International Journal of Agricultural Sciences ☞ DOI:10.15740/HAS/IJAS/16.1/11-16
Volume 16 | Issue 1 | January, 2020 | 11-16 ■ ISSN : 0973-130X Visit us : *www.researchjournal.co.in*

RESEARCH PAPER

Fat, polyphenol and minerals estimate in exotic accessions of cocoa (*Theobroma cacao* L.)

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Abstract : Cocoa is earmarked for its products, made out of fermented and dried beans. Cocoa butter is the major ingredient in chocolate which help us to enjoy the melted chocolate in the mouth. Due to its elevated anti-oxidant property it is used many pharmaceutical and cosmetic products. Fat content above 45 per cent can be considered as a desirable trait and the accessions NA 149 (50.38%), PNG 418 (50.21%) etc. were with high fat content in beans can be selected for further breeding programme. Polyphenols comprise of 12-18 per cent of the total bean weight and it mainly influences the chocolate colour. It is also reported to have high anti-oxidant property. The highest total phenol content was observed in genotypes ICS 41 (11.81%) followed by CRU 12 (11.60%) and IMC 20 (11.27%). Cocoa beverage is considered to be a good source of calcium, potassium and sodium and is very much desirable for health. Genotype JA 10/12 showed highest sodium content of 1.98mg/100g. MATINA 1/7 (2002.07mg/100g) expressed highest potassium content and calcium content was highest for the accession CRU 12 (329mg/100g). Cluster analysis was carried out by using D^2 statistics. All the accession was highly variable and even at 25 per cent similarity level, majority of accessions found to remain as independent units. Breeding for quality is the current trend in cocoa breeding. Hence, the wide variability observed among the exotic genotypes can be exploited for developing superior varieties with improved biochemical properties.

Key Words : Cocoa, *Theobroma cacao* L., Cocoa butter, Polyphenol, Mineral content, Biochemical

View Point Article : Veeresh, S.A., Minimol, J.S., Suma, B., Athira, M.R. and Shilpa, K.S. (2020). Fat, polyphenol and minerals estimate in exotic accessions of cocoa (*Theobroma cacao* L.). *Internat. J. agric. Sci.*, **16** (1) : 11-16, **DOI:10.15740/HAS/IJAS/16.1/11-16.**

Article History : Received : 25.10.2019**; Revised :** 02.11.2019**; Accepted :** 03.12.2019

INTRODUCTION

Cocoa is a significant rainforest tree that was grown by Mayas in Northern parts of America, over 1500 years ago and subsequently dispersed to Central America (Miranda, 1962 and Motamayor *et al*., 2002). Cocoa is earmarked for its products, made out of fermented and dried beans. Cocoa butter is the commercial product obtained from the seeds of cocoa. Cocoa seeds have more fat than any crop apart from coconut. It is the major ingredient for chocolate. Cocoa butter melts in body temperature making us to enjoy the melted chocolate in the mouth. In addition to this it also found application in multiple products like cosmetics, pharmaceuticals etc. Cocoa butter is used as an anti-inflammatory and anti-ageing agent. All these are feasible due to the elevated

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anti-oxidant content present in them (David *et al*., 2011). Polyphenols is present in seeds of cocoa, which gives flavour and colour to the chocolate. The phenolics from cocoa is reported to have protecting capacity against many diseases including cancer in which oxidative stress is a contributing factor (Andujar *et al*., 2012). Mineral content in cocoa beans is very important for product manufacturing and also for the consumer preference. As opined by many scientist (Stienberg *et al*., 2003 and Scapignini *et al*., 2014), most of the minerals affects cardio-vascular system in a positive way.

MATERIAL AND METHODS

Thirty accessions were evaluated for biochemical characters such as fat, polyphenol content and minerals (Na, K, Ca) (Table A). Five ripened pods were harvested from each accession based on the maturity indices. The beans were taken out from all five pods and pooled for analysis. From the lot, twenty beans were taken at random and peeled by using forceps. These beans were dried under sun or in hot air oven until moisture attains an optimum level of 8 per cent. It took seven days to attain optimum moisture condition in sun drying. The dried beans were grind to fine powder form by using a laboratory grinder. Then the powder is used for various biochemical analysis.

Fat content of beans for each accession was estimated by Soxhlet apparatus method given by Sadasivam and Manickam (1996). The cocoa beans were defatted by using petroleum ether to extract the fat using soxhlet apparatus. Ten grams of cocoa bean powder was used for the analysis. Fat settled down due to siphoning of petroleum ether at the round bottom of the flask was weighed and expressed as per cent.

Phenol content was estimated using Folin – Ciocalteau (FC) reagent method proposed by Malik and Singh (1980) 500 mg of defatted cocoa powder was taken for the analysis. The absorbance of the extract was read at 650 nm, using spectrophotometer. Concentration of phenols present in the extract was worked out by substituting the absorbance value thus, obtained, in the calibration equation. The total phenol content was calculated as mg catechol equivalent of phenol per gram of sample.

Minerals like calcium, potassium and sodium present in nibs were also estimated using standard procedures as described by AOAC (2005) with modification. Cocoa bean powder (0.5 g) was used for analysis. The prepared sample solution was subjected to flame photometer to estimate the sodium, potassium and calcium concentration.

Table A: Exotic germplasm used for the study

Sr. No.	Genotypes	Derivations
A1	CRU12	Cocoa Research Unit
A2	ICS 29	Imperial College Selections
A3	ICS 41	Imperial College Selections
A4	MO 109	river Morona
A5	GDL 7	GuaDeLoupe
A6	PA 194	PLAYA ALTA [VEN]
A7	SIAL 339	Selecao Instituto Agronomico do Leste
A8	TARS 31	USA. (UK - Reading Quarantine)
A9	GU 261/P	GUF, French Guiana - CIRAD-CP
A10	LZ 28	Large vuelta Z
A11	NA 149	river NAnay
A12	MATINA 1/7	Matina/R. Matina, CRI
A13	PA 303	PLAYA ALTA [VEN]
A14	PNG 87	Papua New Guinea
A15	PA 156	PLAYA ALTA [VEN]
A16	LX 43	Large vuelta X
A17	POUND 4/B	POUND (collector)
A18	PNG 418	Papua New Guinea
A19	JA 10/12	Ji-pArana
A20	F 303	COL. (UK - Reading Quarantine)
A21	T 85/799	Trinidad
A22	DOM 14	DOMinica
A23	PNG 250	Papua New Guinea
A24	PNG 336	Papua New Guinea
A25	IMC 20	Iquitos Mixed Calabacillo (Mid Channel)
A26	EET 397	Estacion Experimental Tropical
A27	ICS 75	Imperial College Selections
A28	DOM 25	DOMinica
A29	POUND 18	POUND (collector)
A30	POUND 16/A	POUND (collector)

RESULTS AND DISCUSSION

Beans are the major commercial part of cocoa and their quality depends upon the fat, polyphenol and mineral content. Present study showed significant difference in biochemical characters. Result of biochemical characters such as fat content, polyphenol content, mineral content (sodium, potassium and calcium) are summarized in Table 1 and Fig. 1 to 3.

Table 1 : Biochemical characters of cocoa accessions

Genotype	Fat (%)	Polyphenol (%)
CRU12	44.80(49.67)	11.60(19.92)
ICS 29	41.35(43.67)	8.85(17.31)
ICS 41	44.23(48.67)	11.81(20.11)
MO 109	44.22(48.67)	8.06(16.49)
GDL 7	47.86(54.33)	9.08(17.54)
PA 194	45.19(50.33)	8.96(17.42)
SIAL 339	47.67(54.67)	9.45(17.85)
TARS 31	45.19(50.33)	8.51(16.85)
GU 261/P	45.28(50.50)	7.21(15.58)
LZ 28	45.38(50.67)	8.10(16.54)
NA 149	50.38(59.33)	8.35(16.80)
MATINA 1/7	45.76(51.33)	7.56(15.97)
7.56(15.97)	45.38(50.67)	6.34(14.55)
PA 303	45.19(50.33)	9.74(18.18)
PNG 87	45.95(51.67)	8.93(17.39)
PA 156	45.00(50.00)	8.36(16.77)
LX 43	44.80(49.67)	8.56(17.00)
POUND 4/B	50.21(59.00)	9.69(18.14)
PNG 418	44.23(48.67)	7.19(15.56)
JA 10/12	43.08(46.67)	8.40(16.85)
F 303	45.95(51.67)	9.49(17.94)
T 85/799	46.92(53.33)	10.96(19.33)
DOM 14	46.33(52.33)	9.03(17.49)
PNG 250	43.65(47.67)	8.30(16.75)
PNG 336	44.61(49.33)	11.27(19.57)
IMC 20	45.00(50.00)	7.87(16.30)
EET 397	45.00(50.00)	8.88(17.34)
ICS 75	43.27(47.00)	8.12(16.56)
DOM 25	47.48(54.33)	9.47(17.93)
POUND 16/A	45.19(50.33)	8.20(16.65)
CV (%)	4.41	4.83
C.D. (P=0.05)	3.280	1.370

Values in parenthesis are transformed values by using Angular transformation

The genotype NA 149 showed highest value for fat content (50.38%) followed by PNG 418 (50.21%). Among the thirty genotypes fat content ranged from 41.35 per cent in ICS 29 to 50.38 per cent in NA 149. A similar study was conducted by Wood and Lass (1985) and estimated fat content ranged from 45 per cent to 53 per cent. High fat content in cocoa beans is a major attribute to obtain the characteristic flavour and texture in chocolate (Mossu, 1992). Generally fat content was estimated by Soxhlet apparatus method and it ranges from 45-55 per cent in Forestero types in most desirable climatic conditions (Rohan,1963; Reineccius *et al*.,1972; Wood and Lass,1985 and Afoakwa *et al*., 2008). The fat content of the beans in the present study was observed to be slightly lower than that of the reported values. However, reports are there that fat content above 45 per cent can be considered as a desirable trait (Afoakwa *et al*., 2013). In the present study, sixty per cent of accessions expressed fat content above 45 per cent. The accessions like NA 149 (50.38%), PNG 418 (50.21%) etc. with high fat content in beans can be selected for further breeding programme.

The total polyphenol content in the cocoa beans extracts of the exotic genotypes were determined by Folin-Ciocalteau procedure given by Sadasivam and Manickam (1996). Polyphenols comprise of 12-18 per cent of the total bean weight and it mainly influences the chocolate colour (Kim and Keeney, 1984). The highest total phenol content was observed in genotypes ICS 41 (11.81%) followed by CRU 12 (11.60%) and IMC 20 (11.27%) (Table 1). The lowest total phenol content was observed in genotype PA 303 (6.34%). Polyphenol content in cocoa beans was reported to have antioxidant property which is beneficiary to human health (Schinella *et al.*, 2010). Hence, the accession with high polyphenol content can be selected for breeding programme to improve the health benefits of cocoa.

Minerals like sodium, potassium and calcium was estimated using flame photometer. Knowledge about the level of desirable and undesirable mineral content in cocoa beans is very important for the manufactures and consumers. Cocoa beverage is considered to be a good source of calcium, potassium and sodium and is very much desirable for health (Olaofe *et al*., 1987). In their study they reported that cocoa beans have an average of 21µg/ g sodium, potassium of 2480µg/ g and calcium 25.5µg/ g. Present study revealed significant difference in mineral content among the genotypes. Sodium, potassium and calcium content of cocoa beans are summarised in Fig. 1, 2 and 3, respectively. Genotype JA 10/12 showed highest sodium content of 1.98mg/100g followed by TARS 31, GU 261/P and IMC 20 having 1.97mg/ 100g. The lowest sodium content was observed in genotype MATINA 1/7 with 1.31mg/ 100g. Among the thirty accessions, MATINA 1/7 (2002.07mg/ 100g) expressed highest potassium content. The lowest value for potassium was expressed by PNG 250 (848mg/ 100g). Calcium content was highest for the accession CRU 12

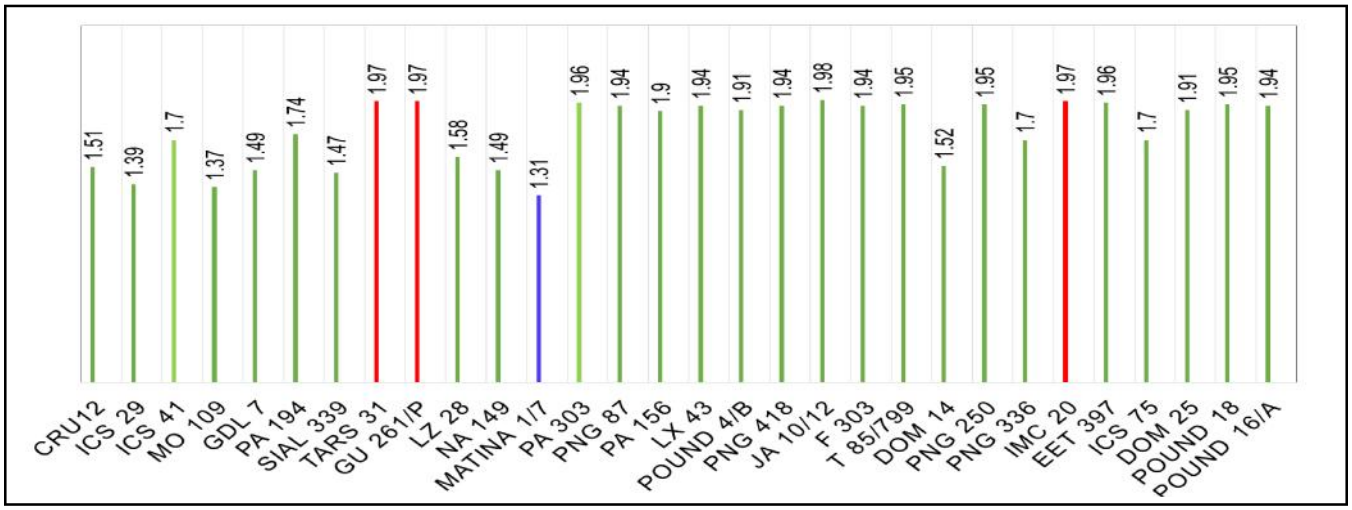

Fig. 1 : Variability in sodium content

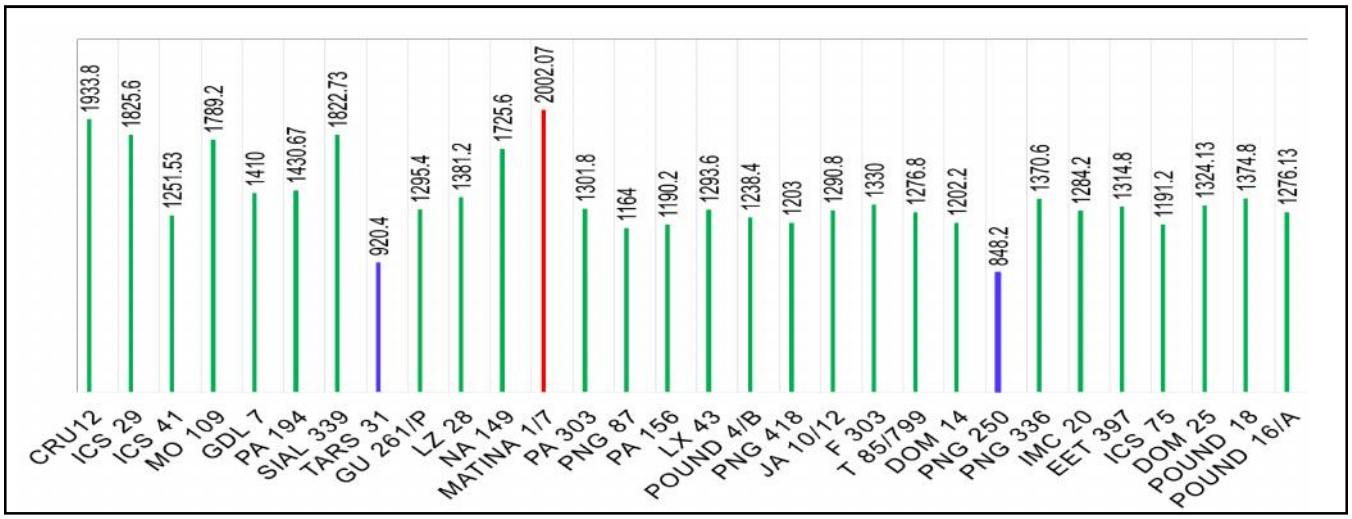

Fig. 2 : Variability in potassium content

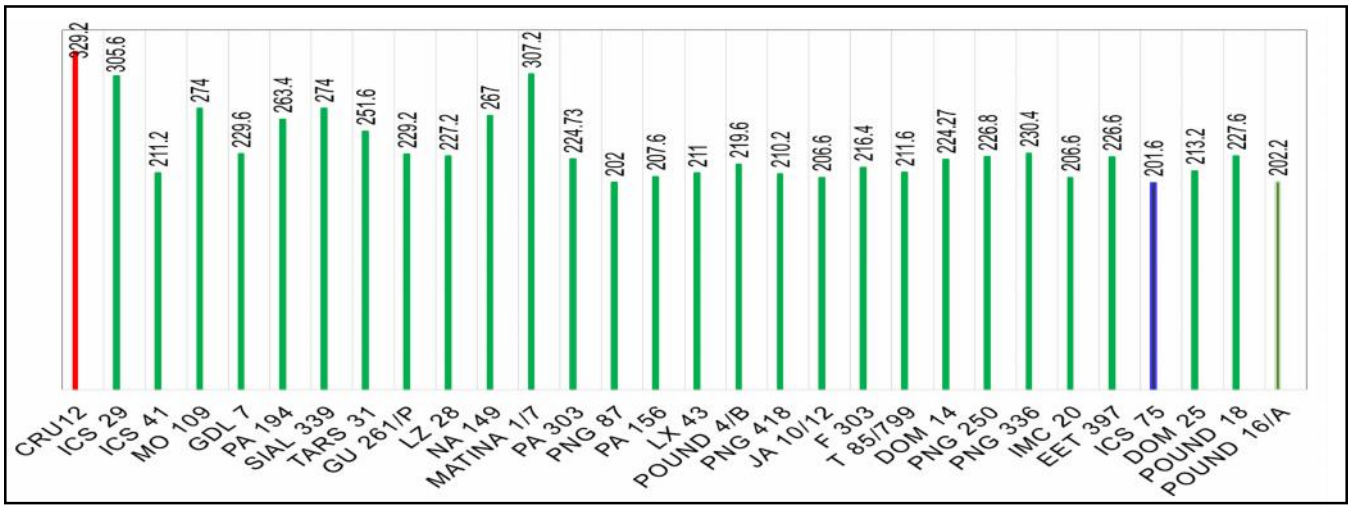

Fig. 3 : Variability in calcium content

(329mg/100g) followed by MATINA 1/7 with 307.20mg/ 100g and ICS 29 with 305.60mg/ 100g. The lowest value for calcium was observed in genotype ICS 75 (201.60mg/ 100g). Afoakwa *et al*. (2013) made an attempt to quantify mineral content in Ghanaian cocoa. The result revealed that sodium content (2.5 to 3.5mg/ 100g) was high and calcium content was low (140.2 to 170.8mg/ 100g) in those accessions when compared with the values of present study.

Cluster analysis was carried out by using D^2 statistics and dendrogram was constructed by Agglomerative method (Day and Edelsbrunner, 1984) and represented in Fig. 4. All the accession was highly variable based on biochemical characters such as fat, polyphenol, sodium, potassium and calcium. Even at 25 per cent similarity level, majority of accessions found to remain as independent units. Hence, clustering was made at 20 per cent of similarity and the details are presented in Table 2 and Fig. 4. In total, 15 clusters were formed

Table 2: Clustering based on biochemical characters in cocoa

Cluster number	No. of genotypes	Genotypes
Cluster I	2	CRU 12, POUND 4/B
Cluster II	1	DOM 25
Cluster III	1	ICS 29
Cluster IV	2	ICS 41, JA 10/12
Cluster V	1	PNG 336
Cluster VI	3	LX 43, ICS 75, EET 397
Cluster VII	4	PA 194, TARS 31,PNG 87, POUND 16/A
Cluster VIII	2	PNG 418, F 303
Cluster IX	2	GU 261/P, IMC 20
Cluster X	2	LZ 28, PA 303
Cluster XI	2	DOM 14, PA 156
Cluster XII	3	T 85/799, PNG 250, POUND 18
Cluster XIII	1	MATINA 1/7
Cluster XIV	2	GDL 7, NA 149
Cluster XV	2	MO 109, SIAL 339

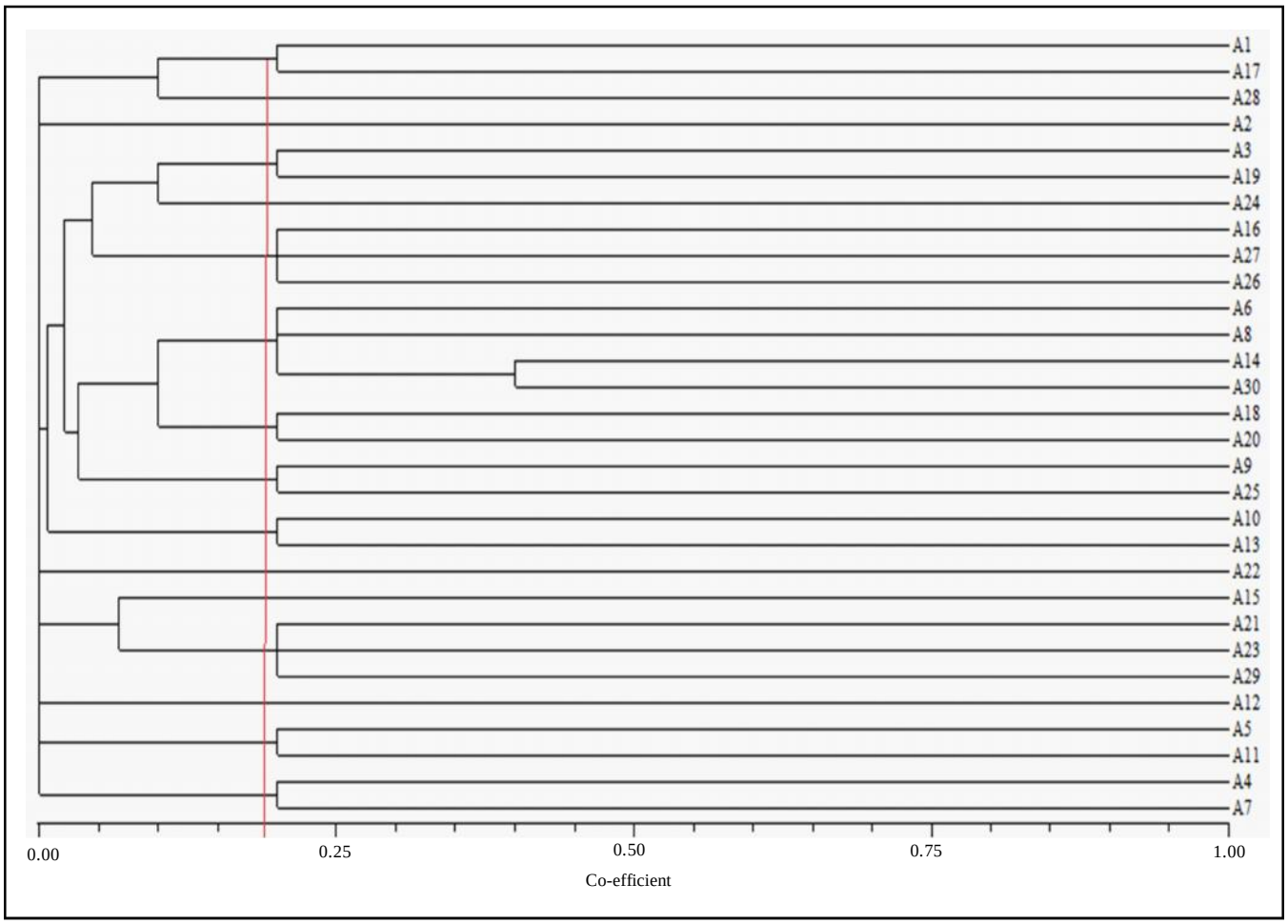

Fig. 4 : Dendrogram based on biochemicalcharacters

and maximum number of genotype was observed in Cluster VII. Asna (2013) had also reported wide variability regarding biochemical characters among exotic accessions.

Breeding for quality is the current trend in cocoa breeding. Hence, the wide variability observed among the exotic genotypes can be exploited for developing superior varieties with improved biochemical properties.

Acknowledgement:

We duly acknowledge Mondelez International Pvt. Ltd. (early Cadbury International Ltd.) for their thirty years of financial support

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