Electronic Journal of Plant Breeding, 7(4): 1105-1109 (December 2016)
ISSN 0975-928X
DOI: 10.5958/0975-928X.2016.00152.6

Research Note

Biochemical characterization of selected accessions of cocoa (*Theobroma cacao* L.)

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(Received: 09 Dec 2015; Accepted: 22 Oct 2016)

Abstract
Fifty clonal accessions of cocoa in the full bearing stage maintained at Cocoa Research Centre, Vellanikkara were evaluated and characterized based on fat and total polyphenol contents. Fat and total polyphenol contents were estimated following standard procedures and the accessions were clustered following unweighted pair group method (Sneath & Sokal, 1973). Wide variability was observed among the accessions for fat and total polyphenol contents. The fat content ranged from 40 to 60 per cent and total polyphenol content from 2.25 to 9.09 per cent. Majority of accessions were remaining as independent units even at one per cent similarity level indicating that they are different.

Keywords
Cocoa, Fat, Polyphenol, Clustering

Cocoa (*Theobroma cacao* L.) is an important beverage crop grown all around the world for the delicious chocolates. Cocoa and cocoa products have received much attention due to their significant fat and polyphenol contents. Cocoa butter is the major commercial product from the seeds of cocoa. Cocoa seeds contain more fat than any other major oil crop other than coconut (Luhs and Friedt, 1994). The fat content of cocoa seeds is found to have a profound influence on the characteristic flavor and commercial value.

Polyphenol content in cocoa is associated with the flavor and colour of chocolate (Kim and Keeney, 1984). The reactions of polyphenol with sugar and amino acids contribute flavor and colour to cocoa beans whereas the alkaloids contribute to the bitterness (Lehrian and Patterson, 1983). Cocoa products namely cocoa liquor, cocoa powder and chocolates may present different levels of antioxidant potentials. Nazaruddin *et al.* (2006) reported that the total polyphenols ranged from 45 to 52 mg/g in cocoa liquor, 34 to 60 mg/g in beans, and 20 to 62 mg/g in powder. The place of origin as well as the method of processing is found to influence the polyphenol content of cocoa products (Jalil and Ismail, 2008).

Keeping in view the above facts, present study was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Thrissur during the period 2011-2013 to evaluate and characterize the fifty clonal accessions of cocoa maintained at Cocoa Research Centre, Vellanikkara based on fat and total polyphenol contents. This will help the plant breeders to screen effective genotypes for the development of lines with high fat and total polyphenol content.

Fifty clonal accessions of cocoa comprising of 40 exotic ones belonging to diverse countries of origin as well as 10 indigenous ones selected from the germplasm maintained by Cocoa Research Centre, Vellanikkara formed the material for the study. The selected accessions which are in the full bearing stage were laid out in completely Randomized block design with three replications. The fat and total polyphenol contents were estimated following standard procedures. The pods were then split open and beans of pods from each accession were pooled. Twenty beans were then selected at random from each accession. After removing the outer slimy layer, the beans were dried to moisture content below 8% either by sun drying or by artificial means. The dry beans were then ground to fine powder using laboratory grinder and the powder was stored in polyethylene bottles for estimation of fat as well as polyphenol contents.

Determination of fat: Cocoa nibs were defatted by extracting the fat with petroleum ether (40-60°C) in a soxhlet extraction apparatus (Sadasivam and Manickam, 1996). Ten grams of the powered sample wrapped in blotting paper was put in the extraction tube of soxhlet extraction apparatus. The total fat present in the sample was extracted along with the siphoning of petroleum ether and was collected in pre-weighed flask of the apparatus. The petroleum ether in the extract was evaporated to dryness. The cream coloured fat left behind after the evaporation of solvent was weighed and expressed as percentage.

Determination of total polyphenols: The powdered defatted bean samples were used for the estimation of total polyphenol content. The defatted samples were extracted exhaustively with methanol in a soxhlet extractor. Phenolic constituents in the

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extract were then estimated colorimetrically by the Folin-Ciocalteau procedure (Sadasivam and Manickam, 1996).

Exactly 250 mg of sample was weighed into a micro soxhlet extractor. The sample was extracted initially using 10 ml petroleum ether (b.p. 40-60°C) for about 30 minutes to remove the fatty matter. The extract was discarded and extraction was continued for another 30 min. with 10 ml methanol. The extract was transferred quantitatively to a 50 ml beaker and solvent evaporated off on a steam bath. The beaker was cooled and the contents were reconstituted in exactly 5 ml of methanol. The extract was taken in an eppendorf tube, centrifuged at 10000 rpm for one minute and stored until analysis.

The detector was calibrated for quantification of total polypheols by the following procedure. The total polyphenols in extracts were assayed in terms of catechin taken as the reference. A series of standard solutions of catechin ranging from 0 to 150 ppm were prepared by suitably diluting the aliquots of a standard solution of catechin prepared by dissolving weighed amount of catechin in measured amount of methanol.

10µl of each of the concentrations were transferred into separate 10 ml test tubes and diluted with 190µl distilled water followed by 500µl Folin-Ciocalteau reagent. After 3 minutes 2 ml of 20 per cent Na_2CO_3 solution was added, mixed well and the test tube was placed in a boiling water bath. After exactly one minute the tubes were removed from the bath and cooled to room temperature under a stream of cold water. The blue colour of the solution was read at 750 nm in a spectrophotometer against reagent blank.

A curve was plotted with concentration of catechin solution pipetted and the absorbance of the corresponding blue coloured solution. From the linear part of the graph, the equation connecting concentration of catechin in the extract analyzed and the corresponding absorbance readings was established.

For estimation of phenols in methanolic plant extracts, 10µl extract was pipetted out into a 10 ml test tube and blue colour was developed in the same manner as done in case of standards and the absorbance was read at 750 nm in a spectrophotometer against reagent blank. Concentration of phenols in the extract (expressed as catechin) was worked out by substituting the absorbance value thus obtained in the calibration equation developed for the purpose.

The fat and total polyphenol content in the samples were calculated and expressed as per cent. Jaccard's similarity coefficients (Jaccard, 1908) among the selected accessions based on fat and total polyphenol contents were worked out using NTSYS pc version 2.1 (Rohlf, 1992). Based on the similarity matrix cluster analysis was performed and dendrogram was constructed by unweighted pair-group method (UPGMA) (Sneath and Sokal, 1973).

The Table 1 summarizes the fat and total polyphenol contents of 50 accessions of cocoa. Significant difference was observed among the accessions based on the Analysis of Variance. The average fat content among the 50 accessions was 51.14 per cent, ranging from 40 per cent in Criollo to 60 per cent in MAR 9, B7 B2 and Calicut local 2. The distribution of fat content among the accessions is shown in Fig. 1. The accessions differed significantly in fat content indicating the availability of genetic variability for this character. In the case of accessions from Konni, the fat content ranged from 44 per cent to 57 per cent. Thirty per cent of the accessions were having a fat content between 50-55 per cent. Nearly 26 per cent of the accessions each, had a fat content between 45-50 per cent and 55-60 per cent (Fig. 1). High fat content of cocoa beans is an important factor deciding the characteristic flavor and aromatic qualities of chocolate (Mossu, 1992). Hence, the cocoa beans from the accessions like MAR 9, B7 B2 and Calicut local 2 with high fat content can be selected for chocolate industry.

The total polyphenol content of the unfermented cocoa samples ranged from 22.55 mg/g to 90.85 mg/g (Table 1). The distribution of total polyphenol content in the evaluated accessions of cocoa is shown in Fig. 2. The accession IMC 54 had the lowest (2.25 %) and B7 B 2 had the highest total polyphenol content (9.09 %). The place of origin as well as the method of processing is found to influence the polyphenol content of cocoa products (Jalil and Ismail, 2008). Very high polyphenol content is undesirable as it can impart bitterness and astringency to the final product and mask the characteristic e flavor of chocolate. However, polyphenols could also contribute different level of antioxidant property beneficial to human health depending on the content. Hence, the accessions with very high polyphenol content can be selected for medicinal purposes.

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 Fig. 3. As depicted in the Fig.3 it can be seen that the accessions are highly variable based on fat and total polyphenol content. Even at one per cent similarity level, majority of accessions were found to be remaining as independent units. The result of this study indicates that a wide variability was available for

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fat and total polyphenol contents among the fifty selected clonal accessions of cocoa. The accessions with high fat content (MAR 9, B7 B2 and Calicut local 2) and high total polyphenol content can be used as parents in cocoa breeding programmes.

Acknowledgment

The first author acknowledges the technical support of the Scientists in Cocoa Research Centre, Vellanikkara and also Dr. Samuel Mathew, Centre for Medicinal and Aromatic Plants Research Station, Odakkali.

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Table 1. Fat and total polyphenol contents in different accessions of cocoa

Sl. No.	Accessions	Fat content (%)	Total polyphenol content (%)
1.	SC 10	42	4.26
2.	COCA 3370-3	57	3.26
3.	AMAZ 10-1	58	4.33
4.	BE 3	46	5.56
5.	AMAZ 15	53	6.89
6.	AMAZ 6-3	57	7.16
7.	AMAZ 3-2	59	3.82
8.	PINA	43	7.11
9.	B 7B2	60	9.09
10.	PA 56	47	4.19
11.	DOM 4	51	5.16
12.	KER 2 E	45	4.02
13.	R (10) (MEX)	52	5.64
14.	B 7 B4	45	5.77
15.	UF 677	58	4.28
16.	GDL 3	55	3.16
17.	B5-7	46	7.19
18.	MAR 9	60	2.84
19.	CLM 90	44	6.66
20.	R (39) (MEX)	54	3.26
21.	B 7 B5	53	4.22
22.	DOM 25	58	6.00
23.	KER 9	45	4.69
24.	LV 28	52	2.60
25.	B7 A6	54	3.06
26.	GU 310	53	4.19
27.	EET 400	43	6.08
28.	IMC 16	48	5.28
29.	EET 397	53	4.50
30.	ICS 95	52	2.39
31.	IMC 67	49	4.93
32.	SCA 6	48	5.05
33.	PA 137	47	6.30
34.	RB 33/3	52	7.22
35.	SPEC 160-9	51	5.59
36.	EQX-3348-44	53	6.59
37.	PUCALA 1	47	2.69
38.	IMC 54	54	2.25
39.	IMC 14	55	4.84
40.	Criollo	40	3.19
41.	Calicut local 1	57	3.05
42.	Calicut local 2	60	5.06
43.	Konni local 1	53	5.59
44.	Konni local 2	48	7.70
45.	Konni local 3	57	3.49
46.	Konni local 4	44	6.03
47.	Konni local 5	56	4.85
48.	Thodupuzha local 1	46	7.53
49.	Thodupuzha local 2	42	6.89
50.	Thodupuzha local 3	55	6.45
	CD	**1.99**	**0.05**

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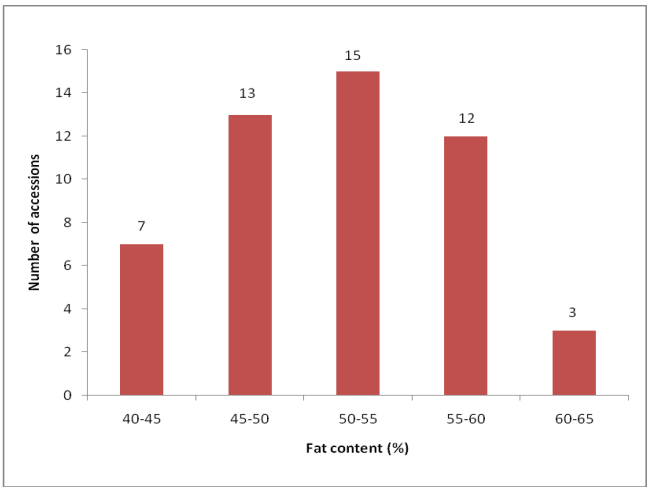

Fig. 1. Distribution of fat content in 50 accessions of cocoa

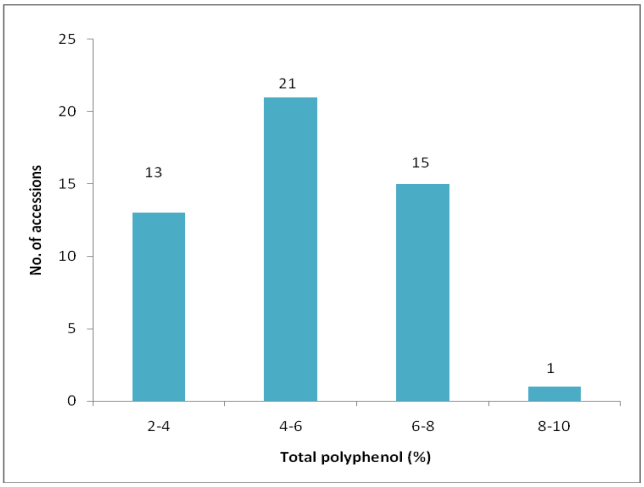

Fig. 2. Distribution of total polyphenol content in 50 accessions of cocoa

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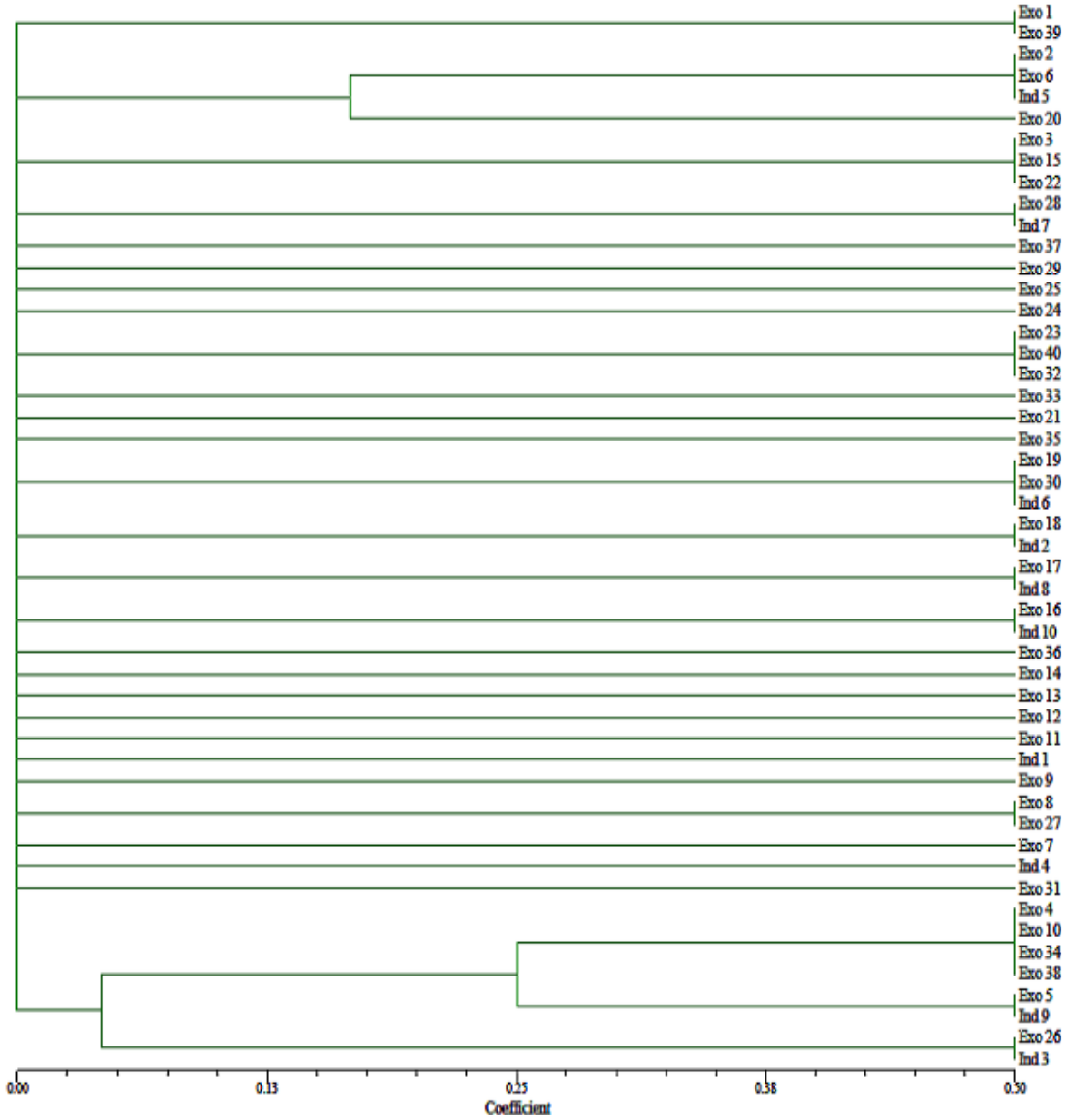

Fig. 3. Dendrogram based on biochemical characters