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Phenotypic diversity of cocoa (*Theobroma cacao* L.) based on morphological characterization descriptors in major cocoa-growing areas of Tanzania

Godwin G. Maro*, Pavithravani B. Venkataramana, Kelvin M. Mtei, Mashamba Lucas Philip

Nelson Mandela African Institution of Science and Technology (NM-AIST), Department of Sustainable Agriculture and Biodiversity Conservation (SABE), Arusha, Tanzania, United Republic of Tanzania

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ABSTRACT

Cocoa (*Theobroma cacao L.*) is an important commercial crop for the livelihoods of the people in Tanzania. Its farming is highly impacted by climate change, pests and diseases, and soil health quality, leading to poor production. This study was conducted in Mbeya (Kyela), Morogoro (Kilombero), and Tanga (Muheza) regions of Tanzania, to assess the farming practices and variations in cocoa morphological characters. Purposive sampling was used to select regions, districts and villages where cocoa was highly grown in Tanzania and simple random sampling technique was used to select the cocoa farmers. Variables such as petiole length, pod length, pod width, number of beans per pod, bean length, and dried bean weight showed significant differences amongst themselves. The coefficient of variation of less than 50%, ranging from 14.60% to 47.56%, indicated moderate variation among the descriptors. The variations exhibited by some of the descriptors might be caused by on-farm practices and mixing of traditional Criollo/Forastero introductions and Trinitario hybrids. Also, the inherent genetic differences between these varieties result in highly diverse morphological characteristics. This study therefore, provides valuable insights in the morphological variations of cocoa which is important for agricultural stakeholders for cocoa farming improvement.

***Corresponding author:** Godwin G. Maro ✉ marog@nm-aist.ac.tz

INTRODUCTION

Cocoa (*Theobroma cacao*) is an important crop for the livelihoods of the people in Tanzania, as it supports around 100,000 people, with the largest production (80%) coming from Kyela in Mbeya region. Cocoa is mainly grown for commercial purposes (Alavaisha, 2020; Nindi *et al.*, 2014) and thus the crop has been potential to reduce poverty and improve people' livelihood in the farming areas. It has been reported that the majority of Tanzanian cocoa farmers employ traditional farming without the application of pesticides (Nyomora *et al.*, 2012). The majority of cocoa growers are smallholder farmers with their fields ranging from 1 to 3 acres (Kalimangasi *et al.*, 2014; Nyomora *et al.*, 2012). The cocoa bean crop is a good small-scale foreign exchange earner and is also an important source of income to smallholder cocoa producers. Cocoa, *Theobroma cacao* L., is a tropical rainforest tree which is believed to have originated in South America. It is one of the most important cash crops in many tropical countries. Trinitario cocoa is mostly farmed on tiny farms, with 14 million farmers earning a living from it (Lachenaud and Motamayor, 2017). The farming of cocoa is impacted by different challenges, for instance, climate change, pests and diseases, and soil health quality, which leads to poor production (Kongor *et al.*, 2024; Kosoe and Ahmed, 2022; Ploetz, 2007; Zhafran *et al.*, 2022). Cocoa is a popular tropical crop all over the world. It is grown largely to produce cacao liquor, butter, and powder for the chocolate industry, owing to its flavour qualities (Kongor and Rahadian, 2024).

The three varieties of cocoa that are known include; Criollo, forastero, and trinitario. The Trinitario kind of cocoa is the most common in Tanzania. Trinitario cocoa is a Costa Rican variety that was initially planted in Ceylon in 1834 and then tried again in 1880 (Monteiro *et al.*, 2009). It was also transferred to Fiji, Madagascar, Samoa, Singapore, and Tanzania during the same period (Bidot *et al.*, 2015). It was first introduced in Tanzania in Morogoro (east of the country) and Mbeya (Southwest). Cocoa grows best at altitudes of 30 to 300 meters above sea level, in climates where temperatures range from 20–28 degrees. Rainfall requirements are determined by the frequency and distribution of rain, as well as the soil's ability to retain water; the least required rainfall is 1000 mm equally spread throughout the year, although 1500–2000 mm is ideal. A deep, well-drained, porous, and humus-rich soil is also required for successful growing. The tree's weak root structure has to be protected from high winds by planting trees around the farm, which would act as a windbreaker. At about three to four years of age, the cocoa trees become mature and start producing fruits in the shape of elongated pods. The pods are available in a range of colours, and in less than six months they become mature (Nyomora *et al.*, 2012).

MATERIALS AND METHODS

Description of the study area

The study was conducted in Mbeya, Morogoro and Tanga regions, where a total of three districts in these regions were involved, including Kyela (Mbeya), Kilombero (Morogoro) and Muheza (Tanga) (Fig. 1). Among these three districts, six (06) villages were purposively selected for this study. Purposive sampling was used to select regions, districts and villages where cocoa was highly grown in Tanzania. Also, simple random sampling technique was used to select the cocoa farmers after being given the list of cocoa farmers from the village extension officer.

Data collection

The sample comprised of a total of 90 sample plants, each 30 samples collected from farmers' fields in Mashewa, Mbigu and Ipinda villages of Tanga, Morogoro and Mbeya regions and was used as the representative samples from the cocoa farming areas in Tanzania.

The morphological descriptors used were adapted from the International Board for Plant Genetic Resources descriptor list for cocoa, and they are key and most discriminative and taxonomically useful (Bekele and Butler, 1998; Engels, 1981; Engels, 1986; Lachenaud *et al.*, 1999; Vásquez-García *et al.*, 2022).

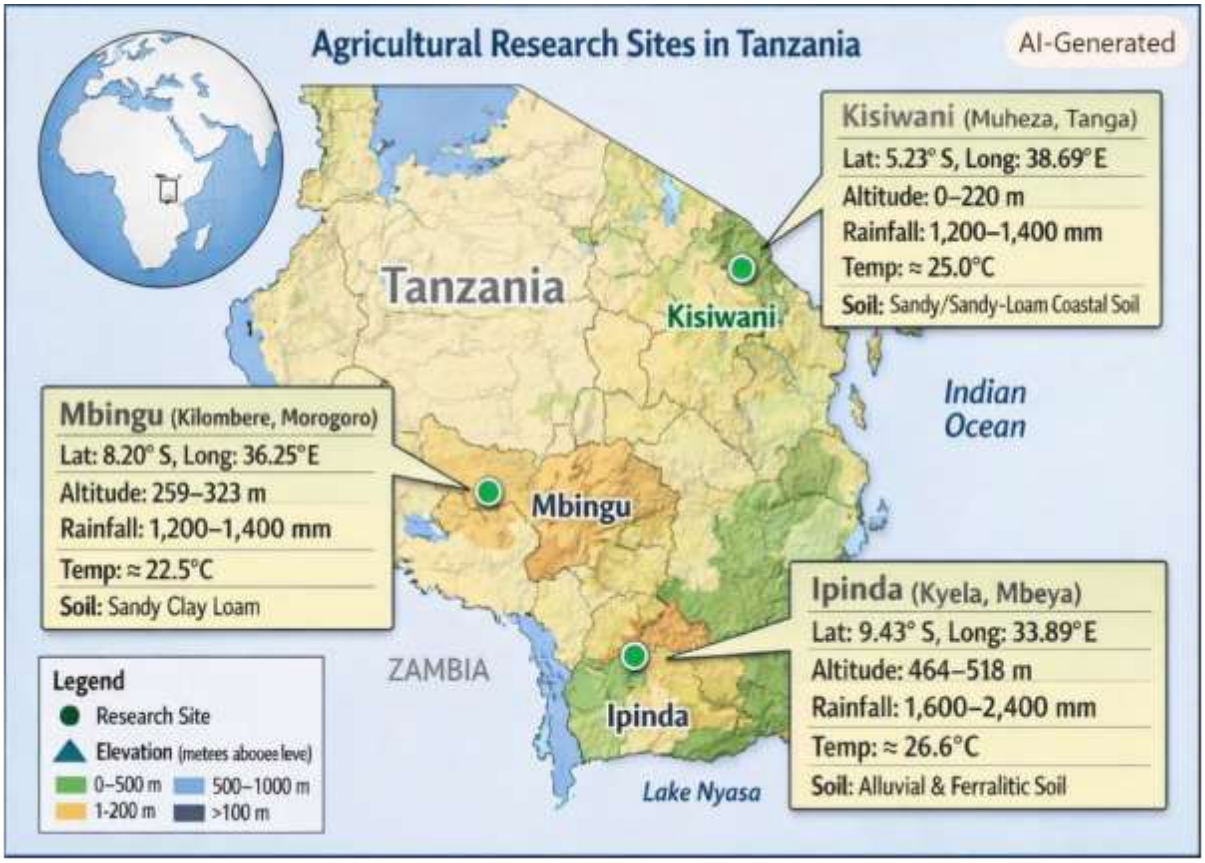


Fig. 1. Map of Tanzania showing the location of the three surveyed cocoa-growing villages

Table 1. Qualitative morphological descriptors studied in cocoa farming areas in Tanzania

Character	Modality
Leaf apex	1=acuminate; 2=apiculate
Leaf base	1=cuneate; 2=obtuse
Leaf flush colour	0=absent; 3=slight; 5=intermediate; 7=intense
Pod shape	1=elliptic; 2=oblong; 3=obovate
Pod colour	1=orange; 2=red; 3=yellow
Pod apex form	1=acute; 2=obtuse; 3=rounded
Pod basal constriction	0=absent; 1=intermediate; 2=slight
Pod surface texture	1=intermediate; 2=rough; 3=smooth
Ridge colour of mature fruit	0=absent; 3=slight; 5=intermediate; 7=intense
Pod ridge pair separation	1=slight; 2=intermediate; 3=wide
Ridge deposition	1=equidistant; 2=paired
Bean shape	1=elliptic; 2=obovate

The inclusion of qualitative descriptors in the assessment of variability is a common practice in taxonomy and is preferable because of their stability of not easily being affected by abiotic factors (Semagn *et al.*, 2010). The descriptors are shown in Table 1. About 12 characters were selected as qualitative morphological descriptors, and 10 quantitative morphological descriptors were used in the study. For leaf morphology, leaf apex, leaf base, and leaf flush colour were visually observed as the qualitative descriptors, while petiole length, leaf length, leaf width and leaf weight were measured as the quantitative descriptors. The length was measured using a meter rule, while the weight was measured using a digital balance.

For the healthy and mature pods, pod colour, pod apex form, pod basal constriction, pod surface texture, ridge colour of mature fruit, pod ridge pair separation, and ridge deposition were selected for characterization and

observed visually and scored as the qualitative characters while pod length, pod width, pod length to width ratio were used as the quantitative descriptors. Cotyledon colour and bean shape were also observed, but cotyledon colour was not scored and included in the analysis, as the only colour observed for the cotyledon was a grey colour. The bean shape was scored, while the number of beans per pod was selected randomly and counted. The bean length was measured with a meter ruler; dried bean weight was obtained after drying the samples in an oven at 105°C for 24-30 hours (Aikpokpodion, 2010), and yield was measured analytical balance.

Statistical data analysis

The descriptive statistics and analysis of variance (ANOVA) were carried out for all quantitative variables. Data were subjected to principal component analysis (PCA) in the SPSS software version 22, with all qualitative and quantitative morphological descriptors included in the analysis to reduce the overall dimensions of the dataset to the major components and to observe the role of morphological characters in putting together the characters. The Eigen values ≥ 1 were used to define variations among the morphological characters. A hierarchical cluster analysis using Ward's method was performed to observe the grouping of the different morphological characters in SPSS vs 22, and the dendrogram was observed for associations among variables. Pearson correlation adjustment was also implemented for correlations among the quantitative variables. Coefficient of variation was computed for assessment of the trends and extent of morphological variations for quantitative characters by the formula;

$$CV\ (\%) = \frac{s}{\bar{x}} \times 100$$

Where;

CV=coefficient of variation;

$\bar{x}$=mean

s=standard deviation

RESULTS

Descriptive statistics and analysis of variance (ANOVA)

The analysis of variance (ANOVA) of the quantitative descriptors showed significant differences for petiole length (P ≤0.001); pod length (P ≤0.003); pod width (P ≤0.038); number of beans per pod (P ≤0.026); bean length (P ≤0.011); dried bean weight (P ≤0.001).

However, no significant differences were observed for leaf length, leaf width, leaf weight and bean width. The coefficient of variation (CV) showed variations of less than 50%. Low coefficient of variations was observed for number of beans per pod (CV=14.60%), followed by bean length (CV=17.46%), leaf length (CV=22.44%); leaf width (CV=23.00%); pod length (CV=25.60%); Moderate variations was observed for dried bean weight (33.94%); bean width (36.12%); Leaf weight (43.41%); pod width (46.46%) and petiole length (47.56%) (Table 2) (Figs. 2 and 3).

Table 2. Descriptive statistics of the cocoa morphological characters

Character	Mean ±SE	CV (%)	P value
Petiole length (cm)	2.42 ±0.12	47.56	***
Leaf length (cm)	31.79 ±0.75	22.44	22.44
Leaf width (cm)	11.03 ± 0.27	23.00	ns
Leaf weight (g)	3.78 ± 0.17	43.41	ns
Pod length (cm)	18.33 ± 0.50	25.60	**
Pod width (cm)	12.90 ± 0.63	46.46	*
No of beans per pod	37.93 ± 0.58	14.60	*
Bean length (cm)	2.01 ± 0.04	17.46	*
Bean length (cm)	2.01 ± 0.04	17.46	*
Bean length (cm)	2.01 ± 0.04	17.46	*
Bean width (cm)	1.21 (0.05)	36.12	ns
Dried bean weight (g)	2.16 (0.08)	33.94	***

Key: ns = non significant, *** = significant at P≤0.001



Fig. 2. Variation among cocoa leaves collected from Mashewa village in Muheza district in Tanga region



Fig. 3. Variation among cocoa pods collected from Mbingu village in Kilombero district in Morogoro region

Table 3. The first five principal components explaining 49.2% of the total variation

Character	PC1	PC2	PC3	PC4	PC5
Eigen value	3.66	2.03	1.97	1.59	1.57
Variation%	16.63	0.304	8.95	7.24	7.13
Leaf weight	-0.731	0.304	0.180	0.356	-0.018
Leaf width	-0.680	0.358	0.170	0.185	0.039
Leaf length	-0.660	0.408	0.205	0.249	0.066
Ridge colour of mature fruit	0.656	0.414	-0.024	0.199	0.068
Pod width	0.586	0.351	0.235	-0.157	0.068
Bean width	0.567	0.104	0.491	0.114	-0.014
Flush colour	0.450	0.148	-0.040	0.144	0.324
Leaf apex	-0.422	-0.062	-0.110	-0.068	-0.343
Pod basal constriction	-0.249	-0.593	-0.058	0.159	0.168
Ridge deposition	-0.081	0.552	-0.313	0.128	0.206
Pods ridge pair separation	0.366	0.435	-0.192	-0.083	-0.260
Dried bean weight	0.036	-0.160	0.616	0.188	0.179
Pod surface texture	0.244	0.084	0.529	0.003	0.045
Petiole length	-0.356	0.134	0.438	0.000	-0.240
Pod colour	-0.015	-0.055	0.065	0.679	0.335
Pod length	-0.178	0.160	0.374	-0.480	0.353
Bean shape	0.374	-0.100	0.021	0.386	-0.369
No of beans per pod	0.153	0.202	0.327	-0.004	-0.508
Pod apex form	-0.105	0.310	-0.406	-0.023	0.505
Bean length	-0.008	-0.150	0.359	-0.342	0.443
Pod shape	0.224	-0.439	0.126	0.171	0.118
Leaf base	-0.324	0.150	0.058	-0.442	-0.065

Principal component analysis (PCA)

The principal component analysis (PCA) of all 22 morphological descriptors revealed that the five principal components (PC1 to PC5) explained 49.2% of the total variation for the morphological descriptors. PC1, PC2, PC3, PC4 and PC5 explained 16.6%, 9.2%, 8.9%, 7.2 and 7.1 respectively. In the first principal component (PC1), the prominent variables were ridge colour of mature fruit, pod width, bean width, flush colour and pod ridge pair separation, while in the second component (PC2), the prominent variable were ridge deposition, pod ridge pair separation, ridge colour, leaf length, leaf width, pod apex form and leaf weight. The third principal component (PC3) explained more dried bean weight, pod surface texture, bean width, petiole length, pod length and bean length while the fourth component (PC4) explained more pod colour, bean shape and leaf weight and in the fifth principal component (PC5), pod apex, bean length, pod length, pod colour were prominent (Table 3).

Relationships among variables

Scatter plots of the first five principal component axes are shown in Fig. 4. According to the plot, there were relationships in most of the cocoa morphological

characteristics, except for some descriptors, such as leaf descriptors, showing high dispersion. The dendrogram from cluster analysis performed on morphological characters based on Euclidean distances shows three distinct clusters, i.e., cluster one (G1), cluster two (G2), and cluster three (G3). Cluster one (G1) consisted of morphological descriptors namely leaf weight, ridge colour of mature fruit, bean width, flush colour, leaf apex, pod basal constriction, ridge deposition, pods ridge pair separation, dried bean weight, pod surface texture, petiole length, pod colour, bean shape, pod apex form, bean length, pod shape and leaf base, making 77.3% of all clusters' characters, while cluster two (G2) consisted of morphological descriptors namely pod length, leaf width and pod width making 13.6% of all the clusters' characters and cluster three (G3) consisted of leaf length and number of beans per pod with only 9.1% of all clusters' characters. Clusters one and two were closely interrelated compared to cluster three, and this shows low variation in morphological descriptors among the cocoa growing regions in Tanzania (Fig. 5).

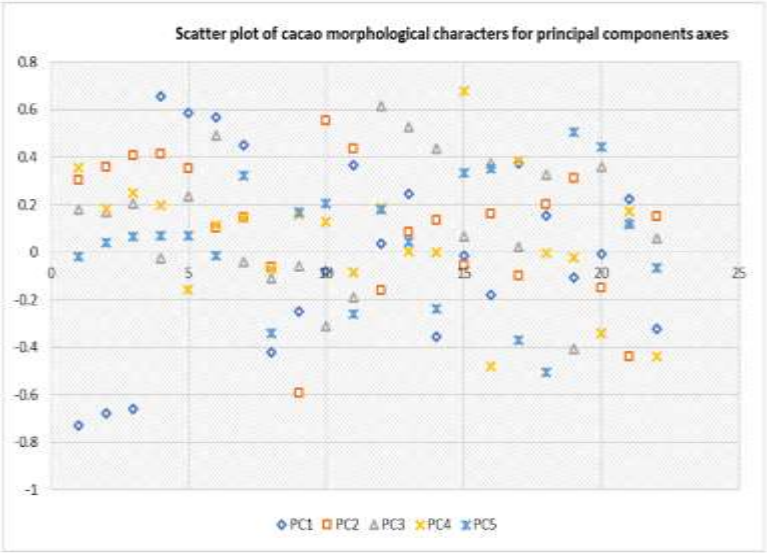


Fig. 4. Scatter plot of cocoa morphological characters on principal component axes 1, 2, 3, 4 and 5 (PC1, PC2, PC3, PC4 and PC5) accounting for 49.2% of total variation

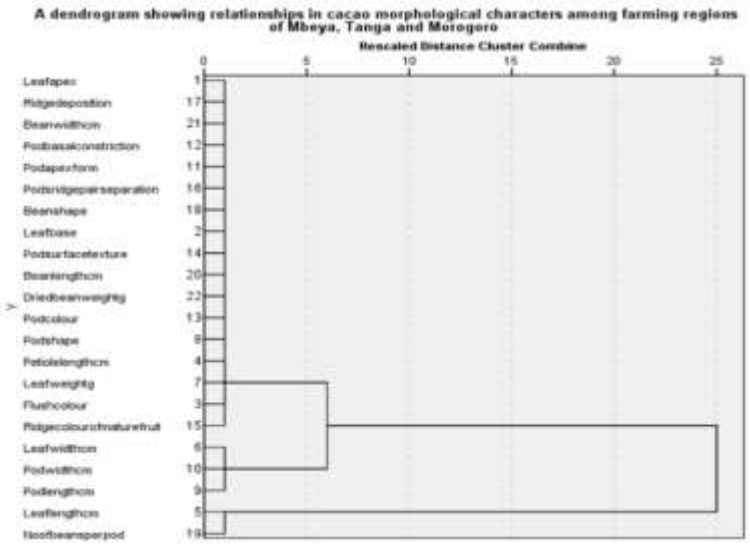


Fig. 5. Cluster analysis showing relationships in cocoa morphological characters among farming regions of Mbeya, Tanga and Morogoro

Table 4. Relationships among cocoa morphological descriptors in Tanzania

Parameters	Petiole length	Leaf length	Leaf width	Leaf weight	Pod length	Pod width	No of beans per pod	Bean length	Bean width	Dried bean weight
Petiole length										
Leaf length	0.261*									
Leaf width	0.257*	0.604**								
Leaf weight	0.252*	0.706**	0.687**							
Pod length	ns	0.208*	ns	ns						
Pod width	ns	ns	ns	-0.313**	ns					
No of beans per pod	ns	ns	ns	ns	ns	ns				
Bean length	ns	ns	ns	ns	0.243*	ns	ns		ns	
Bean width	ns	ns	ns	ns	ns	0.407**	0.215*	ns		
Dried bean weight	ns	ns	ns	ns	ns	ns	ns	0.211*	0.212*	

Correlation analysis

Pearson's correlation was performed to examine the relationship between variables for the quantitative descriptors. It was found that most of the variables showed a significant correlation with each other. The quantitative morphological descriptor, leaf length, showed a significantly high positive correlation with leaf weight (+0.706), followed by leaf width and leaf weight (+0.687), leaf width and leaf length (+0.604). Also, significant positive correlation was shown by pod width with bean width (+0.407), leaf weight with pod width (+0.313), petiole length with leaf length (+0.261), leaf width with petiole length (+0.257), leaf weight with bean width (+0.247), bean length with pod length (+0.243), number of beans per pod with bean width (+0.215), bean width with dried bean weight (+0.211) and leaf length with pod length (+0.208) (Table 4).

DISCUSSION

Morphological variation in cocoa pods, seeds and leaves was assessed to establish the variations among different descriptors. The cocoa quantitative and qualitative descriptors were used in this study to assess the variations and relationships between variables. Significant differences observed in the analysis of variance indicate variations in cocoa among the farming areas in Tanzania. Petiole length, dried bean weight, pod length, pod width, number of beans per pod and bean length accounted for a high percentage of variation in cocoa. According to Bekele *et al.* (2019), these characters explained the variations of more than 50%. Significant variations exhibited by these descriptors indicate diversity, probably due to geographical locations among the growing areas in Tanzania.

Most of the leaf quantitative descriptors, including length, width, leaf length and leaf weight, showed no significant differences. Also, bean width showed no significant differences. These findings agree with Almeida and Valle (2007), who reported that leaf factors were not important discriminative factors. The distribution of cocoa might be responsible for morphological variations in cacao farming. The selection that producers have been carrying out and farming management through cacao cultivation allowed for variations in cocoa trees (Avendaño-Arrazate *et al.*, 2024). Pod descriptors have been a key variable for variation in this study. This is confirmed by Engels (1983) and Lachenaud *et al.* (1999), who stated that pod descriptors were potential and most useful in assessing variability in cocoa. Furthermore, it has been stated that several morphological descriptors, including pod descriptors, have been prone to environmental factors, and thus some variations among groups might be associated with several ecological factors due to different geographical locations (Ballesteros *et al.*, 2015; Efombagn *et al.*, 2009).

According to the principal component analysis (PCA), the first five components (PC1 to PC5) explained 49.2% of the total variability. The ridge colour of mature fruit, pod width, bean width, flush colour, pod ridge pair separation; ridge deposition, ridge colour, leaf length, leaf width, pod apex form, leaf weight; dried bean weight, pod surface texture, petiole length, bean length; pod colour, bean shape, pod length was prominent explaining the variation among variables. The findings are confirmed by other findings of Aikpokpodion (2010), Ballesteros *et al.* (2015) and Bekele *et al.* (2008).

The coefficients of variation (CV) for quantitative descriptors were less than 50% which ranging from 14.60% to 47.56%. Low to moderate coefficient of variations were observed for all quantitative descriptors. The trend from the lowest was the number of beans per pod, bean length, leaf length, leaf width, pod length, dried bean weight, bean width, leaf weight, pod width and petiole length. This is supported by the findings from this study.

The hierarchical classification by Ward's method showed that there was no greater variation depicted by cluster analysis. Most of the descriptors were clustered in cluster 1 (G1), which comprised 77.3% of all morphological descriptors, showing the interrelationships among variables, with cluster 2 (G2) comprising 13.6% of all descriptors and cluster 3 (G3) comprising 9.1% of all descriptors. Cluster 1 had about 17 descriptors, G2 had only 3 descriptors, and G3 had 2 descriptors. The three groups show close associations with low variability among them. It has been reported that when a greater number of distinct clusters are formed, there is greater variability among the descriptors (López-Hernández *et al.*, 2019). The G2 and G3 in the cluster analysis consist mostly of quantitative variables (leaf width, pod width, pod length, leaf length and number of beans per pod). The observable morphological variations might be influenced by environmental and nutritional factors (Botero and Arias, 2018; Perez Garcia and Freile, 2017).

Some factors showed significant positive correlations. The descriptors included leaf length with leaf weight, leaf width and leaf weight, pod width with bean width, leaf weight with pod width, petiole length with leaf length, leaf width with petiole length, leaf weight with bean width, bean length with pod length, number of beans per pod with bean width, bean width with dried bean weight and leaf length with pod length. This is contrary to the findings by Aikpokpodion (2010), who found a significant positive correlation between pod length and pod weight and also between bean weight and pod weight. The large variation observed in this study for cacao grown by farmers indicates a high level of homogeneity in materials maintained on-farm. It is therefore important for the utilization of all recommended morphological descriptors due to the dispersion of some of the descriptors used (Aikpokpodion, 2010).

The scatter plot on principal component axes 1, 2, 3, 4 and 5 (PC1, PC2, PC3, PC4 and PC5) which accounted for 49.2% of total variation showed the clustering of most of the morphological descriptors, the dispersion is not random with some quantitative descriptors with high values of coefficient variations such as leaf length, leaf width, leaf weight, pod colour, pod width, ridge colour of mature pod showing high dispersion form other descriptors. Some morphological characters such as leaf length, pod width, leaf weight and petiole length have been reported to exhibit the highest dispersion from other (Adenuga *et al.*, 2015; Maharaj *et al.*, 2011; Santos *et al.*, 2012).

CONCLUSION

This study provides evidence for morphological variation in cocoa grown in major growing regions in Tanzania. According to the findings from the study, there was a close association of the cocoa trees grown in Mbeya, Morogoro and Tanga. Principal component analysis (PCA), correlation analysis, scatter plot and Cluster analysis have shown close relationships among morphological descriptors. The cluster analysis unveiled the grouping together of a large number of the descriptors. This suggests that probably cocoa grown in these areas originated from the different sources. The variation exhibited by some descriptors might be due to varietal factors and long-term on-farm practices. Plant breeders need to have a collection of genetic resources (germplasm) to support breeding activities.

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