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Feasibility of Cacao (Theobroma cacao) variety authentication based on cotyledon color and biochemical parameters

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Abstract: The distinction between bulk cacao and fine flavor cacao or Philippine Criollo leads to a significant difference in pricing which affects producers and consumers of the crop. Criollo, Forastero, and Trinitario are varieties of cacao recognized internationally. Criollo is considered as the fine flavor variety and is priced higher compared to Forastero and Trinitario. The three varieties have been well studied in the international literature and would have phenotypic and biochemical characteristics that would distinguish one from the other. The Philippines cultivates nine certified varieties of cacao including UF18, BR25, and PBC123. How these match up to the international varieties has not been studied. Farmers have some local practice of identifying criollo, however, they are incorrect based on the results of this study. A project to characterize cacao from different sites in the country based on morphology, biochemistry, and genetics is underway. In order to proceed with the bigger project, this study presents the general biochemical profile of Luzon and Mindanao as well as evaluation of farmer knowledge on cacao varieties. Samples from Metro Manila, Batangas, Quezon, Romblon, South Cotabato, and Davao were analyzed using the cacao bean test and biochemical parameters. The cacao bean test is based on the cotyledon color while caffeine, theobromine, and chlorogenic acid were measured using UV Vis spectrophotometry. Included in this study is the optimized methodology for amplifying trnH-psbA and ITS which are used in determining cacao variety based on genetics. Farmers were correct in identifying non-criollo cacao based on plant and external pod appearance. Batangas and Quezon has lower caffeine and higher theobromine and chlorogenic acid as compared to other provinces in Luzon and Mindanao. The cotyledon color and biochemical concentrations may be correlated with the genetic and aromatic profiles in future studies.

Key Words: Theobroma cacao, bean test, cotyledon color, criollo

1. INTRODUCTION

Theobroma cacao is the source of cocoa and chocolate products. The species originated in the American continent. The first introduction of the species to Asia is in the Philippines during the 1600s (Bartley, 2005). As of 2019, a 60-hectare farm in Davao has garnered two international awards including the Cacao Bean of Excellence shortlist and Heirloom Cacao status. (Malagos Agri-ventures, 2019). However, the Philippines is also a major importer of cacao because the local production does

meet the demand. There is a rising need for updated literature in order for crop yield and quality of both bulk and fine Philippine cacao can be improved. As of writing there are only nine certified varieties and these do not correspond with the international varieties such as criollo, forastero, and trinitario. The discrepancy of local and international varieties causes difficulties in farming practices of producing bulk and fine cacao. This paper presents the morphological, biochemical, and genetic characteristics between Luzon and Mindanao cacao.

2. METHODOLOGY

2.1 Sample collection and sampling sites

Cacao pods were collected from Metro Manila, Batangas, Quezon, Romblon, South Cotabato, and Davao (Figure 1). The beans from the cacao pods were dried after going through the bean test for morphology.

Fig. 1. Map of cacao sampling sites across selected areas in Luzon and Mindanao.

2.2 Farmer Identification and Morphology

Upon collection, the cacao pods were identified as criollo or non-criollo by the farmers. The variety of the cacao pods were validated in the laboratory using the bean test. The cacao bean test is conducted by breaking open the pod and slicing all beans longitudinally the cotyledon color is noted. The ratio of purple to white cotyledon indicates whether it is criollo or non-criollo. The percentage of white cotyledons was noted.

2.3 Biochemical Parameters

The caffeine, theobromine, and chlorogenic acid contents of representative beans from each cacao pod was quantified using UV-Vis Spectrophotometry. The dried beans were crushed into a powder using a mortar and pestle. The powdered beans were weighed into 500mg and transferred into centrifuge tubes. The powdered beans were mixed with 5mL deionized water and boiled for 30 minutes and then centrifuged at 5000rpm for 10 minutes. The supernatant was extracted and transferred into a new centrifuge tube with 3mL of dichloromethane. The tubes were mixed and then centrifuged at 5000rpm for 3 minutes. The extraction with dichloromethane was repeated thice. The organic layer was used to identify the presence of caffeine while the aqueous layer was used to determine theobromine and chlorogenic acid.

2.4 DNA extraction and amplification of trnH-psbA intergenic spacer and Internal Transcribed Spacer (ITS)

Crude DNA extraction was conducted using the KAPA 3G Plant PCR Kit. A 5mm punch of the cacao bean was placed in an extraction buffer consisting of 40ul 50mM Tris-HCl, 40ul 0.1mM EDTA, and 40ul 2% beta-mercaptoethanol. The extraction tube was incubated at -20 degrees Celsius for 5 minutes. The DNA amplification of the trnH-psbA intergenic spacer was conducted using the forward primer 5′-CGCGCATGGTGGATTCACAATCC-3′ and reverse primer 5′-GTTATGCATGAACGTAATGCTC-3′. The DNA amplification of ITS was conducting using the forward primer 5'-GGAAGKARAAGTCGTAACAAGG-3' and reverse primer 5'-GCGTTCAAAGAYTCGATGRTTC-3'. The PCR conditions for both primer pairs were modified from the KAPA 3G Plant PCR Kit. The PCR was performed in a 25ul reaction mixture containing 7.8ul DEPC water, 10ul 2X Buffer, 4ul MgCl2, 0.75ul 10uM forward primer, 0.75ul 10uM reverse primer, 1.5ul of crude DNA, and 0.2ul of Plant DNA Polymerase. The amplicons were resolved in agarose gel electrophoresis.

3. RESULTS AND DISCUSSION

3.1 Farmer Identification and Cotyledon color

None of the pods had 100% white cotyledons indicating that none of the pods were pure criollo. Farmers were correct in identifying non-criollo cacao based on plant and external pod appearance (Table 1). In a study by Cosme, the clustering of genetic and phenotypic data supports the classification of cacao into traditional varieties known as Criollo, Trinitario, and Forastero (Cosme et al, 2016). Cosme reported that Puerto Rican Criollo had 70% white cotyledons ($p<0.0001$).

Table 1. Farmer identification, percentage of white cotyledon, and laboratory validation of samples from selected areas in Luzon and Mindanao.

Location	Farmer identification	% White Cotyledon	Laboratory validation
Romblon	Criollo	0	Non-criollo
South Cotabato	Criollo	50	Non-criollo
Davao	Criollo	7	Non-criollo
Quezon	Criollo	0	Non-criollo
Batangas	Criollo	0	Non-criollo
NCR	Criollo	73	Non-criollo
Batangas	Non-criollo	0	Non-criollo
South Cotabato	Non-criollo	0	Non-criollo
Davao	Non-criollo	0	Non-criollo
NCR	Non-criollo	0	Non-criollo

3.2 Biochemical and Environmental Parameters

Only samples from Batangas and Quezon formed a distinct group implying that Region IV-A CALABARZON has a different biochemical profile from other regions(Figure 1).

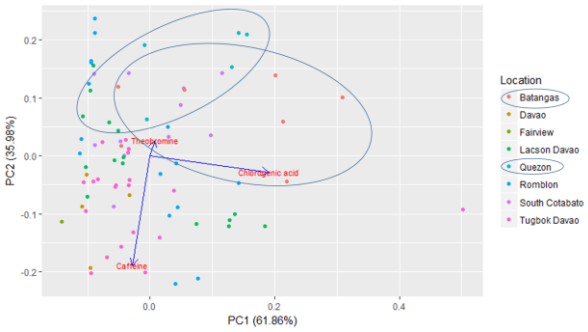

Fig. 2. Principal component analysis of caffeine, theobromine, and chlorogenic acid across selected areas in Luzon and Mindanao.

Batangas and Quezon has lower caffeine and higher theobromine and chlorogenic acid as compared to other provinces in Luzon and Mindanao(Table 2). Davrieux reports that Forastero and Trinitario has a lower caffeine and higher theobromine content than Criollo (Davrieux et al, 2007). Batangas and Quezon cacao may belong to the Forastero group based on caffeine and theobromine content.

Table 2. Biochemical parameters of Batangas and Quezon as compared to all selected sites.

	Caffeine (mg/ml)	Theobromine (mg/ml)	Chlorogenic Acid (mg/ml)
All selected sites	1.313	0.252	0.921
Batangas	0.866	0.393	1.588
Quezon	0.360	0.546	1.372

3.3 DNA extraction and amplification of trnH-psbA intergenic spacer and Internal Transcribed Spacer (ITS)

The trnH-psbA intergenic spacer and ITS were successfully amplified and visualized on gel electrophoresis however, the kilobase pair quantity of trnH-psbA cannot be quantified yet because of the brightness of the band. It is subject to further optimization. The use of trnH-psbA and ITS were suggested in previous studies (Guitierrez-Lopez et al, 2016; Kane et al ,2012)

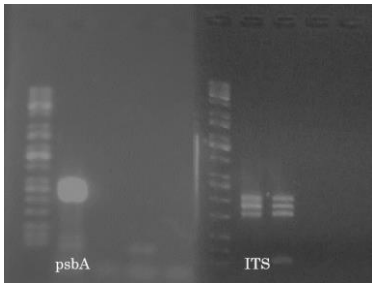

Fig. 3. Gel electrophoresis results of trnH-psbA and ITS

4. CONCLUSIONS

Cacao samples from selected areas in Luzon and Mindanao were analyzed using the cacao bean test and UV Vis spectrophotometry. Farmers were correct in identifying non-criollo cacao based on plant and external pod appearance. Batangas and Quezon has lower caffeine and higher theobromine and chlorogenic acid as compared to other provinces in Luzon and Mindanao. The cotyledon color and biochemical concentrations may be correlated with the genetic and aromatic profiles in future studies.

5. ACKNOWLEDGMENTS

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