EVALUATION OF COCOA BUTTER CONTENT FOR SELECTED COCOA GENETIC MATERIALS IN COCOA RESEARCH AND DEVELOPMENT CENTRE BAGAN DATUK, PERAK

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ABSTRACT – *Malaysian Cocoa Board is collecting and conserving more than a thousand of international cocoa genetic materials in its germplasm collection. These materials are being observed and evaluated for their performance under Malaysia agro-climatic conditions including for cocoa butter content. Cocoa butter is a natural fat which is commonly used in chocolate production, skincare products, pharmaceutical, cosmetic, health and other industries. It is an edible fat extracted from the cocoa beans. At about 50-57% of cocoa seed weight is from cocoa butter. In this paper, cocoa butter content was evaluated from selected cocoa genotypes of the Malaysian Cocoa Board's germplasm collection in Cocoa Research and Development Centre, Bagan Datuk, Perak. ANOVA result revealed that butter content evaluated on the eight selected cocoa genotypes was affected significantly (at p≤0.05) by the genotypes*.

Keywords: Cocoa butter, genotype, germplasm, cocoa beans

INTRODUCTION

Theobroma cacao L. (cocoa tree or chocolate tree) is a perennial tropical tree species in the family Malvaceae, and it grows best under shade (Neela *et al*., 2014; Sumitha et al., 2018). It is native to South and Central America therefore, cocoa tree thrives in tropical zones near the Equator. The cocoa crop is grown for its fruit known as cocoa pod. It contains cocoa seeds, also known as cocoa beans in a sugary pulp (Kongor *et al*., 2016).

Cocoa beans possess a high nutritional value due to their butter content (cocoa butter), which is around 50–57% of a cocoa bean's weight (Steinberg *et al*., 2003). Cocoa butter also called theobroma oil is a natural edible fat extracted from the cocoa bean and is the most expensive ingredient in chocolate due to the high cost of extraction. It is extremely important component for the melting properties of chocolate because it brittles at room temperature and fast liquefies at body temperature. Therefore, it is being used to enhance the texture of the chocolate with mild texture, mouthfeel, pleasant flavour release and shimmer of chocolate products (Liendo *et al*., 1997; Schilchter-Aronhime and Garti, 1988).

Besides that, cocoa butter is also used in the cosmetic and pharmaceutical industries for its moisturizing properties and high antioxidants concentration (Ady, 2009; Batista *et al*., 2016) to protect the skin and improve its elasticity.

Demand for cocoa butter is expected to grow due to increased processing activities of chocolatiers for high-quality chocolates. However, extreme weather due to climate change problems such as fluctuating rainfall, longer dry spells and higher temperature have led to a reduction in cocoa production worldwide. Moreover, the infection of cocoa swollen shoot virus also known as CSSV (Ofori *et al*., 2022) especially in the top two global cocoa-producing countries i.e. Côte d'Ivoire and Ghana also causes a global shortage of cocoa beans.

Genetic materials of cocoa need to be enhanced and explored to search for this valuable cocoa butter content. Malaysian Cocoa Board (MCB) conserving more than 1000 cocoa genetic materials in several MCB research stations throughout Malaysia. The materials are received from the intermediate cocoa quarantine facility maintained at the Reading University, UK. Cocoa materials have been evaluated for their performance under Malaysia agro-climatic conditions including cocoa butter content. The objective of this study is to evaluate the cocoa butter content of the selected cocoa clones from Cocoa Research and Development Centre (CRDC) Bagan Datuk germplasm collection. In this study, the evaluation of the selected cocoa clones is expected to show diversity in cocoa butter content.

MATERIALS AND METHODS

Location of the cocoa genotypes sampling

The location of cocoa genotypes sampling was at germplasm collection block 18B (3.54ºN and 100.51ºE), Malaysian Cocoa Board Research and Development Centre in Bagan Datuk, Perak (Figure 1).

Planting materials

A total of eight cocoa genotypes were selected and utilized for the cocoa butter analysis which include AMAZ 12, IMC 16, IMC 20, IMC 103, MCBC 5, PNG 296, SLA 16 and UF 12 (Table 1).

Figure 1: Block of cocoa gemplasm (18B) at CRDC Bagan Datuk, Perak

Table 1: List of cocoa genotypes evaluated in the study and the related information

Genotype	Source Population	Accession Number	Donor Genebank
AMAZ 12	Reading University, UK	RUQ 334	ICG, T §
IMC 16	Reading University, UK	RUQ 829	ICG, T §
IMC 20	Reading University, UK	RUQ 985	ICG, T §
IMC 103	Reading University, UK	RUQ 862	ICG, T §
MCBC 5	CRDC, MCB, Malaysia	MCBC 5	MCB †
PNG 296	Reading University, UK	RUQ 1299	CIRAD-CP ≠
SLA 16	Reading University, UK	RUQ 1092	ICG, T §
UF 12	Reading University, UK	RUQ 962	ICG, T §

† Malaysian Cocoa Board

§ International Cocoa Genebank (Trinidad)

≠ Centre de cooperation international en recherché agronomique pour le développement ("-CP": Montpellier)

The cocoa trees are about 15 years old, planted in a triangular arrangement at 3 m x 3 m spacing, under 25% shade trees (*Gliricidia macculata*). The trees are maintained with normal agricultural practices for cocoa. Fertilizers are applied at the rate of 250 g per tree using compound fertilizer, Nitrophoska Blue (N P K Mg 12:12:17:2+TE); three times per year. Weeds are controlled using glyphosate – isopropylammonium and glufosinate-ammonium meanwhile deltamethrin and cypermethrin for pests and, metalaxyl and triazole for diseases. Pod sleeving also had been practiced for cocoa pod borer (CPB) control. Maintenance pruning of the trees are carried out every three months to improve air circulation around and within the trees, to reduce the risk of pest and diseases infestation and infection and to form appropriate shape to ease pods harvesting activity.

Experimental design

The experiment was carried out in nested design. Three factors were studied i.e. clones, individual trees and the pods. Nested design was employed with four replications (tree) within clone and three pods within tree.

Sampling procedure

Three healthy pods were sampled randomly for each genotype from four individual trees (Figure 2). The ripe pods were harvested and put in each labelled sack. The cocoa pods were break and the cocoa beans were removed. The beans from each genotype were placed in a separate labelled net bag (Figure 3) so that the mono-clonal beans could undergo the normal shallow box fermentation and processing together with other commercial beans. This method ensures that the beans could be easily separated out after the process. The fermentation and drying process also known as primary processing of the sampled beans were carried out according to standard protocol of MCB.

Figure 2: Some of the cocoa pod samples for cocoa butter analysis

Figure 3: Samples of cocoa beans placed in a separate labelled net bag for cocoa butter extraction

Determination of cocoa butter content

Laboratory analysis consisted of cocoa butter content determination of the selected cocoa genotypes.

Extraction procedures

The shells of the fermented and dried cocoa beans were removed to obtain the cocoa nibs. The nibs were then grounded to small particles. The nibs were hydrolysed with dilute hydrochloric acid (25%) and filtered (Whatman filter paper, 8.0 μm) before extraction processes.

Soxhlet extraction

Cocoa nibs were weighted into the thimble. Then, ethanol (99.5%) was added to a round bottom flask and refluxed for 6 h using Soxhlet apparatus (Figure 4). The extracts were filtered and concentrated under reduced pressure overnight or until dry, according to AOCS 1998 and Roiaini *et al*. (2016).

Figure 4: Soxhlet apparatus for cocoa butter extraction

The yield of cocoa butter was calculated using the formula below according to Roiaini et al. (2016).:

Yield (%) = {[Weight of flask (after) – Weight of flask (before)] / Weight of sample} x 100

Statistical analysis

All the data were subjected to the statistical analysis using the Statistical Analysis Software (SAS), version 9.1.3. The analysis of variance (ANOVA) was calculated using the PROC ANOVA procedure in SAS and means were separated using the Duncan's Multiple Range Test (DMRT). ANOVA was used to determine the significance of variation among genotypes and trees within genotypes. Significant differences ($p<0.05$) between means were determined.

RESULTS AND DISCUSSIONS

Determination of cocoa butter content

The data was obtained for cocoa butter of eight selected cocoa genotype of CRDC Bagan Datuk germplasm collection. Result of the variance analysis (ANOVA) conducted revealed that cocoa butter content evaluated on the eight selected cocoa genotypes were affected significantly (at $p\leq0.05$) by the genotypes. This analysis indicating that there was a variation for butter content among the cocoa genotypes (Table 2). Meanwhile, there was no significant difference in cocoa butter content for the tree within genotype.

According to Roiaini *et al*. (2016), the yield of cocoa butter obtained was about 28.87% using this method.

Table 2: Mean squares in ANOVA table for butter content evaluated on eight selected cocoa genotypes

Source of variation	d.f. †	Mean squares
		Butter content
Genotypes	7	159.86*
Tree / Genotypes	24	58.86 ns
Error	64	41.10
C.V. (%)		14.10

† d.f. = Degrees of freedom; C.V. = Coefficient of variation; *, **, ns = significant at $p\leq 0.05$, significant at $p\leq 0.01$, and non-significant, respectively.

Performance of cocoa genotypes for cocoa butter content

Table 3 shows the results of the comparison of mean values among the eight selected cocoa genotypes for cocoa butter content, using Duncan's Multiple Range Test (DMRT) at $p\leq0.05$. The cocoa butter content of the genotypes ranged from 37.71%– 49.05%. The

mean value of the genotypes for the cocoa butter content was 45.46%.

Among the genotypes, MCBC 5 had the most cocoa butter content with 49.05% followed by UF 12 (48.71%) and IMC 16 (47.02%). Meanwhile, the lowest cocoa butter content was from IMC 20 with 37.71%. Six genotypes i.e. IMC 16, IMC 103, UF 12, AMAZ 12, MCBC 5 and PNG 296 showed no significant difference for cocoa butter content. The variation among the genotypes occurred in IMC 20 and SLA 16. The coefficients of variation for cocoa butter content was 14.10%.

Table 3: Mean performance of eight selected cocoa genotypes for butter content

NO	CLONE	BUTTER CONTENT (%)
1	IMC 16	47.02a
2	IMC 20	37.71b
3	IMC 103	46.38a
4	UF 12	48.71a
5	SLA 16	43.32ab
6	AMAZ 12	44.81a
7	MCBC 5	49.05a
8	PNG 296	46.65a
MEAN		**45.46**
CV		**14.10**

Mean values followed by the same letter in the same column are not significantly different at $p \leq 0.05$, based on DMRT.

CONCLUSIONS

Cocoa butter is a very important property for cocoa-based products especially in chocolate. Thus, it is a vital to produce cocoa planting materials with high cocoa butter content as a source in producing good and high-quality chocolate products. This paper revealed that eight selected cocoa genotypes in the germplasm collection indicating variation in cocoa butter content. Thus, more cocoa genotype in the germplasm collection will be analyzed for cocoa butter content in future for meaningful assessment.

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