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Morphological Diversity of Wild Coffee (*Coffea kihansiensis*) a Potential Coffee Species for Genetic Improvement

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Abstract

Coffee belongs to the genus *Coffea* in the Rubiaceae family, and is mostly grown in the tropical and subtropical regions. The *Coffea* genus comprises 103 species, of which *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* P. (Robusta coffee) are the two most important commercial species. *C. Arabica* is a commercially important high quality coffee with low genetic diversity, while *C. canephora* is cultivated mostly in the northwestern region of Tanzania. Of recent, a discovered wild coffee (*Coffea kihansiensis*) in Tanzania may be a genetic resource for improvement of cultivated coffee. Morphological descriptors from accessions evaluated in different sites at Kihansi wild coffee gardens were carried out using the International Board for Plant Genetic Resources (IBPGR). This work presents morphological diversity of the wild *C. kihansiensis* and elucidating traits for domestication and genetic improvements of coffee.

Keywords: Wild coffee, Diversity, *Caffea kihansiensi*

Introduction

The *Coffea* genus is among the 500 genera with 100 morphologically and genetically distinct species (Hendre et al. 2008, Tshilenge et al. 2009). The Coffea genus belongs to the family Rubiaceae, and *Coffea Arabica* and *Coffea canephora* are the commonly cultivated species in the globe. The origin of the two coffee species is in Africa with Arabica coffee being native to the highlands of south-western Ethiopia (Sylvain 1955) and robusta coffee originated along the East African great lakes. Diversity of coffee genetic resources in form of cultivated and wild coffee species in Ethiopia has been well documented (Labouisse et al., 2008). The two species are the major commercially cultivated types of coffee that contribute about 70% (*Coffea arabica)* and 30% (*Coffea canephora)* of the total coffee production in the world. Globally, the two coffee species are grown in 80 countries in Africa, Asia and South America (International Coffee Organization - ICO 2014, Musoli et al. 2010 and Vega et al., 2008).

Genetic constitution in *Coffea arabica* is tetraploid (2n = 4x = 44) and self-fertile, while Robusta coffee is diploid (2n = 22) and self-incompatible (Tshilenge et al. 2009). There are however, several morphological diversities among the Robusta coffee species that are found along the rift valleys of Tanzania, particularly in the great lake regions.

The *Coffea kihansiensis* early described by Davis and Mvungi (2004), is endemic to the Kihansi gorge forest, southern Udzungwa Mountains. It was listed among the threatened species in that forest (Rija 2014). Furthermore, due to restricted range size and potential threats from human and environmental disturbances, it is listed as threatened species under the IUCN Red List (IUCN 2014). *C. kihansiensis* is likely to be a close wild relative species to cultivated Robusta genotypes and bear a number of characteristics that could be potential for genetic improvement of cultivated coffee varieties. Evidence of utilization of sources of variations from wild coffee relatives for improvement of domesticated species was described by Ng'homa (2014) in improvement for coffee wilt resistance. Therefore, the wild *C. kihansiensis* is a potential genetic resource for coffee improvement, and the exploitation of diversity should be the ultimate objective of genetic resources exploration and conservation.

The International Board for Plant Genetic Resources (IBPGR) has developed various descriptors (passport, management, environment and site, evaluation and characterization) to be used in assessing and evaluating germplasm. Characterization descriptors are used for discriminating among phenotypic characters of germplasm materials. They show distinctive characters of accessions and the highly heritable characters equally expressed in all environments. The morphological characters provide an efficient tool to select the most important characters to be considered in breeding programmes. A number of descriptors have been developed to explorer the variations among the coffee germplasm accessions (IPGRI 1996, Walyaro 2006). The aim of this study was to characterize the morphological diversity as the basis of exploring the potential of this wild species for conservation and genetic improvement of cultivated coffee cultivars.

Material and Methods

This study was conducted at Kihansi gorge located at Udzungwa Mountains southern part of Morogoro region in Tanzania. A survey was conducted in-situ along sites at Kihansi where delineated patterns of natural gardens of wild coffee (*C. kihansiensis*) are distributed over different altitudes along Kihansi gorge distributed in four separate blocks based on altitude and location of the hills, namely; Lower Upper Spray, Upper Spray Forest, Upper Spray Muhalala and Lower Spray wetland. Survey data on selected morphological descriptors (Table 1) were collected randomly from a total of 231 accessions of wild coffee trees for morphological assessment using vegetative stage descriptors and only 64 mature reproductive plants were used for assessment of reproductive stage descriptors.

For characterization purpose, 35 coffee morphological descriptors of agronomic importance were used as described by the International Plant Genetic Resources Institute (IPGRI 1996). The study was conducted in such a way that all the plant growing stages were included in morphological characterization. Both vegetative and reproductive phases of wild coffee plants were used in individual sampling for qualitative and quantitative character analysis according to the IPGRI (1996).

Table 1: List of descriptors for characterization of morphological characteristics of coffee accessions

Descriptor	Reference	Methods of assessment and scales used
1. Plant habit	IPGRI 1996	Measuring the height of coffee trees using 10 metre ruler and scored as follows: 1 = bush (< 5 m- without distinct trunk), 2 = shrub or small tree (<5 m – one or more trunks) and 3 = tree (> 5 m- single trunk)
2. Plant height	IPGRI 1996	Visual estimation: 1 = very short, 3= short, 7 = tall and 9 = very tall
3. Overall appearance at the specific age of plant	IPGRI 1996	1 = elongated conical, 2 = pyramidal and 3 = bushy
4. Branch –ramification number	IPGRI 1996	Average of ramifications scored on five well developed branches

Descriptor	Reference	Methods of assessment and scales used
5. Branching habit	IPGRI 1996	1 = very few branches (primary), 2 = many branches (primary) with few secondary, 3 = many branches (primary) and 4 = many branches (primary) with many secondary and tertiary branches
6. Angle of insertion of primary branches	IPGRI 1996	Observation is done on the main stem (1 = drooping, 2 = horizontal and 3 = semi erect
7. Young leaf colour	IPGRI 1996	1 = greenish, 2 = green, 3 = brownish, 4= reddish, 5 = Bronze and 6 = others if any
8. Leaf shape	IPGRI 1996	1 = obovate, 2 = ovate, 3 = elliptic , 4 = lanceolate and 5 = others if any
9. Leaf apex shape	IPGRI1996	1 = round, 2 = obtuse, 3 = acute, 4 = acuminate, 5 = apiculate, spatulate and 6 = others
10. Leaf width (mm)	IPGRI 1996	Measuring and take the average of five mature (> node 3 from the terminal bud) leaves, measured at the widest part
11. Leaf petiole colour	IPGRI 1996	1 = green , 2 = dark brown, 3 = other
12. Young shoot colour	IPGRI 1996	1 = green, 2 = dark brown and 3 = others
13. Inflorescence position	IPGRI 1996	1 = axillary and 2 = terminal
14. Inflorescence on old wood	IPGRI 1996	0 = absent and 1 = present
15. Number of flowers per axil/ inflorescence	IPGRI 1996, Walyaro 2006	Average of 10 axils, randomly selected from different nodes
16. Inflorescence stalk length(mm)	IPGRI 1996	Average of five inflorescences, randomly selected from different nodes
17. Number of petals per flowers	IPGRI 1996	Average of 10 flowers, randomly selected from different nodes
18. Anther insertion	IPGRI 1996	1 = excluded, 2 = included
19. Number of stamens per flower	IPGRI 1996	Average of 10 flowers, randomly selected from different nodes
20. Fruit colour	IPGRI 1996, Walyaro 2006	Observed on mature fruits: 1 = yellow, 2 = yellow-orange, 3 = orange, 4 = orange-red, 5 = red, 6 red – purple, 7 = purple, 8 = purple-violet, 9 = violet, 10 = black and 11 others
21. Fruit shape	IPGRI 1996, Walyaro 2006	Average of five normal (not caracole) mature fruits: 1 = roundish, 2 = obovate, 3 = ovate, 4 = elliptic,5 = oblong and 6 = others
22. Calyx limb persistence	IPGRI 1996	0 = No and 1 = Yes
23. Fruit length (mm)	IPGRI 1996	Average of five normal mature green fruit, measured at the longest part
24. Fruit width (mm)	IPGRI 1996	Average of five normal mature green fruit measured at the thickest part
25. Pulp thickness	IPGRI 1996	Visual observation in relation to berry/bean. Scoring done using 3 = thin, 5 = intermediate and 7 = thick.
26. Harvest duration	IPGRI1996	Days taken by the crop to ripe
27. Single berry weight	Walyaro 2006	Average of 200 mature fruits
28. Empty fruit rate (%)	IPGRI1996, Walyaro 2006	Scored by floating fruits
29. 100 bean weight (g)	IPGRI 1996	Calculated at (11% moisture) content as follows: (“Bean weight at 0% moisture content” x 100)/

Descriptor	Reference	Methods of assessment and scales used
		("Bean number" x 0.89)
30. Fruit filling coefficient	IPGRI 1996, Walyaro 2006	Ratio of bean number over cherry number. The fruit filling coefficients vary between 0 (sterility) and 2 (complete fertility).
31. Seed length (mm)	IPGRI 1996	Maximum length average of five normal mature seeds
32. Seed width (mm)	IPGRI 1996	Average of five normal mature seeds, measured at the widest part
33. Seed thickness (mm)	IPGRI 1996	Average of five normal mature seeds, measured at the thickest part
34. Seed colour	IPGRI 1996 & Walyaro 2006	Visualized at 11% humidity and scored by using 1 = yellow, 2 = brown purple and 3 = others
35. Seed shape	IPGRI 1996 & Walyaro 2006	1 = round, 2 = obovate, 3 = ovate, 4 = elliptic, 5 = oblong and 6 = others if any
36. Biotic stress susceptibility	IPGRI 1996 &Walyaro 2006	Scored based on a susceptibility scale from 1 to 9: 1 = very low or no visible sign of susceptibility, 3 = low, 5 = intermediate, 7 = high and 9 = very high

Descriptive analysis was performed in order to assess and ascertain groupings of the 231 wild coffee accessions evaluated using vegetative stage and reproductive stage descriptors. Evaluations into different vegetative and reproductive descriptors, overall descriptive mean variations among descriptors were presented using box plots with respective standard deviations from means for each scored descriptor. Furthermore, multivariate analysis was performed using Genstat software 16th edition. Important traits in each principal component that significantly contributed to the variation observed were identified as suggested by Jonson and Wichern (2002).

Results

Distribution and variability of morphological traits

Analysis of variability of morphological parameters (vegetative and reproductive) was performed and presented in box plot (Figure 1). Branching habits, plant habits, fruit length, fruit width, fruit thickness, leaf width, seed width and seed weight show variations among the accessions, and wide distribution were detected corresponding to high standard deviation from the general mean, while angle of insertions of primary branches, leaf apex shapes, leaf shapes, young leaf colour and plant height show no variability among the accessions of wild coffee accessed with little distributed. Seed thickness and seed length show very small insignificant variations among the accession compared to other parameters (Figure 1).

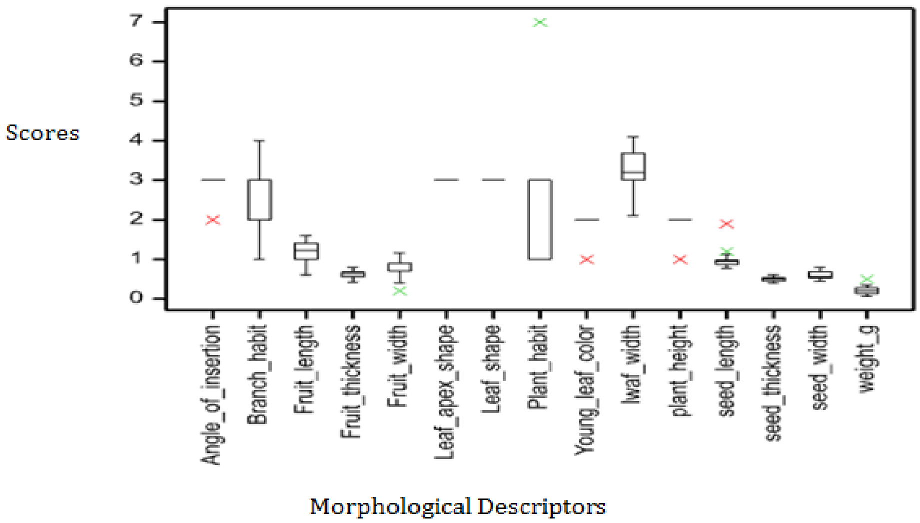

Figure 1: Variations and distribution of Kihansi wild coffee (*C. kihansiensis*) accessions among morphological descriptors assessed.

Distributions of vegetative morphological descriptors

Plant habits

In this study, the 231 wild coffee accessions were distributed into only two categories, shrub and bush types, whereby more than 87% of the observed populations was less than 5 m in terms of height with no distinct trunk and classified as shrub, and the remaining 13% was bush type with less than 5 m but with possession of one or more trunks (Figure 2).

Plant height

Observations showed that four categories of plants in this accession were present distributed as 49% were very short with length less than 0.5 m, 34% were tall plants with characteristics of having lengths between 1 m and 2 m, 12% were very tall plants with highest lengths greater than 2 m, while only 4% were short plants (Figure 3 and Plate 1).

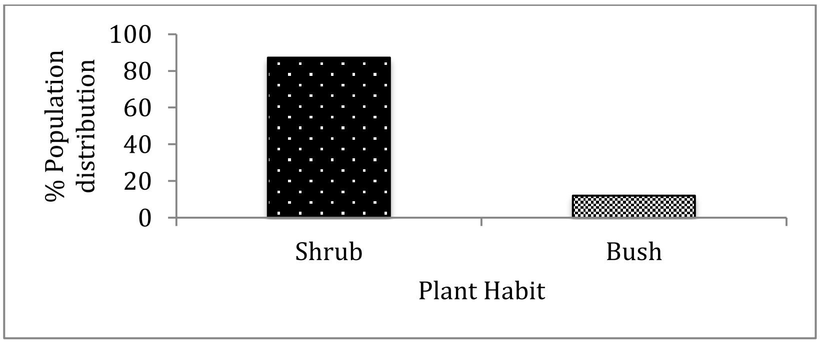

Figure 2: Distribution of Kihansi wild coffee 231 plants population in plant habit morphological traits.

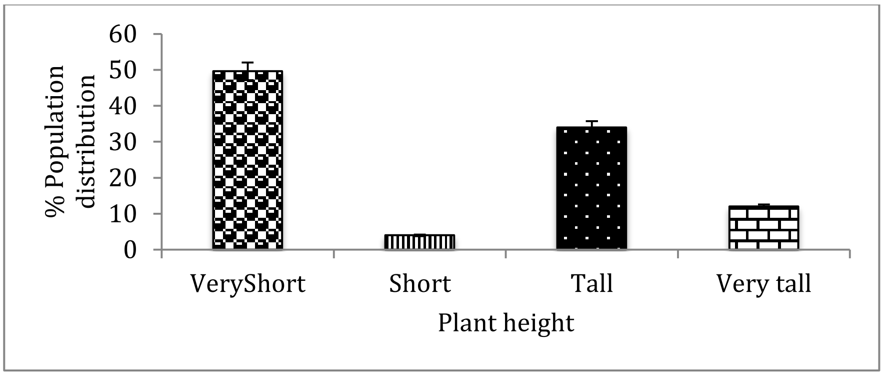

Figure 3: Distribution of 231 accessions of Kihansi wild coffee in different categories of plant height.

(a)

(b)

Plates 1a & b: Plant heights (very short and tall) of Kihansi wild coffee accessions observed.

Branching habits

Based on branching habits distribution, four distinct categories were observed (Figure 4) as distributed by the 231 accessions, whereby 36.4% of the population had many branches with few secondary branches, 32.5% had many primary branches, 17.8% had very few primary branches, while the remaining 13% had many primary branches with many secondary branches as well (Plate 2).

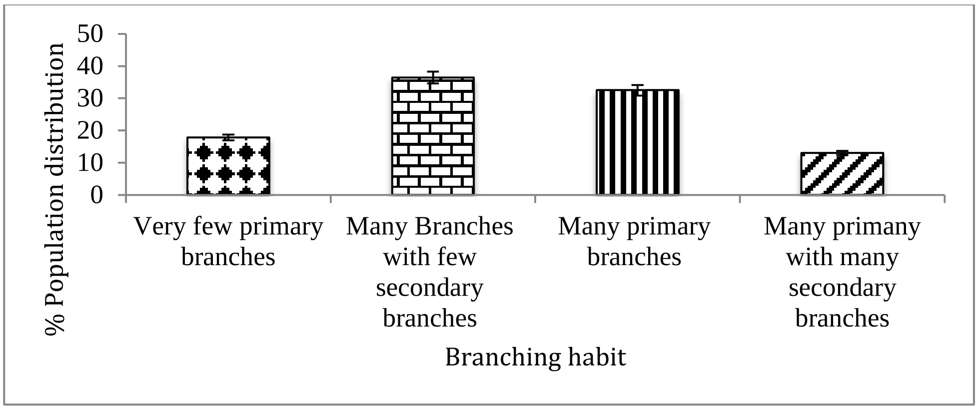

Figure 4: Distributions of 231 accessions of Kihansi wild coffee to categories of branching habits.

Plate 2: Branching habits (many branches(left) and few branches (right)) observed in Kihansi wild coffee accessions.

Angle of insertions of primary branches

Observations showed that more than 66% of the population accessions were characterized as semi-erect in terms angle of branches in its primary branches. Also 33.33% of the population was characterized by having horizontal type of angle of insertions. Over three quarters of the individuals in this population have primary branches which are semi-erect (Figure 5 and Plate 3).

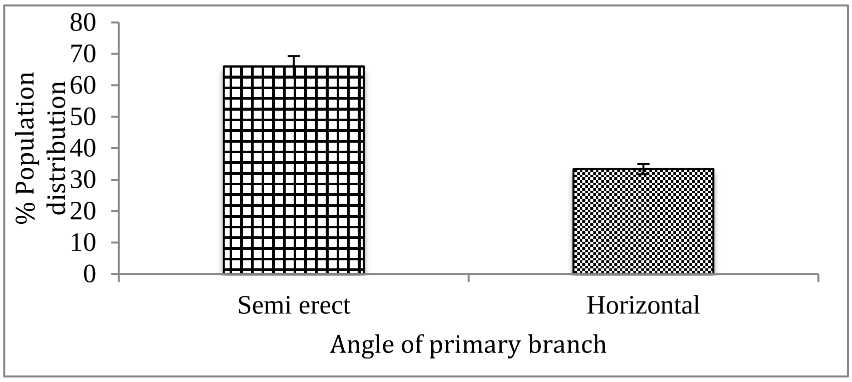

Figure 5: Distribution of population of 231 accessions to angle of insertions to primary branching.

Plate 3: Branching habits (horizontal (left) and semi-erect (Right)) of Kihansi wild coffee.

Young leaf colours

Colours of young leaves were characterized as green by 88.74%, brownish type by 6.5% and the remaining 4.8% were greenish. Young leaf colour dominated in whole population in front of brownish colour and greenish colour (Figure 6 and Plate 4).

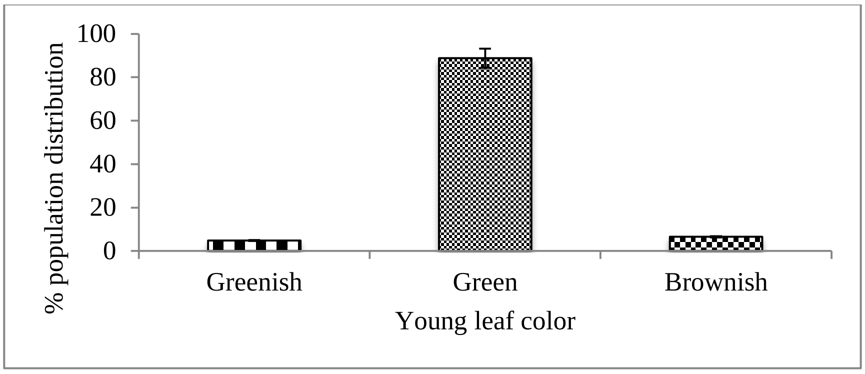

Figure 6: Distribution of 231 accessions of wild coffee to leaf colour morphological characteristics.

Plate 4: Young leaf colours (brownish (left) and greenish (right)) observed in Kihansi wild coffee accessions.

Leaf shapes

Elliptic and ovate shape types were observed (Plate 5) from the population in this study; however, elliptic types of leaves dominated by over 97% and the remaining population of about 3% was observed as ovate type of leaf shapes (Figure 7 and Plate 5).

Plate 5: Different leaf shapes (elliptic-left and ovate-right) observed in Kihansi wild coffee population accessions.

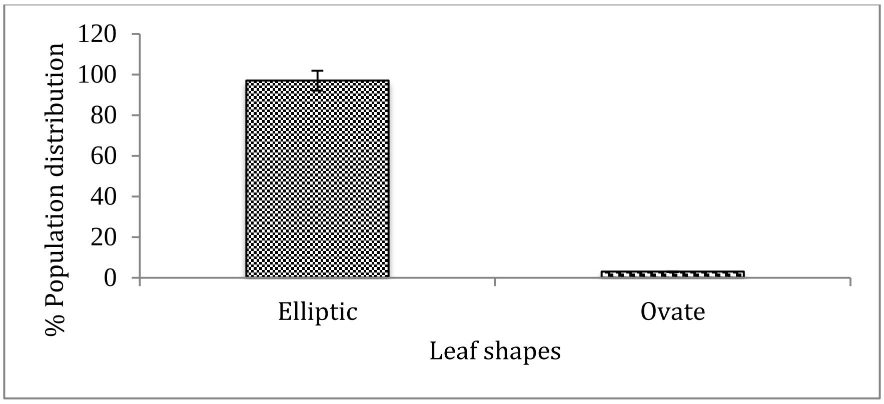

Figure 7: Distribution of Kihansi wild coffee of 231 accessions into leaf shapes morphological characterization**.**

Leaf apex shapes

Fewer number of accessions were characterized into obtuse type of leaf apex shape, which were 6 (1.3%) of the total accession observed under this study. The majority of the accessions observed (225 or 98.7%) were characterized into acute shape of leaf apex **(**Figure 8**).**

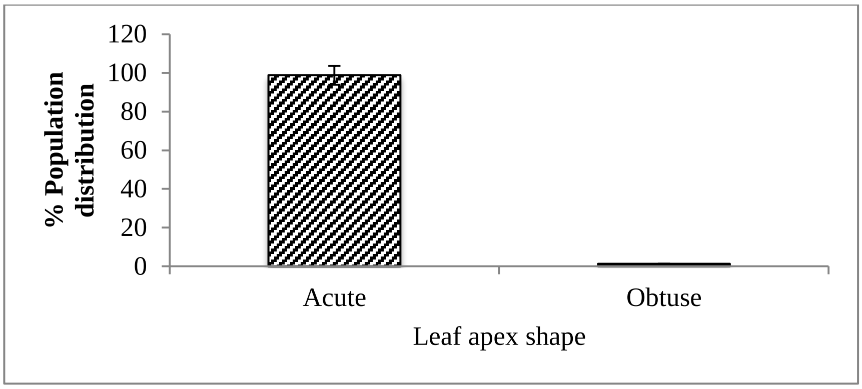

Figure 8: Kihansi wild coffee plant accessions distribution in leaf apex shapes.

Leaf width

Characterization of Kihansi wild coffee species based on leaf width categorized the plants into three different groups, and observed that 80.95% of the accessions had leaf width between 3 cm and 4 cm, 15.15% had mean leaf width of greater than 4 cm, while 3.9% accessions with less than 3 cm mean leaf width were observed (Figure 9).

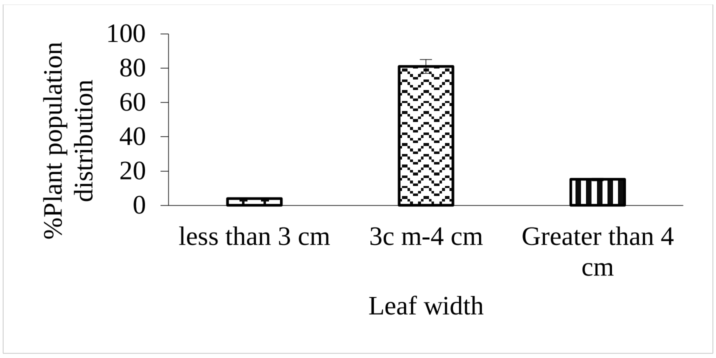

Figure 9: Distribution of Kihansi wild coffee accession to leaf width morphological qualitative traits.

Qualitative reproductive morphological descriptors

Fruit shape

Morphological characterization of fruits accessed in terms of fruit shapes indicated that 66% of total accessions had roundish shaped fruits, while only 34% of the fruits were of oblong shape. High percentage of oblong type fruits were found in the upper wetland plots and remaining found on the other two plots, lower wetland and lower upper (Figure 10 and Plate 6).

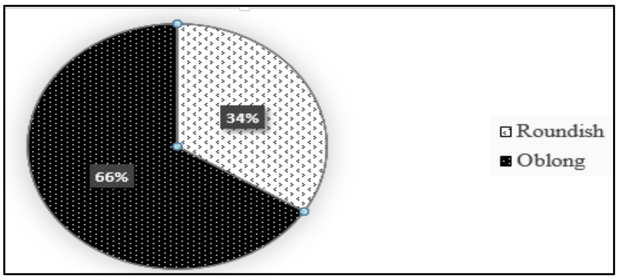

Figure 10: Distribution of wild coffee accessions into fruit shape categories.

(A)

(B)

Plate 6: Fruit shape (A: oblong B: round) of matured Kihansi wild coffee accessions.

Fruit colour

There is no variation of fruits colour among the accessions of Kihansi wild coffee under this study. Only orange fruit colour was observed among the accessions under the observations of this study; however, the fruit colours change during the fruit development from the green fruits then yellowish colour to full yellow and then turn to orange upon maturation (Plate 6 and Plate 7).

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Plate 7: Fruit colour of mature A: unripe, B: matured ripe and C: dried fruits of Kihansi wild coffee.

Inflorescence position

Inflorescence was arranged on the axillary positions of the branches; while no inflorescence on the terminal positions of the branches were observed. Observations also showed the absence of inflorescence in the old wood, demonstrating that 100% of the plants had inflorescence on the primary and secondary branches (Plate 8).

Plate 8: Inflorescence position (axillary) as observed in vertical branches of Kihansi wild coffee plants accessions.

Fruit filling coefficient

The ratio of cherry number over berry number in all the accessions was assessed by counting the bean number in a cherry after removing the outer part (mesocarp or pulp skin). The results showed that 100% of the accessions under this study had complete fruit filling ratio of 1:1 of cherry and bean number of wild coffee fruits, respectively.

Quantitative reproductive morphological descriptors

Variations in fruit dimensions

Observations showed a wide distribution of fruit sizes in length and width. The minimum seed length observed was 6.6 mm, while the maximum fruit length was 16 mm and the overall mean length was 12.2 mm (Table 2). Also, fruit width ranged from 2 mm to 11.6 mm with an overall mean of 8.2 mm, while a narrow distribution of accessions to fruit thickness was observed in a narrow range of 4.2 to 7.9 mm minimum and maximum thickness, respectively.

Small variations between the maximum seed sizes and minimum seed sizes were observed in wild coffee accessions. Seed length, seed width and seed thickness had minimum values of 7.7, 4.5 and 4 mm, respectively, while maximum values of 19, 7.9

and 6 mm, respectively were obtained. Wide variations of distribution of accessions were observed; a very minimum single berry weight was recorded of 0.06 g, and the maximum single berry weight was 0.5 g (Table 2).

Table 2: Fruit and seed dimensions of Kihansi wild coffee accessions indicated by range of minimum value (min) and maximum value (max) with general mean

Parameter (mm, except berry weight)	Min – Max	Mean	Standard deviation
Fruit length (n = 64)	6.0– 16	12.2	
Fruit width (n = 64)	2.0– 11.6	8.2	
Fruit thickness (n = 64)	4.2–7.9	6.2	
Seed length (n = 64)	7.7–19	9.4	
Seed width (n = 64)	4.5–7.9	6	
Seed thickness (n = 64)	4.0–6.0	5.1	
Single berry weight (g) (n = 64)	0.06 – 0.5	0.2	

Comparison a of bean sizes of arabica coffee in relation to Kihansi wild coffee

The coffee seeds are elliptical or egg shaped, possessing the longitudinal furrow on the plane surface and the outer covering is formed by the hard-pale brown endocarp (Eira et al. 2006). Measurements done with many seeds indicate the coffee Arabica seeds are 10 to 18 mm long, 6.5 to 9.5 mm wide (Eira et al. 2006). In comparison to Kihansi wild coffee, the observed results show that the plants had roundish shapes or oblong types of fruits with soft brownish endocarp. Seeds of Kihansi wild coffee were 7.7 to 19 mm long and 4.5 to 7.9 mm wide (Table. 3).

Table 3: Comparison of bean size for *Coffea arabica* L., *Coffea racemose* and *Coffea kihansiensis*

Species of coffee	Seed length (mm)	Seed width(mm)	Reference
C. Kihansiensis	7.7–e 19	4.5–7.9	This study; observed 2017
Coffee arabica L	10–18	6.5–9.5	Eira et al. 2006
Coffee racemose	3–3.5	5–7	Eira et al. 2006

Principal Component Analysis (PCA)

The principal component analysis (PCA) and percentage contribution of each component to the total variations in qualitative morphological traits are presented in Table 4. The results showed that 4 principal components with eigenvalue greater than a unit accounted for 62.52% of the total variations in the populations were obtained with relative discriminating power of PCA as revealed by eigenvalues of 1.64 (highest) in PCA 1 and 1.02 (lowest) in PCA 4. The first principal component accounted about 20% of the total variations, whereby the second PCA accounted 15.86% of the total variations. The highest contributions to the variations were shown in plant growth habits (0.54), plant height (0.51), and branching habits (0.44) in PCA 1, while leaf width (0.68), branching habits (0.42) and angle of insertions of primary branching had the highest components in PCA 2 (Table 4). Young leaf colours (0.52), angle of insertions (0.35) and branching habits (0.32) also showed to be high in contribution to the total variations in PCA 3 (Table 4).

Table 4: Four first principal component (PCA) of Eigen values greater than a unit morphological (vegetative) descriptors

Traits	PC1	PC2	PC3	PC4
Plant habit	0.54	0.14	0.04	0.25
Plant height	0.51	0.16	0.24	0.27
Branching habit	0.44	0.42	–0.32	0.08
Angle of insertion	–0.42	0.37	–0.35	0.22
Young leaf colour	0.19	–0.19	–0.52	–0.32
Leaf shape	0.14	–0.25	0.05	0.84
Leaf apex shape	–0.11	0.28	0.67	–0.06
Leaf width	–0.12	0.68	–0.09	0.08
Eigen value	**1.64**	**1.2**	**1.12**	**1.02**
% variation	**20.29**	**15.86**	**13.92**	**12.65**

Some individual accessions are quite dispersed in a biplot graph (Figure 11); accessions 50, 48, 227 in branching habit; 208, 81, and 209 in leaf width; 228, 219, 140 and 147 in plant habit, while a large proportion of studied accessions show similarities by being concentrated together as indicated in the biplot (Figure 11).

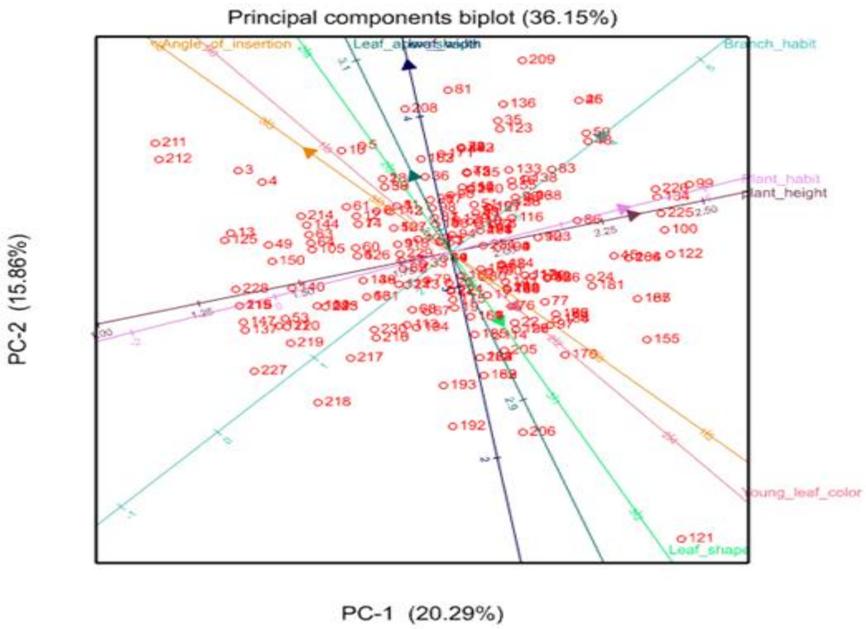

Figure 11: Biplot of two PCA for vegetative morphological descriptors.

Moreover, the results showed that 72.42% of the total variations were contributed by the reproductive morphological descriptors in the first four principal components, whereby PC 1 accounted for 20.29% of the total variations, while fruit length (0.28), fruit thickness (–0.56), and seed width (0.42) contributed greatly than other descriptors in PC1; however, fruit thickness contributed highly but negatively.

PC2 accounted about 15.86% of the total variations with seed length (0.51), seed thickness (0.66), and weight of single berry

(40) contributed more to the total variations compared to other descriptors in this component. In comparative to PC3, 15.17% of the total variations were contributed by this component where fruit width (0.79) and seed width (0.45) had more contribution to the variations in this component. The discriminating power shown by highest Eigen value (1.95) in PC1 and lowest Eigen value (0.96) in PC4 (Table 5).

Table 5: Eigen values and % variations of the four first PC of quantitative morphological (reproductive) descriptors

Traits	PC 1	PC2	PC3	PC4
Fruit length	0.40	0.09	0.28	0.38
Fruit thickness	–0.56	0.13	0.12	0.02
Fruit width	0.20	–0.18	0.79	0.24
Seed length	0.28	0.51	0.21	–0.60
Seed thickness	0.31	0.66	–0.21	0.25
Seed width	–0.42	0.29	0.45	–0.27
Weight (g)	–0.38	0.40	0.01	0.55
Eigen value	1.95	1.16	1.07	0.96
%Variation	27.51	16.30	15.17	13.44

High dispersions of characters in quantitative reproductive descriptors were observed in biplot of the first and second PCA (Figure 12) through scattered numbers of the accessions in different traits though fewer individuals were confined at the centre.

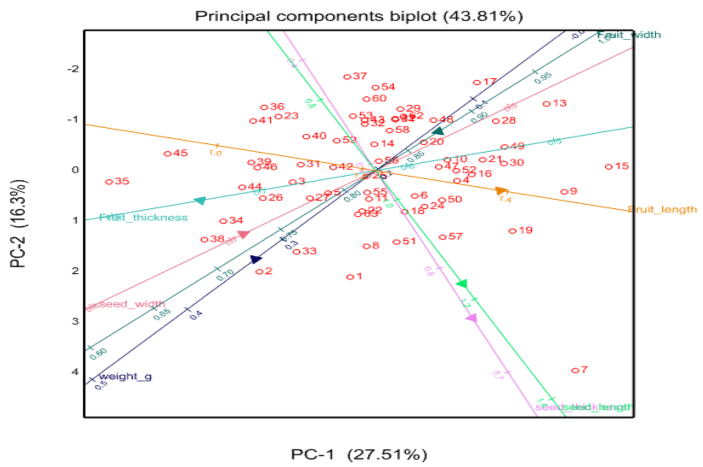

Figure 12: Biplot of first and second principal components of quantitative morphological traits representing reproductive traits.

Correlations of morphological descriptors of *C. kihansiensis*

Among the descriptors, fruit length, fruit width, seed thickness, seed width, fruit thickness and single beery weight showed less and positive correlation. A positive and significance correlation observed between fruit thickness and fruit width (r = 0.4), also was observed between single berry weight and fruit thickness (r = 0.3). However, most of descriptors showed negative correlation with each other as fruit width had negatively correlation with all other parameters (Table 6), seed width had negatively correlations with seed thickness, Fruit thickness and seed thickness also had negatively correlations to each other.

Table 6: Correlations of seed and fruit dimensions as reproductive morphological descriptors

	FL	FW	FT	SL	SW	ST	SBW
FL	1						
FW	0.174	1					
FT	–0.194	–0.194	1				
SL	0.101	–0.168	0.101	1			
SW	–0.116	–0.002	0.405**	0.03	1		
ST	0.2	–0.046	-0.227	0.218	–0.11	1	
SBW	–0.171	–0.049	0.321*	–0.125	0.184	0.047	1

FL = fruit length (cm), FW = fruit width (cm), FT = fruit thickness (cm), SL = seed length (cm), SW = seed width (cm), ST = seed thickness (cm), SBW = single berry weight (g)
*, **Correlation is significant at probability value of 0.05 and 0.01, respectively.

Discussion

Distribution of vegetative morphological characteristics of *C. kihansiensis*

About 87% of the wild coffees in Kihansi are bush type and only 13% of the accessions represented shrubs type. The gardens are dominated by spatial distribution of short plants, tall plants and very tall plants in a population of Kihansi wild coffee (KWC) (Figure 3 and Plate 1). These results can describe the KWC population as dominated by bush type with many short plants and supported Davis and Mvungi (2004) who described the presence of plants with height ranging from 1.5 to 3 m length hence corresponding to these findings. Presence of horizontal and semi-erect types of branches in trees indicated that there are two quite different plant populations existing within the KWC. These were also significantly similar to the results obtained by Davis and Mvungi (2004) who first characterized KWC based on their morphology. They found horizontal to semi-erect, grey to brown and slightly rough types of branches.

Over 88% of the population was characterized to be in green colour in terms of young leaves, the small proportions of accessions were distributed to other groups of leaf colours such as greenish and brownish; this is the indication of young leaf colour being dominant in the population. Very significant variations of plant accessions were observed in leaf shape, however, only two groups of these characters were observed as elliptical like structures dominating the whole population by 97% and ovate type took a portion within the population by 3%. In comparison to the findings obtained by Davis and Mvungi (2004) who characterized the leaf as elliptic to broadly elliptic and sometimes ovate to elliptic obovate with dimensions of 3.5–10 m ×1.5–5.5 cm, the results suggest the presence of diverse accessions in this population. Although this observed difference is phenotypically characterized, further genetic characterization of the KWC population is required to confirm if the variations observed are also due to genetic differences.

Plant height

There were obvious variations in terms of plant height among the surveyed and sampled wild coffee accessions. Majority of the plants in the population were classified as very short (50%) which was a typical distribution of the observed wild coffee plants in Kihansi coffee gardens. The second clusters of plants were tall plants representing 35% of the whole population. There was a scant population of short and very tall plants in the garden. Selection for plant height has agronomic implications related to crop management and harvesting, thus can be used for crop improvement.

Plant growth habits

Majority of the *Coffea kihansiensis* population is dominated by shrub growth habit and very few individuals were observed to grow as bush trees. This can be of interest for regeneration of new productive branches since this coffee bears only few fruits per branch and therefore serves as compensation for fruit yields.

Branching habits and angles of insertion

Branching habits observed in different accessions of wild coffee are skewed to production of many branches with several primary branches. The nature of branching habits is associated with the shrub type of growth habits observed in these plants. The types of branching habits also conform to the number of branches that are productive; they are necessary sites for coffee berry production. Branching habit may be associated with increased number of fruit set per branch.

PCA of vegetative morphology traits

Principle component analyses have shown that the plant habits, plant height, branching habits in PC1 and leaf width in PC2 provide maximum and significant contributions to the total variations and these observed variations were supported by boxplots which showed that branching habits, plant height, leaf width and plant habits had large standard deviations which indicated the wide distributions and deviations of individuals from the mean population.

The biplot of PC1 and PC2 showed individuals within a population of plants concentrated at the centre of the biplot. This close relatedness is the indicator of the similarities among individuals within the population of the KWC, however, some of the individuals observed dispersed far and away from the centre, Accessions close to plant habits line (34, 220 and 99), (206, 208 and 81), close to leaf width line (50, 48, 227 and 217) and close to branching habits show distinctness from other individuals in the population and these are the indicators of distinctness and variability of individuals to some of the parameters.

In light of the results obtained from the PCA and box plot, it may be possible to deduce that more than half (62.52 %) of the variations obtained from vegetative morphological parameters were primarily due to branching habits, plant habits, leaf width and plant height. These results were corresponding to Kebede et al. 2008; Chaparro et al, 2004 and Wrigley, 1988 who evaluated the phenotypic diversity of Ethiopian coffee and reported significant variations attributed by plant habits, branching habits, young leaf colour and number of nodes. In addition, Gessese et al. (2015) reported use of morphological markers to characterize diversity of coffee in southern Ethiopia. Also, a study on morphological diversity of coffee done by Catter (1992) revealed high variations in morphology. These corresponding findings can be used as significant morphological traits in selection in improving productivity of coffee crop, representing great variability of coffee production. The results from this work therefore can be used to identify branching habit, leaf colour, plant height, plant habit, angle of insertion of primary branches, berry size and cherry size.

Reproductive morphological traits

Fruit colour, shape, size and weight

Significant variations in fruit characteristics were observed in terms of fruit colour, ranging from yellowish orange, orange to red fruits. Fruit shapes were oblong and round fruits, whereby round fruits represented the most dominant fruit shapes. There were significant variations (Figure 12 PC1 27.9% and Table 5) in coffee berry weight among accessions in the Kihansi wild coffee, ranging from 0.07 to 0.5 g with an average of 0.28 g. Implying that this coffee produces small fruits in the wild and thus there are variations in fruit characteristics that can be explored for coffee genetic improvement.

Number of fruits per cluster and position of fruits

The coffee collections surveyed indicated that most of the plants produce 2 fruits per cluster and mostly located at the terminal positions of the branches. The deviated position of location of fruits per branch is a trait to explore as the majority of the coffee berries are distributed on the axils of the coffee plants.

Both qualitative and quantitative reproductive traits were assessed; only two distinct groups of accessions had oblong type of fruit shapes and roundish type fruits. However, in terms of fruit colour, only orange fruit colour was observed which developed from the greenish colour to yellowish colour up to orange upon ripening. Significant variations were observed in quantitative traits of this population. Fruit width (0.2–11.6 mm), fruit length (6–16 mm), and fruit thickness (4.2–7.9 mm) showing wide distributions among the accessions with wide ranges (minimum to maximum values). This variability in different traits is a proof to the existence of different accessions within the population (Kathurima et al., 2012) and can be used as a factor for clustering this population. These findings partially agreed with that described by Davis and Mvungi (2004) who found that ellipsoid to ellipsoid-obvoid types of fruit shapes existed in the KWC population with 9.5–12.6 mm × 5–9.2 mm, yellowish to orange berries at maturity. Seeds with 7.7–19 mm × 4.5–7.9 mm (length × width), 4–6 mm thickness were observed in the population, may be due to environmental variations and time in which data were collected, due to the fact that morphological traits are sensitive to environmental factors. These findings are supported by the findings of the previous studies of diversity reported by Cubry et al. (2008) and Musoli et al. (2010).

PCA of reproductive morphological parameters

First, second and third PC with an Eigen value of larger than a unit in reproductive morphological traits accounted about 72.42% of the total variations, whereby fruit length, fruit thickness, seed width and weight of single berry in PC1, seed length, seed thickness and weight of single berry in PC2 as well as fruit width in PC3 contributed more to the total variations observed. It is possible to deduct that selection and clustering of this population could be effectively done if much consideration would be based on reproductive traits including fruit characteristics and yield components (Ermias, 2005). This finding partially agreed with the findings by Tounekti et al. (2017) who worked on diversity of Arabica coffee in Saudi Arabia and found that the first 2 principal components PC1 and PC2 with percentage variations 51.0% and 15.0%, respectively with fruit length, fruit width, fruit thickness and berry weight contribute greatly to the total variations and are used as selection criteria.

According to Chahal et al. (2002), characters with largest absolute values closer to the unit within the first principal components PC1 and PC2 influence the clustering more than those with lower absolute values closer to zero. Also, Olika et al. (2011) have reported that coffee bean length, hundred bean weight, leaf length and leaf width contributed to the variations among Limmu coffee accessions. The existence of morphological diversity among the coffee accessions was further confirmed by many authors who reported the variability of coffee morphologically (Bayetta 1997, Kebede 2008, Gichimu 2010).

Hence, morphological traits mainly those contributed to the PC1, PC2 and PC3 in this study (fruit length, fruit thickness, leaf width, seed width, weight of single berry, plant habit, plant height, and branching habit) could play major roles in classifying and grouping the

KWC accessions into different separate clusters.

Morphological diversity of wild coffee from localized environment in Minziro forests collected from related wild coffee was also demonstrated by Ng'homa (2014). The knowledge of morphological diversity for vegetative and reproductive stages of the wild coffee is very important for conservation and breeding purposes (Musoli et al. 2010). Like other crops, the morphological characteristics provide opportunity for polygenic analysis and demonstrate levels of relatedness among accessions and comparable to cultivated species of coffee (Sureshkumar et al. 2013). The relationships among the morphological characteristics of coffee species are partly due to the involvement of the same sets of alleles that are responsible for regulation of expressions of some important agronomic characters that can have heterotic effect if combined within or between coffee species (Sureshkumar et al. 2013). Following the proper identification of significant variations among agronomic traits, breeders need to select and understand the genetic control of each morphological trait. Therefore, selection and combination of these parameters could have genetic impacts for potential utilization of this wild species to coffee crop improvement of agronomic traits such as disease resistance and/or domestication (Mtenga and Reuben 2012).

Conclusion

With this study, it is concluded that morphological variations existed, especially in reproductive traits (bean size, berry size and shape), to a small extent vegetative traits, also good variations that can be exploited in coffee selection and improvement towards domestication of this wild coffee. Further studies need to be documented on molecular characterization of these same accessions from Kihansi wild coffee gardens. Therefore, further study on DNA sequence of this wild coffee should be done in order to confirm its relatedness with commercial growing coffee, Arabica and Robusta. Most variations are found in individuals within blocks within the gardens.

Finally, holistic conservation strategies should be effectively established specific to each block before the utilization of the KWC to ensure sustainability. Planting of the species into different coffee producing regions in Tanzania should be initiated in selected coffee research institutions. Eventually, adaptation of the wild coffee to local coffee grower areas is expected to be realized in their localities. In long run, this species is expected to be useful in coffee improvement programme and therefore minimizing potential risks of diseases in coffee production (ICO 2002).

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