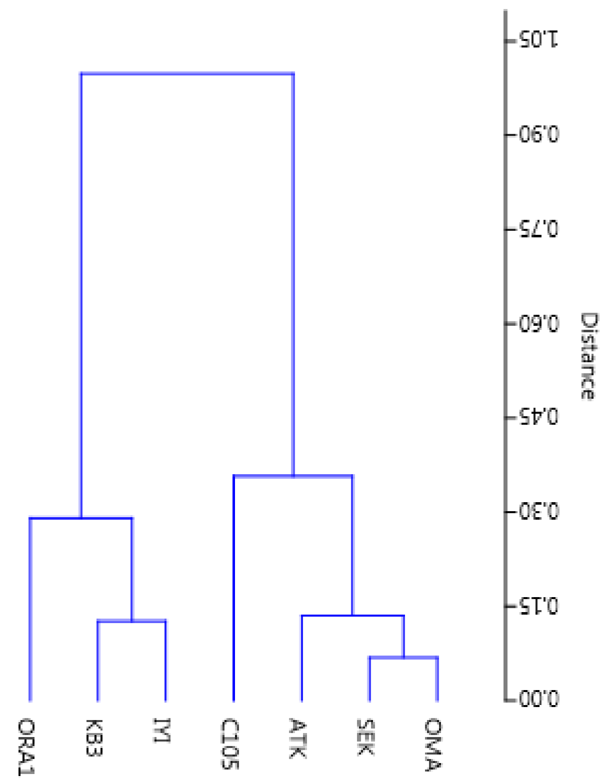

Figure 1. Dendrogram representation for hierarchical clustering of data objects.

leaf length and stem diameter. C105 had the longest in inter node length, but statistically similar with ATK and ORA1. This implies the existence of variability on morphological characters among collected accessions of robusta coffee as reported by (*Olika et al.,* 2011). The observed variations could also be used as a basis for selection of the best accessions as suggested by (Teixeira *et al*., 2013).

Several studies had equally shown that the quantitative characters could be used as a selection criterion for improving the productivity of the crop since they contribute among others to the variability observed within coffee population in different areas (Gessese *et al*., 2015).

All accessions at Ibadan show better performance than the ones at Owena. This shows that differences in morphological characters at different environment indicate an improvement in growing condition of the plants at different environment (Ferráo *et al*., 2017). Ibadan is savanna vegetation, with optimal rainfall support better performance of coffee species and Owena is a tropical rainforest with high rainfall. The robusta coffee prefers warm temperature, as it does not perform well in very high rain fall regimes. Damatta *et al.*, (2006) reported optimal temperatures are required for coffee performance and these changes as phonological stage changes.

As coffee plants grow from one season to the other, it continues to change from vegetative stage to reproductive stage. This was revealed in year 2019 as plants showed better performance in all morphological traits as compared to year 2018. As such characterization of morphological traits becomes important, since genetic diversity is based on the rate of species adaptive and the response of species (Solomon, 2009). This means that selected accessions could be compared for estimation of variability and heritability as suggested by (Mishra *et al.,* 2006).

Cluster analysis grouped accessions into geographical areas of collection North central and Southwest revealing that most of robusta coffee cultivated by farmers within these locations has an associate link of being from the same source of origin or being closely related. Cluster grouping of accessions based on geographical location had shown the existence of diversity among the accessions that could be exploited in robusta coffee in Nigeria.

CONCLUSION

Plants characterization allow efficiently use of time and labour for discriminating of genotypes of different landraces. Analysis of variance at p≤0.05 shows variations between and within robusta coffee landraces collected from farmers and an accession from coffee germplasm. ATK showed best performance than the others accessions. The accessions from the germplasm bank C105 showed higher inter node length than the other accessions. Differences observed on morphological characters between and within coffee collected from different geographical location indicate genetic variability that can be further evaluated using molecular markers. Therefore, further studied for the purpose of increase genetic variance of robusta coffee should be carried out.

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