

Upper Amazon Forastero cacao (*Theobroma cacao* L.) 2: An overview of Parinari clones from a breeder's perspective

F.L. Bekele*, A.D. Iwaro, D.R. Butler and G.G. Bidaisee

Cocoa Research Unit, The University of The West Indies, St. Augustine, Trinidad and Tobago;

**(e-mail: Frances.Bekele@sta.uwi.edu)*

Cocoa provides a source of income for 2 million farmers in developing countries. Accelerated progress in cocoa breeding is required to meet the farmers' needs for high yielding, disease-resistant genotypes. The Parinari (PA) accession group has featured prominently in breeding programmes globally because several of its accessions possess desirable traits including tolerance to Black Pod disease. In this overview, the results of evaluation studies at the International Cocoa Genebank, Trinidad along with an account of the use of PA accessions in several breeding programmes are presented to highlight those of potential value to cacao breeders.

Keywords: Cacao; Characterisation; Cocoa breeding; Evaluation; *Theobroma cacao* L.

Under domestication, three classes of cacao (*Theobroma cacao* L.) are recognised: Forastero (Upper and Lower Amazon and Guianese), Criollo, and Trinitario (Cheesman, 1944; Lachenaud, 1997; Lachenaud *et al.*, 1997). Amazon Forastero trees are hardy and robust, which explains why they currently provide 95% of the world's cocoa. Parinari (PA) clones are classified as Forastero.

Only one-third of the cacao cultivated worldwide involves hybrid and clonal cultivars developed by breeding programmes in Trinidad, Côte d'Ivoire, Ghana, Brazil, Malaysia, Papua New Guinea, Cameroun and other producing countries (Eskes & Lanaud, 2001). There is undoubtedly a need for cocoa breeders to provide new, improved varieties for farmers. Research at the Cocoa Research Unit (CRU), which manages the International Cocoa Genebank, Trinidad (ICG,T), has demonstrated that the genebank contains diverse germplasm that is invaluable for genetic improvement. The goal of CRU's genetic enhancement research is to develop high-yielding, disease-resistant genotypes that will lead to a reduction in the cost of cocoa production and thus benefit farmers (Iwaro *et al.*, 2003). Significant progress has been made with CRU's involvement in the CFC/ICCO/Bioversity International project on

Cocoa germplasm utilisation and conservation: a global approach (1998-2004). This overview was prepared to further facilitate cocoa genetic improvement activities.

Until recently, there was limited access to information on the majority of accessions held in the ICG,T. To address this deficiency and provide valuable information to potential users of the germplasm, morphological characterisation, evaluation and other data are being collated for the 2,300 accessions conserved in the ICG,T (Bekele, 1999). Studies on individual accession groups have been conducted (Bekele *et al.*, 2005; 2006; Sounigo *et al.*, 2005b) and continue to be undertaken at CRU. The PA group has featured prominently in breeding programmes globally, and is the subject of this overview.

The main objective of this study was to identify PA accessions of potential value to cacao breeders. The PA group was also examined to elucidate patterns of association among the accessions based on morphological traits, and to match the pods of individual accessions to the cacao pod type descriptions of the botanist, Dr. F.J. Pound (1933). Pound's descriptions of the PA accessions are recorded in the International Cocoa

Germplasm Database (Wadsworth and Harwood, 2000).

Historical background

Pound (1938) recorded the collection site of the PA clones to be the Loreto Region of Peru, along the lower Río Marañón, Parinari (74.60 W, 4.6S) (Figures 1 and 2). Further collections may have been made in the vicinity of Porvenir between the Boca del Huallaga and Boca del Pastaza (Figure 2). Pods were collected from some 7-20 trees free of WB (Pound, 1938; Lockwood & End, 1993). Six PA morpho-types were distinguished by Pound (1938). The pods varied in appearance from light to pure green. Types 1 to 4 were selected in the region around Parinari. Types 5 and 6 were reportedly collected near Porvenir. The pods of Type 6 were described as having a red tint.

The distinguishing features of the six types are as follows:

- **Type 1** - Similar to Pound's category A for pod shape (Pound, 1933). *Pods are long, generally warty, with a pronounced bottleneck and conspicuous point ("cundeamor").*
- **Type 2** - Similar to Pound's (1933) category C for pod shape. *Similar to Type 1, but lacks the pronounced bottleneck.*
- **Type 3** - Similar to Pound's (1933) category D for pod shape; long oval = D1, short oval = D2. *Pods are shorter than Type 1 and lack both the pronounced bottleneck and conspicuous point*
- **Type 4** - *Smooth lagarta with bottleneck and point, but with shallow furrows.*
- **Type 5** - *Long, warty with pronounced bottleneck and point, but small beans.*
- **Type 6** - *Short, 10-ridged oval, warty pods sometimes with red tint.*



Figure 1: Map of Peru showing the Loreto district, the Huallaga and Pastaza rivers and Parinari where the PA accessions were collected

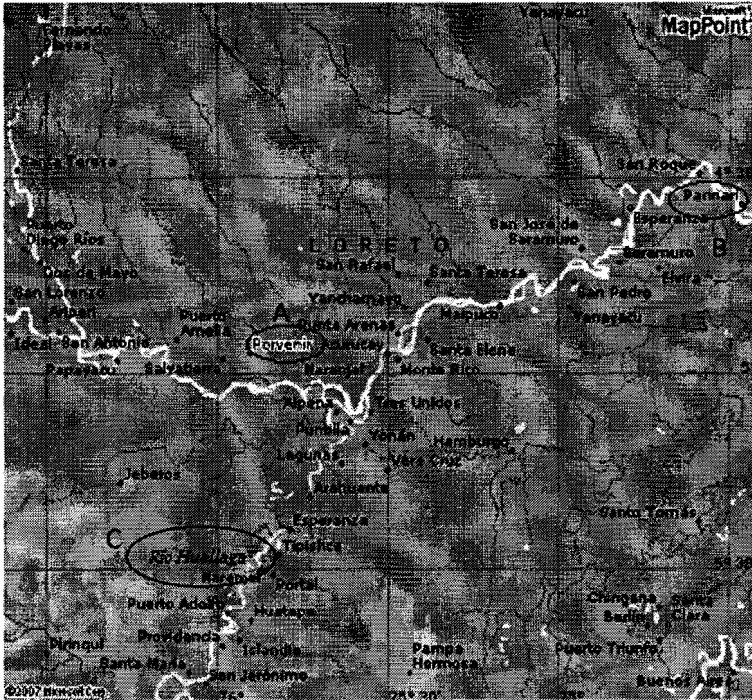


Figure 2: Map of Peru showing Porvenir (A), Parinari (B), the Huallaga River (C) and the Loreto Region

Legend: The Río Marañón flows from west to east and joins the Río Huallaga at Tres Unidos

Utilisation of PA clones in cocoa breeding
 The PA accession group has provided many promising parental genotypes for cocoa breeding. Some of the most widely used include PA 7, PA 107, PA 120, PA 150 and PA 300 (Eskes and Lanaud, 2001; Table 1). Three PA accessions, PA 107, 120 and 150, were included in the international clone trial of the CFC/ICCO/Bioversity International project on *Cocoa germplasm utilisation and conservation: a global approach*. Twelve clones, PA 13, 39, 70, 120, 121, 124, 126, 156, 169, 279, 299, and 303, were included in the CFC/ICCO/Bioversity International project collection comprising 112 accessions selected by Sounigo *et al.* (2005a) based on favourable agronomic traits and resistance to BP and WB diseases. PA 169 was selected for inclusion in the CFC/ICCO/Bioversity International project on *Cocoa Productivity and Quality Improvement, a participatory Approach (2004-2009)* based on its resistance to *Monilia* in tests at Centro Agronómico Tropical de Investigación y Enseñanza

(CATIE), Turrialba, Costa Rica (Eskes, personal communication).

It has also been found to have favourable levels of resistance to one or more species of *Phytophthora* in Brazil (Pires *et al.*, 1993).

Other PA clones have been found useful, but have not been used widely in breeding as those listed in Table 1. In Ghana, PA 146 and 188 were reported to be the most tolerant to Cocoa Swollen Shoot Virus (CSSV) by Adu-Ampomah *et al.* (2005). In Malaysia, PA 13 produced exceptionally high yields in high-density planting trials while PA 127 was notable for high yield at a low planting density (Lockwood and Pang, 1995). PA 76 and 138 were found to be resistant to Vascular Streak Dieback (VSD) in Quoin Hill, Sabah, Malaysia by Chong and Phua (1993). Bong and Phua (1989) had earlier described these two clones as tolerant to VSD. In Brazil, PA 4 was ranked among the top 45 for several agronomic traits including yield (Pires *et al.*, 1993), and PA 16 and 81 were also found promising.

A very high yield (3003 kg ha⁻¹) was observed for PA 82 in Papua New Guinea (Tan, 1993).

Use of PA clones in Trinidad

From 1943, scientists from the Department of Agriculture began evaluation of over 2000 clones from Pound's 1937/38 and 1942/43 expeditions, planted at Marper Estate. Their goal was to select trees combining field resistance to WB with high yield potential and good quality seeds (Montserin *et al.*, 1957). Subsequently, PA 30, 44, 46, 56, 118, 121 along with clones from other accession groups were selected, based on their observed field resistance to WB, as parental genotypes for inclusion in hybrid trials that commenced in 1949. The crosses between PA 44 × PA 46 and PA 30 × PA 121 consistently produced progenies with very low (favourable) pod index values (PI) of less than 16 (defined in Table 3). PA 30 × PA 121 and PA 118 × PA 56 produced some trees with individual wet bean weights of 4.0 g and more. PA 30 × PA 121, planted at 2.4 by 2.4 m, yielded 1606 kg ha⁻¹ of dried cocoa when the trees were only five years old. This was the best performance among several crosses, including SCA 6 × ICS 1, ICS 1 × SCA 6 and SCA 6 × IMC 67, assessed in the field trials.

In CRU's Progeny Trial No. 11, planted in 1968, PA 195 was crossed with B185 and designated AX 368, and PA 30 was crossed with ICS 6. In the germplasm evaluation programme of CRU initiated in 1998 (Iwaro *et al.*, 2003), the PA accession group had the highest proportion (18.1%) of accessions with resistance to BP caused by *Phytophthora palmivora*, combined with favourable PI among six groups studied (Table 2). Among the promising PA clones identified, PA 71 was noted to combine low PI (19.5 pods/kg) with a moderate level of resistance to BP (rating of 5 on a 1 – 8 scale). Among six accession groups (B, ICS, IMC, JA, NA, and PA) for which at least 20 accessions were

evaluated, PA was most outstanding in terms of BP resistance.

Methods

Morphological Characterisation of PA accessions in the ICG,T

Ninety-six PA accessions were observed in terms of 24 morphological traits or descriptors (listed in Table 3), which were adopted from the International Board for Plant Genetic Resources (now Bioversity International) descriptor list for cacao (Anon., 1981; Bekele and Butler, 2000). Data collection was done at the ICG,T and spanned the period 1992-2004. When possible, full samples (Table 3) were collected for each accession at a given time. The ICG,T is situated at the University Cocoa Research Station, Centeno, Trinidad at an altitude of 15 m above sea level.

Shade is provided by trees of *Erythrina* sp. planted 6 m apart, or bananas (*Musa* sp.) placed 4 m apart. The cacao trees are planted 1.8 m apart with typically up to 16 trees per plot for each accession. The soil type is Cunupia fine sandy clay with restricted internal drainage. Over a 30-year period from 1961, the mean annual rainfall in this region was 2,392 mm, and the average temperature 26 °C. The plants are irrigated as necessary during the dry season (January- June) each year.

The methods used for characterisation were described by Bekele and Bekele (1996); Bekele and Butler (2000); Bekele and Bidaisee (2006) and Bekele *et al.* (2006).

Statistical analysis

Descriptive statistics for the descriptors studied were generated using MINITAB Version 14.1 (Minitab Inc., 1997) so that PA accessions of potential value to breeders could be identified.

Table 1 Some of the superior PA accessions identified in the literature

Accession	Attribute(s)
PA 4	Moderately resistant to BP. Pod Index = 26 (Malaysia); 24 (Trinidad & Tobago; Iwaro <i>et al.</i> , 2003). Tolerant to WB (ICGD - Wadsworth and Harwood, 2000).
PA 7	Moderately resistant to BP (CRIG, Ghana). Among best parents at CRIG (Adomako and Adu-Ampomah, 2000). Good female parent in crosses designed for <i>Ceratocystis</i> control in Bahia, Brazil (Ram <i>et al.</i> , 2005).
PA 30	Superior progenies in terms of yield vigour, cropping efficiency and BP resistance in Ghana (Abdul-Karimu <i>et al.</i> , 2005). Less infected by CPB than other clones in Malaysia (Chong and Phua, 1993). Field resistance to WB (MALMR, Trinidad).
PA 46	Moderate to high WB resistance and resistant to BP (MALMR, Trinidad- Montserin <i>et al.</i> , 1957). BP resistance in Trinidad (Latchman <i>et al.</i> , 2000).
PA 71	Moderate resistance to BP in Trinidad and favourable PI (19.5 pods/kg) (Iwaro <i>et al.</i> , 2003).
PA 73	PI = 24 (Malaysia); 24 (ICG,T, Trinidad & Tobago) (ICGD - Wadsworth and Harwood., 2000).
PA 107	PI = 34 (BAL, Malaysia - Lockwood and Pang, 1995; Lockwood <i>et al.</i> , 1994; Lockwood and Pang, 1993); 23 (ICG,T, Trinidad & Tobago) Resistant to BP (Wadsworth and Harwood., 2000).
PA 120	Field resistance to BP in Trinidad (Latchman <i>et al.</i> , 2000). Field resistance to WB in Trinidad (Latchman <i>et al.</i> , 2000). Among 9 of the most resistant to BP of 30 clones tested using a leaf inoculation test at CIRAD, France (Paulin <i>et al.</i> , 2005).
PA 121	PI = 25 (ICG,T, Trinidad and Malaysia). Used in breeding in Brazil (Wadsworth and Harwood, 2000). Field resistance to BP in Trinidad (Latchman <i>et al.</i> , 2000). Field resistance to WB in Trinidad (Latchman <i>et al.</i> , 2000).
PA 150	Resistant to BP (several countries - Paulin <i>et al.</i> (1994); Cilas <i>et al.</i> (1999); Enriquez and Soria (1984); Freeman (1982)). Resistant to all three types of <i>Phytophthora</i> in Brazil (Luz <i>et al.</i> , 1999). Pod Index = 30 (Malaysia - Lockwood and Pang, 1995) (Wadsworth and Harwood, 2000). Among best parents at CRIG along with PA 7 and POUND 7 (Adomako and Adu-Ampomah, 2000; Abdul-Karimu <i>et al.</i> , 2005). Early yielding.
PA 169	PI = 25 (ICG,T, Trinidad). PA 169 × ICS 1 resistant to BP in Jamaica. Field resistance to BP in Trinidad (Latchman <i>et al.</i> , 2000). Field resistance to WB in Trinidad (Latchman <i>et al.</i> , 2000). Resistant to <i>Monilia</i> (Costa Rica) Favourable levels of resistance to one or more species of <i>Phytophthora</i> in Brazil (Luz <i>et al.</i> , 1999).
PA 300	PI = 27 (Malaysia), 21 (ICG,T, Trinidad) (Wadsworth and Harwood, 2000). Dry bean weight = 1.14 g (ICG,T, Trinidad).

PA clone names are suffixed by [PER] in the ICGD; BP – Black Pod disease; CPB – Cocoa Pod Borer; WB – Witches' Broom disease

PI – Pod Index ; ICGD – International Cocoa Germplasm Database

ICG,T – International Cocoa Genebank, Trinidad

Table 2: Distribution of accessions combining low to moderate PI (≤ 25) with high to moderate BP resistance (rating ≤ 5) at the ICG, T

Accession group	Percentage of accessions (%) with favourable PI and BP resistance
PA	18.1
NA	16.7
IMC	5.0
SCA	1.0
POUND	1.0
OTHER	58.2

Source of data: Iwaro *et al.* (2003)

Table 3 Descriptors used for morphological characterisation

Descriptor	State (sample size [n])
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= other.
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, length (cm) [n=10]	
Fruit, width (cm) [n=10]	
Pod, number [n=10]	
Pod, 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 bean weight (total per pod) (g) [n=10]	
Cotyledon length (cm) [n=20].	
Cotyledon width (cm) [n=20].	
Cotyledon weight (g) [n=20]	
Pod index (the number of pods required to produce 1 kg of dried cocoa) [n=10]	

In order to assess the phenotypic variation in this accession group, the coefficient variation (COV) was calculated for the quantitative variables and Shannon Weaver Diversity Index (SWDI) (Shannon & Weaver, 1949), (H), was calculated for the qualitative ones as follows:

$$H = \sum_{i=1}^K P_i \ln P_i, \text{ where } P_i \text{ is the}$$

proportion of the total number of accessions in the i th class, K is the number of phenotypic classes for each descriptor, (Shannon and Weaver, 1949).

Patterns of association among the PA accessions were studied using Principal Component Analysis (PCA) (Mardia *et al.*, 1979; Sneath and Sokal, 1973). The PCA module of MINITAB was used to analyse data generated for 12 qualitative (ordinal) and

12 quantitative (continuous) descriptors. A correlation matrix, which standardised the variables, was used to calculate the principal components. PCA was also used to examine

the level of diversity or variation in the germplasm and to rank the contributions of the variables to it. Score plots of the first two principal components were generated to approximately show the relative groupings of the accessions, give a fair indication of the overall distribution of the data, and uncover any interesting patterns exhibited.

Results

Evaluation of PA accessions from the ICG,T

Descriptive statistics for the phenotypic traits studied for the 96 PA accessions evaluated at

the ICG,T are presented in Table 4 along with information on the phenotypic variation conveyed by the COV and SWDI values. The mean cotyledon weight for the PA accessions was $0.90 \pm 0.014g$. This is somewhat similar to the values obtained by Bekele *et al.* (2006) for the NA and IMC accession groups ($0.89 \pm$

$0.014g$ and $0.89 \pm 0.018g$, respectively), but exceeds the mean cotyledon weights for the MO, POUND and SCA accession groups all collected in Peru (0.78 ± 0.034 , 0.86 ± 0.024 and $0.78 \pm 0.06g$, respectively).

Table 4: Descriptive statistics for 96 PA accessions from the ICG,T

Fruit descriptors			Flower descriptors		
Descriptor	Mean (SD)	CV	Descriptor	Mean (SD)	CV
Fruit/Pod length (cm)	16.56 (0.59)	11.4	Sepal length (mm)	6.9 (0.7)	9.97
Pod width (cm)	7.78 (1.89)	7.2	Ligule width (mm)	2.3 (0.3)	12.3
Fresh bean weight (g)	49.1 (8.03)	16.3	Ovule number	41.6 (4.4)	10.6
Cotyledon weight (g)	0.90 (0.14)	15.5	Style length (mm)	2.2 (0.3)	13.4
Cotyledon length (cm)	2.05 (0.15)	7.3			
Cotyledon width (cm)	1.13 (0.1)	9.7			
Pod Index	30.7 (5.4)	17.7			
Bean number	37.3 (4.24)	10.9			
Descriptor	Mean	SWDI	Descriptor	Mean	SWDI
Mature fruit ridge colour	0.2 (almost no pigment)	0.11	Filament colour	2 (very slight pigment)	0.44
Pod shape	2 (elliptic/oval)	0.36	Ligule colour	3 (slight pigment)	0.39
Fruit/pod basal constriction	3 (strong)	0.34	Pediceal colour	2 (reddish)	0.28
Pod apex form	3 (obtuse)	0.57			
Fruit surface texture	5 (fairly rugose)	0.46			
Pod furrow disposition	2 (paired)	0.02			
Pod furrow pair separation	2 (intermediate)	0.32			
Bean colour	4 (medium purple)	0.30			
Bean shape	2 (elliptic)	0.38			

CV- Coefficient of variation

SD - Standard Deviation

SWDI – Shannon Weaver Diversity Index (Shannon & Weaver, 1949)

Patterns of association among the PA clones

The PA clones studied were widely distributed in the PCA plots generated (Figures 3 and 4). This suggests that a significant amount of phenotypic diversity exists within this accession group. Indeed, the phenotypic diversity of the PA accessions compares favourably with that of 1073 accessions representing 70 accession groups of diverse origin, which are conserved in the ICG,T (Figure 5).

The first two principal components (PC) of Figures 3 and 4 accounted for 33 % of the total variation expressed in the phenotypic

traits (PC 1 - 18.8 %, PC 2 - 14.1 %). The first four principal components accounted for 50% of the variation expressed. The descriptors responsible for most of the variation expressed by the first four principal components are as follows:

- **PC 1** – pod index, fresh bean weight (total), cotyledon weight and length, pod length
- **PC 2** - pod shape, pod surface texture, cotyledon width and weight, bean number, pod apex form
- **PC 3** - sepal length, style length, ligule width, pod shape, pedicel colour,

- PC 4 - bean number, pod basal constriction, ovule number, mature pod ridge colour, pedicel colour

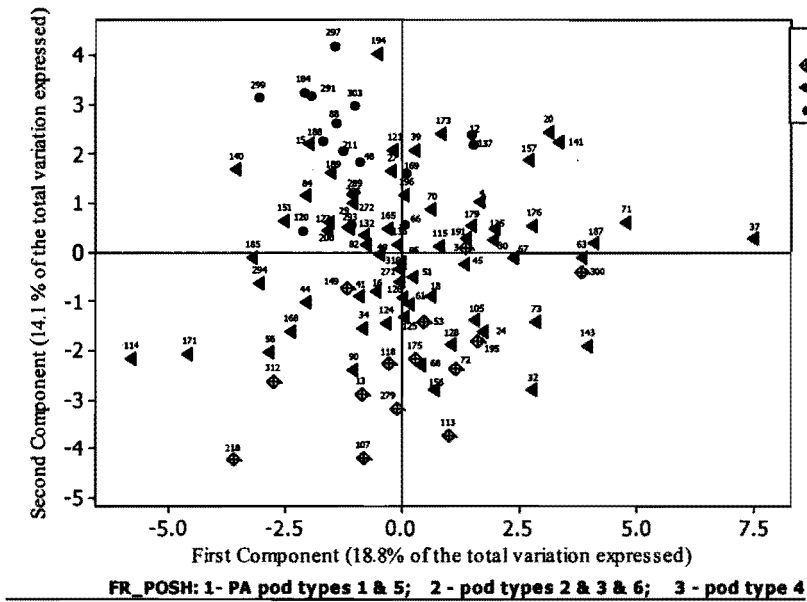


Figure 3 Principal Component Score Plot based on 24 morphological descriptors used to characterise PA accessions at the ICG,T (accessions differentiated according to pod shape)

Pod shape (FR_POSH) 1 – narrow, long with a pronounced basal constriction and pointed apex = PA Types 1 & 5 of Pound (1938); Pod shape 2 - oval or elliptic pods = PA Types 2 & 3 & 6 of Pound (1938); Pod shape 3 - broad oblong or melon-shaped pods = PA Type 4 of Pound (1938); FR_POSH: 1 ○; 2 ▲; 3 ●

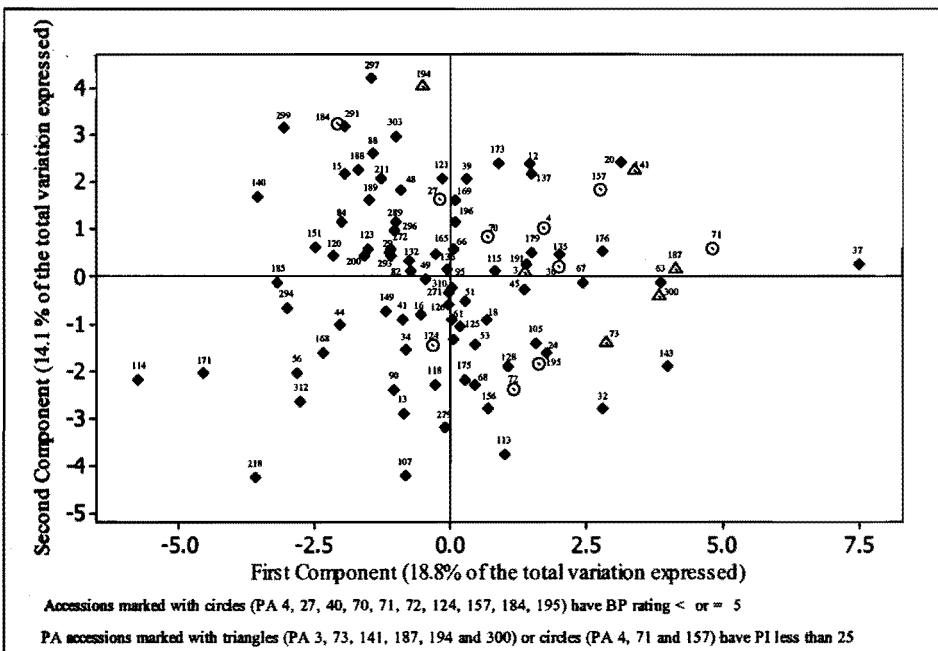


Figure 4 Principal Component Score Plot based on 24 morphological descriptors used to characterise PA accessions from the ICG,T (with promising accessions highlighted)

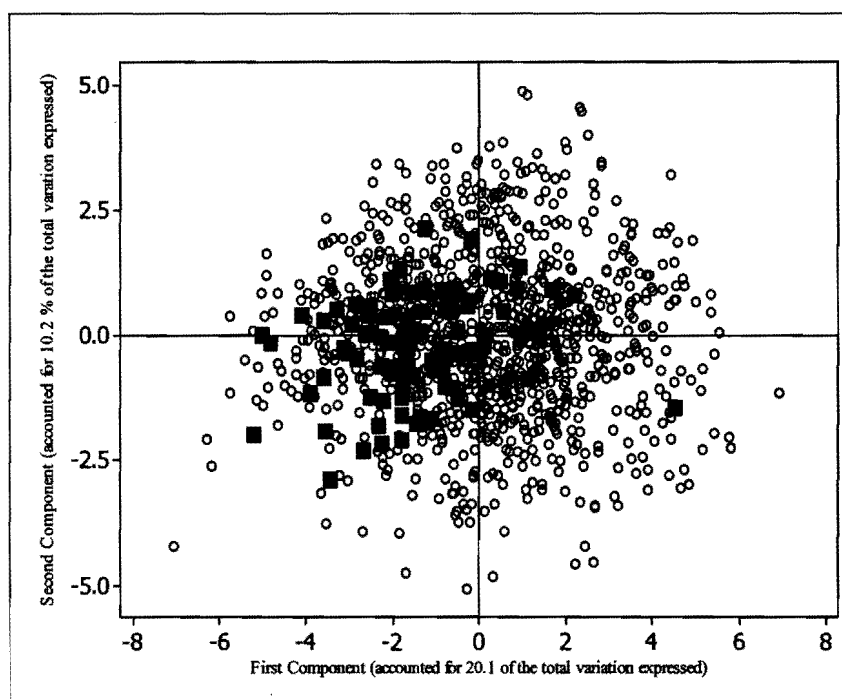


Figure 5 Principal Component Score Plot based on 24 morphological descriptors used to characterise 1169 accessions (including the PA accessions studied) from the ICG,T

- Accessions representing 70 accession groups (excluding PA) from the ICG,T
 ■ PA accessions;

The second principal component clearly separated the clones with narrow, long, oblong, pointed (*cundeamor* type) pods from those with melon or obovate shapes (Figure 3). The oval shaped pods (*Trinitario* types) were widely dispersed in the plot. The observed vertical spread according to pod shape was expected since this variable accounted for most of the variation expressed in PC2.

The clones with the most favourable PI values were separated in a horizontal plane from the rest by the PC1. They were mainly confined to the top right quadrant of Figure 4. This is due to the fact that PI and cotyledon weight accounted for a significant portion of the variation expressed in PC1. Ten of the PA accessions studied combined PI values less than 30 with BP resistance ratings of 5 or lower (Table 5). Forty-four (46 %) of the 96 PA accessions studied had PI values ≤ 30 (Table 6). PA 39 and 169 were among these.

Table 5: Accessions found potentially useful for breeders at the ICG,T

Accession	Trait		
	Cotyledon weight (g)	Pod Index	Rating (with SD) for resistance to BP Disease*
PA 71	1.19	19.5	5 (0.71)
PA 37	1.45	20.0	Susceptible - 7
PA 300	1.14	21.0	Susceptible - 6
PA 63	1.06	21.9	Susceptible - 7
PA 187	1.14	22.5	Susceptible - 7
PA 157	1.17	23.0	3 (1.26)
PA 141	1.17	23.7	Susceptible - 6
PA 3	0.99	24.0	No data available
PA 4	0.99	24.0	4 (0.37)
PA 73	0.90	24.0	Susceptible - 8
PA 194	1.07	24.6	Susceptible - 7
PA 46			1 (0.42)
PA 150			2 (0.58)
PA 30	1.00	27.5	2 (0.96)
PA 136	0.97	30.3	2 (1.50)
PA 124	0.86	29.8	2 (0.83)
PA 95	0.85	30.0	2 (1.18)
PA 218	0.64	36.5	2 (1.16)
PA 125	0.79	22.7	2 (1.63)
PA 70	0.99	29.7	3 (1.18)
PA 27	0.88	29.9	3 (0.95)
PA 120	0.82	25.7	3 (0.79)
PA 195	1.00	26.8	4 (0.00)
PA 72	0.90	26.8	Susceptible - 6

*Source of data: Iwaro et al. (2003)

Table 6 Mean values for fruit descriptor data for PA clones studied at ICG T

Accession	Pod length (cm)	Pod width (cm)	Wet bean weight (g)	Bean number	Cotyledon weight (g)	Bean length (cm)	Bean width (cm)	Pod Index
PA3	18.8 (1.8)	7.9 (0.6)	58.1 (12.2)	42 (8)	0.99 (0.15)	2.11 (0.10)	1.28 (0.08)	24.1
PA4	17.1 (1.5)	8.0 (0.4)	58.0 (8.7)	42 (4)	0.99 (0.11)	2.12 (0.11)	1.25 (0.07)	24.1
PA12	15.7 (1.6)	8.7 (0.4)	54.2 (12.1)	36 (7)	1.02 (0.14)	2.28 (0.12)	1.30 (0.05)	27.2
PA13	18.1 (1.4)	7.4 (0.5)	47.1 (9.7)	42 (6)	0.76 (0.12)	1.98 (0.08)	1.05 (0.06)	31.3
PA15	16.1 (2.4)	7.5 (0.9)	44.1 (12.7)	36 (6)	0.90 (0.21)	1.97 (0.20)	1.12 (0.12)	30.9
PA16	16.7 (1.5)	7.7 (0.6)	46.4 (9.5)	40 (9)	0.86 (0.16)	1.89 (0.27)	1.15 (0.05)	29.1
PA18	13.9 (1.6)	7.2 (1.1)	46.2 (22.4)	39 (5)	0.76 (0.31)	2.08 (0.34)	1.23 (0.20)	33.7
PA20	15.7 (0.9)	8.8 (0.4)	63.7 (6.4)	36 (3)	1.19 (0.09)	2.21 (0.09)	1.34 (0.07)	23.3
PA24	18.0 (1.6)	7.6 (0.5)	56.8 (11.1)	39 (6)	0.93 (0.16)	1.97 (0.18)	1.23 (0.13)	27.6
PA27	13.6 (1.2)	7.5 (0.4)	46.4 (7.9)	38 (6)	0.88 (0.16)	2.08 (0.16)	1.25 (0.07)	29.9
PA29	15.4 (1.2)	7.2 (0.5)	45.2 (8.4)	35 (6)	0.89 (0.15)	2.18 (0.23)	1.13 (0.07)	32.1
PA30	17.4 (1.7)	7.6 (0.7)	54.0 (9.8)	36 (6)	1.01 (0.16)	2.28 (0.18)	1.29 (0.04)	27.5
PA32	18.2 (1.6)	7.8 (0.6)	58.8 (9.2)	44 (4)	0.85 (0.15)	2.17 (0.12)	1.10 (0.07)	26.7
PA34	16.2 (1.2)	7.8 (0.4)	47.8 (8.5)	40 (8)	0.83 (0.10)	1.84 (0.09)	0.96 (0.05)	30.1
PA37	18.3 (1.4)	8.6 (0.4)	69.3 (12.6)	34 (5)	1.45 (0.13)	2.59 (0.14)	1.52 (0.16)	20.3
PA39	14.6 (1.8)	8.0 (1.0)	53.8 (14.6)	39 (6)	0.97 (0.18)	2.18 (0.15)	1.29 (0.08)	26.4
PA41	17.7 (2.2)	7.7 (0.9)	45.3 (8.1)	37 (6)	0.79 (0.22)	2.03 (0.12)	1.00 (0.12)	34.2
PA44	15.8 (1.0)	7.3 (0.7)	37.5 (4.3)	38 (7)	0.75 (0.11)	1.88 (0.13)	1.05 (0.11)	35.1
PA45	16.7 (1.4)	7.9 (0.6)	51.4 (9.0)	37 (6)	0.94 (0.21)	2.03 (0.14)	1.19 (0.09)	28.8
PA48	14.7 (1.4)	7.8 (0.7)	46.2 (10.2)	35 (8)	0.85 (0.12)	2.10 (0.13)	1.14 (0.10)	33.6
PA49	16.9 (1.8)	7.9 (0.4)	47.4 (11.5)	36 (8)	0.81 (0.12)	2.02 (0.16)	1.06 (0.08)	34.3
PA51	17.7 (2.4)	7.5 (0.6)	44.2 (13.1)	34 (9)	0.97 (0.13)	2.12 (0.12)	1.21 (0.07)	30.3
PA53	17.8 (2.0)	6.9 (0.8)	53.0 (14.1)	42 (5)	0.85 (0.31)	2.07 (0.21)	1.19 (0.11)	28.0
PA56	16.7 (1.1)	8.1 (0.4)	47.6 (4.6)	39 (4)	0.80 (0.06)	1.47 (0.09)	0.65 (0.04)	32.1
PA61	16.1 (1.7)	7.4 (0.6)	46.1 (13.1)	41 (3)	0.89 (0.14)	1.95 (0.09)	1.26 (0.08)	27.4
PA63	18.4 (2.8)	8.4 (0.8)	66.8 (16.8)	43 (4)	1.06 (0.16)	2.16 (0.11)	1.13 (0.08)	21.9
PA67	16.2 (2.6)	7.6 (0.8)	55.5 (19.5)	37 (14)	1.07 (0.24)	2.30 (0.16)	1.20 (0.14)	25.3
PA68	17.7 (1.3)	7.8 (0.7)	48.4 (11.6)	45 (3)	0.72 (0.22)	2.10 (0.20)	1.10 (0.09)	30.9
PA70	17.7 (1.0)	8.3 (0.6)	47.6 (7.1)	34 (5)	0.99 (0.09)	2.11 (0.12)	1.23 (0.04)	29.7
PA71	19.4 (1.2)	8.5 (0.6)	66.7 (13.9)	43 (10)	1.19 (0.17)	2.4 (0.17)	1.20 (0.18)	19.6
PA72	18.8 (1.5)	7.8 (0.3)	55.5 (9.9)	41 (5)	0.91 (0.11)	2.13 (0.14)	1.09 (0.08)	26.8
PA73	18.2 (1.9)	8.1 (1.0)	63.2 (14.6)	46 (7)	0.90 (0.12)	2.24 (0.12)	1.13 (0.09)	24.2
PA82	15.6 (1.3)	7.3 (0.5)	50.3 (12.3)	42 (5)	0.78 (0.12)	2.09 (0.12)	1.17 (0.08)	30.5
PA84	15.5 (1.1)	7.7 (0.4)	42.5 (9.3)	27 (5)	1.01 (0.15)	1.98 (0.16)	1.17 (0.11)	36.7
PA88	15.2 (1.5)	7.9 (0.5)	45.7 (9.9)	28 (5)	1.00 (0.18)	1.99 (0.11)	1.23 (0.12)	35.7
PA90	18.3 (1.8)	7.8 (0.6)	42.7 (9.2)	40 (9)	0.76 (0.05)	1.95 (0.09)	0.92 (0.08)	32.9
PA95	17.0 (1.8)	7.7 (0.7)	49.0 (14.7)	39 (8)	0.85 (0.24)	2.19 (0.23)	1.03 (0.09)	30.2
PA105	17.0 (0.9)	7.4 (0.3)	50.9 (7.8)	32 (6)	1.03 (0.16)	2.33 (0.17)	1.19 (0.08)	30.3
PA107	19.7 (1.2)	7.1 (0.3)	47.0 (6.4)	44 (2)	0.75 (0.10)	1.8 (0.08)	0.99 (0.08)	30.3
PA113	19.7 (1.6)	7.4 (0.5)	49.3 (7.1)	42 (4)	0.81 (0.05)	1.91 (0.05)	1.08 (0.03)	29.4
PA114	14.0 (0.9)	6.1 (0.4)	36.6 (11.5)	40 (3)	0.48 (0.22)	1.75 (0.29)	1.10 (0.17)	52.1
PA115	18.9 (1.8)	8.2 (0.5)	55.6 (11.6)	42 (7)	0.90 (0.19)	2.15 (0.16)	1.13 (0.08)	26.5
PA118	18.5 (1.1)	7.5 (0.4)	47.3 (6.0)	38 (5)	0.84 (0.14)	1.97 (0.25)	1.13 (0.08)	31.3
PA120	15.2 (1.8)	7.4 (0.8)	38.7 (10.2)	35 (6)	0.82 (0.12)	2.02 (0.13)	1.11 (0.05)	34.8
PA121	15.2 (2.0)	7.7 (0.6)	44.7 (13.1)	34 (4)	0.96 (0.10)	2.15 (0.10)	1.20 (0.08)	30.6
PA123	15.6 (2.2)	8.0 (0.6)	36.0 (11.1)	29 (6)	0.89 (0.21)	2.08 (0.15)	1.22 (0.08)	38.7
PA124	18.6 (1.9)	7.5 (0.5)	46.0 (8.4)	39 (4)	0.86 (0.16)	1.88 (0.08)	1.09 (0.09)	29.8
PA125	16.5 (2.1)	7.8 (0.5)	48.5 (6.8)	42 (6)	0.79 (0.09)	2.05 (0.12)	1.05 (0.06)	30.1

Table 6 continued...

Accession	Pod length (cm)	Pod width (cm)	Wet bean weight (g)	Bean number	Cotyledon weight (g)	Bean length (cm)	Bean width (cm)	Pod Index
PA126	15.4 (1.9)	6.9 (0.7)	45.7 (16.3)	37 (7)	0.89 (0.23)	2.10 (0.24)	1.21 (0.12)	30.4
PA128	17.1 (1.6)	7.2 (0.5)	51.5 (7.0)	44 (6)	0.79 (0.11)	2.08 (0.12)	1.04 (0.08)	28.8
PA132	14.3 (1.5)	7.3 (0.7)	51.6 (12.9)	40 (4)	0.87 (0.15)	2.06 (0.16)	1.20 (0.12)	28.7
PA135	20.1 (1.6)	8.2 (0.4)	51.0 (10.7)	33 ((7)	1.01 (0.07)	2.17 (0.10)	1.16 (0.05)	30.0
PA136	17.6 (2.1)	7.5 (0.5)	46.7 (9.9)	34 (5)	0.97 (0.12)	2.07 (0.09)	1.12 (0.15)	30.3
PA137	14.3 (1.0)	8.6 (0.3)	49.8 (7.9)	33 (6)	1.08 (0.13)	2.21 (0.11)	1.21 (0.06)	28.1
PA140	12.7 (1.1)	7.4 (0.6)	37.0 (10.4)	27 (4)	0.87 (0.19)	2.03 (0.26)	1.15 (0.13)	42.6
PA141	18.3 (2.7)	9.0 (0.9)	63.1 (10.7)	36 (4)	1.17 (0.15)	2.19 (0.11)	1.31 (0.07)	23.7
PA143	17.2 (2.1)	9.1 (0.7)	55.1 (9.0)	35 (7)	0.97 (0.14)	2.10 (0.13)	1.17 (0.07)	29.5
PA149	18.0 (1.2)	7.7 (0.4)	44.1 (11.9)	34 (8)	0.84 (0.13)	2.09 (0.13)	1.06 (0.08)	35.0
PA151	14.7 (1.0)	7.0 (0.3)	42.7 (6.2)	40 (11)	0.75 (0.18)	1.96 (0.15)	1.03 (0.07)	33.3
PA156	16.8 (1.6)	7.8 (0.6)	48.2 (7.5)	39 (5)	0.79 (0.16)	2.02 (0.13)	1.05 (0.08)	32.5
PA157	16.7 (1.7)	8.5 (0.5)	60.9 (7.5)	37 (3)	1.17 (0.12)	2.24 ((0.13)	1.20 (0.09)	23.1
PA165	18.3 (1.1)	8.2 (0.4)	49.0 (6.5)	37 (7)	0.95 (0.10)	2.07 (0.15)	1.03 (0.08)	28.5
PA168	15.2 (2.7)	7.5 (1.0)	40.7 (15.4)	40 (9)	0.69 (0.26)	1.89 (0.27)	1.03 (0.16)	36.2
PA169	16.0 (1.1)	7.9 (0.5)	47.3 (11.4)	34 (8)	1.01 (0.15)	2.09 (0.14)	1.20 (0.15)	29.1
PA171	15.3 (1.4)	7.1 (0.4)	31.2 (5.7)	42 (7)	0.50 (0.08)	1.89 (0.13)	0.99 (0.06)	47.6
PA173	15.4 (1.4)	7.5 (0.5)	50.6 (12.5)	33 (7)	1.10 (0.08)	2.22 (0.17)	1.28 (0.10)	27.6
PA175	16.7 (1.7)	7.6 (0.3)	57.9 (10.0)	43 (8)	0.88 (0.09)	2.04 (0.15)	0.97 (0.12)	26.4
PA176	18.4 (1.4)	8.3 (0.5)	63.2 (11.1)	40 (5)	0.96 (0.20)	2.17 (0.20)	1.11 (0.07)	26.0
PA179	15.9 (1.4)	7.4 (0.6)	57.8 (17.8)	40 (8)	0.94 (0.24)	2.13 (0.19)	1.26 (0.09)	26.6
PA184	13.4 (1.1)	7.7 (0.4)	46.4 (6.2)	36 (7)	0.95 (0.11)	1.83 (0.11)	1.04 (0.07)	29.2
PA185	14.0 (1.2)	7.0 (0.5)	39.2 (6.9)	33 (5)	0.75 (0.09)	2.17 (0.09)	1.07 (0.09)	40.4
PA187	20.0 (1.8)	9.1 (0.6)	65.3 (13.8)	39 (7)	1.14 (0.14)	2.33 (0.11)	1.13 (0.08)	22.5
PA189	17.1 (2.6)	8.3 (0.6)	46.3 (7.6)	32 (4)	0.89 (0.12)	2.03 (0.15)	1.10 (0.08)	35.1
PA191	19.5 (2.7)	8.7 (0.9)	57.3 (10.8)	36 (6)	1.04 (0.15)	1.89 (0.13)	1.12 (0.05)	26.7
PA194	14.8 (1.2)	7.8 (0.5)	55.4 (14.9)	38 (10)	1.07 (0.10)	2.12 (0.10)	1.26 (0.07)	24.6
PA195	19.3 (1.6)	7.9 (0.6)	54.5 (9.6)	37 (5)	1.01 (0.18)	1.98 (0.19)	1.14 (0.08)	26.8
PA196	14.3 (1.3)	7.4 (0.5)	49.6 (9.1)	37 (6)	0.99 (0.13)	2.00 (0.07)	1.18 (0.08)	27.3
PA200	16.5 (1.1)	7.8 (0.4)	41.5 (8.2)	32 (9)	0.83 (0.09)	1.96 (0.10)	1.08 ((0.03)	37.7
PA211	17.7 (1.4)	8.5 (0.6)	48.8 (9.4)	33 (6)	0.95 (0.12)	1.86 (0.15)	1.07 (0.08)	31.9
PA218	18.8 (1.7)	6.8 (0.7)	33.8 (8.0)	36 (8)	0.64 (0.15)	1.94 (0.16)	0.76 (0.07)	43.4
PA271	16.0 (1.7)	7.7 (0.5)	47.9 (10.4)	39 (11)	0.86 (0.08)	2.03 (0.11)	1.11 (0.07)	29.8
PA272	14.1 (1.8)	8.0 (1.1)	45.4 (9.3)	40 (8)	0.80 (0.10)	1.81 (0.09)	1.08 (0.08)	31.3
PA279	19.0 (1.7)	7.3 (0.9)	45.2 (8.7)	36 (5)	0.87 (0.24)	1.98 (0.19)	1.08 (0.08)	31.9
PA289	17.4 (2.0)	8.0 (0.5)	48.0 (10.7)	34 (6)	0.90 (0.07)	1.98 (0.11)	1.19 (0.07)	32.7
PA291	13.1 (1.1)	7.7 (0.4)	41.5 (6.3)	30 (6)	0.95 (0.07)	2.08 (0.10)	1.18 (0.49)	35.1
PA293	14.8 (1.5)	7.6 (0.8)	48.0 (9.0)	40 (4)	0.78 (0.10)	2.06 (0.13)	1.12 (0.06)	32.1
PA294	14.1 (1.0)	7.2 (0.6)	39.1 (11.8)	36 (7)	0.70 (0.23)	1.88 (0.19)	1.07 (0.07)	39.7
PA296	14.1 (1.6)	7.9 (0.8)	46.2 (10.6)	36 (9)	0.83 (0.14)	2.04 (0.17)	1.19 (0.10)	33.5
PA297	13.8 (1.6)	8.6 (0.8)	44.6 (13.9)	28 (6)	1.00 (0.16)	2.00 (0.21)	1.20 (0.09)	35.7
PA299	13.9 (1.3)	7.3 (0.7)	37.7 (5.8)	31 (3)	0.89 (0.20)	1.83 (0.13)	1.11 (0.08)	36.3
PA300	22.2 (1.5)	8.5 (0.4)	70.9 (7.3)	41 (4)	1.14 (0.11)	2.12 (0.19)	1.18 (0.10)	21.4
PA303	15.5 (2.2)	8.6 (0.8)	45.6 (9.8)	34 (8)	0.94 (0.14)	1.94 (0.18)	1.16 (0.08)	31.3
PA310	15.9 (1.5)	7.5 (0.5)	43.6 (10.8)	37 (7)	0.84 (0.20)	2.00 (0.20)	1.18 (0.11)	32.2
PA312	14.2 (1.4)	6.5 (0.4)	32.7 (10.0)	36 (8)	0.72 (0.15)	2.00 (0.22)	1.09 (0.09)	38.6

Standard Deviation - within parenthesis

Pigmentation of stamen filaments

In order to verify an observation made by Posnette (1945), used as a basis for identifying PA clones, that stamen filaments are unpigmented, this trait was examined in the PA clones under study. Not all of the PA clones were observed to have unpigmented stamen filaments, but that a continuous distribution of scores from absent to almost intense was observed. Thirty-eight (40%) of the accessions had unpigmented filaments while the rest had concentrations that were slight (45 accessions) or moderate (11 accessions) to intense (1 accession). (The effect of light intensity on pigment expression is taken into consideration when observing anthocyanin intensity, and thus a score of 0 was only assigned to an accession when all of the flowers sampled had no pigment.) It is thus not recommended that breeders use stamen filament colour as the sole means of identifying PA clones in germplasm collections.

Summary of results

- ❖ Twenty-four promising PA accessions were identified among the 96 studied, of which some have not been previously used in breeding. Among these, PA 4, 71 and 157 combined low to moderate (favourable) PI with high to moderate BP resistance (rating ≤ 5). PA 4 was ranked among the top clones evaluated for several agronomic traits including yield in Brazil (Pires *et al.*, 1993), was found to be moderately resistant to BP in Côte d'Ivoire (Clement and Sounigo, 1992), and was also reported to have resistance to Witches' Broom disease in Trinidad (Sreenivasan, 1987).
- ❖ PA accessions with large bean weight and low PI (Table 6) were separated from the rest of the accessions by PCA (PC1) (Figure 4), indicating the contribution of these descriptors to the variation expressed.
- ❖ Accessions with long, narrow pods with

attenuate apex form, strong basal constriction and warty surface texture (*cundeamor*) were clearly distinguished by PCA (PC2) from those with melon-shaped pods (Figure 3).

- ❖ The majority (77%) of accessions in this study had pods that matched Pound's (1938) *type* descriptions for PA germplasm, but PA 66, 184, 188, 189 and 303 did not fit into any of Pound's categories.

Discussion and Conclusion

This study demonstrated that the PA group is comprised of phenotypically diverse accessions (Figures 3 and 4) with many useful accessions possessing good values for PI and disease resistance (Figure 4, Tables 1, 5 and 6). Furthermore, the phenotypic diversity of the PA accessions compares favourably with that of 1073 accessions representing 70 accession groups of diverse origin from the ICG,T (Figure 5). This is not unexpected since the PA clones were collected over a relatively large geographic area (Pound, 1943a), and have previously been reported to be diverse in disease reactions and yield potential. Adu-Ampomah *et al.* (2005) observed that the PA accession group contained the widest range of variation for resistance to CSSV among the introductions into Ghana from Trinidad.

It is interesting to closely examine the phenotypic diversity exhibited by this accession group in relation to that of other Upper Amazon Forastero (UAF) groups, (Bekele *et al.* 2008) and a report on such a study, compiled by Bekele *et al.* (2005), has been submitted for publication. It is noteworthy that Bekele *et al.* (2006) found that UAF accession groups studied at the ICG,T were distinct from the Trinitario and Refractario groups studied.

The PA group is perhaps unique among the UAF collected by Pound in terms of its diversity in pod morphology. Indeed, the *Type 1* characteristics (Pound, 1938) are similar to those of the Nicaraguan Criollo typified by ICS (Imperial College Selection)

60. Some relatively heavy beans were also found among accessions in this group, which is uncharacteristic for *Forasteros*. PA 20, 37, 71, 137, 157, 187 and 300 had cotyledon weights of 1.14g or larger.

Pound (1945) observed that the trees above Iquitos on the Rios Ucayali and Marañon are often of immense size for cocoa, and the beans are large and plump though still uniformly dark purple in colour. In this study, data on bean thickness were not available, but the mean cotyledon length and width for the PA accessions were 2.05 cm and 1.13 cm, respectively. This differs from the *small, flat beans* normally attributed to *Forasteros*. The mean cotyledon weight for the PA accessions was 0.90 ± 0.014 g. The mean cotyledon size and weight of the PA accessions, recorded in this study, compare favourably with the mean cotyledon weight (0.97 ± 0.01 g), length (2.16 ± 0.01 cm) and width (1.21 ± 0.004 cm) recorded for 996 accessions representing 70 accession groups and various genetic groups in the ICG,T (Bekele et al., 2004). The latter include Trinitarios, which tend to have larger beans.

Pound's (1938) classification of the PA accessions into various morpho-types was supported by our observations. The majority of the accessions could be classified according to this convention.

The PA clones displaying favourable PI and, in some cases, resistance to BP in this study, were exclusively classified in Pound's (1933) types 1, 4 and 3 categories for pod shape (45.5%, 36.4% and 18.1%, respectively). These results suggest that the clones collected near Porvenir (*Types* 5 and 6) were not sources for the genes involved in the control of the aforementioned traits.

The genetic diversity of the PA group has been assessed using molecular (SSR/microsatellite) markers (Boccarra and Zhang, 2005). A study to compare the resulting classification with that based on morphological traits is planned. Molecular techniques were useful in determining whether accessions, which could not be assigned to one of Pound's (1938) *Type* categories, are in fact mislabelled. The latter include PA 66, 184, 188, 189 and 303.

Boccarra and Zhang (2005) found that PA 66, tree 1, field 5B, plot E 356 ICG,T is mislabelled as it is at Marper Farm, and has a genetic profile distinct from other PAs. Trees 9 and 11 of PA 188 in field 5B, plot F494 of the ICG,T were found to be mislabelled, and do not have the genetic profile typical of PA accessions. In fact, Tree 11 of PA 188 in field 5B has the molecular profile of a Trinitario (and is perhaps rootstock of one of the PA 188 trees at Marper). However, Boccarra and Zhang (2005) found that PA 184 and 303 do have the genetic profile typical of PA accessions. They presented no results for PA 189.

The PA group has undoubtedly provided many promising accessions for cocoa breeding. Some of the most widely used, due to the consistently good traits displayed in different countries, include PA 7, PA 107, PA 120, PA 121, PA 150, PA 169 and PA 300 (Table 1). Other promising accessions, which have not been used widely or at all for breeding, include PA 4, 13, 30, 46, 71 and 73 (Table 1) and PA 157 (Table 5). Sounigo et al. (2005a) selected PA 13, 39, 70, 120, 121, 124, 126, 156, 169, 279, 299, and 303 based on resistance or tolerance to several diseases, including BP and WB, and important agronomic traits including bean size, PI and butterfat content. Twenty-four promising PA accessions were identified among the 96 studied at the ICG,T (Table 5), some of which have not been previously used in breeding. The promising clones include PA 4, 71 and 157, which combine favourable PI and BP resistance, and have been included in CRU's germplasm enhancement programme (Iwaro et al., 2003).

To exploit the wide phenotypic variation observed in this accession group and the promising accessions identified, it is recommended that selected PA parents be incorporated in future recurrent breeding programmes aimed at simultaneous multi-trait improvement. As advocated by Kennedy (1995), a "slow accumulation of good characters over many generations" can be achieved. The Ministry of Agriculture, Trinidad has had significant progress using such a breeding strategy, albeit with a narrow

genetic base (Kennedy *et al.*, 1987), over a period of thirty years beginning in 1949. Genes for resistance to Witches Broom disease (WB) and *Ceratocystis* wilt were incorporated through the use of the clones SCA 6 and IMC 67 (*Forasteros*) as parents, respectively. ICS 1 and 95 (*Trinitarios*) were selected as parents for heavy bearing, large bean weight and flavour (Freeman, 1969). Some of the resulting hybrids have been designated Trinidad Selected Hybrids (TSH). Several TSH clones have been obtained with increased resistance to the aforementioned diseases and favourable agronomic traits, and have demonstrated heterosis. Twelve TSH selections and their progenies have been made available to local farmers. Currently, the TSH breeding programme is being advanced, after a 20-year period of inactivity, using several superior TSH, PA and other UAF and *Trinitario* clones as parents.

This overview has underscored the value of the PA clones from a breeder's perspective. It is envisaged that based on the findings of the various breeding programmes reviewed, the incoming results of the CFC/ICCO/Bioversity International projects, and the evaluation exercises at the ICGT further value will be ascribed to these clones in the process of cacao genetic improvement.

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