__Original Research Article__

Exploring Genetic Variability and Diversity of Cocoa in Exotic Germplasm Collection

ABSTRACT

The current research investigates the genetic variation and diversity in exotic accessions of cocoa to identify materials with desired characteristics as well as important traits contributing to yield and variability. The experiment was conducted at Cocoa Research Centre, Kerala Agricultural University, from 2021 to 2023. The study comprises 23 exotic genotypes in which observations were recorded on quantitative traits on flowers, pods, and beans. Statistical analysis was carried out based on the observations recorded. The morphological evaluation revealed ample variability among the genotypes for all the quantitative characters studied. The average dry bean weight recorded was 1.08 grams, which is a desirable trait in selecting individuals with high processing efficiency for the chocolate industry. Characters such as single bean dry weight, pod value, pod index, and efficiency index have recorded high values of GCV, heritability and genetic advance as percent of the mean. Principal component (PC) analysis showed that the first three PCs with more than one Eigen-value contributed to 92.18 percent of total variability among the germplasm. Yield components viz. pod value, total bean weight pod^{-1} and single bean dry weight significantly contribute to maximum variability among the exotic germplasm. Moreover, genotypes were grouped into four clusters based on their configuration along the first two principal components. Hybridization ~~programmes~~programs involving superior genotypes identified in different clusters as parents can produce high-yielding hybrids. The high values recorded for the genetic parameters by yield components suggest the selection of genotypes based on which can result in better-performing progeny.

Keywords: Theobroma cacao L., Quantitative traits, Correlation, Principal component analysis, Pod value

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a popular beverage crop enjoyed for its bitter beans that serve as the primary raw material for all choco-related confectioneries. It is the most important tree crop in the Malvaceae family and the only source of chocolate. Cocoa originated in the Amazon River basin, which was later referred to as the primary centre of diversity [1]. Over time, the crop spread from Central America to other regions via invaders and traders. Presently, cocoa is grown in Africa, Latin America, south-eastern Asia, and Europe. However, most of the cultivation is confined to the equator due to the specific climatic requirements of the crop. The tropical climate, along with high rainfall and short dry spells in these-area, contribute positively to crop growth. In recent years, the crop has gained the status of a major crop in all growing domains because of its steady demand and reasonable price.

In India, cocoa has been mainly cultivated in the southern states since its introduction in late 1960. Currently, it covers an area of 1,09,300 hectares, with a production and productivity of 29,800 MT and 0.30 MT/ha, respectively [2]. Cocoa has become part of a multi-layered cropping system in major regions, often grown alongside crops like coconut, arecanut, oil palm, and rubber, providing additional income for farmers.

Given its economic and nutritional significance, the selection and utilization of superior genotypes are imperative for optimizing the field performance. Cocoa breeding programmes focus on developing high-yielding varieties which require a diverse germpalasm. However, the lack of detailed agronomic information on germplasm diversity hampers their efficient use. Despite progress in molecular techniques, morphological characterization remains essential for exploiting the genetic potential, as it indirectly assesses the agronomic properties of the crop [3]. Genetic variability analysis helps to identify plant material with desired traits and select the best accessions for improvement programmes. Cacao genetic variability can be understood and utilized through phenotypic characterization using flower, fruit, and seed descriptors, the understanding of which helps in crop improvement.

Multivariate statistical methods are employed to evaluate genetic diversity and family relationships among different genotypes [4]. Among them, principal component analysis (PCA) helps organize various variables into key unrelated factors [5]. It is also helpful in grouping germplasm and identifying major contributors to genetic diversity [6]. This approach facilitates a better understanding of genetic potential for selection and breeding. In this context, this study aims to evaluate the genetic background of exotic genotypes based on quantitative traits, with the goal of identifying superior genetic stocks for future breeding activities.

2. MATERIALS AND METHODS

The study was conducted at the Cocoa Research Centre, Kerala Agricultural University. Twenty-three exotic genotypes (table 1) imported from the University of Reading, UK, and field planted during 2009 were used for the study. The experiment was laid out in a completely randomized design with five replications. Pods and fresh flowers were collected from steady-bearing cacao trees, pointing to a less heterogeneous environment for the age of the tree, and observations were taken.

Table 1. List of genotypes

Sl. No.	Genotype
G1	POUND 7/B
G2	CRU 111
G3	ICS 5
G4	GV 5.1
G5	GV 145 UK
G6	ICS 40
G7	TRD 44
G8	SC 20
G9	COCA 3308/A (CHA)
G10	GEBP 914/AF (ADR)
G11	GEBP 617/AF (ADR)
G12	GEBP 180/AM (ADI)
G13	BE 3
G14	CC 11
G15	EQX 69
G16	GU 114/P
G17	PBC 123
G18	MAN 15-2

G19	MAN 15-60
G20	GU 2691/V
G21	PA 56
G22	SC 1
G23	UF 221

Fresh flowers were collected during morning hours, and observations were made using the stereo microscope model Leica EZ4. For pod characterization, ten ripened pods (two per replication) were collected and their average values were used for statistical analysis. The average value of twenty peeled and dried beans to moisture content below eight per cent was taken as the single bean dry weight. The economic parameters such as pod value, pod index, and efficiency index were calculated as per the standard formula. Pod value is the total weight of dried beans obtained pod^{-1} [7]. The pod index estimates the number of pods required to get one kilogram of dry beans [8], whereas the efficiency index indicates the pod weight required to get one gram of dry beans [9]. Ranking of genotypes was carried out by scoring based on eight yield attributing characters. Computation of genetic parameters, correlation analysis and PCA were carried out using the software GRAPES [10].

3. RESULTS AND DISCUSSION

Information about variability and genetic relatedness among elite breeding materials is a major component of plant breeding programmes [11]. In the study, the genetic analysis of the exotic cocoa germplasm based on quantitative characters revealed the presence of ample variability among the genotypes. The genotypes exhibited the highest significant variation for all the traits assessed in the study (table 2). The distribution of single dry bean weight showed that more than 50 percent of the total population had weight above the minimum prescribed value of 1.00 grams [12] and, with the highest of 1.86g. Number of beans pod^{-1} is an important factor in tracing variability as well as a determinant of crop yield [13]. Among the genotypes, the trait ranged from 26.10 to 48.80, and the lowest and highest values were recorded by genotypes PA 56 and MAN 15-2, respectively. Pod value, the yield component had a mean value of 41.26g. The maximum pod value was recorded for TRD 44 (64.31g), while the minimum was for GU 2691/V (23.42g). Another economic parameter, the pod index, which gives an indirect measurement of yield, ranged between 15.62 and 42.72. Among the 23 genotypes, the efficiency index recorded a mean value 12.25. Ranking of genotypes based on scores obtained for the eight morphological characters, UF 221 and CC 11 ranked first, followed by ICS 40. Meanwhile, PA56 and GU 2691/V scored inferior among the studied germplasm.

Table 2. Mean performance of genotypes for yield contributing characters

Genotype	FD	OB	NB	TBWT	SBDWT	PV	PI	E
G1	11.79	1.19	44.20	130.91	0.86	38.01	26.35	11.36
G2	12.05	0.93	37.80	141.64	1.60	60.68	16.59	7.32
G3	14.90	0.88	40.20	150.82	1.44	57.93	17.31	9.93
G4	12.64	0.98	41.90	100.25	0.89	37.21	26.92	10.83
G5	10.45	1.01	44.80	107.12	0.91	40.91	24.71	9.64
G6	11.89	0.90	40.10	146.90	1.61	64.31	15.62	8.83
G7	9.37	1.03	38.20	110.94	0.80	30.39	33.02	12.26
G8	13.70	0.99	43.10	141.82	1.18	50.78	19.78	10.97
G9	12.14	0.96	29.60	92.34	1.09	32.16	31.19	16.58
G10	12.83	1.27	43.90	124.66	0.88	38.56	26.04	16.57
G11	10.09	0.90	38.30	81.55	0.84	32.32	31.01	9.77
G12	9.82	0.96	47.60	98.97	0.78	37.00	27.11	9.56
G13	10.37	1.13	48.20	141.97	1.12	53.93	18.72	10.75
G14	15.13	0.99	43.70	151.67	1.18	51.09	19.70	9.09
G15	15.57	1.16	36.15	118.11	1.20	43.25	23.20	11.48
G16	10.66	1.00	42.90	95.08	0.71	30.49	33.13	16.73
G17	12.29	1.01	42.10	117.41	1.23	51.77	19.36	9.84
G18	12.70	1.10	49.80	112.92	0.81	40.27	25.23	11.85
G19	12.86	0.94	43.60	119.92	1.04	45.30	22.55	11.83
G20	12.51	0.96	28.40	72.63	0.83	23.42	42.72	15.45
G21	10.95	0.82	27.30	84.37	0.92	25.04	40.08	18.59
G22	11.57	0.90	37.20	119.11	1.28	47.46	21.10	11.84
G23	15.56	1.00	35.90	143.04	1.58	56.64	17.76	8.75
CV	1.53	10.52	7.31	9.25	5.36	8.80	8.24	15.00
CD	0.31	0.18	4.89	18.09	0.10	6.29	3.45	2.93
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

(P < .01 – characters are significant at 1% level of significance)

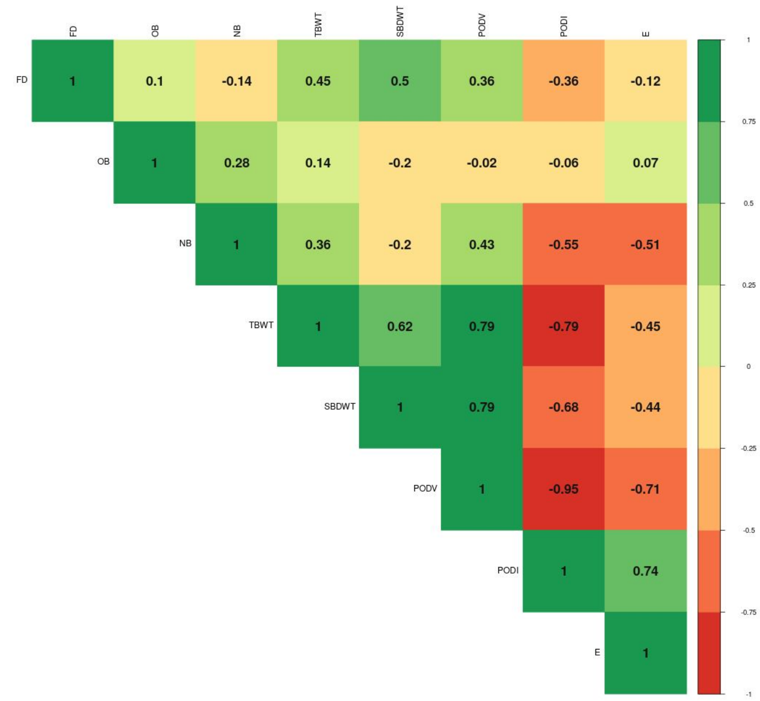
FD – flower diameter, OB – ovary breadth, NB – number of beans pod^{-1}, TBWT – total bean weight pod^{-1}, SBDWT – single bean dry weight, PV – pod value, PI – pod index, E – efficiency index

Validation of genetic parameters for the selected traits will aid in the selection of parents for further breeding activities as it ensures genetic gain. Among the genetic parameters, the values of PCV and GCV were high (>20%) for single bean dry weight, pod value, pod index, and efficiency index. In terms of broad sense heritability, all characters exhibited high values except ovary breadth (table 3). Similar results were reported earlier [13]. High values of GCV and heritability indicates less influence of external environment in their expression. However, the trait ovary breadth showed a moderate heritability. Single bean dry weight, pod value, pod index, and efficiency index showed high PCV, GCV, heritability, and genetic advance in the studied germplasm, indicating additive gene action and hence can be used as a selection criterion for future breeding programmes.

Table 3. Genetic parameters of yield contributing characters

Character	Mean	Range	PCV	GCV	H_b^2 *(%)*	GAM
Flower diameter (mm)	12.25	9.37-15.57	14.76	14.68	98.90	30.08
Ovary breadth (mm)	1.00	0.82-1.27	14.22	9.60	45.50	13.34
Number of beans pod^{-1}	40.22	27.30-49.80	18.50	15.44	69.70	26.56
Total bean weight pod^{-1} (g)	117.57	72.63-151.67	21.64	19.56	81.70	36.43
Single bean dry weight (g)	1.08	0.71-1.61	26.32	25.65	95.00	51.51
Pod value (g)	41.26	23.42-64.31	27.97	25.43	82.60	47.61
Pod index	26.30	15.62-42.72	30.62	28.42	86.10	54.34
Efficiency index (%)	12.25	7.32-18.59	29.68	24.62	68.80	42.07

The number of seeds pod^{-1} is primarily influenced by the number of ovules ovary^{-1}, the fertility of the ovules, and the compatible nature of the tree [14]. Here, the correlation analysis (figure 1) revealed a positive correlation of ovary breadth with the number of beans pod^{-1}. Similarly, the flower diameter exhibited a moderate positive correlation with total bean weight pod^{-1} as well as single bean dry weight [15]. The positive association, along with high heritability and GAM of these characters, indicates that the selection of genotypes having larger flowers for breeding programmes brings a high yield. In the correlation analysis, the trait pod value showed a very strong and strong negative correlation with the pod and efficiency indexes, respectively. This association indicates that, similar to the pod index, the low value of the efficiency index is a preferred characteristic for obtaining a better yield. Selection of genotypes were performed earlier [16] based on inter-character associations for good yield.



Comment [EH2]: Mention the significant correlation

Fig. 1. Correl<u>ation</u>~~ogram~~ of yield attributing components

Comment [EH3]: Mention the significant correlation

FD – flower diameter, OB – ovary breadth, NB – number of beans pod^{-1}, TBWT – total bean weight pod^{-1}, SBDWT – single bean dry weight, PV – pod value, PI – pod index, E – efficiency index

Principal component analysis (PCA) was carried out using eight quantitative characters. In PCA, eigenvalues reflect the amount of variation explained by each principal component (PC). The eigenvectors for each trait are presented in table 4. The first three axes explained 90.28 per cent of the total variation across the eight traits used to describe the germplasm, with eigenvalues of 4.48, 1.68, and 1.06, respectively. The first PC alone accounted for 55.99 per cent of the total variation, highlighting its importance in distinguishing between exotic germplasm. Hence, the traits loaded on it play significant roles and are the most important in describing the variation among the cacao clones since PCA measures axes along which variation between genotypes is maximized [17].

In this study, the first principal component was primarily loaded by pod value (0.46), total bean weight per pod (0.44), and single bean dry weight (0.39), while the efficiency index (-0.37) had a negative effect, hence reducing diversity. The second PC was influenced mainly by the number of beans per pod (0.65) and ovary breadth (0.59). Meanwhile, the third PC was mainly loaded by flower diameter (0.68). Key yield related traits, such as pod value, total bean weight pod^{-1}, and single bean dry weight, were contributing to maximum variation among the genotypes, as they were highly loaded in the first principal component. A similar trend in variability by pod value was already reported [18].

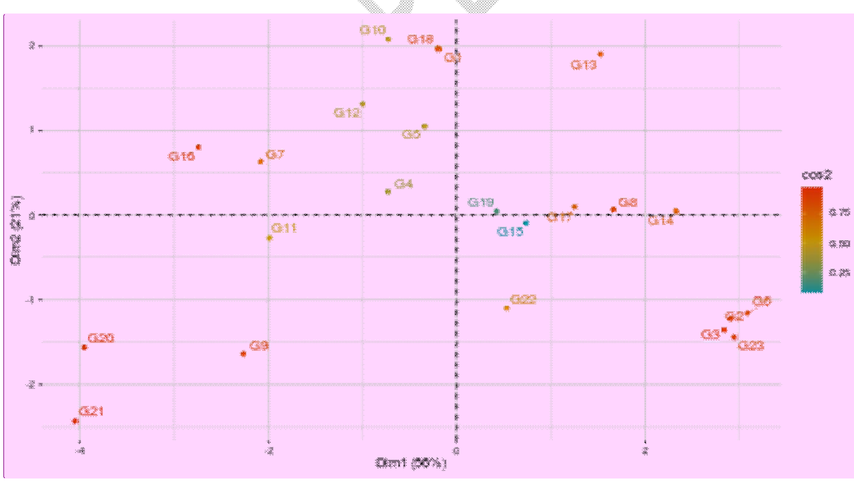
The configuration of genotypes along the first two axes, which accounted for 76.99 per cent of the total variation, is plotted in figure 2. The figure shows the clear separation of genotypes, allowing for grouping genotypes based on their positions in the PCA biplot [19]. In this study, based on their distribution along the first two axes, genotypes G2, G6, G3, and G23 can be clustered together, while G20 and G21 form the second cluster. The third group consists of genotypes G17, G8, and G14. Another cluster includes G10, G18, G12, G5, G1, and G4. The clustering observed corresponds to the quantitative data collected. However, the rest of the genotypes did not fit into these four clusters as they exhibited distinct natures for the traits studied.

Comment [EH4]: For better representation cluster analysis can be done

Table 4. Eigen values, loading of variables on principal components and percentage contribution of variance by principal components

Variables	PC1	PC2	PC3	PC 4
FD	0.25	-0.21	0.68	0.62
OB	0.03	0.59	0.54	-0.22
NB	0.19	0.65	-0.23	0.18
TBWT	0.44	0.08	0.14	-0.31
SBDWT	0.39	-0.40	0.04	-0.30
PV	0.46	-0.06	-0.08	-0.21
PI	-0.46	-0.08	0.07	0.09
E	-0.37	-0.07	0.41	-0.55
Eigen value	4.48	1.68	1.06	0.38
Percentage contribution to variance	55.99	20.99	13.29	4.74
Cumulative percentage of variance	55.99	76.99	90.28	95.01

FD – flower diameter, OB – ovary breadth, NB – number of beans pod^{-1}, TBWT – total bean weight pod^{-1}, SBDWT – single bean dry weight, PV – pod value, PI – pod index, E – efficiency index



Comment [EH5]: Add figure of trait contribution

Fig. 2. Configuration of exotic genotypes in first two principal components

4. CONCLUSION

In short, the results revealed significant genetic variation in the germplasm regarding yield-related traits. The presence of distinct variability among germplasm ensures the evolutionary survival of cocoa and provides opportunities for enhancing the crop by focusing on traits exhibiting significant variation. In the present study, among the 23 genotypes evaluated, UF221 and CC11 performed better based on the yield attributes and can be exploited further in crop improvement programmes. Based on the values of genetic parameters and correlation, characters such as pod value, pod index, efficiency index and single bean dry weight should give emphasis while considering these genotypes for developing genetic stocks. Moreover, among these four, pod value and single bean dry weight have the major contribution to the total variability of this exotic population, which was evident from the principal component analysis and hence can be fixed as the selection criteria.

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