International Journal of Innovations in Engineering and Technology (IJIET)
http://dx.doi.org/10.21172/ijiet.194.02

Genetic Relationships of Fourteen Prominate Cocoa Varieties in Southern Vietnam assessed by RAPD analysis

Lam Thi Viet Ha
Food Biotecnology Department, Faculty of Agriculture and Applied Biology, Cantho University, Vietnam

Ha Thanh Toan
Biotechnology Research and Development Institute, Cantho University, Vietnam

Truong Trong Ngon
Biotechnology Research and Development Institute, Cantho University, Vietnam

Abstract- This work focuses on establishing the genetic diversity of the fourteen popular cocoa cultivars in the south of Vietnam using the Random Amplified Polymorphic DNA (RAPD) technique. All detected amplification products were encoded into a binary matrix, and a dendrogram was generated using the program NTSYS-PC 2.1. The dendrogram shows the genetic distances of 14 popular cocoa cultivars based on their DNA polymorphisms. The similarity matrix was subjected to cluster analysis by un-weighted pair grouped methods for arithmetic mean (UPGMA). The genetic clustering of 14 cocoa cultivars showed genetic differences ranging from 0 to 41%. At around 59% similarity value, fourteen popular cocoa cultivars could be divided into two clades A and B. Clade A had twelve (12) cultivars (TD1, TD2, TD3, TD5, TD6, TD7, TD8, TD9, TD11, TD12 and TD13), and clade B consisted of 2 cultivars (TD14 and TD15). Clade B performed at a homologous rate of 77% and had a higher similarity value than clade A (69%) which separated into three sub-group AI, AII and AIII. Two RAPD primers used in this study were amplified and typically generated major amplification products. These results make a significant contribution to the Vietnamese cocoa breeding and selection activities. The experiment presents RAPD technique as a useful method to determine the genetic relationships of cocoa cultivars and other cultivars in Vietnam.

Keywords – Genetic diversity, Genetic clustering, RAPD, *Theobroma cacao* L.

I. INTRODUCTION

Random Amplified Polymorphic DNA (RAPD), first described by Williams et al. (1990) and Welsh and McClelland (1991), is one of the most popular DNA marker systems since it is simplicity. It does not require prior sequence knowledge and it is highly suitable for quick DNA fingerprinting (Stift et al., 2003). In addition, RAPD is a rapid method to classify the genetic diversity of many plant species such as rapeseed (Yu et al., 2004), sesame (Ercan et al., 2004; Salazar et al., 2006; Pham et al., 2009), and rice (Kanawapee et al., 2011). RAPD technique has some advantages such as low development cost, low level of training and low cost per assay, which in particular is important for countries with less resource (Pham et al., 2009). Therefore, this RAPD method has been commonly used in Vietnam to date (Hien et al., 2010; Hien et al., 2012; Pha et al., 2013; Vuong et al., 2014; Hoa et al., 2014; Thanh, 2014; Nghia et al., 2015; Huong et al., 2016).

Currently, Vietnam mainly focuses on the investment of fourteen (14) popular cocoa cultivars for exportation purpose since their bean quality is on par with other top cocoa exporting countries such as Ghana and Ivory Coast (Phuoc, 2009; Ha et al., 2015a). Therefore, in recent decades, projects relating to the breeding and selection of these 14 popular cocoa cultivars with high-yielding, disease-resistant and well-adapted traits have been invested to develop cocoa cultivars in southern Vietnam. Previous studies mainly investigated the chemical properties of beans of those popular cultivars in Mekong Delta River (Tran et al., 2010; Tran and Hoang, 2011; Nguyen et al., 2011). To the best of our knowledge, the research on Vietnamese cocoa genetic diversity has not been published yet. A molecular study of cocoa cultivars will be useful to clarify the genetic relationships of Vietnamese cocoa cultivars.

The genetic diversity in crop species can be established by morphological or agronomic characteristics, which is associated with a strong influence from environmental elements (Pham et al., 2009). DNA methods overcome this limitation because they are not influenced by the environment (Pham et al, 2009). The results of chapter 2 verified the diversity of morphology characteristics of 63 cocoa collections in Vietnam including these 14 popular cultivars. However, the genetic differentiation of these cultivars was not established yet. Hence, the work studies on the genetic differentiation of these 14 popular cocoa cultivars by RAPD methods.

II. Materials and Methods

2.1 Sampling collection

Mature leaves of fourteen popular cocoa cultivars were collected at two provinces DakLak (Highland) and BenTre (Mekong Delta). The cocoa leaves were packed in paper bags, and stored in a cold box, which had ice blocks to maintain low temperature.

2.2 DNA extraction

DNA extraction was performed as described in the CTAB-SDS method.

2.3 RAPD primers

The two RAPD primers were described by Petiard and Crouzillat (2004) for cocoa products.

Primer 1: 5 '- CCCACACGCA - 3'

Primer 2: 5 '- CAGACCGACC - 3'

2.4 PCR reaction

PCR amplification assays were carried out in a final reaction volume of 50 µL containing 5 µL of extracted DNA product, and 45 µL of master mix consisting of 18.75 µL bidistilled water, 25 multiplex PCR buffer, and 1.25 µL of primer. The positive control consisted of 5 µL of DNA soya solution.

The PCR amplifications were performed in a GeneAmp PCR System 9700 (Applied Biosystems) using the following program for RAPD primer: initial denaturation at 94ºC for 2 min, 45 amplification cycles of denaturizing at 95ºC for 1 min, primer annealing at 37ºC for 1 min, and extension at 72ºC for 1 min followed by a final extension step of 10 min at 72ºC.

2.5 DNA electrophoresis

PCR products were separated based on length using polyacrylamide gel electrophoresis. A 30% polyacrylamide gel was run at 50V, 400 mA in 990 minutes (16.5 hours) at 25ºC, stained with 1 ng/µL of ethidium bromide solution, and visualized with a UV transilluminator system (Bio-RAD Gel Doc, 2000), resulting in a digital image from the Quantity One software program. Lambda DNA/HindIII marker was used to estimate the size of the DNA fragments.

2.6 Genetic analysis method

The major DNA bands observed after electrophoresis were coded in binary matrix and clustered in genetic groups by NTSYS 2.1 based on UPGMA method (Rohlf, 2000).

III. Result and Discussion

The two RAPD primers evaluated on the fourteen popular cocoa cultivars generated a total of 456 amplification products. RAPD primer one (1) similarly resulted a total of 279 bands for all 14 samples (Figure 1). RAPD primer two (2) similarly resulted a total of 177 bands for all 14 samples (Figure 2). The highest number of amplification product counted of a sample in the primer 1 and primer 2 was 22 (TD14) and 17 (TD10) bands, respectively (Figure 1 and Figure 2). The lowest number of amplification product counted of a sample in the primer 1 was 3 (TD8) and 10 (TD9) bands. Similarity estimation ranged from 0.40 for TD8 compared to TD14 to 0.90 for TD1 compared to TD9. After analyzing the genetic diversity by UPGMA method, the genetic distance indexes between 14 cocoa cultivars were calculated. The cocoa cultivars were divided into two clades coded as A and B based on the similarity percentage at 59% (Figure 3). The polymorphic bands of the primer 1 were higher than that of the primer 2 which resulted in higher of the polymorphic percentage at 82% and 71%, respectively.

Clade A consisted of 12 cultivars and clade B contained two cultivars. More specifically, clade A acquired the greatest genetic similarities with three sub-clades (AI, AII and AIII) at 77% similarity, only TD8 was separated in a single clade with 74% similarity. Sub-clade AI had three clades comprising of six cultivars (TD1 and TD9; TD2 and TD6; TD5 and TD7), and genetically ranged from 83 to 90%. Sub-clade AII had two clades comprising of two cultivars TD3 and TD12 belonging to the first clade and a single clade with TD11 at 77% similarity. The third one, sub-clade AIII had only one variety TD8. Two cultivars TD1 and TD9 indicated the closest relation at 90% similarity. Clade B included of two cultivars TD14 and TD15 and this group share 74% similarity with group A.

.

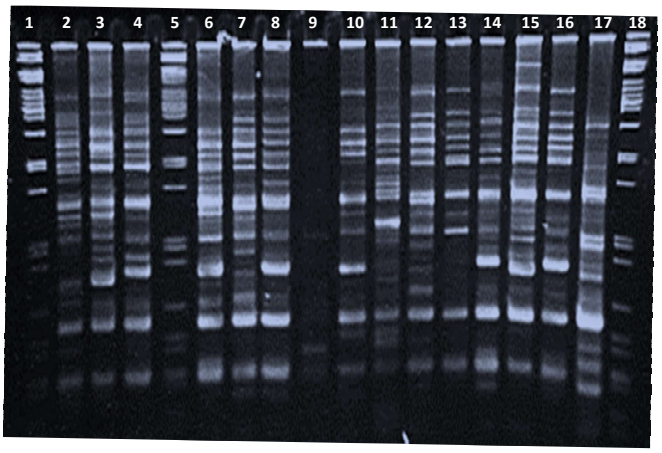

Figure 1. PCR results of 14 TD cocoa cultivars using RAPD primer 1. LamdaHind III standards (lane 1, 5, 18); TD1 to TD3 (lane 2-4); TD5 to TD15 (lane 6-16); positive control-soya DNA (lane 17)

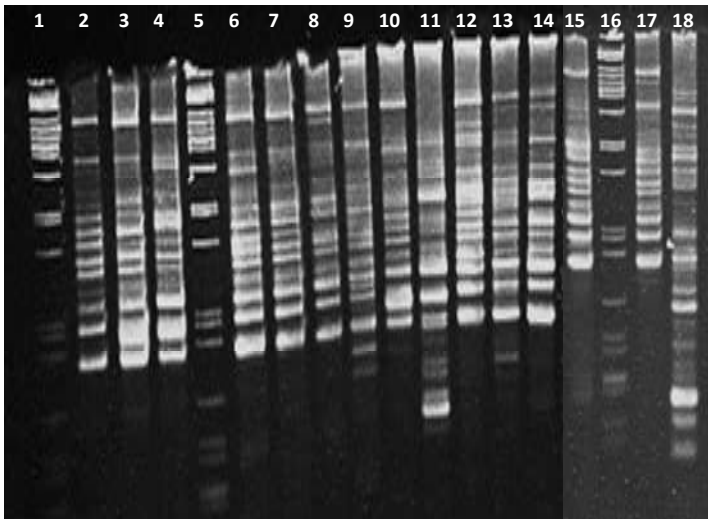

Figure 2. PCR results of 14 TD cocoa cultivars using RAPD primer 2. LamdaHind III standards (lane 1, 5, 16); TD1 to TD3 (lane 2-4); TD5 to TD15 (lane 6-16); positive control-soya DNA (lane 18)

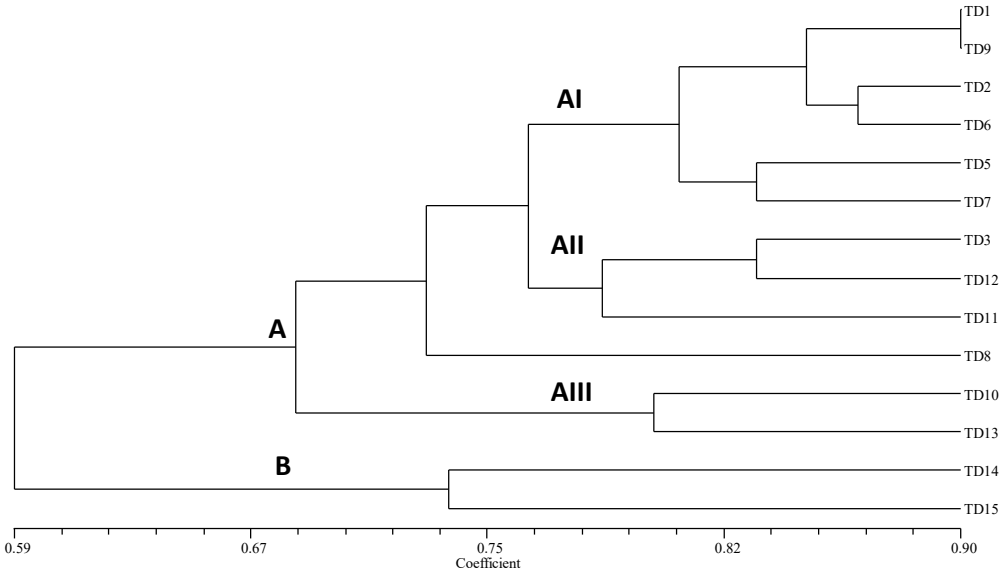

Figure 3. Dendrogram showing the genetic relationship of 14 popular cocoa TD cultivars using UPGMA cluster analysis based on RAPD primers 1 and 2 pattern combination.

Table 1. Two genetic clades of 14 cocoa TD cultivars based on NTSYS analysis

Clade	Sub-clade	Cultivar
A	AI :	TD1 and TD9, TD2 and TD6, TD5 and TD7
	AII:	TD3 and TD12, TD12
	AIII:	TD10 and TD13
	TD8	
B		TD14 and TD15

DNA fragments were separated by polyacrylamide gel (PAA) electrophoresis. This PPA gel was used due to its high resolving power and more bands are produced (Stift et al., 2003). The results showed 75% polymorphism average value. Moreover, the results indicated a high level of polymorphism among 14 popular *T. cacao* cultivars. The fourteen popular cocoa cultivars cultivated in the southern region of Vietnam showed a homologous genetic diversity of up to 32%. Based on the dissimilarity, genetic groups were clustered into two major groups of which group A was further divided into 3 sub-groups with 12 cultivars, and group B had two cultivars. Group A with 12 cultivars shared a closed relationship at 76% similarity while group B shared the genetic similarity at 74% (2 cultivars TD14 and TD15) (Figure 3).

Fourteen prominent cocoa varieties cultivated in the southern region in Vietnam acquire a highly distant genetic up to 41%. Based on the dissimilarity, genetic groups are clustered into 3 major groups which group A is further divided into 3 sub-groups with 10 varieties, group B and C have 2 varieties for each. Group B has TD10 and TD13 sharing 80% genetic similarity; Group C has 2 TD14 and TD15 sharing 73% genetic similarity ; Group A is branched into 3 sub-groups AI, AII và AIII at the level of 73% genetic similarity. Sub-group AI is further cladded into AI_1 and AI_2 : AI_1 consisting of TD1, TD9, TD6 and TD2, sharing 86%-90% similarity. AI_2 has TD5 and TD7, sharing 84% similarity. Sub-group AII có has TD3, TD12 and TD1 sharing 78.5%. Only AIII is left with TD8.

Group A has 10 varieties (TD1, TD2, TD3, TD5, TD6, TD7, TD8, TD9, TD11, TD12) that are cladded into 3 sub-groups AI (), AII() and AIII () based on genetic similarity percentage ??? respectively. . These variesties virtually share a similar phenotype of pod and leaf colour which are in green, except for 2 varieties TD3 and TD6, they genetically level at 76% similarity but morphologically differentiate from the rest, the pod and leaf colour of these

two express in reddish purple. Nevertheless, this finding is consistent to Lam (Lâm et al., 2015), TD3 and TD6 were taxonomically classified in the Criollo hybrid group. Accordingly, other varieties TD5, TD7, TD9 and TD11 also have the same percentage of 76% and morphogically analysed as the Forastero group.

IV.CONCLUSION

In the present work, we presented the successful use of RAPD markers to analyse genetic relationship of 14 popular cocoa cultivars in Vietnam. When combined with RAPD technique and electrophoresis in polyacrylamide gels, more polymorphic patterns were generated. As a result, the RAPD primer 1: 5 '- CCCACACGCA - 3' and primer 2: 5 '- CAGACCGACC - 3' used in this study yielded highly polymorphic patterns in two clusters A and B (59% similarity). Two primers have possible use in descendant identification and population differentiation analysis of *T. cacao* cultivars in southern Vietnam. These results contribute to the breeding, selection, and classification of cocoa cultivars for large-scale cultivation projects and scientific research. This RAPD experiment performed the simplest genetic analysis to clarify the genetic relationships of Vietnamese popular cocoa cultivars. Currently, the RAPD method has been successfully applied for genetic diversity study of many plants, especially in Mekong River Delta, the famous fruit production region in Vietnam.

REFERENCES

[1] Chen, T., Ng, C., Wang, C. and Shyu, Y. (2007). "Molecular identification and analysis of Psidium guajava L. from indigenous tribes of Taiwan", Journal of Food and Drug Analysis, vol *15*(1), 82.

[2] Geleta, M., Bekele, E., Dagne, K., and Bryngelsson, T. (2010). Phylogenetics and taxonomic delimitation of the genus Guizotia (Asteraceae) based on sequences derived from various chloroplast DNA regions. *Plant systematics and evolution*, *289*(1-2), 77-89.

[3] Lachenaud, P., Paulin, D., Ducamp, M. and Thevenin, J. M. (2007). Twenty years of agronomic evaluation of wild cocoa trees (Theobroma cacao L.) from French Guiana. Scientia horticulturae, 113(4), 313-321.

[4] Lannér, C. (1998). Relationships of wild Brassica species with chromosome number 2 n= 18, based on comparison of the DNA sequence of the chloroplast intergenic region between trnL (UAA) and trnF (GAA). Canadian Journal of Botany, 76(2), 228-237.

[5] Lam Thi Viet Ha, Lore Vanlerberghe, Ha Thanh Toan, Koen Dewettinck and Kathy Messens. (2015). Comparative Evaluation of Six Extraction Methods for DNA Quantification and PCR Detection in Cocoa and Cocoa-Derived Products. Food Biotechnology, 29,1–19.

[6] Lam Thi Viet Ha, Phùng Thị Hằng, Trần Nhân Dũng, Hà Thanh Toàn, Koen Dewettinck and Kathy Messens. (2015). Đặc điểm hình thái giải phẫu mô các giống Cacao chủ lực miền Nam Việt Nam. CanTho Journal of Science, 36(A), 47-56.

[7] Nguyễn Bảo Vệ, Trần Văn Hâu and Lê Thanh Phong. (2011). Giáo trình cây công nghiệp dài ngày, Cantho Publication. 9-16.

[8] Petit, R. J., Demesure, B. and Dumolin, S. (1998). cpDNA and mtDNA primers in plants. In Molecular tools for screening biodiversity. Springer Netherlands, pp. 256-261.

[9] Pham Hong Duc Phuoc. (2009). Kỹ Thuật Trồng Cacao ở Việt Nam. Vietnam Agriculture Publication.1-46.

[10] Shri. Mohan Jain and P.M. Priyadarshan. (2009). Breeding Plantation Tree Crops. Springer, New York, USA. 589-591.

[11] Smulders, M. J. M., Esselink, D., Amores, F., Ramos, G., Sukha, D. A., Butler, D. R and van Loo, E. N. (2008). Identification of cocoa (Theobroma cacao L.) varieties with different quality attributes and parentage analysis of their beans. IGENIC Newsletters, 12, 1-13.

[12] Taberlet, P., Gielly, L., Pautou, G. and Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology, 17, 1105-1109.

[13] Trần Nhân Dũng. 2011. Thực tập Kỹ thuật Sinh học phân tử. Vietnam Agriculture Publication. pp. 4-9.

[14] Trần Văn Hâu and Hồ Thị Ngân. (2011). Kết quả bước đầu về bình tuyển cây ca cao (*Theobroma cacao* L.) đầu dòng tại Cần Thơ. Tạp chí NN&PTNT chuyên đề giống cây trồng, vật nuôi, tập 1, tháng 6/2011: 120-127.

[15] Wood, G. A. R., and Lass, R. A. (2008). Cocoa. John Wiley & Sons, London, UK. 29-87.