Morphological variability and taxonomic affinity of cocoa (*Theobroma cacao* L.) clones from Central Sulawesi, Indonesia

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ABSTRACT

Cocoa (*Theobroma cacao* L.) is one of the most important export commodities of Indonesia, with Sulawesi becomes the largest cocoa production centers in Indonesia. Since the majority cocoa production comes from Sulawesi Island, there is high diversity of cocoa clones cultivated in this area. The objective of this study was to explore morphological variability and to assess taxonomic affinity of cocoa clones cultivated by smallholder farmers in four districts of Central Sulawesi, Indonesia. Nineteen cocoa clones from the districts of Sigibiromaru, Palolo, South Parigi and Sausu were used in this study. Fourteen morphological characters of leaves, fruits, and beans were examined. Cluster analysis were done to determine taxonomic affinity between the clones. Results of this study indicated that there was high morphological variability, mainly in fruit or pod characters. Cluster analysis on 14 morphological characters resulted in the grouping of the clones into two main clusters. The grouping of cocoa clones did not correspond to the geographical origin of samples. It is concluded that taxonomic affinity was determined by similarities of morphology, especially fruit and bean characters.

KEYWORDS: Agro-morphology, cocoa, phenetics, plant taxonomy.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is native to the tropics and has been widely cultivated in South and Central America, Africa, Southeast Asia and some other tropical countries [1]. Between 2007 and 2011, Indonesia was ranked as the second top producers of cocoa beans in the world, after Côte d'Ivoire [2]. With the area of cultivation over 1.5 million hectares, cocoa bean is one of the important agricultural export products of Indonesia, and majority of its production was from smallholder farmers [3]. One of the largest cocoa production centers in Indonesia is Sulawesi Island, particularly in South Sulawesi, Central Sulawesi and Southeast Sulawesi.

Three horticultural races of cultivated cocoa was recognized, namely the Criolo, Forastero, and Trinitario [4]. From historical classification record, *T. cacao* is divided into two sub-types based on morphological characters and geographical origin, the *T. cacao* ssp. *cacao* which is known as Criollo, and *T. cacao* ssp. *sphaerocarpum* known as Forastero. The third race, Trinitario, is a hybrid group with intermediate agronomical traits between Criolo and Forastero [5]. The cocoa clones cultivated in Indonesia belong to Forastero and Trinitario.

Cocoa plantations in Indonesia are dominated by traditional farming system owned by smallholder farmers. In managing their plantations farmers select and develop local clones by themselves, and thus variations between clones are mainly found in their physiological adaptations to local environment. In such a case, accurate identification and characterization of local clones become a crucial issue. Morphological and genetic characterization is thus essential to detect clone duplicates for efficient farming management. Moreover, identification and characterization of cocoa clones are indispensable for conservation of germplasms and development of new varieties. Accordingly, this study was addressed to explore morphological variability and to assess taxonomic affinity of cocoa clones cultivated at traditional plantations in four districts of Central Sulawesi, Indonesia.

MATERIALS AND METHODS

Plant materials used in this study were individual plants from cocoa plantations in Sigibiromaru, Palolo, South Parigi and Sausu Districts of Central Sulawesi, Indonesia. Three mature individual plants from different clones were selected as samples from each plantation. Observation on morphological characters was done on leaves, fruits, and beans, based on IBPGR standard

cocoa descriptors as used in previous studies [6, 7]. Ten samples of each organ were observed and measured for qualitative and quantitative characters. Assessment of taxonomic affinity was done using numerical taxonomic methods. The level of similarity between samples was calculated using percent similarity. The resulting similarity matrix was subjected to cluster analysis using unweighted pair group method using arithmetic averages (UPGMA). Principal component analysis was performed to identify the role of morphological characters in the grouping of cocoa clones. Cluster analysis and principal component analysis were done using Multivariate Statistical Program version 3.1 [8].

RESULTS AND DISCUSSION

Field observations on cocoa plantations from District Sigibiromaru, Palolo, South Parigi and Sausu showed there were 19 clones of cocoa cultivated in the area (Table 1). Samples of leaves, pods (fruits), and beans (seeds) of 19 cocoa clones under study showed morphological variability (Fig. 1).

Detail observation of these organs resulted in 14 morphological characters for cluster analysis (Table 2). Most of them were

Tabel 1. Cocoa clones used in this study

No.	Clone	Sample origin (Disrict)
1.	ICCRI 1	Sigibiromaru
2.	ICCRI 2	Sigibiromaru
3.	ICCRI 3	Sigibiromaru
4.	Sidondo	Sigibiromaru
5.	Panter	Sigibiromaru
6.	Irian	Sigibiromaru
7.	Hibrida	Sigibiromaru
8.	M 01	Sigibiromaru
9.	Sulawesi 1	Palolo
10.	Sulawesi 2	Palolo
11.	ICS 60	Palolo
12.	Palolo	Palolo
13.	Bobo	Palolo
14.	M 045	South Parigi
15.	M 05	Sausu
16.	45	Sausu
17.	Merah	Sausu
18.	Hijau	Sausu
19.	06	Sausu

Table 2: Morphological variability of leaves, pods (fruit), and beans on 19 cocoa clones

No.	Character	Morphological variability
1.	Leaf apex	acuminate, apiculate
2.	Leaf base	cuneate, obtuse, truncate
3.	Pod shape	obovate, elliptic, oblong
4.	Pod length	medium, long
5.	Pod width	medium, wide
6.	Pod apex form	acute, rounded, obtuse
7.	Pod basal constriction	absent, slight, intermediate
8.	Pod color	yellow, orange, red
9.	Pod surface texture	smooth, intermediate, rough
10.	Pod's ridge prominence	slight, intermediate, pronounced
11.	Pod's primary ridge separation	slight, intermediate
12.	Bean shape	ovate, elliptic, oblong
13.	Cotyledon color	light purple, deep purple
14.	Bean number per pod	few, numerous

qualitative characters. The use of qualitative characters in taxonomy is quite common, since these characters tend to be stable and are not easily affected by environmental changes [9]. Flower characters did not show any variations between clones, and thus was not included in the analysis of taxonomic affinity. The morphology of the cocoa flower is shown in Fig. 2. The flowers grow on stem or major branches (cauliflower), solitary or in clusters, with white sepals, yellowish white petals, purple stamens and staminodes (sterile stamens). A study on the characterization of cocoa morphology [10] noted that cacao flowers only showed a slight difference in petals color, either pink or white. The flower morphological characters observed in this study were uniform among samples, and therefore they were not used in the analysis of taxonomic relationship. This is in line with several morphological diversity studies of cocoa reported by authors from different region, in which flower characters were not included in the characterization or evaluation of cocoa plants [11,12,13].

The observed morphological variations in leaves were leaf apex, which varied from acumintae to apiculate, and leaf base, namely cuneate, obtuse, and truncate. Variations in leaf characters had been reported on six species of cocoa in Brazil [14], in which three quantitative characters showed the highest variation, i.e. petiole length, leaf area, and leaf weight. Meanwhile, pods (fruits) has the highest variability, with 9 out of 14 the observed characters were varied. These results were consistent with the study on morphological diversity on cocoa in Cameroon [15], in which differences between cocoa clones originating from southern and northern Cameroon were in quantitative characters on fruits. Similarly, [16] reported that fruit qualitative traits and bean qualitative traits were important and accounted for the agro-morphological variability observed in 184 accessions of cocoa from the farmer's fields and filed genebank collections in Nigeria.

The role of fruit and seed characters in taxonomy has been confirmed in many different species. In *Geranium*, seed morphology was proven to be significant for infrageneric, specific or infra-specific delimitations [17]. A study on five varieties of *Capsicum annuum* found that both qualitative and quantitative characters of fruits are of systematic value because they are consistently different in the taxa studied [18]. Fruit and seed characters also play an important role in taxonomy of the genus *Galium* [19].

Results of this study indicated that there was high morphological variability among traditional cocoa clones cultivated in Central Sulawesi. Documentation of morphological variability is very important as a baseline data for the inventory of intraspecific diversity of clonal plants with high potential to be developed into superior hybrids. The importance of documenting such information is necessary to determine the level of variation in field-grown cocoa in order to provide information for future breeding strategies, on-farm selection, and conservation efforts [18].

Result of cluster analysis on 19 cocoa clones based on 14 morphological characters was presented as phenogram in Fig. 3. Two major groups were formed, with the first and second clusters

Figure 1: Morphology of leaves (a), beans (b), and pods (c) representing variability among 19 cocoa clones under study

Figure 2: Morphology of cocoa flowers

were consisted of 9 and 10 clones, respectively. The first cluster was composed of cocoa clones characterized by smooth surface texture and pronounced ridge prominence. The second cluster was consisted of clones with characteristics of rough surface texture and slight or intermediate ridge prominence.

The grouping of clones into two clusters was clearly indicated the similarity of morphological characters, especially in their fruits (pods). In addition, the grouping pattern was not corresponding to the origin of samples. This result obviously showed that morphological similarities and differences between clones are inherent characteristics, and was not influenced by environmental factors.

The taxonomic affinity between clones and the grouping patterns generated from phenetic approach using cluster

analysis in this study showed the important role of pods in recognizing differences between clones. The role of pod characters in differentiating cocoa clones or varieties has been reported from previous studies. A study on the phenotypic and agro-economic traits among Trinidad selected hybrid varieties of cocoa revealed that wet bean weight, pod index, pod width, number of beans and cotyledon weight contributed significantly to the separation of the varieties [20]. The role of pod and bean characters in the discrimination and classification of cocoa genotypes has been mentioned before [21]. Moreover, a study on morphological characterization on elite cocoa trees found that leaf, pod, and bean traits were useful in differentiating cocoa genotypes [22].

Based on the result of principal component analysis as shown in Table 3, fruit morphological characters were noticeably has a considerable role in discerning differences and grouping of 19 cocoa clones, as indicated by high loading on axis 1, that are >0.3. Result of principal component analysis is therefore support the clustering pattern observed in the phenogram from cluster analysis. Three fruit characters that have major role in the grouping of samples were pod rugosity, pod's ridge prominence, and pod apex form. Meanwhile, the difference between samples within one cluster on phenogram was also influenced by fruit characters, as indicated by their loading on axis 2, namely pod shape, pod color, and pod basal constriction.

The application of principal component analysis has been reported in morphological diversity studies for identifying and assessing the contribution of morphological characters to variability of *Oryza sativa* varieties [23]. Similarly, PCA was employed to identify morphological characters which have important contribution in the discrimination of *Coix lacryma-jobi* accessions for selection and utilization in breeding program [24].
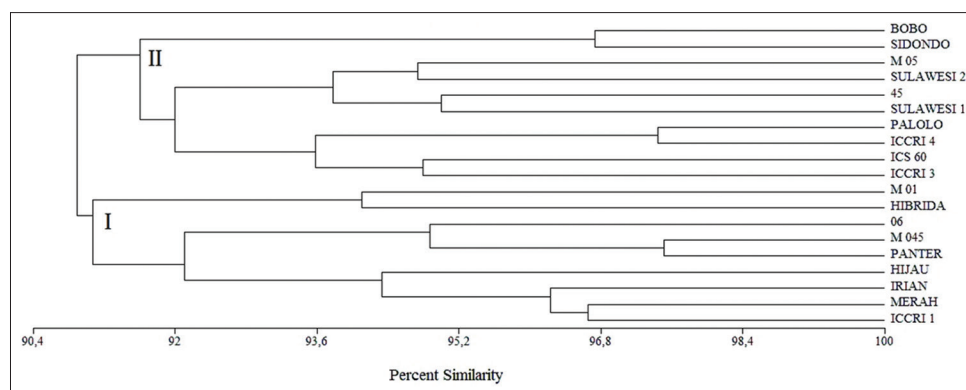
Figure 3: The UPGMA phenogram of 19 cocoa clones based on 14 morphological characters

Table 3: PCA variable loadings of 14 morphological characters

Characters	Axis 1	Axis 2
Leaf apex	-0,19	-0,35
Leaf base	0,25	0,04
Pod shape	0,07	-0,49
Pod length	-0,02	0,04
Pod width	-0,09	0,01
Pod apex form	0,31	0,19
Pod basal constriction	-0,13	0,30
Pod colour	0,20	0,44
Pod rugosity	0,63	0,05
Pod's ridge promonence	0,48	-0,46
Pod's primary ridge separation	-0,07	0,10
Bean shape	-0,13	-0,31
Cotyledon colour	-0,06	0,01
Bean number per pod	-0,29	-0,04
Eigenvalues	0,19	0,14
Percentage	24,84	18,57
Cumulative percentage	24,84	43,41

This study provides scientific evidence on the morphological variability of various cocoa clones cultivated in Central Sulawesi. The taxonomic affinity between cocoa clones resulted from this study is useful in predicting genetic diversity, and provides a basis for developing superior clones through plant breeding programs.

CONCLUSION

Cocoa clones cultivated in Central Sulawesi have high morphological variability, especially on fruit or pod characters. Taxonomic affinity between clones based on cluster analysis is not based on geographical origin of samples, which means the morphological variability found in 19 cocoa clones is an inherent characteristics and not influenced by environmental factors where they grow.

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