Short Scientific Report

Journal of Plantation Crops, 2014, 42(2): 246-251

Variability analysis in bean characters of selected accessions of cocoa (*Theobroma cacao* L.)

A.C. Asna*, K.T. Presannakumari, J.S. Minimol[1] and S. Krishnan[2]

Department of Plant Breeding & Genetics, College of Horticulture, Kerala Agricultural University, Vellanikkara, Kerala, India

[1]Department of Plant Breeding & Genetics, Cocoa Research Center, KAU, Vellanikkara, Kerala, India

[2]Department of Agricultural Statistics, K AU, Vellanikkara, Kerala, India

(Manuscript Received: 19-03-14, Revised: 21-05-14, Accepted: 27-06-14)

Keywords: Bean weight, cocoa, clustering, flat bean

Cocoa (*Theobroma cacao* L.) is an important beverage crop grown all around the world for the delicious chocolates. Cocoa beans form the economic part and bean size is an important factor deciding the yield of cocoa (Soria, 1978). The morphological and structural characters of beans often exhibit ample amount of variations among accessions (Adewale *et al*., 2010). Variations in colour, size and weight of beans are related to the race, cultivar group and place of origin. Significant variations among the different accessions of cocoa for the traits related to dried bean may provide trustworthy criteria for selection. In the present study, the variability in bean traits and the genetic diversity among the forty accessions were evaluated.

Forty clonal accessions of cocoa, belonging to diverse countries of origin, selected from the germplasm maintained at Cocoa Research Centre, Vellanikkara served as the material for the study. Among the forty accessions, twelve belonged to Ecuador (COCA 3370-3, AMAZ 10-1, AMAZ 15, AMAZ 6-3, AMAZ 3-2, B5-7, CLM 90, LV 28, EET 400, IMC 16, EET 397, EQX 3348-44), eight to French Guiana (PINA, B7 B2, KER 2 E, B7 B4, B7 B5, KER 9, B7 A6, GU 310) seven to Peru (PA 56, IMC 67, SCA 6, PA 137, PUCALA 1, IMC 54, IMC 14) five to Trinidad & Tobago (DOM 4, GDL 3, MAR 9, DOM 25, ICS 95), three to Brazil (SC 10, BE 3, RB 33/3), two to Costa Rica (UF 677, Criollo), two to Mexico (R (10) (MEX), R (39) (MEX)) and one to Columbia (SPEC 160-9). All these accessions, laid out in RBD with 3 replications are in the steady bearing stage (9 years).

Fifteen mature pods (5 from each replication) of uniform size and maturity were harvested from each accession for recording observations. Total wet weight of beans pod^{-1}, number of beans pod^{-1} and number of flat beans pod^{-1} were recorded for each accession. Fifteen beans were then selected at random from each accession. The outer slimy layer was removed before taking observations on wet bean characters. The wet weight, length, breadth and thickness of peeled beans as well as the colour of cotyledons were recorded for the beans of each accession. The length, breadth and thickness of peeled beans were measured using vernier caliper following the method suggested by Kaushik *et al.* (2007). The colour of cotyledons were recorded based on the descriptor for cocoa (Bekele and Butler, 2000). The dry weight of peeled beans was taken after oven drying the beans to moisture content below 8 per cent.

The means per replication for each of the eight quantitative characters were then generated accessions wise and subjected to Analysis of Variance. The descriptive statistics *viz.,* mean, standard deviation, standard error, phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic gain for these

**Corresponding Author: asna.ac@gmail.com*

eight bean characters were then computed. Pearson correlation coefficients were calculated to understand the relationship between pairs of morphological traits.

The genetic associations among the accessions were estimated by Jaccard's similarity coefficients (Jaccard, 1908) using NTSYS pc version 2.1 (Rohlf, 1992). Cluster analysis was performed based on the similarity matrix and dendrogram was constructed by unweighted pair-group method (UPGMA) (Sneath and Sokal, 1973) for the accessions evaluated.

Wide variability was observed among the accessions for cotyledon colour (Table 1). In the descriptor, only six states *viz.,* white, grey, light purple, medium purple, dark purple and mottled are given for cotyledon colour. However, in the present investigation mixture of different coloured beans was also observed in the pods of certain accessions. Hence, an additional score of seven was used to represent the mixed types. Light purple coloured cotyledons were observed in the accessions IMC 67 and EQX-3348-44. Medium purple coloured cotyledons were present in twelve accessions. Majority of accessions were having beans with dark purple coloured cotyledons. The accessions SC 10, GU 310, AMAZ 15, AMAZ 6-3, R (10) (MEX) and Criollo exhibited the presence of mixture of beans having cotyledon colour ranging from white to dark purple within a single pod. The typical Criollo types are often characterized by the presence of white beans alone as reported by Wood (1975). Hence, the presence of mixed beans in the pods of the accession Criollo evaluated in the present study is an indication of the variability crept in by natural out crossing.

Table 1. Variability in cotyledon colour among the 40 accessions of cocoa

Bean colour & grade	No. of accessions	Name of accessions
Light purple (3)	2	IMC 67, EQX-3348-44
Medium purple (4)	17	COCA 3370-3, BE 3, AMAZ 3-2, UF 677, B7 B2, MAR 9, CLM 90, KER 9, B7 B5, R (39) (MEX), EET 400, IMC 16
Dark purple (5)	24	AMAZ 10-1, PINA, PA 56, DOM 4, KER 2E, B7 B4, GDL 3, B5-7, IMC 14, IMC 54, PUCALA 1, SPEC 160-9, RB 33/3, EET 397, ICS 95, SCA 6, PA 137, DOM 25, LV 28, B7 A6
Mixed (7)	7	SC 10, AMAZ 15, AMAZ 6-3, GU 310, R (10) (MEX), Criollo

The mean values of the different bean quantitative characters for the 40 accessions are presented in Table 2. The flat bean content of the pods is expressed as a percentage of the total number of beans pod^{-1}, for easiness of comparison among the accessions. The selected accessions of cocoa differed significantly with respect to the eight quantitative characters reflecting the heterogeneity among these accessions. The total wet bean weight pod^{-1} was the highest for the accession EET 400 (206.08 g) and the lowest for the accession KER 2E (42.21 g). The number of beans pod^{-1} ranged from 22.93 (SPEC 160-9) to 49.27 (IMC 54). The flat bean content was the highest in accession LV 28 (12.60%) followed by AMAZ 6-3 (10.42%) (Table 2). The pods of accession EQX-3348-44 were found to be devoid of flat beans. The flat beans in cocoa are considered to be developing from the unfertilized ovules. Hence, the presence of flat beans is taken as an undesirable character in cocoa. The crop improvement programmes in cocoa aims to reduce the number of flat beans in the pods. The wet weight of peeled bean ranged from 1.17 g in Criollo to 2.00 g in SPEC 160-9 and dry weight from 0.58 g in Criollo to 1.72 g in SPEC 160-9 respectively among forty exotic accessions of cocoa evaluated.

Bean size is one of the most important components of yield in cocoa (Soria, 1978). Significant variability was observed among the accessions evaluated for bean size represented by length, breadth and thickness (Table 2). Monteiro *et al.* (2009) has remarked that cocoa accessions with dry bean weight, with peel, higher than 1 g or dry bean weight, without peel, higher than 0.8 g are superior**.** Among the evaluated accessions KER 2E, CLM 90, KER 9, IMC 54 and Criollo failed to satisfy the international standard (Table 2) in the

Table 2. Mean values of bean characters of 40 accessions of cocoa

Accessions	Total wet bean wt. pod^{-1} (g)	No. of beans pod^{-1}	Flat beans pod^{-1} (%)	Peeled bean (mm)			Wt. of peeled bean (g)	
				Length	Breadth	Thickness	Wet	Dry
SC 10	150.19	33.66	3.25	19.77	11.03	5.95	1.77	1.53
COCA 3370-3	187.57	30.66	4.16	19.30	9.14	6.77	1.75	1.37
AMAZ 10-1	125.67	39.00	2.18	16.96	8.02	4.10	1.23	0.83
BE 3	132.20	35.73	3.43	15.71	8.89	4.78	1.34	0.94
AMAZ 15	150.22	36.13	1.45	18.24	9.43	7.25	1.63	1.25
AMAZ 6-3	110.90	33.27	10.42	20.90	10.25	4.70	1.72	1.39
AMAZ 3-2	124.46	35.13	2.96	15.78	8.65	5.45	1.33	1.01
PINA	146.59	42.33	1.56	17.28	9.82	4.89	1.41	1.07
B7 B2	135.47	36.47	3.70	18.49	10.65	6.51	1.88	1.49
PA 56	116.74	38.13	4.68	16.56	9.55	3.95	1.29	0.89
DOM 4	136.25	24.07	0.82	15.56	9.01	4.88	1.39	0.92
KER 2 E	42.21	41.67	0.64	13.89	8.84	4.17	1.39	0.75
R(10) (MEX)	131.70	49.20	0.81	21.29	12.57	6.29	1.95	1.68
B7 B4	114.57	37.87	0.53	17.42	9.39	7.30	1.59	1.26
UF 677	158.73	42.80	1.09	20.82	11.95	4.61	1.76	1.35
GDL 3	148.44	29.60	0.67	17.43	10.49	4.96	1.54	1.18
B5-7	98.99	34.87	1.89	18.83	8.89	5.96	1.70	1.16
MAR 9	129.99	38.27	1.03	17.71	10.25	5.44	1.64	1.28
CLM 90	115.09	42.73	1.54	14.37	7.62	3.22	1.36	0.71
R (39) (MEX)	135.26	32.27	2.03	18.24	10.79	5.89	1.74	1.21
B7 B5	87.32	40.27	0.81	16.98	9.43	5.65	1.43	0.97
DOM 25	124.48	33.07	0.39	15.68	8.29	5.28	1.30	0.93
KER 9	102.77	38.53	1.71	15.72	7.84	3.90	1.34	0.71
LV 28	128.97	35.60	12.60	19.63	11.76	4.37	1.66	1.16
B7 A6	95.98	39.20	1.01	16.09	8.83	4.02	1.33	0.87
GU 310	152.83	37.93	1.74	19.02	9.76	4.29	1.55	1.18
EET 400	206.08	47.07	0.15	20.90	9.92	6.78	1.72	1.37
IMC 16	149.22	41.20	1.13	19.12	9.69	4.05	1.41	1.01
EET 397	144.84	36.93	0.73	17.22	9.69	5.36	1.53	1.03
ICS 95	129.20	40.80	0.49	18.68	11.44	5.56	1.67	1.35
IMC 67	186.80	36.80	1.42	17.18	9.18	4.15	1.43	1.10
SCA 6	95.24	37.20	1.92	18.34	7.63	4.77	1.35	0.81
PA 137	99.77	31.07	1.49	20.22	9.69	5.01	1.35	1.07
RB 33/3	64.59	39.80	0.50	21.36	9.82	3.20	1.41	1.07
SPEC 160-9	152.01	22.93	0.82	18.34	10.48	5.79	2.00	1.72
EQX-3348-44	75.25	44.80	0.00	18.48	9.09	3.36	1.39	0.88
PUCALA 1	107.52	48.27	0.14	15.51	9.76	3.81	1.53	1.03
IMC 54	118.07	49.27	0.14	17.81	8.02	4.36	1.33	0.73
IMC 14	96.10	49.07	0.41	18.12	8.89	2.85	1.45	1.13
Criollo	119.21	35.53	2.92	12.83	7.05	2.25	1.17	0.58
CD	23.68	5.97	0.65	1.45	0.77	0.56	0.14	0.13

Table 3. Descriptive statistics of exotic accessions of cocoa

Character	Range		Mean	SD	SE	PCV	GCV	H^2	GG	Genotypic
	Minimum	Maximum				(%)	(%)	(%)	(%)	F-values
Total wet bean wt. (g)	42.21	206.08	125.88	31.33	4.95	13.43	24.69	86.47	23.92	14.61**
No. of beans pod^{-1}	22.93	49.27	37.98	6.10	0.964	17.36	14.37	68.51	24.51	8.92**
No. of flat beans pod^{-1}	0.00	5.13	0.79	0.98	0.154	131.68	121.68	85.38	231.61	13.33**
Length of peeled bean (mm)	12.83	21.36	17.79	2.03	0.322	13.16	11.21	94.04	25.51	17.24**
Breadth of peeled bean (mm)	7.05	12.57	9.54	1.22	0.193	13.43	12.49	86.47	23.93	23.01**
Thickness of peeled bean (mm)	2.25	7.30	4.90	1.19	0.188	25.03	24.03	92.15	47.51	37.13**
Wet wt. of peeled bean (g)	1.17	2.00	1.52	0.20	0.322	14.17	13.05	84.89	24.78	19.56**
Dry wt. of peeled bean (g)	0.58	1.72	1.10	0.27	0.042	25.03	24.02	92.07	47.47	35.46**

** Level of significance at p<0.01.

*PCV & GCV (Sivasubramanian & Madhavamenon, 1973) - Low: Less than 10%, Moderate: 10-20%, High: More than 20%

*H^2 (Johnson *et al.,* 1955) - Low: Less than 30%, Moderate: 30-60%, High: More than 60%

*GG (Johnson *et al.,* 1955) - Low: Less than 10%, Moderate: 10-20%, High: More than 20%

case of dry weight of peeled bean. Most of the accessions involved in the present study are satisfying the international standard. Pound (1932) and Enriquez and Soria (1966) revealed that yield expressed as dry or wet weight of bean is a highly variable character and it varied from 0.5 g to 2.5 g depending on the accession. However, in the present study the range observed was from 0.58 g to 1.72 g only. This can be due to the differences in the accessions used in the present study. Among the accessions evaluated R (10) (MEX) was found to be a desirable one with low flat bean content pod^{-1} (0.81%) coupled with high number of beans pod^{-1} (49.20) and dry weight of peeled bean (1.68 g). This was followed by EET 400.

The descriptive statistics *viz.,* range (minimum & maximum), mean, standard deviation (SD), standard error (SE), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2) and genetic gain (GG) for the eight quantitative characters studied in 40 accessions of cocoa are presented in Table 3. Moderate to high phenotypic and genotypic coefficients of variation were observed for the various bean characters as per the classification proposed by Johnson *et al.* (1955). Among the bean characters evaluated, number of flat beans pod^{-1} exhibited maximum variability as indicated by its high PCV (131.68%) and GCV (121.68%). The length of peeled bean exhibited the highest heritability value (94.04%) and the number of flat beans pod^{-1} showed the highest genetic gain (231.61%). The high amount of variability coupled with high heritability (85.38%) and high genetic gain for flat beans pod^{-1} indicates the considerable scope for selection programmes for improving the

Table 4. Pearson correlation coefficients of seven morphological traits on cocoa beans

	Total wet bean wt.	No. of beans pod^{-1}	No. of flat beans pod^{-1}	Length of peeled bean	Breadth of peeled bean	Thickness of peeled bean	Wet wt. of peeled bean	Dry wt. of peeled bean
Total wet bean wt.	1	.423 **	.054 ns	.315 **	.248 **	.406 **	.439 **	.420 **
No. of beans pod^{-1}		1	-.126 ns	.055 ns	.069 ns	.035 ns	.004 ns	.070 ns
No. of flat beans pod^{-1}			1	.227 **	.245 **	-.005 ns	.213 **	.198 **
Length of peeled bean				1	.711 **	.365 **	.688 **	.780 **
Breadth of peeled bean					1	-.457 **	.713 **	.777 **
Thickness of peeled bean						1	.571 **	.613 **
Wet wt of peeled bean							1	.883 **
Dry wt. of peeled bean								1

population based on this character. All the other bean characters also exhibited high heritability according to the classification proposed by Johnson *et al.* (1955). High broad sense heritability is a good indicator of reliability for genetic improvement of phenotypic traits (Adewale *et al.,* 2010). The high broad sense heritability obtained in this study indicates that the contribution of the environmental factor to the phenotypic variances of these eight quantitative traits in cocoa is low.

The correlation coefficients among the different bean characters are presented in Table 4. The total wet bean weight is positively and significantly correlated with the number of beans pod^{-1}, length, breadth, thickness, wet weight and dry weight of peeled bean. The number of flat beans pod^{-1} was found to have no significant correlation with any of the other variables. The bean length had a positive and significant correlation with the bean width and thickness (r = 0.711 and 0.365 respectively). However, the correlation between bean width and its thickness was negative and significant. The wet and dry weight of peeled bean had positive and significant correlation with all the characters except number of flat beans pod^{-1}. The very high positive correlation of the weight of the bean to its length, breadth and thickness depicts the significance of length, breadth and thickness in determining the bean weight.

Agglomerative hierarchical clustering was performed using the Jaccard's similarity co-efficient matrix by unweighted pair group method (Sneath & Sokal, 1973) and the resulting dendrogram is presented in Figure 1. The accessions are highly variable based on the eight quantitative characters

Table 5. Group of accessions having 100 per cent similarity

Group	Accessions
I	AMAZ 10-1, IMC 54
II	EET 397, PUCALA 1
III	AMAZ 6-3, EET 400
IV	AMAZ 3-2, Criollo
V	DOM 4, KER 2E
VI	B7 B4, RB 33/3, IMC 14, GDL 3
VII	UF 677, IMC 16
VIII	SCA 6, PA 137
IX	BE 3, KER 9
X	PINA, B5-7, CLM 90, R (39) (MEX), GU 310
XI	MAR 9, R (10) (MEX), B7 A6
XII	AMAZ 15, IMC 67

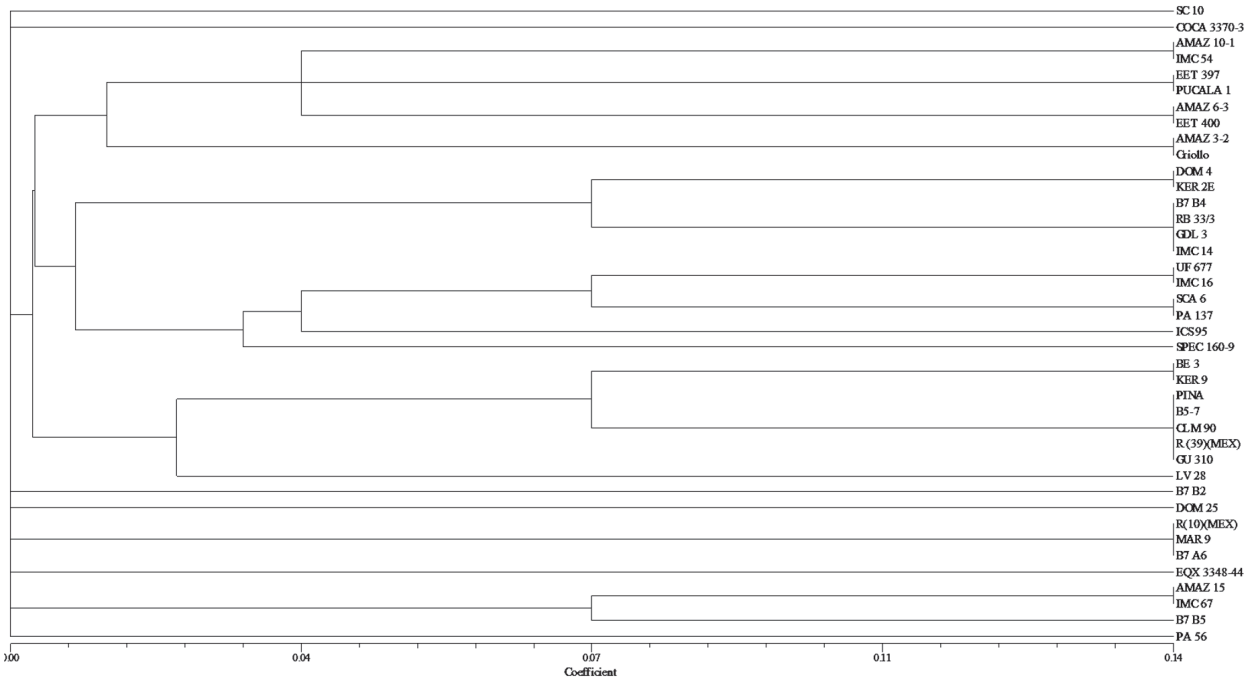

Fig. 1. Dendrogram based on similarity coefficient among 40 accessions of Cocoa

of beans. The accessions SC 10, COCA 3370-3, B7 B2, PA 56 and DOM 25 were found to be distinct from all other accessions as they were found to remain as independent units even at one percent similarity level. These accessions belonged to different countries of origin also. So these accessions can be used in breeding programmes for getting hybrids superior in bean traits. Some of the accessions evaluated were found to be exactly identical as per the dendrogram (Fig. 1) and the list is presented in Table 5.

The forty clonal accessions of cocoa belonging to diverse countries of origin in the present study showed wide variability for the bean characters evaluated. The high variability coupled with high heritability and genetic gain for flat beans pod^{-1} points to the possibility of improving the population following selection programmes. Five accessions *viz.,* SC 10, COCA 3370-3, B7 B2, PA 56 and DOM 25 belonging to Brazil, Ecuador, French Guiana, Peru, and Trinidad & Tobago respectively were found to be distinct in cluster analysis indicating the possibility of their use in hybridization programmes.

Acknowledgement

The financial assistance given by Kerala Agricultural University, Vellanikkara for the MSc. (Ag.) programme of the first author is acknowledged.

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