Genetic diversity of some peanut cultivars based on SSR and rapd molecular markers in Vietnam

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Abstract

RAPD and SSR markers have been used for analyzing the genetic diversity of 30 individuals of different peanut varieties collected from different geographical locations in Vietnam. Analysis of PCR products of 26 RAPD primers showed a total of 273 amplified fragments, 235 fragments of them were polymorphic. On the other hand, SSR markers analysis cleared that there were 44 alleles resulting from 12 SSR primer pairs, 38 alleles of them were polymorphic. Phylogenetic analysis proved the genetic similarity and relationship between the 30 peanut individuals analyzed. Genetic similarity ranged from 0.660 between L14 - QNGAI and L14-L20 to 0.881% between DU-L27 and LDH09-L19066 base on RAPD and SSR markers. The observed number of alleles (na), effective alleles (ne), Nei's gene diversity (h) and Shannon's information index (I) were observed at the individuals level as 1.868, 1.395, 0.239 and 0.371 respectively.

These results showed the abundant genetic variability in the individuals of different peanut varieties. Nei's gene diversity investigation showed that genetic diversity was mainly found within geographical populations. The results of UPGMA analysis showed that geographical isolation is an important factor in the observed genetic differentiation. A similarity tree based on the combination between RAPD and SSR shows that two main clusters, the first main cluster contained L14 and LACDOBG while the second contained the remaining twenty eight Peanut cultivars. Phylogenetic analysis showed the genetic distance and the genetic similarity among the 30 Peanut cultivars. These results may be due to the origin of the cultivars and all peanut cultivars may result from the true species peanut and its inter-hybridization.

Keywords: Peanut, Genetic, Diversity, Molecular marker, Phylogenetic, RAPD and SSR.

Introduction

Plant genetic resources are critical in the diversity and development of agricultural production. The evaluation of funding is important in the protection and sustainable use of plant resources, Gene analysis is crucial not only for identifying distinct varieties/species but also for understanding the genetic relationships between them in order to preserve genetic diversity. Peanut (*Arachis hypogaea* L.) (2n = 4x = 40) is one of the most important oil crops in the world, widely grown in many regions, from the Americas, Africa and Asia with an annual cultivated area over the globe to nearly 22.2 million hectares with an output of approximately 35 million tons, the average yield is 15.5 quintals/ha[6,9,12,14,15,21].

In Vietnam, groundnut is one of the main crops. Peanut is both a food plant and a good soil plant. Peanut is an export crop that brings high income to farmers. Among the 100 peanut-growing countries in the world, Vietnam ranks 10th in terms of area. Among the 25 peanut-growing countries in Asia, Vietnam ranks fifth in the planted areas after India, China and Myanmar and Indonesia. Peanut trees are planted everywhere from the Southeast provinces to the Northern mountainous areas. Groundnut area accounts for 28% of the total annual industrial crop area[2].

Even though peanut is an important crop economically and nutritionally, narrow genetic diversity and a deficiency of polymorphic DNA markers in the public database have hindered genetic mapping and the application of molecular breeding in cultivated peanut. Nevertheless, the peanut research community still lacks adequate tools and resources for peanut genetic and genomic research and breeding and therefore, for expanding our basic knowledge of the genetic control of complex traits[12].

Recently, the explosive development of methods and techniques in the field of molecular biologies such as RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSR (simple sequence repeat) and DNA barcodes has been used to assess genetic variability and conduct evolutionary studies in different crops[1,2,5,7,10,17,18] and techniques have resulted in excellent tools that may be immediately used to biodiversity conservation research.

Molecular biology techniques have advantages in the ability to identify diversity at the gene level, creating a basis for assessing the value of preserving the genetic resources of individuals within the same species or between different species, which is the basis for subspecies classification, new species discovery and evolutionary relationships between species. These methods overcome the disadvantages of phenotypic-based genetic diversity assessment. Compared to other kinds of markers such as RFLPs, RAPDs and SSR markers are co-dominant and multi-allelic in inheritance,

have a higher level of DNA polymorphism in cultivated peanuts and are easier to amplify with less DNA quantity and low cost[11,13]. Recent studies have shown that SSRs can detect more polymorphism in cultivated peanut than RFLP, RAPD and AFLP[1,5,10].

In recent years, a large number of SSR markers for peanut have been developed from genomic DNA libraries and expressed sequence tags (EST) with the goal of providing sufficient sequence resources for developing a critical mass of DNA markers for the community[7,8], making it feasible to use SSRs to construct a genetic linkage map.

In this study, the RAPD and SSR markers indicator was used to study the genetic diversity of local peanut varieties and imported quality varieties, popularly sown in Vietnam, helping to provide useful information for the study of selecting and breeding high-quality disease-resistant peanut variety.

Material and Methods

Plant Materials: In this study, a total of 30 individuals of different peanut varieties were collected from the different geographical locations of Vietnam. The origin of each individual is mentioned in table 1. All 30 peanut varieties were grown in net house, Institute of Biotechnology, Hue University.

DNA Isolation: Total DNA was extracted and purified from fresh leaves of each peanut variety by CTAB (N-cetyl-N, N, ntrimethylammonium bromide) method as described by Cuc et al[3] with slight modifications: 200 mg leaves were cut into small pieces and ground with 500 µL buffer CTAB extraction (2% CTAB, 100 mM Tris-HCl, 20 mM EDTA and 1.4 M NaCl, pH 8.0), supplemented with 2% β-mercaptoethanol, 1% polyvinylpyrrolidone and incubated at 60°C for 60 min. DNA samples were purified in two stages.

Stage 1: Total DNA from each leaf sample was purified twice with the same volume of chloroform: isoamylalcohol (24:1) and precipitated with 2/3 volume of isopropanol at -20°C and centrifuged at 10000 rpm for 10 minutes to obtain a precipitate and then re-dissolved in 100 µL of TE buffer (10 mM Tris-HCl, 1 mM Na2EDTA). Remove RNA with 100 µg/µL RNase A at 37˚C for 60 min.

Stage 2: The solution containing total DNA will be purified for the second time with 1/2 volume of phenol + 3/4 volume of chloroform:isoamylalcohol (24:1) (conducted twice). Total DNA in the solution will be precipitated with 1/10 sodium acetate and 2.5 ethanol -20˚C for 60 minutes and centrifuged at 6000 rpm for 10 minutes to collect the precipitate to dry at room temperature for 20 min, DNA corpuscles were re-dissolved in TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0).

Table 1
List of samples used in the study

S.N.	Sample code	Sampling location	S.N.	Sample code	Sampling location
1	DU	Tay Nguyen	16	BF10	Bac Giang
2	L27	Gia Lai	17	L21090	Agricultural Science Institute of North Central Vietnam (ASINCV)
3	BVMAI	Bacgiang Agriculture and Forestry University	18	L19082	Agricultural Science Institute of North Central Vietnam (ASINCV)
4	PHUYEN	Phu Yen	19	QNGAI	Quang Ngai
5	CNC	Agricultural Science Institute for Southern Coastal Central of Vietnam	20	LYTN	Tay Nguyen
6	L11096	Bacgiang Agriculture and Forestry University	21	LDH09	Agricultural Science Institute for Southern Coastal Central of Vietnam
7	FVL01	Nghe An	22	SELANG	Quang Nam
8	CUCHT	Ha Tinh	23	L19066	Bacgiang Agriculture and Forestry University
9	SENNA	Nghe An	24	L20	Agricultural Science Institute of North Central Vietnam (ASINCV)
10	LDH01	Agricultural Science Institute for Southern Coastal Central of Vietnam	25	L19097	Bacgiang Agriculture and Forestry University
11	TL03	Institute of Biotechnology, Hue University	26	QNAM01	Quang Nam
12	L14	Thua Thien Hue	27	SERAN	Tay Nguyen
13	BF7	Bac Giang	28	SEDARAN	Ha Tinh
14	L19	Quang Binh	29	L2100	Bacgiang Agriculture and Forestry University
15	LACDOBG	Bacgiang Agriculture and Forestry University	30	L21088	Bacgiang Agriculture and Forestry University

The concentration and purity of total DNA were determined by spectrophotometry on a NanoDrop ND-1000 (Thermo, USA) and 1% agarose gel electrophoresis. The DNA solution was stored at -20°C for use in PCR-RAPD and PCR-SSR reactions.

PCR Amplification: The RAPD and SSR primers were selected from common RAPD and SSR primers reported to have been used in other plants. The screened primers were then used to amplify all samples studied. The screening of RAPD and SSR primers for all individuals shows that 26 RAPD primers (University of British Columbia) and 12 SSR primers[16,25] (Tables 3 and 4) were selected based on high polymorphism, maximum variability and good reproducibility of the fragments (alleles) generated. All primers were synthesized by IDT company (USA).

The PCR-RAPD reaction mixture consisted of 10 µL 2x PCR Master Mix (GoTaq Green Master Mix 2X, Promega, USA), 20 pmol of random primers, 50 ng of total DNA and sterile distilled water enough for a total reaction volume of 20 µL. PCR program conditions consisted of 94°C for 5 min, then 35 cycles of 94°C for 1 min followed by 36°C for 1 min and 72°C for 3 min and one cycle at 72°C for 10 min.

The PCR-SSR amplifications were conducted in a total volume of 20 µl solution containing 50 ng of genomic DNA, 10 µL 2x PCR Master Mix (GoTaq Green Master Mix 2X, Promega, USA), 10 pmol each of forward and reverse primers and sterile distilled water enough. PCR program used for the amplification consisted of a cycle of 95°C for 5 min, 40 cycles of 95°C for 45s followed by annealing step at 60°C for 30 s with -1°C/cycle for 10 cycles, then at 50°C for 30 cycle, then at 72°C for 10 min and one cycle at 72°C for 7 min.

DNA Electrophoresis: Amplified PCR products were separated by electrophoresis in 3.5% agarose gels, run in 0.5×TBE buffer for 4 h at 120 V and stained with 0.5 mg/L of ethidium bromide. A photographic record was taken under UV transilliminator (using Gell documentation camera).

Data Analysis: Based on the results of electrophoresis, bands that are present (which are clear, undistorted bands) will be numbered "1", if not present (or are too faint) will be numbered "0". The size of each PCR product band was estimated based on the standard marker. Based on POPGENE (version 1.32), diversity indices were identified including Nei's gene diversity (h), Shannon's information index (I), observed number of alleles (na), effective number of alleles (ne)[26]. Besides, the data in the form of a logical matrix is fed into the NTSYSpc 2.1 program to build a phylogenetic tree using the UPGMA algorithm, with a distance matrix established based on on SM similarity coefficient (Simple Matching, SM ∈ [0;1])[23].

Results and Discussion

Total DNA extraction results: Fresh leaf samples of 30 individuals of different peanut varieties were extracted and purified by CTAB method as described by Cuc et al[3].

The electrophoresis results in figure 1 show that the total DNA extracted from the leaves of the studied samples gives a single, clean, unbroken, clear band. Quality DNA is guaranteed to be used as a raw material for further experiments (Figure 1).

Peanut Cultivars genetic characterization based on RAPD and SSR-PCR products: Out of a total of 30 individuals of different Peanut Cultivars used in PCR amplification of 26 RAPD primers, a total of 4452 DNA fragments were obtained. Different individuals have a different number of DNA fragments formed, ranging from 129 to 163 fragments, the mean 148.400 DNA fragments per sample.

BVMAI has the most higher number of amplification fragments with created 163 DNA fragments (accounting for 3.661% of the total number of DNA fragments formed) followed by individual QNAM01 having 157 fragments of DNA, accounting for 3.527% of the total number of fragments formed. Individuals with the same number of DNA fragments formed were L19, L20 and L19097 (with 156 fragments of DNA, accounting for 3.504% of the total number of fragments formed).

The individual with the least number of DNA fragments obtained was L14 with 129 DNA fragments accounting for 2.898%. Thus, the number of amplification fragments obtained in individuals of different peanut Cultivars for the 26 studied primers gave a high rate (Table 2).

Meanwhite, SSR-PCR products performed on 30 individuals of different peanut Cultivars with 12 primers showed that a total of 717 alleles were formed.

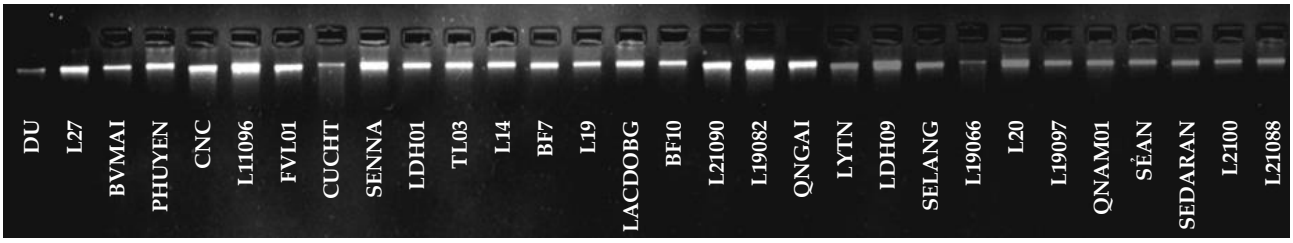

Figure 1: Electrophoresis of total DNA from fresh leaf samples of 30 individuals of different peanut varieties

Table 2
List of 30 analyzed Peanut Cultivars and their total genetic characterization based on RAPD and SSR markers

Individual	Total number of fragments	Total number of alleles	Individual	Total number of fragments	Total number of alleles
DU	154	24	BF10	147	26
L27	150	29	L21090	155	26
BVMAI	163	24	L19082	150	28
PHUYEN	151	22	QNGAI	149	28
CNC	142	22	LYTN	144	22
L11096	148	19	LDH09	137	28
FVL01	147	23	SELANG	149	26
CUCHT	145	18	L19066	144	26
SENNA	141	20	L20	156	28
LDH01	151	19	L19097	156	28
TL03	154	20	QNAM01	157	23
L14	129	22	SERAN	146	23
BF7	141	16	SEDARAN	147	26
L19	156	18	L2100	150	31
LACDOBG	142	26	L21088	151	26
Total of RAPD	**2214**	**-**	**-**	**2238**	**-**
Total of SSR	**-**	**322**	**-**	**-**	**395**

The number of alleles between different individuals is different, ranging from 16 to 31 alleles, with an average of 23.900 alleles per sample. Individual L2100 gave the highest number of alleles formed (31 alleles, accounting for 4.324% of the total number of formed alleles). The individual with the smallest number of alleles is BF7 (16 alleles, accounting for 2.232% of the total number of alleles formed) (Table 2).

RAPD Analysis: Research results in table 3 show that there are 272 DNA fragments amplified detected from 26 random primers, of which 235 are polymorphic DNA fragments, accounting for 86.461%. The mean number of DNA fragments amplified per peanut individual and polymorphic DNA fragments amplified per primer observed were 9.038 and 10.462 respectively. The DNA fragment size ranges from 170-2800 bp. The primers RAPD such as UBC#303, UBC#304, UBC#310, UBC#311, UBC#312, UBC#314, UBC#315, UBC#317, UBC#320, UBC#322, UBC#323, UBC# 325, UBC#336, UBC#338, UBC#343, UBC#351, UBC#356, UBC#361, UBC#364 and UBC#380 for the maximum number of amplified Individual 30/30, accounted for 100% with the number of DNA fragment ranging from 6 to 19 fragments followed by primers UBC#302, UBC#306, UBC#308, UBC#354 and UBC#374 for the number of individuals successfully amplifying 29/30 individuals, accounting for 96.67% with the number of DNA fragments formed ranging from 6 to 14 fragments. Primer UBC#385 with the lowest number of successful PCR amplification was 27/30 individuals (accounting for 90%).

The percentage of polymorphic fragments obtained per RAPD primer ranged from 50 to 100% (Table 3). Analysis of the genetic diversity coefficient of individuals in the population (h) based on the RAPD indicator showed a large genetic diversity in the studied samples. Of the 26 random RAPD primers used in the study, UBC#325 showed the highest diversity with a mean h = 0.377 followed by primer UBC#317 (h = 0.371). The lowest diversity was in primer UBC#303 (h = 0.072). The diversity factor in each random primer ranged from about 0.072 to 0.377, with a mean of 0.232. Shannon's information index (I) shows the corresponding result ($I_{UBC\#303}$ = 0.137 and $I_{UBC\#325}$ = 0.554, mean = 0.326) (Table 4).

The observed number of alleles (na) for the 26 RAPD primers used in the genetic diversity assessment of the peanuts population, ranged from 1.00 to 2.000 and reached a mean value of 1.869 ± 0.338. The RAPD primers with the highest observed allele value were UBC#302, UBC#311, UBC#317, UBC#320, UBC#325, UBC#380, UBC#306, UBC#385, UBC#313, UBC#315, UBC#322, UBC#336 correspond to the value na = 2. The RAPD primer for the lowest observed allele value is UBC#310 corresponding to na = 1.500. In addition, the effective number of alleles (ne) between primers also varied, ranging from 1.085 to 1.681 and reaching the mean value of 1.381 ± 0.350 in which UBC#313 gives the highest value corresponding to ne = 1.558 and primer UBC#310 gives the lowest obtained value corresponding to ne = 1.349 (Table 4). Primer UBC#313 showed the greatest genetic diversity for 30 peanut cultivars out of 26 RPAD primers.

According to a study by Nei[20], the greater is the number of amplified DNA fragments, the greater is the ability to distinguish patterns on the phylogenetic tree, in which the minimum number of polymorphic fragments is 50% to be able to build a pedigree tree exactly[20]. With 26 primers RAPD used, we have obtained 235 polymorphic DNA fragments from 30 individuals of different cultivars of peanut to serve for genetic diversity research and

phylogenetic tree building. Therefore, the data obtained after analyzing 26 RAPD primers is sufficient for this study.

On the other hand, in our study, the polymorphism percentage (86.461%) increased compared to Raina et al[22], Dwivedi et al[4] and Suryadi et al[24] as 42.7%, 18.74% and 7.55% respectively of polymorphism among selected peanut cultivars using the same RAPD technique[4,22,24].

The genetic similarity matrix results are in harmony with those obtained by Dwivedi et al[4] who reported that the genetic similarity values among selected groundnut germplasm ranged from 59.0% to 98.8 % with an average of 86.2%. NaguibNemat et al[19] reported that the genetic similarity among peanut cultivars ranged from 0.68 to 0.92 with an average of 0.8. (in our study ranged from 0,666 to 0,910, mean = 0,788).

SSR Analysis: The total number of alleles produced by twelve SSR primer pairs was 44 alleles, there are 38 polymorphic loci accounting for 86.111%, with size alleles ranging from 150 to 650 bp. The mean number of alleles created per peanut individual and polymorphic alleles per primer observed were 3.667 and 3.167 respectively. GM613 primer gave the highest alleles number per loci (7 alleles). Meanwhile, GM621, GM695 and AHCW0698 primers gave the lowest alleles number per loci (2 alleles). The observed number of alleles (na) and an effective number of alleles (ne) ranged from 1.500 (GM695 primer) to 2.000 (GM613, GM617, GM621, GM693, AHCW0698, AHCW2506 and AHCW3758 primers) and 1.071 (GM695 primer) to 1.882 (AHCW2506 primer) respectively.

The genetic diversity coefficient (h) according to Nei[20] ranged from 0.062 (GM695) to 0.468 (AHCW2506), mean = 0.238. Shannon's Information index (I) shows the corresponding result (I_{GM695} = 0.122 and $I_{AHCW2506}$ = 0.661, mean = 0.428). The number of polymorphic loci obtained at different primers will give different results, ranging from 1 to 7 alleles, mean = 3.667 allele per primer. Primer GM613 gave the most highly polymorphic loci (7 alleles, h = 0.288, I = 0.453) (Figure 2 and table 4) and primer GM695 gave the lowest polymorphic loci (1 allele, h = 0.062, I = 0.122). The most highly h and I (0.468 and 0.661, respectively) were obtained from AHCW2506 primer as presented in table 4.

Study by Cuc et al[2] showed that sixty four groundnut varieties were selected for the study with 50 SSR markers. A total of 78 alleles were detected. Of the 50 markers used for screening, only 9 polymorphic markers were used. Polymorphic information content (PIC) values ranged from 0.0 to 0.75, with an average of 0.18. Genetic similarity coefficient of 64 studied groundnut varieties ranging from 0.57 to 0.97[2]. This result is similar to the result of our study (genetic similarity coefficient ranging from 0.488 to 0.955). Meanwhile, Wang et al[25] have been identified polymerase chain reaction (PCR) primer pairs designed for 4340 novel SSR markers. 210 new SSRs were validated using 24 A. hypogaea varieties. Of the 210, 191 (91%) yielded PCR products with 37 (18%) identifying polymorphisms. The 37 polymorphic SSR markers detected 146 alleles (2–10 alleles per locus) and the average polymorphic information content was 0.403 (with a range of 0.077 to 0.819)[25]. The results of this study are similar to the results obtained from our study (Table 5).

Another study showed that the primer pairs were designed for fifty-six different microsatellites, 19 of which showed polymorphism among the genotypes studied. The average number of alleles per locus was 4.25 and up to 14 alleles were found at one locus. This suggests that microsatellite DNA markers produce a higher level of DNA polymorphism than other DNA markers in cultivated peanut[11]. These results show a higher number of alleles than our results when using 12 polymorphic primers for 30 different peanut varieties in Vietnam.

Cuc et al[3] show that 104 (61.2%) primer pairs yielded scorable amplicon and 46 (44.2%) primers showed polymorphism among 32 cultivated groundnut genotypes. The polymorphic SSR markers detected 2 to 5 alleles with an average of 2.44 per locus. The polymorphic information content (PIC) value for these markers varied from 0.12 to 0.75 with an average of 0.46. Based on 112 alleles obtained by 46 markers, a phenogram was constructed to understand the relationships among the 32 genotypes.

Phylogenetic Analysis: The information based on RAPD and SSR data was used to calculate the similarity and the genetic distance between the analyzed 30 peanut cultivars. Similarity matrix (data dose not presented) indicated that genetic similarity among analyzed peanut cultivars ranged 0.660 between L14 - QNGAI and L14 - L20 and 0.881% between DU-L27 and LDH09-L19066.

Similarity tree based on the combination between RAPD and SSR (Figure 3) shows that the first main cluster contained L14 and LACDOBG, while the second main cluster splits into two sub-clusters, the first sub-cluster consisted of LYTN, LDH09, L19066, L20, L19097, L2100, L21088, QNAM01, SELANG, SERAN and SEDARANG. Otherwise, the second sub-cluster included two groups, the first group included L11096, FVL01, CUCHT, SENNA and LDH01, while the second group included two sub-groups, the first sub-group contained DU, L21, BVMAI and PHUYEN and the second subgroup contained CNC, BF10, TL03, BF7, L19, L21090, L19082 and QNGAI.

The second sub-group contains two branches, the first branch consists of CNC and BF10 while the second branch included two- branchs, the first sub-branch contained TL03, BF7, L19 and the second sub-branch contained L21090, L19082 and QNGAI. Phylogenetic analysis showed the genetic distance and the genetic similarity among the 30 Peanut cultivars (Figure 3).

Table 3
Number of cultivars and number of amplified fragments in each primer RAPD

Primer	Sequences 5' to 3'	Number of amplified cultivars	The rate of amplified cultivars (%)	Number of total amplified fragments	Number of polymorphic fragments	Size range (bp)	Polymorphism (%)
UBC#302	ACTTCCTCCA	29	96,67	10	10	170-1700	100
UBC#303	GGTCTCCTAG	30	100	11	6	300-1400	54.55
UBC#304	CCTCACCTGT	30	100	10	9	250-1700	90
UBC#306	CGGAGAGCGA	30	100,00	14	14	200-2450	100
UBC#308	CCGGCATAGA	30	100,00	13	12	380-1600	92.31
UBC#310	GCCGCTACTA	30	100	6	3	280-1000	50
UBC#311	GACATCTCGC	30	100	8	8	250-2200	100
UBC#312	ACAGGGAACG	30	100	10	9	230-1600	90
UBC#314	TCTAAGCTCG	29	97	5	5	300-1050	100
UBC#315	ATACGGCGTC	30	100	9	9	250-1300	100
UBC#317	GCGAACCTCC	29	97	13	13	200-2500	100
UBC#320	AACGCGTAGA	29	97	9	9	300-1500	100
UBC#322	ATGGCAAAGC	27	90	9	9	250-1050	100
UBC#323	TGGACCACCC	30	100	19	15	200-2800	78.95
UBC#325	TCCCGAACCG	30	100	8	8	350-1250	100
UBC#336	CACGGCTGCG	29	97	9	9	360-1200	100
UBC#338	TGACGCGCTC	30	100	10	7	200-1600	70
UBC#343	CTAGGGGCTG	30	100	14	9	440-2250	64.29
UBC#351	CTCCCGGTGG	30	100	12	11	250-1400	91.67
UBC#354	CTAGAGGCCG	30	100,00	6	3	450-1500	50
UBC#356	GCGGCCCTCT	30	100	11	11	350-2000	100
UBC#361	GCGAGGTGCT	30	100	13	7	400-1900	53.85
UBC#364	GGCTCTCGCG	30	100	13	11	350-1700	84.62
UBC#385	GGTCAACCCT	27	90,00	8	8	300-1700	100
UBC#374	AGGAGTGAGA	30	100,00	9	7	300-2000	77.78
UBC#380	ACCGGGAACG	30	100	13	13	200-2500	100
Mean			**98.590**	**10.462**	**9.038**	**170-2800**	**86.461**
Total				**272**	**235**		

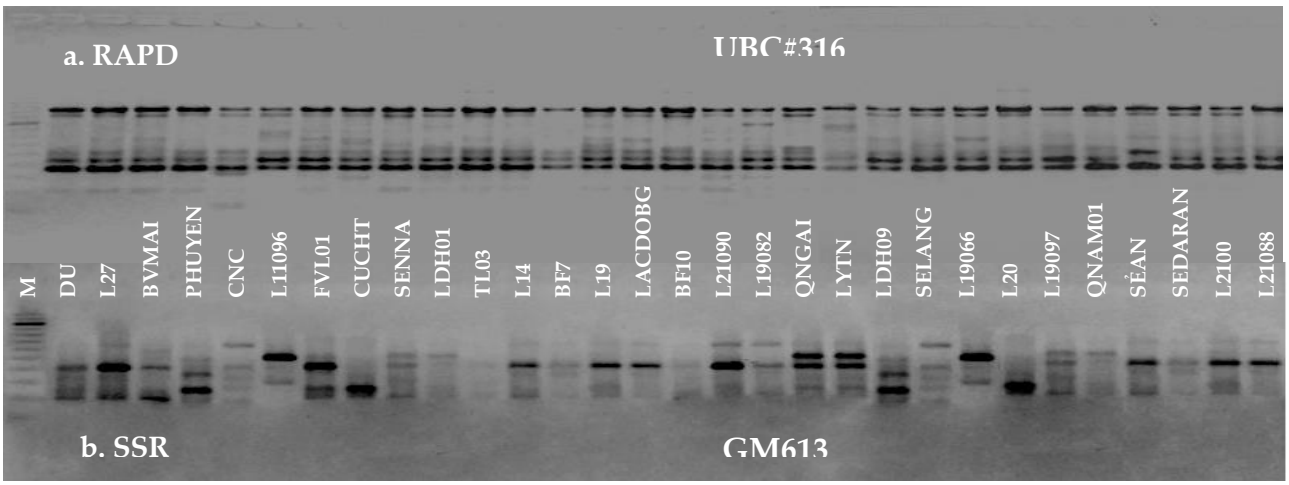

Figure 2: Dendrogram of PCR amplified products of: a) RAPD primer UBC#316; b) Microsatellite GM613 with different 30 Peanut cultivars

Table 4
Number of cultivars and number of amplified fragments in each primer SSR

Primer	Sequences 5' to 3'	Repeat motif	The rate of amplified cultivars (%)	Number of alleles	Number of polymorphic alleles	Size range (bp)	Polymorphism (%)
GM613	F: GCGTGAAATGAGTGTTGTGAG	(AT)21	100	7	7	190-500	100
	R: CATAGCCACCATAGACACCAAA						
GM617	F: CCTCTTCTTGATTTCGTGCTG	(TTA)6	100	5	5	150-480	100
	R: GAAACATACATTCCTCTTGCATCA						
GM621	F: ACCAACGATGCTGCTGATAAC	(AT)9	96.667	2	2	300-400	100
	R: ATCCTCAACCCATGCTTTCTT						
GM623	F: CAGGATGAACAGGCACAGAAT	(TAT)16	100	4	3	300-500	75
	R: ATGAACAATTGCGATTTGGAC						
GM655	F: GAAGACTATTGCACCCTCCAA	(CT)10+(CA)7	100	3	2	400-650	66.667
	R: CGGCACTGAATAGAGCAATGT						
GM693	F; ATGTATAGTGGCGGATCCAAT	(TA)12	66.667	3	3	400-480	100
	R: TTTTGAAGTATTCTCTTTTTCAACA						
GM695	F: TGGTCGCAGATAGTATTTCTCCT	(ATA)19	100	2	1	400-480	50
	R: TGGAATTTGAATCGCACTCTT						
AHCW0698	F: GGAACTGAAGCCCCACACAT	(TC)6	93.333	2	2	300-400	100
	R: CACAACACCATCGCCATCAC						
AHCW1250	F: TCATCGTCCTTCTTTGTCCTCT	(TC)6	100	6	4	280-500	66.667
	R: GGATTGGGAGGGAACGAAGG						
AHCW2133	F: AGCTACTATTCACTGAAACCATCA	(TAA)7	100	4	3	240-480	75
	R: TGTCCGAAATTTGGCAACCG						
AHCW2506	F: ACCTGCTGTTTTAACTCCACA	(AGA)5	66.667	3	3	400-520	100
	R: GGTGGAACAAAACAAGCAGGA						
AHCW3758	F: CTAGGAAACACAGCCCTGGG	(AGA)6	66.667	3	3	350-480	100
	R: TCACCAATTCCTCGTCGCTC						
Mean			**90.833**	**3.667**	**3.167**		**86.111**
Total				**44**	**38**		

Note: Primers SSR: GM613, GM617, GM621, GM623, GM655, GM693, GM695 reference from Li et al[16] and Primers SSR: AHCW0698, AHCW1250, AHCW2133, AHCW2506 and AHCW3758 reference from Wang et al[25].

Table 5
Diversity indices in each primer

Locus	Sample Size	na*	ne*	h*	I*
RAPD					
UBC#302	30	2.000	1.417	0.252	0.394
UBC#304	30	1.900	1.454	0.283	0.434
UBC#311	30	2.000	1.415	0.259	0.408
UBC#317	30	2.000	1.659	0.371	0.545
UBC#320	30	2.000	1.340	0.222	0.363
UBC#325	30	2.000	1.681	0.377	0.554
UBC#374	30	1.778	1.207	0.136	0.232
UBC#380	30	2.000	1.550	0.329	0.497
UBC#308	30	1.923	1.551	0.310	0.461
UBC#323	30	1.789	1.538	0.302	0.443
UBC#343	30	1.857	1.541	0.311	0.461
UBC#306	30	2.000	1.456	0.286	0.447
UBC#351	30	1.917	1.179	0.126	0.226
UBC#354	30	1.500	1.286	0.161	0.238
UBC#385	30	2.000	1.416	0.254	0.397
UBC#356	30	1.909	1.147	0.102	0.190
UBC#361	30	1.538	1.161	0.107	0.180
UBC#364	30	1.846	1.275	0.169	0.275
UBC#312	30	1.900	1.355	0.228	0.363
UBC#313	30	2.000	1.331	0.216	0.356
UBC#315	30	2.000	1.295	0.220	0.371
UBC#303	30	1.545	1.085	0.072	0.137
UBC#310	30	1.500	1.111	0.088	0.157
UBC#322	30	2.000	1.350	0.225	0.365
UBC#336	30	2.000	1.645	0.358	0.525
UBC#338	30	1.700	1.271	0.178	0.285
SSR					
GM613	30	2.000	1.450	0.288	0.453
GM617	30	2.000	1.589	0.342	0.509
GM621	30	2.000	1.221	0.178	0.319
GM623	30	1.750	1.357	0.217	0.331
GM655	30	1.667	1.465	0.267	0.391
GM693	30	2.000	1.688	0.368	0.537
GM695	30	1.500	1.071	0.062	0.122
AHCW0698	30	2.000	1.345	0.250	0.413
AHCW1250	30	1.667	1.401	0.231	0.345
AHCW2133	30	1.750	1.435	0.271	0.409
AHCW2506	30	2.000	1.882	0.468	0.661
AHCW3758	30	2.000	1.688	0.368	0.537
Mean of primer RAPD		**1.869**	**1.381**	**0.232**	**0.362**
St. Dev of primer RAPD		**0.338**	**0.350**	**0.177**	**0.238**
Mean of primer SSR		**1.864**	**1.480**	**0.283**	**0.428**
St. Dev of primer SSR		**0.347**	**0.358**	**0.177**	**0.239**
Mean of primer RAPD+SSR		**1.868**	**1.395**	**0.239**	**0.371**
St. Dev of primer RAPD+SSR		**0.339**	**0.352**	**0.178**	**0.239**

Note: *na = Observed number of alleles; *ne = Effective number of alleles; *h = Nei's (1973) gene diversity;
*I = Shannon's Information index.

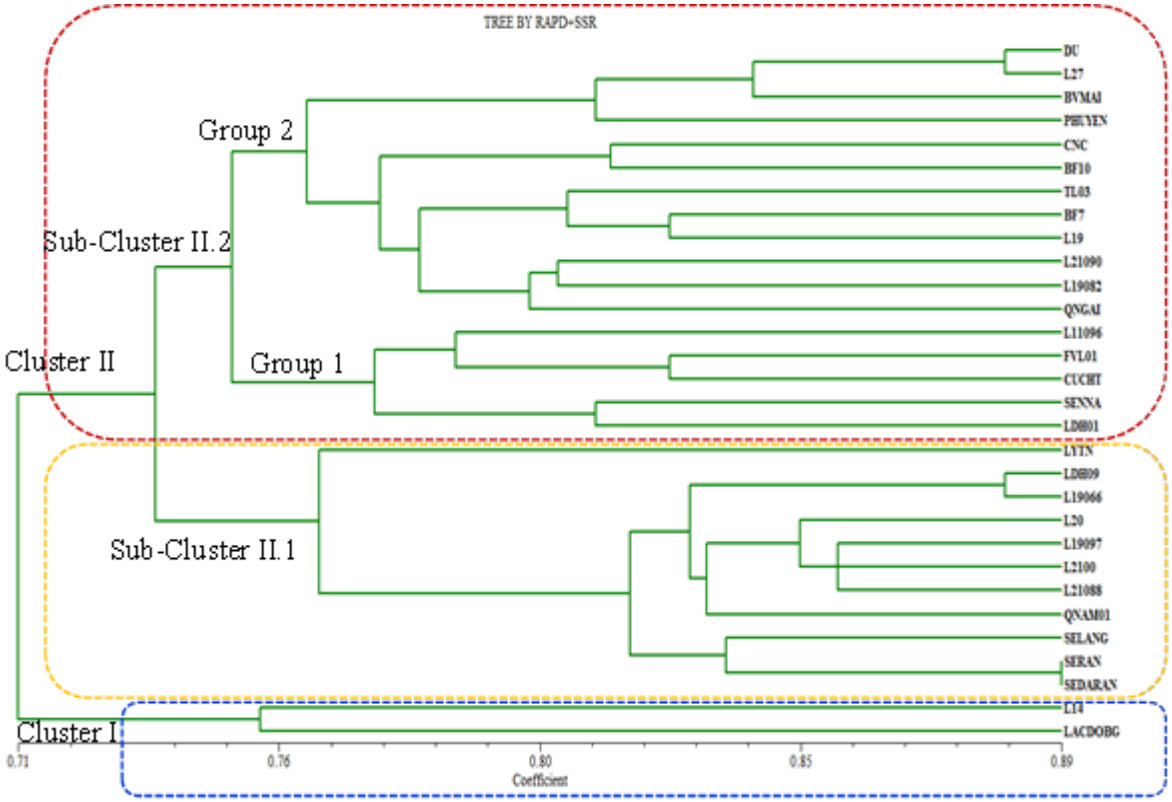

Figure 3: UPGMA tree showing the relationship among 30 cultivars of peanut (*Arachis hypogaea*) base on marker RAPD and SSR

Conclusion

In conclusion, we hereby reported that molecular characterization, marker polymorphisms and genetic diversity analysis of 30 peanut cultivars collected from different locations in Vietnam using RAPD and SSR markers provided a strong method for generating potential marker diagnostics for cultivar analysis. A total of 26 RAPD and 12 SSR markers revealed a mean of 86.461% and 86.111% of polymorphism respectively.

Further, genetic diversity was determined via UPGMA cluster analysis using covariance of samples and genetic distance showing good genetic relatedness between 30 peanut cultivars, diversity and genetic boundary due to different geographical distances. Thus, the study proves that RAPD and SSR markers for genetic diversity studies are good biomarkers in this study.

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