

# Examining phenotypic diversity and economic value of cacao (*Theobroma cacao* L.) conserved at the International Cocoa Genebank, Trinidad to support improvement in cocoa yield globally

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Morphological characterization of cacao germplasm, conserved at the International Cocoa Genebank Trinidad (ICGT), can facilitate global utilization of the rich cacao genetic diversity maintained therein. There are several superior genotypes (primary and enhanced) at the ICGT that combine favourable yield potential, seed size, seed mass and other desirable traits. These genotypes and the diverse cacao collection are at the disposal of the international cocoa community. They can be used to improve cacao planting material to increase production and productivity and, subsequently, the living income generation within rural cocoa communities. This article underscores the value of morphological characterization data and the exploitation of cacao germplasm diversity in the genetic improvement of cacao. It also considers the value of diverse cacao genetic resources in safeguarding the sustainability of the global cocoa and chocolate industry. The results of descriptive statistical analysis, ANOVA and Principal Component Analysis of 1586 characterized cacao accessions demonstrated the considerable phenotypic variation evident in this sample of cacao germplasm. Thirty-two cacao accessions with favourable yield potential, recorded as pod index of 17 or less and other aforementioned useful traits of economic interest, were identified as putatively valuable cultivars and parental types for cacao improvement globally.

**Keywords:** Cacao, cocoa, conservation, economic value, enhancement, germplasm, phenotypic diversity, yield potential

Cacao (*Theobroma cacao* L.), of the family Malvaceae *sensu lato*, subfamily Byttnerioideae (Alverson et al. 1999), is a perennial, Neotropical plant. 'Cocoa' is the dried and usually fermented fatty seed of the cacao tree, which is used in the preparation of chocolates. The crop was domesticated in South and Central America in pre-Columbian times. In 1753, Linnaeus designated its scientific name, *Theobroma*, meaning 'food of the gods'. Currently, a burgeoning global cocoa and chocolate industry is associated with cocoa production. The International Cocoa Organisation (ICCO 2020) forecasted grinding of 4.78 million tons of cocoa for 2019-2020. Markets and Markets Analysis (Markets and Markets 2018) assessed the worth of the global cocoa market as US \$2.1 billion, in 2018, and projected that the chocolate market, in 2019, would be worth US \$131.7 billion. The global chocolate market value is expected to approach

\$171.6 billion by 2026 (Research and Markets 2020).

The cacao tree only grows in humid tropical climates. Cocoa is currently an important agricultural commodity in West Africa, which produces over 70% of the world's supply, as well as in South-East Asia, Latin America and the Caribbean. It is cultivated by over 5 million growers in 50 countries and supports the livelihood of 40-50 million people (Bekele and Phillips-Mora 2019). Progress in cacao breeding has been hindered by a long generation cycle, limitations in land availability for large-scale breeding trials, the narrow genetic base of parental types utilized in breeding to date and challenging abiotic and biotic stress factors, including several major diseases (Bekele and Phillips-Mora 2019). Despite the fact that the acreage of harvested cocoa in Africa has increased, the yields per hectare have

plateaued. Based on the agricultural efficiency indicators, it is likely that the West African cocoa producers may not be able to supply cocoa sustainably to meet the growing global demand for bulk cocoa (Hawkins and Chen 2016). There is thus still an urgent need to increase cocoa productivity and production through breeding for increased yield, yield efficiency, disease and pest tolerance and climate resilience (Bekele and Phillips-Mora 2019).

Cacao germplasm has been traditionally conserved in field collections or genebanks rather than in seed banks since the cacao seed is recalcitrant (it cannot be stored for long periods due to its sensitivity to dehydration and temperature variation). The Cocoa Research Centre (CRC), Trinidad, manages the International Cocoa Genebank Trinidad (ICGT), which is one of two international field collections of cacao germplasm in the public domain. Diverse cacao germplasm was assembled through at least nine cacao collecting missions (Bekele et al. 2006; Bekele and Phillips-Mora 2019), and approximately 2200 cacao accessions are currently conserved at the ICGT. The other universal cacao collection is maintained at the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica (Bekele and Phillips-Mora 2019).

At the CRC, a high priority is accorded to the characterization, evaluation and utilization of the conserved cacao germplasm. Measures of yield potential and other agronomic and economic traits of special interest to breeders have been routinely recorded since 1990 (Bekele et al. 2020). More than 1500 accessions have been characterized, to date, in terms of 25 flower and fruit descriptors (characters), including those of agronomic and economic interest such as seed or bean number, dried cotyledon mass (seed without testa/shell) and pod index (a measure of yield potential, which is the number of pods required to produce 1 kg of dried cocoa without testa) (Bekele et al. 2020).

It has been established that bean mass,

bean number and pod index are moderately heritable (Toxopeus 1972) and are good yield indicators. A low pod index is also desirable since it is normally associated with moderate to large bean size (dried individual cotyledon mass of at least 1 g), which is preferred by chocolate manufacturers.

With this background information, the objectives of this study were to:

1. Examine variation in the expression of 25 morphological traits recorded for 1586 accessions from the ICGT; and to
2. Evaluate these accessions in terms of economically important characters such as cotyledon mass, bean number and pod index (PI) to support improvement in cocoa yield.

In addition, accessions expressing one or more desirable trait(s) were identified to facilitate the search for promising accessions to include in future global cocoa research and enhancement or breeding programmes. Furthermore, fifteen (15) of the Germplasm Enhancement for Black Pod (GEBP) progeny, generated during the Common Fund for Commodities/International Cocoa Organisation/Bioversity International project entitled Cocoa germplasm conservation and utilization: a global approach (1999-2004) (Iwaro et al. 2009; 2010), were evaluated during this cacao phenotypic characterization exercise.

## Materials and methods

In this study, morphological characterization data for 1586 cacao accessions, representing 80 accession groups of diverse origin from the ICGT (Iwaro et al. 2003; Bekele et al. 2006), were collated according to the convention of Bekele et al. (2006) and Bekele and Butler (2000). The 25 descriptors used (Table 1) were selected from the International Board for Plant Genetic Resources (now Bioversity International) descriptor list for cocoa (Anon. 1981) based on analyses described by Bekele et al. (1994) and Bekele and Butler (2000).

They have been used for routine characterization of cacao accessions at the ICGT (Bekele and Bekele 1996; Iwaro et al. 2003; Bekele et al. 2003; 2006; 2008 a and b; 2020) and were found to be the most discriminative and taxonomically useful and preclude redundancy. They were also selected for ease of observation, reliability of scoring, and, in the case of seed characters, agronomic and economic value.

All of the accessions conserved at the ICGT have assigned alpha-numeric codes, which are internationally adopted (International Cocoa Germplasm Database (ICGD) <http://www.icgd.rdg.ac.uk/>). The phenotypic data of the 1586 accessions, studied herein, are accessible via the ICGD. The soil, climatic and husbandry practices at the ICGT are described by Bekele et al. (2020).

Descriptive statistics for each descriptor studied were generated using MINITAB version 16. The data for seed bean number, cotyledon mass and pod index were log transformed to allow for the skewness of the data (non-normal

distribution). Analysis of Variance (ANOVA) was then performed using the General Linear Model in Minitab to compare means for the recognized conventional classes of cacao (Chesman 1944), studied in terms of the aforementioned quantitative traits of economic value, using the Tukey-Kramer t-test to allow for unequal sample sizes (Minitab Inc. 2000; Dunnett 1980).

Principal Component Analysis (PCA) was employed to examine the phenotypic diversity and relatedness in the germplasm studied. PCA was performed on a correlation matrix of the data recorded using MINITAB version 16. Data for the 25 descriptors used (Table 1) were first standardised to eliminate the effects of different scales of measurement. Special attention was paid to the identity of noticeably divergent genotypes with superior traits such as low pod index, which may be useful for achieving heterotic combinations (Toxopeus 1972), where characteristics of the offspring are superior to those of the parents.

Table 1: Descriptors used for morphological characterization - their states and sample sizes (n)

Descriptor	State
Flower, anthocyanin intensity in column of pedicel	1=green, 2=reddish, 3=red [n=10].
Flower, sepal length (mm) [n=10]	
Flower, anthocyanin intensity on ligule	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Flower, ligule width (mm) [n=10]	
Flower, anthocyanin intensity in filament	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Flower, style length (mm) [n=10]	
Flower, ovule number [n=10]	
Fruit, shape	1= oblong, 2= elliptic, 3=obovate, 4= orbicular [n=10], 5= oblate or other (specified).
Fruit, basal constriction	0=absent, 1=slight, 2=intermediate, 3=strong, 4=wide shoulder [n=10]
Fruit, apex form	1=attenuate, 2=acute, 3=obtuse, 4=rounded, 5=mammillate, 6=indented [n=10]
Fruit, surface texture (rugosity or degree of wartiness)	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Fruit, anthocyanin intensity in mature ridges	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Fruit, ridge disposition	1=equidistant, 2=paired [n=10]
Fruit, primary ridge separation	1=slight, 2=intermediate, 3=wide [n=10]
Fruit, pod wall hardness [n=10]	3 = < 2.0 MPa    5 = 2.0 to 2.49 MPa    7 ≥ 2.5 MPa
Fruit, length (cm) [n=10]	
Fruit, width (cm) [n=10]	
Seed, number [n=10]	
Seed, shape	1=oblong 2=elliptic 3=ovate
Seed, cotyledon colour	1=white, 2=grey, 3=light purple, 4=medium purple, 5=dark purple, 6=mottled [n=40]
Wet seed/bean mass (total) (g) [n=10]	
Cotyledon (seed minus testa) length (cm) [n=20].	
Cotyledon width (cm) [n=20].	
Cotyledon mass (g) [n=20]	
Pod index [n=10]	

## Results

### *Evaluation*

Descriptive statistics for the 1586 accessions studied in terms of bean number, cotyledon mass and colour and pod index are presented in Table 2. The significant phenotypic variation displayed, in terms of these traits, is evident from the coefficients of variation, which all exceeded 10.0%, apart for seed number in the enhanced genotypes (GEBP accessions) of which there were only 15.

The progeny of the Germplasm Enhancement Programme for Black Pod Resistance (GEBP) had the highest mean bean number observed among the study cohort ( $43.6 \pm 0.7$ ) compared to the overall mean of  $38.7 \pm 0.15$ . However, the mean cotyledon weight was the lowest for the GEBP accessions ( $0.79 \text{ g} \pm 0.04$ ) and consequently, these enhanced selections had the second highest mean pod index value ( $30.2 \pm 1.53$ ) after the Forastero group ( $30.6 \pm 0.3$ ) (Table 2). The penultimate mean pod index value indicates that an average of 30 or more fruits/pods from GEBP accessions are required to produce 1 kg of dried cocoa. This mean pod index, relative to the ideal of 20 or less, is therefore not favourable, but is offset by the enhanced seed number ( $43.6 \pm 0.7$ ) in the GEBP progeny.

Thirty-seven percent (588) of the 1586 accessions studied had moderate (fairly favourable) pod index values of less than or equal to 25. Refer to Table 3 for the top 32 accessions. Among the 32 accessions with pod index values of 17 or less, accessions from the Trinitario class of cacao (Cheesman 1944; Bekele et al 2006; 2020) accounted for 25% (8/32) and had the most favourable mean pod index of 25.1. Forasteros accounted for 9.4 % (3/32), and Refractarios for 18.8 % (6/32). Notably, accessions of mixed/various, unclassified or unknown origin, designated as

‘U’, accounted for 46.9 % (15/32) of the favourable accessions in terms of yield potential. Among the latter group of superior accessions with low pod index, the accession group, “CRU”, accounted for 14 of the 15 accessions. These unclassified accessions have thus been proven to have significant value in terms of yield potential and seed/bean mass.

In addition to favourable yield potential (PI of  $25.1 \pm 0.3$ ), the Trinitarios studied had significantly higher cotyledon mass, length, width, pod length and ligule width (data not shown) than the other germplasm studied. They also had the lowest mean anthocyanin content in the seed cotyledons compared to Forasteros, Refractarios and accessions of unknown origin (Table 2).

It is noteworthy that UF 11 and 12 [selections made in Costa Rica and derived from seeds imported from Trinidad (Johnson et al. 2007; 2009)] are among the top three accessions with pod index values of 13.9 and 14.9, respectively. The accession CC 10, from Costa Rica, was the second-best clone in terms of pod index (Table 3). However, the latter accession was reported to show a “high incidence” of Black Pod disease in Brazil despite being one of the top 45 clones for yield per tree (Pires et al. 1993).

### *Utilization*

Divergent accessions, based on PCA, with superior traits such as low pod index ( $\leq 25$ ), which may be useful for achieving favourable (heterotic) combinations in mating designs (Toxopeus 1972), are presented in Figure 1 and include:

B 17/20 [POU], CC 10, CRU 5B/25, CRU 63, CRU 122, CRU 138, CRU C 12/6, DE 36/A, ICS 43, ICS 60, ICS 68, ICS 75, JA 5/35, LP 4/39, MOCO 1/2, RIM 106, SM 9, TRD 35, TRD 42, UF 11 and UF 12 (refer to Figure 1- upper and lower right quadrants).

Table 2: Descriptive statistics for cotyledon colour, bean number, cotyledon mass and pod index

Variable	Conventional class of cacao	Number of observations	Mean $\pm$ Standard Error	Coefficient of variation
Cotyledon colour	Forastero (F)	565	4.59 $\pm$ 0.02	11.8
	Trinitario (T)	229	<b>4.35</b> $\pm$ 0.04	<b>14.8</b>
	Refractario (R)	604	4.50 $\pm$ 0.02	13.2
	Unknown (U)	174	4.54 $\pm$ 0.05	13.8
Seed/Bean number	F	565	39.4 <sup>bc</sup> $\pm$ 0.3	<b>16.3</b>
	T	229	38.4 <sup>bc</sup> $\pm$ 0.3	12.5
	R	604	37.4 <sup>cd</sup> $\pm$ 0.2	14.2
	U	174	40.7 <sup>a</sup> $\pm$ 0.4	13.3
	Improved <sup>β</sup>	15	<b>43.6</b> $\pm$ 0.9	7.7
Cotyledon mass (g)	F	565	0.88 <sup>d</sup> $\pm$ 0.007	19.6
	T	229	<b>1.09<sup>a</sup></b> $\pm$ 0.01	20
	R	604	1.03 <sup>bc</sup> $\pm$ 0.008	17.9
	U	174	1.02 <sup>bc</sup> $\pm$ 0.02	<b>24.2</b>
	Improved	15	<b>0.79 <math>\pm</math> 0.04</b>	19.4
Pod index	F	565	30.6 <sup>c</sup> $\pm$ 0.3	24.7
	T	229	<b>25.1<sup>a</sup></b> $\pm$ 0.3	20.5
	R	604	27.4 <sup>b</sup> $\pm$ 0.3	26.0
	U	174	25.8 <sup>a</sup> $\pm$ 0.6	<b>26.7</b>
	Improved	15	30.2 $\pm$ 1.53	19.6

**Legend:** \* Means with different letters are significantly different at P = 0.0001 according to the Tukey-Kramer *t*-test  
 Note: The improved germplasm, represented by fewer than 20 selections, was not compared with the other groups  $\beta$  = GEBP progeny

Table 3: The 32 most promising accessions of the 1586 studied, based on pod index, cotyledon mass and seed/bean number

Accession	Conventional classification	Cotyledon mass (g)	Seed/Bean number	Pod index
UF 11	Trinitario	<b>1.84</b>	39	13.94
CC10	Unknown	<b>1.75</b>	39	14.65
UF 12	Trinitario	<b>1.77</b>	38	14.87
CRU 116	Unknown	1.54	43	15.10
CRU 147	Unknown	1.43	46	15.20
CRU 153	Unknown	1.60	41	15.24
TRD 35	Trinitario	1.59	41	15.34
CRU 122	Unknown	1.47	44	15.46
JA 5/36	Refractario	1.40	46	15.53
CRU 34	Unknown	1.37	47	15.53
ICS 60	Trinitario	<b>1.64</b>	39	15.63
CRU 4A/4	Unknown	1.48	43	15.71
CRU 138	Unknown	1.35	47	15.76
JA 5/7	Refractario	1.41	45	15.76
CRU 4A/11	Unknown	1.51	42	15.77
ICS 68	Trinitario	1.26	50	15.87
ICS 43	Trinitario	<b>1.64</b>	38	16.05
CRU 51	Unknown	1.41	44	16.12
CRU 5B/25	Unknown	1.51	41	16.15
CRU 38	Unknown	1.26	49	16.20
CRU 154	Unknown	1.54	40	16.23
SILECIA 8 (synonym EET395)	Trinitario	1.40	44	16.23
CRU 73	Unknown	1.23	50	16.26
CRU 35	Unknown	1.43	43	16.26
LCT EEN 261/S-4	Forastero	1.41	43	16.49
PA 205	Forastero	1.41	43	16.49
AM 1/85	Refractario	1.54	39	16.65
CLM 59	Refractario	1.50	40	16.67
MOQ 2/29	Refractario	1.38	43	16.85
IMC 10	Forastero	1.02	<b>58</b>	16.90
ICS 5	Trinitario	1.37	43	16.98
JA 5/35	Refractario	1.37	43	16.98

The most unfavourable accessions in terms of yield potential, seed/bean number, seed/bean mass and size are presented in Table 4. These

18 accessions will not be useful for improving yield, but may possess other favourable traits such as disease resistance.

Table 4: The most unfavourable accessions (18) of the 1586 studied, based on seed/bean number, cotyledon mass, length and width and pod index

Accession	Conventional classification	Seed/Bean number	Cotyledon mass (g)	Cotyledon length (cm)	Cotyledon width (cm)	Pod Index
B 9 /10-35 [POU]	Refractario	22	0.49	1.58	0.84	92.76
GU 339/M	Forastero	23	0.61	1.90	1.07	71.28
SCA 11	Forastero	27	0.56	1.96	0.91	66.14
B 9/10-28 [POU]	Refractario	36	0.44	1.77	0.94	63.13
SPEC 41/6-29	Forastero	23	0.69	2.16	1.09	63.01
GU 219/F	Forastero	26	0.62	1.82	1.08	62.03
DE 53/B	Unknown	28	0.58	1.81	1.05	61.58
CL 27/96	Refractario	17	0.98	2.04	1.29	60.02
CL 19/31	Refractario	26	0.65	1.91	1.06	59.17
NA 92	Forastero	21	0.83	1.58	1.08	57.37
NA 39	Forastero	22	0.82	1.79	1.13	55.43
LCT EEN 411	Forastero	32	0.57	1.91	0.83	54.82
ELP 30/S5	Forastero	26	0.71	1.99	1.08	54.17
NA 706	Forastero	28	0.67	2.00	1.15	53.30
NA 154	Forastero	28	0.68	1.72	1.00	52.52
PA 114	Forastero	40	0.48	1.75	1.10	52.08
CL 13/27	Refractario	35	0.55	1.81	1.26	51.95
PA 134	Forastero	39	0.50	1.70	0.95	51.28

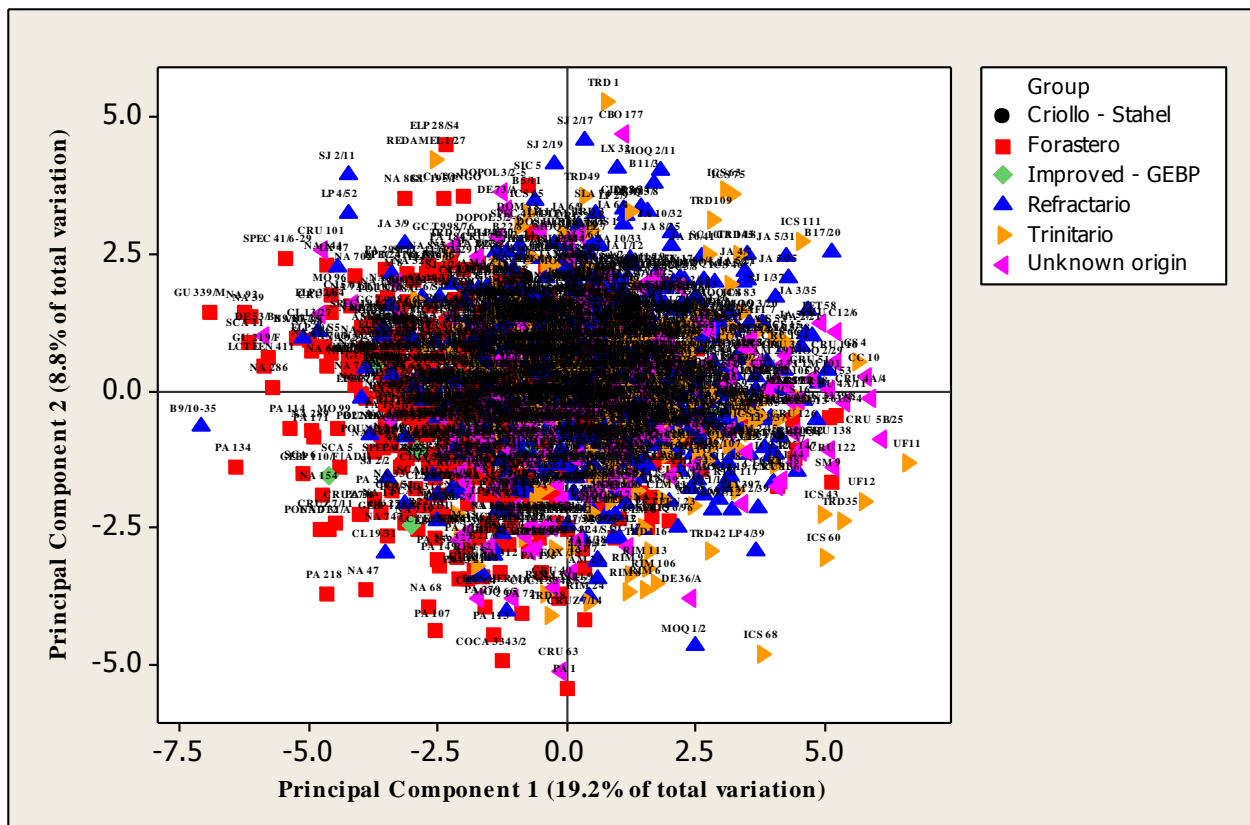


Figure 1: Principal Component plot of 1586 accessions based on 25 morphological descriptors

Table 5: Contributions of variables studied, measured by vector loadings, to the phenotypic variation revealed by Principal Component Analysis of 1586 cacao accessions

Variable/descriptor	Principal Component 1 Vector Loadings	Principal Component 2 Vector Loadings
FL_Sepal length	0.206	0.062
FL_Ligule width	0.137	0.015
FL_Ovule number	-0.030	-0.030
FL_Style length	0.115	0.002
FL_Ligule colour	0.003	0.012
FL_Filament colour	0.030	-0.015
FL_Pedicel column colour	-0.003	0.080
FR_Mature Pod ridge colour	0.101	0.054
FR_Pod shape	0.067	0.461
FR_Pod basal constriction	0.015	-0.409
FR_Pod apex form	0.027	0.359
FR_Pod surface texture	-0.007	-0.402
FR_Pod furrow disposition	0.001	-0.029
FR_Pod furrow separation	0.008	0.238
FR_Pod wall hardness	0.012	0.022
FR_Cotyledon colour	0.011	-0.050
FR_Seed shape	0.003	-0.060
FR_Pod length	0.218	-0.460
FR_Pod width	0.350	0.083
FR_Wet bean mass	0.415	-0.045
FR_Seed number	0.092	-0.095
FR_Cotyledon mass	-0.395	-0.003
FR_Cotyledon length	0.382	0.070
FR_Cotyledon width	0.316	0.116
Pod index	-0.394	0.057

Legend: FL – flower FR – fruit

The first two Principal Components only accounted for 28% of the total phenotypic diversity expressed within this large group of accessions (Figure 1). The variables/descriptors, which accounted for major contributions to the variation expressed by Principal Component 1, as measured by the vector loadings (Morrison 1967), were total wet bean weight, pod index, cotyledon mass, cotyledon length, pod width, cotyledon width, pod length and sepal length (Table 5). Fruit shape, pod length, fruit basal constriction, fruit surface texture and fruit apex form contributed most to the variation expressed by Principal Component 2 (Table 5). The relationships among 220 accessions with favourable pod index of  $\leq 21$  and seed number of 38 or more, based mainly on genetic grouping according to

the convention of Motamayor et al. (2008), are represented in Figure 2. It is noteworthy that among these superior accessions, Trinitarios were well represented, compared to the other genetic and classification groups studied. Some separation between the Trinitarios and the Refractario and Forasteros of the Iquitos group (based on the conventional classification as described by Bekele et al. 2006) was also evident. However, there was a generally heterogeneous association of the 'genetic' groups, as defined by Motamayor et al. 2008, based on phenotype (Figure 2).

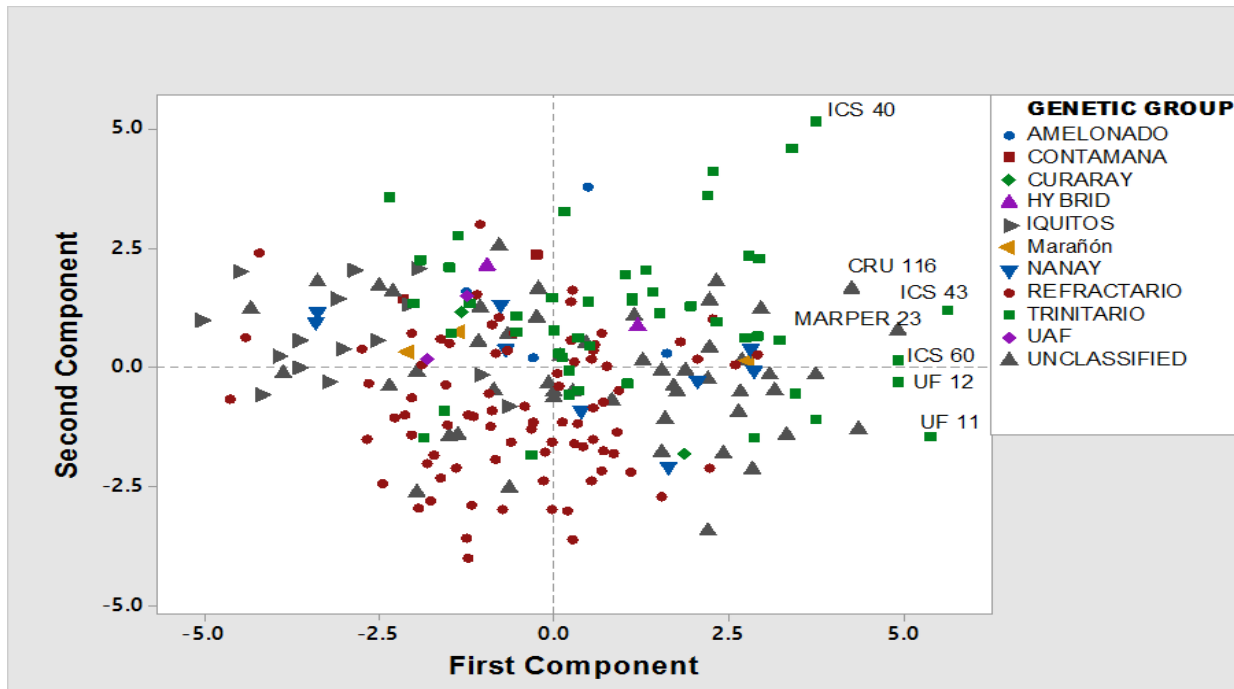


Figure 2: Principal Component plot depicting the phenotypic relationships among accessions studied from various genetic and other groups with favourable pod index ( $\leq 21$ ) and seed number of 38 or more

Legend: UAF = Upper Amazon Forastero

## Discussion

The agronomic and economic value of this large and diverse sample of cacao germplasm, located at the ICGT, has been highlighted by this study. Thirty-seven percent (37%) or 588 of the 1586 accessions studied had moderate (fairly favourable) pod index values of less than or equal to 25 and some of these have previously demonstrated good levels of Witches' Broom and Black Pod disease tolerance (Iwaro et al. 2003; 2009; 2010). The ICGT is thus a good source of potentially high-yielding genotypes. The 32 ICGT accessions, combining favourable good yield potential (low pod index of less than or equal to 17) and large cotyledon mass and seed/bean number (Table 3), are potential candidates for inclusion in future cacao germplasm enhancement or improvement programmes. The relatively good performance of germplasm of unknown origin, observed in this study, underscores the importance of conserving diverse germplasm

despite the fact that some accessions may have become mislabelled or have little or no available passport data.

The Trinitario accessions, which are cultivated and not wild germplasm (Bekele et al. 2006), have distinguished themselves with favourable yield potential (low pod index values), and significantly higher cotyledon mass, length, width, pod length and ligule width (data not presented) compared to the other germplasm studied. This was supported by observations made by Bekele et al. (2020) and is in keeping with the conclusion of Doebley et al. (2006) that "cultivated species generally have larger fruits or seeds compared to their wild ancestors, indicating that fruit and seed size are major agronomic traits that have been selected in crops during their domestication." None of the poor performing accessions were Trinitarios (Table 4). The Trinitarios also had the lowest mean anthocyanin pigment concentration in the cotyledons compared to Forasteros,



Refractarios and accessions of unknown origin (Table 2). Since an absence or low concentration of anthocyanin pigment in cotyledons is associated with fine or flavour cocoa (Wellensiek 1931), Trinitarios such as GS 10 and ICS 16 may prove useful for improvement of flavour profiles in enhanced germplasm and breeding programmes of fine flavour cocoa producing countries such as Trinidad and Tobago, as stated by Bekele et al. (2020).

Considerable phenotypic diversity was evident among the germplasm studied, based on the results of Principal Component Analyses, presented in Figures 1 and 2, and the coefficients of variation in Table 2. Molecular profiling (genotyping) of all of the accessions conserved at the ICGT, combined with morphological characterization, can provide a clearer appreciation of the diversity (phenotypic and genetic) contained therein. Genotyping would be particularly useful to elucidate the genetic profiles of the “Unclassified” accessions, which had favourable yield potential. Motilal et al. (2011) resolved some of the identity issues at the ICGT using the former technique.

Among the 13 most taxonomically useful cacao descriptors or variables used for phenotypic characterization in this study are 12 fruit and seed traits (Table 5). This observation was supported by other studies at the ICGT (Bekele et al. 1994, 2006, 2008a and b, 2020). These traits are of agronomic and economic importance and were also most useful in explaining the phenotypic diversity expressed in the 1586 accessions phenotyped in this study (Figures 1 and 2, Table 5).

The genetic gain in the GEBP progeny, in terms of resistance to Black Pod disease, has been demonstrated by Iwaro et al. (2009; 2010). The performance of the 15 GEBP progeny evaluated in this study, in terms of pod index (as an indicator of yield potential) and seed/bean number, was favourable. The GEBP progeny had the highest mean seed/bean number among the study cohort ( $43.6 \pm 0.9$ )

[overall mean of  $38.7 \pm 0.15$ ] (Table 2). The acceptable, though not highly favourable, yield potential of the GEBP selections evaluated (mean pod index of  $30.2 \pm 1.53$ ), relative to the overall mean of  $28.08 \pm 0.18$  (Table2), demonstrated the benefit of utilizing phenotypic and agronomic data for 452 accessions (Iwaro et al. 2003; Bekele et al. 2003), along with BP resistance data, when selecting suitable parents for the GEBP crossing scheme or mating design, described by Iwaro et al. (2009; 2010).

## Conclusion

The superior bean number of the 15 enhanced (GEBP) progeny, characterized in this study, warrants that the remaining progeny from this enhancement programme be screened in terms of yield potential. GEBP progeny combining good yield potential and tolerance to Black Pod disease may be used by plant breeders in Trinidad and Tobago and other cocoa producing countries across the globe as a source of Black Pod disease resistance and favourable yield potential genes, which can be incorporated into locally adapted commercial varieties, as outlined by Bekele and Phillips-Mora (2019). Successful outcomes of further cacao improvement, using these enhanced genotypes, will negate the need for heavy fungicide applications that are expensive, may pose risks to human health and are often deleterious for the environment, and will thus facilitate more sustainable cocoa production systems (Iwaro et al. 2010; Bekele and Phillips-Mora 2019).

However, it must be noted that Bartley (1967) commented on the inability to always predict the performance of clones as parents in breeding programmes. He stated that the yield of a clone was often no guide to its value as a parent. This makes the conventional process of selection cumbersome, time-consuming and expensive since it requires mass screening of progeny and recurrent selection to facilitate identification of superior genotypes.

Consequently, genomic approaches such as genome-wide association studies, genomic prediction and genomic selection (Crossa et al. 2017) should be pursued to expedite the process. Furthermore, genetic distance estimates, based on performance and molecular data (Lanaud et al. 2003), may also be used to identify prospective parental types and hybrids with putative heterosis for yield and other quantitative traits of economic importance.

A reduction in the cost of cocoa production for farmers through the development of high-yielding, resistant cultivars is the anticipated outcome of CRC's germplasm characterization, evaluation and utilization/ enhancement efforts. The application of the results, presented herein, can secure higher and more sustainable cocoa yields, through cultivation and breeding using the recommended genotypes along with good agricultural practices.

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