International Journal of Genetics
ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 11, Issue 11, 2019, pp.-667-670.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000226

Research Article

AGRO-MORPHOLOGICAL CHARACTERIZATION OF COCOA (*THEOBROMA CACAO* L.)

SHILPA K.S., MINIMOL J.S.*, SUMA B. AND CHITHIRA P.G.
Cocoa Research Centre, Kerala Agricultural University, Vellanikkara, Thrissur, 680656, Kerala, India
*Corresponding Author: Email - minimoljs@gmail.com

Received: November 05, 2019; Revised: November 25, 2019; Accepted: November 26, 2019; Published: November 30, 2019

Abstract: Cocoa (*Theobroma cacao* L.), being an introduced crop in the country and the material available has evolved from very limited population estimate of variability in a breeding population forms major step. Twenty cocoa hybrids were evaluated for the variability based on morphological characters. Eleven qualitative characters including colour of young leaf (flush), three floral characters (pedicel, sepal and petal colour), six pod characters (pod shape, unripe pod colour, ripe pod colour, pod apex, pod base and pod rugosity) and colour of bean were considered for the study. Hybrids showed wide variability in most of the characters considered. Diversity analysis was carried out using NTSYS pc version 2.1 software. Based on the similarity matrix, cluster analysis was done and a dendrogram was constructed by Unweighted Pair-Group Method (UPGMA). Thirteen clusters were obtained at 60 per cent similarity coefficient expressing the huge amount off variability present among the hybrids.

Keywords: *Theobroma cacao, Hybrids, Morphological characterisation, Diversity analysis*

Citation: Shilpa K.S., *et al*., (2019) Agro-morphological Characterization of Cocoa (*Theobroma cacao* L.). International Journal of Genetics, ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 11, Issue 11, pp.- 667-670.

Academic Editor / Reviewer: Vipul N Kapadia, Utpal Roy, I S Chakrapani

Introduction

Cocoa (*Theobroma cacao* L.), the source of fashionable delicacy chocolate is believed to be originated in the Amazon region of South America. Cadbury India Private Limited (now renamed as Mondelez International) introduced cocoa to India as a profitable mixed crop in coconut and arecanut plantations as part of their commercialization strategy. At present, 18920 tonnes of cocoa is produced in India in an area of 82940 hectare [1]. *Theobroma cacao* L. was initially classified under the family Sterculiaceae [2], however based on the research using molecular data it was reclassified under the family Malvaceae [3]. Cocoa calls for a long and dynamic breeding programme [4] especially due to its perennial nature. Two major activities included in plant breeding are identifying natural genetic variation from available germplasm or creating genetic variation and selection from the variable population based on our objective. Characterisation of breeding population is essential to select suitable genotypes for strong breeding programmes. Morphological traits which are highly heritable are helpful for characterising germplasm in plants [5, 6]. Malhotra and Apshara [7] stated that expression of crop diversity is estimated from different indicators of variability and among them morphological traits are particularly important for cataloguing and characterisation. The present study was conducted to characterise and analyse the diversity within a hybrid cocoa population based on major qualitative morphological traits.

Materials and Methods

Twenty cocoa hybrids which were in the steady bearing stage, selected from the Comparative Yield Trial (CYT), planted during 2008 at Cocoa Research Centre (CRC), Kerala Agricultural University served as the material for the study. Fifteen pods (five/ plant) were collected from three plants per replication and observations were recorded. Experiment was replicated in two trials. The observations were taken on eleven qualitative characters, *i.e.* colour of young leaf (flush), three floral characters (pedicel, sepal and petal colour), six pod characters (pod shape, unripe pod colour, ripe pod colour, pod apex, pod base and pod rugosity) and colour of bean. Outer mucilage covering of beans were removed using forceps to evaluate bean colour. The descriptor developed by Bekele and Butler [8] was used for recording observations. Diversity analysis was carried out using NTSYS pc version 2.1 software [9], the genetic associations among the genotypes were evaluated and estimated by Jaccard's similarity coefficient [10]. Based on the similarity matrix, cluster analysis was done and a dendrogram was constructed by Unweighted Pair-Group Method (UPGMA) [11].

Results and Discussion

For describing and classifying genotypes, Smith [12] regarded morphological characterisation as the first step. Many scientists considered morphological and agronomic characters of cocoa pod, bean and flowers in order to analyse diversity among genotypes [13-16]. In the present study observations were recorded on qualitative characters of flush leaf, flower, pod and bean of twenty hybrids and presented in [Table-1] and [Table-2]. Out of twenty hybrids, eight (40%) exhibited green flush colour. Greenish red (PIV 45.4, PIII 2.3, VSDI 10.13,VSDI 30.8 and PIV 26.8) and reddish green (VSDI 33.4, PIV 58.6, PIV 56.9, VSDI 11.11and PIV 31.9) coloured flush were shown by five hybrids each. Two hybrids (SIV 5.15 and VSDI 23.21) were having red coloured flush which represented ten percent of the total hybrids. Kochhar [17] studied the variation in flush colour of cocoa and revealed that it ranged from light green to red. Bartley [18] reported young leaf colour as a distinct trait in cocoa. In the case of flower three qualitative characters were considered (colour of pedicel, colour of sepal and colour of petal). Except colour of petal, other two showed variation among the hybrids. Green and reddish coloured pedicel was observed among the hybrids. Twelve hybrids (60%) were having green coloured pedicel, whereas eight (40%) had reddish pedicel. Veeresh [19] reported that green coloured pedicel is most common in Forastero cocoa. Hybrids considered in the study exhibited two types of sepal colour and they are cream and greenish cream. Hybrids PIV 45.4, SIV 1.10 and PIV 19.9 were having greenish cream sepal and the rest seventeen exhibited cream coloured sepals. Enriquez and Soria [20], Lachenaud *et al*. [14] and Efombagn *et al*. [21] in their studies revealed that flower characters allow detection of variability in different

Table-1 *Variability in leaf and flower characters among twenty cocoa hybrids*

Hybrids	Flush colour	Pedicel colour	Sepal colour	Petal colour
PIV 45.4	Greenish red	Green	Greenish cream	Cream
PIII 2.3	Greenish red	Green	Cream	Cream
PIV 59.8	Green	Green	Cream	Cream
SIV 10.11	Green	Green	Cream	Cream
VSDI 10.13	Greenish red	Green	Cream	Cream
SIV 1.10	Green	Reddish	Greenish cream	Cream
PIV 60.9	Green	Reddish	Cream	Cream
PII 12.11	Green	Green	Cream	Cream
SIV 5.15	Red	Reddish	Cream	Cream
VSDI 33.4	Reddish green	Reddish	Cream	Cream
VSDI 23.21	Red	Reddish	Cream	Cream
PIV 58.6	Reddish green	Reddish	Cream	Cream
PIV 56.9	Reddish green	Reddish	Cream	Cream
VSDI 30.8	Greenish red	Green	Cream	Cream
VSDI 11.11	Reddish green	Green	Cream	Cream
SIV 1.6	Green	Green	Cream	Cream
PIV 19.9	Green	Green	Greenish cream	Cream
PIV 26.8	Greenish red	Reddish	Cream	Cream
PIV 31.9	Reddish green	Green	Cream	Cream
VSDI 29.9	Green	Green	Cream	Cream

Table-2 *Variability in pod and bean qualitative characters of twenty cocoa hybrids*

Hybrids	Pod shape	Unripe pod colour	Ripe pod colour	Pod apex	Pod base	Pod rugosity	Bean colour
PIV 45.4	Cundeamor	Dark green	Greenish yellow	Acute	Slight	Intense	Medium purple
PIII 2.3	Amelonado	Dark green	Greenish yellow	Obtuse	Slight	Intermediate	Dark purple
PIV 59.8	Cundeamor	Intermediate	Yellow	Acute	Strong	Intense	Medium purple
SIV 10.11	Amelonado	Intermediate	Yellowish green	Mammelate	Slight	Slight	Light purple
VSDI 10.13	Cundeamor	Intermediate	Yellow	Acute	Intermediate	Intermediate	Light purple
SIV 1.10	Amelonado	Intermediate	Yellowish green	Obtuse	Slight	Slight	Medium purple
PIV 60.9	Cundeamor	Intermediate	Yellow	Acute	Intermediate	Slight	Medium purple
PII 12.11	Amelonado	Dark green	Greenish yellow	Obtuse	Slight	Intermediate	Medium purple
SIV 5.15	Angoleta	Intermediate	Yellow	Acute	Slight	Intermediate	Dark purple
VSDI 33.4	Amelonado	Dark green	Yellowish green	Obtuse	Slight	Slight	Dark purple
VSDI 23.21	Cundeamor	Dark green	Yellowish green	Acute	Intermediate	Intermediate	Medium purple
PIV 58.6	Cundeamor	Intermediate	Yellowish green	Obtuse	Strong	Slight	Dark purple
PIV 56.9	Amelonado	Purplish green	Yellow	Obtuse	Absent	Slight	Medium purple
VSDI 30.8	Amelonado	Light	Yellow	Obtuse	Slight	Intermediate	Dark purple
VSDI 11.11	Cundeamor	Dark green	Yellow	Attenuate	Intermediate	Intermediate	Dark purple
SIV 1.6	Angoleta	Dark green	Yellow	Attenuate	Strong	Intense	Mixed
PIV 19.9	Angoleta	Intermediate	Yellowish green	Obtuse	Slight	Slight	Dark purple
PIV 26.8	Amelonado	Intermediate	Yellowish green	Attenuate	Absent	Slight	Light purple
PIV 31.9	Cundeamor	Intermediate	Yellow	Attenuate	Intermediate	Slight	Medium purple
VSDI 29.9	Angoleta	Intermediate	Yellow	Obtuse	Slight	Intermediate	Dark purple

cocoa cultivars. The descriptor given by Bekele and Butler [8] explains about five different pod shapes in cocoa. However, the hybrids included in the present study exhibited only three types of variation in pod shapes such as cundeamor (40%), amelonado (40%) and angoleta (20%). Calabacillo and criollo shaped fruits were not observed. Cundeamor is characterised by intensively ridged and warty pods with bottle neck. Amelonado fruits are melon shaped. Angoleta are deeply ridged and warty with square shape at stalk end. Pod apex form was obtuse in majority (45%) of the hybrids. Acute (30%) and attenuate (20%) types of apex were also observed. Five hybrids with Cundeamor pod shape were having acute pod apex which reveals that pod shape and apex form has certain relation. Minimol *et al*. [22] and Sujith *et al*. [23] also reported same type of results. Pod basal constriction was also observed in all the twenty hybrids and they were grouped into four classes such as absent, slight, intermediate and strong. No pods were observed with wide shoulder. Majority of hybrids had slight basal constriction (50%). Five hybrids (25%) exhibited intermediate and three (15%) were having strong basal constriction. Basal constriction was found to be absent in hybrids PIV 56.9 and PIV 26.8. Rubeena [24] reported that 45 percent of hybrids observed by her were having acute pod apex while 75 percent were with slight basal constriction. According to Thi *et al*. [25] in cocoa, pod colour and shape are the most significant characters which distinguish genotypes. Cocoa hybrids used in the present study found to be variable in unripe pod colour; dark green (35%), intermediate green (55%), light green (5%) and purplish green (5%) colours were observed. All the four types of unripe pod colours given in the descriptor of Bekele and Butler [8] were observed among the hybrids. The hybrid VSDI 30.8 showed light green coloured unripe pod and the hybrid PIV 56.9 exhibited purplish green coloured pod. Similar to colour of unripe pod, ripe pod colour also showed variability. It was determined by analysing the colour of ridges and furrows. Three variants of colour were observed in case of ripe pod which are yellow (50%), yellowish green (35%) and greenish yellow (15%). Pod rugosity is the measure of roughness of the pod surface and it can be absent, slight, intermediate or intense as per the descriptor of Bekele and Butler [8]. Hybrids devoid of pod rugosity were not observed among the material used for the present study. Nine hybrids were having pods with slight rugosity. Intermediate rugosity was observed in eight hybrids and the rest three showed intense rugosity. According to Kochhar [26] and Malhotra and Apshara [7] smooth and slight rugous pod surface is the feature of Forastero genotypes. Cocoa genotypes showed difference in colour of peeled bean or cotyledon. Bekele and Butler [8] categorized colour of cotyledons as white, grey, light purple, medium purple, dark purple, mottled and mixed. Light purple (15%), medium purple (40%), dark purple (40%) and mixed (5%) beans were observed among the hybrids used for the present study. This result is on par with the findings of Veeresh [19]. Diversity analysis will reveal the true amount of variability present in a population [27]. Hence based on Jaccard's similarity coefficient, Agglomerative hierarchical clustering was done using UPGMA method [11]. Ten qualitative characters of cocoa including leaf, flower, pod and bean traits were considered and dendrogram was constructed and presented in [Fig-1]. Based on the ten qualitative characters the twenty hybrids were grouped into thirteen clusters at 60 percent similarity coefficient. The clusters along with the hybrids are presented in [Table-3].

Table-3 *Clustering based on qualitative characters of cocoa hybrids*

Cluster No.	No. of hybrids	Hybrids
Cluster I	1	PIV 45.4
Cluster II	3	PIII 2.3, PII 12.11, VSDI 30.8
Cluster III	2	SIV 5.15, VSDI 29.9
Cluster IV	1	PIV 59.8
Cluster V	1	SIV 1.6
Cluster VI	4	VSDI 10.13, VSDI 11.11, PIV 60.9, PIV 31.9
Cluster VII	1	VSDI 23.21
Cluster VIII	1	SIV 10.11
Cluster IX	1	SIV 1.10
Cluster X	1	PIV 19.9
Cluster XI	1	PIV 26.8
Cluster XII	2	VSDI 33.4, PIV 58.6
Cluster XIII	1	PIV 56.9

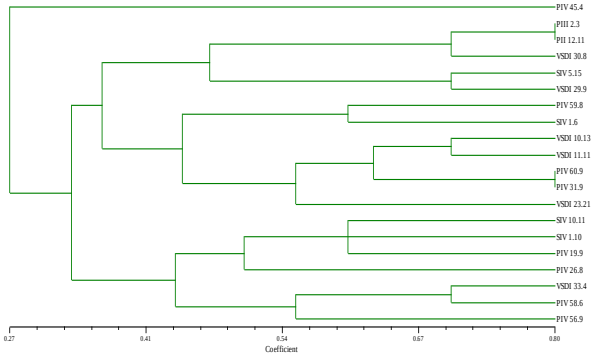

Fig-1 Dendrogram based on similarity coefficient among twenty cocoa hybrids

Cluster VI is the largest one with four hybrids; VSDI 10.13, VSDI 11.11, PIV 60.9 and PIV 31.9. All the four hybrids included in cluster VI are having five similar characters like colour of pedicel (green), colour of sepal (cream), pod shape (cundeamor), ripe pod colour (yellow) and pod apex shape (obtuse). In cluster II three hybrids were present and in cluster III and XII two hybrids were grouped. Hybrids PIII 2.3, PII 12.11 and VSDI 30.8 are included in cluster two and they show similar colour of pedicel (green), colour of sepal (cream), pod shape (amelonado), shape of pod apex (obtuse) and pod basal constriction (slight). Cluster III included SIV 5.15 and VSDI 29.9 having angoleta shaped pods with intermediate unripe pod colour, yellow ripe pod colour, slight basal constriction and intermediate rugosity. Beans of these hybrids were dark purple in colour. Nine hybrids showed no similarity with other hybrids for these ten characters and they includes genotypes PIV 45.4, PIV 59.8, SIV 1.6, VSDI 23.21, SIV 10.11,SIV 1.10, PIV 19.9, PIV 26.8 and PIV 56.9 and they were placed in separate clusters i.e. I, IV, V, VII, VIII, IX, X, XI and XIII respectively. Wide variability was noticed among the hybrids as indicated by more number of clusters. Genotypes with suitable characters can be selected and utilized for further breeding programme.

Application of research: Genotypes present in different clusters shows more diversity and selecting genotypes from diverse clusters as parents in further breeding programme results in genetic gain.

Research Category: Diversity analysis

Acknowledgement / Funding: Authors are thankful to Mondelez International (earlier Cadbury India Ltd.) for their continuous association and financial support for 30 years. Authors are also thankful to Cocoa Research Centre, Kerala Agricultural University, Vellanikkara, Thrissur, 680656, Kerala, India

***Research Guide or Chairperson of research: Dr Minimol J S**
University: Kerala Agricultural University, Vellanikkara, Thrissur, 680656, Kerala
Research project name or number: Genetic analysis of cocoa (*Theobroma cacao* L.) hybrids and screening superior hybrids for major biotic stress

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Cocoa Research Centre, Thrissur, 680656

Cultivar / Variety name: Cocoa (*Theobroma cacao* L.)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References:

[1] Anonymous (2018) *Directorate of Cashewnut and Cocoa Development.*
[2] Purseglove J.W. (1974) *Tropical crops: monocotyledons and dicotyledons. Longman Publications*.
[3] Alverson W.S., Whitlock B.A., Nyffeler R., Bayer C., Baum D.A. (1999) Phylogeny of the core Malvales: evidence from *ndhF sequence data. American Journal of Botany*, 86(10), 1474-1486.
[4] Malhotra S.K. and Hubbali V. N. (2016) *Cashew and Cocoa: Production to Marketing, Directorate of Cashewnut and Cocoa Development, Kochi*.
[5] Klug W. S. and Cummings M.R. (1994) *Concepts of genetics. Macmillan College Publishing Company*.
[6] Sounigo O., Bekele F., Christopher G., Bidaisee Y., Umaharan R. (1997) *Cocoa Research Unit Annual Report. The University of the West Indies, St. Augustine, Trinidad*.
[7] Malhotra S.K. and Apshara E.S. (2017) *Indian Journal of Genetics and Plant Breeding*, 77(2), 199-213.
[8] Bekele F. and Butler D.R. (2000) *Working procedures for cocoa germplasm evaluation and selection*. IPGRI, Montpellier, France.
[9] Rohlf F.J. (1992) NTSYSpc. *Numerical taxonomy and multivariate analysis system Version 2.0. Department of Ecology and Evolution, State University of New York*.
[10] Jaccard P. (1908) *Bulletin de la Société vaudoise des Sciences Naturelles* 44, 223-270.
[11] Sneath P. H. and Sokal R. R. (1973) *Numerical taxonomy. Freeman*.
[12] Smith J. S. C. (1989) *Maydica*, 34, 151-161.
[13] Bekele F. and Bekele I. (1996) *Crop Science*, 36(1), 57-64.
[14] Lachenaud P., Bonnot F., Oliver G. (1999) *Genetic Resources and Crop Evolution*, 46(5), 491-500.
[15] Iwaro A.D., Bekele F.L., Butler D.R. (2003) *Euphytica*, 130(2), 207-221.
[16] Bekele F.L., Bekele I., Butler D.R., Bidaisee G.G. (2006) *Genetic Resources and Crop Evolution*, 53(5), 933-948.
[17] Kochhar S.L. (1998) *Economic botany in the tropics*. MacMillan India Ltd.
[18] Bartley B.G.D. (2005) *The genetic diversity of cacao and its utilisation. CABI Publishing*.
[19] Veeresh S.A. (2017) *M.Sc. Thesis, Kerala Agricultural University*.
[20] Enriquez G.A. and Soria J.V. (1967) *Tropical Agriculture*, 44(2), 117-123.
[21] Efombagn M.I.B., Sounigo O., Nyassé S., Manzanares-Dauleux M., Eskes A. B. (2009) *Journal of Plant Breeding and Crop Science*, 1(6), 258-264.
[22] Minimol J.S., Amma S.P., Krishnan S., Vasudevan N.R. (2011) *Seminar on strategies for enhancing productivity of cocoa. CPCRI*.
[23] Sujith S.S., Minimol J.S., Ajmal P.M., Suma B. (2017) *Journal of Tropical Agriculture*, 55(1), 81-86.
[24] Rubeena M. (2015) *M.Sc. Thesis, Kerala Agricultural University*.

[25] Thi L., Ha V., Nusantor B., Philip R.A., Anh H.P., Toan T.H., Messens K., Dewettinck K. (2016) *Can Tho University Journal of Science*, 4, 81-86.
[26] Kochhar S.L. (1986) *Tropical Crops: A Textbook of Economic Botany. 2nd ed., MacMillan Publishers Ltd.*
[27] Sun W., Yuan X., Liu Z., Lan S., Tsai W., Zou S. (2019) *Plos one*, 14(7), e0219046.