Omonrice 11: 143-147 (2003)

SHORT COMMUNICATION

GENETIC EVIDENCE FOR THE ORIGIN OF CAYENNE PINEAPPLE

Nguyen thi Lang, Pham thi Be Tu and Bui Chi Buu

ABSTRACT

The genus Annanas comosus L. (Merrill), Bromeliaceae, is an important crop in Vietnam for domestic consumption and export. In this study, genetic variation in 30 germplasm accessions of Cayenne pineapple with Queen as a control was analysed using RAPD marker. UPGMA analysis showed overall that domesticated and genetically coherent groups. Population level patterns of RAPD variation for pinapple (Cayenne) related to other pineapple, suggest that those populations developed from natural populations of the cultivated cayenne under freely crossing to create a complex populations

Keywords. Genetic diversity, RAPD, NTSYSpc, UPGMA

Genetic study has been conducted to evaluate the different hypotheses on the origin of Cayennes. Genetic analysis with molecular markers has proven useful for identifying the origins of cayennes crop relatives, including the possibility of introgression of crop alleles into wild population. Therefore, we have used RAPD analysis to characterize the genetic diversity of Cayenne populations in Mekong Delta and compare it with the diversity of the cultivated Khom and Thom. This comparison provides evidence for the evolutionary origin of Mekong Delta annanas

Materials and methods

RAPD diversity was assayed from 30 acessions of the Cayenne pineapple. Samples were obained from SOFRI and CLRRI gene bank (table 1)

Table 1: List of Cayenne pineaple used for RAPD assay

No.	Name	Origin	No.	Name	Origin
1	ThomNhat	Long Dinh	16	ThomThailanMC	LongDinh
2	UcI	Long Dinh	17	ThomTrungAn	Long Dinh
3	KhomTayNinh	CLRRI	18	ThomBT39	CLRRI
4	UcII	Long Dinh	19	ThomKienGiang	LongDinh
5	Codevoire	Long Dinh	20	ThomBT24	CLRRI
6	DailoaBL	Long Dinh	21	ThomBT28	CLRRI
7	MontiquiI	Long Dinh	22	ThomBT22	CLRRI
8	Thomphung	Long Dinh	23	ThomBT23	CLRRI
9	ThomBT9	CLRRI	24	ThomBT25	CLRRI
10	KhomHaNoi	CLRRI	25	ThomBenLuc	LongDinh
11	ThomTayNinh	CLRRI	26	ThomBT27	CLRRI
12	ThomThaiLan	Long Dinh	27	ThomBT32	CLRRI
13	ThomDN17	Long Dinh	28	ThomBT29	CLRRI
14	DailoanI	Long Dinh	29	ThomBT30	CLRRI
15	ThomLamDong	Long Dinh	30	ThomBT31	CLRRI

DNA extraction for PCR analysis

DNA suitable for PCR analysis was prepared using a simplified miniscale procedure (Lang 2002). A piece of young rice leaf (2 cm) was collected and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf was ground using a polished glass rod in a well of a Spot Test Plate (Thomas Scientific) after adding 400 µl of extraction buffer (50 mM Tris-HCl pH 8.0, 25mM EDTA, 300mM NaCl and 1% SDS). Grinding was done until the buffer turned green which is an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of the extraction buffer was added and mixed into the well by pipetting. Around 400 µl of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized using 400 µl of chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA precipitated using absolute ethanol. DNA was air-dried and resuspended in 50 µl of TE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0). An aliquot of 1 µl is sufficient for PCR analysis.

PCR amplification

PCR amplification components and conditions were done based on the methods used by Lang 2002. The PCR reaction mixture contained 20-50 nanogram (ng) template DNA, 50ng of each primers, 0.05 mM dNTP's, 1xPCR buffer (10mM Tris pH 8.4, 50mM KCl, 1.8mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 unit of *Taq* DNA polymerase in a total volume of 20 µl. Template DNA was initially denatured at 93^{o}C for 5 minutes followed by 35 cycles of PCR amplification using the following parameters: 1minute denaturation of 93^{o}C, 1minute primer annealing at 37^{o}C and 2 min primer extension at 72^{o}C. Completion of primer extension was alllowed by a final 8 min incubation at 72^{o}C.

An aliquot of 10 µl of the PCR product was routinely taken for gel electrophoresis to determine if amplification was successful. When the primers detected an amplicon length polymorphism, the samples were readily scored. The PCR products or the DNA fragments produced by restriction digestion were resolved electrophoretically on 1.2% agarose gel in 1 X TBE buffer.

Data analysis

Standard population genetic parameters were used to estimate genetic polymorphism and population genetic structure for individul accessions and groups of accessions, including the proportion of polymorphic loci(P), the mean number of alleles among all loci (A) and among polymorphic loci (A p) and estimated heterozygosity (H). Genetic distances were computed according to Nei (1975). Computation were facilitated by the PC- based program NTSYS (Rohlf 1992).

RESULTS

Phenotyping

There are many pineapple varieties cultivated by farmers in their small patchy farmlands. Some of the varieties are good fruits quality, but are low yielding. Very popular varity is Cayennes so-called "Tho", another is "Khom" concerning "Queen pineapple". They were distinguished by thorn presence in leaf edges, and fruit shape. Khom and Tho naturally flower from April and havesting from June to July.

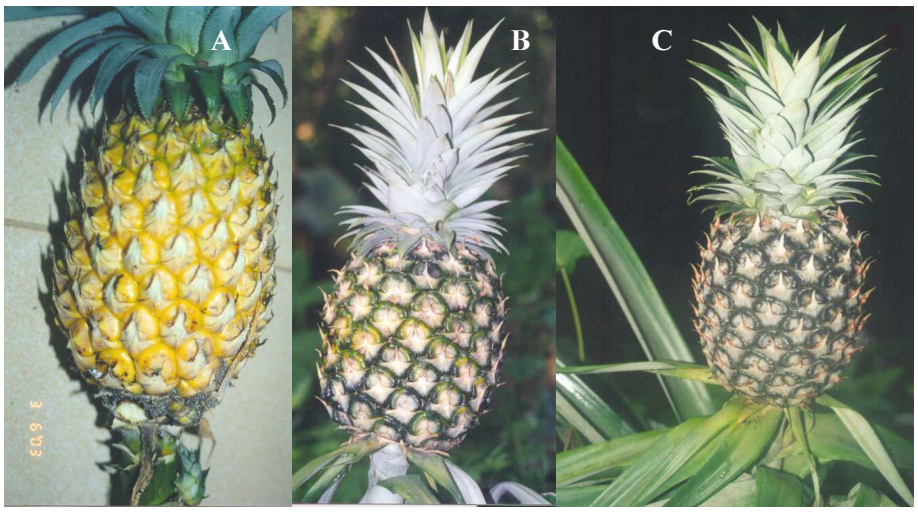

Figure 1: A Khom Cam Giang (Tay Ninh) , B: Tho Ben tre and C: Cayenne

Figure 2: A: Cayenne from Ben Tre and B: Cayenne from Can Tho

Genotyping

Of 17 primers, we resolved 16 loci (approximately 1.4 loci per primer) and 60 alleles (approximately 3.8 alleles per locus). Allele frequencies for individual populations were appropriate to individual. Figure 3 shows the amplification patterns generated using the primer RAPD 5 in 30 individuals from Cayenne.

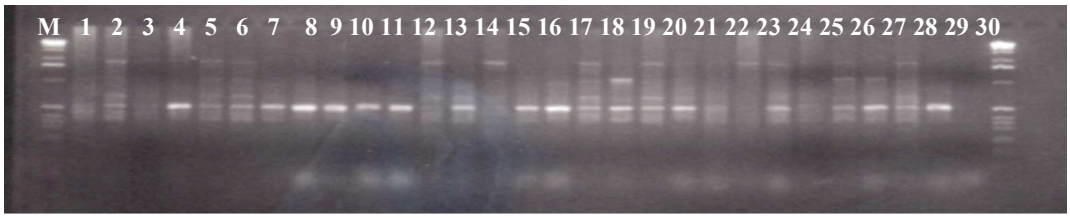

Figure 3: RAPD patterns obtained on the garose gel : lane 1 –30: Cayenne pineapple exhibited different genotypes

A UPGMA dendrogram was constructed to elucidate the genetic relationships among 30 pineapples based on Nei's (1975) genetic distances. The dendrogram generally separates the accessions according to their taxonomic designations. All "Khom" accessions are separated into one cluster. "Thom Ben tre" acession 39, 23, 27 and Thai pineapple are clustered together into a common subcluster. One cluster representing collections from Bentre (acc. 22, 24, 25, 28, 29, 30, 31, 32) is closely related to Thom Ben luc. Thom Uc I and Uc II from (Australia) were in the same cluster

Table 2. List of RAPD markers used to analyse genetic diversity of pineapple

Primer	Sequence 5'----------3'	No. of bands detected	Size of products (kb)
RAPD 1	GGTGCGGGAA	2	0.3 - 0.2
OPA_03	GCTCAGCCAC	4	0.4 - 0.3
OPA_04	AATCGGGCTG	5	0.3 - 2.5
OPA_10	GTGATCGCAG	6	0.2 - 1.5
OPA_13	CAGCACCCAC	8	0.5 - 2.5
OPC_06	GAACGGACTC	4	0.3 - 1.7
OPC_11	AAAGCTGCGG	4	0.3 - 1.3
OPC_15	GACGGATCAG	5	0.3 - 2.5
OPD_02	GGACCCAACC	4	0.3 - 1.3
OPD_03	GTCGCCGTCA	4	0.2 - 1.3
OPD_07	TTGGCACGGG	4	0.3 - 2.5
OPD_08	GTGTGCCCCA	4	0.2 - 1.2
OPD_13	GGGGTGACGA	5	0.5 - 1.2
RAPD 2	GTTTCGCTCC	5	0.7 - 1.6
RAPD 3	GTAGACCCGT	3	0.2 - 3.0
RAPD 4	AAGAGCCCGT	4	7.5 - 1.5
RAPD 5	AACGCGCAAC	6	0.5 - 1.8
RAPD 6	CCCGTCAGCA	2	1.1 - 2.5

UPGMA dendrogram of systematic relationships among 30 populations based on Nei's (1975), genetic distance derived from allele frequencies at 17 polymorphic RAPD loci were recognized. Genetic evidence suggests that these Cayenne pineapples have at least three different origins.

Table 3. Genetic divesity analysis of seven major cluster in collected pineapples

Cluster	N	A	Ap	P	H	U
Thom BT	34.5	2.1	3.0	0.55	0.33	33
Thom Uc	2.0	1.8	2.5	0.46	0.12	28
Thom Phap	2.0	1.5	2.3	0.4	0.34	24
Thom Dai Loan	2.0	2.7	3.17	0.72	0.22	43
Thom Thai Lan	3.0	2.7	2.0	0.72	0.27	43
Thom Nhat	1.0	2.4	1.1	0.62	0.33	37
Khom	2.0	1.5	2.0	24	0.14	24

N: average number of plants sampled per accession.;
A: average number of allele per locus
Ap: average number of alleles per polymorphic locus;
P: proportion of loci polymorphic.;
H estimated heterozygosity;
U: muber of unique alleles per group with the Cayenne alleles.

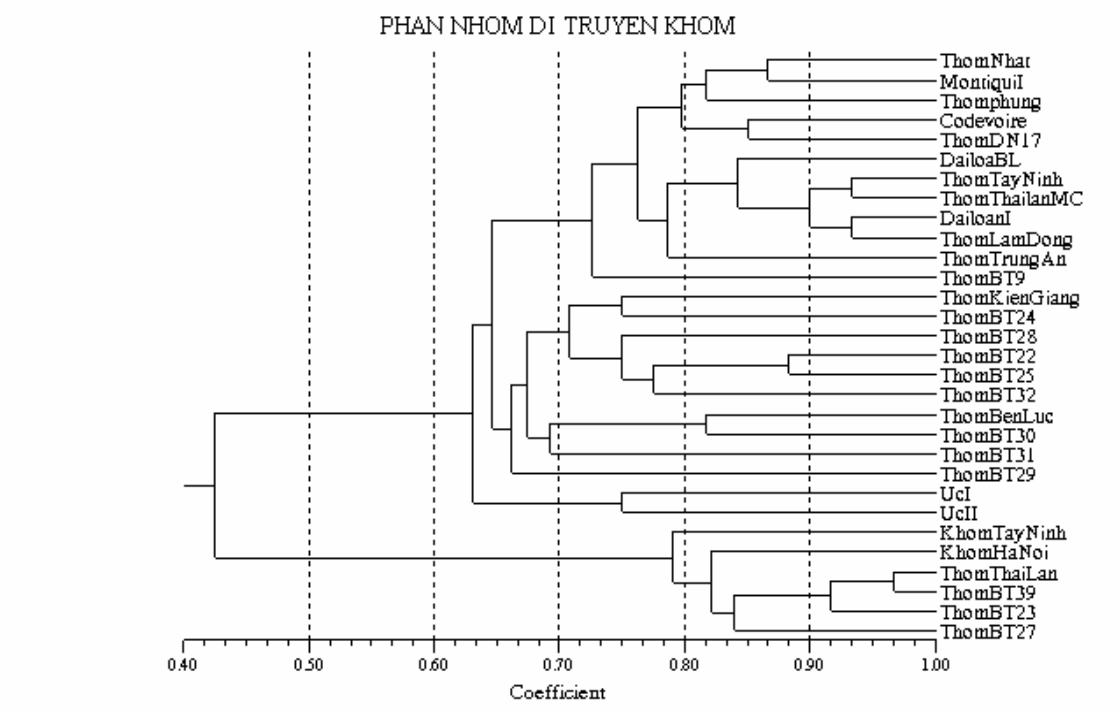

Figure 4: Dendrogram of collected pineapple samples through NTSYS-pc analysis.

Acknowledgements

Thanks are due to Mr Pham ngoc Lieu, Vice Director of SOFRI for supply breeding materials; Dr Hoang Chung Lam, Vegetable and Fruit Research Institute, Hanoi for his support to the study.

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SUMMARY IN VIETNAMESE

Phân tích đa dạng di truyền các giống dứa cayenne

Dứa Cayenne có nhiều nguồn gốc du nhập khác nhau được thu thập từ các địa phương, từ Viện Cây Ăn Qủa Long Định, Viện Lúa ĐBSCL, với số mẫu giống phân tích là 30, sử dụng khóm Bến Lức (nhóm Queen) làm đối chứng. Phân tích kiểu gen bằng RAPD marker tại 17 loci cho thấy có thể chia tập đoàn này thành 7 nhóm kiểu gen khác nhau. Ngay cả trong một quần thể cũng thể hiện sự không đồng nhất về kiểu gen. Nghiên cứu này cần được tiếp tục để hiểu rõ nội dung một cách hệ thống hơn